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Original scientific article

IDENTIFICATION OF GENETIC DIVERSITY IN WILD PEAR (*Pyrus Elaeagrifolia* Pall.) GENOTYPES COLLECTED FROM DIFFERENT REGIONS OF TURKEY WITH SSR MARKER SYSTEM

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Uzun A., H. Pinar, M. Yaman, M. A. Yigit, Y. Cakiroglu, A. Karakaya, M. Uysal, G. Ozturk, K.r U. Yilmaz, K. Gurcan, S. Ercisli (2022). *Identification of genetic diversity in wild pear (Pyrus elaeagrifolia pall.) genotypes collected from different regions of Turkey with SSR marker system.* - Genetika, Vol 54, No.1, 109-118.

Turkey with diverse ecologies is among the unique countries in terms of plant species and diversity. Among these plant species, naturally growing wild pears (*Pyrus elaeagrifolia* Pall.) are resistant to chlorosis and drought and could be used in rootstock development programs. In present study, genetic diversity in 96 wild pear genotypes collected from 11 different provinces (Kayseri, Ankara, Kahramanmaraş, Adana, Nevşehir, Konya, Isparta, Denizli, Uşak, Afyonkarahisar, Eskişehir) and regions of Turkey through selection was investigated with the use of SSR (Simple Sequence Repeat) molecular marker system. Present analyses carried out in ABI (Applied Biosystem) 3500 capillary electrophoresis system revealed 93 scorable and all polymorphic bands, thus polymorphism rate was 100%. In UPGMA (Unweighted pair group method with arithmetic mean) dendrogram of wild pear genotypes, similarity index values varied between 0.20-0.83 and a large variation was observed among the genotypes. Present finding may have significant contributions to further studies to be conducted for preservation of gene sources and breeding of wild pear genotypes.

Keywords: Wild pear, *Pyrus elaeagrifolia* Pallas, molecular marker, genetic diversity

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INTRODUCTION

Turkey has a diverse climate ranging from sub-tropical to terrestrial and different ecological and geographical conditions nationwide. Thus, it has a great diversity in plant species and genetic resources (SIMSEK *et al.,* 2010a; UZUN *et al.,* 2017; PINAR *et al.,* 2019). Therefore, several fruit species both naturally grow and economically cultured throughout the country. Wild pear is among the naturally growing fruit species of Anatolia. Wild pears belong to Rosaceae family and are endemic species in Southeastern Europe and Ukraine (AYGUN and DUMANOGLU, 2015; KAVAK and KECECI, 2019). Scientifically, wild fruit is one of 22 *Pyrus* species and wild fruit and almond-leafed wild pear taxa belong to *Pomoideae* sub-family of *Rosaceae* family (ANŞIN and ÖZKAN, 1993; HLAING *et al.,* 2019).

Wild pear is highly adapted to dry climate conditions. Fully xerophyte wild pear trees with a deep rooting system have a potential to be used as rootstock especially in commercial pear culture (ÖZÇAĞIRAN *et al.,* 2005; YILMAZ *et al.,* 2015). Wild pear fruits in their habitats are freshly consumed or dried-consumed or they are used in brine and fruit syrup production (YERLITURK *et al.,* 2008). Just because of biochemical structure, wild pear fruits have a high-water holding capacity, thus have a healing characteristic in treatment of diarrhea (BAYTOP, 2004).

Increasing world population have resulted in reduction of natural resources and put some natural species under danger (SENGUL *et al.,* 2014; SNEP and CLERGEAU, 2020). Wild pears are among these endangered species (YILMAZ *et al.,* 2015). Besides, for plant breeding purposes, genetic resources should be collected and taken under protection (SIMSEK *et al.,* 2010b; KOCSISNÉ *et al.,* 2020). While collecting plant species, various molecular, morphological, and biochemical marker systems able to distinguish one from the another are used (KUMAR *et al.,* 018; URWAT *et al.,* 2019). Among these markers, molecular techniques without environmental effects are the most reliable (YAMAN, 2021).

