Вестник Томского государственного университета. Биология. 2019. № 45. С. 34–46

БОТАНИКА

UDC 615.322:582.998.1:581.4/.9
doi: 10.17223/19988591/45/2

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Population structure, resource potential and diagnostic features of raw materials of *Saussurea controversa* DC.*

The life form and the ontogenesis of the perspective medicinal plant Saussurea controversa DC. (Asteraceae) are described. The species is considered as polycentric, perennial, summer-green, herbaceous polycarpic half-rosette plant with rhizome. The aerial part of the plant includes the polycyclic rosette vegetative and elongated generative shoots. In the ontogenesis, four periods and nine ontogenetic states are distinguished. The ontogenetic and vital structures of four populations were studied in the vicinity of Efremkino village (Republic of Khakassia). Populations are incomplete with accumulation of virginal individuals and predominance of individuals of intermediate and high classes of vitality. Analysis of the distribution and resource research in the population with the highest density and area makes it possible to classify the species as the 3rd category of resource species due the necessity to observe the regime of sustainable use. The diagnostic features of the S. controversa's raw material are the structure of the middle vein of the leaf which includes a large number of vascular-fibrous beams and the abaxial epidermis containing stomata of the anomocytous type and 4 types of trichomes.

The paper contains 3 Figures and 27 References.

Key words: *Saussurea; medicinal plant; life form; resource; epidermis.*

Acknowledgments: *This study was supported by the Tomsk State University competitiveness improvement programme.*

Introduction

Genus *Saussurea* DC. of Asteraceae family includes 350-410 species according to various sources [1, 2]. Many of them are widely used in Siberian, Chinese, Indian, Mongolian and Tibetan traditional medicine [3], and are characterized by a high content of terpenoids and flavonoids [4-7]. For example, *S. controversa* DC. has been used in folk medicine for a long time: the decoction was used against glaucoma, pulmonary diseases, as a hemostatic, antirheumatic and for gastrointestinal diseases; water infusion of roots was drunk against headache and as a hemostatic agent for uterine bleeding. Fresh leaves have a wound-healing effect, especially with purulent wounds; ethereal root extract exhibits antibacterial activity. Vitamin C, caffeic acid, flavonoids, rubber, triterpenic saponins are found

* The paper was translated by the authors.

in the aerial parts of *S. controversa* plants [8]. The raw materials of this species are the leaves collected during the flowering period.

Currently, researchers of Siberian State Medical University are investigating the ability of medicine from *S. controversa* to positively influence bone repair processes in osteomyelitis. Leaves of *S. controversa* contain a large number of different biologically active substances (BAS): flavonol glycosides-quercetin-7-O-α-L-rhamnoside-3-O-β-D-glucoside, quercetin-3-O-β-D-diglucoside-O-α-L-rhamnoside, rutin and others; oxycinnamic and oxybenzoic acids - chlorogenic, cinnamic, syringic; phenylpropanoids - syringin, syringorezinol; macronutrients: Ca, K, Mg, P [4, 9]. The polysaccharide complex is represented by water-soluble polysaccharides, pectin substances and hemicelluloses [4]. These BAS are molecular carriers of antioxidant, membrane protective, antimicrobial, anti-inflammatory, and immunomodulating activity [10]. Thus, the raw material of this type is a promising candidate for the development of plant-based products that are effective in the pathologies of bone tissue, including infectious genesis [11].

Saussurea controversa is a boreal mountainous Ural-Siberian species with a disjunctive geographical range. The species is common in the Urals; it is marked on the eastern limit of the Ural fragment of the range near Chelyabinsk and near Kamensk-Uralsky. The Siberian part of the range covers the south-east of Western Siberia (Tomsk Region, Novosibirsk Region, Kemerovo Region, Altai Republic), the south of Eastern Siberia and the north of Mongolia [12]. The species grows mainly in the forest area on the meadow-steppe, gravelly, stony slopes of different exposures, in the forests, on the meadows, sometimes rises to the subalpine zone. *S. controversa* confines to specific habitats while forms abundant populations [13]. This suggests that the species can be attributed to the category of resource species, and its harvesting is permissible. *S. controversa* is planned to be included in the pharmacopeia [11]. Therefore, it is important to assess the state of natural populations as well as to screen the approximate reserves of this species to provide the pharmacological industry with high-quality raw materials and to conserve the natural habitats.

