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Anatomic-Morphological and Biochemical Characteristics of *Patrinia intermedia* (Horn.) Roem. et Shult in Conditions of the South-East of Kazakhstan

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Abstract: *Patriniaintermedia* (Horn.) Roem. etShultis widely known medicinal plant and with the ever-increasing demand for medicinal raw material Patrinia average, our task was to study the peculiarities of its anatomic-morphological structure in conditions of the South-East of Kazakhstan and identify biochemical and molecular-genetic characteristics. As a result of anatomic-morphological study of overground and underground parts of *Patriniaintermedia* found differences in the location and sizes of differentiated cells in both of the studied plant populations of Patrinia.In biochemical studies were identified differences in the nature of changes in the activity of soluble proteins, depending on the body of the plant and the population studied plants *Patriniaintermedia*. The sequencing of the plants *Patriniaintermedia* (Horn.) Roem. etShult growing in the territory of South-Eastern Kazakhstan allowed to determine the nucleotide sequence ITSI - 5.8S, rDNA - ITSII regions rDNA with 100% of the species identity.

Key words: Patriniaintermedia · Morphology · Anatomy · Biochemistry · Sequencing

INTRODUCTION

Wild-growing useful plants - the source of diverse natural resources for many sectors of the industries and medicine, has long cause an inexhaustible interest to researchers, invariably are very popular because they are less toxic available and their action is time-tested. The need of medicinal vegetative raw materials is constantly growing and is far ahead of its production.

The increased demand for wild medicinal plants, which are an indispensable source of physiologically active substances, pectin, sugars, vitamins, organic acids. In this connection there is the problem of finding and inventory of plant resources, their rational use, as well as a comprehensive study of the useful properties of certain perspective for the economic development of the species. Since the territory of our Republic in relation to plant resources is not well studied and unevenly, are currently at work in the unexplored regions, implementation of which will contribute to the provision of health and other branches of national economy of the Republic of Kazakhstan a stable raw material base [1-5].

The subject of study botanical resources is, on the one hand, plants as a source of raw materials and with another - plant resources in general. It should be noted that of all the wealth of flora, including about 300 thousand species of higher plants, uses only one hundredth of a part that can be applied in the form of medicines, food supplements, cultivated and commercialized. The study of unique and peculiar flora and use of its representatives in medicine, cattle breeding, the food industry is very topical at present. In the search for plants that have a therapeutic effect, used two principles - the accumulated experience of folk medicine and Botanical relationship, which consists in studying the families and genera, among which there are representatives of official plants with known pharmacological effect.

One of the most long been used in medicine is a family of Valerianov, numbering over 200 species. Representatives of this family are applied in both Western and Eastern traditional medical practices. The most known and used since ancient times to the present time, is a genus Valerian. Less well known genus Patrinia, has long

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been used in Korean and Tibetan medicine. The most studied species such as Patrinia average and Siberian. They are used extensively, along with preparations of Valerian. For Patrinia average developed temporary Pharmacopeia. Recognizing their high sedative, soothing efficiency, it should be noted restricted area Patrinia medium and small reserves of raw biomass of both species. In practical medicine and veterinary practice need to be sedated means, in connection with the strengthening of the socio-economic and environmental stresses in animals and man.

Based on information of folk medicine and phylogenetic kinship with the genus Valerian, promising to study as a potential medicine *Patriniaintermedium*. The use of little-explored species as a medicine requires assessment made by taking into account the range and resources, growing conditions, physical and chemical characteristics of raw materials and biological activity of plants [6-18].

Provides a preliminary checklist of medicinal plants of Kazakhstan, drawn up on the basis of the analysis of floristic, resource study, phytochemicals, ethnobotanical and other sources, which has over 1,500 species of vascular plants from 134 families. List of medicinal plants of Kazakhstan allows carrying out scientific and predictable search for promising for practical use of medicinal plants of the Republic [19].

Conservation and rational use of vegetation resources of our planet is now a global problem of interstate level. The ongoing process of climate change endangers the preservation of the natural vegetation and the human environment. Therefore, in modern conditions «...the inventory of natural plant resources both at regional and national levels, together with General replenished with new information about the useful properties, is the basis for the development of a science-based algorithm sustainable use of plant riches»[20].Fauna of Kazakhstan is characterized by a rich gene funds and unique reserves of useful plants, primarily wild species with medicinal properties, significant part of which is promising for use in investigations of chemical composition and biological activity of the metabolites primarily, biologically active substances, representing science-intensive and competitive products that are in increasing demand on the world market.

Medicinal plants are valuable raw material for production of phytopreparations with a wide spectrum of pharmacological and therapeutic actions that are quick, do not possess cumulative properties and to a lesser extent accompanied by undesirable side effects. Actively developing worldwide research in chemistry of natural compounds are constantly increasing number of medicinal species. Only in recent years and only in Kazakhstan, received new effective medical drugs from Kazakhstan aconites, delphinium, meadow rue, motherwort, harmala shrub and a number of others.