Number of studies on morphological and biochemical characteristics and propagation performance of wild pear species and rootstocks is highly limited and a comprehensive molecular marker study for genetic relationships in present wild pear population haven't been conducted, yet. In present study, genetic diversity in 96 wild pear genotypes, largely growing in different regions of Turkey and collected through selection, was aimed to determine with the use of SSR marker system.

MATERIAL AND METHOD

Present plant materials were collected from 11 different provinces (Kayseri, Ankara, Kahramanmaraş, Adana, Nevşehir, Konya, Isparta, Denizli, Uşak, Afyonkarahisar, Eskişehir) with widespread wild pear populations and composed of 96 different genotypes in Table 1. Temperature and drought resistance and adaptation to different soil conditions were taken into consideration while selecting the wild pear genotypes. Since the regions where the genotypes were selected were different, the climate and soil characteristics also varied.

Pests and disease-free, newly burst young shoot leaves were used for DNA isolation of present wild pear genotypes. Leaves were brought to laboratory and DNA isolation was practiced in accordance with modified CTAB protocol of DOYLE and DOYLE (1990) method (GÜLSEN *et al.,* 2009). DNA quality and quantity measurements of the genotypes were

determined through running the samples in 1% agarose gel. DNA samples were preserved a t–20 °C until the analyses.

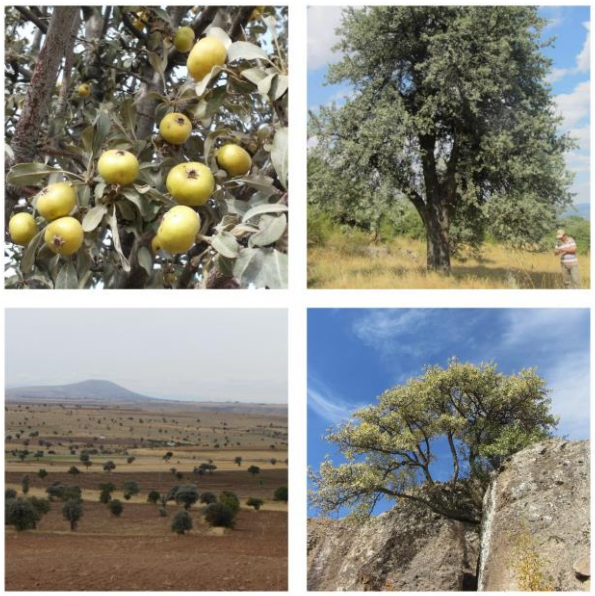

Fig. 1. Wild pear genotypes used in this study (taken by Aydın Uzun)

Fig. 2. *Provinces from where wild pear genotypes were collected (source: wikipedia.org)*

Table 1. List of wild pear genotypes and collected region

Genotype No	Province	Genotype No	Province	Genotype No	Province	Genotype No	Province
2	Kayseri	28	Kayseri	59	Ankara	85	Denizli
3	Kayseri	29	Kayseri	60	Ankara	86	Uşak
4	Kayseri	31	Adana	61	Ankara	87	Uşak
5	Kayseri	32	Adana	62	Ankara	88	Isparta
6	Kayseri	33	Adana	63	Ankara	89	Isparta
7	Kayseri	34	Adana	64	Ankara	90	Konya
8	Kayseri	35	Adana	65	Ankara	91	Konya
9	Kayseri	36	Adana	67	Ankara	92	Konya
10	Kayseri	37	Nevşehir	68	Eskişehir	93	Konya
11	Kayseri	38	Nevşehir	69	Eskişehir	94	Konya
12	Kayseri	39	Nevşehir	70	Eskişehir	95	Konya
13	Kayseri	40	Nevşehir	71	Eskişehir	96	Konya
14	Kayseri	41	Nevşehir	72	Eskişehir	97	Konya
16	Kayseri	42	Nevşehir	73	Afyonkarahisar	98	Konya
17	Kayseri	43	Nevşehir	74	Afyonkarahisar	99	Konya
18	Kayseri	44	Nevşehir	75	Afyonkarahisar	100	Konya
19	Kayseri	45	Nevşehir	76	Afyonkarahisar	E1	Isparta
20	Kayseri	48	Kayseri	77	Afyonkarahisar	E2	Isparta
21	Kayseri	52	K. Maraş	78	Afyonkarahisar	E3	Isparta
22	Kayseri	53	K. Maraş	79	Afyonkarahisar	E4	Isparta
23	Kayseri	54	K. Maraş	81	Afyonkarahisar	E5	Isparta
24	Kayseri	55	K. Maraş	82	Afyonkarahisar	E6	Isparta
26	Kayseri	57	K. Maraş	83	Denizli	E7	Isparta
27	Kayseri	58	Ankara	84	Denizli	E13	Isparta

