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# Genetic Signs of Interspecific Polymorphism of *Ephedra* L. Species in Kazakhstan Flora

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**Abstract:** Genetic features of 7 kind's ephedra, growing in the south Kazakhstan are investigated. It is established that from 20 primers only two Pr-15 and Pr-23 can be used for interspecific research. For 4 kinds ephedra species-specific fragments are revealed.

**Key words:** Ephedra · Interspecific polymorphism · Genetic signs · DNA · Primer · Aplikon · Specific markers

## INTRODUCTION

The flora of Kazakhstan is rich with economically important kinds of plants. Botanists do the big work on their systematization, mapping, to the description of conditions of growth and an estimation of their efficiency. However on revealing of genetic, physiological and morphological and biochemical features of various populations of valuable kinds while it are not enough complex researches. Their knowledge is very important for selection of plants from perspective populations, to planning of preparations and restoration of biocoenosis. Besides, it represents the big theoretical interest for studying of an orientation of change of genetic and biochemical signs in populations of economically valuable kinds growing in various ecological conditions [1, 2].

In Kazakhstan grow 7 kinds of ephedras: horsetail ephedra - Ephedra equisetina Bge., a. medium - *E. intermedia* Schrenk., a Bordered - *E. lomatolepis* Schrenk., a. cone-bearing - *E. strobilacea* Bge., e. Double - eared - *E. distachya* L., a. One-seed - *E. monosperma* C.A., a. Regelevskya - *E. regeliana* Florin. One of them - a. Bordered is endemic, 4 kinds are considered medicinal, vitamins [3, 4].

## MATERIALS AND METHODS

**Allocation of DNA from Needles Ephedra:** 100 mg of a dry vegetative material pounded in a mortar together with 20 mg of an oxide of aluminum, then received homogenate transferred to test tubes of type of Eppendorf and 700 mcL BECOMING-BUFFER, containing 50 mm tris-HCl, pH 8,0; 0,7 M NaCl; 10 mm EDTA; 1 %-s' (on weight) cetyltrimonium ammonium bromide (BECOMING) and 20 mm 2-merkaptoetanol. Received homogenates abstracted 15 mines at 65°C, periodically mixing test tube contents.

After 15 mines extraction, test tubes cooled at room temperature and added equal volume of a mix chloroform: isoamyl pure alcohol (24:1), with the subsequent hashing and centrifugation at 8000g at room temperature.

Water phases transferred to a pure test tube and to the remained deposit added 200 mcL buffers for homogenization and spent re - extraction. The received water phases united, added 0.1 volumes of 10 % BECOMING and carefully mixed and deprotenized mix chloroform: capryl alcohol (24:1).

After centrifugation the supernatant fluid selected in a pure test tube of type eppendorf. For DNA sedimentation to the received water phase added equal volume of the buffer for the sedimentation containing 50мM tris - HCl pH 8,0; 10 mm EDTA; 1 % (on weight) BECOMING. After two-hour incubation a deposit consisting of a complex nucleonic acid: BECOMING besieged in baket - a rotor at 1500g during 10-15 mines at room temperature. The received deposit dried and dissolved in 1мл 1M NaCl. After full dissolution of a deposit in a test tube added equal volume cold isopropylalcohol. Through 60 mines of a test tube centrifugation and the received deposit was washed out

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by 70 % the ethanol cooled to-20S0, for removal of traces detergent. Deposit dried and dissolved in the sterile, deionized water. Cleanliness of the received preparations of DNA checked electrophoretic division in agarose gel [5, 6]. Technique of carrying out of the RAPD-analysis.

At carrying out PCR of reactions used twenty 10-chlennyh casual primers, most suitable of them was the following: Pr-3 CCGAATTCGC; Pr-12 CCGGCACGCA; Pr-15 GCCTCGCCCA; Pr-23 GGTGCCGTAC. The reactionary mix for PCR in volume contained 20 microns 2.5ед. Tag- polymerase (Bion, Russia), 10mm tris-HCL pH 8.3; 50мM KCL; 0.01 % Tween 20; on 100 mlM dATP, dGTP, dCTP and dTTP ("Pharma", Sweden); 1mkM primers; 4mM MgCl and to 25 ng DNA.

