

Anatomical and Morphological Stem Features of two *Haloxylon* Species (Chenopodiaceae Vent.) of Drought Stress, Kazakhstan

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The territory of Kazakhstan lies mainly in zones of deserts and semideserts. Soil degradation is all over the territory as a result of human intervention in vegetation. The representatives of Chenopodiaceae Vent. genus (*Haloxylon aphyllum* Minkw., *Haloxylon persicum* Bng.) are natural sandbinders in northern deserts of Kazakhstan. This article concerns adaptation traits of plants of drought stress. The article states the anatomical and morphological characteristics of representatives of Chenopodiaceae family: *Haloxylon aphyllum*, *Haloxylon persicum*. It was identified that there are similarities and differences in the anatomy of the stem of both species. The main difference is the number of layers of the epidermis: one layer in *Haloxylon aphyllum*, 2 layers in *H. persicum*, size of epidermis, chlorenchyma, parenchyma. Comparative characteristics of the two species of *Haloxylon* show some features of adaptation of psammophytes (*Haloxylon persicum*) and halophytes (*Haloxylon aphyllum*). Increased size of epidermal tissue and chlorenchyma of *Haloxylon persicum* stem, as well as increased size of parenchyma and crystalliferous cells of *Haloxylon aphyllum*. Features of the anatomical structure of the vegetative organs of plants adequately reflect their adaptation to growing conditions. Studied species are plants-C₄, by type of CO₂ fixation, as a mechanism of adaptation to hot and dry climate. *Haloxylon persicum* is regarded as a genetic derivative to *H. aphyllum*.

Key words: *Haloxylon aphyllum* Minkw., *Haloxylon persicum* Bng., ecological adaptation, psammophyte, halophyte, epidermis.

Big part of Central Asia lies in arid and semiarid zones. In Kazakhstan 70% of territory is prone to desertification (Eisfelder *et al.*, 2012, 2013; Lioubimtseva and Adams, 2004; Lioubimtseva *et al.*, 2005, 2009).

Soil degradation and desertification are main ecologic issues of this country. Therefore, Kazakhstan is an important object of study (ADB, 2010). Environmental degradation was defined as one of major threats by High Level Panel on

Threats, Challenges and Change, United Nations Organization (Eisfelder, 2014).

As a result of high rates of desertification it is necessary to restore disturbed lands and provide ecological recovery of natural vegetation. The main purpose of restoration is to use viable populations. It will help to restore functions and processes of ecosystems, prevent erosion and protect biological diversity (Bradshaw, 1987).

It is widely accepted that efficiency of ecological restoration depends on the selection of local plants, especially in stress conditions (Knapp and Rice, 1994; Linhart, 1995; Lesica and Allendorf, 1999).

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Chenopodiaceae family includes about 100 genera and more than 1500 species which are broadly spread on arid and subtropical lands (Heywood, 1978). *Haloxylon* (Chenopodiaceae Vent.) genus comprises 10 species where on the territory of CIS countries and in semideserts of Central Asia there are only 3 species (*Haloxylon aphyllum* Minkw., *Haloxylon persicum* Bng., *Haloxylon ammodendron* C.A. Mey. Bunge. *Haloxylon aphyllum* (local name -Black saxaul) and *Haloxylon persicum* (local name -White saxaul) have a vast growing area in deserts of Iran-Turanian region and Arab peninsula. *Haloxylon ammodendron* can be only found in the east of Kazakhstan and in Junggar province. This species mainly grow in the area of the Gobi Desert in Central Asia (Lavrenko, 1962).

Saxaul forests directly affect environmental situation creating favorable conditions for human life and farming. Owing to natural domination saxaul can be considered as ecologically key species. Saxaul forests serve the following functions of ecosystem: biomass production, sand stabilization; minimization of soil drifting; microclimate mitigation which ensures growth and development of associated plants (for example, *Carex physodes* M. Bieb.); setting habitat conditions for animals. And, desert forests are a food supply for farming and energy-efficient biofuel for local population (Buras *et al.*, 2012).