Different SSR primers were used in this study. Pre-tests were conducted on these primers and 7 primers yielded a band. The band-yielding primers were identified as AT565, AT550, AT532, AT565 red and FAM 1,2,3. PCR outcomes were run in ABI 3500 capillary electrophoresis system and allele lengths were determined. These polymorphic primers were marked as fluorescent labeled [6-FAM, VIC, NED, PED (Applied Biosystems)]. Primer labeling and fragment analysis were conducted in accordance with the report of SCHUELKE**,** (2000). Initially, cultivars and types were amplified with the labeled primers in PCR. The PCR products, 4 of labeled primers, were pooled based on label color (6-FAM, VIC, NED, PED) and length (100-200 SSRs; 200-300 SSRs; 300-400 SSRs). About 1 µl PCR product taken from 4 primer products were completed to 80 µl with gradient water and loaded onto multiplex structures and ABI 3500 system.

Following the imaging processes, band existence was scored as (1), inexistence was scored as (0) and non-amplification was scored as (9). Data obtained with the use of NTSYSpc 2.1 software were analyzed, similarity matrix was generated with the use of DICE, (1945) method

and a dendrogram was generated for wild pear genotypes in accordance with UPGMA method. For each marker used in present study, total number of bands, number of polymorphic bands and polymorphism ratios were determined. The formula of (number of polymorphic bands x 100 / Total number of bands) was used while calculating polymorphism ratio.

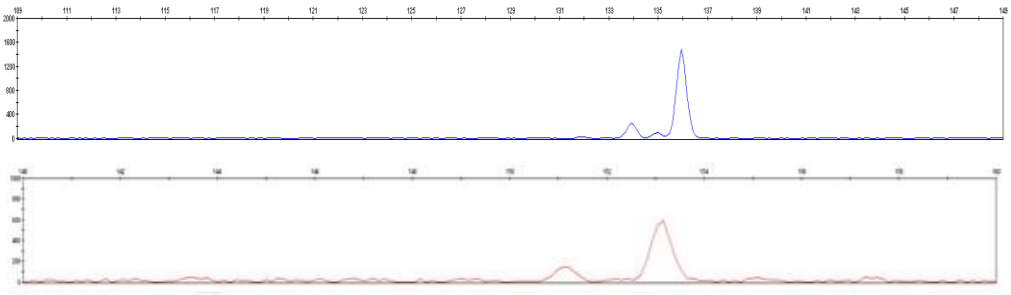

Fig. 3. ABI image of AT565 and FAM 1 primers (taken by Mehmet Yaman)

RESULTS AND DISCUSSION

Seven different SSR markers were used to determine genetic relationships in wild pear genotypes collected from different provinces, a total of 93 scorable bands were obtained and all of them were polymorphic. The greatest number of polymorphic bands (25 bands) was obtained from AT565 primer, and the lowest number of polymorphic bands (8 bands) was obtained from AT565 Red primer. Average polymorphism ratio per marker was identified as 100%. Molecular marker analyses are quite limited in wild peer species. In a study conducted on wild peer genotypes with ISSR marker system, mean polymorphism ratio was reported as 82.8% (YIĞIT, 2017). GENCER *et al.* (2018) conducted a RAPD analysis on pear genotypes and reported the mean polymorphism ratio as 63.2%. OUNI *et al*. (2020) used SSR marker analysis and reported mean polymorphism ratio of pear genotypes as 94%. The reason for the difference between the previous studies and the current study is that the genotypes used in the study were different.

