BIOMETRIC AND ANATOMICAL STUDY OF THE SPECIES POLYGONUM SACHALINENSE F.SCHMIDT

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Abstract. *The anatomical structures of the species Polygonum sachalinense, from the collection of the Botanical Garden, grown in the pedo-climatic conditions of the Republic of Moldova, were studied. The microscopic examination performed on cross sections and surface preparations revealed specific anatomical features: the presence of calcium oxalate druses and starch granules, collenchyma and sclerenchyma in stems, multicellular protective hairs and multicellular glands with browned content in leaves. The biometric study performed on a set of morphological indices (stem height, stem diameter, number of internodes, length of internodes, number of ramifications, number of leaves, length and width of leaves) indicates that P. sachalinense accumulates large amounts of biomass.*

Key words: *Polygonum sachalinense, biometry, anatomy, specific structures.*

INTRODUCTION

The species Polygonum sachalinense F. Schmidt is native to East Asia (Sakhalin, Kuril, Hokkaido and Honshu Islands, North and South Korea). It is also known as: Reynoutria sachalinensis (F. Schmidt) Nakai, Fallopia sachalinenses (F. Schmidt) Ronse Decr., Pleuropterus sachalinensis (F. Schmidt) H. Gross, Tiniaria Sachalinensis (F.Schmidt) Janch., its synonyms [5, 8, 14]. The common names of this species, in Russian, are гречиха сахалинская, горец сахалинский, сибирский бамбук, in Romanian – hrişca de Sahalin and in English – giant knotweed and Sakhalin knotweed. P. sachalinense is a gynodioecious species, with hermaphrodite and female flowers on separate plants, both types of plants coexist in a population. Genetically, the populations of P. sachalinense can be tetraploid (2n=4x=44), hexaploid (2n=6x-66), octoploid (2n=8x=88) [7, 8], and sometimes – dodecaploid (2n=12x=132). The species named P. sachalinense is known as having a number of chromosomes 2n=44 and 2n=66, and named Fallopia sachalinensis – 2n=32, 44, 132 [1, 6].

P. sachalinense is an herbaceous perennial plant, growing up to 3-4 m tall, in its native countries, with underground organs represented by extensively spreading rhizomes, with thin adventitious roots and with erect fistulous stems. Young roots are white and elastic. At the beginning of the growing season, the stems are herbaceous, then become lignified [4, 9, 10]. In optimal conditions, the Sakhalin knotweed reproduces vegetatively (by segments of rhizomes and aerial stems) and by seedlings, both in its native countries and in the Republic of Moldova [12, 14].

P. sachalinense grows spontaneously in the Far East. It is a promising plant, which contains high amounts of proteins, macro- and micronutrients, vitamins and biologically active substances (alkaloids, phytoestrogens, phenolic compounds, including tannins, phlavanoids) [5, 20]. This species was introduced in the collection of the Botanical Garden (I) of the ASM by A. Teleuta, in 1982, from North Ossetia (Agricultural Institute, Vladikavkaz). Amino acids play an important role in the metabolism of living organisms, and the fresh mass of Sakhalin knotweed is rich in amino acids, it contains high amounts of glutaminic acid, aspartic acid and lysine, and the list continues with another 13 amino acids [19]. P. sachalinense is one of the most important sources of resveratrol, which is a natural phytoalexin, and of its glycosides. Resveratrol inhibits the formation and growth of cancer cells [21]. The plant can be cultivated for feed, energy, medicinal, ornamental and food purposes (you can eat the young twisted leaves and young shoots, which resemble those of asparagus) [17]. An important advantage of P. sachalinense plants is their longevity; they grow on the same land for 10-15 years, maintaining high biological productivity [18].

The purpose of this study has been to determine the biometric and morpho-anatomical features of P. sachalinense plants grown in the pedo-climatic conditions of the Republic of Moldova.

MATERIALS AND METHODS

Plants of P. sachalinense, the variety Gigant (approved and registered in the Catalogue of Plant Varieties of the Republic of Moldova, in 2012), from the collection of the Plant Resources Laboratory of the Botanical Garden (I) ASM, from which, leaves and stems were harvested at the beginning of flowering phase, served as biological material for research. The biometric study was performed on mature plants by analysing a set of indices: plant height, number of leaves, length and width of leaves, number of internodes, length of internodes, number of ramifications, according to the classic methodical indications [16]. The obtained data were systematized and processed statistically, according to the following parameters: average, dispersion, standard deviation, coefficient of variation and standard error.

