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Genetic diversity of pear cultivars using SSR markers and their reactions to pear rust (*Gymnosporangium fuscum*)

Armut çeşitlerinin SSR markörlerine göre genetik çeşitliliğinin belirlenmesi ve Armut memeli pasına (*Gymnosporangium fuscum*) reaksiyonları

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ABSTRACT

Commonly grown in different ecological conditions of Turkey and the world, pear (*Pyrus communis* L.), as a plant species, has a long cultivation history in Turkey. However, its unique genetic resources are in danger of extinction due to changes in the agro-ecosystem and genetic erosion. In addition, pear rust caused by *Gymnosporangium fuscum* is one of the significant diseases of pear. Severe economic losses have been reported in some pear orchards near the extensive juniper populations. In the present study, the genetic diversity and sensitivity level to pear rust (*Gymnosporangium fuscum*) of 25 local and commercially important pear varieties grafted on seedling and Quince A (QA) was determined using 13 SSR primers. The selected varieties were clustered into two major groups that were closely related. The SSR markers provided reliable genotyping and demonstrated their usefulness for identifying pear genetic diversity. The difference between years and rootstocks according to disease severity rates was found to be statistically significant. Although none of the pear varieties assessed in these experiments were resistant to rust, the disease severity of the pear varieties of QA rootstock was generally higher than that of the seedling varieties.

INTRODUCTION

Pear belongs to Rosaceae, grown in different ecological environments and has a prominent global economic value as a pome fruit tree species. In recent years, an increase has been recorded in the production of pear in many countries. Turkey is the fifth-largest pear producer in the world following China, Argentina, Italy, and the United States. Worldwide pear production is approximately 24 million tonnes, of which Turkey produces 503.004 tonnes (FAO 2017, Lombard and Westwood 1987, Wu et al. 2013). Some pear species are in danger of extinction because of poor agriculture practices, ecosystem changes, genetic erosion

and pathological diseases such as pear rust, fire blight, pear decline. With the advancement in molecular marker technology (AFLP, SSR, RAPD, etc.), research are applying various types of molecular markers for determining genetic diversity, origins of the cultivars, and relationships, for classifying cultivars and species identification (Barakat et al. 2011, Hokanson et al. 1998, Fang and Roose 1997, Gianfranceschi et al. 1998, Koller et al. 1993, Kong et al. 2011, Oliveira et al. 2010, Uzun et al. 2011). The determination of genetic variability and relationships among pear cultivars has been reported by using DNA-based genetic markers,

such as SSRs, RFLP, AFLP and RAPD (Bao et al. 2007, Iketani et al. 1998, Monte-Corvo et al. 2000, Oliveira et al. 1999, Wünsch and Hormaza 2007, Yamamoto et al. 2001).

Simple sequence repeats (SSR) have been used for a long time for determining the genetic diversity and variety identification in homozygous species, for instance, apple, citrus, cherry, rice, wheat, eggplant, and tomato (Aranzana et al. 2002, 2003a, Bouhadida et al. 2007, Cheng et al. 2009, Dirlewanger et al. 2004, Li et al. 2008, Sosinski et al. 2000,; Wünsch et al. 2006). To date, approximately 500 SSRs have been developed and mapped for the Prunus genus (Aranzana et al. 2003b, Dirlewanger et al. 2004, Howad et al. 2005), showing their importance in genetic studies. Representative SSRs with full coverage are required to detect diversity and identify plant species (Infante et al. 2008). In addition, SSR markers are generally preferred, as they can efficiently detect the traits such as co-dominance, polymorphism, and transferability (Brini et al. 2008, Erfani et al. 2012, Hokanson et al. 2001, Powell et al. 1996). As a novel tool, SSR markers have been used to reveal genetic diversity in many pear cultivars (Cao et al. 2007, Kimura et al. 2003, Zhang et al. 2007). However the reports of SSR regarding the pear rust (*G. fuscum*) are still low and there is a need to identify the potential SSR markers, which could be helpful in the breeding of resistant pear varieties.

Pear rust (*G. fuscum*) is an obligate parasite that causes moderate disease in pears. This parasite alternates between species of Pyrus and Juniperus and completes its lifecycle by over-wintering on twigs of some Juniperus species, such as *Juniperus oxycedrus* L., *J. excelsa* Bieb (Anonymous 2008). During spring rains, horn-shaped telial structures on Juniperus species swell and produce teliospores. The teliospores release basidiospores, which are transported by wind to the pear trees (Agrios 2005, Juhasova and Praslieka 2002). *G. fuscum* infects the leaves mostly, along with branches and fruits. It can reduce the pear yield by up to 100% (Agrios 2005, Anonymous

2008). Some fungicides have shown effective against *G. fuscum*, however, pesticide usage has well known negative environmental effects and accordingly humans. Therefore, the determination and development of disease-resistant cultivars offer a suitable alternative to the application of fungicides.

In the present study, we investigated the commercially important pear cultivars (25 cultivars) in Turkey in terms of their genetic diversity and the response of the pear cultivars grafted on the QA and seedling rootstocks to the pear rust (*G. fuscum*) by using different SSR markers.

MATERIALS AND METHODS

A collection belongs to Fruit Research Institute in Egirdir, Isparta-Turkey was used. The responses of commercially important 25 pear cultivars grafted on both seedling rootstock and QA to the pear rust were investigated during the field experiment from 2008 to 2009, followed by SSR marker analysis.

DNA extraction

DNA was extracted from 25 randomly selected young leaves of each cultivar. Extraction was carried out by using the DNA Extraction Kit (Qiagen, Roche). The aliquots (10 µl) of DNA from each sample were loaded on a 0.8% agarose gel to check the quality, and stocks DNAs were stored at -20 °C for further analysis.

