


SSR-based evaluation of genetic diversity in populations of *Agriophyllum squarrosum* L. and *Agriophyllum minus* Fisch. & Mey. collected in South-East Kazakhstan

Y. Genievskaya¹, D. Karelova¹, S. Abugalieva^{1,2}, P. Zhao³, G. Chen³, Y. Turuspekov^{1,2} 

¹ Laboratory of molecular genetics, Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan

² Department of biodiversity and bioresources, al-Farabi Kazakh National University, Almaty, Kazakhstan

³ Key laboratory of stress physiology and ecology in cold and arid regions, Northwest Institute of Eco-Environment and Resources, Gansu, China


 e-mail: yerlant@yahoo.com

Abstract. The development of informative polymorphic DNA markers for poorly studied genera is an important step in population analyses of living organisms, including those that play very important ecological roles in harsh environments, such as desert and semi-desert area. Examples of those poorly studied desert species are *Agriophyllum squarrosum* L. and *Agriophyllum minus* Fisch. & Mey. However, a recent RNA-sequencing project in *A. squarrosum* has proposed a large set of hypothetical SSR (simple sequence repeat) markers. In this work, 11 novel polymorphic SSRs were found due to the screening of 24 randomly selected SSRs for three populations of *A. squarrosum* and one population of *A. minus*. The analysis of 11 SSRs revealed 16 polymorphic loci in two *Agriophyllum* species, 8 polymorphic loci within three populations of *A. squarrosum*, and 6 polymorphic loci in the population of *A. minus*. Statistical analyses showed high interspecific, but relatively low intraspecific genetic diversity. The phylogenetic clusterization and population structure analysis have demonstrated a clear segregation of *A. minus* from *A. squarrosum*, as well as the separation of population 1 from populations 2 and 3 of *A. squarrosum*. Thus, we identified the set of novel and informative SSR markers suitable for the study of genetic diversity in *Agriophyllum*.

Key words: sand rice; *Agriophyllum squarrosum*; *Agriophyllum minus*; SSR markers; genetic diversity; population structure.

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Оценка генетического разнообразия с использованием SSR-маркеров в популяциях *Agriophyllum squarrosum* L. и *Agriophyllum minus* Fisch. & Mey., собранных на юго-востоке Казахстана

Ю.А. Гениевская¹, Д.С. Карелова¹, С.И. Аbugалиева^{1,2}, П. Жао³, Г. Чен³, Е.К. Туруспеков^{1,2} 

¹ Институт биологии и биотехнологии растений, Алматы, Казахстан

² Кафедра биоразнообразия и биоресурсов, Казахский национальный университет им. аль-Фараби, Алматы, Казахстан

³ Ключевая лаборатория по физиологии стресса и экологии в холодных и засушливых регионах,

Северо-Западный институт экологической среды и ресурсов, Гансу, Китай

 e-mail: yerlant@yahoo.com

Аннотация. Разработка информативных полиморфных ДНК-маркеров для малоизученных родов – необходимое и важное условие для эффективного популяционного анализа живых организмов, в том числе играющих важную экологическую роль в суровых климатических условиях, в частности в пустынях и полупустынях. Примерами подобных малоизученных пустынных видов являются *Agriophyllum squarrosum* L. и *Agriophyllum minus* Fisch. & Mey. В недавнем проекте по РНК-секвенированию *A. squarrosum* было найдено большое количество гипотетических SSR (простые повторяющиеся последовательности) маркеров. В настоящей работе в результате скрининга 24 случайно отобранных SSR-маркеров для трех популяций *A. squarrosum* и одной популяции *A. minus* обнаружены 11 новых полиморфных SSR-маркеров. Изучение этих 11 SSR-маркеров показало наличие 16 полиморфных локусов для двух видов *Agriophyllum*, 8 полиморфных локусов среди трех популяций *A. squarrosum* и 6 полиморфных локусов для популяции *A. minus*. Статистический анализ выявил высокое межвидовое, но относительно низкое внутривидовое генетическое разнообразие. Филогенетический анализ и анализ структуры популяций позволили обнаружить явное разделение на уровне видов, а

также дивергенцию популяции 1 *A. squarrosum* относительно популяций 2 и 3 данного вида. Таким образом, нами идентифицирован набор новых и информативных SSR-маркеров, подходящих для изучения генетического разнообразия *Agriophyllum*.