Currently, as a result of human impact it was identified that saxaul is spread only on 25% of potential areal in Kazakhstan. About three thirds of potential areal of saxaul forests were destroyed or they deteriorated comparing to potential areal (Thevs *et al.*, 2013).

Having studied for many years growth of *Haloxylon*, its development, morphology, and seed germination it became possible to identify a number of special features of its biology. The sources contain much information about morpho-biological study of *Haloxylon aphyllum* (Ol'shevskii, 1935).

Chenopodiaceae family consists of a big number of C4 species where dominant plants of deserted territories of Kazakhstan hold a specific place. Many researchers studied anatomy structure of this genus (Kadereit *et al.*, 2003; Pyankov *et al.*, 1999, 2000; Sage *et al.*, 1999; Sage 2001; Su Pei-Xi, 2005; Ramazannejad, 2006; Zhibin, 2011; Heklau, 2012).

A big number of Chenopodiaceae stem anatomy descriptions was published by Gregory (1994, 2009). Plenty anatomy researches belong to Russian scientists (Arcichovskij & Osipov, 1934; Butnik, 1983; Vasilevskaja, 1955, 1965; Novruzova & Chapari, 1974; Timonin 1987a & b).

In order to provide theoretical basis for recommendations on restoration of natural ecosystems in deserted areas and provision of stability of natural ecosystems it is necessary to study the anatomy structure of green stems of two species of Chenopodiaceae genus (*Haloxylon aphyllum*, *H. persicum*), analysis of similarities and differences of morphometric indices and adaptabilities of the species under study.

MATERIALS AND METHODS

The research was carried out on the territory of Ili-Balkhash region, situated within the borders of Almaty city region, Balkhash-Alakol hydro-ecological region (Fig.1). The material of the research was collected in the framework of experimental fieldworks carried out on the territory of Almaty city region in Ili-Balkhash region, valley of the Ili River during summer time.

Four plots were examined on the territory of the Ili River estuary. A representative of Chenopodiaceae family (*Haloxylon persicum*) was collected on plots I and II. *Haloxylon aphyllum* was gathered as a research material on plots III and IV (Table 1).

According to soil and geomorphological, climatic, and geobotanical conditions the territory under study belongs to coastal salt plain region. Relief of the territory is typically hummocky and contains ridges, melting into plain and hills. Among hills and ridges there are quite high ridges (10-20 m) and quite low sand deposits up to 10 m high. Spots of dry-type playas are found where hills and ridges are considered as occasional sand hills (0.5 – 2.5 m high). Dry beds and drainless lowlands are typical to Bakanass plain. According to soil-geographical regionalization the territory belongs to the zone of grey-brown soils which in their turn belong to modern desert crust soils (Gvozdeva, 1960).

The climate is extremely continental. The moderate temperature of the coldest month of the year (January) in ten years varies between minus

11 and minus 13 degrees Celsius. In the hottest month of the year (July) the moderate temperature is plus 26 degrees Celsius. The duration of frost-free season is 153 days. The annual amount of atmospheric sediments is not more than 200 mm. The moderate degree of saturation is 61%. Winds blow predominantly from the east. Average wind speed is 2.5 m/s (Bedarev, 1985).

The dominant species of vegetation is *Haloxylon aphyllum*. The following wood and shrub species are also there: *Halimodendron halodendron*, *Populus deversifolia*, *Calligonum aphyllum*, *Eurotia ceratoides*, *Astragalus ammodendron*, etc. *Artemisia terrae albae*, *Stipa Hohenackeriana*, *Agropurum sibiricum* and some other of them are typical species of grass vegetation. The grass canopy consists of *Artemisia solgorica*, *Echinopa albicalis*, etc. Considerable part of the grass canopy is represented by ephemeral plants (Bedareva, 2009).

Experimental trees were selected according to age and other evaluation indices. Thus, there was measured height of experimental tree, its crown diameter, root collar diameter, as well as stand density and ground water level (GWL) (Table 2) were identified. In order to do a morpho-

anatomical structure study samples were taken from at least three trees of each species.

