

**Studying the polymorphism of different *Agrimonia L.* populations growing in the south-eastern Kazakhstan**A.N. Kaliyeva<sup>1</sup>, G.U. Dyuskaliev<sup>1</sup>, A. Newsome<sup>2</sup>, R.K. Zhexembiyev<sup>1</sup>, M.S. Kurmanbayeva<sup>1</sup>, A.K. Mussaeva<sup>1</sup><sup>1</sup>Department of Biology, Kazakh State Women's Teacher Training University, Aiteke bi str., 99, Almaty, 050000, Republic of Kazakhstan<sup>2</sup>Mississippi Valley State University, Itta Bena, USA

**Abstract.** There are various methods of genetic marking aimed at preserving and expanding the gene pool of plants which are of medical or agricultural importance and at the genetic monitoring of their natural population. The RAPD analysis can be a kind of rapid method for detecting a genetic polymorphism and genomic marking in population research. The results showed that each species of *Agrimonia L.* under analysis is at a considerable genetic distance (more than 0.4). This is an evidence of significant differences between them. The RAPD revealed a high level of intraspecific polymorphism in different populations of *Agrimonia asiatica Juz.* and the considerable distinctions of their DNA-profiles versus another species *Agrimonia pilosa Ldb.*

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**Keywords:** *Agrimonia L.*, population, RAPD analysis, amplification, primers, genomic DNA

**Introduction**

In practical health care all over the world, there is an increasing interest to herbal medicinal products and the centuries of experience in use of them for therapy. In health care, the need for herbal medicines grows lately. Medicinal plants become very popular worldwide in the form of food additives, herbal drugs and the main sources of pharmaceuticals. About 70-80% of the world population apply the methods of traditional medicine for various diseases and dispositions [1,2].

In recent decades, the interest for searching and introducing new medicinal plants in Russia and abroad. Genus *Agrimonia L.* is one of the promising groups for obtaining valuable crude drugs [3]. One representative of the genus, *Agrimonia eupatoria*, is best studied from the viewpoint of pharmacology. Its aerial part procured at the beginning of flowering is used in medicine. The plant contains tannins, essential oil, glycosides, mineral salts, bitter stuff, flavonoids, vitamins and traces of alkaloids. *A.eupatoria* is included in the pharmacopeia and is used in scientific medicine in Europe in North America [4].

In Kazakhstan, there are two species of *Agrimonia L.*: *A.asiatica Juz.* and *A.pilosa Ldb.* [5]. In particular, scientists found out that *A.asiatica Juz.* includes a large amount of tannins which have an antimicrobial and anti-inflammatory value. Besides, there were found a considerable volume of flavonoids which are food antioxidants [6]. The goal of this study is to assess the level of intraspecific and interspecific differences of the natural populations of *A.asiatica Juz.* and *A.pilosa Ldb.* with the help of RAPD marking.

**Materials and methods**

The samples of *Agrimonia asiatica Juz.* were collected in three different populations (1-3) growing in the village of Koldy (30 km from Almaty, at an altitude of 832 m above sea level), in The Central Botanical Garden of Almaty (at an altitude of 856-906 m above sea level) and near the hole Butakovka (22 km from Almaty, at an altitude of 1870 m above sea level), respectively. The samples of *Agrimonia pilosa Ldb.* population were collected in the village of Saty (270 km from Almaty, at an altitude of 1300 m above sea level).

The isolation of total DNA from herbal material was performed by the CTAB method [7]. The concentration of isolated DNA was measured by spectrometry using instrument NanoDrop 2000c (Thermo Scientific, USA) with wave length 260 nm. The purity of obtained DNA-specimens was checked on the basis of OD<sub>260</sub>/OD<sub>280</sub> ratio and was equal to 1.6–1.9. Moreover, the quality of genomic DNA was checked by electrophoresis in 1% agarose body with further coloration in the solution of ethidium bromide.

The amplification of genomic DNA was performed with the help of thermal cycler Eppendorf Mastercycler ep gradient S (Eppendorf North America, Germany) in the Scientific Research Institute for Issues in Biology and Biotechnology of the Al-Farabi Kazakh National University. For RAPD-PCR analysis, the following five OPA primers were chosen: 5'- CTGTACCCCC - 3'; 5'- ACGCGCCAGG - 3'; 5'- ACTCGGCCCC - 3'; 5'- GGCCCCATCG - 3' и 5' - ACGGCGGCTC - 3'. The amplification reaction was performed in reaction mixture with volume 25 µl containing 2 MW of

