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A first insight into genetic diversity of Jerusalem artichoke accessions collected from different regions of Turkey assessed by ISSR markers

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Abstract

The study aimed to assess genetic diversity among Jerusalem artichoke (*Helianthus tuberosus* L.) accessions using ISSR (Inter Simple Sequence Repeat) markers. Twenty-five Jerusalem artichoke accessions collected from the different regions of Turkey have been used for molecular analysis. Nine primers used in the study produced a total of 62 bands. A total 57 of these are polymorphic. 57 of them resulted in polymorphic. Allele's average number was 6.33. Polymorphic information content (PIC) varied between 0.219 and 0.340. Examined accessions were classified into two main groups in a dendrogram. Genetic similarities varied from 0.001 to 0.815, with an average of 0.292. Also, Principal Coordinate Analysis (PCoA) of molecular data was conducted. These findings present valuable diversity data for recognizing the Jerusalem artichoke germplasm, preserving genetic richness, and determining parents for genetic enhancement.

Keywords: Genetic diversity, Gene-Pool, *Helianthus tuberosus* L., ISSR

Introduction

Jerusalem artichoke is classified in the Helianthus genus of the *Asteraceae* family. Helianthus genus is of American origin and has around 50 species currently grown in the World.The worldwide distribution of these species is limited. Sunflower (*Helianthus annuus* L.) and Jerusalem artichoke (*H. tuberosus* L.) are the most prominent species of this genus (Heiser, 1978). The sunflower is grown as an oilseed crop in general, while Jerusalem artichoke is grown as a vegetable, fodder crop, and as a reserve of inulin for food and industrial purposes (Heiser, 1978). This plant is grown for edible tubers. The root length varies between 1 and 3 meters and is an important part of the plant's biomass. Jerusalem artichoke can tolerate yearly rain varying from 31 to 282 cm with an average temperature scale of 6.3-26.6 °C and a pHof 4.5-8.2. Even if temperatures sub-zero kill the leaves and stems of the Jerusalem artichoke, the tubers can survive for a long time without being damaged by frost. Tolerance of tubers to low temperatures allows them to be stored under the ground during the cold winter until harvested. Jerusalem artichoke should ideally be planted in well-drained soil. Besides, good aeration of the soil before planting is advantageous throughout the cultivation because of its aggressive growth. Another advantage of rapid growth is the low pesticide need and the increase in the amount of biomass per area. The high biomass rate per area leads to good resistance to pests. The tubers of Jerusalem artichoke includes different kind of vitamins, minerals. Also it contains the complex carbohydrate inulin which can all promote good health in humans. Because of these properties, Jerusalem artichoke is considered not only as a human or animal food but also as a promising plant for ethanol production (Kays et al., 2007).

Turkey has a rich genetic diversity for many plant species. Preservation of this richness will enable both the improvement of new varieties andthe transfer of genetic resources to future generations (Balkaya et. al., 2001). The rich genetic diversity of Turkey's geography has great potential for crop breeding programs. Local accessions have been considered as the initial population for the development of many new commercial varieties. In addition to these local varieties, wild relatives of plant species are also used in breeding programs. However, for different reasons, this wealth is at risk of extinction. To reduce this risk, studies have been carried out within the framework of the National Program for Conservation of Plant Genetic Resources / Diversity since the 1960s (Tan, 2010). The low genetic diversity in breeding programs limits the rate of genetic progress expected fromsome crops. The use of germplasm stored in gene

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banks as a source of genetic diversity strongly influences the development of high yield varieties (Broughton et al., 2003). Despite all these efforts, a comprehensive characterization study has not been carried out in Turkish Jerusalem Artichokes' genetic resources. In a study conducted by the same author between the years 2018-2020, Jerusalem artichokes samples were collected from different cities of Turkey. During these trips, a predominance of traditional family agriculture was observed. Also, all farmers emphasized that they cultivate Jerusalem artichoke in very small areas such as 100 or 200-meter square. The main advantage emphasized by the local farmers was that Jerusalem artichoke isvery easy to cultivate (Hanci and Tuncer, 2019).