The anatomical study was performed on cross sections of the stem and leaf, and on superficial preparations of clarified materials with NaOH 3% and chloralhydrate [11].

The sections were examined under microscopes A. Kruss Optronic and Micros (Austria) with digital camera connected to computers, at the Pharmacognosy and Pharmaceutical Botany department of the State University of Medicine and Pharmacy "Nicolae Testemitanu".

RESULTS AND DISCUSSIONS

Plant biometry. Under the pedo-climatic conditions of Moldova, the plants of sp. P. sachalinense are developed as herbaceous perennial plant, which can reach a maximum height of 6 m. The growing season begins with the development of dormant buds at the base of the stem, changing from reddish-brown to pink, starting to form green leaves.

The morphological research denotes that, in the pedo-climatic conditions of the Republic of Moldova, P. sachalinense develops a root system consisting of 1st, 2nd and 3rd order adventitious roots, which appear on the rhizomes and their function is to store reserve substances (fig.1). The young roots develop absorbent hairs, which appear in the 3rd or 4th day of seed germination.

Fig. 1. The aspect of P. sachalinense plants: A – the underground part of the plant in the first year of vegetation; B – plants at the beginning of the growing season; C – plants in the flowering phase (June).

The phenological observations and the biometric analysis (plant height, number of leaves, length and

width of leaves, number of internodes, length of internodes and number of ramifications) were performed dynamically during the entire vegetative season, and the results are presented in Table 1.

The Sakhalin knotweed developed vegetative shoots that grew slowly until the middle of April. At the beginning of the growing season, the shoots were green and then turned brown and lignified. In April-July, the plants were characterised by an intense and rapid development (a stem grew from 38.9 cm to 534.7 cm). The number and the length of internodes changed as the plants grew, their number varied from 4-6, in April, to 30-37, in July. The length of internodes reached in July the highest values – 25 cm. The development of the lateral ramifications, of 1st, 2nd and 3rd order, occurred later, in mid-May, in a period of 45-50 days from the beginning of the vegetative season. At the end of July 2016, the maximum plant height was recorded – 580±60.51 cm and the maximum ramification of the stem – 12 units.

Table 1
Biometric analyses of the shoots of P. sachalinense during the growing season, April-July 2016

Date	Biometric indices	Statistical parameters					
		Min.	Max.	Average	Standard error (Sx)	Standard deviation (δ)	Coefficient of variation (CV)
15.04	Height of the stem (cm)	28	63	38.9	±3.26	10.29	26.45
	Diameter of the stem (cm)	2	4	2.6	±0.22	0.7	26.92
	Number of internodes	4	6	4.8	±0.20	0.63	13.13
	Length of internodes (cm)	6	8	6.7	±0.22	0.68	10.15
	Number of ramifications	-	-	-	-	-	-
	Number of leaves	3	7	4.6	±0.5	1.58	34.35
	Width of leaves (cm)	7	14	9.5	±0.69	2.17	22.84
	Length of leaves (cm)	8	13	9.5	±0.58	1.84	19.37
15.05	Height of the stem (cm)	260	315	290.3	±5.11	16.14	5.56
	Diameter of the stem (cm)	2	4	2.7	±0.37	1.17	43.33
	Number of internodes	13	24	14.6	±0.73	2.32	15.89
	Length of internodes (cm)	8	18	11.6	±1.03	3.24	27.93
	Number of ramifications	-	-	-	-	-	-
	Number of leaves	12	15	13	±0.36	1.15	8.85
	Width of leaves (cm)	17	22	18.7	±1.70	5.38	28.77
	Length of leaves (cm)	20	28	23.8	±1.18	3.73	15.67
15.06	Height of the stem (cm)	389	470	450	±10.34	32.68	7.26
	Diameter of the stem (cm)	3	6	4.4	±0.30	0.96	21.82
	Number of internodes	19	24	22.2	±0.66	2.10	9.46
	Length of internodes (cm)	11	19	15	±0.84	2.67	17.8
	Number of ramifications	3	6	4.6	±0.27	0.84	18.26
	Number of leaves	31	38	36	±0.68	2.16	6.00
	Width of leaves (cm)	26	32	29.3	±0.60	1.89	6.45

