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Full Length Article

Genetic Characterization of *Rheum ribes* (Wild Rhubarb) Genotypes in Lake Van Basin of Turkey through ISSR and SSR Markers

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Abstract

Rheum ribes L. (wild rhubarb) is one of the less known plants to the world and the only species from the *Rheum* genus present in Turkey. In this study, one *R. rhabarbarum* (as check genotype) and 80 *R. ribes* genotypes were collected from different geographical locations of Turkey for the investigation of diversity and genetic structure using ISSR (Inter Simple Sequence Repeat) and SSR (Simple Sequence Repeats) markers. SSR markers reflected higher (100%) polymorphism as compared to the ISSR marker. However, ISSR markers produced higher average Polymorphism Information Content (PIC) value (0.805) than the SSR markers (0.724). A Similar range of (PIC) values with ISSR markers was found greater (0.935-0.395) as compared to the range of SSR makers (0.88-0.47). Using Jaccard similarity index, genetic distance was measured for both markers and average genetic distance was found to be higher with ISSR markers as compared to the SSR markers. Neighbor-joining analysis clustered genotypes into 3 groups for both marker systems. During this study some distinct genotypes like *R. rhabarbarum*, YYUERC19, YYUERC09 and YYUMER65 were investigated that can be used as candidate parents for the development of *R. ribes* L. varieties. Structure analysis grouped the genotypes according to altitude by clustering genotypes having at more than 2000 m in one group and genotypes less than 2000 m altitude in another group. Genetic variations observed in this study can be applied to investigate various traits of interest for the *R. ribes* L. breeding. © 2019 Friends Science Publishers

Keywords: *Rheum ribes* L.; Genetic diversity; ISSR; SSR; Turkey

Introduction

Rheum ribes L. commonly known as wild rhubarb is a hardly perennial plant belonging to *Polygonaceae* family. This family is native to Turkey, Iran and some other surrounding countries (Bazzaz *et al.,* 2005). Genus *Rheum* contains 60 species, most of them distributed in Asia and central Asia are considered as the center of diversity of this genus. *Rheum ribes* L. is one of the most diverse and widely distributed species of *Rheum* genus. *R. ribes* L. is distributed in Turkey, Iran, Pakistan, Afghanistan, Iraq, Armenia and Lebanon (Bazzaz *et al.,* 2005). *R. rhabarbarum* L. is generally considered a tetraploid, 2n = 44; however, other *Rheum* species are 2n = 22, 44 and 66 (Ruirui *et al.,* 2010). *R. ribes* L. contains perennial thick rhizomes and leaves of this plant are green or reddish green in color (Turkmen *et al.,* 2005). Its generative parts (seed stalks) are mainly consumed as a vegetable and the rhizome of plant has great importance for medicinal uses (Özcan *et al.,* 2007). This plant is mainly used to treat the diarrhea, hypertension, obesity and diabetes (Krishnaiah *et al.,* 2011). Wild rhubarb is a rich source of vitamin A, B and C (Heshmat *et al.,* 2008) and also contains good concentrations of flavonoids, aloe-emodin-8-O-glucoside, sennoside A, physcion-8-O-glucoside, rhaponticin, physcion and chrysophanol (Oktay *et al.,* 2007). Similarly, various studies have been conducted to evaluate the antiviral (Hudson *et al.,* 2000), antifungal (Sardari *et al.,* 2009), antibacterial (Bazzaz *et al.,* 2005; Alaadin *et al.,* 2007) and antioxidant (Oktay *et al.,* 2007; Krishnaiah *et al.,* 2011) effects of this plant.

Turkey is considered as the one of the centers of agriculture and center of origin and distribution centers for the different crops due to its very important geographic location (Sensoy *et al.,* 2007; Baloch *et al.,* 2017). Wild rhubarb is present at 1800 m to 2800 m altitude in the rocky countryside of the Anatolia region of Turkey and Iran (Tartik *et al.,* 2015). Among the 60 species of genus *Rheum*, *R. ribes* L. is the only species that is adapted to Turkish geographical conditions (Cullen, 1966).

Genetic diversity is crucial for crop breeding,

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conservation and improvement, and to meeting societal demand for food security (Alghamdi *et al*., 2007a, b). From this perspective, it is thus crucial to understand the structure and evolution of crop species and their wild relatives (Sensoy *et al*., 2007; Baloch *et al*., 2017). Various studies have been conducted to evaluate the genetic structure and diversity in the members of *Polygonaceae* family through the usage of different molecular marker systems (Kuhl and DeBoer, 2008; Zhang *et al.*, 2008; Chen *et al*., 2009; Hu *et al*., 2010, 2011; Wang, 2011).

ISSR and SSR markers have been most commonly used for the determination of genetic diversity in *R. tanguticum* (Chen *et al.*, 2009; Hu *et al.*, 2010). However, information about the diversity and genetic structure of wild rhubarb is very scare and demanding the attention of researchers to explore this plant to the world. Such types of studies are helpful for conservation and management strategies as well as in the selection of diverse germplasm lines for the cultivation and future improvement of crops. Moreover, inferences of these studies can be vital for the taxonomic implications. Thus, a gap is present requiring answers to issues such as the level of genetic diversity present in natural populations of wild rhubarb and how these genetic variations are distributed in different geographical areas of Turkey. Considering these aspects, the main aim of present study was to investigate the genetic diversity and population structure of 80 wild rhubarb genotypes in Lake Van Basin of Turkey using ISSR and SSR markers.