To use any gene source in breeding studies, the distribution of the genetic similarity of the cultivated species and their wild relatives must be known in detail. This can be possibly more effective by various methods, such as the use of technologies based on DNA polymorphism. DNA molecular markers are widely used to detect differences and similarities between accessions or to identify similarities or differences between varieties and their parents (Karaca et al., 2002). Today, molecular markers can be used in many plant species for several purposes such as genotypic identification, systematic and characterization, QTL (Quantitative Trait Loci) mapping, marker-assisted selection, and conservation of genetic resources (Vardar-Karlıtepe et al., 2010). Despite the important economic potential, the genetic diversity of the Turkish Jerusalem artichoke remained undiscovered yet, concerning traits such as DNA-based variation. This investigation aimed to assess the genetic diversity of Turkish Jerusalem artichoke accessions using ISSR markers for the first time. These genetic similarity data will guide the selection of parents in crossbreeding programs to be implemented in the future.

Materials and Methods

Twenty-five locally grown Jerusalem artichoke tubers, which were collected from eleven cities of Turkey (Table 1), were used as plant material. These accessions are preserved in the Hanci's cool-season vegetable collection under the Erciyes University. None of these materials are commercial varieties. Tuber characteristics of 15 accessions have been described and published previously during the establishment of this collection. High variabilitywas detected for tuber color, with five classes: red (7 accessions); light red (3 accessions); fawn (1 accession); light yellow (3 accessions); yellow (11 accessions). Also, high variation was observed in tubers weight ranging between 6,03 g (40/03) to 118,55 g (50/02). Accessions 50/02, 19/03, and 50/01 were distinct from all other ones due to high tuber weight (Hanci and Tuncer, 2019). Some of the collected tubers were planted in 10-liter pots in November 2018. The other part was stored in the refrigerator and planted in the same size pots in March 2019.

Table 1. Jerusalem artichoke samples assessed in the study and their geographic locations

Code Number	Geographic location	Coordinate	Code Number	Geographic location	Coordinate
06*1	Ankara / Beypazarı	40°09'45.7"N 31°55'27.5"E	50*1	Nevşehir / Gülşehir	38°44'25.7"N 34°37'57.5"E
06*2	Ankara / Bala	39°32'41.8"N 33°07'26.0"E	50*2	Nevşehir / Avanos	38°42'52.2"N 34°50'41.1"E
07*1	Antalya / Alanya	36°34'26.8"N 31°59'33.6"E	50*3	Nevşehir / Avanos	38°43'04.8"N 34°51'49.9"E
19*1	Çorum / Sungurlu	40°10'05.9"N 34°23'02.8"E	50*4	Nevşehir / Ürgüp	38°37'37.2"N 34°54'52.0"E
19*2	Çorum / Sungurlu	40°10'32.7"N 34°26'37.4"E	55*1	Samsun / Vezirköprü	41°07'56.9"N 35°27'09.1"E
19*3	Çorum / Feruz	40°42'57.0"N 34°52'35.5"E	55*2	Samsun / Havza	40°59'14.8"N 35°38'10.2"E
19*4	Çorum /Center	40°34'25.5"N 34°57'34.4"E	58*1	Sivas / Gemerek	39°11'42.2"N 36°04'54.1"
19*5	Çorum / Bayat	40°38'41.0"N 34°15'33.6"E	61*2	Trabzon/ Ortahisar	41°00'41.3"N 39°36'21.0"E
38*1	Kayseri / İncesu	38°37'38.3"N 35°11'56.4"E	66*1	Yozgat/ Center	39°49'15.7"N 34°48'19.0"E
38*2	Kayseri / Pınarbaşı	38°44'34.8"N 36°25'49.9"E	66*2	Yozgat / Boğazlıyan	39°48'05.4"N 34°47'25.3"E
40*1	Kırşehir /Center	39°09'10.1"N 34°10'00.3"E	66*3	Yozgat / Boğazlıyan	39°47'53.9"N 34°45'43.2"E
40*2	Kırşehir / Center	39°11'43.3"N 34°08'56.6"E	77*1	Yalova / Center	40°39'31.0"N 29°16'30.8"E
40*3	Kırşehir / Mucur	39°02'51.5"N 34°26'28.3"E			