Plants were botanized and fixed for structure analysis. Fixation was done in 70% spirit by Strasburger-Flemming technique (spirit, glycerin, water 1:1:1). Anatomic specimens were prepared by means of microtome with TOC-2 freezing device. Slices were mounted into glycerin in accordance to conventional procedures (Prozina, 1960; Permyakov, 1988; Barykina et al., 2004). Anatomic slices were 10-15 micron thick. In order to do quantitative analysis morphometric indices were taken by means of software for micrography. Photomicrographs were taken with MC-300 microscope (Zoom $\times 180$). For statistical data processing mathematical statistics method and Microsoft Excel were applied.

RESULTS AND DISCUSSION

Table 3 shows similarities and differences of saxaul species. It is based on published data and research works of different scientists (Ol'shevskii, 1935; Gvozdeva, 1960; Lavrenko, 1962; Bedareva, 2009).

Analysis of morphological characteristics

Table 1. Information about the plots

Plot	Name of species	Region under study	Coordinates	Landscape type	Analysis of anthropogenic effect
I	<i>Haloxylon persicum</i>	Kazakhstan, Almaty city region, Ili-Balkhash region, valley of the Ili River	45°21 N74°28 E	Hilled ridges	Moderate
II			45°24 N74°25 E	Alluvial sands	Sand barchans of human origin
III	<i>Haloxylon aphyllum</i>		45°02 N74°43 E	Salt plain	Low
IV			45°05 N74°37 E	Inter-ridge	Light forest of saxaul, cut-over land

Table 2. Growing condition, evaluation indices of experimental trees

S No.	Evaluation indices of an experimental tree				GWL [m]	Stand density [tree/ha]	Associated species of plants
	Age [years]	Height [m]	Root collar diameter [cm]	Crown diameter [m]			
I	8±2.3	0.7±0.3	5-6±3.1	1.2±0.3	7.5	1000	<i>Carex physodes</i> M. Bieb.,
II	18±2.7	1.5±0.2	8±4.8	1.4±0.2	8	1500	<i>Eremurus nderiensis</i> Regel.
III	20±4.3	3.0±0.2	20±3.9	2±0.5	4.6	950	<i>Krascheninnikovia ceratoides</i> L.
IV	5-6±2.3	0.5±0.3	4±2.4	0.8±0.2	3.7	780	Gueldenst., <i>Artemisia terrae-albae</i> Krasch.

Table 3. Comparative morphological characteristics of *Haloxylon* genus types

S. No.	Morphological characteristic	<i>Haloxylon aphyllum</i> (Minkw.) Iljin	<i>Haloxylon persicum</i> Bunge ex Boiss. and Buhse
1	Taxonomy	phylum class order family genus specie Magnoliophyta Magnoliopsida Caryophyllales Chenopodiaceae <i>Haloxylon</i> <i>aphyllum</i> (Minkw.) Iljin	phylum class order family genus specie Magnoliophyta Magnoliopsida Caryophyllales Chenopodiaceae <i>Haloxylon</i> <i>persicum</i> Bunge ex Boiss. & Buhse
2	Life-form	Small tree	Large shrub
3	Size	From 2-5m up to 8-10 m tall	4—5 (6) m tall
4	Root collar diameter	50-60 cm	20—30 cm
5	Root system	It is up to 4.1 m long in alkaline soils, 7.3 m long in partly fixed hummocky sands, and up to 10 m deep in soils with deep underground water deposits	Root system is lateral or with a tap root, and up to 10-11 m deep
6	Trunk	Bark is dark-grey. Wood is heavy, of high density, short grain, and of darker color in the center	Bark is whitish or light-grey. Wood is hard, short grain, unmanageable
7	Stems	Sappy, dark-green, bent, faintly acidic to taste	Fresh sprays are dryish, pale-green, bitter to taste
8	Leaves	Leaves are opposite and reduced to primordia. Base of leaves has short curly whitish hair	Leaves are very small, with delta-shaped scales, awl-shaped, with white glumal brim
9	Flowers	Flowers are small-seized, bisexual, solitary, based in axils of small scale-like leaves which are shorter than a flower itself. Bracts have glumaceous edges. Flowers have 5 stamens. Ovary has 4-5 fugacious stigmas	Flowers are bisexual, plain, solitary or multiple, having two small bracts. Perianth is glumaceous, with 5 rounded loculars. There are 5 stamens. Style has 2-5 short stigmas
10	Fruit	Type – Dry, Seed Strophiole - Wings. Fruit is impressed on top. Wings have rounded or cuneate roots. Seeds are flat, with spiral embryo	Type – Dry, Seed Strophiole - Wings. Fruit is an obloid, winged, slightly fleshy nut. It contains planispiral embryo greening in testa
11	Habitat	Modern and ancient valleys and estuaries of rivers; degradations of sand ridges; it forms heavy bed form heavy bed	Sand deserts: predominantly in sands, depression beds, banks and tops of sand hummocks. It does not
12	Ecological group	Xerophyte, light demander, halophyte, psammophyte salt-tolerant than <i>Haloxylon aphyllum</i>	Typical psammophyte, strong light demander, drought-tolerant, less
13	Uses	Feeding, technical	Rock amendment, sand stabilizing