Modern assessment of species diversity drug flora and its resource potential is particularly relevant in terms of the sovereign Kazakhstan, when the production of medical preparations from medicinal plants is directly connected with the provision of pharmaceutical raw plant materials. Despite a long history of use and phyto-chemical study of medicinal plants, up to the present time, Kazakhstan has not carried out the inventory of medicinal flora, there is no a sufficiently full and analytical data, reflecting its current state and perspectives of the study.

Inventory of species of medicinal flora, aggregate data on their use and localization in the territory of Kazakhstan should facilitate search of natural compounds and creation of modern herbal remedies, promote the formation of a stable raw material base and, at the same time, contribute to the development of security measures for prevention of genetic losses unexplored part of the potentially useful plants. The initial step in the inventory of the research is formation of an annotated list of medicinal plants.

This list includes more than 1500 species of medicinal plants. The list includes Latin, Russian, Kazakh names of species, as well as its use in practical or folk medicine. For convenience of species of plants are arranged in alphabetical order according to generally accepted in the Botanical world practice the Latin names. The compilers of the list, consider the preliminary information, since the study of biodiversity of Kazakhstan flora continues [21-31].

Objects of research:Within the family Valerianaceae Batsch. there is differentiation and specialization of species on the morphology of the flower and fruit. The plants of this family is most noticeable difference in the morphology of the flower is the number of stamens, which varies from 4 to one, although there is a message about the availability of five Patrinia Juss [32]. This group of plants is in the direction of reducing the number of stamens. M.J. Donoghue with co-workers [33], referring to a large database of chloroplast analysis in combination with ITS sequencing of the region, concluded that initially

decrease in the number of stamens from four to three occurred in the common ancestor of the family Valerianaceae.Then there were two independent additional reduce the number to two stamens (species of the genus Fedia) and to single stamen (species of the genus Centranthus). A Cup of species of the family or permanent (sheet, for example, Nardostachys; reduced to a small cloves, for example, Fedia and Valerianella; or in the form of Pappus of Centranthus and species Valeriana)or completely absent.B.Eriksen [34] did the hypothesis that the full reduction of a cup took place independently and several times within a site (on the example of some Latin American species Valeriana). Moreover, the degree of reduction of the two barren abaxile cameras of various species of the family is also heterogeneous symptom - from strongly reduced to a more advanced that found in several species Valerianella and Valeriana. Modification of both these characteristics, namely, cups and fruitless cameras, well correlate with the value of the dispersion. The characteristic feature of this group of plants of the family Valerianaceae is that in most species, including Triplostegia, there are such chemical compounds, as iridoids [35].

With the help of which micro molecular chemo plant systematics studies, on the one hand, the relationship between species and, on the other hand studying their chemical characteristics [36]. In the field of chemo systematics value characterization iridoids is of great importance in plant classification and phylogeny.

The plants collected Patriniaintermedia (Patriniaintermedia (Horn.) Roem. etShult.), growing in Kaskelenand Talgar gorges of ZailiyskiyAlatau. Pitch range distribution Patrinia quite broad - from 800 to 2500 m above sea level. Collection of plants of the species of concern was held in May-June, in the flowering period of development.

On the basis of the material recorded in 70% ethanol solution, held anatomic-morphological study of medicinal plants Patriniaintermedia according to the standard technique.

MATERIALS AND METHODS

Methods: For anatomical studies were prepared temporary medicine overground and underground vegetative organs according to the standard technique for structural research of vegetable objects. Anatomical sections above-and underground parts of plants were made with the help of microtome with freezing device TOC-2 and manually.

Temporary preparations vegetable slices were concluded in glycerin and converted into permanent slides, then lay in Canada balsam in accordance with generally accepted methods. The thickness of the anatomical slices was 10-15 microns. It is prepared more than 3500 temporary and permanent preparations for micro photography and holding morphometric analysis.

Methods for biochemical studies: We conducted a division of the composition of the soluble proteins plants: P. intermedia native method of electrophoresis in SDS-PAGE by B.J. Davis [37].

For electrophoretic analysis of soluble proteins nonspecific esterase (EST) and cathodic peroxidase (PER) were used buffer extracts from freshly picked leaves, stems and roots of plants. For separation of the EST and the PER used triglycinecontinuous buffer solution, pH 8.3. Staining of proteins in gels is histochemical by incubation in special mixtures of colouring by common methods with minor modifications.

The protein concentration was determined by the method of Bradford on optical density protein solution at a wavelength of 595 nm on the digital UV-VIS spectrophotometer PD-303UV (Apel, Japan).

The total content of alkaloids and other chemicals in the plant samples was determined by the standard technique.

Molecular-genetic methods of research: isolation of genomic DNA from underground and above-ground organs of plants was conducted by STAV - method with some modifications.