Mean number of bands and total number of bands per primer was identified as 13.28. Mean bp lengths varied between 126-218 (Table 2). ZHU *et al.* (2009) conducted ISSR analysis on China-originated pear genotypes and reported mean number of bands and total number of bands per primer respectively as 10.81 and 9.37 and mean bp lengths as between 200 – 2000. KALKIŞIM *et al.* (2016) conducted RAPD analysis on pear genotypes and reported mean number of bands and total number of bands per primer respectively as 8.5 and 7.87. Differences from the present findings were attributed to different marker system used in present study.

Values for effective alleles (Ne) ranged from 1.064 (AT565) to 1.219 (FAM 1) (average 1.14), for Shannon's information index (I) from 0.116 (AT565) to 0.241 (FAM 1) (average 0.196), for expected heterozygosity (He) from 0.055 (AT565) to 0.143 (FAM 1) and for unbiased expected heterozygosity (uHe) from 0.055 (AT565) to 0.144 (FAM 1) (average 0.108) (Table 3). For all these parameters except for the q value, FAM 1 primer had the highest values whereas AT565 had the lowest values.

Table 2. List of SSR primers, their base length, number of total (TFN) and polymorphic fragments (PFN), rate of polymorphism (PR)

Primers	Bp	TFN	PFN	PR (%)
AT565	148-178	25	25	100
FAM 1	130-143	7	7	100
FAM 2	186-218	14	14	100
FAM 3	126-163	14	14	100
AT565 Red	157-174	8	8	100
AT532	186-210	11	11	100
AT550	186-210	14	14	100
Mean	126-218	13.28	13.28	100
Total	-	93	93	-

Table 3. SSR primers studied, their estimated allele frequency (p and q), number of effective alleles (Ne), Shannon's information index (I), expected (He) and unbiased expected heterozygosity (uHe)

Primers	p	q	Ne	I	He	uHe
AT565	0.030	0.970	1.064	0.116	0.055	0.055
FAM 1	0.137	0.863	1.219	0.241	0.143	0.144
FAM 2	0.053	0.947	1.116	0.185	0.095	0.095
FAM 3	0.059	0.941	1.132	0.187	0.101	0.102
AT565 Red	0.108	0.892	1.191	0.209	0.123	0.123
AT532	0.074	0.926	1.167	0.225	0.125	0.126
AT550	0.064	0.936	1.142	0.209	0.112	0.112
Mean	0.075	0.925	1.140	0.196	0.107	0.108

Similarity index values of wild pear genotypes varied between 0.20-0.83. OUNI *et al*. (2020) conducted a study on local pear genotypes of Tunusia with SSR markers and reported similarity index valeus as between 0.2-1.00. JIE *et al.* (2019) conducted a study on pear genotypes with SSR markers and reported similarity index valeus as between 0.24 – 1.00. Present findigns comply with those earlier ones. The cophenetic correlation coefficient, indicating the correlations between similarity index and dendrogram, was identified as r = 0.81. A cophenetic coefficient of between 0.8-0.9 indicate a well correlation between the similarity index and the dendrogram (MOHAMMADI and PRASANNA, 2003). Present value (0.81) indicated that there was a high correlation between the similarity index and the dendrogram and present dendrogram well represented the similarity index values.

In present UPGMA dendrogram generated with SSR markers, wild peer genotypes were divided into 2 main groups (Figure 4). The first main group was also divided into 2 sub-groups and only the genotypes E1, 71, E6 and 64, collected from Isparta, Ankara and Eskişehir provinces, were palced into these group. Remaining 96 genotypes were placed into the second main group. The second groups was also divided into 2 different sub-groups and the genotypes 33, 70 and 72 collected from Adana and Eskişehir provinces were placed into one of these sub-groups. Remaning genotypes were clustered in the other sub-group. The genotypes 38 and 44

collected from Nevşehir province were identified as the closest genotypes with a similarity index of 0.83. Present sub-groups were partially shaped around the geographical origins of the genotypes, but the genotypes were generally placed into the groups in a mixed fashion. Such differences among the genotypes were attributed to open-pollination of the wild pear genotypes in the nature and evolutions in genetic structure of the genotypes since these genotypes were seed-propaged ones. QUEIROZ *et al.* (2019) investigated genetic diversity in Portugal-originated pears with the use of SSR markers and placed the genotypes into two main groups. BACCICHET *et al.* (2020) conducted a study to determine the variations among 170 pear genotypes with the use of SSR markers and indicated that this marker system was quite practical in finding out the variations among the genotypes. ERFANI *et al.* (2012) conducted a study on 47 different per genotypes with the use of 28 different SSR markers and indicated that relevant outcomes were sufficient in separation of the genotypes. Present findings comply with the findings of those earlier studies.

