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Molecular study of *Berberis iliensis* M. Pop. and *Berberis sphaerocarpa* Kar. et Kir. wild populations in South-East Kazakhstan using ISSR markers

B. iliensis is a rare endangered plant species with decreasing natural area. Interspecific hybrids *B.iliensis* × *B. sphaerocarpa* are even more rare phenomenon. In this work 6 populations of *B.iliensis,* 4 populations of *B. sphaerocarpa* and a single *B. oblonga* population are analysed. DNA from barberry leaves was extracted using SDS method. ISSR analysis was conducted with 5 primers. UPGMA tree and STRUCTURE diagram of 11 populations were constructed. Obtained results show relations between *B.iliensis* and *B. sphaerocarpa* in pure and hybrid populations and lay a first basis for further investigation of Kazakhstan barberry species.

Key words: *Berberis iliensis, Berberis sphaerocarpa, Berberis oblonga,* ISSR.

Introduction

Berberis L. is the largest genus of *Berberidaceae* family including approximately 500 species, growing in Eurasia, North-East Africa, North and South America [1]. Most of them are widely used as food, medical and decorative plants. Some species are cultivated worldwide. The number of wild species of *Berberis* growing in Kazakhstan varies from 6 to 9 accordingly to different authors [2, 3]. *Berberis iliensis* M. Pop is a rare endemic species with decreasing natural area under protected stutus [4]. As a part of protecting measures a study of wild populations is conducted by laboratory of dendrology of Institute of Botany and Phytointroduction (Almaty, Kazakhstan) since 2009. During field studies a population containing plants with uncommon traits was discovered in Temirlik gorge (Ketmen mountains). These individuals were identified as inter-specific hybrids between *B. iliensis* and *B. sphaerocarpa* [5]. Such hybrids were not described previously. They are of particular interest, because *B.iliensis* and *B. sphaerocarpa* belong to different genus sections, *Integerrimae* and *Heteropodae*, respectively, and examples of hybridization between species from these sections are unknown. Hybrids of *B.iliensis* and *B. sphaerocarpa* are more rare than *B.iliensis* itself. So, it is important to conduct comprehensive research of barberries diversity in Kazakhstan on a level of populations as well as species in order to evaluate inter-specific genetic variation and clarify taxonomic status of local species. It is particularly important in order to protect such endangered species as *B.iliensis* and its rare hybrid forms from extinction. In this work, we performed analysis of six *B. iliensis* and four *B. sphaerocarpa* populations. Three populations of *B. iliensis* from Temirlik gorge and right banks of the Big Usek and Usek rivers were assumed to be hybrid populations with *B. sphaerocarpa.* Also, one population of *B. oblonga* Schneid. was analyzed in order to compare its kinship to *B. iliensis* and *B. sphaerocarpa.*

Materials and methods

Leaves of 134 plant samples in total were collected from wild populations in Almaty region (Table 1, Fig. 1). Harvested plant materials were stored at -80°C. DNA was isolated from frozen leaves by SDS method as described in [6]. Quantity and quality of extracted DNA were evaluated by electrophoresis in 1% agarose gel and measured by spectrophotometer NanoDrop2000 (Thermo Scientific, EU) at wavelengths 260, 280, 230 nm. 65 samples with optimal quantity and quality of DNA were selected for further analysis.

PCR was performed in 25 μl of reaction mix containing 2.5 mM of magnesium chloride (3.0 for

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ISSR), 10 mM of deoxyribonucleozide triphosphates, 10 mM of each ISSR primer, 0.5 unit of *Taq* DNA polymerase in standard 1x Taq buffer by Fermentas (Thermo Scientific, EU). 5 ISSR primers from [7] were used (Table 2). Amplification of ISSR markers was ran with following program: 1.5 min initial denaturation at 94°C, then 45 cycles of denaturation at 94°C for 40 s, annealing at specific temperature for each primer for 45 s, elongation at 72°C for 1.5 min, and final extension at 72°C for 15 min. PCR products were resolved by electrophoresis in 1,8% agarose gel in SB buffer with voltage 80 V for 3,5 h, stained by ethidium bromide and visualized in ultraviolet rays.