Ключевые слова: кумарчик песчаный; кумарчик малый; *Agriophyllum squarrosum*; *Agriophyllum minus*; SSR-маркеры; генетическое разнообразие; структура популяции.

Introduction

Xerophytes and psammophytes (plants with adaptations to survive in water-deficiency environment) become the main plants in the desert region. Despite the harsh environmental conditions, many of these plants have adapted and became a part of complex and diverse desert ecosystems. Due to the wide distribution of desert territories, the study of its wild flora is very important for the ecological prediction and conservation of biodiversity. The Central Asia region, including Kazakhstan, is having numerous large and small sand deserts, such as Gobi, Taklamakan, Karakum, Kyzylkum and Moynkym. Deserts and semi-deserts occupy more than half of Kazakhstan territory and keep growing (Issanova et al., 2015). The major representatives of wild desert flora here are herbaceous plants, subshrubs, shrubs and subtrees. The list of dominating species includes genera *Artemisia*, *Salsola*, *Ferula*, *Arthrophytum*, *Calligonum*, *Ammodendron*, *Haloxylon*, and *Agriophyllum* (Rachkovskaya et al., 2003). Many of them are endemic for the Central Asian and Kazakhstan deserts.

Agriophyllum squarrosum L. and *Agriophyllum minus* Fisch. & Mey. are important desert species in Kazakhstan. They belong to the tribe Corispermaceae within the subfamily Chenopodioideae of the family Chenopodiaceae (Kühn, 1993). The genus *Agriophyllum* includes five species, and four of them, including *A. squarrosum*, grow in Kazakhstan (Ageeva et al., 1960). *A. squarrosum* is also widely spread in all Central Asia territory, Caucasus, and China. Local people in the sandy desert regions of China consume the seed of the species during periods of food shortage, and refer to the plant as 'shami' in Chinese, which translates as 'sand rice' (Chen et al., 2014). Sand rice is an example of psammophyte perfectly adapted for harsh desert environmental conditions. Morphologically it is a shrub-like plant with a height ranging from 20 to 100 cm. The stem of sand rice at the young plant stage is firm, branched, green, and covered with short hairs. Leaves are small, sessile, green, and usually linear. Small flowers are organized in a spike-like inflorescence. *A. squarrosum* blossoms in the late summer and early fall, after that ovoid shape seeds are formed. Seeds of sand rice are very light and covered by a thin husk. After ripening, the husk is cracked into two parts, and seeds are easily dispersed by the wind. The root system is represented by a long taproot penetrating deep into the soil to access stored moisture, and almost equally long lateral roots branching near the soil surface and helping to fix plant in loose sand. Although, there is a lack of information related to the structure of the sand rice's genome, the transcriptomic analysis of *A. squarrosum* ($2n = 18$) had showed presence of 67741 unigenes and approximately 43 % of them were annotated (Zhao et al., 2014).