Measurement of concentrations of selected DNA was held on spectrophotometric device NanoDrop 1000 (Thermo Scientific, USA) at a wavelength of 260 nm. With the purpose of documentary identification of isolated DNA electrophoresis was carried out in 1% agarose gel with 100V within 15 minutes.

Total DNA was used for PCR with different RAPD primers to identify genetic markers of plants Patriniaintermedia (Horn.) Roem. etSchult. In our work we used the following RAPD primers: ORA 12, ORA 14, ORA 15, ORA 17, ORA 19 and ORC 6, ORC 8, ORC 6 and ÎÐN 8, ÎÐN 15 and ÎÐN 19. Mode RAPD amplification consisted of pre denaturation at 950C for 5 minutes and 30 cycles of denaturation at 950C 30 sec. annealing at 370C for 45 sec. and elongation at 720C 1 minutes Last elongation was held with the 720C within 7 minutes.

Amplification of the spatial sections of ribosomal DNA, namely ITSI and ITSII regions was conducted using specific primers: forward ITSI and reverse ITSIV (Synthol, Russia), kindly provided by the laboratory of

cell selection and biotechnology NCBSupervisory Committee Ministry of Education and Science of the Republic of Kazakhstan. Polymerase chain reaction (PCR) was carried out on the instrument DNA Engine Tetrad 2 Cycler (Bio-Rad, USA). The reaction mixture was: 10 x solution dNTPs (2 mmol/l of each nucleotide) 2,5µl, 10 x PCR buffer 2,5 µl, DNA target (0,02 micrograms/µl) 5 µl, Taq DNA polymerase 1U, 10 pmol/µl primers 0.5 µl. Total volume of the mixture is brought up to 25 µl water free of nucleases.Temperature conditions of amplification of the following: pre denaturation at 950C for 5 minutes, denaturation at 950C 30 seconds, annealing at 550C 1 min and elongation at 720C 1 minutes. Only mode of amplification consisted of 35 cycles. Last elongation was also when 720C within 7 minutes for visualization of PCR products held by electrophoresis in 2% agarose gel with 120V for 25-30 minutes. Drawings gels were photographed under UV light with the help of photo documentation of the system Gel-Doc 2000 (Bio-Rad, USA). As a DNA markers used solution DNA ladder (100 bp, Fermentas).

Elutedfrom the gel and cleaned the PCR products were sequenced using a set of BigDye terminator v3.1 sequencing kit (Applied Biosystems, USA) on the instrument 3730 DNA analyzer (Applied Biosystems, USA) according to the Protocol of the manufacturer. Analysis of the results of sequencing carried out using computer programs Vector NTI Advance 10 (Applied Biosystems, USA) and Sequence Scanner v1.0 (Invitrogen, USA), as well as database NCBI GenBank (USA). Dendrogram was constructed carried out using the method Neighbor Joining (Max Seq difference 0,75). All experiments were carried out in 2-4 repeated.

RESULTS AND DISCUSSION

Patriniaintermediais a perennial herb, root system which is a modified escape - rhizome of embryonic axillary buds which can grow from 1 to 9 floriferous stems (Fig. 1).

Stem plants Patriniaintermediaover the entire length covered with very fine hairs and bears 2-5 pairs of stem (Fig. 2 - 2) pinnately dissected leaves and a few basal leaves (Fig. 2-3). The inflorescence is a Patriniaintermediathyroid umbel with a cup and inconspicuous yellow corolla (Figure 2 - 1) and at the bottom of each umbel there are a couple bracts oblong-prolonged form. Fruit length of 4 - 4.5 mm.

According to one-source literature stem plants Patriniaintermediareaches a height of 25-50 cm and according to other sources - 25-70 cm, depending on the degree of development of its root system (Figure 2 - 4).

Unlike Talgar plant populations Kaskelen patriniain termedia plant population was less developed root system (Fig. 3).

Morphometric study patriniaintermedia showed that the height of the plants Talgar gorges were higher than Kaskelen population of these plants (Table 1).

Weight aboveground parts Talgar plant populations Patrina was 21-183 grams and the weight of the aerial part Kaskelen plant population was 9-22.2 grams. In addition, both populations of plants P. intermedia different in the number and length of internodes. The average number and length of internodes patriniaintermediaTalgar plant population was more than Kaskelen (Table 1).

