Characterisation of some *Ribes* L. accessions from Turkey based on SSRs patterns

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***Abstract*—** *The variability of SSRs patterns were analysed for taxonomical delimitations including intra specific variations in 7 Ribes alpinum, 2 Ribes bieberstenii and 1 Ribes uva-crispa accessions from their natural populations. The total amplified produces of 10 SSRs primers were 172 between 50 and 330 bp (average of 17.2 bands per primer), of which 157 bands were polymorphic between Ribes accessions, corresponding to 91.2 % genetic diversity. The number of bands for each SSRs primer varied from 6 to 32. Segregations of Ribes accessions at specific and intraspecific levels were accomplished showing taxonomical and phylogeographical relations. Obtained results can be used as complementary data in characterizations of Ribes gene pool in Anatolia and selection of the germplasms suitable for crop improving.*

***Keywords*— Ribes, SSRs, taxonomy, variation.**

I. INTRODUCTION

Genus *Ribes* L. from Grossulariaceae has about 200species and native throughout the temperate regions of the Northern Hemisphere. The centers of diversity is in temperate north America, Eurasia and Andes. The greatest number of species occurs in north America (ca.70). 16 species are distributed in Europe. A few of the European species are found in Mediterranean region of northwestern Africa. The plant is a shrub having alternate, simple, usually palmately lobed, exstipulate or with small stipules adnate to the petiole. Inflorescence racemose. Flowers hermaphrodite or dioecious, actinomorphic, epigynous. Hypanthium conspicuous, oftencoloured. Sepals 4-5. Petals 4-5, free. Stamens 4-5, alternating with the petals. Ovary inferior, with 2 carpels; placentation parietal; ovules numerous; styles 2,joined below. Fruit a berry with persistent calyx at apex; seeds with copious endosperm (Chamberlain, 1972). Traditionally, currents (*Ribes*) and gooseberries (*Grossularia*) have been treated as distinct groups by Janczewski (1907) and Berger (1924). On the other hand, monophyletic origins of *Ribes* and *Grossularia* have been suggested based on 18-26S nuclear r DNA ITS and ETS regions and from the chloroplast psbA-trnH intergenic spacer (Schultheis et al. 2004). Although numerous subgeneric classifications have been proposed, nine subgenera are recognised in *Ribes* (Janczewski, 1907; Berger 1924; Messinger *et al.*,1999). In the infrageneric analysis of *Ribes* using restriction site polymorphisms in two cp DNA regions including rbcL to accD and rpoC1 to rpoC2, eight section were reported to be monophyletic (Messinger*et al.*, 1999).Based on ITS sequence data, several major clades in *Ribes* referred as *Berisia*, *Calobotrya*, *Coreosma* and *Grossularia* were revealed and broader circumscriptions suggested in some subgenera (Senters and Soltis, 2003). Six species namely, *Ribes rubrum* L., *Ribes biebersteinii* Berl. Ex. DC, *Ribes nigrum* L., *Ribes uva-crispa* L., *Ribes alpinum* L., *Ribes orientalis* L. were recorded in flora of Turkey (Chamberline, 1972) in addition to *Ribes anatolica* Behçet spec. nov. Published as a new species recently (Behçet, 2001). This genus has large distribution zone across a range of habitats and plant types in Turkey. A large number of investigations on the wild species and cultivars of *Ribes* were reported on biological activities and nutritional qualities of the fruits as potential functional food such as essential oils (Kampuss *et al.*, 2008), fatty acids and fruit juice characteristics (Del Castillo *et al.*, 2004), antioxidants contents (Moyer *et al.*, 2002), fatty acid compositions of the seed oils (Del Castillo *et al.*, 2002; Özcan, 2013), antiviral activities (Sekizawa, 2013), health promoting effects etc. (Wu *et al.*, 2004). Commercially important species is *R.nigrum*L. which is mainly used for juice production, where thehigh levels of ascorbic acid and anthocyanins are valued.On the other side, potential use of molecular markers including microsatellites (SSRs) (Scott,2001; Brennan *et al.*, 2002; Wood head *et al.*, 2003; Cavanna *et al.*, 2009), AFLPs, ISSRs and RAPDs markers (Lanham and Brennan, 1999; Lanham *et al.*, 2000; Brennan *et al.*, 2002; Korbin *et al.*, 2002;Brennan *et al.*, 2009)have been previously reported in the characterizations of the germplasm collections of *Ribes*. AFLP, SSR (genomic and EST-derived) and SNP markers were also used in constructing of genetic linkage map of *Ribes nigrum* L. which allows the dissection of important quantitative traits and also the identification of candidate genes controlling specific characters in marker-assisted breeding programs (Brennan *et al.*, 2008). A few numbers of studies were published on this genus distributing in the native range of Turkey. Some morphological characteristics of the vegetative organs and fruits (Eyduran and Ağaoğlu, 2007), and AFLP profiles of some currant cultivars were reported (İpek *et al.*, 2010).In the flora of Turkey, morphological descriptions and