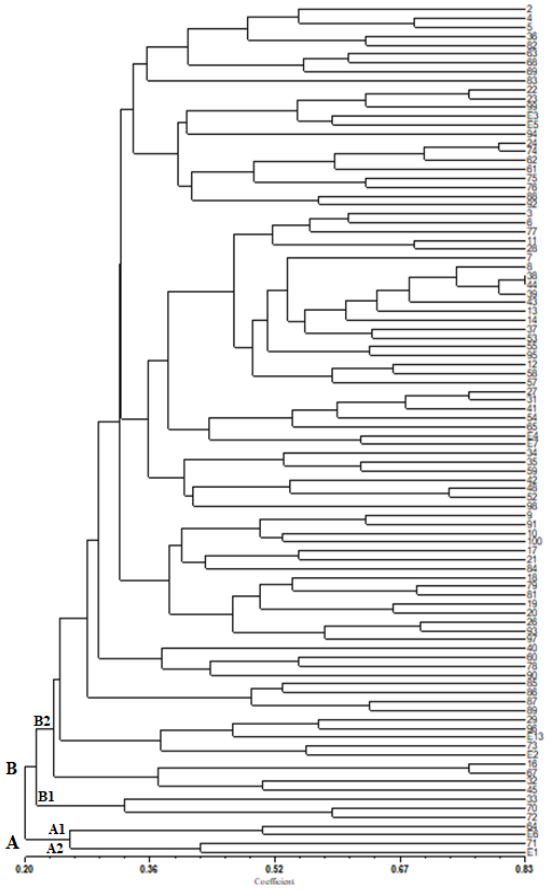

Fig. 4. Dendrogram for 96 wild pear genotypes constructed with SSR markers

As to conclude, identification of genetic diversity plays a significant role in preservation of naturally growing wild pear genotypes and bringing them into use in breeding programs. In present study, genetic diversity in wild pear genotypes collected from different regions of Turkey was identified with the use of SSR marker system. There were quite many differences among the present genotypes. It was concluded that SSR marker system could reliably be used in identification of genetic diversity among the genotypes. Present findings may have significant contributions to further breeding programs to be conducted with intraspecific or interspecific hybridizations of these genotypes.

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IDENTIFIKACIJA GENETSKE RAZNOLIKOSTI POMOĆU SSR MARKERA KOD GENOTIPOVA DIVLJE KRUŠKE (*Pirus Elaeagrifolia* Pall.) PRIKUPLJENIH IZ RAZLIČITIH REGIJA TURSKE

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Izvod

Turska sa raznolikom ekologijom spada među jedinstvene zemlje u pogledu biljnih vrsta i raznovrsnosti. Među ovim biljnim vrstama, prirodno rastuće divlje kruške (*Pirus elaeagrifolia* Pall.) su otporne na hlorozu i sušu i mogu se koristiti u programima razvoja podloga. U ovoj studiji, istražena je genetska raznolikost u 96 genotipova divlje kruške sakupljenih iz 11 različitih provincija i regiona Turske (Kajseri, Ankara, Kahramanmaraš, Adana, Nevšehir, Konja, Isparta, Denizli, Ušak, Afjonkarahisar, Eskišehir) upotrebom SSR molekularnih markera. Dosadašnje analize sprovedene u sistemu kapilarne elektroforeze ABI (*Applied Biosistem*) 3500 otkrile su 93 polimorfne trake, tako da je stopa polimorfizma bila 100%. U UPGMA dendrogramu genotipova divlje kruške, vrednosti indeksa sličnosti su varirale između 0,20-0,83 i primećene su velike varijacije među genotipovima. Sadašnji rezultati mogu imati značajan doprinos daljim proučavanjima koje će se sprovoditi za očuvanje izvora gena i oplemenjivanje genotipova divlje kruške.

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