Amplification was performed in a programmable thermostat Cyclo Temp 6 (CTM, Russia) in the following conditions: 2 cycles: a denaturation - 95°, 3 mines; annealing-30°, 1 mines; synthesis-72°, 1min and 35 cycles at values of corresponding processes 94°, 1min; 37°, 1 mines and 72°. Electrophoresis spent in 8 % nom polyacrylamide gel and analyzed by means of firm Bio-Rad device Gel-Doc in passing ultra-violet light at length of a wave from 260 to 360 nanometers.

**Definition of a Genetic Distance:** Genetic distances RAPD - spectra have been calculated by software package Quantity One-4.1.1 (GelDoc, BioRad) and constructed genealogical dendrogram with the help of non - balanced pair-grouped method with arithmetic averaging - UPGMA.

Thus, by us it is shown that used in cluster work the condition analysis of amplicons an UPGMA-method in RAPD-spectra and primers reliably enough proves authentic distinctions of specific structure of sort *Ephedra* L. Used in work of primers reliably enough differentiate kinds of plants of sort *Ephedra* L. Proceeding from the received results it is visible that the factor of cluster similarities of plants of this sort lies in limits from 0.38 till owing to what it is possible to assume that two kinds - E intermedia and E. *Equisetina* differ from each other genetically.

Application of nucleotide and amino-acid markers has allowed connecting the received results with earlier received data on studying of dynamics of accumulation of alkaloid substances. It is known that in our samples of research, in particular, in E intermedia dominate d-pseudoephedrine and E. Equisetina - l - ephedrine. Other kinds of plants of sort Ephedra L., growing in republic Kazakhstan is intermediate.

Revealing of polymorphism RAPD - markers at different kinds of ephedra allows bringing an attention to the question on them grouping and exacting identification. This technology can have the big practical value. So, for four kinds of ephedra are defined individual species-specific marked fragments, which can be used, further in definition with exsiccatae material. Revealing RAPD - polymorphism at different intraspecific forms of ephedra allows speaking about possibility of use of these markers in genetic mapping for authentic ordering kinds of ephedra.

The big actual material on phytochemical research and biochemistry of 7 kinds of ephedras is saved up. On an example of horsetail ephedra it is shown that the maintenance in green branches decreases in the dampest and the habitat in dry years and during the dry period of summer [5-8] raises in rather dry conditions.

Proceeding from it, a main objective of our research was studying of interspecific polymorphism and biochemical features of plants of sort *Ephedra* L. is the light of last achievements of biochemistry, chemistry of natural connections. For finding-out of this question we used molecular markers: DNA structure.

Earlier by us distinctions between populations of horsetail ephedra to morphological signs are shown. Authentic distinctions between populations of horsetail ephedra are shown on biochemical markers (on componental structure of the peroxidase which are not specific esterases, sour phosphatase and DNA structure). Used in work primers reliably enough differentiate populations E.equisetina Bge., [6-7].

In the present work we result results of research of interspecific polymorphism of DNA of plants of sort *Ephedra* L.

The phenomenon of polymorphism of DNA does possible the permission of questions at issue of systematization and phylogeny in different taxonomic groups of plants. Among existing of molecular - genetic methods of studying of polymorphism of DNA the central place occupies PCR - the analysis based on application of any oligonucleotide is directed on turned repeating sequences (Randomly Amplified Polymorphic DNA - RAPD) [8, 9].

RAPD - the analysis is widely used for studying of genetic polymorphism of plants. Now the RAPD-technology is fulfilled on variety of agricultural crops: cabbage, onions, grapes, a potato, a tomato, carrots, a string bean and barley. It is widely used in studying vegetative genom at designing of genetic cards, the analysis of genetic structure of population, genotyping, marking of signs and also in selection programs for fast identification selection of important signs [10].