Materials and Methods

Plant Material and DNA Extraction

A total of 80 *R. ribes* L. genotypes with 1 *R. rhabarbarum* L. as control genotype were collected from 4 different locations of Lake Van Basin of Turkey (Table 1). Fresh leaves of each genotype were collected and preserved in zip-lock bags with silica gel until DNA extraction was performed. DNA from stored leaves samples was performed according to modified CTAB protocol of Doyle and Doyle (1990) with some modifications of Baloch *et al.* (2016).

ISSR Amplification

Initially, a total of 66 ISSR primers were screened on randomly selected 8 wild rhubarb genotypes. These ISSR primers were synthesized by Shanghai Sangon Biological Engineering Technology and Service (China), according to the primer set published by University of British Columbia, Canada (UBC set No.9) (Wang, 2011). Out of these 66, those 13 primers were selected for amplification of all genotypes that resulted in easily identified polymorphisms (Table 2). PCR reaction was carried out by following the Hu *et al.* (2010). PCR reaction contains 2.5 μL 10 ×buffer (Tris–HCl (pH 8.3) 100 m*M*; KCl 500 m*M*), 0.1 m*M* dNTP, 3.0 m*M* $MgCl_2$, 10 pmol primer, 0.75 U Taq DNA polymerase (5 U/μL; Thermo Scientific, catalog number EP0402) and 30 ng template DNA in a final volume of 25 μL. Amplification was performed in a T-100 thermocycler (Bio-Rad, USA) by maintaining initial denaturation at 94°C for 5 min, followed by 38 cycles of 20 s at 94°C, 60 s at 50–59.7°C (depending on primers used; Table 2) and 80 s at 72°C with a final extension for 6 min at 72°C (Hu *et al*., 2010). PCR products were detected by electrophoresis on 1.2% (w/v) agarose gel using 1×TAE buffer for 2.5 h; the gel was stained with ethidium bromide after the electrophoresis and visualized under UV Imager Gel Doc XR+ system (Bio-Rad, USA) light and later photographed. A 100-bp ladder (Thermo Scientific) was used as a molecular weight marker.

SSR Amplification

A total of 10 SSR primers (Zhang *et al.*, 2008) were used in this study according to their ability to successfully amplify all rhubarb plants with greater reproducibility of their product (Table 2). For amplification, a total volume of 25 μL was prepared by following the Zhang *et al.* (2008) containing 10–15 ng genomic DNA,1x reaction buffer (Takara), 250 m*M* of each dNTP, 20 m*M* of each primer, and one unit of Taq DNA polymerase (Takara). For amplification, Thermal Cycler (Bio-Rad USA) was programmed by maintaining one cycle of 95 C for 3 min as denaturation temperature; 95ºC for 30 s, 46ºC for 30 s, 72ºC for 30 s, 37 cycles and final extension at 72ºC for 10 min, one cycle. A 100-bp ladder (Thermo Scientific) was used as a molecular weight marker.

PCR products were evaluated by capillary electrophoresis. Calibration of the QIAxcel screen electrophoresis system (QIAGEN) was done with the help of the QIAxcel screen gel version 1.4 software. 25-500 bp (19 ng/mL) size marker and 15-3000 bp alignment marker (19 ng/mL) were utilized for band screening of the PCR products. The OL1200 method was utilized in the capillary gel electrophoresis system to determining the size of the amplicon. The injection time in the software was set to 15 sec. High-Resolution DNA 1200 sample kit was utilized.

Data Analysis

Amplified SSR fragments were analyzed using GeneMapper software v3.7 (Applied Biosystems), by following its provided user manual. For ISSR markers, amplified DNA fragments were scored as binary data (1/0), indicating the presence or absence of a marker in the genomic representation of each sample respectively and only consistently reproducible bands were scored. ISSR markers were screened by their reproducibility and Polymorphism Information Content (PIC) value and only those markers were considered having PIC value greater than 0.1. Statistical and clustering algorithms were implemented in R

Table 1: Passport data of 80 wild rhubarb genotypes and *R. rhabarbarum* cultivar