	Length of leaves (cm)	33	41	38	±0.73	2.31	6.03
15.07	Height of the stem (cm)	391	580	534.7	±21.10	66.65	12.46
	Diameter of the stem (cm)	3	7	5	±0.42	1.33	26.6
	Number of internodes	30	37	33.8	±0.68	2.15	6.36
	Length of internodes (cm)	17	25	21.7	±0.76	2.45	11.29
	Number of ramifications	10	12	11.1	±0.23	0.73	6.57
	Number of leaves	49	69	58.7	±1.95	6.17	10.46
	Width of leaves (cm)	30	35	33.2	±0.49	1.55	4.67
	Length of leaves (cm)	40	44	42	±0.49	1.56	3.71

The number of internodes and their growth also confirmed the intense development of the plant; their number grew from 4, in mid-April, to 37, at the beginning of July, during that period (April-July) the length of internodes varied from 6.7±0.22 cm to 21.7±0.76 cm.

The plants have simple, petiolate, alternate leaves, with ovate blade, cordate (heart-shaped) at the base, with slightly wavy, crenate margin and acuminate apex. The leaves from the basal part of the stem started withering and falling in June, and those from the middle and upper part of the plant remained until the end of the growing season. In the flowering phase, the highest number of leaves on a plant was of 69±1.95, and they reached a maximum size (44±0.49 cm long and 35±0.49 cm wide), developing an impressive leaf area.

The biometric study, carried out using millimetre paper, showed that the leaf area of a mature plant, in the flowering phase, varied between 27728 and 48288 cm2 and was determined by the height and the degree of branching of the stem, as well as by the number and the size of leaves.

The flowers are actinomorphic, small (0.5-0.8 cm in diameter), with simple perianth, gathered in panicle inflorescences. The androecium consists of eight free stamens, with flattened, smooth filaments. The stigma is three lobed, and the ovary – superior. The fruit is dry, indehiscent, three-sided achene with pointed tip, shiny, brown; the pericarp is not fused with the seed coat. The seeds are about 2-3 mm long and 1-1.5 mm wide. One gram contains about 1047 seeds of Sakhalin knotweed. The seeds cannot be stored a long time, because they lose their potential to germinate [2].

The morphological analysis of the development of the shoots of P. sachalinense, during the growing season, has shown that this species has a high potential for producing green mass. This feature makes it more advantageous as compared with other, traditional species of forage plants.

Stem anatomy. The stem in cross-section is costate, with 6-13 angles. The following histological zones are easily distinguished in the cross-section of a stem: the epidermis, the primary cortex and the central cylinder. The epidermis consists of a single layer of tangentially flattened cells, covered with a well-defined cuticle. The exodermis consists of an angular collenchyma, which is developed very well at the angles. The mesoderm is formed of 4-5 layers of parenchymal cells with starch granules and with sporadic calcium oxalate druses. The endodermis consists of a layer of tangentially elongated cells (Fig. 2).

The central cylinder occupies the largest volume in the cross section of the stem. The conducting vascular bundles are collateral open, arranged in a circle, bounded by narrow medullary rays (2 layers of cells, which are rich in starch granules). In every vascular bundle, there are 3-4 woody vessels with large diameter. The stem is also characterized by the presence of the lignified sclerenchyma, which forms a ring of 8-10 layers of cells, interrupted by the medullary rays from the external part of the vascular bundles. The woody sclerenchyma consists of only 4-6 layers of lignified cells. The medullary parenchymal cells are large, rich in starch granules, without intercellular spaces. The internal epidermis also consists of a single layer of tightly packed cells, but they are smaller than in the external epidermis.