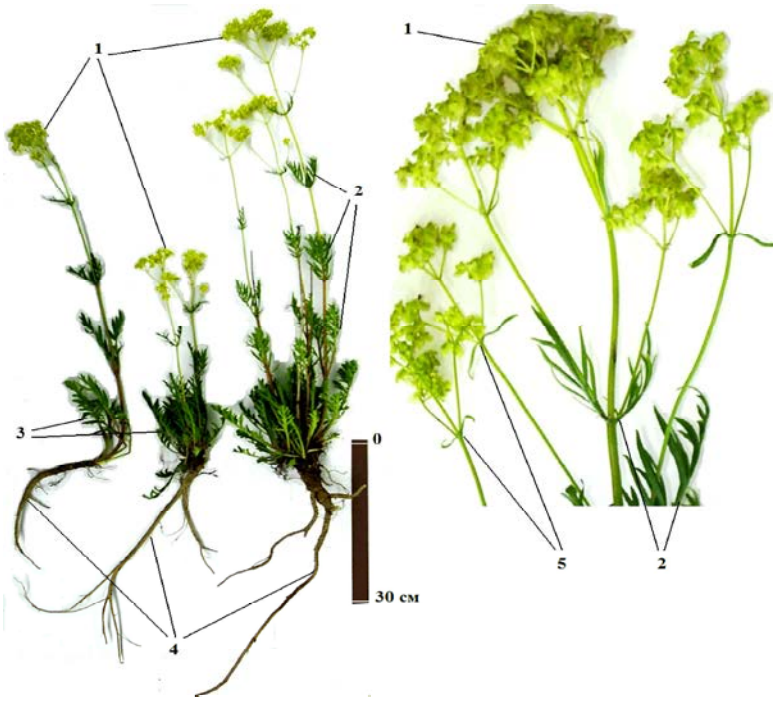
Talgarpatriniaintermedia plant population is located directly along the floodplain. Talgar near large boulders (Fig. 4 A), often washed with river water. Kaskelen plant population P. intermedia is located on the dry-stone river sediments (Fig. 4 B).

Thus, the detected morphological differences between populations Kaskelen and Talgar plants patriniaintermedia due to differences in the conditions of their habitat, in particular temperature conditions, moisture content and soil substrates.

Anatomical and morphological features of plants Patriniaintermedia (Horn.) Roem. etSchult. Results of the study the anatomical structure of the stem plants patriniaintermedia are shown in Figure 5. Characteristic of dicotyledonous herbaceous plants stem in cross section consists of a layer of the epidermis (E), cells of the primary cortex (PC) and the central cylinder. The outer walls of the epidermal stem cells of plants are covered with a layer of cutin and the layer of the epidermis consists of tightly adjoining cells. Below that is a layer of mechanical tissue - collenchyma. Initial stem cortex of plants represented patrinia 4-5 layers of parenchyma cells. Among the cells of the primary cortex of the stem plant P. intermedia highlighted one or two layers of cells hlorenhimnyh. Ends primary cortex of the stem cells of plants patrinia pronounced endoderm. The central cylinder of the stem is surrounded by plants patrinia 1-3 layers sclerenchyma cells, which are adjoined vascular bundle cells.

Unlike Talgar plant populations (Fig. 5A), in a population of plants Kaskelenpatriniaintermedia(Fig. 5 B) layers of sclerenchyma cells of the stem had a sinuous arrangement around the central cylinder and more concave to the inner parts in places where xylem rays. Vascular bundles of the stem plants merge with each other, forming a continuous type of structure of the central cylinder. Moreover, the stem xylem parenchyma distributed asstrand rays which cells lent from the exterior

Fig, 1: Flowering stems P. intermedia (Horn.) Roem. etSchult. Talgar plant populations



1 - inflorescence, 2 - stem leaves, 3 - basal leaves, 4 - rhizomes, 5 - bracts

Fig. 2: Generative species of P. intermedia (Horn.) Roem. etSchult. Talgar plant populations

Table 1: Morphological features of P. intermedia (Horn.) Roem. etSchult. from different populations of plants ZailiyskiyAlatau.

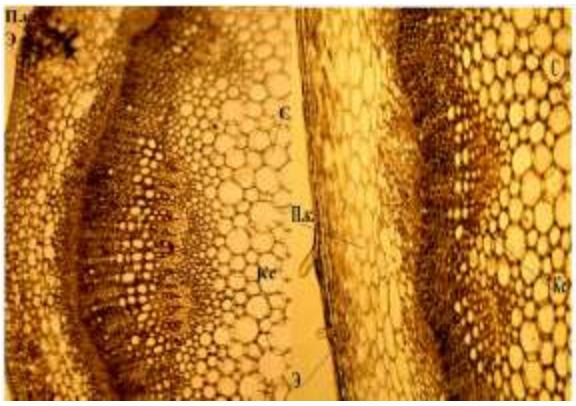
Place for the collection of plants	Plantheight, cm	Numberofinternodes,pieces	Internodelength, cm	Rhizomelength, cm
Talgarpopulation	58,9 ± 8,01	5,0 ± 0,73	10,7 ± 1,43	29,5 ± 5,37
Kaskelenpopulation	54,6 ± 11,80	4,0 ± 0,55	9,9 ± 1,37	-

Fig. 3: Generative species of P. intermedia (Horn.) Roem. etSchult. Kaskelen plant populations

(a) (b)