identification key of taxa based on some variable morphological traits were presented (Janczewski, 1907). Although limited number of *Ribes* species have been recorded in Turkey, it is critical to revise the gene pool of this genus having broad range of distribution in Anatolia with using additional and reliable parameters.

In the framework of this study, it was aimed to determine infra generic variations within *Ribes* accessions based on SSRs patterns providing also additional data for selection and characterization of valuable genotypes in Anatolia, having usage potential in food and phytopharmaceutical industry.

II. MATERIALS AND METHODS

2.1 Plant materials

Ten accessions of three *Ribes* species, namely *Ribes alpinum* L., *Ribes bieberstenii* Berl., *Ribes uva-crispa* L. were collected from their natural populations in A2, A7 and A8 grid squares in northern part of Anatolia between June and August. Herbarium materials of the accessions were prepared and deposited in ISTF herbarium. Samplings were documented in Table 1. Collected leaf specimens were transported to the laboratory in polypropylene bags and kept in -18 ℃ until analysed.

TABLE 1
LOCALITIES OF THE SAMPLINGS OF *RIBES* ACCESSIONS

Accession number	Taxa	Localities
ISTF 40811	*Ribes alpinum* L.	Demirkapı köyü, Uzungöl, Çaykara, Trabzon, forest mountain
ISTF 40812	*Ribes alpinum* L.	Cumalıkızık, Bursa
ISTF 40813	*Ribes alpinum* L.	Ambarlık vilage, Rize
ISTF 40814	*Ribes uva-crispa* L.	Ambarlık vilage, Rize
ISTF 40815	*Ribes bieberstenii* Berl.	Ambarlık vilage, Rize
ISTF 40816	*Ribes alpinum* L.	Demirkapı vilage, Uzungöl, Çaykara, Trabzon, rocky slopes, 2000 m.
ISTF 40817	*Ribes alpinum* L.	Demirkapı vilage, Uzungöl, Çaykara, Trabzon, rocky slopes, 2000 m.
ISTF 40818	*Ribes alpinum* L.	Pazar vilage, Atacanlar district, Rize
ISTF 40819	*Ribes alpinum* L.	Otopazar vilage, Soykanlar district, Rize
ISTF 40820	*Ribes bieberstenii* Berl.	Güzelyayla vilage, Kiraz mezrası district, Maçka, Trabzon

2.2 DNA extraction

Genomic DNA analysis was carried out on the leaf samples of 10 *Ribes* accessions by using Plant DNA extraction Mini Kit (Intron Biotechnology, Inc.). According to this protocol, the mortars sterilized in autoclave before pulvarization were cooled with liquid nitrogen for not adhering the surface of the specimens and easy pulvarizing. 50 mg of leaf tissue mortar and pestle with liquid nitrogen and allocated into eppendorf tubes with 1.5 ml. 390 µl lysis buffer, 7 µlenhancer solution, 20µl Proteinaz K and 5 µl RNase were added into the test tubes. After vortex, the test tubes closed with paraffine were incubated in water bath in 65 ^{0}C for 30 minutes. During incubation, test tubes were rotated for each 5-7 minutes. And then, the test tubes with 100 µl precipitation solution were incubated in the ice for 5 minutes and santrifuged in 13000 rpm for 5 minutes. Supernatants transported into new test tubes. 650 µl buffers for each tube for binding spin column was added into supernatant solution. 650 µl from this mixture was transferred into the collecting tube containing spin column and santrifuged in 13000 rpm for 1 min. The solution remaining in collecting tube was removed. Spin column was taken to new collecting tube and 700 µL washing solution added. After centrifugation in 13000 rpm for 1 min., remaining phase was removed, and spin column was transferred into new collecting tube. 700 µl washing solution was added and centrifugated in 13000 rpm for 1 min. Remaining phase in collecting tube was removed and spin column was placed into eppendorf tube with 1.5 ml. Lastly, spin column with 50µl elution buffer was centrifuged in 13000 rpm for 1 min., and the contents (gDNA) were stored in -20^{0}C. After DNA extraction, all genomic DNAs from the collected specimens including 1 µl loading dye and 8 µl dH_2O were run in 1% agarose gel togather with ladder cut with λ-Hind III (2 µl λ -DNA+ 10 µl dH_2O) at 80V for 40 min. (Figure 1).