Throughout history, sand rice was used for diverse purposes. Aboveground organs (stem and leaves) are eaten by both wild animals and livestock of farmers in arid and semi-arid regions,

especially in Western Kazakhstan on camel pasture. Historically, nutritious seeds of sand rice were an alternative to cereals not surviving in hot deserts. In China and Mongolia, the local villagers consume sand rice seed in a variety of dishes. There are many reports about the rich nutrition value of *A. squarrosum* seeds close to its widely-used as food quinoa seeds (Chen et al., 2014). However, sand rice is not a domesticated plant species with several agriculturally unfavorable traits, such as fragile spikes and light seeds. Still, the works on possible domestication of sand rice as a novel crop are reported (Chen et al., 2014). In addition to nutrition purposes, sand rice had found its application in medicine. Back in the days, it was used as antipyretic and analgesic medicine (Gong et al., 2012; Chen et al., 2014). It was reported that the extract of *A. squarrosum* decreases blood glucose levels in type 2 diabetic mice and has the potential for further medical researches (Saqier et al., 2019). Sand rice is useful in combat against shifting sands (Wen-Ming et al., 2004). Climate change and human activities led to the growth of sand desert areas and the migration of sand dunes to agricultural territories. The structure of the sand rice root system and its ability to form seed banks in active sand dunes allow the fixation of the sand surrounding the plant. Thus, *A. squarrosum* has a tremendous potential together with other psammophytes to be used in a large-scale sand fixation (Liu et al., 2007; Ma, Liu, 2008). This species is an interesting model for different studies of morphology and physiology of desert plants. For example, earlier *A. squarrosum* was already used for the study of growth under drought conditions (Mo et al., 1997; Huang et al., 2008) and for the study of fertilizer effect on psammophytes under different rainfall conditions (Yuan et al., 2019).

Endemic species with great economic potential like sand rice are an interesting subject for genetic and molecular researches. One of the most common methods utilized for the studies on biodiversity conservation, population and phylogenetic studies of wild plant species is the usage of molecular DNA markers (Nybom, 2004). Examples of successful application of the most common DNA markers in plants include random amplification of polymorphic DNA (RAPD) (Nybom, Bartish, 2000), amplified fragment-length polymorphisms (AFLP) (Zhang C. et al., 2018), and other nuclear and chloroplast DNA markers (Abugalieva et al., 2017; Almerikova et al., 2018; Turuspekov et al., 2018). Nuclear ribosomal internal transcribed spacer (nrITS) region and five chloroplast DNA (cpDNA) fragments have been used earlier for the study of population dynamics of *A. squarrosum* in China (Qian et al., 2016). The *maturase K* (*matK*) gene of the chloroplast genome and nrITS were used for comparison of *A. squarrosum* and *A. minus* populations in two regions of Kazakhstan (Genievskaia et al., 2017). The literature survey suggests that there is a limited information on the study of *Agriophyllum* species by using SSR markers. However, a recent RNA-sequencing

Table 1. Geographical locations of sampling sites

Sampling site	Species, population	Number of plants	Coordinates	Altitude, m
South-East Kazakhstan, Zhambyl region, Moyynkum desert	<i>A. squarrosum</i> , pop1	10	44°34'55.1"N 76°58'30.3"E	411
South-East Kazakhstan, Zhambyl region, Moyynkum desert (near Birlik village)	<i>A. squarrosum</i> , pop2	25	44°40'56.9"N 76°41'49.7"E	407
	<i>A. squarrosum</i> , pop3	19	44°41'36.2"N 76°44'53.5"E	407
	<i>A. minus</i> , pop1	20	44°42'08.3"N 76°44'19.3"E	403



Fig. 1. *A. squarrosum* (on the left) and *A. minus* (on the right) in Moyynkum desert.

project of *A. squarrosum* populations in China has suggested several thousands of potential SSR markers for this species (Zhang J. et al., 2018).

In this study, we selected 24 SSR markers from this *Agriophyllum* genome resequencing project and used them for the assessment of genetic diversity within and among populations of *A. squarrosum*, and between *A. squarrosum* and *A. minus*.

Materials and methods

Plant material. In total, leaf samples of four wild *Agriophyllum* populations were collected in South-East Kazakhstan and used for the analysis (Table 1). The list included three populations of *A. squarrosum* and one population of *A. minus* sampled in Moyynkum desert of Almaty region in South-East Kazakhstan (Fig. 1). Population 1 of *A. squarrosum* was collected in 2016, while the populations 2 and 3 of *A. squarrosum* and population 1 of *A. minus* were collected in 2019. The distances between populations were at least four kilometers, and plants within the population were sampled in at least 50 meters apart.