Collection of medicinal plants often cause depletion of their populations and lead to species extinction and biodiversity reduction [14, 15]. To avoid local extinction of medicinal plants, one should cultivate target species or conduct harvesting with rules of sustainable use [16, 17]. It is possible after investigation of plant life history and population ecology [18-20]. The aim of this work is to estimate the state of natural populations, to assess the resource potential of *S. controversa*, and to identify the diagnostic features of this type of raw material for further use in medicine.

Materials and methods

The description of the life form and the study of the ontogenesis of *S. controversa* were carried out in the field. We dug, examined, and laid in the morphological herbarium all the individuals from counting plots for further

study in the laboratory [21, 22]. In total, about 350 specimens of individuals in all ontogenetic conditions were collected in the studied habitats. We additionally studied specimens stored in the P.N. Krylov Herbarium (TK).

The material for the study of the ontogenetic and vital structure was collected in the Republic of Khakassia (Shirinsky district, vicinity of Efremkino village (N 54.4669527°, E 89.445091°) in four populations (P) of *S. controversa* under different environmental conditions in 2017 (Table 1). We consider that a coenotic population is the one located within the limits of a sustainable biocenosis. The ontogenetic structure of populations was considered as the percentage ratio of groups of individuals at different ontogenetic states for which ontogenetic spectra were compiled. For the compilation of these spectra, the assessment of vitality and the calculation of the density of populations in plant communities, transects were laid in a regular way and divided into counting plots of 1/16 to 1 m² depending on the density of populations. We analyzed the vitality structure according to the method of YA Zlobin [23, 24]. We selected characters that most fully revealed the vitality of the individual. Then, limits of the intermediate class were determined, so the individuals were divided into 3 classes of vitality (a - high, b - intermediate and c - low).

On the basis of the division into classes, histograms of the vital spectra were constructed. The following indicators were taken from each individual: the number of leaves, the length and width of the leaf blade, the length of the stem, the caudex dimensions (length and width). To assess the vitality of *S. controversa*, we used features of virginal (sterile fertile) individuals: length, width of the leaf blade, and the number of leaves. The intermediate class of vitality was distinguished by establishing the upper and lower limits of each of the features used to assess vitality.

Table 1

Habitat characteristics of *Saussurea controversa*

P	Altitude, m / exposure	Phytocenosis features
1	580 / SE	Left bank of the Belyi Iyus river. Edge of the birch-aspen forest (*Betula pendula* Roth + *Populus tremula* L.). Motley grass meadow. TPC = 100%. Slope angle ≈ 3°. *Rubus saxatilis* L., *Vicia cracca* L. and other species prevail in the herbal cover
2	624 / NE	Right bank of the Belyi Iyus river. Edge of the larch forest (*Larix sibirica* Ledeb.). TPC ≈ 70%. Slope angle ≈ 2°. Both *Fragaria vesca* L. and *Carex duriuscula* C.A. Mey. prevail in the grassy cover
3	527 / NE	Left bank of the Belyi Iyus river. Edge of the birch and larch forest (*B. pendula*, *L. sibirica*). TPC ≈ 80%. *Padus avium* Mill and *Salix* sp. are in the undergrowth. Slope angle ≈ 2°. *Carex pediformis* C.A. Mey. prevails in the grassy cover
4	749 / NW	Right bank of the Belyi Iyus river. Edge of the birch and larch forest. TPC ≈ 60%. Slope angle ≈ 7°. *S. controversa* and *Bupleurum multinerve* L. prevail in the grassy cover

Note. TPC - Total projective cover.

The key plot for the calculation of resource indicators was laid in P4. 100 virginal specimens were collected and weighed in fresh and dried state, and then the coefficient of drying of the raw material was calculated. With the help of the Garmin ETREX 10 GPS navigator, we measured the area of the key plot. By extrapolating the mean yield (dry mass of virginal individuals per 1 m^2) to the obtained area of the population, we calculated the biological reserves of raw materials in this population.