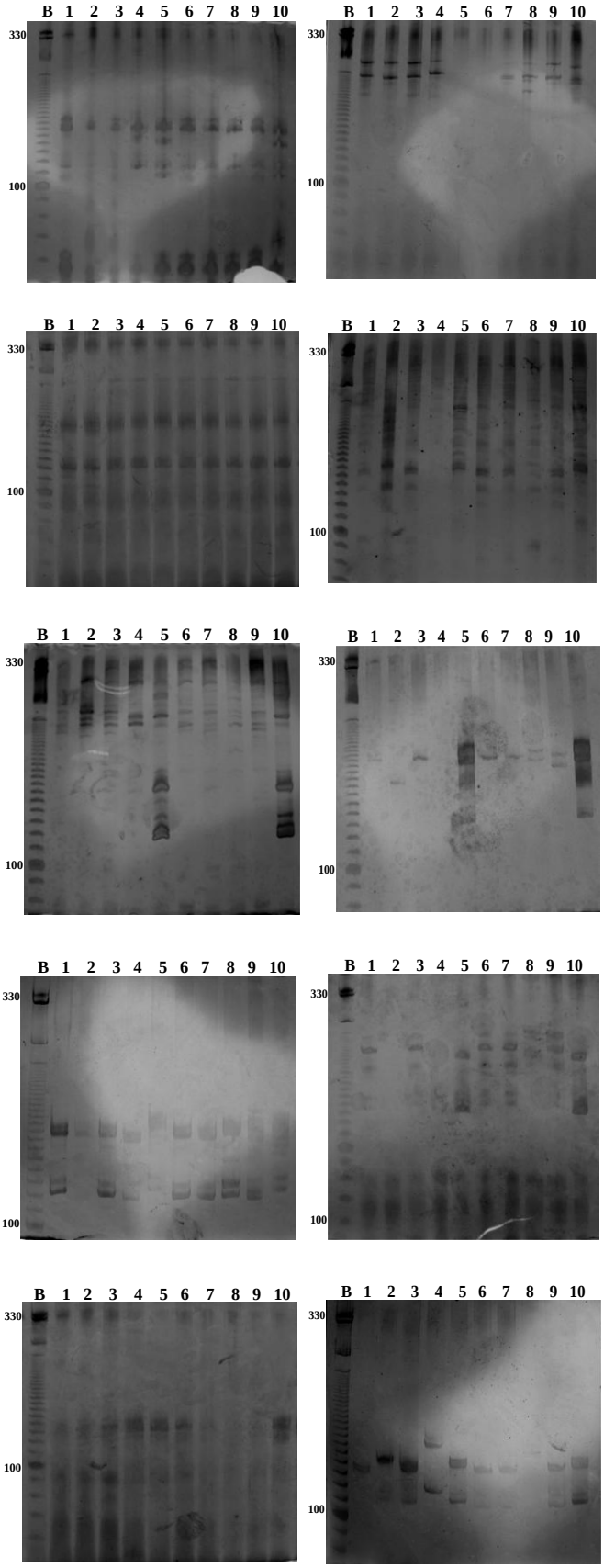

FIGURE 1. SSRS BAND PATTERNS PRODUCED WITH PER PRIMERS INDIVIDUALLY (S1-S10).