RAPD - the technology is the unique tool, allowing spending the express train - identification of any organisms. For this purpose it is possible to use one of universal primers and there was a possibility of detailed studying of structure genom separate organisms and genetic structure of population and kinds of any organisms on the loci which earlier aren't subject to the analysis. Revealing on a number of primers PCR polymorphism can be used as genetic markers of grades, populations, individuals, clones and other intraspecific structures. With the help of cluster analysis methods carry out construction dendrogram, distinctions reflecting degree or similarity between RAPD - spectra of investigated objects that is important for studying inter - and intraspecific mutual relations. Methods UPGMA are most widely used: not weighed pair grouped a method with arithmetic averaging.

At the decision of specific targets of comparison of genomes with the help RAPD - a method there is a choice primers problem, allowing receiving informative enough, i.e. containing polymorphic fragments, RAPD - spectra.

## RESULTS AND DISCUSSION

From 20, before used at the analysis of ephedra primers genomes, only 4 give polymorphic fragments of DNA and are quite suitable for revealing of genetic polymorphism at ephedra, both in one kind and between different kinds. Each of investigated kinds and population of ephedra had specific spectrum RAPD - the products, characterized by certain quantity of fragments, in their sizes and degree of expressiveness [11].

All used primers effectively provided synthesis of specific and reproduced sets of fragments (amplicons). The number of amplicons depending on used primers made from 5 to 20, their sizes varied within 100-2000. All investigated kinds had specific RAPD - spectra, excellent number amplicons, their sizes and expressiveness degree on electrophoregramme (Figs. 1-4).

For definition of interspecific polymorphism have been used primers Pr-15 and Pr-23. From received amplicons only two, the size 100 and 350 are constitutive for all kinds of ephedra. For 4 kinds fragments which can be considered as species-specific markers are revealed. For E.monosperma specific markers are amplicons with the size 400 and 450. The greatest numbers of unique fragments in the sizes from 120 to 1100 are revealed for *E. distachya*. At kind *E. strobilacea* two fragments in the size 130 and 2200 specific to this kind are defined. For *E. equisetina* three are defined in a video specific

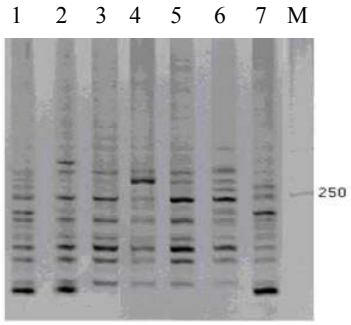


Fig. 1: RAPD-spectra, received from PR-15
*1- E. intermedia; 2- E. Regeliana;*
*3- E. lomatolepis; 4- E. monosperma;*
*5- E. distachya; 6- E. strobilacea;*
*7 E. equisetina.*

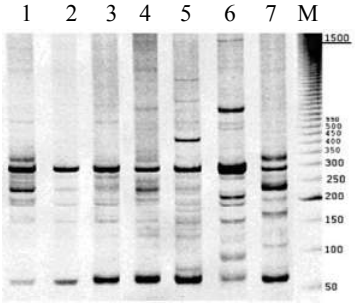


Fig. 2: RAPD-spectra, received from PR-23
*1- E. intermedia; 2- E. Regeliana;*
*3- E. lomatolepis; 4- E. monosperma;*
*5- E. distachya; 6- E. strobilacea;*
*7- E. equisetina.*

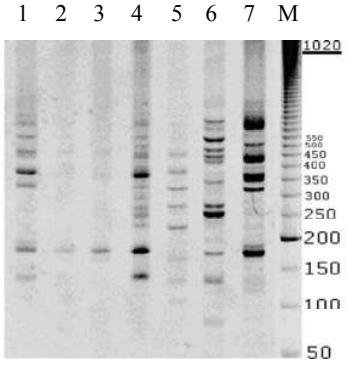


Fig. 3: RAPD- spectra, received from PR-3
*1- E. intermedia; 2- E. Regeliana;*
*3- E. lomatolepis; 4- E. monosperma;*
*5- E. distachya; 6- E. strobilacea;*
*7- E. equisetina.*