The ISSR band patterns were scored as «1» for presence and «0» for absense. Distance matrix based on Jaccard coefficients was calculated and visualized using FAMD 1.2 and MEGA 4 software [8, 9]. Bayesian MCMC analysis was performed on STRUCTURE software using admixture model with no prior location information; 100,000 cycles were done for both burn-in period and MCMC algorithm; 20 iterations for each *K* from 2 to 15 were run [10]. Results for each *K* over 20 runs were aligned by CLUMPP [11] and visualized by DISTRUCT [12]. The true *K* value was obtained by Evanno method as implemented in STRUCTURE HARVESTER website [13].

Table 1 – Analyzed populations of *B.iliensis, B. sphaerocarpa* and *B. oblonga.*

Species	Place	North longitude	East latitude	Altitude, m
Berberis iliensis	Akzhar	44° 56,320’	75° 48,628’	370
	Bakanas	44° 46,350’	76° 19,200’	395
	Darbazakum	43° 58,820’	79° 36,918’	505
	Right bank of Usek river	44° 27,461’	79° 49,326’	1170
	Right bank of the Big Usek river	44° 27,800’	79° 49,340’	1250
	Temirlik	43° 17,050’	79° 12,252’	1080
Berberis sphaerocarpa	Altyn Emel	44° 11,429’	78° 33,711’	1300
	Big Kirgyzsay	43° 19,174’	79° 31,220’	1470
	Turgen	43° 21,420’	77° 37,865’	1220
	Talgar	43° 13,762’	77° 16,848’	1570
Berberis oblonga	Aksu Dzhabagly	42° 19,727’	70° 19,727’	1460

Table 2 – ISSR primers used for analysis [7]

Primer ID	Length	Sequence	Annealing temperature
201274	14	5’ – CACACACACACARY – 3’	40°C
201275	14	5’ – CACACACACACARG – 3’	42°C
201276	16	5’ – AGAGAGAGAGAGAGYC – 3’	48,2°C
201277	14	5’ – GTGTGTGTGTGTYR – 3’	40°C
201278	14	5’ – GTGTGTGTGTGTAY – 3’	40°C

Results and discussion

101 bands in total were obtained, 20.4 per primer in average, 99 of them are polymorphic. 32.0±7.0 band presences per individual were observed. Data were analyzed and dendrogram was constructed with unweighted pair group method with arithmetic mean (UPGMA) (Fig. 2). Three distinct clusters are observed. The first cluster corresponds to three *B. iliensis* populations from valley of Ili river. Populations from delta of Ili river (Akzhar and Bakanas sites) form one mixed subcluster, and a population from Darbazakum forms distinct subcluster. The second cluster corresponds to *B. sphaerocarpa* populations with one *B. oblonga* population. The third cluster combine three *B. iliensis* populations which are

considered as hybrid (from Temirlik gorge and the right bank of Big Usek river) and a population from the right bank of Usek river. STRUCTURE analysis was performed, and the true number of clusters was estimated as K=2 by highest ΔK parameter (Evanno method). The second high value of ΔK was at K=6. The STRUCTURE diagram for two clusters demonstrate clearly distinct groups of pure *B. iliensis* and *B. sphaerocarpa* individuals (Fig.3). Putative hybrid populations demonstrate mixed origin from two species with prevalence of *B. iliensis*, and this supports an assumption about interspecific hybridization. *B. oblonga* demonstrate predominant relation with *B.sphaerocarpa* cluster. With increase of K complexity of population structure also increases. The diagram for six clusters shows hybrid populations having specific cluster membership. The Altyn Emel population of *B.sphaerocarpa* and Darbazakum population of *B. iliensis* are clearly distinct from other populations of their species, respectively.

Figure 1 – Location of 11 barberry populations used for analysis. 1 – Bakanas, 2 – Akzhar, 3 – Altyn Emel, 4 – right bank of Usek river, 5 –right bank of the Big Usek river, 6 – Darbazakum, 7 – Big Kirgyzsay, 8 – Temirlik, 9 – Turgen, 10 – Talgar, 11 – Aksu Dzhabagly.