Table 2. Various parameters of ISSR primers observed in the study

Marker	Tm (°C)	Band Size (bp)	Total number of alleles	Number of polymorphic alleles	% Polymorphism	Observed Heterozygosity	PIC*
DBDA$(CA)_7$	45	370-990	3	3	100	0.435	0.340
$(CT)_8$TG	48	210-1590	10	9	90	0.336	0.279
$(CA)_8$R	45	420-1090	8	7	70	0.292	0.249
VHV$(GT)_7$G	45	250-890	4	4	100	0.332	0.279
HVH$(CA)_7$T	45	90-320	3	3	100	0.250	0.219
$(AG)_7$YC	45	130-790	7	6	86	0.269	0.233
HVH$(TCC)_7$	60	1200-1790	7	6	86	0.304	0.258
BDB$(CA)_7$C	45	120-990	15	14	88	0.329	0.273
$(CA)_8$YG	45	110-800	5	5	71	0.417	0.330

*PIC: polymorphism information content

Bulked samples of four tubers of the same accessions were used for DNA isolation using an EasyPure® Quick Gel Extraction Kit (TransGen Biotech Co., Ltd, China). Tissue samples were taken from twenty days old fresh shoots and frozen in liquid nitrogen. Then these frozen tissues have disintegrated until they turned into a powder-like appearance using a tissuelyser (TissueLyser II Retsch, Qiagen Retsch GmbH., Hannover, Germany). DNA was obtained following the procedures specified by the producers of the kit. The DNA quality was examined on 1.3 % agarose gel.

A set of nine ISSR (Inter Simple Sequence Repeat) markers were preferred for their powerful polymorphism, according to the conclusions of previous studies (Wangsomnuk et al., 2011). Polymerase chain reaction (PCR) was performed using a thermal cycler model SC 300 (Australia). Each reaction tube (30 µL) contained 15 µL of 2X AmpMasterTMTaq PCR Mix (components: Taq DNA polymerase 1.25U; dNTP mixture 100 × 4 = 400 µL; reaction buffer with 1.25 mM MgCl2 1X; and loading dye&stabilizer 1X) and 25 ng/µL template DNA. The reaction conditions were of 95˚C for 2 min; then 45 cycles at 94 ˚C for 30 s, annealing temperature 45, 49 or 51˚C for 30 s, and 72˚C for 90 s; and a final extension at 72˚C for 5 min (Tan, 2010). After the amplification, PCR products were electrophoretically analyzed in 4 % agarose gel in 1X TBE buffer.

For data analysis, each scorable band was defined as a single locus/allele. The loci were scored as present (1) or absent (0), and the bi-variate 1-0 matrix was generated genetic distances were calculated using PAST3 software (Wangsomnuk et al., 2011). Cluster analysis was performed on molecular data using the Unweighted Pair Group Method with Arithmetic Means (UPGMA). The dendrogram was formed using Dice's value. The polymorphic information content (PIC) and observed heterozygosity (H) were calculated to define the informativeness and the discriminatory capacity of each ISSR marker using the PICcalc software (Mornkham et al., 2010). PIC was defined using the formula (Hildebrand et al., 1992):

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2\, p_j^2$$

where pi and pj are the population frequency of the ith and jth allele, respectively.

Genetic similarities between accessions were also determined with a coefficient based on the proportion of shared alleles and a principal coordinate analysis (PCoA) using the PAST3 software.

Results and Discussion

A total of 62 different alleles were identified using nine ISSR primers in 25 Jerusalem artichoke accessions (Table 2). Fifty-seven of these alleles were polymorphic (92 %). The size of the alleles varied from 90 to 1790 bp. The number of polymorphic alleles varied between three and 14. The most distinguished polymorphic alleles were obtained from marker BDB(CA)7C. The markers DBDA(CA)7 and HVH(CA)7T produced just three polymorphic alleles. The mean value of polymorphic alleles per locus was 6.33. The PIC values ranged from 0.219 to 0.3405. Observed heterozygosity varied from 0.250 to 0.435. The abundance of allelic variation is of importance concerning both evolutionary and breeders' aspects (Wouw et al., 2010). Genetic similarities between Jerusalem artichoke accessions varied from 0.001 to 0.815, with an average of 0.292. The highest genetic similarity value (0.815) was found between accessions 40*3 and 66*1 (Figure 1), which were collected from the Kırşehir and Yozgat provinces, respectively. The other genetically close accessions are 61*2 and 50*3 (collected from Trabzon and Nevşehir province, respectively). But, all these accessions are not phenotypically similar. UPGMA analysis showed that the accessions were divided into two main groups. (Figure 1). No correlation was observed between the distributions in these groups and the geographical origins. The Group-I was consisted of five accessions. Allmembers of this group were collected from different provinces. There is no ecological similarity between these collection sites. For example, although accession 61*2 is collected from a very humid environment, accessions 66*3, 40*3, 50*3, and 19*1 were collected from central Anatolian cities with an arid climate. The genetic similarity value of these accessions were 0.408.