SSR-PCR amplification

In the current study, 13 SSR primer pairs (Table 1) were amplified and used according to the method previously described by Yamamoto et al. (2002a, 2002b, 2002c) with some minor modifications. Amplifications were done using a Thermal Cycler (Biorad C1000, Germany) as follows: 50 ng template DNA, 0.3 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl pH 9.0, 220 pmol each primer, 0.5 mM dNTP, 2.5 U Taq DNA polymerase (Takara Ex), in 25 µl final volume.

Table 1. Primer pairs used for SSR analysis (Yamamoto et al. 2002a, 2002b, 2002c)

SSR name	Forward primer sequence (5':3')	Reverse primer sequence (5':3')
NB102a	TGT TAT CAC CTG AGC TAC TGCC	CTT CCT CTT TAT TTG CCG TCT T
CH01D08	CTCCGCCGCTATAACACTTC	TACTCTGGAGGGTATGTCAAAG
NB105a	AAA CAA CCG ACT GAG CAA CAT C	AAA ATC TTA GCC CAA AAT CTC C
NB106a	GTA CGT CGA CAT GAG AGA G	TCT CTT GTT CCT TCC TGC AC
NB109a	ATG CTC TAT AAA ACC CAC CTA CC	AGA GGG ACC ATT GTG TTA TTG TAT
NH002b	GGAGTCAGCGGCAAAAAAAG	CCCCTCCCTCCTCTTATTGT
NB113a	ATG AAA TAT GTC GTG TTG CCC TTA G	CCC TTC CTC AGC ATG TTT CCT AGA C
NH019b	GAG ATG GAG TAG TAA AGA AGA AGG	ACG ACA TAG TGA AAA CAG AAG
NH015a	TTGTGCCCTTTTTCTACC	CTTTGATGTTACCCCTTGCTG
NH009b	CCGAGCACTACCATTGA	CGTCTGTTACCGCTTCT
NH013a	GGTTTGAAGAGGAATGAGGAG	ATTGACTTTAGGGCACATTTC
NH015a	TTGTGCCCTTTTTCTACC	CTTTGATGTTACCCCTTGCTG
KA16	GCCAGCGAACTCAAATCT	AACGAGAACGACGAGCG

Table 2. Pear rust disease scale value (Anonymous, 1996)

Scale value	Symptom
0	No infection
1	2 spots smaller than 10 mm and 1 spot larger than 10 mm
2	3 spots smaller than 10 mm and 2 spots larger than 10 mm
3	4 spots smaller than 10 mm and 3 spots larger than 10 mm
4	5-15 spots, small/large
5	16-20 spots, small/large
6	21-30 spots, small/large
7	More than 31 spots

For amplification, PCR was carried out with an initial denaturation at 94 °C for 3 min, followed by 33 cycles of denaturation at 94 °C for 40 s, annealing at 58 °C for 30 s, elongation at 72 °C for 1 min and a final extension at 72 °C for 10 min. PCR products were separated by electrophoresis on 2% High-Resolution Agar (LONZA Metaphor Agorase). Bands were scored as '0' and '1', for absence or presence in each cultivar, separately. The total number of bands (TNB), the number of the polymorphic band (NPB) and polymorphism for each primer combination were determined. Polymorphism information content (PIC) that was relevant to primers was calculated according to the formula (Smith et al. 1997).

$$PBI=1-\sum fi^2$$

fi = Frequency band

The polymorphism rate was calculated according to the following equation.

$$\text{Polymorphism rate (\%)} = (\text{Number of polymorphic bands}) / (\text{Total number of bands}) \times 100$$

The data matrix was recorded using the NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.11, Exeter Software, Setauket, N.Y., USA, Rohlf, 2000). The similarity indices were calculated according to the method of Dice (1945) and the dendrogram has been established according to the method of UPGMA (Unweighted Pair-Group Method with Arithmetic Average). Principal component analysis (PCA) on the two-dimensional chart presents the distances between genotypes and the calculation of 'eigen' value revealed the total variation in the components that are made by using the same program.

Pathogenicity assessment

The pear rust severity on selected twenty-five cultivars grafted on both QA and seedling rootstock cultured was evaluated under natural epidemic conditions through two subsequent years (2008–2009). The experiment was conducted in a completely randomized design (CRD) with four replications (Anonymous 1996). The pear varieties, grafted on QA, were planted at a distance of 6x3 m while the pear varieties grafted on seedling rootstock were planted with a distance of 6x6 m. No fungicide treatments were applied during the experiment period. The determination of the pear rust severity was made

by using the one-hundred randomly collected leave in both years using a scale from 0-7 (Table 2). The disease severity was identified as the percentage in each replication according to the Townsend-Heuberger formula (Unterstenhöfer 1963). Means were calculated using Fisher's LSD at the 5% level of significance. The variance was analyzed using SPSS statistical software (SPSS 2004).

RESULTS AND DISCUSSION

The size range (bp), the total number of bands (TNB), polymorphic band number (NPB), polymorphism rate (PO), and polymorphism information content (PIC) values are presented in Table 3. Results revealed that the average number of bands per SSR primer pairs was 2.07, the average number of polymorphic bands was 1.76, and polymorphism was 76.9%. NH002b and NB102a primer pair only amplified in the single-band. The NH013a SSR primer produced the highest polymorphism information content (0.74), and the overall average of PIC was recorded as 0.45 (Figure 1).

