Species of *Fragaria L.* (Rosaceae) genus of Central, Northern and Eastern Mongolia

Sergey Baturin[1*], *Nasanjargal* Tovuudorj[2], *Elena* Filipenko[1], *Renchinmyadag* Tovuudorj[3] and *Ganbold* Jigmed[2]

[1]Federal Research Center Institute of Cytology and Genetics the Siberian Branch of the Russian Academy of Sciences, 630090 Lavrent'ev av., 10, Novosibirsk, Russian Federation
[2]Mongolian University of Life Sciences, Agronomy and Plant Protection Department, 17024, Ulaabaatar, Mongolia
[3]Institute of Geography and Geo-ecology, Mongolian Academy of Sciences, 15170, Ulaanbaatar, Mongolia

Abstract. Numerous publications of Russian and Mongolian researchers report the only species *Fragaria orientalis* Losinsk to be typical for Mongolia. However, by generally accepted taxonomy of *Fragaria* genus, the species *F. orientalis* is a tetraploid (2*n*=4*x*=28) which occupies a small area within Eastern regions of Russia and North-Western China. To clarify the specific belonging of strawberry, 11 *Fragaria* samples were collected in Central, Northern and Eastern parts of Mongolia. Species were identified by morphological characters and molecular marker of alcohol dehydrogenase adh1 gene. The results of the specific belonging study in *Fragaria* samples reveal two diploid species *F. viridis* and *F. mandshrica* growing in Mongolia.

1 Introduction

Modern taxonomy of *Fragaria* L. genus includes at least 20 wild species, three spontaneous interspecific hybrids species and two cultivated hybrid species [1-3]. All these species form the polyploid series of *Fragaria* genus: 2*n*=2*x*, 4*x*, 5*x*, 6*x*, 8*x* and 10*x*, where *x*=7 [4, 5]. The diploid group includes 12 species, namely *F. vesca* L., *F. viridis* Duch., *F. mandshurica* Staudt, *F. bucharica* Losinsk, *F. iinumae* Makino, *F. nipponica* Makino, *F. nilgerrensis* Schlect., *F. chinensis* Losinska, *F. daltoniana* J. Gay, *F. nubicola* Lindl., *F. pentaphylla* Losinsk и *F.* × *bifera* Duch. The diploid species are widespread in South-Eastern Asia: there grow 8 of them in China [6], but also well represented in Siberia and Far East of Russia [7].

Numerous publications of Russian and Mongolian scholars report that *F. orientalis* Losinsk is the only one strawberry species growing in Mongolia [8-13]. However, there are indications to one more species in the Northern part of the country – *F. viridis* Duch. [14], which is also mentioned in the list of plants of Mongolia The Catalogue of Life Partnership [15]. According to generally accepted taxonomy of *Fragaria* genus, *F. orientalis* is a

* Corresponding author: SO_baturin@mail.ru

tetraploid species (2*n*=4*x*=28) and it occupies a rather small area within Eastern regions of Russia including Primorsky Krai, Khabarovsk Krai, Jewish Autonomous Oblast and Amur Oblast, and of China in Heilongjiang province. In China along the Amur River valley, *F. orientalis* meets with *F. mandshurica*, but different chromosome number prevents hybridization, so mixing of the species does not occur [16]. For Mongolian territory it is accepted to state only *F. mandshurica* growing there [1, 6, 16-18]. In this connection, our study aimed to clarify the specific belonging of wild-growing strawberry plant material collected in different aimags of Central, Northern and Eastern Mongolia.

2 Materials and methods

Eleven wild growing *Fragaria* samples collected in Mongolia were studied and included to the *Fragaria* collection of the Laboratory of Plant selection of Mongolian University of Life Sciences, Ulaanbaatar. Sites of plant collecting are presented in Table 1.

Table 1. List of *Fragaria* samples.

