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Some Features of the Valuable Desert Plant of Kazakhstan Flora *Cistanche ambigua*

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Abstract

Morphological and biochemical features of the deserts plant Cistanche ambigua studied. It was shown features of life cycle and particular qualities of the anatomical structure of root, peduncle, stem, and nodule. The main physiologically active substances of Cistanche were isolated and identified. The differences between the populations in composition of soluble proteins, polypeptides, nonspecific esterase, acid phosphatase and peroxidase were revealed. It has been shown that Cistanche is not annual, but perennial plant. Seminal way is the main method of reproduction. However, vegetative way is also widespread.

Keywords: *Cistanche ambigua; Morphological and Biochemical Features; Deserts Plant*

Introduction

Flora of Kazakhstan is an inexhaustible source of biologically active substances. Wild vegetation of Kazakhstan is used very restrictedly due to the poor knowledge of plants' biochemical composition. This is especially true for such valuable plant as *Cistanche ambigua* (CA). This species is extremely popular among the founders of medicines. About 10 new compounds are already isolated and identified from *Cistanche*. One of the main suppliers of this plant in the world is Kazakhstan, although it is not used in the country. For obtaining primary knowledge about population polymorphism we have tried to study some of the morphological and biochemical features of Moinkum's and Mangyshlak's populations of CA.

Methods

Enzymes separation. Component composition of the enzymes was determined by isofocusing method in the plate of PAG. PH 3,5 - 9 ampholytes were used. Plate's size was 160 x 160, track width - 4 mm. 15 - 20 µl of extract was applied to each track depending on the activity. The amperage of 20 mA per plate, isofocusing duration - 3 hours. The enzyme was extracted from the needles using 0.05M acetate buffer with pH 5.2. Peroxidase components were revealed using 0.2 mM benzidine in 0.2 acetate buffer with pH 5.2. Peroxidase activity is maximal at this pH value.

Non-specific esterase stained in the gel using Jaaska method. After isofocusing the gels were incubated for 30 min at room temperature in 0.2M maleate buffer with pH 6.0 - 6.5 to reduce the pH of the gel, then stained in a mixture containing 2M fresh hexanitrated basic fuchsine and 0.5 mg/ml of α-naphthyl acetate in 0.1M maleate buffer with a final pH value 6.0 - 6.5.

Acid phosphatase was determined using a method of Jaaska. The gels were incubated for 30 minutes in 0.2M maleate buffer with pH 5.2 to reduce the pH of the gel. The gel was then transferred to a reaction mixture containing 0.25 mg/ml of 1-naphthylphosphate and 0.001M hexanitrated basic fuchsine in 0.1M maleate buffer with pH 5.0 [1-8].

Chemical composition

The qualitative composition of *Cistanche*'s extracts were determined by gas chromatography method using mass selective detector (MSD). For this, dried inflorescence and stems were milled and extracted with hot ethanol. 200 - 500 mg of weighed substance was taken, and 5 - 10 ml of ethanol was added to the substance and then mixed. Then the mixture was shaken on a shaker for 30 - 40 minutes, treated for 10 - 15 minutes in the ultrasonic bath and centrifuged.

Chromatography was performed using the following parameters:

Injector temperature = 280°C

Volume (application) of injection - 1 µl, Split = 6: 1

MSD interface temperature = 300°C

Flow of the carrier gas (helium) = 1.5 mL/min, Mode: Constant flow

Column: DB-5MS, 0.25 mm * 30m * 0,25 mkm of Agilent firm

Temperature mode of thermostat columns: 7°C/min

80°C ---> 320°C (5 minutes). Analysis time 39, 29 minutes.

Scan of substances was carried out automatically by comparing the spectrum of chromatogram integrated peak of the sample with the library spectrum.

Equipment used: Gas chromatograph: Agilent 6890N and mass selective detector: Agilent MSD 5973. Used software: Program Chem-Station © Agilent Technologies; Library of spectra: NIST Mass Spectral Database.