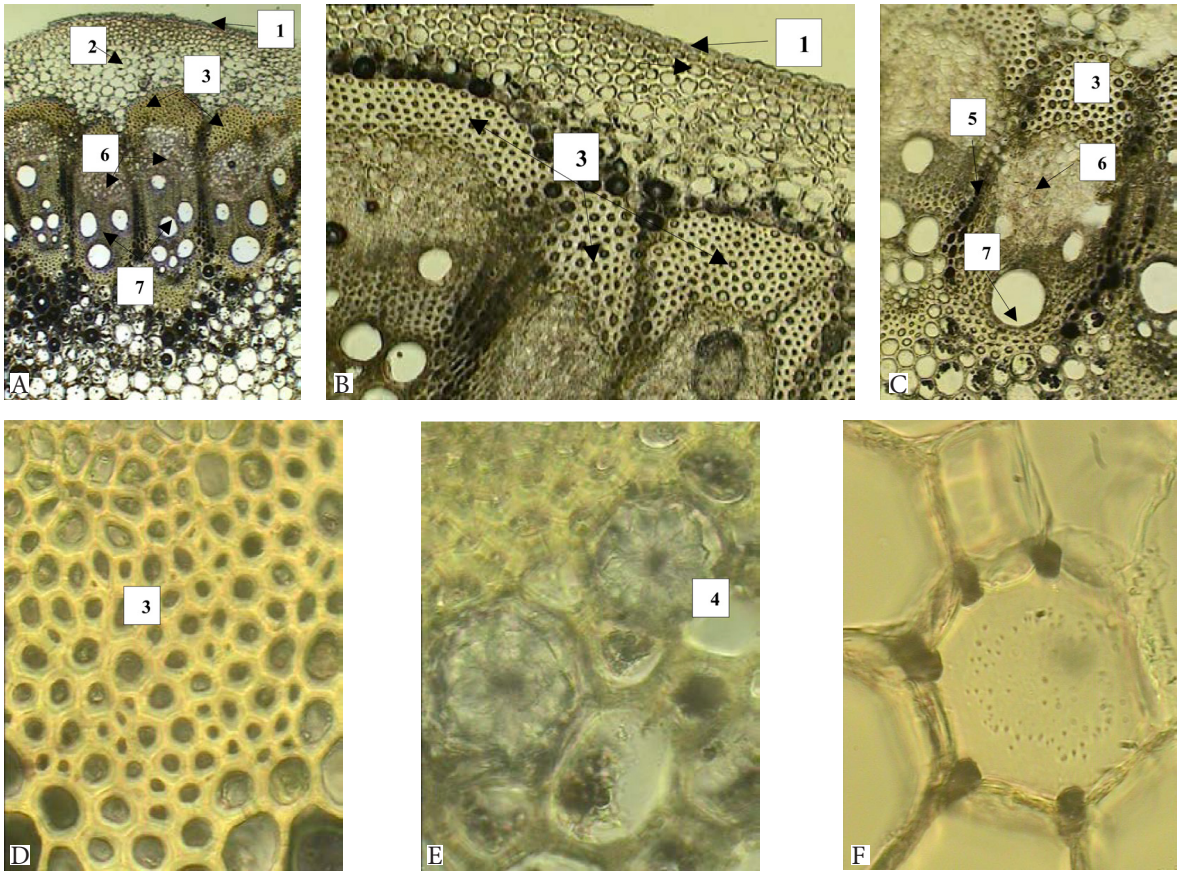

Fig. 2. The anatomy of the stem: A, B, C – cross sections; D – sclerenchymatous layer (bast fibre), x400; E – parenchyma with calcium oxalate druses, x400; F. – punctuated cell wall of parenchyma, x400; 1 – epidermis; 2 – angular collenchyma; 3 – sclerenchyma; 4 – calcium oxalate druses; 5 – medullary rays; 6 – phloem 7 – xylem.

Leaf anatomy. In the cross section of the leaf blade we distinguish: the upper epidermis, the dorsiventral mesophyll and the lower epidermis. The epidermis consists of slightly flattened, tightly packed parenchymal cells, with specific structures (stomata, protective hairs and glands). The leaves are amphistomatic with anomocytic stomata. On surface preparations, the upper epidermis differs from the lower one. The cells of the upper epidermis are larger, the cell walls are slightly wavy and the number of stomata is lower than in the lower epidermis. On the lower epidermis, the stomata are more rounded in shape and smaller, are numerous, and the epidermal cells are characterized by markedly wavy cell walls, giving it a mosaic appearance (Figure 3).

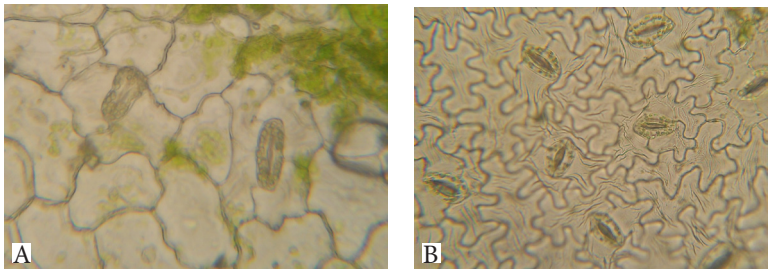

Fig.3. Epidermis of the leaf blade; A – upper, B – lower, x400.