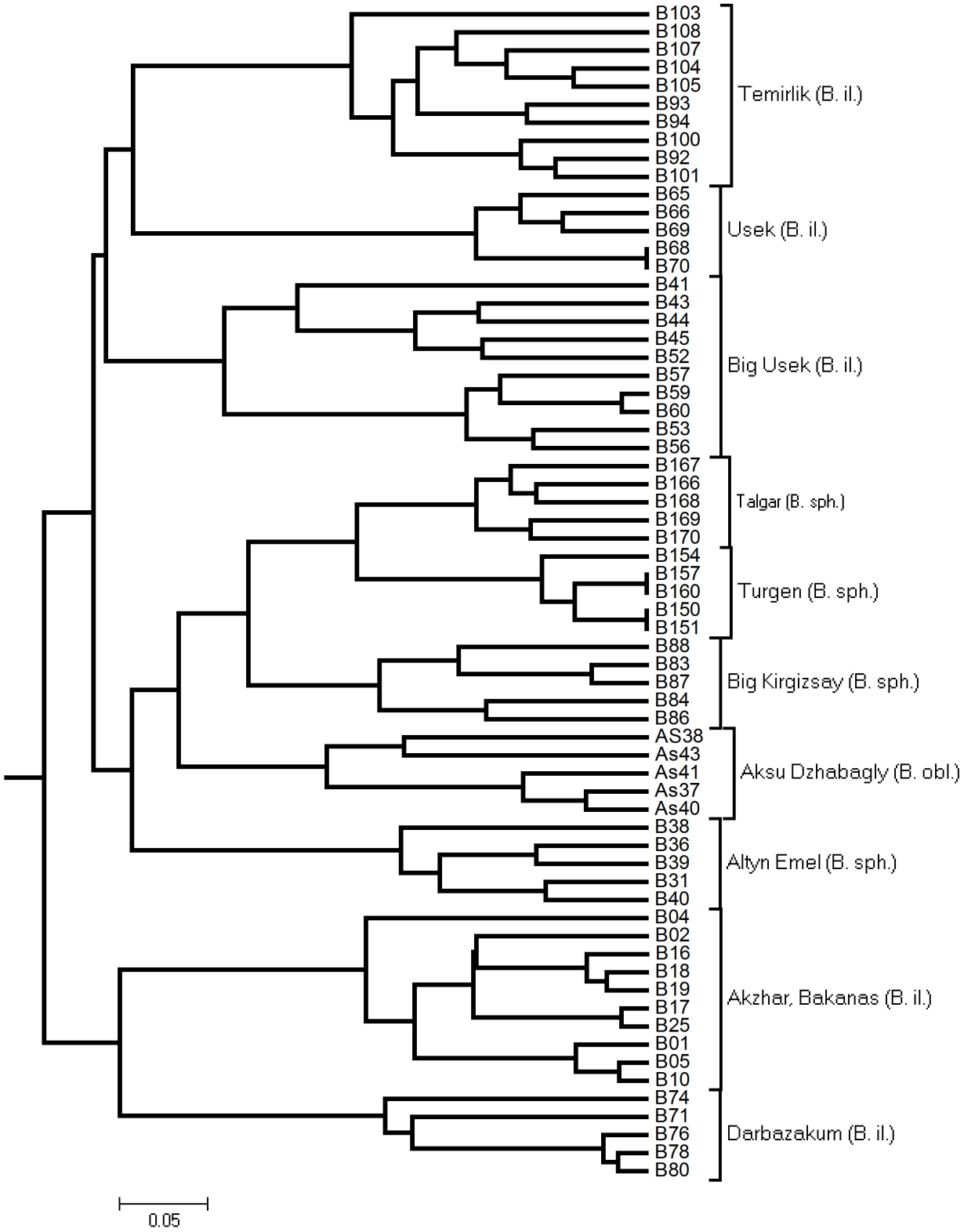

Figure 2 – UPGMA dendrogram of 65 individuals from 11 barberry populations.