№	Sample registration number	Aimag	Region of Mongolia	Geographical coordinates of the site of collecting and alttitude (H), m
1	18-2	Ulaanbaatar	Central	N 47°52'34", E 106°53'44", H=1424 m
2	18-3	Tuv	Central	N 47°39'26", E 107°43'49" , H=1716 m
3	18-4	Ulaanbaatar	Central	N 47°52'27", E 106°53'30", H=1509 m
4	18-7	Arkhangai	Central	N 46°21'57", E 101°42'28", H=1948 m
5	18-8	Arkhangai	Central	N 46°21'57", E 101°42'28", H=1948 m
6	18-9	Bulgan	Northern	N 49°28'22", E 105°06'16", H=1107 m
7	18-10	Bulgan	Northern	N 49°28'22", E 105°06'16", H=1107 m
8	18-11	Tuv	Central	N 48°13'54", E 106°18'51", H=1390 m
9	18-12	Khentii	Eastern	N 48°10'62", E 108°57'51", H=1624 m
10	18-13	Selenge	Northern	N 49°46'18", E 107°35'47", H=761 m
11	18-14	Khentii	Eastern	N 48°35'28", E 110°30'96", H=1219 m

In wild-growing *Fragaria* plants, species were identified with keys and descriptions proposed by G. Staudt [16]. Beyond that, in order to identify the species by bio-morphological characters, PCR-fragments of Mongolian samples were compared with the ones of *Fragaria* representatives growing in wild in Siberian Region: *F. vesca* - sample № 15-7/3 (Irkutsk Region, Slyudyanka; N51°38.104′ E103°42.160′ H=665 м), *F. viridis* – sample № 16-17 (banks of the Kuyum River, Chemalsky District, the Altai Republic; N51°31.05′, E86°02.18′ H=486 м), *F. mandshurica* – sample № 3 (near the town of Yitulihezhen, Yakeshi, Inner Mongolia, China; N50°30.57′, E122°24.47′). Sample № 3 was kindly provided by Prof. J.J. Lei from *Fragaria* species collection of Shenyang Agricultural University [6]. Botanical distribution maps for species were drawn on ArcView 10.5.

For species identification of wild-growing *Fragaria* samples from Mongolia, we used gene alcohol dehydrogenase *adh*1 as a molecular marker. This is the first protein-coding gene in *Fragaria*, whose nucleotide sequence was established [19]. For phylogenetic and population researches, high informativeness is provided by application of this gene [2, 21] due to the presence of introns in its structure which are known to be exposed to rapid evolutional changes [20]. To identify the species of wild-growing samples, there were used

specific primers *adh*-2F and *adh*-3R complementary to *adh*1 locus allele of the II linkage group in *F. vesca*, earlier developed and successfully applied in studies on phylogenetic relationships in *Fragaria* genus [21, 22]. In *F. vesca* genome, nucleotide sequences of an exon and intron of the second allele *adh*2 significantly differ from *adh*1 allele and that is exactly why application of specific primers to *adh*1 allele helps to avoid misamplification with *adh*2 allele [23].

DNA from strawberry leaves was extracted using Genomic DNA Purification Kit (Fermentas). For PCR, special primers for alcohol dehydrogenase (*adh*1) were synthesized: *adh*-2F 5' CCAAGGTACACATTCTTTTTTC 3' and *adh*-3R 5'GTCACCCCTTCACCAACACTCTG 3'. PCR reaction mixture contained: 50-100 ng of plant DNA and 600 nM concentration of primers in a buffer of 65 mM Tris HCl, (pH 8.9), 24 mM $(NH_4)_2SO_4$, 3 mM $MgCl_2$, 0.5% Tween 20, and 200 µm dNTP, adding Taq-polymerase "hot start" (produced by ICBFM, Sib.Br. of Rus.Sci.Acad.) 1 unit per reaction. After preliminary heating at 95°C for 15 min, there followed 30 cycles: 95°C for 1 min, 58°C for 90 s and 72°C for 1 min. Amplified DNA fragments were separated by electrophoresis in 2% agarose gel and 1x TAE buffer.

Chromosome number in seedlings calculation was performed at root tips by staining chromosomes with lacto-propionic orcein [24].