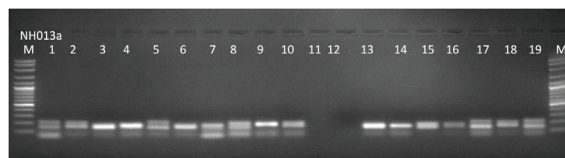


Figure 1. PCR amplification patterns obtained with the NH013a primers for accessions pear cultivars, M: 100 bp DNA Ladder Plus

The NTSYS analysis results were recorded as 0.73 for pear varieties clustered into two major groups (Figure 2). PCR analysis demonstrated the similarity interval as 0.73-1.00. P. Crassane, BP. Morettini, Santa Maria, and Beurre Hardy pear varieties were clustered in the first group. The others were clustered in the second group with a similarity of 0.76. Results showed that the overall genetic similarity was ranged from 73 to 100%. According to the SSR results, the highest genetic similarity within the varieties was determined among B.P Morettini-Santa Maria (Figure 2, Figure 3). It was proved that SSR was efficient by giving some accurate results in genotyping and may be used for the determination of genetic diversity in pear. Furthermore, genetic diversity is a good source to provide the materials for breeding programs. Many numbers of literature reported the genetic

Table 3. Size range (bp), total number of bands (TNB), polymorphic band number (PBN), polymorphism rate (PR) and polymorphism information content (PIC) values in SSR pairs

SSR primer pair	Size range (bp)	TNB	PBN	PO(%)	PIC
NB105a	130-190	2	2	100	0.433299
NB113a	130-165	2	2	100	0.575414
CH01D08	280-310	2	2	100	0.419761
NB106a	110-150	2	1	50	0.497449
NH002b	180	1	0	0	0
KA16	238-290	2	2	100	0.485651
NH015a	130-136	2	1	50	0.488166
NB102a	180	1	0	0	0
NH009b	130-159	2	2	100	0.487796
NH020a	120-150-180	2	2	100	0.608127
NH013a	180-200-219	3	3	100	0.743924
NH019b	150-180-210	3	3	100	0.605263
NB109a	160-200-210	3	3	100	0.453031
Total		27	23	1000	5.80
Mean		2.07	1.76	76.9	0.45

diversity of pear varieties in many countries using a various number of microsatellite SSR primers loci (Erfani et al. 2012, Ghosh et al. 2006, Volk et al. 2006, Wunsch and Hormaza 2007). Xie et al. (2010) collected 94 peach varieties from the Zhejiang province of China and analyzed them using 34 polymorphic SSR markers. The dendrograms showed two major clusters. The inheritance analysis revealed 94% of the selected varieties were individually identified.

Similar report from Aranzana et al. (2003a) assessed 212 peach varieties using 16 different SSR markers (9 SSR loci are in common with our study). According to the results, the mean number of alleles observed was 7.3. In another similar report, the mean number of the allele was recorded as 4.2 using 41 SSRs in 27 peach varieties (Dirlewanger et

al. 2002). Wunsch et al. (2006) detected it as 3.5 in a set of 85 local Spanish peach genotypes based on polymorphic SSRs while it was detected as 3.1 by Cheng et al. (2009). On the other hand, Sosinski et al. (2000) and Bouhadida et al. (2007) observed it as 2.6 and 2.3 in 28 scion peach varieties with 10 SSRs and 30 peach accessions using 20 polymorphic SSRs, respectively. These results showed that honey peach varieties of the Fenghua had a low genetic diversity. Xie et al. (2010) reported a higher level of genetic diversity in introduced varieties than that of the Fenghua local varieties. This could be related to the sources of the samples; the introduced varieties were obtained from various locations whereas the Fenghua accessions were derived from certain parental materials a result of inbreeding. Over 90% of alleles

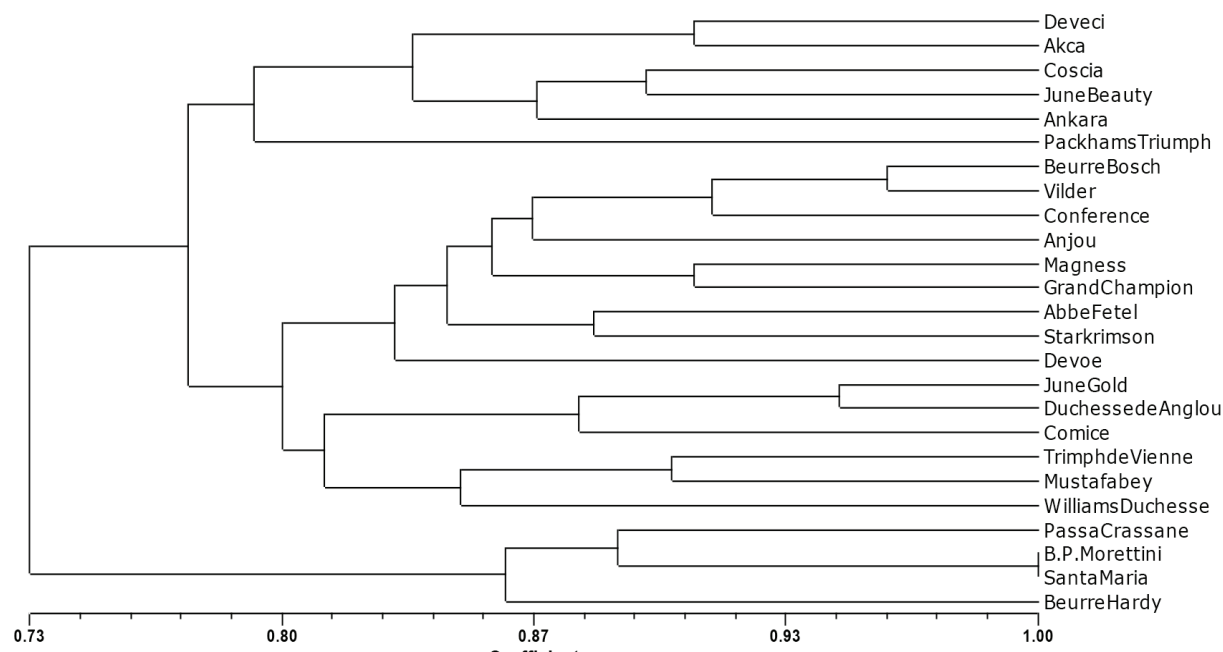


Figure 2. Dendrogram of 25 pear cultivars accessions generated by the data from 13 SSR primers