The study of diagnostic features of *S. controversa*'s raw materials was carried out on dried material collected in 2016 and 2017 in various geographic locations: 1) Irkutsk region, vicinity of Nizhniy Cochergat village (N 52.1353794°, E 105.2810168°); 2) Republic of Khakassia, vicinity of Mendol village (N 54.290370°, E 89.660009°); 3) Krasnoyarsk Region, vicinity of Ingol Lake (N 55.545632°, E 88.860249°). In the laboratory of structural and molecular analysis of plants (TSU), 90 temporary preparations of the adaxial and abaxial epidermis of the leaf were prepared, as well as cross sections of 70 μm in thickness through the leaf blade and leaf stalk. We clarified preparations with glycerol and examined using Zeiss Lab A1 with a 5-megapixel color photo/video camera Axio Cam ERc 5s. Description of the epidermis was carried out according to the classification by NA Aneli [25]. The stomatal index was calculated by the formula:

$$I_s = N_s \times 100\% / (N_e + N_s),$$

where N_s is the number of stomata on the area unit, N_e is the number of main cells of the lower epidermis per unit area.

Results and discussion

We consider *S. controversa* as the polycentric perennial summer-green herbaceous semi-rosette polycarpic plant with polycyclic rosette vegetative and elongated generative shoots. The underground shoot system is represented by a short rhizome forming epigeogenically after the pulling of the basic part of the rosette shoot into the soil by the taproot or adventitious roots. Young adult individuals sometimes form elongated rhizomes from the buds on maternal rhizomes. These rhizomes are situated under the soil surface at the depth of 5-10 cm. When their apical buds reach the soil surface, the above ground parts of individuals are formed. Such individuals correspond with the immature and virginal individuals of the seed origin. As a result, the clone including the vegetative progenies forms after their separation from maternal individuals. Four periods (latent, pre-fertile, fertile, and post-fertile) and nine ontogenetic states – seedlings (s), juvenile (j), immature (im), virginal (v), young fertile (f1), middle-aged fertile or mature (f2), old fertile (f3), subsenile (ss), and senile (s) were distinguished in the ontogenesis (Fig. 1). The marks of ontogenetic states are the following: the presence of cotyledons, the length and width of the primary or secondary taproot, the presence, number and size of adventitious roots on the rhizome; the degree of necrosis of the secondary taproot and rhizome, the number

and location of rosette vegetative and semi-rosette (elongated) generative shoots, the number, size and shape of the leaves, the number of anthodia in a complex inflorescence.