DNA extraction, amplification and SSR marker assessment. Five young leaves from each sample were dried in silica gel. The total genomic DNA was extracted from dry leaf

tissues using CTAB method (Doyle, 1991). The quality and concentration of extracted DNA were assessed via spectrophotometric test, and 1 % agarose gel electrophoresis.

Twenty-four pairs of SSR markers and their primers (Supplementary Table)¹ were selected from 6150 SSRs in the sequence of *A. squarrosum* genome reported by Zhang J. and co-authors (2018). SSR motives and expected sized of alleles were obtained from the same source.

Annealing temperature (T_a) for primer pairs and concentration of reagents in a PCR reaction mix were determined empirically. All successful PCR reactions were performed in total 16 μ l volumes, including 4 mM of each dNTP, 2 mM of $MgCl_2$, 6.4 mM of primer mix (forward+reverse), 1.6 U of Taq polymerase and 50 ng of DNA. The amplification was performed in Veriti Thermocycler (Applied Biosystems, Foster City, CA, USA) with an initial denaturation step at 94 °C for 3 minutes, followed by 40 cycles of 94 °C for 30 seconds, optimized T_a °C (see Suppl. Table) for 45 seconds and 72 °C for 1.5 minutes. The final extension step was at 72 °C for 10 minutes. The PCR products were separated on 6 % polyacrylamide gel (PAG). The SSR profile image was

¹ Supplementary Table is available in the online version of the paper: <http://www.bionet.nsc.ru/vogis/download/pict-2020-24/appx10.pdf>

captured using the GelDoc gel documentation unit (Bio-Rad Laboratories, Hercules, CA, USA). Allele sizes were estimated visually based on the size of ladder bands and the control sample on each gel.

Statistical analysis. Nei's genetic diversity indices were calculated via POPGENE software ver. 1.32 (Yeh et al., 1997); the polymorphism information content (PIC) (Botstein et al., 1980) was calculated as follows: $PIC = \sum(1 - p_i^2)$, where p_i is the frequency of the i -th band or percentage of individuals in which the fragment is present. Principal coordinates analysis (PCoA) and unweighted pair group method with arithmetic mean (UPGMA) hierarchical clustering were performed based on genetic distances among populations and species. PCoA plot was made via GenAlEx (Genetic Analysis in Excel) ver. 6.5 software (Peakall, Smouse, 2012), UPGMA analysis was performed in R statistical software environment (R Core Team, 2018). Bayesian clustering was based on the use of the STRUCTURE software ver. 2.3.4 (Pritchard et al., 2000). The value of K was set from 1 to 5 with five iterations for each value of K . Both, length of burn-in period, and the number of Markov Chain Monte Carlo (MCMC) repeat after burn-in was set at 100000. Web-tool STRUCTURE HARVESTER (Earl, von Holdt, 2011) based on Evanno's method (Evanno et al., 2005) was used to determine the best fit value of K . The molecular variance (AMOVA) test was calculated using GenAlEx software.

Results

Performance of SSR markers in *Agriophyllum* species

Initially, 24 SSR markers were chosen for the analysis (see Suppl. Table), however, successful amplification was performed for 18 markers only. Five of 18 SSRs were multilocus markers, while other 13 SSRs were single-locus. In total, 18 markers allowed identification of 23 loci (Table 2), of which 16 were polymorphic and suitable for the analysis (Table 3).

The screening of sixteen SSR loci has allowed the identification of 43 alleles in the analysis of three populations of *A. squarrosum* and one population of *A. minus*. The molecular sizes of alleles in loci were ranged from 143 to 342 bp. The number of alleles in the study of all four populations varied from 2 to 4.