		Coordinates		
Genotype name	**Collection site**	**Altitude**	**Latitude (N)**	**Longitude (E)**
YYU-ERC-01	ERÇEK- Karakoç Village Irgat Mountain	1983	38 36' 23,41	43 44' 12, 28"
YYU-ERC-02	ERÇEK- Karakoç Village Irgat Mountain	2019	38 36' 22, 52	43 44' 10,2"
YYU-ERC-03	ERÇEK- Karakoç Village Irgat Mountain	2015	38 36' 23,14	43 44' 10,2"
YYU-ERC-04	ERÇEK- Karakoç Village Irgat Mountain	2016	38 36' 23, 23	43 44' 7,83"
YYU-ERC-05	ERÇEK- Karakoç Village Irgat Mountain	2018	38 36' 23,26	43 44' 6,37"
YYU-ERC-06	ERÇEK- Karakoç Village Irgat Mountain	2064	38 36' 23,21"	43 44' 2,62"
YYU-ERC-07	ERÇEK- Karakoç Village Irgat Mountain	2066	38 36' 23,46"	43 44' 1,27"
YYU-ERC-08	ERÇEK- Karakoç Village Irgat Mountain	2081	38 36' 22,62"	43 44' 0,01"
YYU-ERC-09	ERÇEK- Karakoç Village Irgat Mountain	2076	38 36' 22,02"	43 43' 58,22"
YYU-ERC-10	ERÇEK- Karakoç Village Irgat Mountain	2083	38 36' 21,76"	43 43' 57,77"
YYU-ERC-11	ERÇEK- Karakoç Village Irgat Mountain	2083	38 36' 21,54"	43 43' 57,77"
YYU-ERC-12	ERÇEK- Karakoç Village Irgat Mountain	2082	38 36' 21,53"	43 43' 55,39"
YYU-ERC-13	ERÇEK- Karakoç Village Irgat Mountain	2126	38 36' 18,25"	43 43' 55,39"
YYU-ERC-14	ERÇEK- Karakoç Village Irgat Mountain	2128	38 36' 18,12"	43 43' 54,3"
YYU-ERC-15	ERÇEK- Karakoç Village Irgat Mountain	2147	38 36' 12,69"	43 43' 50,44"
YYU-ERC-16	ERÇEK- Karakoç Village Irgat Mountain	2138	38 36' 12,24"	43 43' 50,98"
YYU-ERC-17	ERÇEK- Karakoç Village Irgat Mountain	2122	38 36' 10,01"	43 43' 50,53"
YYU-ERC-18	ERÇEK- Karakoç Village Irgat Mountain	2117	38 36' 11,01"	43 43' 50,7"
YYU-ERC-19	ERÇEK- Karakoç Village Irgat Mountain	2128	38 36' 11,19"	43 43' 50,73"
YYU-ERC-20	ERÇEK- Karakoç Village Irgat Mountain	2119	38 36' 11,05"	43 43' 507,3"
YYU-BAH-21	BAHÇESARAY	1925	38 0' 29,67"	42 44' 45, 74"
YYU-BAH-22	BAHÇESARAY	1960	38 0' 31, 26"	42 44' 31,17"
YYU-BAH-23	BAHÇESARAY	1960	38 0' 31,21"	42 44' 31,18"
YYU-BAH-24	BAHÇESARAY	1960	38 0' 31,32"	42 44' 30,95"
YYU-BAH-25	BAHÇESARAY	1960	38 0' 30,92"	42 44' 31,68"
YYU-BAH-026	BAHÇESARAY	1970	38 0' 30,52"	42 44' 31,45"
YYU-BAH-27	BAHÇESARAY	1980	38 0' 30,08"	42 44' 31,47"
YYU-BAH-28	BAHÇESARAY	1980	38 0' 30,08"	42 44' 31,47"
YYU-BAH-29	BAHÇESARAY	1985	38 0' 29,71"	42 44' 32,48"
YYU-BAH-30	BAHÇESARAY	1985	38 0' 29,48"	42 44' 32,39"
YYU-BAH-31	BAHÇESARAY	1990	38 0' 29,33"	42 44' 32,54"
YYU-BAH-032	BAHÇESARAY	1985	38 0' 29,62"	42 44' 32,57"
YYU-BAH-33	BAHÇESARAY	1985	38 0' 29,6"	42 44' 32,85"
YYU-BAH-34	BAHÇESARAY	1980	38 0' 29,64"	42 44' 33,07"
YYU-BAH-35	BAHÇESARAY	1975	38 0' 29,85"	42 44' 33,26"
YYU-BAH-36	BAHÇESARAY	1970	38 0' 30,17"	42 44' 33,57"
YYU-BAH-37	BAHÇESARAY	1965	38 0' 30,01"	42 44' 33,72"
YYU-BAH-38	BAHÇESARAY	1960	38 0' 30"	42 44' 33,91"
YYU-BAH-39	BAHÇESARAY	1960	38 0' 30"	42 44' 33,91"
YYU-BAH-40	BAHÇESARAY	1960	38 0' 30,31"	42 44' 34,03"
YYU-MUR-41	Doğangün Village- MURADİYE	2245	38 45' 28,41"	43 45' 1,25"
YYU-MUR-42	Doğangün Village- MURADİYE	2250	38 45' 27,94"	43 45' 1,18"
YYU-MUR-43	Doğangün Village- MURADİYE	2255	38 45' 27,56"	43 45' 2,15"
YYU-MUR-44	Doğangün Village- MURADİYE	2265	38 45' 25,46"	43 44 59,14"
YYU-MUR-45	Doğangün Village- MURADİYE	2280	38 45' 22,66"	43 44' 54,96"
YYU-MUR-46	Doğangün Village- MURADİYE	2290	38 45' 20,92"	43 44' 54,67"
YYU-MUR-47	Doğangün Village- MURADİYE	2335	38 45' 18,58"	43 44' 54,46"
YYU-MUR-48	Doğangün Village- MURADİYE	2340	38 45' 16,69"	43 44' 53,73"
YYU-MUR-49	Doğangün Village- MURADİYE	2350	38 45' 15,83"	43 44' 53,92"
YYU-MUR-50	Doğangün Village- MURADİYE	2360	38 45' 15,66"	43 44' 53,24"
YYU-MUR-51	Doğangün Village- MURADİYE	2360	38 45' 15,69"	43 44 53,23"
YYU-MUR-52	Doğangün Village- MURADİYE	2370	38 45' 14,38"	43 44' 53,11"
YYU-MUR-53	Doğangün Village- MURADİYE	2370	38 45' 13,74"	43 44' 53,34"
YYU-MUR-54	Doğangün Village- MURADİYE	2395	38 45' 13,01	43 44 51,63"
YYU-MUR-55	Doğangün Village- MURADİYE	2395	38 45' 12,53"	43 44' 52,42"
YYU-MUR-56	Doğangün Village- MURADİYE	2395	38 45 12,71"	43 44' 52,32"
YYU-MUR-57	Doğangün Village- MURADİYE	2395	38 45' 12,93"	43 44' 52,64"
YYU-MUR-58	Doğangün Village- MURADİYE	2395	38 45' 12,46"	43 44' 53,11"
YYU-MUR-58	Doğangün Village- MURADİYE	2395	38 45' 12,32"	43 44' 53,83"
YYU-MUR-60	Doğangün Village- MURADİYE	2420	38 45 10,82"	43 44' 53,12"
YYU-MER-61	Erek Mountain	2110	38 29' 50,76"	43 29' 0,76"
YYU-MER-62	Erek Mountain	2110	38 29' 50,39"	43 29' 0,76"
YYU-MER-63	Erek Mountain	2095	38 29' 49,45"	43 29' 0,45"
YYU-MER-64	Erek Mountain	2145	38 29' 46,58"	43 28' 55,7"
YYU-MER-65	Erek Mountain	2145	38 29' 44,17"	43 28' 54,53"
YYU-MER-66	Erek Mountain	2145	38 29' 44,9"	43 28 53,78"
YYU-MER-67	Erek Mountain	2150	38 29' 45,54"	43 28' 54,42"
YYU-MER-68	Erek Mountain	2165	38 29' 44,31"	43 28 54,365"
YYU-MER-69	Erek Mountain	2135	38 29' 39,82"	43 28' 54,46"
YYU-MER-70	Erek Mountain	2135	38 29' 39,82"	43 28' 54,46"
YYU-MER-71	Erek Mountain	2135	38 29'39,82"	43 28' 54,46"
YYU-MER-72	Erek Mountain	2135	38 29' 40,62"	43 28' 54,07"
YYU-MER-73	Erek Mountain	2145	38 29' 40,16"	43 28' 54,56"
YYU-MER-74	Erek Mountain	2145	38 29' 39,25"	43 28' 54,36"
YYU-MER-75	Erek Mountain	2145	38 39' 39,45"	43 28' 54,33"
YYU-MER-76	Erek Mountain	2155	38 29' 39,09"	43 28' 54,56"
YYU-MER-77	Erek Mountain	2155	38 29' 39,09"	43 28' 54,56"
YYU-MER-78	Erek Mountain	2165	38 29' 38,13"	43 28' 54,41"
YYU-MER-79	Erek Mountain	2165	38 29' 38,43"	43 28' 54,23"
YYU-MER-80	Erek Mountain	2165	38 29' 37,98"	43 28' 54,33"
Rheum rhabarbarum	Cultivar			