Figure 1. Dendrogram of all 25 Jerusalem artichoke accessions, generated for polymorphic loci through nine ISSR markers using UPGMA.

Group-II consisted of 20 accessions. The genetic similarity value of these accessions was found to be 0.370. In this group, as in the previous one, the geographic origin was not determinant. The only interesting point in this group is that all red tuber colors' accessions (19*1, 06*1, 19*4, 50*2, 58*1, 66*1, 66*2) are placed in this cluster (Hanci and Tuncer, 2019). Within this group, 40*2 (collected from Kırşehir province) is located in a different branch compared to the other members. However, no phenotypic features could be observed to explain the reason for this. The ISSR data explained 38.19 % of thevariation in the principal coordinate analysis by coordinates 1 and 2. The first coordinate explained 20.73 %, the second coordinate explained 17.46 % by PCoA analysis (Figure. 3).

At the end of thesame analysis, the first six principal coordinates showed 68.45 % of the total variation. The PCoA graph indicated that 25 Jerusalem artichokeaccessions did not separate as main groups and there was partially similarity between the patterns constructed by the UPGMA cluster. The most extensive investigations concerning the DNA-based genetic relationships among Jerusalem artichoke accessions were performed by Wangsomnuk et al., 2011. In this study, 147 Jerusalem artichoke accessions collected from nine countries were examined using 30 RAPD (Random Amplified Polymorphic DNA) markers. Thirteen markers produced 357 scorable bands. It was observed that 94.3% of these bands were polymorphic (Wangsomnuk et al., 2011). Also, low genetic richness was detected among wild and cultivated accessions. These results are consistent with the findings of this study. In this study, 57 polymorphic bands with nine ISSR markers were obtained. The ratio of these bands to total bands is 92 %.