In total, eight polymorphic loci were observed in *A. squarrosum*, six polymorphic loci were found in *A. minus*, and 16 loci had demonstrated polymorphism between two species (see Table 3). In total, 20 alleles were identified exclusively in *A. squarrosum*, 16 alleles were exclusive for *A. minus*, and 7 alleles were found in both species. The average number of alleles per locus in polymorphic loci was 2.69 ± 0.70 for both species, 2.25 ± 0.16 for *A. squarrosum*, and 2.17 ± 0.17 for *A. minus*, respectively.

Nei's index and PIC value were calculated separately for polymorphic SSR loci of *A. squarrosum*, *A. minus*, and jointly for two *Agriophyllum* species (see Table 3). The genetic distance-based PCoA plot suggested a clear separation of *A. minus* from populations of *A. squarrosum*, as well as distinguishing of the population 1 from populations 2 and 3 in *A. squarrosum* (Fig. 2, a). The UPGMA tree had also demonstrated two clusters corresponding to *A. squarrosum* and *A. minus*. The portion of samples in population 1 of *A. squar-*

Table 2. The characteristics of SSR loci successfully amplified in *A. squarrosum* and *A. minus*

No.	Locus	Annealing temperature (T_a), °C	Number of alleles	Range of alleles sizes, bp
1	AGS-02	46	1*	237
2	AGS-03	60	2	266–273
3	AGS-05	42	1*	280
4	AGS-07	46	1*	182
5	AGS-09.1	46	4	178–210
6	AGS-09.2	46	3	264–273
7	AGS-10	46	1*	280
8	AGS-11	54	1*	200
9	AGS-13	46	1*	275
10	AGS-16.1	39	3	159–186
11	AGS-16.2	39	3	284–305
12	AGS-17.1	33	2	228–238
13	AGS-17.2	33	2	287, null allele
14	AGS-18	39	3	176–245
15	AGS-20.1	33	3	298–323
16	AGS-20.2	33	2	359, null allele
17	AGS-23	54	2	170–182
18	AGS-24	46	1*	108
19	AGS-26	54	3	183–199
20	AGS-27.1	46	2	182, null allele
21	AGS-27.2	46	4	229–342
22	AGS-29	42	2	298–328
23	AGS-30	51	3	143–167

* Monomorphic loci excluded from further analysis.

rosum was clustered together with populations 2 and 3, while the remaining part formed a separate subcluster (see Fig. 2, b). Bayesian distance-based analysis of the structure among two *Agriophyllum* species was congruent with the UPGMA clusterization and also indicated that $K = 3$ is an optimal number of groups in the study (see Fig. 2, c).

AMOVA results for the set of data containing all *Agriophyllum* samples revealed that the majority (88 %) of the genetic diversity in *Agriophyllum* exists between two species rather than within them (Table 4).

Genetic diversity in *A. squarrosum* populations

Part of this study was focused on the assessment of the genetic diversity within and between populations of *A. squarrosum*. In total, 26 alleles were identified in eight SSR loci. The largest number of unique alleles was found in population 1 (8 alleles), while population 2 had only one unique allele (Table 5). The largest values of Nei's index (0.27 ± 0.16) and PIC value (0.25 ± 0.09) were observed in population 1.

Table 3. Assessment of genetic diversity in populations of *Agriophyllum* species based on SSR marker analysis