2.3 SSR Reaction

Amplification reaction was carried out in 20 µl volume including 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 320 µM dNTP mixture (dATP, dGTP, dCTP ve dTTP),25 µM, 25 ng/µl DNA for each primer pair (F and R) and 3U Taq DNA polymerase (Intron Biotechnology, *İ*-Taq™) (Table 2.). An optimized program (Brennan et al., 2002) was used in SSR-PCR reaction (MJ Research INC PTC-200). After denaturation of genomic DNA at 94˚C for 5 min., PCR reaction comprise of 7 cycle at 94 ˚C for 30 sec., with decreasing 1 ˚C at each cycle from 65˚C to 58˚C for 30 sec., at 72˚C for 30 sec., at 94˚C for 30 sec., at 58˚C' for 30 sec., at 72˚C for 30 sec. and at 72˚C for 7 min. as last extension reaction.

TABLE 2
DNA CONCENTRATIONS FOR EACH SAMPLES

Ribes	DNA concentrations (ng/µl)
1	200
2	190
3	150
4	50
5	300
6	175
7	140
8	50
9	175
10	250

2.4 Polyacrylamide Gel Electrophoresis (PAGE)

Amplification products dyed with ethidium bromide were loaded firstly on 2.5 % agarose gel prepared in 1x TAE buffer for determining the existence of bands before loading on the electrophoresis system and photographed digitally under UV light. After this process, SSR-PCR products were run on polyacrylamide gel (6%) and visualized with silver stain. PCR product with 6 µl and PAGE loading dye with 1.5 µl (Ambion; cat. No. 8546G) were transferred into eppendorf tube with 0.5 ml and centrifuged shortly for mixing and precipitation of the samples. Obtained samples were prepared for loading to gel after denaturation at 95 °C for 5 min. and taking into ice. For preparation of polyacrylamide gel, 4.2 g urea, 0.5 ml 10X TBE, 1.875 ml %40 acrylamide solution (19:1 acrylamide:bisacrylamid Sigma) were dissolved in beaker and total gel volume completed into 10 ml with bidistilled water. After adding 6.25 µl N,N,N′,N′-Tetramethylethylenediamine (TEMED) and 25 µl, % 25 ammoniumpersülfate (APS), prepared gel without polymerizing was load on the system with syringe. 1X TBE running buffer was added into upper and lower buffer loading part of the system after polymerizing the gel, and run at 300 V for 40 min. After completing the electrophoresis, gel was taken and shaked at 75 rpm until blue color of the running dye was disappeared in 10 % acetic acid. For removing of acetic acid, gel was shaked in distilled water two times for 2 min., taken into silver nitrate and keeped in dark for 30 min. After completing of dying, the gel placed in distilled water for a short time was shaked by transferring into freshly prepared developing solution up to bands appeared. The gel transferred into new developing solution was shaked in 10 % acetic acid for 2-3 min. for fixation of gel after good appearing of the bands belonging to ladder and the samples. The gels shaked in distilled water were evaluated by taking their photographes. Images of the amplification products obtained from each primer were demonstrated in the figures (Figure 1).

2.5 Statistical analysis

Gel patterns obtained with each SSR primer were scored for the presence (1) or absence (0) to create binary matrices. Dendrogram based on SSR data for genetic relationships between genotypes was constructed using the unweighted pair-group method with arithmetic average (UPGMA) and Jaccard coefficient of similarity was employed in calculation of distance based on co dominant markers resulting from SSR analysis though the software (MVSP 3.1).

III. RESULTS AND DISCUSSION

DNA concentrations for each samples were documented in Table 2. The highest genome sizes were observed in *Ribes bieberstenii* (250 and 300ng/µl) while the lover levels in *Ribes uva-crispa* and an accession of Ribes *alpinum* (50 ng/µl). The

other accesions of last species exhibited moderate genome sizes between 140-190 ng/µl. Ten SSRs have been screend for amplification and polymorphism (Table 3).