software. Genetic distance between wild rhubarb genotypes was measured by using Jaccard similarity index and a neighbor-joining cluster was constructed by applying the genetic distance coefficients with the R software (Paradis, 2012).

In order to obtain a clear picture of the wild rhubarb genotypes in this study, the Bayesian model-based clustering algorithm was implemented in the STRUCTURE software. In the structure program, number of populations (K) were determined through the Admixture and shared allele frequencies model in the range from 1-12. For each independent runs, initial burn-in period was set to 500 with 500,000 MCMC (Markov chain Monte Carlo) iterations. Most suitable value of K was evaluated through the ΔK method as implemented in Structure Harvester (Evanno *et al.*, 2005).

Results

Genetic Diversity and Population Structure based on ISSR

Within the investigated population of 80 *R. ribes* L. genotypes with 1 *R. rhabarbarum* L. genotype, 13 most polymorphic ISSR primers resulted in a total of 120 scorable bands. Among these 120 bands, a total of 119 (99.16%) were found polymorphic with an average of 9.15 polymorphic fragments per primer (Table 3). The average number of bands per primer was 9.23 and the minimum number of bands was produced by the primer Sola2, while a maximum number of bands were produced by primers 834, 5F and Sola1. All primers generated 100% polymorphism except the Sola6 primer which produced one monomorphic band. These 13 ISSR primers resulted in a high level of polymorphism in 80 *R. ribes* L. genotypes and 1 *R. rhabarbarum* L. genotype with an average of 98.72. The maximum value of polymorphism information content (PIC) was 0.93 produced by the primer 834 (Table 3). Sola3 was the primer that resulted in the minimum PIC value (0.395). The average PIC value per 13 ISSR markers for 80 wild rhubarb genotypes was 0.805.