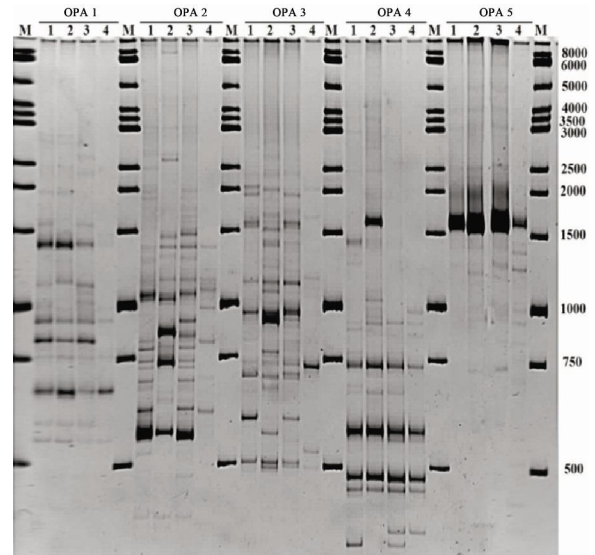
MgCl<sub>2</sub>, 0.2 MW of each dNTP; 2.5 μm of primer; 0.625 units of Taq-polymerase (Thermo scientific, Latvia), 1x buffer from an appropriate set and 20 ng of the genomic DNA. The PCR mode consisted of DNA denaturation during 60 s with 42°C; DNA synthesis – 90 s with 72°C with the number of cycles 30 and pre-denaturation – 5 min (94°C). The final cycle of elongation was performed during 5 min with 72°C. The resultants was separated by electrophoresis in 11% polyacrilamide gel (PAAG) and photographed with a professional highly sensitive gel imaging system Infinity-1500/36M. The size of amplified fragments was identified relative to DNA-marker 1 kb GeneRuler (Thermo scientific, Latvia).

The statistical analysis included compiling binary matrixes for each primer in which the fragments with the same molecular weight were “present” (1) or “absent” (0) at the electrophoregram. Each RAPD fragment was considered as a separate genetic locus. The software package POPGENE v. 1.32 [8] helped to identify the main factors of population genetic structure on the basis of the total matrix of RAPD spectra. These were such factors as: the percentage of polymorphic fragments (PPF), the visible number of alleles per locus (Ao), the effective number of alleles per locus (ne), the expected heterosigosis (He) and the Shannon information index [9]. The unweighted pair group method with arithmetic mean (UPGMA) was used in the analysis of relationships between populations and tree diagram construction on the basis of Nei’s genetic distances (D) [10] with the help of Treeconw program.

## Results and discussion

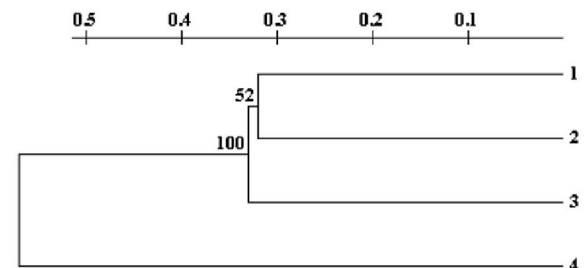
Figure 1 shows DNA-profiles of three different populations of *A. asiatica Juz.* (see tracks 1-3) and one natural population of *A. pilosa Ldb.* (see track 4) growing in the south-eastern Kazakhstan.

One can see at Figure 1 that the DNA-profiles of different populations of both species differed by length and the number of PCR fragments. The amplification of five DNA samples with five OPA primers resulted in presentation fragments with length from 200 to 3500 bps. It was shown that the DNA fragments of *A. asiatica Juz.* obtained by the used primers and having size ≤720, 750, 900, 1200 and 1400 bps are not polymorphic. At the same time, the other amplicons were present or absent depending on a DNA sample and thus can be considered polymorphic. The RAPD analysis revealed a high level of intraspecific polymorphism in different populations of *A. asiatica Juz.* and considerable differences of their DNA-profiles versus *A. pilosa Ldb.*



**Figure 1:** The RAPD-patterns of *A. asiatica Juz.* and *A. pilosa Ldb.*: 1-3 are the DNA samples of *A. asiatica Juz.* growing in the village of Koldy, in the Central Botanical Garden of Almaty and near the hole Butakovka, respectively; 4 is the DNA samples of *A. pilosa Ldb.* Growing in the village of Saty; M is a DNA marker 1 kb GeneRuler.

Figure 2 shows the phylogenetic tree constructed with the help of RAPD marking results in order to assess the level of interspecific and intraspecific differences in the natural populations of *A. asiatica Juz.* and *A. pilosa Ldb.*



**Figure 2:** Tree diagram based on the RAPD profiles of *A. asiatica Juz.* and *A. pilosa Ldb.* populations: 1-3 are the DNA samples of *A. asiatica Juz.* growing in the village of Koldy, The Central Botanical Garden of Almaty and near hole Butakovka, respectively; 4 – are the DNA samples of *A. pilosa Ldb.* Growing in the village of Saty.

The tree diagram shows that each of the analyzed species of *Agrimonia L.* is at a significant genetic distance (more than 0.4). This is an evidence of significant differences between them. At the same

time the calculation of genetic distances in three populations of *A. asiatica Juz.* based on the RAPD analysis showed the difference within the limits of 48% with genetic distance within 0.3-0.33.

So this article contains the results of PCR analysis with RAPD primers of different populations of *A. asiatica Juz.* and *A. pilosa Ldb.* The enzymatic analysis allowed detecting the interspecific markers of genus *Agrimonia L.*, while the RAPD analysis, besides, revealed the intraspecific differences in three *A. asiatica Juz.* populations under study.

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