A - Talgar population, B - Kaskelen population

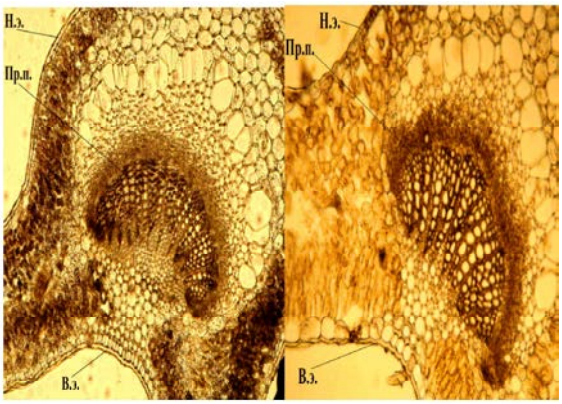
Fig. 4: Generative individuals patrinia growing on different soil substrates



(a) (b)

Fig. 5: The anatomical structure of plant stems P. intermedia from various gorge Zailiyskiy Alatau

to the interior of the cylinder. The core of the stem of plants represented patrinia large thin-walled parenchymal cells (Fig. 5).



A - Talgar population, B - Kaskelen population. E - epidermis; PC - Primary cortex; Xy - xylem, C - core

Fig. 6: The anatomical structure of the leaves of plants P. intermedia from various gorge Zailiyskiy Alatau

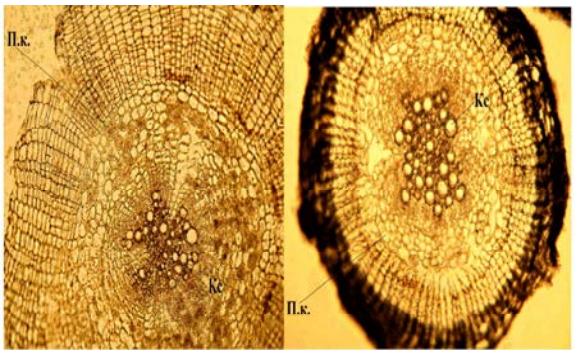
Lamina plants P. intermedia on cross-section is composed of cells of the epidermis, mesophyll and vascular bundles. Cells of the upper and lower epidermis sheet vary in the number, shape and size, their constituent cells. Cells of the upper epidermis of a leaf plate larger than the lower ones.Palisademesophyll consists of one-two layers of cells. Water-stocking cells sheet large, thin-walled (Fig. 6).

Conducting system of the lamina blade plant P. intermedia, consisting of phloem and xylem cells and is surrounded by tufts of 1-3 layers of sclerenchyma cells. Moreover, in comparison with the population Talgar plants (Fig. 6 A), xylem rays strand sheet Kaskelenpatriniaintermedia plant population (Fig. 6 B) were larger.

Root plants P. intermedia outside is covered with secondary covering cloth - periderm. Root hairs are absent. Under the cover of the root tissue patrinia located several layers of parenchyma cells of the primary cortex. A cross section of the plant root clearly visible annual rings of growth. In the center there are elements of primary root xylem and plant populations have Kaskelenpatrinia average (Fig. 7 B) large parenchymal cells of the xylem more than Talgar (Fig. 7 A). It is known that, since the virginal state, in the cells of the primary cortex plants accumulates many inclusions. If plants Valerianaofficinalis L. in young generative state has no inclusions, then the plant P. intermedia presence of a large number of inclusions is saved to the old generative state.

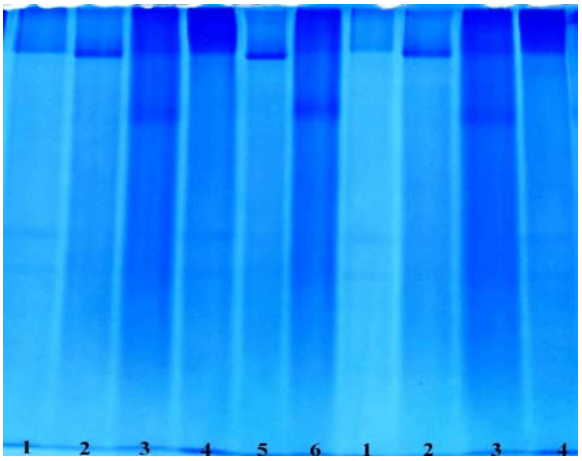
Table 2: Quantitative content of chemical substances in rhizomes Patriniaintermedia (Horn.) Roem. etShult.