Genetic distances between wild rhubarb genotypes were calculated on the basis of shared-allele distances. The highest genetic distance was 0.90 present between YYUERC19 and *R. rhabarbarum* L. genotypes followed by the *R. rhabarbarum* L. and YYUERC02 genotypes having 0.89 genetic distance. Minimum genetic distance was 0.09 present between YYUMER70 and YYUMER71 genotypes and followed by the YYUERC05 and YYUERC10 genotypes having 0.26 genetic distance. Average genetic distance between 80 *R. ribes* L. and 1 *R. rhabarbarum* L. genotype was 0.60. By applying Neighbor Joining analysis based on the Jaccard genetic distance, all genotypes grouped into three clusters A, B and C (Fig. 1). Group B was found smaller by containing 10 genotypes and Group C was bigger group by clustering 58 genotypes; while 13 genotypes

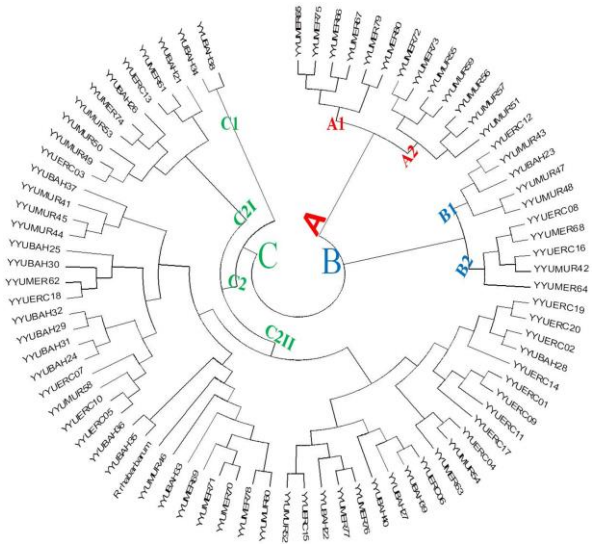

Fig. 1: Neighbor joining clustering of 80 wild rhubarb genotypes and *R. rhabarbarum* cultivar using ISSR markers

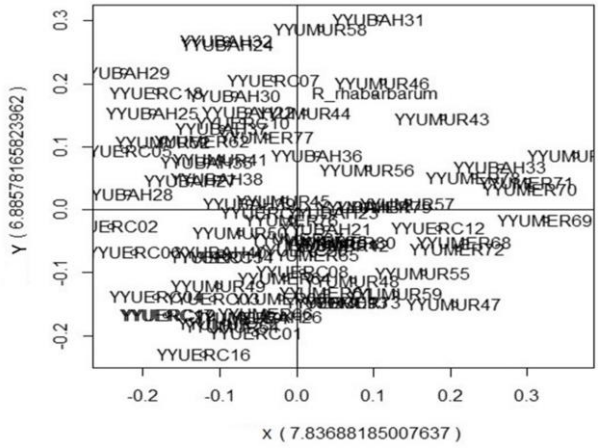

Fig. 2: Jaccard similarity analysis of 80 wild rhubarb genotypes and *R. rhabarbarum* cultivar using ISSR and SSR markers

clustered in group A. *R. rhabarbarum* L. clustered in the C2II and found close to the YYUMUR46 genotype. Principal coordinate analysis (PCoA) was performed which clearly discriminated the genotypes according to their collection sites (Fig. 2). ISSR data was also used for the genetic structure determination using the Bayesian clustering model implemented in the computer software STRUCTURE and maximum observed ΔK value was 2. STRUCTURE analysis divided studied material into two groups (Fig. 3).

Genetic Diversity and Population Structure based on SSR

A total of 10 SSR primers were used in this study that

Table 2: Name, sequence and annealing temperature of ISSR and SSR primers used for the investigation of diversity and genetic structure of wild rhubarb