The mesophyll is differentiated into palisade and spongy tissue. The palisade layer is situated beneath the adaxial (upper) epidermis, represented by two layers of elongated cells, with a large number of chloroplasts, located perpendicularly to the upper epidermis of the leaf blade. The spongy parenchyma is located behind the lower epidermis and consists of lobed cells, with fewer chloroplasts and with intercellular spaces. In the cells of spongy parenchyma, there are ergastic inclusions – druses of calcium oxalate. At the border of palisade and spongy parenchyma, the leaf mesophyll is perforated by collateral vascular bundles. Next to the largest vascular bundles, angular collenchyma is present.

The large, unbranched, multicellular tector hairs, formed of 3-5-7 uniseriate cells, covered with thick, sometimes corrugated cuticle, are characteristic of the leaf epidermis of Sakhalin knotweed. On the epidermis, there are also smaller multicellular tector hairs, with thick, but smooth cuticle. There are more protective hairs on the lower epidermis of the leaf blade than on the upper one, where they are found only along the veins. Some multicellular (6-8 cells) glands with browned content were found only on the lower epidermis (Fig. 4)

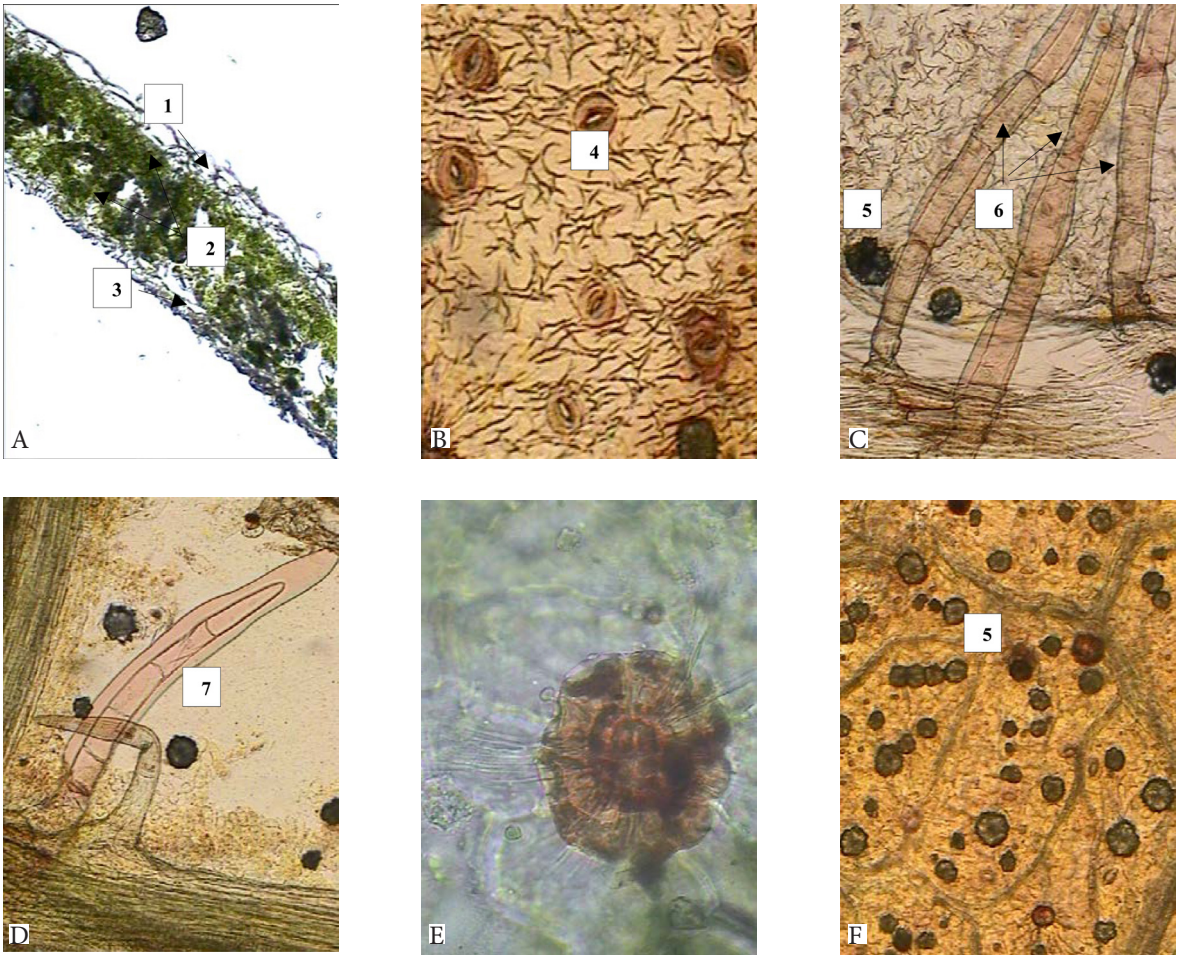

Fig. 4. Leaf anatomy: A. – Cross section of the leaf blade, x100; B, C, D. – lower epidermis, (surface preparation) x400; E, F – upper epidermis x400. 1 – upper epidermis x 100; 2 – differentiated mesophyll; 3 – lower epidermis; 4 – stomata; 5 – calcium oxalate druse; 6 –multicellular protective hairs with corrugated cuticle, 7 – protective hairs with smooth cuticle.

The data obtained as a result of the morphological study, carried out on a set of morphological indices, denotes that, in the Republic of Moldova, Sakhalin knotweed grows rapidly and has high capacity to produce very large amounts of biomass, expressed by the large leaf area, which determines a high photosynthetic potential that would allow the biosynthesis and the accumulation of natural chemical compounds.

The results of the anatomic study performed on surface preparations and cross sections of stems and leaves of P. sachalinense, analysed thought scientific literature data [15,19], allowed the elucidation of specific anatomical structures for plant organs: in stems – the presence of the calcium oxalate druses, the angular collenchyma at the angles, the sclerenchymatous ring and the collateral open vascular bundles; in leaves – the lower epidermis with cells with wavy walls, the amphistomatic leaf blade with anomocytic stomata, which are small and numerous in the lower epidermis, the two types of unbranched, multicelular protective hairs with corrugated and smooth cuticle, located mainly along the veins, the multicelular glands with browned content found in the lower epidermis, the dorsiventral mesophyll with calcium oxalate druses.