shows the following common factors: *Haloxylon* genus types are quite similar in structure of stems, leaves, fruits. Species differ in life-forms and ecological conditions of habitat. Comparative analysis of *Haloxylon persicum* and *Haloxylon aphyllum* shows specific adaptation traits of psammophytes and halophytes. All these traits determine adaptation of plants to conditions of halomorphic and alkaline soils.

As a psammophyte and halophyte, *Haloxylon aphyllum* grows in ancient valleys of rivers, in degradations of sand ridges, takyr-like soils, and stabilized sands. Whereas *Haloxylon persicum* grows in more severe conditions: in sand deserts, sandy soils, unfixed sands, and tops of sand hummocks.

Transverse sections of green stems were used for identification of similarities and differences between *Haloxylon aphyllum* and *Haloxylon persicum* on anatomic level (Fig. 2).

According to the analysis of received data, transverse section of a stem consists of epidermis, palisade chlorenchyma, parenchyma cells, and vascular cylinder.

Cuticle covers stem from outside protecting it from mechanical defects and extreme temperatures. Epidermis consists of one (*H. aphyllum*) or two layers (*H. persicum*) of cells. Epidermis serves specific functions and is characterized not only by micromorphologic form of stem but also by protection function for palisade chlorenchyma cells.

However, different number of epidermic layers probably depends on severe growth conditions of *Haloxylon persicum* (two layers of epidermal cells). Therefore, water loss decreases and stems become tougher. Hypodermis layer comes after epidermis. It serves mechanical function. Hypodermis layer of *Haloxylon aphyllum* is distinct and is developed in entire layer (Fig. 3, 4). Owing to several layers of epidermis *Haloxylon persicum* has hypodermis not as an entire layer but as single inclusions (Fig. 5, 6).

Palisade chlorenchyma layer follows epidermis cells. It is well known that essential and mostly common feature that is acquired in the process evolutionary adaptation to dry environment conditions is reduction of lamina. However, number of layers of mesophyll increases in plants. Palisade tissue becomes especially well

Table 4. The size of tissues of assimilating shoots of *Haloxylon* genus

Species	Sample [plot]	Epidermis thickness [micron]	Number of epider-mis layers	Chlorenchy-ma thickness [micron]	Crystallife-rous cells in chlorenchy-ma [micron]	Sheath thickness [micron]	Parenchyma thickness [micron]
<i>Haloxylon persicum</i>	I	12.79±2.066	2	25.405±3.48	10.31±1.155	7.061±1.498	79.563±5.499
	II	13.627±3.05	2	28.038±5.02	12.735±1.74	8.97±1.218	80.527±6.043
<i>Haloxylon aphyllum</i>	III	7.675±1.697	1	23.628±2.596	15.309±1.288	8.61±1.288	99.698±1.354
	IV	10.53±1.403	1	17.609±3.571	10.454±0.976	8.088±1.073	143.985±8.51