TABLE 3
PRIMERS USED IN SSRS ANALYSIS

	Code	Sequences of the primers (5'-3')
S1	e1-O01 F	CCT TTC CAG AGA AAA CTC AAA CA
	e1-O01 R	AAG TAT GGG AAC AAC GGC AG
S2	e2-L15 F	GAAGCCAGCAGAGAAGAAGC
	e2-L15 R	TCAACGCTCTTCTTCGACCT
S3	e3-M04 F	CTT ACC CAC CCC ACC ACC
	e3-M04 R	TGT GTT CTC ATC AGA GAC TTT CG
S4	g1-B02 F	CGA CTT CAT CGC TCT CCT CT
	g1-B02 R	CCA TTG ATT TGG TGA GGG T
S5	g1-F04 F	ATC ACC TTG ATT TTG GGT CG
	g1-F04 R	GGG ATG GAT TTG AGG GTT TT
S6	g1-H09 F	CCC AAA CAA ACG GAA CTC TG
	g1-H09 R	AAT GAT GGA CCC ACC ACT TG
S7	g1-P05 F	CCA AGA GCC CCA ACA CTA AC
	g1-P05 R	ATG GAA CTG CAC CTG GTT TC
S8	g2-G12 F	GTG ACC CAC CTA AAC CGT CC
	g2-G12 R	GGA GTG GAG GGT TGG AAA AT
S9	g2-J11 F	GAA CCA AAC CGA TCG AAG AA
	g2-J11 R	GCC GAC ACT ATG GTA AGG GA
S10	g3-A17 F	GTT CCA GAT TGC CAA AGT CG
	g3-A17 R	GGA GGA GGA GAG AGT GGC TT

The majority of the band positions in polyacrylamide gel electrophoresis varied between *Ribes* accessions.10 primers used were informative generating distinctly robust band patterns varying in numbers Samples of SSRs analysis are shown in Figure 1. Selected SSRs primers produced large numbers of polymorphic bands. The total amplified produces of 10 SSRs primers was 172 between 50 and 330 bp (average of 17.2 bands per primer), of which 157 bands were polymorphic between *Ribes* taxa, corresponding to 91.2 % genetic diversity. 15 bands (8.72 %) was monomorphic. The number of bands for each SSRs primer varied from 6 to 32. Correlation matrix of *Ribes* accessions using data obtained from the band patterns were documented in table Table 4.

TABLE 4
SIMILARITY MATRIX OF *RIBES* TAXA

	1	2	3	4	5	6	7	8	9	10
1	1,000									
2	0,425	1,000								
3	0,624	0,491	1,000							
4	0,355	0,282	0,437	1,000						
5	0,373	0,336	0,406	0,325	1,000					
6	0,563	0,370	0,519	0,347	0,449	1,000				
7	0,495	0,382	0,454	0,330	0,367	0,679	1,000			
8	0,505	0,480	0,464	0,333	0,402	0,558	0,567	1,000		
9	0,513	0,423	0,500	0,340	0,425	0,580	0,641	0,646	1,000	
10	0,414	0,381	0,489	0,341	0,691	0,445	0,380	0,423	0,477	1,000
	1	2	3	4	5	6	7	8	9	10

Correlation coefficients are between0.282 and 0.691 (average 0.454), and significantly correlated with each other's ($p<0.05$). In the dendrogram constructed based on obtained data, two main clad have been occurred representing two accessions of *Ribes bieberstenii* from different localities in the first clad, and 6 accessions of *Ribes alpinum* in the second clad. Northwestern accession of *Ribes alpinum* having isolated locality exhibited separate positon from the northeastern accessions of this species in the same main clad. *Ribes uva-crispa* was located in the distinct clad of the dendrogram. Under the second clad, each doubled branch of *Ribes alpinum* represents the genotypes collected from nearby localities, apart from number 1 and 3 accession. The dendrogram using SSRs data showed a clear distinction between *Ribes* accessions (Figure 2.).Based on these observations, segregations at specific and intraspecific levels of *Ribes* accessions were suggested. Some specific primers can be picked up for sorting population and species differences, and selecting high yield-genotypes in *Ribes*.