No.	Locus	<i>A. squarrosum</i>				<i>A. minus</i>				<i>A. squarrosum</i> + <i>A. minus</i>			
		na	ne	Nei	PIC	na	ne	Nei	PIC	na	ne	Nei	PIC
1	AGS-03	2	1.85	0.46	0.35	2	1.11	0.10	0.09	2	1.99	0.50	0.38
2	AGS-09.1	2	1.04	0.04	0.04	3	2.06	0.52	0.46	4	1.81	0.45	0.41
3	AGS-09.2	2	1.04	0.04	0.04	2	1.84	0.46	0.35	3	1.80	0.45	0.40
4	AGS-16.1	Monomorphic*				2	1.11	0.10	0.09	3	1.71	0.42	0.34
5	AGS-16.2	3	1.22	0.18	0.17	2	1.11	0.10	0.09	3	1.19	0.16	0.15
6	AGS-17.1	Monomorphic*				Monomorphic*				2	1.63	0.39	0.31
7	AGS-17.2	Monomorphic*				Monomorphic*				2	1.63	0.39	0.31
8	AGS-18	2	1.43	0.30	0.26	Monomorphic*				3	2.22	0.55	0.49
9	AGS-20.1	Monomorphic*				2	1.21	0.18	0.16	3	1.69	0.41	0.34
10	AGS-20.2	Monomorphic*				Monomorphic*				2	1.65	0.39	0.32
11	AGS-23	Monomorphic*				Monomorphic*				2	1.66	0.40	0.32
12	AGS-26	2	1.04	0.04	0.04	Monomorphic*				3	1.69	0.41	0.34
13	AGS-27.1	Monomorphic*				Monomorphic*				2	1.65	0.39	0.32
14	AGS-27.2	3	1.46	0.32	0.29	Monomorphic*				4	2.29	0.56	0.50
15	AGS-29	Monomorphic*				Monomorphic*				2	1.65	0.39	0.32
16	AGS-30	2	1.34	0.25	0.22	Monomorphic*				3	2.13	0.53	0.46
	Mean	2.25	1.30	0.20	0.18	2.17	1.41	0.24	0.21	2.69	1.77	0.42	0.36
	SEM	0.16	0.10	0.06	0.04	0.17	0.17	0.08	0.07	0.18	0.07	0.02	0.02
	Number of plants	54				20				74			
	Number of populations	3				1				4			

Note. na – number of alleles per locus; ne – number of effective alleles; Nei – Nei’s genetic diversity index; PIC – polymorphism information content; SEM – standard error of mean. * Monomorphic loci were not considered for the calculation of mean and SEM values.