Results and Discussion

Biological and ecological characteristics of Kazakhstani *Cistanche* species

Genus *Cistanche* (*Cistanche* Hoffmgy et Link) of the family Orobanchaceae (Orobanchaceae Vent), order - Tubiflorae, class -Dicotyledonae, is presented in Kazakhstan by three types: 1. *C. flava*, 2. *C. salsa*, 3. *C. ambigua*, a synonym for *Cistanche deserticola* (Ma 1960). Biochemical composition of *Cistanche*, especially of Kazakhstani species, are poorly studied. They do not have chlorophyll, but often contain other pigments in a large amount. Thus, *C. salsa* contains red pigment and alkaloids - up to 0.332% [9-11].

Cistanche is a parasite, which fasten to the roots of *Haloxylon, Calligonum* or *Tamarisk* and absorbs its nutrients [12]. It has not its own root system. Its fruiting body and stem with flowers usually are used. In non-scientific literature the term of *Cistanche*'s root or herb is used, meaning the plants' stem or stolon. The plants usually grow in the desert preferring small hills 225 - 1150m, sharply continental climate and a lot of sand and sun.

Cistanche or in Chinese - Rou Cong Rong grows in Mongolia and China in Kansu, Tsinghai, Sinkiang, Xinjiang provinces. Plants are usually used in spring, mainly *Cistanche deserticola*. This species is listed in many Chinese tea collections mentioned in the literature. *C. salsa* could also be picked, but it is not as widespread and used in Chinese medicine. *C. salsa* could be used to produce the yellow pigment.

Cistanche ambigua: Glandulous and pilous parasites lacking chlorophyll and living on the roots of Haloxylon. It has a simple unbranched stem with seated bare or almost bare squama that ends with spiky inflorescences. Commonly it grows in clay solonetzic desert steppes. They can parasites on plants of *Anabasis* and *Salsola* generas, but prefer *Haloxylon*.

***Cistanche* salsa:** Perennial plant, 10 - 40 cm in height, more or less pilous, its stem is thick, with 5 - 20 mm thickness in the middle, inflorescence is short cylindrical, or cylindrical, sometimes strongly shortened, covering squamous are ovate or oblong lanceolate, on the back and edge are pilous, of 2 - 3.5 cm long, bracts are linear and oblong, blunt, with almost equal calyx, which are pilous on the edge and 9 - 14 mm in length. Corolla is blunt and campanulate, 25 - 35 mm long, with pale yellow tube and purple limb, sometimes it is entire light yellow. Stamens at the base are pilous, anther is 3 - 4 mm long, strongly pilous. Stigma is thick, slightly sinuate, a box opens with 2, rarely with 3 leaves. It grows in clay solonetzic desert steppes. Parasitizes on plants of *Anabasis* and *Salsola* generas, but prefers *Haloxylon*.

Anatomical structure: The study of the anatomical structure of parasite's fruiting body accreted into a single knot of *Cistanche* and *Haloxylon* root tissues was conducted. We consider that this is perennial plant. But in flora of Kazakhstan mentioned that it is annual plant. Seeds germinate in spring, reach the host roots, penetrate it and begin to absorb nutrients, gradually gaining biomass. It hibernates and in spring throws the peduncle, when there is a sufficient biomass. Stolon can persist in the soil until the following year in bad weather or other conditions. It has a short strong stem, seated alternate squama, absorbed in the soil, only peduncle is above the surface. Due to parasitic lifestyle roots are modified in so-called haustoria. It penetrates into the host roots with their help. Primary root of seedling performs this function in *Cistanche*. Tuberous formation called nodule is developed from a part of the seedling in the place of the implantation corresponding to hypocotyl. Nodule stores up nutrients, lays down fertile cormus and secondary haustoria forming organs, which provide vegetative reproduction of the parasite. Underground stem begins to form on the next year. Pedicels, stems, roots, nodules of *Cistanche salsa*, *ambigua* and *flava*, growing near river Bakanas on the roots of *Haloxylon*, were taken for the study of the anatomical structure of samples, fixed in 70% ethanol and then stored in ethanol-glycerol solution.

It has been found, that pedicle of *Cistanche* is oval on the cross section. Epidermal cells are covered with cuticle, elongated, pressed from the both sides, the side walls are lignified. Cortical parenchyma is loose, there are wide viewing intercellular spaces. Vascular bundles of the central cylinder are located under the endoderm. Since peduncle is erect, all its vascular bundles are identical and arrange ring. They are weak developed, only one or two vessels are visible on xylem part. Cambium absents (procambium). The core of the pedicel is hollow in the middle.