developed what allows plants to resist first of all soil drought (Vasilevskaya, 1955). Increasing number of layers and also density of palisade tissue in some ways balances small size of photosynthetic organs. But on the other side, having small area for photosynthesis plants have to get macronutrients. This happens due to development of palisade parenchyma from the both side of stem. As palisade tissue is the most highly-productive type of tissue and it makes main contribution to photosynthesis (Vasilevskaya, 1965).

Further on, there is a layer of cells of vascular bundle sheath (kranz anatomy). Saxaul leaves are reduced very much, and annual fresh stems serve main C_4 photosynthetic function (Pyankov *et al.*, 1999). These stems have the same origin as true induviae (apical meristem of shoot apex) (Fahn and Arzee, 1959). Carbon dioxide fixation takes place in chloroplasts of mesophyll as a result of CO_2 addition to phosphoenolpyruvate producing the four-carbon acids – oxaloacetate and malate. This is a ‘carbon fixation pathway’. plants grow in southern regions and are adapted to hot and dry climate (Pyankov *et al.*, 1999).

Conducting bundles fuse among themselves creating complete central cylinder. Pith consists of big passage parenchyma cells. Layer of parenchyma cells, as well as chlorenchyma contains crystalliferous cells (druses). Salt crystals are considered metabolic-waste products of plant bodies, especially in xerophytes. Probably, crystals favor increase of osmotic pressure of enchylema (Fig. 5, 6).

Vascular cylinder includes xylem and phloem. In *Haloxylon persicum* types xylem vessels are symmetrized having cambium sheath. While xylem vessels of *Haloxylon aphyllum* are not symmetrized and cambium sheath appears as entire layer, both species of *Haloxylon* genus have sclerenchyma in vascular cylinder.

Specific features of anatomy structure of vegetative organs of plants including stem organization quite clearly show the way they are adaptive to growth conditions.

Though *Haloxylon persicum* and *Haloxylon aphyllum* grow in similar habitats and are members of one ecological group of psammophytes, they have similarities as well as obvious differences in anatomy structure.



Fig. 1. Location of the study area

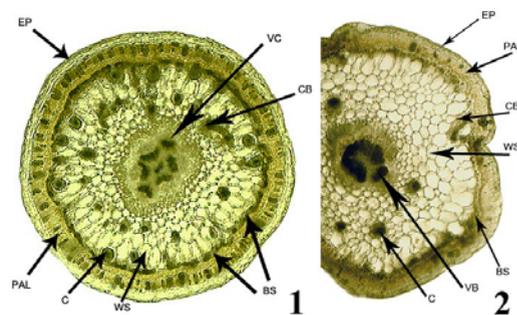


Fig. 2. Comparative anatomy structure of assimilating shoots of *Haloxylon persicum* (1), *Haloxylon aphyllum* (2). EP- epidermis, PAL – palisade chlorenchyma, C – crystalliferous cells, BS – cells of vascular bundle sheath, WS – water storage tissue, VC – vascular cylinder, CB – cortical bundle, VB – vascular bundle

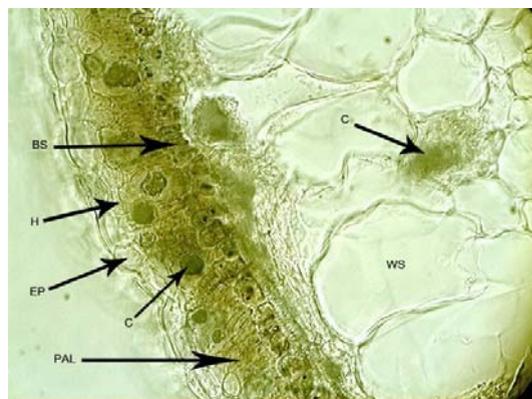


Fig. 3. Anatomy structure of assimilating shoots of *H. aphyllum*. EP- epidermis, H- hypodermis, PAL- palisade chlorenchyma, C – crystalliferous cells, BS- cells of vascular bundle sheath, WS – water storage tissue, VC – vascular cylinder, CB – cortical bundle, VB – vascular bundle, S – sclerenchyma