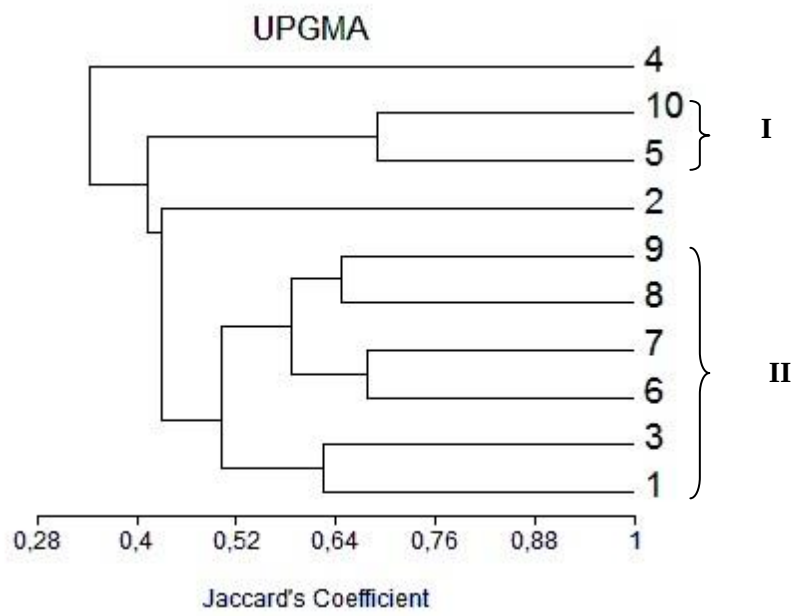

FIGURE 2. UPGMA DENDROGRAM OF 10 *Ribes* TAXA BASED ON SSRS PATTERNS USING JACCARD COEFFICIENT. THE NUMBERS (1-10) IN THE DENDROGRAM CORRESPOND THE ACCESSION NUMBERS IN TABLE 1.

The variability of SSRs patterns of 7 *Ribes alpinum*, 2 *Ribes bieberstenii* and 1 *Ribes uva-crispa* accessions representing different habitat conditions were analysed as the first report from Turkey in order to observe range of variation of this trait between different accessions as a complementary strategy to traditional phenotyping approach. Obtained results based on ten SSRs markers were evaluated from the points of taxonomical delineations, intraspecific variations and segregation of populations of the species. SSRs band patterns may also be useful for contribution to the selection of high yield genotypes which have high product potential for complementary source of phytochemicals in the nutraceutical industries. In our observations, six accessions of *Ribes alpinum* collected from A7 and A8 grid squares in northeast Anatolia have been clustered in the same clad of the dendrogram supported by the similarity matrix showing high correlation coefficients between related accessions. Three subclades in the nearby populations reflect strict genotypic relations between the accessions. On the other hand, distant and geographically isolated population of this species located in A 2 (A) grid square covering a part of northwest Anatolia constituted a separate branch apart from the northeastern populations. *Ribes alpinum* have a large distribution zone in Southern and Norhern Europea including Scandinavia, Ukraina, Caucasus in additon to Morocco as a distinct population (Euro+Med PlantBase home.mht). Large variation in phenotypic and genotypic traits in this species is expected considering to its adaptations against various habitat conditons. *Ribes alpinum* can be separated from closely related species *Ribes orientale* with its elongate and acute buds, and glabrous fruits. Distributional patterns of these species coincide with each others in the northeastern part of Anatolia. High level of intraspecific variations in *Ribes alpinum* detected firstly with SSRs analysis may explain its adaptation capacity to different climatic and environmental conditions. Hence, northwestern accession of this species exhibited distinct profile from the others implying its probable taxonomically subspecific status. But it is needed to evaluate the marker information combined with data on morphological, phenological