ISSR	Sequence	Annealing temp (°C)	SSR	Sequence	Annealing temp (°C)
816	CACACACACACACACAT	50.0	Rta001	F:GTATGCTATTATTGTGGTTGGAC; R:CAGCGGAATCATGAATTAGTAAC	50
834	AGAGAGAGAGAGAGAGCTT	54.0	Rta 002	F:GAATCACAAACAAAGCTTACCC; R:CATATGTTGCTTGTATGTATGGC	52
4F	$(GA)_8YC$	54.8	Rta 003	F:AAAGCCATCCAAATCGAAGC; R:CTACAGAGGCAAGACAATCAAC	50
5F	$(GA)_8YG$	54.8	Rta 004	F:AAGCGTGTGGTGTGCTGAGAG; R:CACAGTTTGAACCATTTAAACAC	48
6F	$(AG)_8YT$	52.6	Rta 005	F:CCGAAGTCCAAGTAGGGGTCC; R:CACCAAACCCACTTCAACCAC	54
7F	$(AG)_8YC$	54.8	Rta 006	F:CAGCGTAATCACGACTTAGAAC; R:GAGTGTGTATGACGTGTTGATG	52
8F	$(AC)_8YA$	52.6	Rta 007	F:GGGTAGTCCCCTTTGAGGTTGTAG; R:TGCATGCCTGCAGGTCGACG	52
10F	$(GT)_8YC$	54.8	Rta 008	F:AGCAGAATCAATTCACGTTCAC; R:CAATATGTGCTTAGATTTGGC	47
Sola-1	BDB-(ACA)5	50.0	Rta 009	F:TTGAGGCATTGCGTGTGAGC; R:ACACAATCCTTTGTCTCATATGC	52
Sola-2	$DD\text{-}(CGA)_5$	56.8	Rta 010	F:GAGCTCGGTACCCGGGGATC; R:TGCAGGTCGACGATTTTTAAGGC	52
Sola-3	$DBH\text{-}(CGA)_5$	59.0			
Sola-5	$DBD(AC)_7$	51.1			
Sola-6	$BDB\text{-}(CAC)_5$	59.7			

Table 3: ISSR and SSR primers with total and polymorphic band and PIC value obtained in this study

ISSR primers	Polymorphic band	Total band	PIC	Polymorphism (%)	SSR Primers	Polymorphic band	Total band	PIC	Polymorphism (%)
816	9	9	0.75	100.00	Rta-01	12	12	0.89	100.00
834	14	14	0.94	100.00	Rta-02	8	8	0.86	100.00
4F	10	10	0.90	100.00	Rta-03	3	3	0.60	100.00
5F	14	14	0.89	100.00	Rta-04	2	2	0.48	100.00
6F	13	13	0.90	100.00	Rta-05	3	3	0.65	100.00
7F	12	12	0.89	100.00	Rta-09	7	7	0.80	100.00
8F	6	6	0.77	100.00	Rta-10	6	6	0.80	100.00
10F	9	9	0.87	100.00					
Sola1	14	14	0.93	100.00					
Sola2	3	3	0.63	100.00					
Sola3	4	4	0.40	100.00					
Sola5	6	6	0.82	100.00					
Sola6	5	6	0.78	83.33					
Mean	9.15	120	0.81	98.72		5.86	41	0.72	

produced a total of 41 scorable bands. All of these 41 bands were found polymorphic with an average of 5.86 polymorphic fragments per primer (Table 3). Maximum numbers of polymorphic bands (12) were produced by the Rta01 primer and minimum 2 bands were produced by the Rta04 primer. All primers were found to be 100% polymorphic and no monomorphic banding patterns were produced by these primers. PIC values ranged between 0.478-0.88 and the average PIC value was 0.72 (Table 3). Maximum PIC value (0.88) was observed with Rta01 primer and followed by the Rta02. Minimum PIC value was recorded with the primer Rta04.

Genetic distance between 80 *R. ribes* L. and 1 *R. rhabarbarum* L. genotype was also calculated and average genetic distance was 0.53. Maximum genetic distance 0.96 was present between the YYUERC09 and YYUMER65 genotypes followed by the *R. rhabarbarum* L. and YYUERC10 genotypes having 0.89 genetic distance. Minimum genetic distance between 80 *R. ribes* L. and 1 *R. rhabarbarum* L. genotype was 0.06 present between YYUERC06 and YYUMUR59 genotypes followed by the YYUERC12 and YYUMER63 genotypes having 0.07 genetic distances.

Neighbor-Joining analysis based on Jaccard genetic distance, all genotypes grouped into three clusters A, B and C (Fig. 4). Cluster A was smallest among these by clustering only a single genotype YYUMER65. Group C was found larger group by clustering 44 genotypes and 36 genotypes grouped in the group B. Group B was further grouped into 2 groups B1 and B2. Subgroup B2 was found larger by clustering 30 genotypes and only 6 genotypes grouped into B1 subgroup. Similar to B group, C group also further grouped into 2 subgroups C1 and C2. C2 sub group was larger group by clustering 33 genotypes and 11 genotypes grouped into C1 subgroup. Similar to ISSR markers, PCoA analysis was also performed which also clustered the genotypes on the basis of their geographical locations (Fig. 5). SSR data was also used for the determination of genetic structure of 81 *Rheum* genotypes by using the Bayesian clustering model implemented in the computer software STRUCTURE and Maximum observed ΔK value was 2. STRUCTURE analysis divided 81 *Rheum* genotypes into two groups (Fig. 6).