Stem: Reduction of laminas leads to a significant reduction of the evaporating surface and underdevelopment of fibrovascular bundles in *Cistanche*. The stem is converted into a body, which is adapted to store water. The outer part of the epidermis is covered with a thick cuticle. Mechanical tissue absents. Cortical parenchyma is permeated by vascular bundles of the pedicel. Stem tissue is homogeneous and mainly consists of thin-walled parenchyma permeated with underdeveloped vascular bundles. Each beam has a little vessels, they all are narrow- banded and have primitive structure, due to the lack of leaves, which in its development stimulate the formation of fibrovascular bundles of the stem. The core consists of thin-walled parenchyma cells.

Rhizome: Rhizome is a perennial shoot and generally is thicker than stem part. It is covered with periderm from the outside. The core in the rhizome is developed in connection with the main function - storage of reserve substances in the rhizome developed core. It consists of a round, thin-walled parenchyma cells, with small intercellular spaces between them. The core for the volume and capacity prevails over the other tissues of rhizomes. Cells with inclusions that do not exist in other tissues of *Cistanche*, are found in the latter.

Nodule: The predominance of parenchyma in nodules over the other cells even more clearly seen than in the rhizome. Just as in the stems of succulent plants the whole mass of the tuber, except the cover (periderm) performed by storage tissues, permeated with underdeveloped and primitive built bundles of the conduction system.

Significant differences in the anatomical structure of plants from different populations and two studied species were not found, probably due to the same parasitic way of life.

The vegetation period of the stem part is 10 - 20 days in April and May. After completion of flowering stem becomes slimy and plant dies quickly. A lot of worms could be found in the body of the dying stolon. Dry stems of *Cistanche* with seeds can be seen for a long time on the surface. It was unclear - if it is annual or perennial plant, as it is impossible to imagine that from such a small seed can form such a large fruiting body and peduncle in a short time.

To answer this question, we studied the aerial and underground parts of CA. For this purpose, in April, 30 plants of *Cistanche* were taken near the village of Moinkum in Jambul region from different populations. Plants were parasitized on the roots of Haloxylon. The vegetation is rather monotonous, but the soil, where the studied populations were grown, had varied chemical composition. At the same time, *Cistanche* salsa and *ambigua* were sampled from 18 populations in the Mangistau region. The differences between the Moinkum and Mangistau populations on the accumulation of biomass and morphometric indicators are found. Mangistau's *Cistanche* has low productivity and growth processes, unlike Moinkum's *Cistanche*. These plants' species in Mangistau parasitize on the roots of *Tamarix*. It was found that seed germination begins in June. The plants are then affixed to the host's roots and begin to gain biomass. By the end of the growing season *Cistanche* gains enough mass to form the peduncle for the next spring. Thus, *Cistanche* is two, rather than one year plant, as noted in the literature. Interestingly, that rotting offstolon of *Cistanche* forms new plant on the place of affixing to the host plant roots. Complete and flowering plant is forming the following year. The mechanism of formation of new plants is still being studied (Table 1 and 2). There are significant differences in structure of stolons between *Cistanche ambigua* from Mangyshlak and Moinkum regions. In Moinkum it is loose and has slightly sweet taste. Mangyshlak's *Cistanche* has fibrous structure of stolons. After drying, its size is reduced slightly. It has slightly bitter taste. In our opinion, this *Cistanche* is unsuitable for the manufacture of medicines and belongs to the species - *Cistanche* salsa (Figure 1-3).