and biochemical traits to estimate the taxonomical position of this accession by large samplings. High resolution characteristics of SSRs assay may be useful in the segregation of the populations and determination of intraspecific variations in *Ribes* accessions. Obtained results can also be assessed to identify targeted genotypes and may have potential utility in the breeding programs. On the other hand, *Ribes uva-crispa* is the first segregated clad from the investigated accessions proving early divergency from the group in phylogenetic processes. Thus, in the identification key of *Ribes* reported in the flora of Turkey, this species separate from the other species distributed in Turkey with its flowers located in axillary clusters of 1-3 and the existence of spines. The other species having flowers in racemes are different from *Ribes uva-crispa* in morphology and SSRs profiles. It was reported that this species is native in west, central and south Europe, Caucasia and northern Iran. It is extensively cultivated for its fruit (Gooseberry-in Turkish 'Bektaşi üzümü') and widely naturalized elsewhere. In Turkey probably native in N.E. Anatolia (Chamberline, 1972), in addition to northwest and middle Anatolia. Two accessions of *Ribes bieberstenii* from distinct localities have been clustered in the same clad showing highest correlation coefficients, apart from the other accessions. In the dichotomic key of Turkish *Ribes* taxa, campanulate hypanthium and densely ciliate features of the margins of sepal was reported as key characteristics for this species and located in distinct position in the key. Parallel with morphological observations, both accessions in this species constituted a distinct clad togather divided into two subclad, reflecting early divergency from the group including *Ribes alpinum*. *Ribes bieberstenii* has native areal in northeast Turkey, Caucasus and northwest Iran as Euro-Siberian element. Type of infloresence as a basic synapomorphic character in the identification key separate the *Ribes* species into two groups including *Ribes uva-crispa* and the others is bearing flowers in racemes. In the raceme bearing group, another key characters between species are dioecious or hermaphrodite features of the flowers, shape of buds and hypanthium, colors and hairy or glabrous features of the fruits, existence of the glands in the leaves. In regarding group, *Ribes alpinum* with dioecious flowers differs from *Ribes bieberstenii* which has hermaphrodite flowers as the synapomorphic trait separating raceme bearing group. Parallel with the morphological features, it is possible to see such relations between the accessions in the dendrogram. It was reported that SSRs markers are favourable in establishing genetic profiles and diversity, exploring genetic relatedness between accessions and fingerprints of cultivars in addition to map quantitative trait loci (QTL) (Carvanna *et al.*, 2009). SSR marker analysis have been successfully employed in the management of germplasms (Palmieri *et al.*, 2013) and selecting a core collection for the Northern European *Ribes*germplasm including *R. nigrum*, *R. rubrum*, *R. uva-crispa* (Antonius *et al.*, 2012).

Our observations based on SSRs patterns as a tool capable of monitoring and evaluating the whole genome in the absence of phenotype supported the taxonomical associations of *Ribes* species established with traditional phenotyping approach. Furthermore, obtained data in the present sudy provide consistent and additional information for intraspecific variations in *Ribes* gene pool. Because Turkey has great potential for the diversity of *Ribes* populations, large scanning is needed to explain taxonomical circumscriptions and phylogeographical relations, in addition to preparing databank collection for preserving genetic resources and the selection of valuable alleles for improving the crop plants in the field.

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REFERENCES

[1] Antonius, K.,Karhu1, S., Kaldma, H., Lacis, G., Rugenius, R.,Baniulis, D., Sasnauskas, A., Schulte, E., Kuras, A., Korbin, M., Gunnarsson, A., Werlemark, G., Ryliskis, D.,Todam-Andersen, T., Kokk, L., and Järve K. 2012. Development of the Northern European Ribes core collection based on a microsatellite (SSR) marker diversity analysis. Plant Genetic Resources: Characterization and Utilization **10(1)**: 70–73.

[2] Behçet, L. 2001. A new species of *Ribes* L. (Grossulariacea) from east Anatolia, Turkey. Turkish Journal of Botany **25**:103-105.

[3] Berger, A. 1924. A Taxonomic Rewiew of Currants and Gooseberries. Techn. Bull. New York State Agr. Exp. Sta.109-118.

[4] Brennan, R., Jorgensen, L., Woodhead, M. and Russell, J. 2002. Development and characterisation of SSR markers in*Ribes* species. Molecular Ecology Notes **2**:327–330.

[5] Brennan, R. 2008. The development of a genetic linkage map of blackcurrant(*Ribes nigrum* L.) and the identification of regionsassociated with key fruit quality and agronomic traits. Euphytica **161**:19–34.

[6] **Brennan, R.M., Jorgensen, L., Gordon, S.L., Loades, K.W., Hackett, C.A. and Russell, J.R.** 2009.The development of a PCR-based marker linked to resistance to the blackcurrant gall mite (Cecidophyopsis ribis Acari: Eriophyidae).*Theoretical and Applied Genetics**118**:205-211*.

[7] Cavanna, M., Torello Marinoni, D., Beccaro, G.L. and Bounous, G. 2009. Microsatellite-Based Evaluation of *Ribes* Spp. Germplasm. Genome **52(10)**:839-848.

[8] Chamberlain, D.F. 1972. *Ribes*. *In:* Davis, P.H. (ed.),Flora of Turkey and the East Aegean Islands.Vol. **4**. University Press, Edinburgh, pp. 261-263.