No	Indicators	Theaveragevalue, %	Standarddeviation, ±%
1	The content of extractives (valeric acid)	33,5	1,05
2	Totalashcontent	9,8	0,30
3	The ash content, insoluble in 10% hydrochloric acid	1,1	0,05
4	Organicimpurity	0,3	0,01
5	Contentofmineralimpurities	0,1	0,05



A - Talgar gorge B-Kaskelen gorge. E - epidermis; Pc - Primary cortex; Xy - xylem, C - core

Fig. 7: The anatomical structure of the plant root P. intermedia from various gorgeZailiyskiyAlatau



1 - leaves (Kaskelen Gorge), 2 - stems (Kaskelen Gorge) 3 - inflorescence (Kaskelen Gorge) 4 - leaves (Talgar Gorge), 5 - stems (Talgar Gorge) 6 - inflorescence (Talgar Gorge)

Fig. 8: ElectrophoregramPAGE component composition of the soluble proteins of plants patrinia average (Patriniaintermedia (Horn.) Roem. Et Shult.)

The results of the anatomical and morphological study of the surface and underground parts of P. intermedia suggest differences in the location and size of the differentiated cells were found in both Talgar and Kaskelen plant populations Patrina.

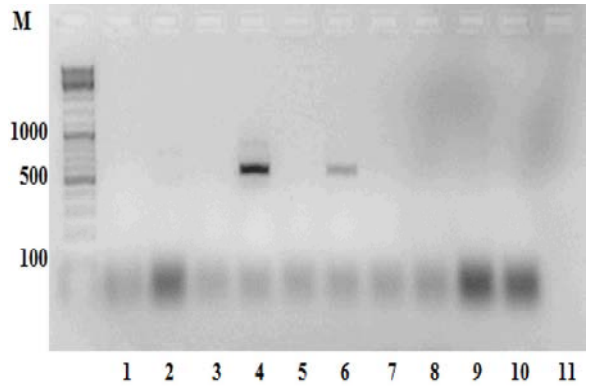
Differences were that the plant population in Talgarpatrinia layers of the stem cells had sclerenchymatous more rounded, whereas Kaskelen plant populations of stem cells sclerenchyma layers had a sinuous arrangement around the central cylinder and more concave to the inner parts in places where xylem rays. Further, leaf xylem rays Kaskelen plant populations were larger than Talgar plant populations Patrinia. PatriniaKaskelen plant population of large cells of the xylem parenchyma root more than Talgar. The resulting anatomical differences may be due to different environmental conditions studied populations of plants.

Biochemical and molecular genetic features Patriniaintermedia (Horn.) Roem. etShult. It is known that the underground part of the "Stone valerian" (patriniaintermedia) contain 12-13% saponins, tannins 1.4%, 0.17% essential oils and 0.13% nitrogen bases. Also calming effect of water infusions patrinia found that his crude extracts have angiogenic activity. Biochemical analysis of rhizomes patriniaintermedia (Table 2) was conducted in accordance with VFS RK 42-451-2001.

Plants P. intermedia amount of soluble proteins fractionated zones (Fig. 8), depending on the sample taken was 4-6. Area with electrophoretic mobility Rf = 0,02 is specific to the leaves of P. intermediaRoem. etShult. Talgar population. Low-mobility zones Rf = 0,036, 0,048 and 0,11 detected in stems P. intermedia (Horn.) Roem. etShult. Kaskelen and Talgar population. In both population samples inflorescences patrinia identified specific zones with Rf = 0,23, absent in other samples studied plants.

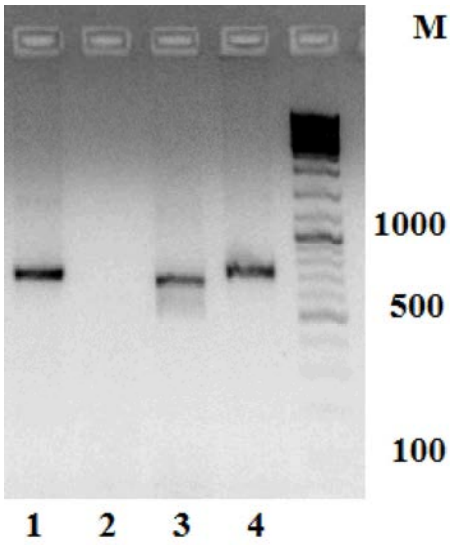
So, when electrophoretic definitions were identified differences in the nature of changes in the activity of the protein markers depending on the body of plants and from the study population Patriniaintermedia that allows to speak about the existence of some specific differences between samples of plants.

When electrophoretic division in 2% agarose gel (Fig. 9) products RAPD amplification were also differences, which concerned the length of the PCR products PatriniaintermediaRoem. etShult. and depend on used primers. So, as a result of PCR primer OPN 15 visualized amplicons ≤600 and >800 gel (Fig. 9, track 4). On 6 track (Fig. 9, track 6) when working with primer ORA



M - DNA molecular weight markers, bp, 1 - PCR products Patriniaintermedia (Horn.) Roemet. Shult. primer OPK 6 2 - too, but with a primer OPN 8, 3 -, too, but with a primer ORN 8 4 - too, but with a primer ORN 15, 5 -, too, but with primer OPA 12, 6 -, too, but with primer OPA 14, 7 -, too, but with primer OPA 15, 8 -, too, but with primer OPA 17, 9 -, too, but with a primer ORN 19, 10 -, too, but with primer OPA 19, 11 - negative control (H2O).