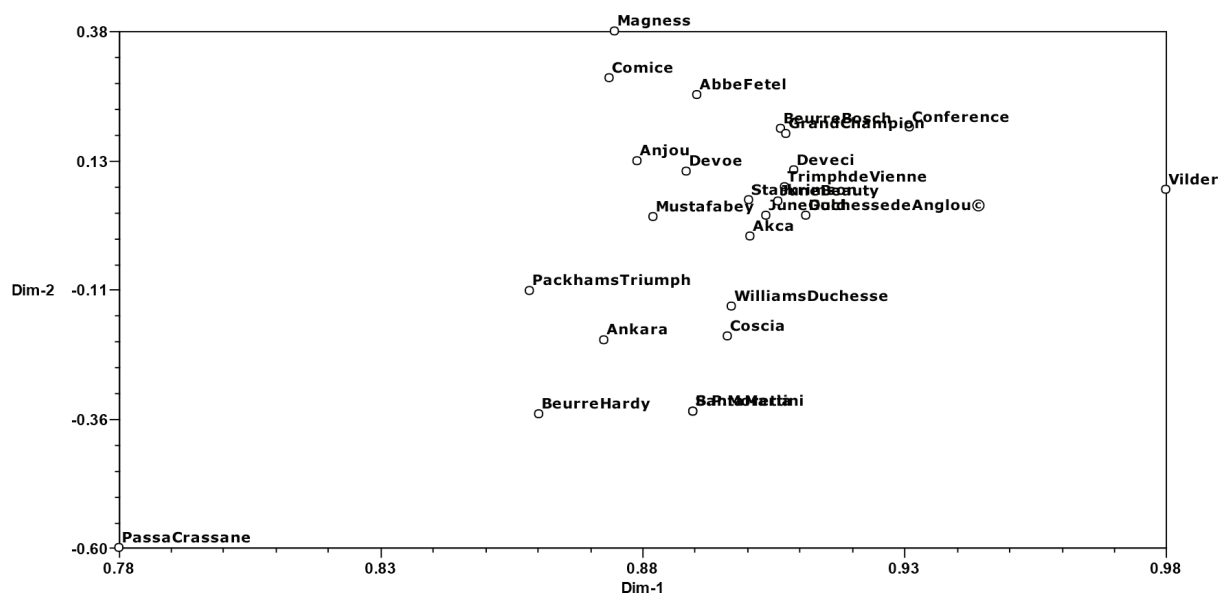


Figure 3. Biplot (the first two principle coordinate analysis) of 25 pear varieties accessions generated by the data from 13 SSR primers

were similar in local varieties, and in one accession 28 out of 34 loci were homozygous. In addition, the findings show the necessity of making full use of the introduced varieties as parental materials to enrich genetic diversity in breeding programs.

Screenings based on the SSR have been performed for various large collections of European pear (Ahmed et al. 2010, Bassil and Postman 2009, Bassil et al. 2008, Brini et al. 2008, Fernández-Fernández 2010, Miranda et al. 2010, Sisko et al. 2009, Urbanovich et al. 2011, Wünsch and Hormaza 2007, Xuan 2008, Yakovin et al. 2011). However, most of the studies showed a large number of mislabelling even in well-characterized fruit tree collections (Schouten et al. 2012).

In this study, the related cultivars were Santa Maria and B.P. Morettini. Turkish local varieties Deveci, Ankara and Akça clustered with Coscia, June Beauty cultivars. Yet, Mustafa Bey clustered with Trimhde Vienne cultivar. Akçay et al. (2009) determined that June Beauty and Akça had similar pomological characteristics. However, Deveci had more fruit weight than June Beauty and Akça. İkinci and Bolat (2016) compared to Akça, Coscia, Deveci and Dr. Jules Guyot pear cultivars about yield and other parameters that although there was a similarity with yield, fruit weight of pear cultivars were not similar. Mustafa Bey pear cultivar has small fruit (49.7 g) (Bostan and Acar 2012). The similar results were obtained with Wünsch and Hormaza (2007) as they used seven microsatellite loci (SSR) which were developed in apple for the identification of 63 European pear cultivars. Wilder and Conference

cultivars nested at the same cluster with other cultivars; Abbe Fetel, Comice, Passa Crasana, Packham's Triumph B.P. Morettini, Grand Champion T. de Vienne were close to each other. However, Beurre Hardy was nested different groups than other cultivars as our results. Brini et al. (2008) used Comice, Conference and Passa Crassane cultivars with 25 local Tunisian cultivars to identification using SSR primers and they reported that Comice, Conference and Passa Crassane cultivars nested at the same cluster. Passa Crassane cultivar nested at different cluster Comice and Conference nested the same cluster in the present study. Obtained SSR results consistent with some previous studies.

The pear rust severity on commercially important twenty-five cultivars grafted on both QA and seedling rootstock cultured was evaluated for two subsequent years and the data of 2008 presented in Table 4 demonstrates that the disease severity between the varieties was found statistically important ($p < 0.05$) and all of the pear varieties have different sensitivity levels against to pear rust disease. The lowest disease severity on seedling rootstock and QA rootstock are observed in variety A. Fetel (5.75%) and variety P. Triumph (8.08%) and variety Ankara (11.30%), respectively. The highest disease severity was observed in variety Akça (46.88%), variety Magnes (41.88%) and variety Comice (37.97%) on QA rootstock and variety Magnes (42.63%), variety Mustafa Bey (41.25%) and variety Akça (37.23%) on seedling rootstock. Overall, the Mean Disease Severity of Varieties (MDSV) on QA rootstock was 23.34% while MDSV on seedling rootstock was 21.18%.