3 Results and discussion

In Central, Northern and Eastern Mongolia (Fig. 1), strawberry is a very popular wild berry [10, 11, 13]. Wild strawberry occurs in Khentii, Khangai, Mongol Altai, Mongol Daguur Biosphere Reserve [8].

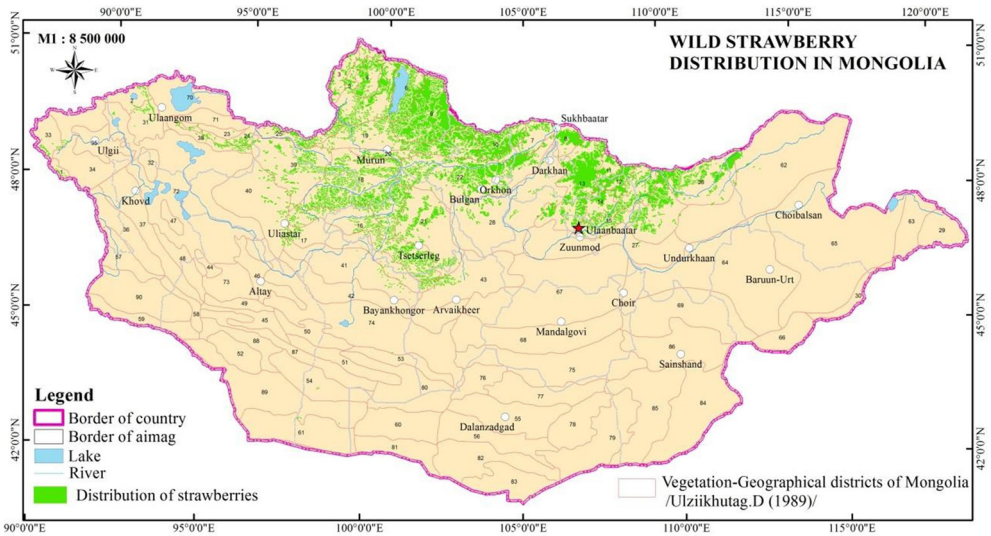

Fig. 1. The wild strawberry distribution in Mongolia.

It grows in 14 of 21 Mongolia's aimags and most widely wild strawberry is spread in Khuvsgul, Bulgan and Selenge aimags comprising the Northern part of Mongolia (Table 2). Distribution of wild strawberry species in Mongolia was mainly taken according to the data of V.I. Grubov [8], which based on the botanical-geographical regionalization made by N. Ulziikhutag [25].

Table 2. The wild strawberry distribution area and percentage in Mongolia.

Aimag	Area (km^2)	Percentage (%)
Ulaanbaatar	909.0066	0.06
Dornod	1029.064	0.07
Khentii	9981.921	0.64
Tuv	7958.095	0.51
Selenge	15007.18	0.96
Darkhan-uul	583.1363	0.04
Orkhon	222.9639	0.01
Uvurkhangai	1595.049	0.10
Bulgan	15 881.89	1.02
Bayankhongor	497.381	0.03
Arkhangai	10565.06	0.68
Khuvsgul	36566.08	2.34
Zavkhan	8232.512	0.54
Bayan-ulgii	600.2874	0.04
Total	109629.6	7.1

In all the collected in Mongolia samples of *Fragaria*, somatic chromosome number calculation proved them to be diploid, 2n=14. Identification of species by bio-morphological characters separated the samples as belonging to two known diploid species*:* *F. mandshurica* Staudt and *F. viridis* Duch. Besides, there were representatives of both species at once in some of collected sample sets. Those were samples № 18-7 and 18-8 from Arkhangai aimag, 18-9 and 18-10 from Bulgan aimag.