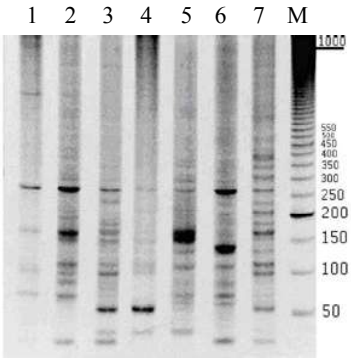


Fig. 4: RAPD- spectra, received from PR-12
*1- E. intermedia; 2- E. Regeliana;*
*3- E. lomatolepis; 4- E. monosperma;*
*5- E. distachya; 6- E. strobilacea;*
*7- E. equisetina.*

Table 1: A condition matrix of ephedra's amplicons

| | Calculation Method | | | | Dice Coefficient | | |
|---|---|---|---|---|---|---|---|
| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1 | 100 | 70.6 | 85.7 | 60.9 | 48 | 63.6 | 52.6 |
| 2 | 70.6 | 100 | 75 | 44.4 | 60 | 58.8 | 42.9 |
| 3 | 85.7 | 75 | 100 | 54.5 | 58.3 | 66.7 | 44.4 |
| 4 | 60.9 | 44.4 | 54.5 | 100 | 46.2 | 52.2 | 40 |
| 5 | 48.0 | 60 | 58.3 | 46.2 | 100 | 48 | 27.3 |
| 6 | 63.6 | 58.8 | 66.7 | 52.2 | 48 | 100 | 21.1 |
| 7 | 52.6 | 42.9 | 44.4 | 40 | 27.3 | 21.1 | 100 |

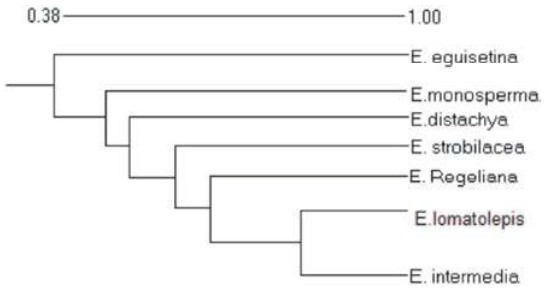


Fig. 5: The cluster analysis of amplicons' condition by UPGMA-method in RAPD-spectra of 7 kinds of ephedras

fragment on 150, 250 and 3000 accordingly. Presence of these species-specific amplicons is confirmed in several independent experiments.

For a quantitative estimation RAPD - polymorphism and level definition of divergence between kinds of the received matrix of a condition of amplicons have been processed by software package Quantity One-4.1.1 (GelDoc, BioRad) and are presented in the form of a matrix of conditions of binary signs in which presence or absence in RAPD - spectra identical on the size of amplicons was considered as a condition 1 and 0 accordingly and it was translated in percentage variation parities. The data is presented in Table 1.

**Accordingly:** Presence of these species-specific amplicons is confirmed in several independent experiments.

1- *E. intermedia*;
2- *E. Regeliana*;
3- *E. lomatolepis*;
4- *E. monosperma*;
5- *E. distachya*;
6- *E. strobilacea*;
7- *E. equisetina*.

On matrixes of conditions that software package had been calculated matrixes of distinctions. The factor of Zhakkarda Dj = Na + Nb/Na + Nb + Nab, where Na - number of the strips which are available in a spectrum and, but absent in a spectrum b, Nb – number of strips in a spectrum b, but not in a spectrum and and Nab - number of strips, the general for both spectra was thus used. Proceeding from this matrix, non - balanced pair - grouped clusters by the method (UPGMA) has been constructed by dendrogram genetic relationship between different kinds (Fig. 5).

## CONCLUSION

On the basis of the received data it is visible that kinds of *E. intermedia* and *E. lomatolep* is which form one cluster with a factor of cluster similarities 0.86 owing to what it is possible to assume about their genetic affinity are closely related. *E. equisetina*, having low (0.38) factor of cluster similarities to them, genetically is the most remote from these two kinds. Intermediate position occupy *E. Regeliana* (0.73), *E. strobilacea* (0.63), *E. distachya* (0.54) and *E. monosperma* (0.40).

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