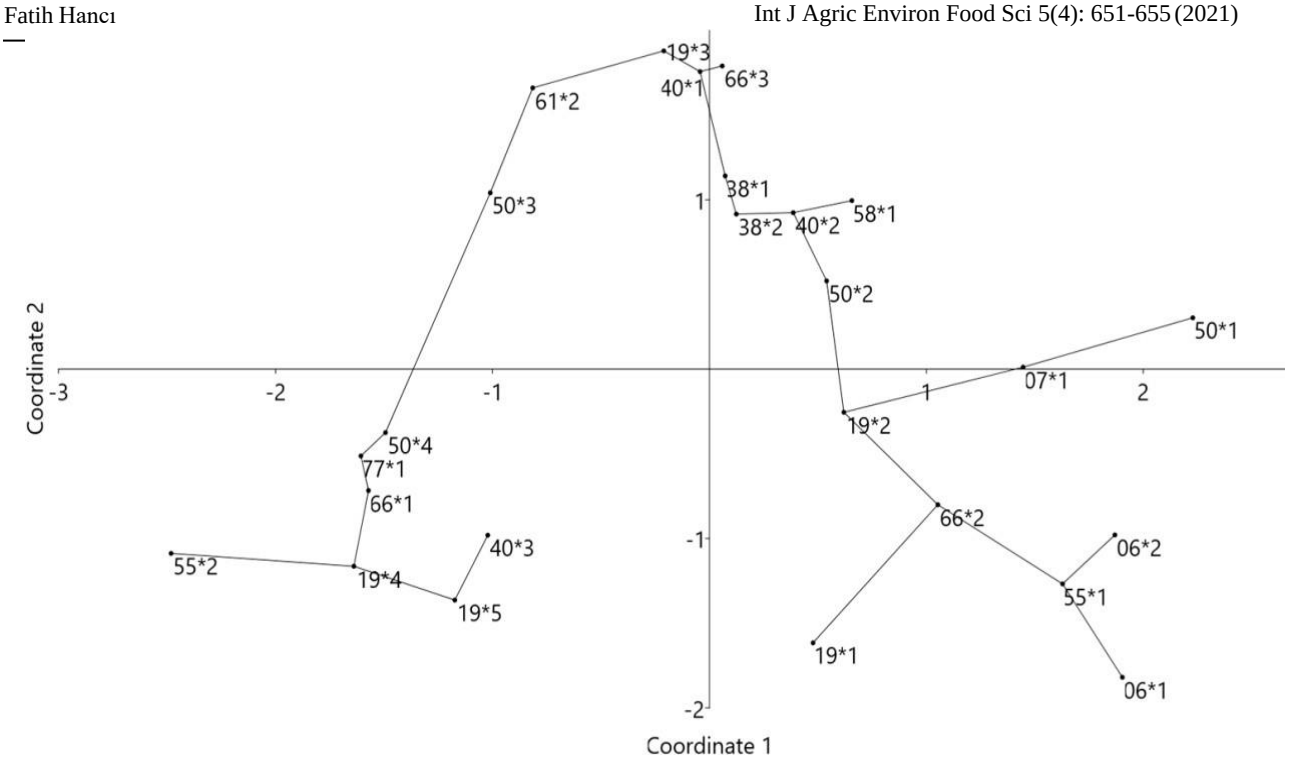

Figure 3. Distribution of Jerusalem artichoke accessions based on principal coordinate analysis

In another study, (Wangsomnuk et al., 2011) the efficacy of RAPD, ISSR, and SRAP (Sequence Related Amplified Polymorphism) marker in characterizing the Jerusalem artichoke accessions was compared. Forty-seven different Jerusalem artichoke accessions were used in that study. A total of 92 (80%) polymorphic bands from six ISSR primers; 296 (87.1 %) polymorphic bands from 13 RAPD primers; and 194 (88.6 %) were detected from nine combinations of SRAP primers. The polymorphism rate obtained from this study (92 %) was found to be high. (Wangsomnuk et al., 2011).

Mornkham et al. (2012) developed 43 EST-SSR markers using 40,362 Jerusalem artichoke ESTs. Then they tested these EST-SSR markers in six Jerusalem artichoke populations. At the end of the study, these SSR loci showed a large genetic richness between accessions. In this study, allele numbers range from 2 to 7, with an average of 3.95 alleles per loci. However, the number of polymorphic alleles per loci was higher in our study (6.33). The reasons for this difference are assumedto be different marker types (dominant ISSR and co-dominate EST-SSR) and the size of the gene pool examined (Jung et al., 2014).

Conclusion

This study contains the first investigation carried out to exhibit the DNA-based genetic diversity between 25 Jerusalem artichoke accessions collected from different geographical localities of Turkey. For this purpose, nine ISSR markers were used, and these markers were found to be quite successful according to the obtained polymorphic band ratio and polymorphism information content (PIC) values. The genetic richness was expected to be narrow because the Jerusalem artichokes are generally vegetatively propagated, and that their homeland is continental America. The richness of genetic diversity observed in this study may be explained as follows: (a) These plants have been grown in very different ecological regions of Turkey for many years, and variations may have arisen through natural selection; (b) the emergence of new genotypic variations as a result of generative reproduction, even in low probability; (c) and spontaneous mutations may have caused genetic variation. The findings of this study can be used by breeders in the selection of various parents for future breeding programs because the particular core subsets can promote the association mapping of genes controlling ecologically relevant features such as inulin, oil characters, and disease resistance [Brown, 1989; Wangsomnuk et al., 2011). However, further characterization studies in this gene pool must continue to establish an effective selection scheme. For this purpose, the concentration of different compounds, such as inulin, will be determined under the same project. Also, studies on the use of high biomass for different purposes are ongoing.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

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Data availability

Not applicable.

Consent for publication

Not applicable.

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