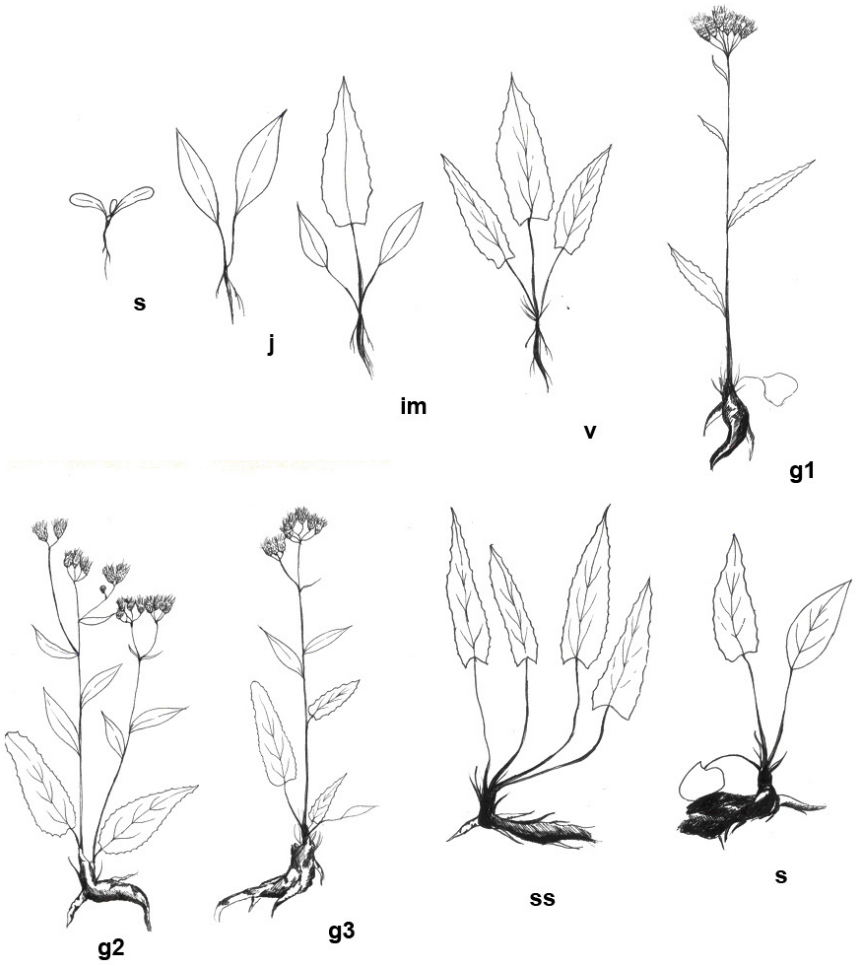

Fig. 1. Ontogenesis of *Saussurea controversa* with seed origin
(s - seedling; j - juvenile; im - immature; v - virginal; g1 - young fertile;
g2 - middle-aged fertile; g3 - old fertile; ss - sub-senile; s - senile)

Seedlings (*s*) are characterized by the presence of two obovate cotyledons, about 5 mm long and 4 mm wide.

Juvenile individuals (*j*) have 2-3 true lanceolate leaves of juvenile type, 2-3 cm long and about 1.5 cm wide. It is characterized by loss of connection with the seed.

Immature individuals (*im*) are distinguished by the presence of traits and properties, transitional from juvenile plants to adults. An individual at this state has 2-3 leaves of lancet-shaped form (as in an individual at the juvenile state), and is also characterized by the presence of the adult leaves (narrower, obtuse, not heart-shaped at the base; 5 cm long and 2 cm wide).

Virginal individuals (*v*) are characterized by the appearance of basic features that are typical of adults (adult leaves, shoots, and root system). The processes of senescence are almost not expressed.

Young fertile individuals (*f1*) are distinguished by the appearance of the first generative organs. The number of anthodia in the inflorescence is about 7; the process of growth prevails over senescence (necrosis of the secondary taproot, adventitious roots and rhizome is not expressed). The number of leaves is about 6, they are 5.5 cm long and 2.5 cm wide.

Middle-aged fertile (mature) individuals (*f2*) are characterized by balancing the processes of growth and senescence. Rhizome starts branching by forming shoots from lateral buds. The number of shoots is 2-3 in average. There are about 8 leaf plates, their average length is about 7 cm and width is 2.5 cm. The number of anthodia is about 8-9.

Old fertile individuals (*f3*) are characterized by the predominance of senescence processes over the new growth. Generative function, root and shoot formation processes decrease sharply. The degree of necrosis of the rhizome is expressed moderately. The evidence of this state of individual is a great number of "stumps" being the remnants of last year's generative shoots.

Sub-senile individuals (*ss*) are distinguished by predominance of the processes of senescence over the growth, the absence of generative shoots, and the simplification of the aerial parts to the immature state. The degree of necrosis of the rhizome is significant. When an individual consists of several shoots, its fragmentation sometimes occurs by splitting into separate particles.

Senile individuals (*s*) are characterized by the extreme simplification of the life form and the manifestation of juvenile features of the organization. New growth is completely absent.

Reproduction in *S. controversa* populations is supported both by the seed and vegetative methods. The fruit is single-seeded achene.

According to the average population density of *S. controversa*, relatively favorable conditions can be judged for the growth of this species (Table 2). Lower density of individuals is observed in P1 and P3. Ecological conditions of habitats and competition of related species do not contribute to successful seed reproduction of individuals.

Populations of *S. controversa* are incomplete. Spectra are bimodal (P1-P3) or with three "peaks" (P4). Virginal individuals predominate in all populations. Secondary "peaks" fall on subsenile (P1, P2), juvenile (P3) and the old fertile ontogenetic states (P4) (Fig. 2). In P3, there is also an accumulation of old fertile individuals (a third-degree "peak"). This indicates that seed reproduction

in populations occurs irregularly, and the species is characterized by "waves of renewal". The predominance of virginal individuals in populations with high abundance, apparently, is a consequence of vegetative propagation.

Table 2

Density of *Saussurea controversa* populations

P	*n*	*M*±*SE*	*ST*	S^2
1	6	2.8±0.4	1.0	1.0
2	28	123.4±0.9	4.9	24.5
3	40	1.3±0.4	2.6	6.6
4	29	113.6±0.8	4.2	17.7

Note. *n* - Sample, *M* – Mean, *SE* - Standard error, SD - Standard Deviation, S^2 - Dispersion.