Number of populations	General height	Height of the flower	Weight of the flowering part G	Weight of the middle part g	Weight of the stolon g	Diameter of the lower part, cm	Diameter of the middle part, cm	Diameter of the upper part, cm
1	70	25	292	448	614	6,3	5,2	2,7
2	82	23	216	265	870	5,3	3,2	
3	81	20	150	167	395	3,6	2,3	
4	60	20	233	240	765	4,3	3,7	
5	54	25		200	220	3,6	2,2	
6	70	28	170	255	250			
7	55	14		230	490			
8	50	35			520			
9	65	23		235	470			
10	96	45		140	340			
11	39	12		304	296			
12	75	18		407	249			
13	44	13		218	218			
14	43	11		164	143			
15	67	19		349	245			
16	62	11		306	228			
17	43	11		179	153			
18	45	7		245	138			

Table 1: *Some characteristics of the populations of Mangistau's Cistanche salsa.*

Population	Height of the plant, cm	Weight of the plant g	Length of the inflorescence, cm	Weight of the inflorescence, g	Weight without the inflorescence, g
Bakanas	82	663	35,8	186	476
Moinkum, 1	71,7	411,7	37,9	142,3	268,3
Moinkum, 2	103	1084	48,08	365	718
Mangyshlak	46,17	293	25,8	116,7	185

Table 2: *Productivity of different populations of Cistanche, 2005.*

Figure 1: *Moinkum's Cistanche, top.*

Figure 2: *Mangistau's Cistanche in nature (top), haustoria on the root of tamarisk (bottom).*

Figure 3: *Mangistau's samples of Cistanche under natural conditions.*

Some biochemical features of *Cistanche:* Component composition of peroxidase, nonspecific esterase, phosphatase, soluble proteins of *Cistanche*'s stolons.

Composition of isoenzymes and proteins previously has not been studied in this plant species. There are methodological difficulties of work with *Cistanche* - stolon consists almost of 95 - 98% of the water, but has small amount of high-molecular compounds. Another organ is the radicle (haustoria) of 1 - 3 cm long, almost lignified and is unsuitable for research. Leaves are absent. There are a lot of small flowers on the peduncle. From our previous experience we know that the flowers are heterogeneous in composition of enzymes and proteins. They are not suitable for chemo systematic research of populations. Based on this, we did not use flowers, and for comparative studies of populations we took the middle part of the stolon.

One of the most sensitive indicators to a variety of environmental factors is a peroxidase. Practically, change in the activity and the component composition of the enzyme is observed under the influence of any damaging effect of environmental factors. Component composition of peroxidase reliably characterizes the status of plant populations. In this connection, using isofocusing method we studied component composition of stolon's peroxidase in 9 different populations of *Cistanche*. Peroxidase isoforms were separated by isofocusing method. Isopoints of components have pH value ranging from 4 to 10. The number of components depending on the population ranges from 13 to 21. The most heterogeneous range has been found in Mangyshlak's sample. The number of components it reaches 21. The general scheme of components' position is the same for all samples. The differences are identified by component with alkaline and neutral pH. Mangyshlak and 30 populations have more components with neutral pH, and 26 (flowers) and 28th (stolons) - with alkaline pH. Usually, flowers have richer enzyme spectrum than stolons (Figure 4).

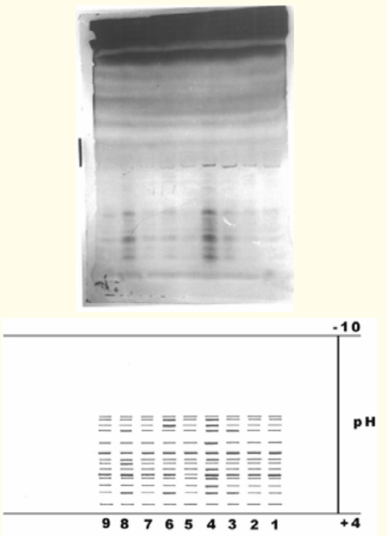

Figure 4: *Component composition of nonspecific esterase of Cistanche's stolons and flowers. Isofocusing was performed in PAG-plates within pH value 4 - 9. 1 - 2 - 4th population, 3 - 4 -26th population, 5 - 6 - 28th population, 7 - 8 - the 30th population of Moinkum's Cistanche (the even flowers), 9 - Mangistau's population (stolon).*

Component composition of nonspecific esterase was studied using isofocusing method. Isoelectric points of the enzyme were placed in a pH range 4 - 6. Number of components remained the same - 14.26th and 28th populations had a high activity of components. Isopoints of active components in 26th population are located in the pH range 4.8 - 5.2, and in 28 population - within 5.7 - 6.0. The most interesting is that the enzyme's spectrum in Mangistau's and Moinkum's populations, as well as spectrum of stolons and flowers were similar (Figure 5).