Discussion

Turkey contains a large diversity of plants and has played an important role in the distribution of different crops due to its geographical conditions (Baloch *et al.,* 2017; Yaldiz *et al.,* 2017; Arystanbekkyzy *et al.,* 2018). Medicinal plants are

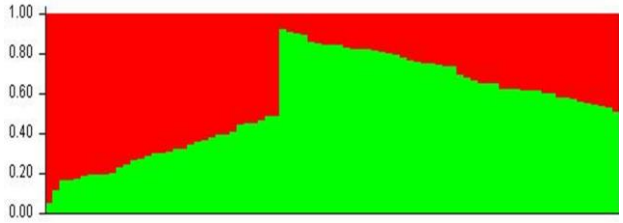

Fig. 3: Population structure analysis of wild rhubarb genotypes and *R. rhabarbarum* cultivar using ISSR markers

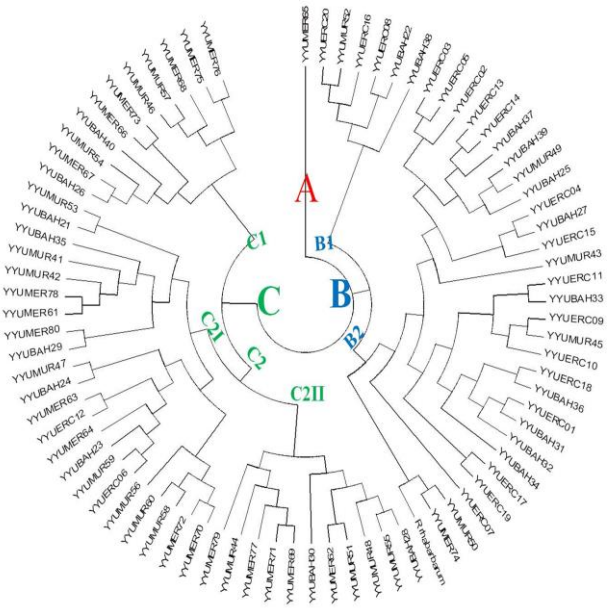

Fig. 4: Neighbor joining clustering of 80 wild rhubarb genotypes and *R. rhabarbarum* cultivar using SSR markers

very popular among the Turkish people and some of them are included in their daily food (Ugulu *et al.,* 2009; Nadeem *et al.,* 2018). Besides their occurrence in huge numbers, a good number of medicinal plants are not well familiar to world and members of *Polygonaceae* are very less known to world instead of containing high medicinal importance. Various scientists tried to explore these plants to the world; however, in front of economically important crops, studies conducted on these medicinally important plants are almost nothing to explore their importance, effectiveness, population structure and level of genetic diversity. Wild rhubarb (*R. ribes* L.) is less known to the world and to our best knowledge this is very first report claiming its genetic diversity and structure through the application of two different molecular markers.

A total of 80 Turkish *R. ribes* L. genotypes and 1 control cultivar (*R. rhabarbarum* L.) collected from various geographical locations in Lake Van Basin of Turkey were used as study material. The total number of polymorphic bands produced by the ISSR markers were greater as compared to SSR markers. The average number of

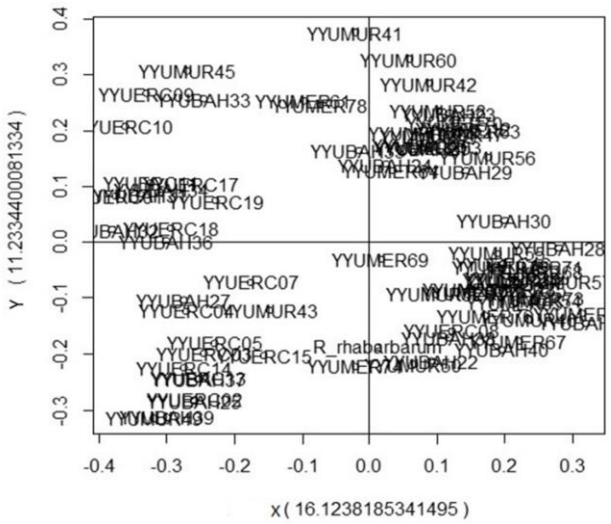

Fig. 5: Jaccard similarity analysis of 80 wild rhubarb genotypes and *R. rhabarbarum* cultivar using SSR markers

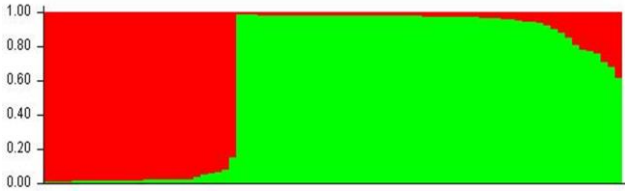

Fig. 6: Population structure analysis of wild rhubarb genotypes and *R. rhabarbarum* cultivar using SSR markers

polymorphic bands per primer were found to be greater with ISSR markers as compared to SSR markers. However, SSR markers reflected higher (100%) polymorphism compared to 98.72% polymorphism observed with ISSR markers. Wang *et al.* (2012) and Hu *et al.* (2014) used the ISSR markers to investigate the genetic diversity in the different species of *Rheum* genus and both of these studies resulted higher number of bands as compared to obtained in this study. One of the possible reasons behind the higher number of bands may be due to a larger number of samples and presences of samples from different species. However, Wang *et al.* (2012) obtained fewer polymorphic bands (180/189) as compared to this study (119/120). Polymorphism obtained with the application of ISSR markers was much higher compared to Hu *et al.* (2014) which observed 93.87%. For the SSR markers, the total numbers of bands produced by Gilmore *et al*. (2014) were found to be greater compared to this study. However, the level of polymorphism with SSR markers was found to be greater in this study as compared to Gilmore *et al.* (2014).