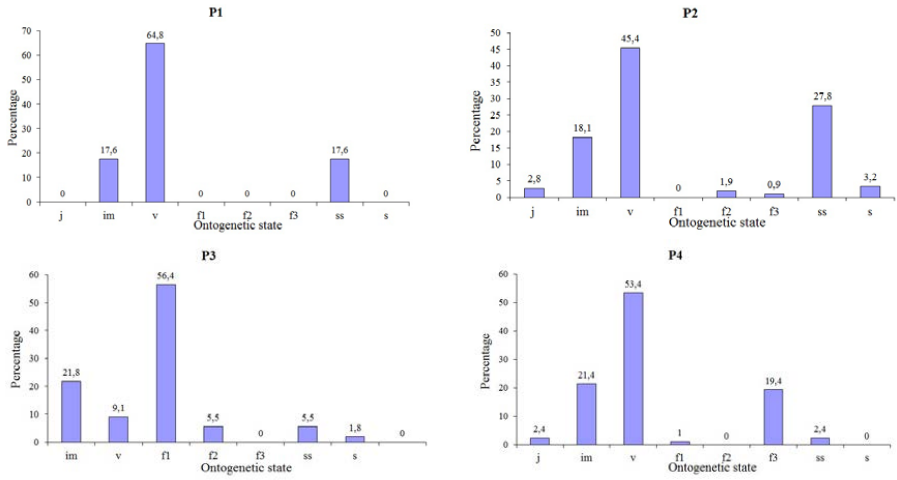

Fig. 2. Ontogenetic spectra of populations of *Saussurea controversa* (P - Population, s - seedling; j - juvenile; im - immature; v - virginal; g1 - young fertile; g2 - middle-aged fertile; g3 - old fertile; ss - subsenile; s - senile)

Senile individuals are represented only in P2. This indicates that the ecological conditions of the habitat are quite favorable, since the senile individual is characterized by the ultimate simplification of the life form, and survival in adverse conditions is difficult for it. Blooming fertile individuals were not detected; there are interruptions in flowering, which also contributes to the irregularity of seed production. Single blooming middle-aged fertile individuals were noted only in P2.

There is predominance of individuals of the intermediate class of vitality in P1 and P3 (Table 3 and Fig. 3). Despite the low density of these populations, indicating the high level of competition at the state of seedling fixation, the more adult individuals reach average size and do not experience oppression from one another.

Table 3

Borders of vitality classes for features of virginal individuals of *Saussurea controversa*

Character	Vitality class		
	low	intermediate	high
P1			
Length of the greatest leaf blade, cm	≥7.0	10.11-7.10	≤10.2
Width of the greatest leaf blade, cm	≥3.54	4.92-3.55	≤4.93
Number of leaves	≥2.28	2.27-3.97	≤3.98
P2			
Length of the greatest leaf blade, cm	≥9.81	10.53-9.82	≤10.54
Width of the greatest leaf blade, cm	≥3.12	3.38-3.13	≤3.39
Number of leaves	≥2.01	2.33-2.02	≤2.34
P3			
Length of the greatest leaf blade, cm	≥8.66	10.93-8.67	≤10.94
Width of the greatest leaf blade, cm	≥3.18	3.96-3.19	≤3.97
Number of leaves	≥1.88	2.43-1.89	≤2.44
P4			
Length of the greatest leaf blade, cm	≥9.21	10.11-9.22	≤10.12
Width of the greatest leaf blade, cm	≥2.78	3.03-2.79	≤3.04
Number of leaves	≥2.11	2.41-2.12	≤2.42