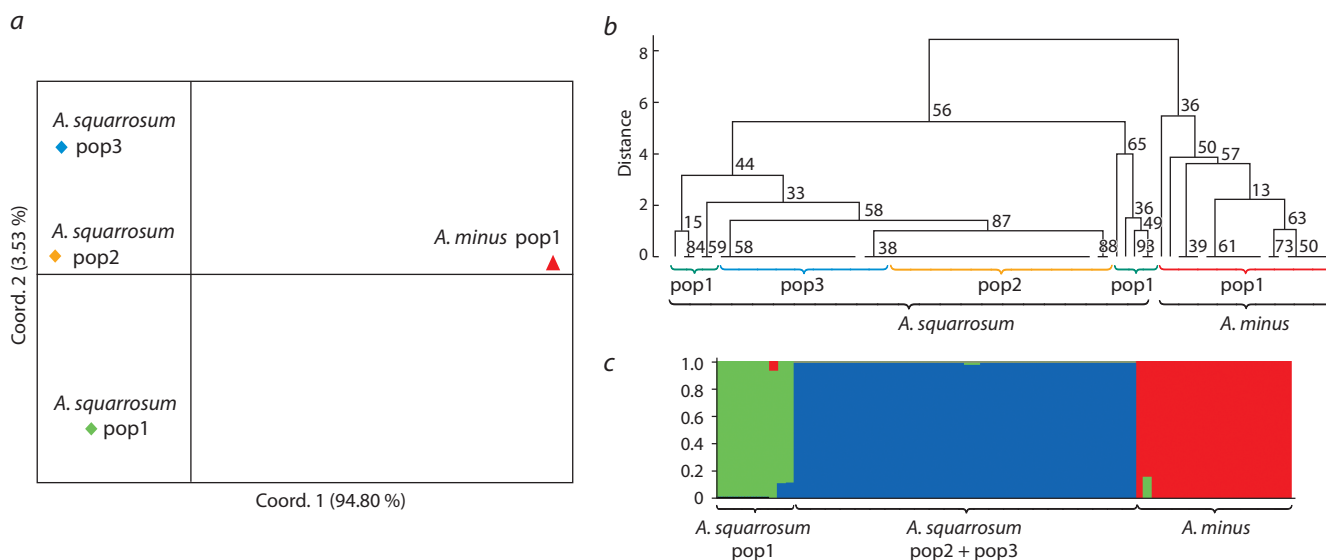


Fig. 2. Population structure among studied *Agriophyllum* species.

a, PCoA of three *A. squarrosum* and one *A. minus* populations; b, UPGMA dendrogram showing clusters among 74 samples of both species; c, Bayesian clustering of 74 *Agriophyllum* samples at $K = 3$.

Table 4. AMOVA in two species of *Agriophyllum*

Source	df	SS	MS	Est. Var. (%)	<i>p</i>
Between species	1	435.289	435.289	14.840 (88)	0.001
Within species	72	151.941	2.111	2.110 (12)	0.047
Total	73	587.230		16.951 (100)	

Note. df – degree of freedom; SS – sum of squares; MS – mean squares; Est. Var. – estimated variance; *p* – significance level.

Table 5. Average values of diversity statistics for eight polymorphic SSR loci across three *A. squarrosum* populations

<i>A. squarrosum</i>	nea	ne	na	Nei	PIC
Population 1	8	1.43 ± 0.32	1.88 ± 0.35	0.27 ± 0.16	0.25 ± 0.09
Population 2	1	1.02 ± 0.06	1.13 ± 0.35	0.02 ± 0.05	0.02 ± 0.05
Population 3	0	1.03 ± 0.09	1.13 ± 0.35	0.03 ± 0.07	0.02 ± 0.06

Note. nea – number of alleles exclusive for population; na – number of alleles per locus; ne – number of effective alleles; Nei – Nei's genetic diversity index; PIC – polymorphism information content.

Table 6. AMOVA among and within three populations of *A. squarrosum*

Source	df	SS	MS	Est. Var. (%)	<i>p</i>
Among populations	2	29.185	14.592	0.771 (34)	0.002
Within populations	51	77.556	1.521	1.521 (66)	0.003
Total	53	106.741		2.292 (100)	

Note. df – degree of freedom; SS – sum of squares; MS – mean squares; Est. Var. – estimated variance; *p* – significance level.

The AMOVA for *A. squarrosum* had shown a higher molecular variance within populations than the variance among populations (Table 6).

Discussion

The availability of informative DNA markers is advantageous for the study of rare species, their population structure, and for the development of a proper conservation strategy to prevent their extinction (Adams, Turuspekov, 1998; Kramer, Havens, 2009). SSR markers are one of those types of DNA markers that reliably utilized in population structure analysis due to their hyper-variability, co-dominance, and high reproducibility (Aitken et al., 2005). Successful applications of SSR markers for the identification, classification, and taxonomy of Chenopodiaceae species have been reported previously (Borger et al., 2008; Prinz et al., 2009; Sampson, Byrne, 2012; Nachtigall et al., 2016). However, for the genus *Agriophyllum*, there were no previous studies with the use of SSRs. The situation was changed recently due to the RNA-sequencing project of *A. squarrosum* accessions, and a large number of hypothetical SSR markers were suggested for population studies (Zhang J. et al., 2018). Hence, in this work 24 SSRs were randomly selected for the assessment of three populations of *A. squarrosum* and one population of *A. minus* collected in South-East Kazakhstan. The assessment of selected SSR markers resulted in the identification of 11 novel SSR markers that allowed the identification of 16 polymorphic loci in two *Agriophyllum* species (see Table 3). The large percentage of molecular variance (see Table 4) and a large

genetic distance (see Fig. 2) observed between *A. squarrosum* and *A. minus* indicated good discriminative power of studied SSR markers and confirmed a significant genetic difference reported between these species earlier (Genievskaia et al., 2018). Fifteen SSR loci used for both species were in intermediate diversity group with PIC values ranged between 0.32 and 0.50, and Nei's diversity index ranged between 0.39 and 0.56 (see Table 3).