Table 4. The rust disease (*Gymnosporangium fuscum*) severity of the pear varieties grafted on QA and seedling rootstock in 2008-2009 (%)

Variety	2008		LSD	2009		LSD
	QA	Seedling		QA	Seedling	
Akça	46.88±3.55	37.23±1.61	6.74	42.79±1.43	34.29±1.77	3.94
Conference	24.44±1.54	18.88±2.32	4.83	21.10±1.52	12.60±1.51	3.72
Comice	37.97±2.87	26.17±2.88	7.04	12.07±1.01	15.13±1.43	3.03
P.Crassane	35.77±2.18	21.71±2.53	5.79	31.45±0.75	38.86±1.77	3.33
S.maria	26.36±2.13	21.07±2.33	5.46	7.97±1.53	8.92±1.16	3.32
B.Hardy	29.01±1.59	23.42±1.37	3.63	17.23±1.21	20.88±1.16	2.90
Mustafa Bey	29.68±3.08	41.25±1.60	6.02	28.35±1.52	15.67±1.02	3.17
Morettini	19.67±1.40	17.28±1.22	3.22	15.91±1.83	9.17±0.75	3.43
Williams	16.27±1.36	15.63±1.43	3.42	13.44±1.29	5.55±2.05	4.20
Magnes	41.88±2.65	42.63±2.42	6.22	43.22±1.75	39.48±1.17	3.65
Anjou	29.87±2.98	19.88±1.40	5.71	5.37±0.95	4.81±1.26	2.73
Starcrimson	17.02±1.54	26.87±1.46	3.68	19.31±1.44	8.58±1.58	3.71
A.Fetel	17.46±0.70	5.75±1.06	2.20	12.79±1.27	9.61±1.71	3.70
T.De Vienne	28.12±0.83	29.03±1.07	2.35	21.25±1.71	9.44±2.30	4.97
Devoe	14.17±1.02	15.17±1.96	3.82	18.29±1.26	14.02±1.58	3.51
D.Angouleme	23.35±2.13	24.58±2.57	5.78	16.15±1.16	9.87±2.04	4.07
J.Beauty	13.28±1.87	12.16±2.18	4.97	12.58±1.50	15.32±2.45	4.97
G.Champion	21.48±1.48	22.74±2.61	5.20	12.74±1.22	6.53±2.08	4.17
Ankara	11.30±1.12	17.96±1.77	3.62	21.53±1.04	19.16±1.54	3.21
Coscia	15.12±1.74	16.13±1.29	3.75	13.43±0.63	13.58±2.27	4.09
B.Bosc	22.78±1.39	17.29±1.85	4.00	9.42±1.00	10.38±0.79	2.21
P.Triumph	16.61±1.65	8.08±2.44	5.10	11.32±1.82	11.68±1.52	4.11
J.Gold	15.34±3.32	15.94±2.41	7.10	13.92±1.31	12.22±1.77	3.81
Wilder	18.09±2.51	14.89±1.96	5.52	14.40±1.32	4.35±0.84	2.72
Deveci	11.75±0.58	11.69±1.17	2.26	9.96±1.50	9.47±1.78	4.04
LSD	4.09	3.89		2.70	3.27	

The data from 2009 in Table 4 shows that the differences between disease severity were statistically important ($p < 0.05$). The lowest disease severity was found in variety Wilder (4.35%), variety Anjou (4.81%) and variety Williams (5.55%) on seedling rootstock while variety Magnes (43.22%), variety Akça (42.79%) and variety P. Crassane (31.45%) on QA rootstock were the highest disease severity. In general, MDSV on QA rootstock was 18.25% while MDSV on seedling rootstock was 14.49%. In a comprehensive study, the differences in the response of pear cultivars, Mramornaya, Zengale, Mlievskaya Rannaya, Belorusskaya Pozdnyaya, and Vizhnica grafted on rootstock Pyrodwarf, to the pear rust was evaluated Prokopova (2011). The cultivars Vizhnica and Zengale showed the highest severity against disease, whereas the sensitivity of cultivars Mlievskaya Rannaya, Mramornaya, and Belorusskaya Pozdnyaya against pathogen was less than the others. The results of Prokopova (2011) indicate that the cultivars evaluated in the trial were susceptible against pear rust.

It was indicated that there are suggestions of less susceptible cultivars which are thought to be Bunte Juli, Concorde, Clapps Liebling, Condo, and Trevoux. Cultivars

as Conference, Verdi and Cascade are on the other hand considered to be highly susceptible (Fitzner and Fischer 2005). The European pear rust severity on twenty-five cultivars of different origins was evaluated five years by Lâce and Bankina (2013). The severity of disease did not show significant differences among tested pear cultivars; ranging from 0.8 and 0.9 points on average (cvs. Liva, Duhmyanaya, and Harrow Delight) to 1.4 points on average (cvs. Mlievskaya Ranyaya, Fritjof, Conference, Belorusskaya Pozdnyaya, Zengale, BP-8965, Bere Kievskaya, Concorde, Condo, and Mramornaya).

It is important to consider that fungicides do not serve complete protection against infections, particularly in cold and humid conditions. Also, they are not usually effective unless they are timed properly and combined with accurate agricultural practices (Agrios 2005). In addition, the use of pesticides has negative effects on useful insects, microorganisms, plants, water, and even human health. For this reason, the development of resistant varieties appears as the best and eco-friendly solution for controlling the disease and consequently the loss in yield and production. The results obtained in this study are thought to be important

and will provide the necessary information and materials for the resistant pear rust breeding programs.

In this study, the genetic diversity and the response of these pear cultivars to pear rust (*Gymnosporangium fuscum*) of 25 pear cultivars grafted on seedling and Quince A (QA) was examined using 13 SSR primers. The used SSR markers provided reliable genotyping and demonstrated their usefulness for identifying pear genetic diversity. The results of the orchard experiment proved the natural infection level of the pear rust and none of the pear varieties used in the experiments was not resistant to rust disease. The means of disease severity varieties on QA rootstock were generally higher than the varieties on the seedling. The development of the pear varieties on QA rootstock was slow compared to the varieties on the seedling rootstock. To establish intensive pear orchard, the varieties grafted on dwarf rootstocks are preferred in Turkey. Dwarf orchard trees are more sensitive to disease caused by the high relative humidity due to high canopy. Avoiding the diseases caused by rust can be possible if the varieties on seedling rootstock used in the orchard where the rust disease is often observed. The present study is the first study in this subject matter and further research is needed for biological testing against pear rust races.