The following features are common ones for the majority of psammophytes:

1. stem form (rounded);
2. crystalliferous cells in parenchyma;
3. entire chlorenchyma, crystals in chlorenchyma;
4. elongated parenchyma cells around vascular cylinder;
5. cortex cells;
6. vascular cylinder, 4 different layers, every vascular bundle is surrounded with thick layer of sclerenchyma cells;
7. well developed palisade parenchyma;
8. well developed sclerenchyma.

The main distinction of saxaul species is a number of epidermis layers, size of epidermis, chlorenchyma, parenchyma (Table 4). Epidermic layer thickness increases due to epidermal differentiation of *Haloxylon persicum*. *Haloxylon persicum* has epidermis layer 12.79 – 13.62 micron thick comparing to *H. aphyllum* with 7.67-10.53 micron. Increase of number of epidermis layers defines ecologic valence of species to severe temperature conditions. Chlorenchyma size of *Haloxylon persicum* (25.405 – 28.038 micron thick) is bigger than one of *H. aphyllum* (17.609 – 23.628 micron thick). Chlorenchyma is a tissue functioning in photosynthesis in species of *Haloxylon* genus which according to the structure and nature of chemical activity during fixation corresponds to type of photosynthesis. The general concept of - pathway is that it is possessed by evolutionarily younger taxons which have high temperature optimum of photosynthesis, high rates of light saturation, and water use efficiency (Shamsutdinov, Ubaidullayev and Shamsutdinova, 2014). According to V.I. Pyankov (Shamsutdinov, Pyankov, *et al.*, 2014) number of plants (in a row: barchans sands – stabilized sands – takyr-like soils – saline soils) has increased from 16.5% to 70%. The same row shows deterioration of soil conditions: increase of water deficiency, degradation of soil agrophysical properties, salt content rise.

Parenchyma thickness varies in species. Thus, *Haloxylon aphyllum* has higher thickness of parenchyma layer (99.69 – 143.98 micron) than *Haloxylon persicum* does (79.56 – 80.52 micron). Probably, the reason is that *H. aphyllum* grow in more favorable conditions where underground

waters run close to the surface. As a result of more available moisture water-storing tissue of stems develops better. Also, high number of crystalliferous cells in anatomy structure of stems of *Haloxylon aphyllum* is explained by more salt-affected habitat than one of *Haloxylon persicum*.

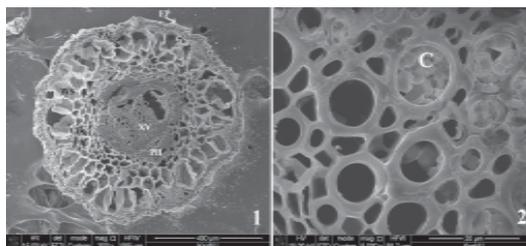


Fig. 4. Scanning electronic microscope microphotograph of anatomy of *H. aphyllum* assimilating shoots (1), arrangement of crystalliferous cells in cortical bundle (2). EP- epidermis, PAL – palisade chlorenchyma, S – water storage tissue, CB – cortical bundle, PH – phloem, XY- xylem, C – crystalliferous cells

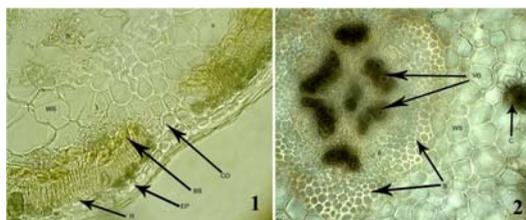


Fig. 5. Anatomy structure of assimilating shoots of *H. persicum* (1), Vascular cylinder of stem (2). EP- epidermis, H- hypodermis, PAL – palisade chlorenchyma, C – crystalliferous cells, BS – cells of vascular bundle sheath, WS – water storage tissue, CB – cortical bundle, Ñ – collenchyma, VB – vascular bundle, S – sclerenchyma