The results of specific identification of the samples by alcohol dehydrogenase adh1 gene in studied *Fragaria* representatives is presented in Fig. 2. There can be clearly seen the specific traits of each *Fragaria* sample under the study. The electrophoregramme profiles show that for both diploid strawberry species (*F. viridis* and *F. vesca*), presence of the only one PCR fragment is typical, although, the fragments are significantly different by length. For *F. viridis* typical fragment length is 446 bp, while for *F. vesca* – 582 bp (Fig. 2, tracks 1 and 2, correspondingly). That matches the results of earlier studies on phylogeny analysis in *Fragaria*, including *F. vesca* as well [2]. Moreover, DNA amplified fragments of *F. viridis* and *F. vesca* in our experiment were of the same length as PCR fragments in the study conducted by T. Davis & al. [22]. Amplification with *F. mandshurica* DNA also resulted in the only one PCR fragment (Fig. 2, track 3). However, the size of amplified DNA fragment was slightly bigger than of PCR *F. vesca* fragment (Fig. 2, tracks 1 and 3). In comparison to *Fragaria* species growing in Siberian region, amplification with DNA of wild-growing samples from Mongolia showed the presence of these two species among Mongolian samples. In samples №№ 18-7, 18-8, 18-10, 18-12, and 18-13, the major band of the electrophoregramme is equal by length to the PCR-fragment of *F. viridis* (Fig. 2, tracks 2, 7, 8, 10, 12, and 13), and in №№ 18-2, 18-3, 18-4, 18-9, 18-11, and 18-14 – to the one of *F. mandshurica* (Fig. 2, tracks 3, 4, 5, 6, 9, 11, and 14). Consequently, comparison of amplified fragment profiles of plants from Mongolia testify to the presence of two species *F. viridis* and *F. mandshurica* and that corresponds to the data on identification by bio-morphological characters.

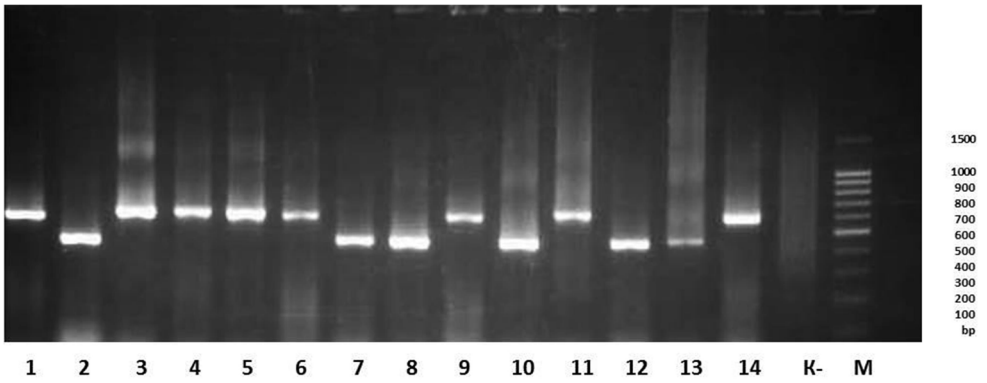

Fig. 2. The electrophoregramme of PCR fragments in 2 % agarose gel. Tracks: 1 – *F. vesca*; 2 – *F. viridis*; 3 – *F. mandshurica*; 4 – № 18-2; 5 – № 18-3; 6 – № 18-4; 7 – № 18-7; 8 – № 18-8; 9 – № 18-9; 10 – № 18-10; 11 – № 18-11; 12 – № 18-12; 13 – № 18-13; 14 – № 18-14; M – DNA length marker (produced by Evrogen)

Results of the study prove growing of two diploid species *F. viridis* and *F. mandshurica* in the territory of Central, Northern and Eastern Mongolia (Fig. 3). Moreover, representatives of both species can grow side by side, as testified by samples from Bulgan aimag (samples №№ 18-9 and 18-10).