The pubescent leaves, protected by 2 types of protective hairs with thick cuticle and glands with browned content, the presence of calcium oxalate druses and other structural indices constitute the structural adaptive potential of plants to unfavourable conditions and ensure the ability to adapt to the specific pedo-climatic conditions of the Republic of Moldova, which are different from those of Far East (Eastern Asia) – the native area of P. sachalinense.

Thus, due to its morphological features and biometry of the most important indices for productivity (leaf area, height and ramification of the stem), P. sachalinense species, the variety Gigant, is a forage crop with certain advantages under the pedo-climatic conditions of the Republic of Moldova and can be recommended for cultivation on large areas in order to obtain fodder for animals and biomass for energy production.

CONCLUSIONS

1. In the conditions of the Republic of Moldova, P. sachalinense is a vigorous plant with a large leaf area (48288 cm2), the maximum height – 580 cm, 8-10 ramifications, the highest number of leaves – 69, the maximum size of a leaf (44 cm long and 35 cm wide), which constitute a high photosynthetic potential that would ensure the capacity to accumulate large quantities of green biomass.
2. The following zones are specific to the anatomy of the stem of Sakhalin knotweed: the epidermis, which consists of a single layer, the cortex with collenchyma and storage parenchyma with starch granules, calcium oxalate druses, the sclerenchymatous layer and the central cylinder with collateral open vascular bundles, interrupted by amylogenous medullar rays.
3. The anatomy of the leaf is characterised by: the dorsiventral mesophyll, the amphistomatic leaf blade, the anomocytic stomata, the multicelular protective hairs of two types with corrugated and smooth cuticle, more numerous on the lower epidermis, the multicelular glands with browned content and presence of calcium oxalate druses.
4. The biometric data regarding the main morphological characteristics (plant height, ramifications, number and length of internodes, number of leaves, leaf area etc.) allow recommending the cultivation of the species P. sachalinense (variety Gigant) on large areas, under the pedo-climatic conditions of the Republic of Moldova.

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