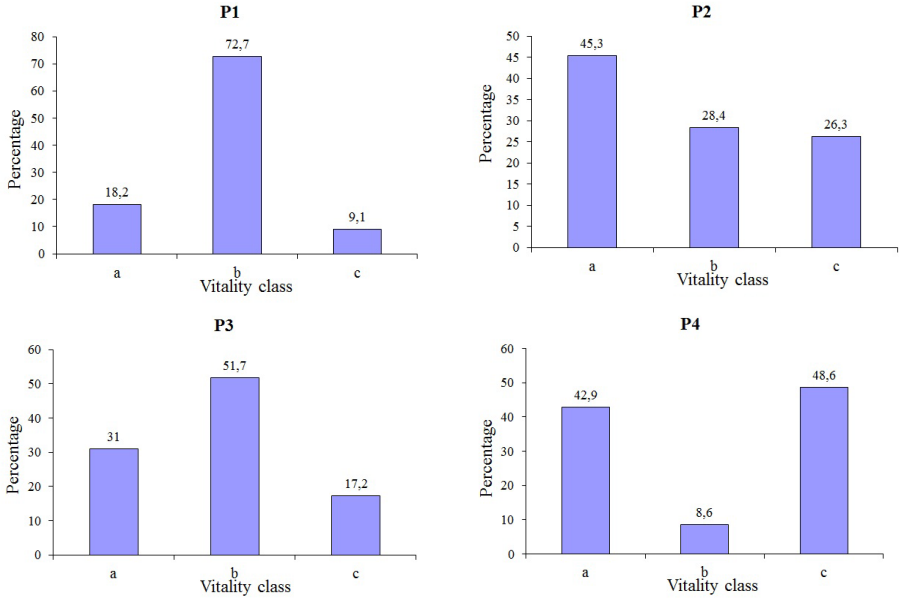

Fig. 3. Vitality structure of populations of *Saussurea controversa*
(P - Population, vitality classes: a - High, b - Intermediate, c - Low)

P2 is characterized by the predominance of individuals of high vitality class. Consequently, the maximum ecological density of this population has not yet been reached. Habitat conditions contribute to successful competition with other species in this phytocenosis and propagation of seedlings.

In P4 (key plot), individuals of both high and low vitality classes prevail. This suggests that in this population the maximum ecological density has been reached for the species, habitat resources are used most fully and efficiently, and the size is reduced in some individuals. This is a case of intraspecific competition.

The area of the key plot is 2666 m^2. Interest in the quality of medicinal raw materials are the most abundant adult (here virginal) individuals. With a population density of 113.6 pcs/m^2 and a share of virginal individuals of 45%, the biological reserve of raw materials in the key plot was 56 kg. It is obvious that a one-time collection of such a quantity of raw materials from this population is unacceptable since this will lead to irreversible changes in its composition and size. We assume that the frequency of harvesting should be no less than 3-4 years, and we recommend harvesting 25% of adult (virginal) individuals of the population. Thus, one time in 3-4 years from the key area one can get 14 kg of dry raw material. At the moment, it can be argued that *S. controversa* belongs to the 3rd category of resource species. Strict observance of the regime of sustainable use is necessary for the harvesting of such species [26]. Raw material of *S. controversa* can also be obtained on plantations since the results of a long-term introduction experiment with this species conducted in the Siberian Botanical Garden of Tomsk State University indicate the possibility of its successful cultivation in the south of Tomsk Region [27].

The anatomical structure of *S. controversa* leaves indicates xerophytization. The leaves on the abaxial side are densely covered with trichomes which form white-felt fluff. The outer walls of the cells of the adaxial epidermis are thickened and covered with a thick layer of wrinkled cuticle. Hypostomatic dorsoventral leaves have a single-rowed well distinguishable palisade and 5-6-rowed spongy mesophyll (Fig. 4, *A*).

In the spongy mesophyll, the intercellular spaces are placed. There are 4 types of trichomes on the abaxial epidermis:

1) Long 2-cell scrawny trichomes consisting of one small cell at the base and the second main long cell. These trichomes make up the pubescence on the abaxial epidermis (Fig. 4, *A, F*);

2) 2- and 3-celled capitate trichomes (Fig. 4, *A*);

3) 4-celled trichomes with three rounded cells at the base with inclusions and one apical long plague-shaped cell (Fig. 4, *C*);

4) multicellular fragile trichomes with pedestal or barrel-shaped base consisting of 4-7 cells (Fig. 4, *E*).

The collateral conductive bundles are reinforced from the upper and lower sides by sclerenchyma strands. Up to 14 conductive bundles passes in the leaf stalk. The main vein of the leaf on the underside is reinforced with collenchyma (Fig. 4, *B*).