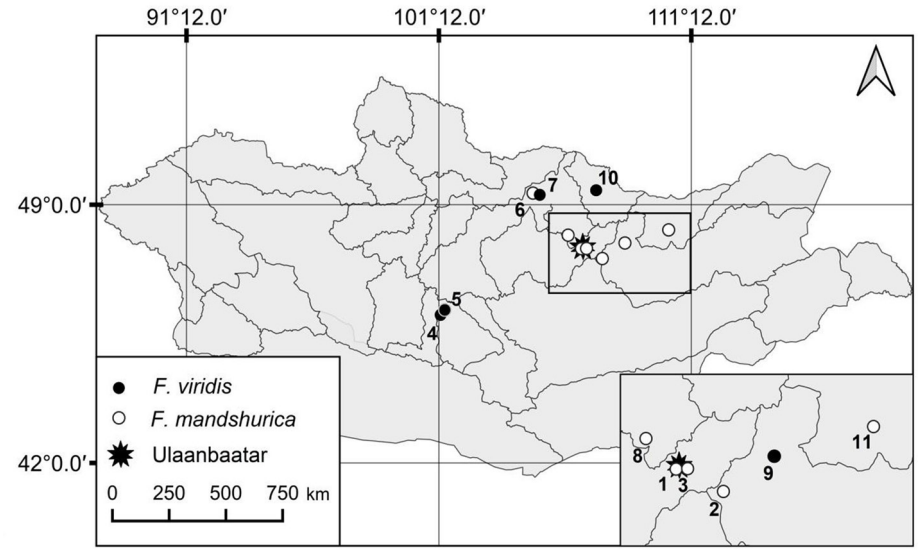

Fig. 3. Distribution of studied *Fragaria* samples on the map of Mongolia: 1 - № 18-2; 2 - № 18-3; 3 - № 18-4; 4 - № 18-7; 5 - № 18-8; 6 - № 18-9; 7 - № 18-10; 8 - № 18-11; 9 - № 18-12; 10 – № 18-13; 11 – № 18-14.

F. viridis and *F. mandshurica* growing in Mongolia does not contradict the data of English-language literature [1, 13, 14, 15, 16, 17]. On the contrary, publications in Russian and Mongolian languages mention *F. orientalis* instead of *F. mandshurica*. The problem of confusion of these two specific names is the central focus of a large experimental paper by G. Staudt [16] and he clearly shows the difference between *F. mandshurica* and *F. orientalis* and points their areas. Such a mistake in *F. mandshurica* identification in the territory of Mongolia is caused by similarity of many morphological characters used for

specific identification due to phylogenetic closeness of these species. G. Staudt [16, 17, 18] supposed *F. orientalis* to be a tetraploid descendant of *F. mandshurica*. However, molecular-genetical analysis testifies that *F. mandshurica* is one of the three potential diploid donors along with *F. vesca* and probably, *F. bucharica* [26]. And yet, RFLP analysis of chloroplast DNA refutes the fact that *F. mandshurica* is a mother donor of *F. orientalis* [27]. Nevertheless, the main morphological character to distinguish *F. mandshurica* and *F. orientalis* is flower type: flowers of *F. mandshurica* are monoclinous, while in the form of *F. orientalis* flowers dioecy is realized; main cytological characters, in turn, are diploid chromosome number ($2n$=14) and less in size pollen grains in *F. mandshurica* [16]. Analysis of ability to crossing between *F. mandshurica* (samples collected in Yakutia) and *F. orientalis* revealed reproductive isolation of the two species, as very few seeds developed and the seedlings grown from obtained scarce seeds all turned to be triploid and consequently, sterile [7].

Thus, our study on specific belonging of *Fragaria* samples from Central, Northern and Eastern parts of Mongolia proves growing of two diploid species *F. viridis* and *F. mandshurica* there.

Authors are grateful to Prof. J.J. Lei for kindly provided standard *F. mandshurica* Staudt sample from *Fragaria* species collection of Shenyang Agricultural University. The authors sincerely thank Tatyana Kolomiychuk for the translation of this article. This study was conducted according to budget project RF № 0324-2019-0039-C-01.