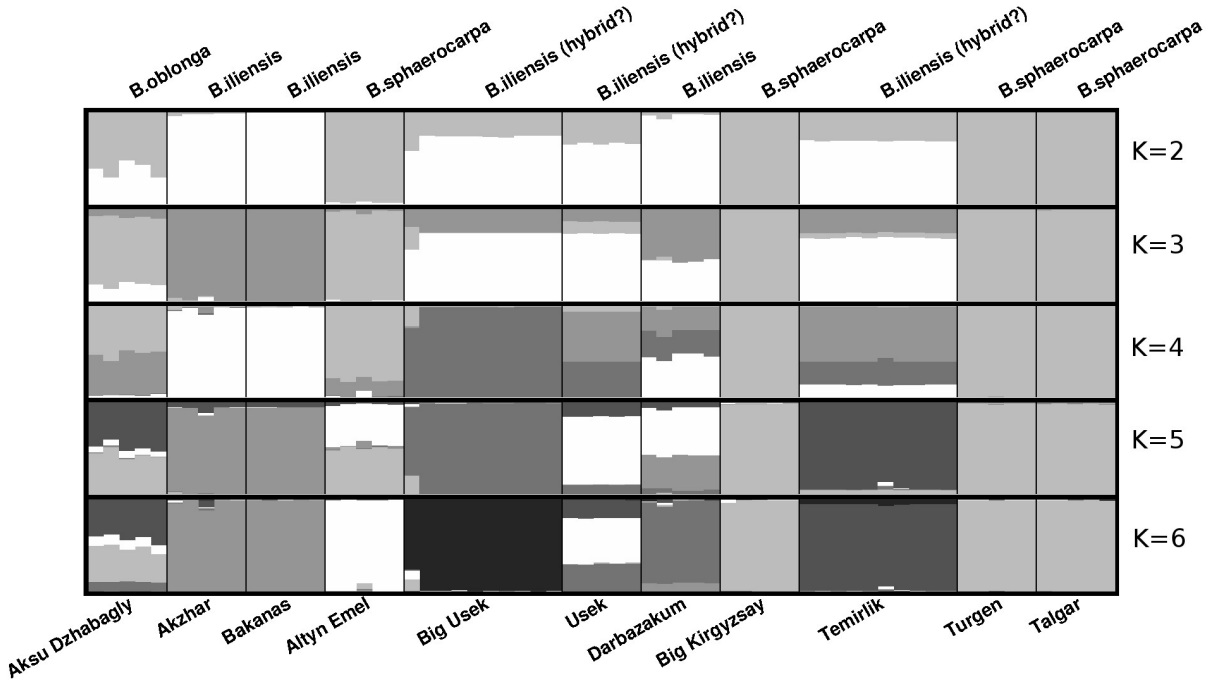

Figure 3 – STRUCTURE diagram of 11 barberry populations for *K* from 2 to 6.

Previously we obtained a proof of a hybridization between *B. iliensis* and *B. sphaerocarpa* in Temirlik population using ISSR markers (unpublished data). Results of this work support assumption about interspecific hybridization in three *B. iliensis* populations. However, there is a contradiction between results obtained using different approaches. Accordingly to UPGMA dendrogram of Jaccard distance coefficient, hybrid populations are more closely related with *B. sphaerocarpa,* but bayesian approach implemented in STRUCTURE shows predominant *B. iliensis* origin of hybrid populations. Both methods shows high similarity of *B. oblonga* population with *B. sphaerocarpa,* however data from one population are not sufficient for firm conclusion about relationship between these species. Ambiguity of data about population structure of *B. iliensis* and *B. sphaerocarpa* and their hybrid populations is probably due to low reliability of such multilocus dominant markers as ISSR and restricted sample of individuals. So, it is important to conduct further research with more reliable methods, and the present study is initial step of investigation of *Berberis* diversity in Kazakhstan on individual, population and specific levels.

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