ÖZET

Armut, Türkiye ve dünyanın farklı ekolojik koşullarında yaygın olarak yetiştirilen bir bitki türüdür. Armut yetiştiriciliği Türkiye'de uzun bir geçmişe sahiptir, ancak bu eşsiz genetik kaynakların tarımsal ekosistemdeki ve genetik erozyondaki değişimler nedeniyle nesli tükenme tehlikesi bulunmaktadır. *Gymnosporangium fuscum*'un neden olduğu armut memeli pas hastalığı, armut ağaçlarının önemli hastalıklarından biridir ve aynı zamanda ardıc ağaçlarında da bulunur (*Juniperus oxycedrus* L. ve *J. excelsa* Bieb.). Ardıc popülasyonlarının yakınındaki bazı armut bahçelerinde ciddi ekonomik kayıplar görülmüştür. Bu çalışmada, Çöğür ve Quince A (QA) üzerine aşılınmış 25 armut çeşidinin genetik çeşitliliği 13 SSR primer kullanılarak belirlenmiştir ve bu armut çeşitlerinin armut memeli pası (*Gymnosporangium fuscum*)'na karşı reaksiyon seviyeleri incelenmiştir. 25 ticari açıdan önemli armut çeşidi iki ana grup içinde yakın ilişkili olarak ele alınmıştır. Bu SSR markörleri armut genetik çeşitliliğin tanımlanmasında faydalı ve güvenilir olarak kullanılabileceğini göstermiştir. Hastalık şiddeti oranlarına göre yıllar ve anaçlar arasındaki fark istatistikî yönden önemli bulunmuştur. Hastalık tüm armut çeşitlerinde görülmüş ve hiçbir armut çeşidi hastalığa karşı dayanıklı olarak değerlendirilmemiştir. QA anaçlı armut çeşitlerinde çöğür anacına göre hastalık şiddeti daha yüksek tespit edilmiştir.

Anahtar kelimeler: armut, armut memeli pası, genetik çeşitlilik, SSR

REFERENCES

- Ahmed M., Anjum M.A., Khan M.Q., Ahmed M.J., Pearce S., 2010. Evaluation of genetic diversity in *Pyrus germplasm* native to Azad Jammu and Kashmir (Northern Pakistan) revealed by microsatellite markers. African Journal of Biotechnology, 9 (49), 8323-8333.
- Agrios G.N., 2005. Plant pathology (Fifth Edition). ISBN0-12-044565-4, California, USA, 574-576.
- Akçay M., Büyükyılmaz M., Burak M., 2009. Marmara Bölgesi için ümitvar armut çeşitleri. IV. Bahçe Dergisi, 38 (1), 1-10.
- Anonymous, 1996. Procedures of plant protection products registration. Ministry of Food Agriculture and Livestock General Directorate of Agricultural Research and Policy, Ankara, Turkey.
- Anonymous, 2008. Plant protection pesticides technical instructions, Vol. 4, Ministry of Food Agriculture and Livestock General Directorate of Agricultural Research and Policy, Ankara, Turkey. 9-12 p.
- Aranzana M., Pineda A., Cosson P., Dirlewanger E., Ascasibar J., Cipriani G., Lezzoni A., 2003a. A set of simple-sequence repeat (SSR) markers covering the Prunus genome. Theoretical and Applied Genetics, 106, 819-825.
- Aranzana M.J., Carbó J., Arús P., 2003b. Using amplified fragment-length polymorphisms (AFLPs) to identify peach cultivars. Journal of the American Society for Horticultural Science, 128 (5), 672-677.
- Aranzana M.J., Garcia-Mas J., Carbo J., Arús P., 2002. Development and variability analysis of microsatellite markers in peach. Plant Breeding, 121 (1), 87-92.
- Bao L., Chen K., Zhang D., Cao Y., Yamamoto T., Teng Y., 2007. Genetic diversity and similarity of pear (*Pyrus* L.) cultivars native to East Asia revealed by SSR (simple sequence repeat) markers. Genetic Resources and Crop Evolution, 54 (5), 959-971.
- Barakat M.N., Al-Doss A.A., Elshafei A.A., Moustafa K.A., 2011. Identification of new microsatellite marker linked to the grain filling rate as indicator for heat tolerance genes in F2 wheat population. Australia Journal of Crop Science, 5, 104-110.
- Bassil N., Postman J., Hummer K., Dolan S., Lawliss L., 2008. Molecular fingerprints identify historic pear trees in two U.S. national parks. Acta Horticulturae, 800, 417-422.
- Bassil N., Postman J., 2009. Identification of European and Asian pears using EST-SSRs from *Pyrus*. Genetic Resources and Crop Evolution, 57 (3), 357-370.