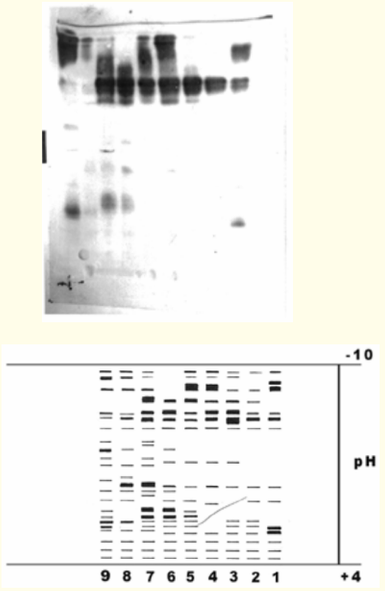

Figure 5: *Component composition of peroxidase of Cistanche's stolons and flowers. Isofocusing was performed PAG-plates in the pH range 4 - 9. 1 - 2 - 4th population, 3 - 4 -26th, 5 - 6 - 28th population, 7 - 8 - the 30th population of Moinkum's Cistanche (the even flowers), 9 - Mangistau's population (stolon).*

Component composition of acid phosphatase is also heterogeneous and varies between studied populations. The number of components in the spectrum depending on population and organ characteristics ranged from 7 to 11. Typically, the number of components in the spectrum of stolon variety is more than of the flower. The smallest number of components in the spectrum of Mangistau sample was 7. Stolon lacks alkaline and part of neutral components (Figure 6).

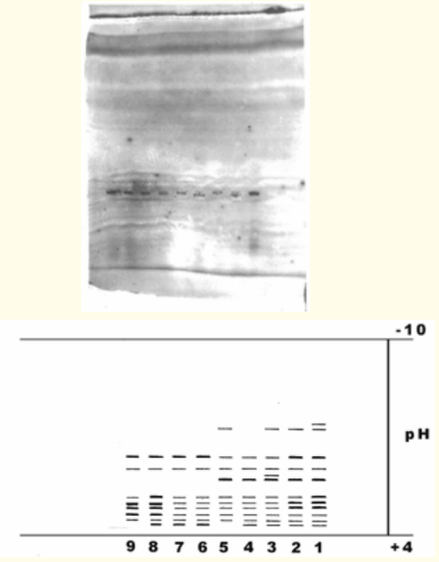

Figure 6: *Component composition of acid phosphatase of Cistanche's stolons and flowers. Isofocusing was performed PAG-plates in the pH range 4 - 9. q - 2 - 4th population, 3 - 4 -26th, 5 - 6 - 28th population, 7 - 8 - the 30th population of Moinkum's Cistanche (the even flowers), 9 - Mangistau's population (stolon).*

Number of activity zones of *Cistanche*'s soluble proteins (Figure 7) ranges from 2 to 8. The sample of 1stpopulation of Moinkum differs from the rest samples by the presence in peduncles of distinct alkaline activity zone (pH 9 - 9,5). Proteins 2 and 3 are common to all samples. More zones are detected in inflorescences (7 - 8), but less - in stolons (2 - 4) depending on the studied part of the plant.

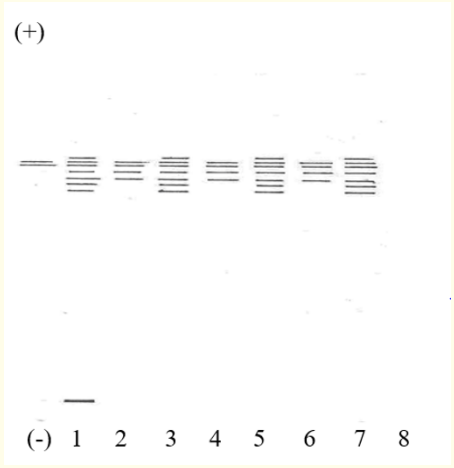

Figure 7: *The scheme of isofocusing (pH 3.5 - 9.5) in PAGE of soluble proteins of Cistanche's stolons and peduncles: 1- Cistanche's stolon, Moinkum, 1st population; 2- Cistanche's peduncles, Moinkum village, 1st population; 3- Cistanche's stolon, Moinkum, 2nd population; 4- Cistanche's peduncles, Moinkum village, 2nd population; 5- Cistanche's stolon, Moinkum village, 3rd population; 6- Cistanche's peduncles, Moinkum village, 3rd population; 7- Cistanche's stolon, Bakanas village; 8- Cistanche's peduncle, Bakanas village.*