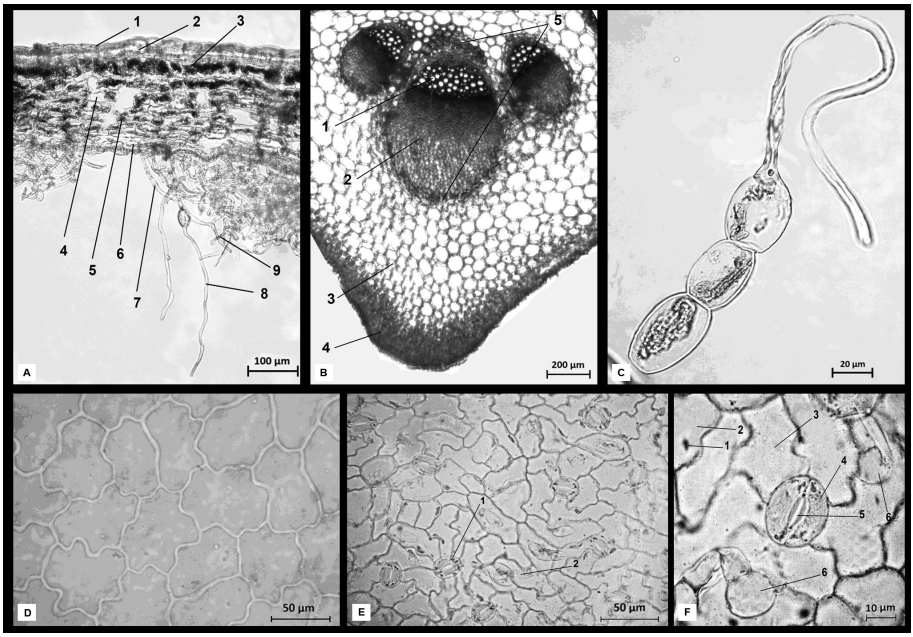

Fig. 4. Anatomical structure of the leave of *Saussurea controversa*
(A. Cross-section through the leaf blade *Saussurea controversa* (× 10): 1 - Cuticle, 2 - Adaxial epidermis, 3 - Palisade mesophyll, 4 - Intercellular spaces, 5 - Spongy mesophyll, 6 - Abaxial epidermis, 7 - Covering scrawny trichome, 8 - 4-celled trichomes with inclusions, 9 - Capitate trichome; B. Cross-section through the leaf stalk (a -× 5): 1 - Xylem, 2 - Phloem, 3 - Parenchyma, 4 - Collenchyma, 5 - Sclerenchyma of vascular fibrous bundles; C. 4-celled trichome with inclusions (× 40); D. Adaxial epidermis epidermis (× 40); E. Abaxial epidermis (× 40): 1 - Stomatal apparatus, 2 - Pedestal base of trichome;
F. Stomatum on the adaxial epidermis (× 100): 1 - Nucleus, 2 - Main cell of the epidermis, 3 - Accompanying cells of stomata, 4 - Sniffing cells of stomata, 5 - Stomatal crack, 6 – Capitate) (Photo was made by MN Shurupova, 21.11.2018)

Cells of the adaxial and abaxial epidermis of the leaves are curtain-walled of the 1st order (Fig. 4, *D, E*) while the main cells of the adaxial epidermis are much larger (3-4 times) than the abaxial (Fig. 4, *D, E*). The stomata are anomocytic, multidirectional, wide-oval shaped, 23.6 ± 0.2 and 19.3 ± 0.2 µm in length and width (Fig. 4, *F*), respectively. The stomatal index is 25.8 ± 0.6%.

Conclusion

Saussurea controversa belongs to polycentric perennial summer green herbaceous semi-rosette polycarpic plants with rhizomes. Reproduction occurs both by seeds and clone. Four periods and nine ontogenetic states were distinguished in ontogenesis. Populations of this species in the Republic of Khakassia are incomplete with the accumulation of virginal individuals and the predominance of

individuals of the intermediate and high classes of vitality. *Saussurea controversa* should be attributed to the 3rd category of resource species, harvesting of which requires strict adherence to the regime of sustainable use. The diagnostic features of the *S. controversa* raw material are the structure of the leaf stalk with a large number of vascular fibrous bundles, and the abaxial epidermis containing the stomata of anomocytic type and trichomes of four types.

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Received 05 December 2018; Revised 15 February 2019;
Accepted 12 March 2019; Published 21 March 2019

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For citation: Shurupova MN, Parshina EP, Reshetov YaE, Krasnobaeva AA, Avdeeva EYu, Belousov MV. Population structure, resource potential and diagnostic features of raw materials of *Saussurea controversa* DC. *Vestnik Tomskogo gosudarstvennogo universiteta. Biologiya = Tomsk State University Journal of Biology*. 2019;45:34-46. doi: 10.17223/19988591/44/2