- Bouhadida M., Casas A.M., Moreno M.A., Gogorcena Y., 2007. Molecular characterization of Miraflores peach variety and relatives using SSRs. *Scientia Horticulturae*, 111 (2), 140-145.
- Bostan S.Z., Acar Ş., 2012. Ünye’de (Ordu) yetiştirilen yerel armut çeşitlerinin pomolojik özellikleri. *Akademik Ziraat Dergisi*, 1 (2), 97-106.
- Brini W., Mars M., Hormaza J.I., 2008. Genetic diversity in local Tunisian pears (*Pyrus communis* L.) studied with SSR markers. *Scientia Horticulturae*, 115, 337-341.
- Cao Y.F., Liu F.Z., Gao Y., Jiang L.J., Wang K., Ma Z.Y., Zhang K.C., 2007. SSR analysis of genetic diversity of pear cultivars. *Acta Horticulturae Sinica*, 34, 305-310.
- Cheng Z., Huang H., 2009. SSR fingerprinting Chinese peach cultivars and landraces (*Prunus persica*) and analysis of their genetic relationships. *Scientia Horticulturae*, 120 (2), 188-193.
- Dice L.R., 1945. Measures of the amount of ecologic association between species. *Ecology*, 26 (3), 297-302.
- Dirlewanger E., Cosson P., Howad W., Capdeville G., Bosselut N., Claverie M., Laigret F., 2004. Microsatellite genetic linkage maps of myrobalan plum and an almond-peach hybrid-location of root-knot nematode resistance genes. *Theoretical and Applied Genetics*, 109, 827-838.
- Dirlewanger E., Cosson P., Tavaud M., Aranzana M., Poizat C., Zanetto A., Arús P., Laigret F., 2002. Development of microsatellite markers in peach (*Prunus persica* (L.) Batsch) and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). *Theoretical and Applied Genetics*, 105 (1), 127-138.
- Erfani J., Ebadi A., Abdollahi H., Fatahi R., 2012. Genetic diversity of some pear cultivars and genotypes using simple sequence repeat (SSR) markers. *Plant Molecular Biology Reporter*, 30, 1065-1072.
- Fang D.Q., Roose M.L., 1997. Identification of closely related Citrus cultivars with inter simple sequence repeat markers. *Theoretical and Applied Genetics*, 95, 408-417.
- FAOSTAT 2017. Retrieved in January, 17, 2019 from <http://faostat.fao.org> (accessed date: 17.01.2019).
- Fernández-Fernández, F., 2010. Final report of Defra project GC0140 ‘Fingerprinting the national apple and pear collections’. <http://randd.defra.gov.uk/> (accessed date: 17.01.2019).
- Fitzner S., Fischer M., 2005. Bewertung von Pyrus-arten auf Befall mit Birnengitterrost (*Gymnosporangium sabinae* Dicks.). *Erwebs – Obstbau*, 47, 37-39.
- Ghosh A.K., Lukens L.N., Hunter D.M., Strommer J.N., 2006. European and Asian pears: simple sequence repeat-polyacrylamide gel electrophoresis based analysis of commercially important North American cultivars. *HortScience*, 41 (2), 304-309.
- Gianfranceschi L., Seglias N., Tarchini R., Komjanc M., Gessler C., 1998. Simple sequence repeats for the genetic analysis of apple. *Theoretical and Applied Genetics*, 96, 1069-1076.
- Hokanson S.C., Lamboy W.F., Szewc-McFadden A.K., McFerson J.R., 2001. Microsatellite (SSR) variation in a collection of Malus (apple) species and hybrids. *Euphytica*, 118, 281-294.
- Hokanson S.C., Szewc-McFadden A.K., Lamboy W.F., McFerson J.R., 1998. Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a *Malus domestica* Borkh. core subset collection. *Theoretical and Applied Genetics*, 97, 671-683.
- Howad W., Yamamoto T., Dirlewanger E., Testolin R., Cosson P., Cipriani G., Arús P., 2005. Mapping with a few plants: using selective mapping for microsatellite saturation of the Prunus reference map. *Genetics*, 171 (3), 1305-1309.
- Iketani H., Manabe T., Matsuta N., Akihama T., Hayashi T., 1998. Incongruence between RFLPs of chloroplast DNA and morphological classification in east Asian pear (*Pyrus* spp.). *Genetic Resources and Crop Evolution*, 45, 533-539.
- İkinci A., Bolat İ., 2016. Comparison of yield, fruit quality and leaf nutrient content of some pear cultivars. *International Multidisciplinary Congress of Eurasia*, 2, 208-219.
- Infante Espiñeira R., Martínez Gómez P., Predieri S., 2008. Quality oriented fruit breeding: Peach [*Prunus persica* (L.) Batsch]. *Journal of Food Agriculture and Environment*, 6 (2), 342-356.
- Juhasova G., Praslieka J., 2002. Occurrence and harmful effects of *Gymnosporangium sabinae* (Dicks) winter in Slovak republic. *Plant Protection Science*, 38 (3), 89-93.
- Kimura T., Iketani H., Kotobuki K., Matsuta N., Ban Y., Hayashi T., Yamamoto T., 2003. Genetic characterization of pear varieties revealed by chloroplast DNA sequences. *The Journal of Horticultural Science and Biotechnology*, 78 (2), 241-247.
- Koller B., Lehmann A., Mcdermott J.M., Gessler C., 1993. Identification of apple cultivars using RAPD markers. *Theoretical and Applied Genetics*, 85, 901-904.
- Kong H.J., Moon J.Y., Nam B.H., Kim Y.O., Kim W.J., Lee J.H., Kim K.K., Kim B.S., Yeo S.Y., Lee C.H., 2011. Molecular characterization of the autophagy-related gene Beclin-1 from the olive flounder (*Paralichthys olivaceus*). *Fish and Shellfish Immunology*, 31 (2), 189-195.