Fig. 9: Electrophoretic separation of DNA amplification products Patriniaintermedia (Horn.) Roem. etShult. in 2% agarose gel



1, 4 - PCR products, length of ≈ 750 bp was obtained from DNA isolated from the roots C. clematidea (Shrenk) Clark; 2 - negative control (H2O) 3 - PCR products, length of ≈ 680 bp was obtained from DNA isolated from leaves Patriniaintermedia; M - molecular weight markers, bp

Fig. 10: Electrophoretic separation of amplification products Patriniaintermedia (Horn.) Roem. etShult. 2% agarosegel

Table 3: Results of the comparison of the nucleotide sequence of ITS region rDNA Patrinia intermedia database NCBI GenBank

The object of research	Registration number of the sample in GenBank	Species name of the sample registered in GenBank	Maximumsimilarity	E value	Identity%
Patriniaintermedia	EU591961.1	*Patriniascabiosifolia*	952	0.0	97%
	EU591960.1	*Patriniavillosa*	935	0.0	97%
	AY236191.1	*Patriniatriloba*	839	0.0	94%
	AY792824.1	*Patriniagibbosa*	771	0.0	92%
	AJ426557.1	*Patriniaintermedia*	407	6e-116	100%
	AJ426558.1	*Patriniaintermedia*	302	3e-84	100%

14 were found PCR products in length >550 P.N. PCR products Patriniaintermedia were not detected when you add in reaction primers ORC 6 and 8, OPN 8 and 19, as well as ORA 12, 15, 17 and 19 (Fig. 9, tracks 1 and 2, 3 and 9, 5, 7, 8 and 10, respectively).

In addition, conducted polymerase chain reaction DNA samples plants PatriniaintermediaRoem. etShult. to amplify the ITS region of rDNA results of which are shown in Figure 10. For comparison, the sizes of the amplification products to agarose gel wells 1 and 4 have the PCR products obtained using the plant DNA C. clematidea (Shrenk) Clark and 3 well gel - PCR products obtained with the addition of the DNA recovered from Patriniaintermedia (Horn.) Roemet. Shult. On electrophoregram (Fig. 10) shows that the length of the amplification products of samples patriniaintermedia molecular weight less than that of samples codonopsis and is ˜ 680 base pairs (bp).

The resulting PCR amplicon after consecutive training procedures has been sequenced and the detecting device gave chromatographic pattern matching portion comprising ITSI - 5.8S rDNA - ITSII regions plants Patriniaintermedia. In the chromatogram (Fig. 11) shows a portion of the nucleotide sequence of ITS region rDNA plants Patriniaintermedia (Horn.) Roem. etShult. sequencingobtained by amplification with a primer and a commercial reverse ITSIV nucleotides labeled with different fluorescent dyes.

The results of processing the received sequence reveal the nucleotide sequence ITSI - 5.8S, rDNA - ITSII regions rDNA plants Patriniaintermedia (Horn.) Roem. etShult (Annex A):