When species were analyzed separately, 8 out of 16 loci were polymorphic for *A. squarrosum*, and 6 loci were polymorphic for *A. minus* (see Table 3). The average diversity in *A. minus* was slightly higher than in *A. squarrosum* and lower than the average interspecies diversity (see Table 3). Thus, *A. squarrosum* and *A. minus* have maintained a low level of genetic diversity. It has been demonstrated that annuals like *Agriophyllum* or short-lived perennials usually demonstrate low levels of genetic diversity compared with long-lived and outcrossing species (Austerlitz et al., 2000). However, the limited amount of samples in studied species (particularly in *A. minus*), their close geographical locations, and a relatively small number of polymorphic SSR markers used for the analysis could influence obtained results.

The study of the genetic diversity within *A. squarrosum* suggested that 66 % of the total variation was within and 34 % between populations (see Table 6), indicating that the difference of population 1 from populations 2 and 3 was rather substantial. The presence of relatively high diversity within populations may be explained by the geographical proximity of *A. squarrosum* populations used in this study (see Table 1),

which may cause a high rate of gene flow in a limited area (Conner, Hartl, 2004). Populations 2 and 3 of *A. squarrosum* had demonstrated an extremely low level of genetic diversity in comparison with population 1 (see Table 5), but the fact that samples from each population clustered together using multiple analyses (PCoA, UPGMA, Bayesian clustering) (see Fig. 2) indicates that there is a high level of heterogeneity in the species. The grouping of 5 samples from population 1 on the UPGMA tree and on the STRUCTURE bar plot into separate subcluster in the entire species cluster may indicate the presence of some barriers between groups of samples within population 1 interfering gene flow. Therefore, even if no physical obstructions were found during the sample collection, probably, two groups in population 1 may be considered as subpopulations (Waples, Gaggiotti, 2006). This assumption is also supported by the relatively higher genetic diversity in population 1 comparing with the other two *A. squarrosum* populations (see Table 5).

Conclusions

In this study, sequences of 24 pairs of oligonucleotides for SSR markers were randomly selected from *A. squarrosum* genome resequencing project, and used for the study of genetic diversity in the genus *Agriophyllum*. Hence, it is the first report exploring the performance of novel SSR markers in the genetic analysis of this genus. The study revealed 16 polymorphic loci in eleven SSR markers using two *Agriophyllum* species, 8 polymorphic loci within three populations of *A. squarrosum*, and 6 polymorphic loci within the population of *A. minus*. Statistical analyses showed high interspecific, but relatively low genetic diversity in populations 2 and 3 of *A. squarrosum*. The phylogenetic clusterization and analysis and population structure analysis demonstrated clear segregation of *A. minus* from *A. squarrosum*, as well as the separation of population 1 of *A. squarrosum* from populations 2 and 3. As a result, we identified the set of novel and informative SSR markers suitable for the study of genetic diversity in *Agriophyllum* species. These results provide an important contribution to the population study and approaches for the development of conservation mechanisms for *Agriophyllum* species.

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ORCID ID

Y. Genievskaya orcid.org/0000-0001-5987-2952
D. Karelova orcid.org/0000-0002-2767-4350
S. Abugalieva orcid.org/0000-0002-9748-507X
P. Zhao orcid.org/0000-0003-4955-0989
G. Chen orcid.org/0000-0002-3687-4012
Y. Turuspekov orcid.org/0000-0001-8590-1745

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