- Láce B., Bankina B., 2013. Evaluation of European pear rust severity depending on agro-ecological factors. *Research for Rural Development* 1, 6-12.
- Li T.H., Li Y.X., Li Z.C., Zhang H.L., Qi Y.W., Wang T., 2008. Simple sequence repeat analysis of genetic diversity in primary core collection of peach (*Prunus persica*). *Journal of Integrative Plant Biology*, 50 (1), 102-110.
- Lombard P.B., Westwood M.N., 1987. Pear rootstocks. In: *Rootstocks for fruit crops*. Rom R.C., Carlson R.F. (eds.). John Wiley and Sons, New York, USA, 145-183.
- Miranda C., Urrestarazu J., Santesteban L.G., Royo J.B., Urbina V., 2010. Genetic diversity and structure in a collection of ancient Spanish pear cultivars assessed by microsatellite markers. *Journal of the American Society for Horticultural Science*, 135 (5), 428-437.
- Monte-Corvo L., Cabrita L., Oliveira C., Leitão J., 2000. Assessment of genetic relationships among *Pyrus* species and cultivars using AFLP and RAPD markers. *Genetic Resources and Crop Evolution*, 47, 257-265.
- Oliveira C.M., Mota M., Monte-Corvo L., Goulao L., Silva D.L., 1999. Molecular typing of *Pyrus* based on RAPD markers. *Scientia Horticulturae*, 79, 163-174.
- Oliveira L.O., Venturini B.A., Rossi A.A.B., Hastenreiter S.S., 2010. Clonal diversity and conservation genetics of the medicinal plant *Carapichea ipeacuanha* (Rubiaceae). *Genetics Molecular Biology*, 33 (1), 86-93.
- Powell W., Morgante M., Andre C., Hanafey M., Vogel J., Tingley S., Rafalski A., 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding*, 2 (3), 225-238.
- Prokopova B., 2011. The severity of European pear rust depending on pear cultivars. *Scientific Works of the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry and Lithuanian University of Agriculture*. *Sodininkyste ir Darzininkyste*, 30 (2), 43-50.
- Schouten H.J., van de Weg W.E., Carling J., Ali Khan S., McKay S.J., van Kaauwen M.P.W., Wittenberg A.H.J., Koehorst-van Putten H.J.J., Noordijk Y., Gao Z., Rees D.J.G., van Dyk M.M., Jaccoud D., Considine M.J., Kilian A., 2012. Diversity arrays technology (DArT) markers in apple for genetic linkage maps. *Molecular Breeding*, 29 (3), 645-660.
- Sisko M., Javornik B., Siftar A., Ivancic A., 2009. Genetic relationships among Slovenian pears assessed by molecular markers. *Journal of the American Society for Horticultural Science*, 134 (1), 97-108.
- Smith J.S.C., Chin E.C.L., Shu H., Smith O.S., Wall S.J., Senior M.L., Mitchel S.E., Kresovich S., Tiegle J., 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. *Theoretical and Applied Genetics*, 95, 163-173.
- Sosinski B., Gannavarapu M., Hager L.D., Beck L.E., King G.J., Ryder C.D., Abbott A.G., 2000. Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. *Theoretical and Applied Genetics*, 101 (3), 421-428.
- Unterstenhöfer G., 1963. The basic principles of crop protection orchard trials. *Pflanzenschutz-Nachrichten, Bayer*. 1963/3. 155-156 p.
- Urbanovich O.Y., Kazlouvskaia Z.A., Yakimovich O.A., Kartel N.A., 2011. Polymorphism of SSR alleles in pear cultivars grown in Belarus. *Russian Journal of Genetics*, 47, 305-313.
- Uzun A., Yeşiloğlu T., Polat İ., Aka-Kaçar Y., Gülşen O., Yıldırım B., Tuzcu Ö., Tepe S., Canan İ., Anil Ş., 2011. Evaluation of genetic diversity in lemons and some of their relatives based on Srap and SSR Markers. *Plant Molecular Biology Reporter*, 29, 693-701.
- Volk G.M., Richards C.M., Henk A.D., Reilley A.A., Bassil N.V., Postman J.D., 2006. Diversity of wild *Pyrus communis* based on microsatellite analyses. *Journal of the American Society for Horticultural Science*, 131, 408-417.
- Wu J., Wang Z., Shi Z., Zhang S., Ming R., Zhu S., Khan M.A., Tao S., Korban S.S., Wang H., 2013. The genome of pear (*Pyrus bretschneideri* Rehd.). *Genome Research*, 23, 396-408.
- Wünsch A., Hormaza J.I., 2007. Characterization of variability and genetic similarity of European pear using microsatellite loci developed in apple. *Scientia Horticulturae*, 113 (1), 37-43.
- Wünsch A., Carrera M., Hormaza J.I., 2006. Molecular characterization of local Spanish peach [*Prunus persica* (L.) Batsch] germplasm. *Genetic Resources and Crop Evolution*, 53 (5), 925-932.
- Xie W.G., Zhang X.Q., Cai H.W., Liu W., Peng Y., 2010. Genetic diversity analysis and transferability of cereal EST-SSR markers to orchardgrass (*Dactylis glomerata* L.). *Biochemical Systematics and Ecology*, 38 (4), 740-749.
- Xuan H. 2008. Identifying european pear (*Pyrus communis* L.) cultivars at the KOB by using apple SSRs. *Acta Horticulturae*, 800, 439-445.
- Yakovin N.A., Fesenko I.A., Isachkin A.V., Karlov G.I., 2011. Polymorphism of microsatellite loci in cultivars and species of pear (*Pyrus* L.). *Russian Journal of Genetics*, 47, 564-570.

Yamamoto T., Kimura T., Sawamura Y., Kotobuki K., Ban Y., Hayashi T., Matsuta N., 2001. SSRs isolated from apple can identify polymorphism and genetic diversity in pear. *Theoretical and Applied Genetics*, 102, 865-870.

Yamamoto T., Kimura T., Sawamura Y., Manabe T., Kotobuki K., Hayashi T., Ban Y., Matsuta N., 2002a. Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears. *Theoretical and Applied Genetics*, 106, 9-18.

Yamamoto T., Kimura T., Sawamura Y., Manabe T., Kotobuki K., Hayashi T., Ban Y., Matsuta N., 2002b. Simple sequence repeats for genetic analysis in pear. *Euphytica*, 124, 129-137.

Yamamoto T., Kimura T., Shoda M., Ban Y., Hayashi T., Matsuta N., 2002c. Development of microsatellite markers in the Japanese pear (*Pyrus pyrifolia* Nakai). *Molecular Ecology Notes*, 2 (1), 14-16.

Zhang D., Shu Q., Teng Y.W., Qiu M.H., Bao L., Hu H.J., 2007. Simple sequence repeat analysis on genetic assessment of Chinese red skinned sand pear cultivars. *Acta Horticulturae Sinica*, 34, 47-52.

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