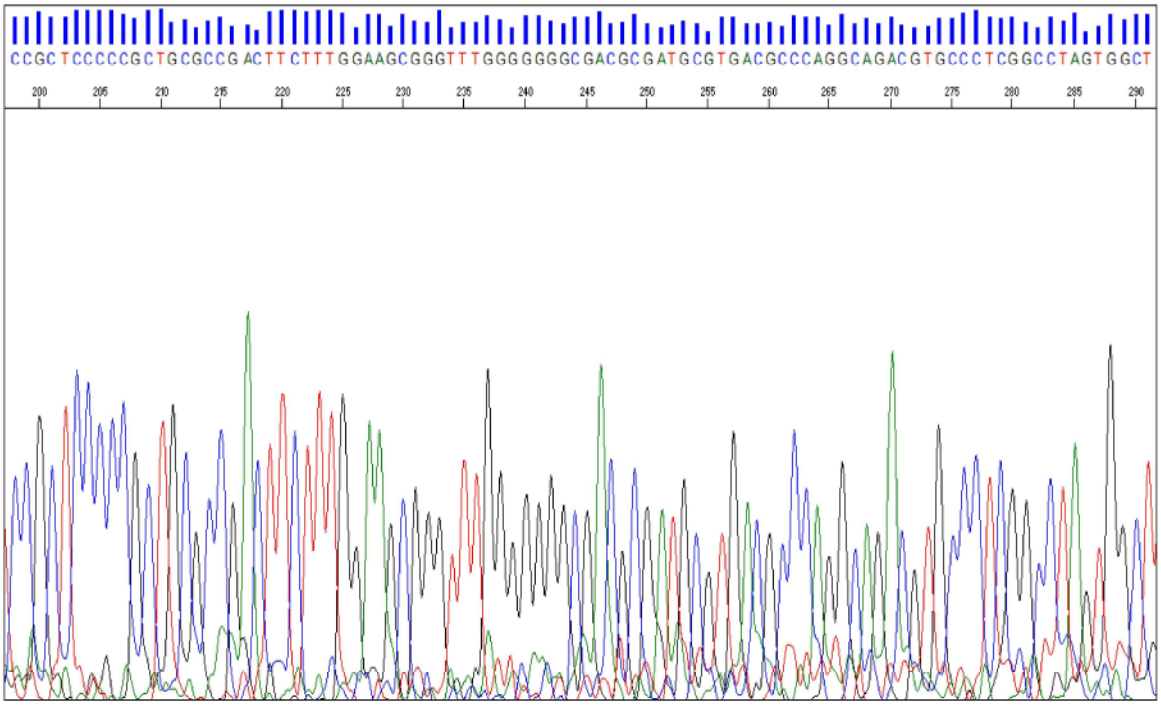


Fig. 11: The chromatogram of the nucleotide sequence of ITS region of rDNA plants Patriniaintermedia (Horn.) Roem. etShul

- 1aacgcacgacgcgataagagggctgtttcgaccaccactcgtcgt gacgtccgccgccag
- 61ggactcgattttaggccagccgagcccggagacccgggaggc cattgtccgctcccccgc
- 121tgcgccgacttctttggaagcgggtttgggggggcgacgcgat gcgtgacgcccaggcag
- 181acgtgccctcggcctagtggcttcgggcgcaacttgcgttcaaa gactcgatggttcacg
- 241ggattctgcaattcacaccaagtatcgcatttcgctacgttcttcat cgatgcgagagcc
- 301gagatatccgttgccgagagtcgtttgtgtttcttccggtgggtc gcgttcgcccaaccc
- 361gcgccgcgaacgggccggttgggttggcgctcgcctcctgatt tcttgttccttggcgcg
- 421gatcgcgccgggggttcggtttgcggtcgcggagccggaagg caccgctaccatgggatca
- 481ccggcctcgcggccgacgtccccgtgtacgaacgtgttcgcg ggtcgttctgctgtgcag
- 541 gtttcgacaatgatccttccgcaggttcaccctacggaag

The nucleotide sequence of the equalized (Annex F) through the database GenBank and comparative analysis (Table 3) showed that the obtained sequence of length of 580 base pairs ITSI - 5.8S, rDNA - ITSII regions rDNA plants Patrinia intermedia (Horn.) Roem. et Shult. identical to 97% sequencer following types: Patrinia scabiosifolia and Patrinia villosa, as well as the types of Patrinia triloba and Patrinia gibbosa with the identity of 94%and 92%, respectively. Part completely sequencing ITSI - 5.8S, rDNA - ITSII regions of plants Patrinia intermedia identical to 100% with sequencer ITSI sample AJ426557.1 and ITSII sample AJ426558.1 plants Patrinia intermedia.

The dendrogram was constructed using the nucleotide sequence obtained Patriniaintermedia (Horn.) Roem. etShult. (lcl 3173) and sequences of rDNA ITS regions samples registered in GenBank. Despite the 97 percent identity of our sample sequences and lcl 3173 ITSI - 5.8S rDNA - ITSII rDNA regions and types PatriniascabiosifoliaPatriniavillosa form a separate subcluster, as Patriniatriloba and Patriniagibbosa with 94% and 92% identity, which were on the separately standing branches.

CONCLUSIONS

The results of the anatomical and morphological study of the surface and underground bodies of P. intermedia suggest differences in the location and size of the differentiated cells were found in both Talgar and Kaskelen plant populations Patrina.

Differences were that the plant population in Talgarpatrinia middle layers of the stem cells had sclerenchymatous more rounded, whereas Kaskelen plant populations of stem cells sclerenchyma layers had a sinuous arrangement around the central cylinder and more concave to the inner parts in places where xylem rays. Further, leaf xylem rays Kaskelen plant populations were larger than Talgar plant populations Patrina.PatriniaintermediaKaskelen plant population of large cells of the xylem parenchyma root more than Talgar. The resulting anatomical differences may be due to different environmental conditions studied populations of plants.

When electrophoretic definitions were identified differences in the nature of changes in the activity of soluble proteins, depending on the body of the plant and the population studied plants Patriniaintermedia. Identified polymorphic zone of activity of the protein data allow to speak about the existence of specific differences between plants in different populations, as well as the possibility of their identification.

Moreover, when the RAPD amplification found two out of ten used primer, which gave three specific fragment of the Kazakhstan population Patriniaintermedia. The sequencing of the plants Patriniaintermedia (Horn.) Roem. etShult growing in the territory of South-Eastern Kazakhstan allowed to determine the nucleotide sequence ITSI - 5.8S, rDNA - ITSII regions rDNA with 100% of the species identity and this sequence is registered in the database GenBank, USA.

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