Polypeptide composition: Electrophoresis of water-soluble proteins under denaturing conditions has shown differences in the polypeptide composition of *Cistanche*'s stolons. Number of components ranges from 18 to 24 in polypeptide spectrum of different *Cistanche*'s samples. Polypeptides spectrum, the most sharply distinguished from the others, is in the sample from populations 7 and 8 there are 26 - 27 components in spectrum. Their molecular weight ranges between 20 and 102 kDa. Molecular weight of new components is 89, 81 kDa.

Amylase (alpha and beta): We have also studied isozyme composition of amylase in different samples of *Cistanche*. We did not detect the presence of amylase activity in stolons. Component composition of alkaline phosphatase and glucose-6-phosphate dehydrogenase could not be determined due to methodical features.

Chemical composition: Three samples of different origins were taken for the work: 1 - from the Taklimakan Desert (China), 2 - from the Moinkum desert (near the village Bakanas), 3 - from the Moinkum desert (near the village Moinkum). Samples were taken during the flowering period, dried, pre-cut longitudinally into small slices. Qualitative assessment of *Cistanche*'s extracts was performed using gas chromatography method with mass selective detector (MSD). Investigations were carried out on Agilent equipment.

Column chromatography has shown that the sample from China №1 has 281 components, №2 - from the village Bakanas - 263 components, №3 - from the village Moinkum - 207 components. Identification by global library of chemical compounds has shown the presence of large amounts of carbohydrates and phenolic compounds. The most heterogeneous range of compounds has been detected in the Chinese sample. Close to the Chinese sample is the sample of Bakanas. Both of them are characterized by vegetation on relatively unsalted sandy soil. Moinkum's *Cistanche* growing near the village Moinkum was grown in areas with higher salt content. However, we should not assume that the number of components from the spectrum is determined by the soil composition. Population genetics may also play a role.

The following compounds were identified with close probability to 100%: 2 (5H) -Furanone, Glycerin, Cyclobutanol, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 2 (3H) -Furanone, dihydro-4-hydroxy-, 2 (3H) -Furanone, dihydro-4-hydroxy-, (S) -5-Hydroxymethyl-2 [5H] -furanone, 2-Methoxy-4-vinylphenol, 2- Propenoic acid, 3- (3,4-dimethoxyphenyl) -, methyl ester, n-Hexadecanoic acid (Palmitic acid), 9,12-Octadecadienoic acid (Z, Z) - (Linoleic acid), Oleic Acid, Octadecanoic acid (Stearic acid), ç-Sitosterol.

Comparison of *Cistanche*'s composition with those in the literature has shown that the composition of the components is similar. However, nobody earlier has detected such a wide range of components in *Cistanche*. For example, medicinal species of ephedra and licorice have fewer components in the spectrum. It's weird, as *Cistanche* is parasite with simplified metabolism. The accumulation of such a wide range of components is likely not associated with 100% parasitism of *Cistanche* on *Haloxylon* (*Tamarisk*), but connected to their synergism.

Conclusion

Thus, we have shown features of life cycle and particular qualities of the anatomical structure of root, peduncle, stem, and nodule. The main physiologically active substances of *Cistanche* were isolated and identified. The differences between the populations in composition of soluble proteins, polypeptides, nonspecific esterase, acid phosphatase and peroxidase are shown. Formula of *Cistanche*'s physiologically active substance have been selected. It has been shown that *Cistanche* is not annual, but perennial plant. Seminal way is the main method of reproduction. However, vegetative way is also widespread, by laying down the bulb in the place of affixing of parasite's haustoria to the root of *Haloxylon*.

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