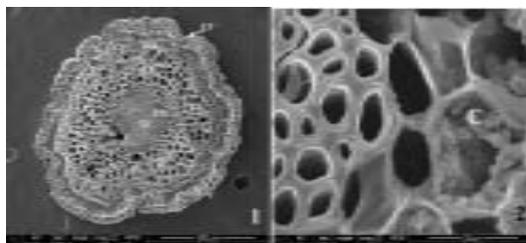


Fig. 6. Scanning electronic microscope microphotograph of anatomy of *H. persicum* assimilating shoots (1), arrangement of crystalliferous cells in water storage tissue (2). EP- epidermis, PAL – palisade chlorenchyma, S – water storage tissue, CB – cortical bundle, PH – phloem, XY- xylem, C – crystalliferous cells

It stands salty level of underground waters (up to 40 gr/l) and in terms of mineral accumulation rate it belongs to group of plants for alkaline soil (sodium) (Rodin, 1963).

According to I.P.Petrov (Petrov, 1936) ecologic differences of white and *Haloxylon aphyllum* are based on edaphic factors. Therefore, specific nature of their distribution on the territory is not a consequence of world zonation but a result of edaphic differences of habitat.

L.Ya.Kurochkina (1978) agrees with Petrov's point of view and considers formations of white and *Haloxylon aphyllum* forests as 'ecologic-phytocenotic variants' (psammophytic, halomesoxerophytic and petrophytic) which replace each other in different ecologic conditions. For this reason she assigns them under one vegetation type.

The outstanding association of *Haloxylon persicum* forests with sand nonsaline substratum allows to consider *Haloxylon persicum* as a genetic derivative of *H. aphyllum*, its ecologically specific blind branch (Bykov, 1968) which features rest in physiological and anatomy characters. *Haloxylon persicum* has three layers of epidermis, deeply buried stomatal mechanisms, and high osmotic pressure. These factors ensure drought-resistance of this species comparing to *H. aphyllum*, and widespread occurrence of *Haloxylon persicum* in sand massifs (Rachkovskaya *et al.*, 2003).

Consistent identification of functional and structural features of plants allows to project climatic habitat and potential environmental opportunities. This approach can be applied both for projection of changes of vegetation under changing climate and development of high-efficiency agrophytocenoses in different natural and climatic zones (Shamsutdinov, Pyankov *et al.*, 2014).

The best possible spatial combination of plants taking into consideration their phonological rhythms and mechanisms of adaptation to cycles of drought and soil salinity provides guarantee to higher plants to survive and to accumulate biomass on degraded grazing lands. Coexistence of drought-resistant species in accordance to a wide range of salt content in combination with limited water quantity is an essential factor in development of landscape design and methods of rehabilitation of

degraded grazing lands suffered from salt in soils (Shuyskaya, 2012).

CONCLUSIONS

Studied species are plants-, by type of fixation, as a mechanism of adaptation to hot and dry climate. Different number of epidermic layers probably depends on severe growth conditions of *Haloxylon persicum* (two layers of epidermal cells). Therefore, water loss decreases and stems become tougher. Hypodermis layer comes after epidermis. Parenchyma thickness varies in species. *Haloxylon aphyllum* has higher thickness of parenchyma layer than *Haloxylon persicum* does. The reason is that *H. aphyllum* grow in more favorable conditions where underground waters run close to the surface. Layer of parenchyma cells, as well as chlorenchyma contains crystalliferous cells (druses). This feature is considered as a mechanism for adaptation to saline soils. Thus, the results of the research can become a framework and evidence of possibility to create a culture to be more resistant in sense of vegetative reclamation and to have more broad ecologic variability.

Thus, the results of the research can become a framework and evidence of possibility to create a culture to be more resistant in sense of vegetative reclamation and to have more broad ecologic variability.

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