[9] del Castillo,M.L.R., Dobson, G., Brennan, R. and Gordon, S. 2002. Genotypic Variation in Fatty Acid Content of Blackcurrant Seeds. Journal of Agricultural and Food Chemistry **50(2)**:332-335.
[10] del Castillo,M.L.R. 2004. Fatty Acid Content and Juice Characteristics in Black Currant (*Ribes nigrum* L.) Genotypes. Journal of Agricultural and Food Chemistry **52(4)**:948-952.
[11] Eyduran, S. and Ağaoğlu, Y. 2007. Ankara (Ayaş) Koşullarında Yetiştirilen Frenk Üzümü Çeşitlerinin Bazı Pomolojik ve Bitkisel Özellikleri. Tarim Bilimleri Dergisi,Ankara Üniversitesi Ziraat Fakültesi **13 (3)**:293-298.
[12] Ipek, A., Barut, E., Gulen, H. andIpek, M. 2010. Genetic Diversity Among Some Currants (*Ribes* Spp.) Cultivars as Assessed by AFLP Markers. Pakistan Journal of Botany **42(2)**:1009-1012.
[13] Janczewski, E.1907. Monographie des groseilliers, Ribes L.Me´moires de la Socie´te´ de physique et d'histoire naturellede Gene`ve **35**:199–517.
[14] Kampuss, K.,Christensen, L.P. andPedersen, H.L. 2008. Volatile Composition of Black Currant Cultivars. Proceedings of the IXth Internatıonal *Rubus* and *Ribes* Symposium, Book Series: Acta Horticulturae **777**:525-529.
[15] Korbin, M., Kuras, A. and Zurawicz, E. 2002. Fruit Plant Germplasm Characterisation Using Molecular Markers Generated in RAPD and ISSR-PCR. Cellular and Molecular Biology Letters **7 (2b)**:785-794.
[16] Lanham, P.G. and Brennan, R.M. 1999. Genetic characterization ofgooseberry (*Ribes grossularia* subgenus *Grossularia*)germplasm using RAPD, ISSR and AFLP markers. Journal of Horticultural Science and Biotechnology**74**:361–366.
[17] Lanham, P.G., Korycinska, A. and Brennan, R.M. 2000. Geneticdiversity within a secondary gene pool for *Ribes nigrum*L. revealed by RAPD and ISSR markers.Journal of Horticultural Science and Biotechnology**75**:371–375.
[18] Messinger, W., Hummer, K. andListon, A. 1999.*Ribes* (Grossulariaceae) phylogeny as indicated by restriction-site polymorphisms of PCR-amplified chloroplast DNA.Plant Systematics and Evolution**217**(3-4):185-195.
[19] Moyer, R.,Hummer, K.,Wrolstad, R.E. andFinn, C. 2002. Antioxidant Compounds in Diverse *Ribes* and *Rubus* Germplasm.Proceedings of the Eighth International *Rubus* And *Ribes* Symposium, vols: 1 and 2, Book Series: Acta Horticulturae **585**:501-505.
[20] Özcan, T. 2013. Accumulation patterns of some seed oil components from wildsources of Turkey. Natural Product Research**27(1)**:54-60.
[21] Palmieri, L.,Grando, M.S., Sordo, M., Grisenti, M., Martens, S. andGiongo L. 2013. Establishment of molecular markers for germplasm management in a worldwide provenance *Ribes* spp. Collection. Plant Omics **6(3)**:165-174.
[22] Scott, K.D. 2001. Microsatellites derived from ESTs and their comparison with those derived by other methods.*In:*Henry, R.Y. (ed.),Plant genotyping: the DNA fingerprinting of plants. CABI, Cambridge, MA**.**
[23] Schultheis, L.M. andDonoghue, M.J. 2004. Molecular phylogeny and biogeography of *Ribes* (Grossulariaceae), with an emphasis on gooseberries (subg. *Grossularia*). Systematic Botany**29(1)**:77-96.
[24] Sekizawa, H. 2013. Relationship between polyphenol content and anti-influenza viral effects of berries. Journal of the Science of Food and Agriculture **93**:2239-2241.
[25] Wu, X., Gu, L., Prior, R.L. and Mckay, S. 2004. Characterization of anthocyanins and protoanthocyanidins in some cultivars of *Ribes*, *Aronia* and*Sambucus* and their antioxidant capacity. Journal of Agricultural and Food Chemistry **52(26)**:7846-7856.