References

1. K. E. Hummer, N. Bassil, W. Njuguna, Wild Crop Relatives: Genomic and Breeding Resources, Temperate Fruits (Springer-Verlag, Berlin Heidelberg, 2011) 17-44
2. L. M. DiMeglio, G. Staudt, H. Yu, T. M. Davis, PLoS ONE, **9**, 7 (2014)
   M. K. Sobczyk, The Genomes of Rosaceous Berries and Their Wild Relatives (Springer International Publishing AG, part of Springer Nature, 2018) 11-23
3. M. Rousseau-Gueutin, A. Gaston, A. Aïnouche, M. L. Aïnouche, K. Olbricht, G. Staudt, L. Richard, B. Denoyes-Rothan, Mol. Phylogenet. Evol, **51**, 515–530 (2009)
4. O. K. Kamneva, J. Syring, A. Liston, N. A. Rosenberg, BMC Evolutionary Biology. **17,** 180 (2017)
5. J. J. Lei, L. Xue, H. P. Dai, M. Q. Deng, Acta Horticult. **1049**, 289-294 (2014)
6. N. B. Sukhareva, Apomixis and its meaning for evolution and selection, **30**, 165-175 [in Russian]
7. V. I. Grubov, Identification Guide of the Vascular Plants of Mongolia (with atlas) (The Science, Leningrad, 1982) 443 [in Russian]
8. R. V. Kamelin, I. A. Gubanov, Sh. Dariymaa, E. Gandbold., Botancal Journal, **77**, 4, 10-21 (1992) [in Russian]
9. N. Altansukh, Mongolia: country report to the FAO international technical conference on plant genetic resources, (Leipzig, 1996)
10. D. Damiran, Circular of Information No. 3. (Union, OR, USA: Eastern Oregon Agricultural Research Center, 2005)
11. Ch. Dulamsuren, M. Hauck, M. Mühlenberg, IAVS, Applied Vegetation Science **8**, 149-154 (2005)
12. U. Magsar, O. Kwon, B. Badamdorj, K. Nyamsuren, I. Tuvshintogtokh, N. Batkhuu, M. Tovuudorj, S. Khadbaatar, O. Batlai, Y. Cho, J. Pak, M. Kim, S. Choi, H. Kang, H.

Lkhavgadorj, Introduction. The Handbook for Traditional Knowledge on Biological Resources. Mongolia**, 1**, 631 (2015)

13. I. A. Gubanov, Systematic Notes on Flora of Outer Mongolia, Vascular Plants (VALANG, Moscow, 1996) 136 [in Russian]
14. M. Hassler, Species 2000 & ITIS Catalogue of Life, 2019 Annual Checklist (Naturalis, Leiden, the Netherlands, 2019) http://www.catalogueoflife.org/annual-checklist/2019
15. G. Staudt, Bot. Jahrb. Syst. **124,** 397-419 (2003)
16. G. Staudt, Acta Horticult. **265**, 23-33. (1989)
17. G. Staudt, Acta Horticult. **842**, 71-84 (2009)
18. D. J. Wolyn, G. Jelenkovic, Plant Mol. Biol. **14**, 855–857 (1990)
19. R. L. Small, R. Cronn, J. F. Wendel, Aust. Syst. Bot. **17**, 145–170 (2004)
20. G. Staudt, L. M. DiMeglio, T.M. Davis, P. Gerstberger, Bot. Jahrb. Syst. **125**, 53-72 (2003)
21. T. M. Davis, H. Yu, J. Hered. **88**, 215–221 (1997)
22. T. M. Davis, M. E. Shields, Q. Zhang, D. Tombolato-Terzic´, J. L. Bennetzen, A. Pontaroli, H. Wang, Q. Yao, P. Sanmiguel, K. Folta, BMC. Plant Biol. **10**, 81 (2010)
23. N. Preeda, T. Yanagi, K. Sone, S. Taketa, N. Okuda, Scientia Hort. **114**, 133-137 (2007)
24. N. Ulziikhutag. *Survey of vascular plant of Mongolia* (Ulsiin Khevleliin Gazar, Ulaanbaatar, 1989)
25. O. K. Kamneva, J. Syring, A. Liston, N. A. Rosenberg, BMC Evolutionary Biology. **17**, 180 (2017)
26. W. Njuguna, Development and Use of Molecular Tools in Fragaria (Oregon State University, 2010)