PIC values represent the broader picture for the assessment of diversity as compared to the number of obtained bands because it takes account of the relative frequencies of each band (Cömertpay *et al.,* 2012). Hence, results obtained during this study leads to the selection of

greater polymorphic markers in order to reduce the number of required loci for precise genotype characterization. During this study, ISSR markers produced higher average PIC value as compared to SSR markers. Similarly, the range of PIC values with ISSR markers was found to be greater (0.935-0.395) compared to the range of SSR markers (0.88-0.47). The range and average PIC values obtained by Tabin *et al.* (2016) were far less using ISSR markers compared to this study. Polymorphism obtained in this study was much greater compared to Tabin *et al.* (2016).

To explore the phylogenetic relatedness among 80 Turkish wild rhubarb genotypes, genetic distances were measured using Jaccard similarity index. ISSR markers reflect higher genetic distances as compared to SSR markers. The maximum genetic distance was present between YYUERC19 and *R. rhabarbarum* L. genotypes. These genotypes reflect the presence of a higher level of variations between them and can be used as candidate parents for breeding of wild rhubarb. However, as compared to the ISSR markers, SSR markers expressed the greater range (0.06-0.96) of genetic distances compared to ISSR markers with 0.09-0.90. The maximum genetic distance with SSR markers was present between YYUERC09 and YYUMER65 genotypes, suggesting these two genotypes might be used for future breeding activities.

To get a clear picture of genetic diversity, Neighbor Joining analysis was applied that clustered all samples in to three main groups A, B and C for both marker systems. Within ISSR markers, group C was the larger and group B was smaller. It was clearly understandable that geographical locations played a key role in the clustering of genotypes with the ISSR markers. Previous studies have also showed the clustering of samples according to their geographical location and our result using ISSR markers were found in line with the Wang (2011) and Wang *et al.* (2012) where they used ISSR markers with various *Rheum* species. Main group A contains most of the genotypes collected from Erek Mountain; however, it also contains genotypes like YYUMUR51, YYUMUR56, YYUMUR57 and YYUMUR59 collected from Doğangün Village-Muradiye. Main group C was further subdivided into C1 and C2 and the C1 subgroup contains only two distinct genotypes YYUBAH38 and YYUBAH34 belonging to same geographical location. Subgroup C2 was further grouped into C2I and C2II and contained 56 genotypes. However, it was also observed that some genotypes belonging to other group also clustered into another group. Group B was found most diverse group of study by clustering genotypes from all locations. Results of this study was found in line with the Tabin *et al.* (2016) working on the various *Rheum* species with the ISSR markers.

Neighbor joining analysis based on the Jaccard genetic distance was also applied for the SSR markers that grouped all genotypes into three main groups A, B and C. Group A was the smallest, clustering a single genotype, YYUMER65. Maximum genetic distance was also present between YYUMER65 belonging to group A and YYUERC09 present in group B with SSR markers. YYUMER65 was found to be very distinct from all other genotypes this genotype is a candidate to start breeding activities. Group C was the largest group that was further sub-grouped into two sub groups, C1 and C2. Most of the genotypes grouped according to their geographical location similar to the ISSR markers. Similar to ISSR marker, same patterns of genotypes grouping with other groups was also observed. Group B contained most genotypes from different geographic locations. However, in group C, most of the genotypes belonging to similar locations grouped together. Gilmore *et al.* (2014) also used SSR markers to investigate the genetic structure of various *Rheum* species and they found the clustering of genotypes on the basis of their geographical locations and we also found similar results in case of genotype clustering. To understand the level of diversity more clearly, PCoA analysis was performed for the both marker systems which clearly supported the results of UPGMA based clustering by grouping all genotypes mainly according to their geographical regions with very few exceptions. Genotypes belonging to YYUBAH and YYUMUR locations mostly clustered with each other's in ISSR PCoA, while genotypes from YYUERC and YYUBAH locations were found together in SSR PCoA. For both markers system, *R. rhabarbarum* was also mixed with the *R. ribes* and did not make divergence from it. Possibly, we used only single cultivar, so it will be much interesting to include more numbers of *R. rhabarbarum* cultivars in order to investigate the level of variations and their phylogenetic relationship with *R. ribes*. Population structure analysis grouped the 80 *R. ribes* L. genotypes in to two groups A and B at K=2 for both ISSR (Fig. 3) and SSR (Fig. 6) markers. For the both markers systems, altitude of samples collection sites played an effective roles in their clustering. Green color in structure analyses for both marker systems represent the genotypes at greater than the 2000 m and less than 2000 m are present in other group represented with red color.

Conclusion

This study comprehensively explored the efficiency of ISSR and SSR markers and 100% polymorphism was observed with SSR markers. Both marker systems reflected that geographical locations played an effective role in the clustering of genotypes, and *R. rhabarbarum* L., YYUERC19, YYUERC09 and YYUMER65 were found to be distinct genotypes that can also be used as parents for the breeding of this wild vegetable, which is also medicinally important.

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