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Anatomy and compositions of seeds of the walnut *Juglans L*

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Abstract

Anatomy and morphological parameters of epidermal cells of the seed coat of various species of the genus *Juglans L* were studied using the ion-scanning electron microscope (SEM) Quanta 200 3D, the Carl Zeiss microscope and the computer program VideoTest 5.0. The structural features of the seed coat and the kernel and the interspecies differences in the parameters of the epidermal cells were revealed. The results can be used as additional diagnostic indications of the genus *Juglans L*. In addition, elemental compositions were detected using the SEM Quanta 200 3D and chemical compositions were determined using Russian standard methods (GOST).

Keywords: seed, walnut, *Juglans L.*, epidermis, cells, chemical composition

1. Introduction

There are only a few studies on the structure of the seeds of the genus *Juglans L* [2, 3, 4]. The purpose of this work was to investigate the possibility of using anatomical features of the structure of seeds for supplemental literature in systematics and phylogeny of the genus *Juglans L*, as well as to determine elemental and chemical compositions of the seed kernel. Species of this genus have high nutritional values [1, 5]. The useful properties of walnuts are due to the following reasons: 1- Availability of vitamins and minerals; 2- Availability of biologically active substances; 3- Availability of monounsaturated fats [7].

2. Materials and methods

The seeds of four species of the genus *Juglans L* were used as research materials: *Juglans regia*, *Juglans mandshurica*, *Juglans nigra*, *Juglans cinerea*. The seeds were collected in the botanical garden of the Bryansk State Academy of Engineering and Technology and in the training and experimental forestry. Anatomical study and elemental compositions of the kernel of the seeds were carried out using the ion-scanning electron microscope in the Center of Collective Use of Scientific Equipment of Belgorod State National Research University. Anatomy and morphological parameters of the epidermal cells of the seed coat of various species of the genus *Juglans L* were studied using the SEM Quanta 200 3D, the Carl Zeiss microscope and the program VideoTest 5.0. Chemical compositions of the kernel of the seeds were analyzed following the instructions in GOST 10846 – 91 (for protein) and GOST 29033 – 91 (for fat).

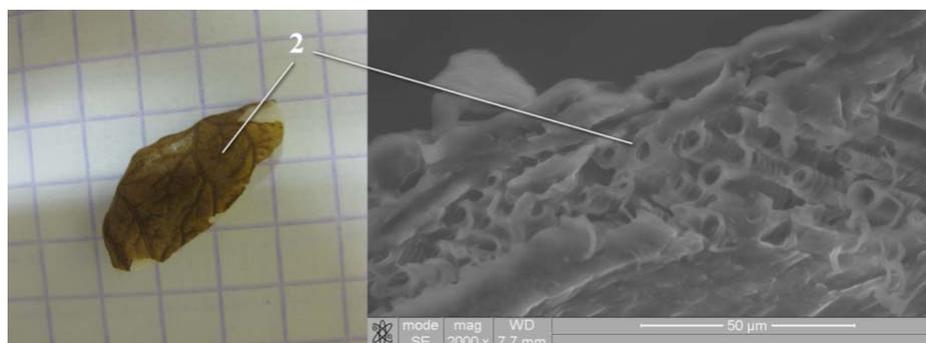


Fig 1: Appearance (left) and transverse section (right) of the seed coat of the *Juglans mandshurica* (symbol 2 – vascular bundles)

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3. Results and discussion

3.1 Anatomical structure of the seed coat and the kernel of species of the genus *Juglans* L

The presence of large and round stomata on the surface of the seed coat of the genus *Juglans* L was detected (Fig. 2-3). Besides stomata, pores were also found on the surface of the

epidermis of the genus *Juglans regia* (Fig. 4). The epidermis is filled by vascular bundles (Fig. 1, 5, 7, 9). The seed coat consists of two layers. Aleurone grains and oil droplets were found in the longitudinal section of the cells of the seed kernel (Fig. 6, 8, 10).

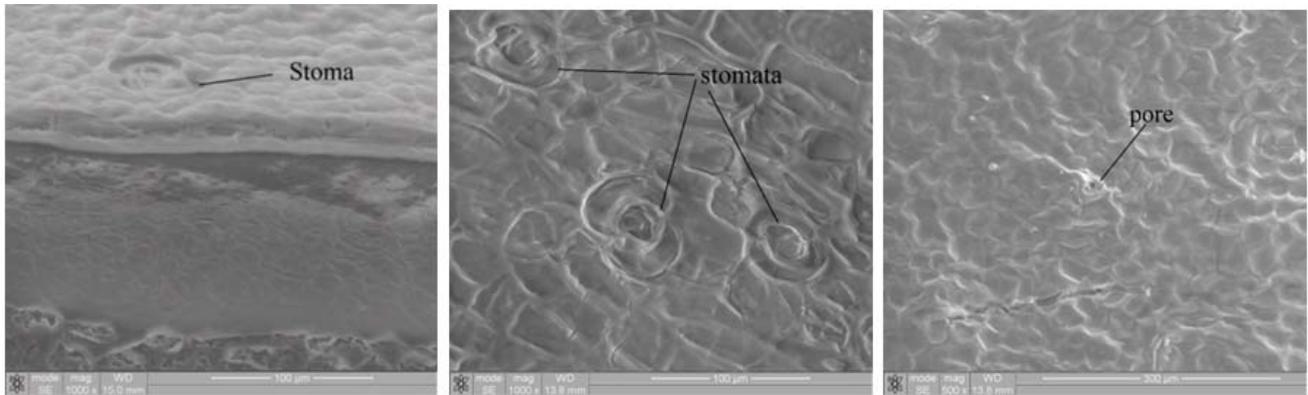


Fig 2-4: Surface of the seed coat of the genus *Juglans* L

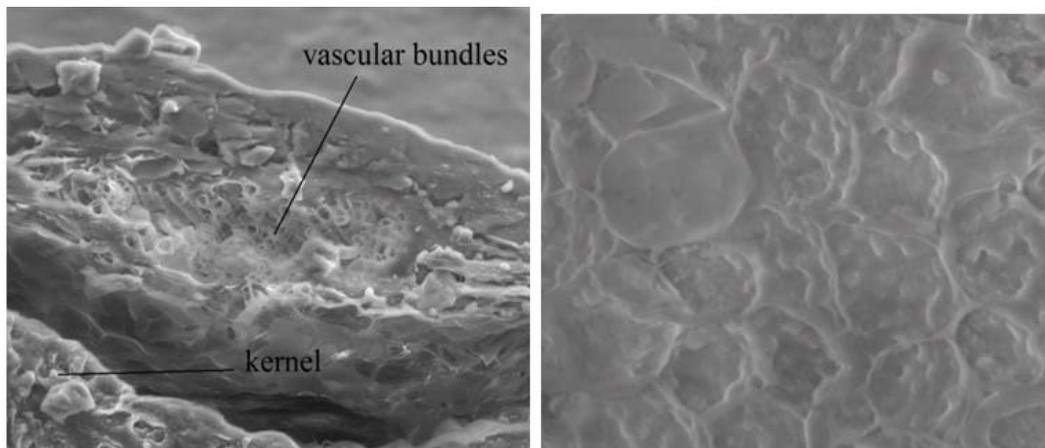


Fig 5: Transverse section of the seed kernel of the genus *Juglans regia*.; **Fig 6:** Longitudinal section of the seed kernel of the genus *Juglans regia*

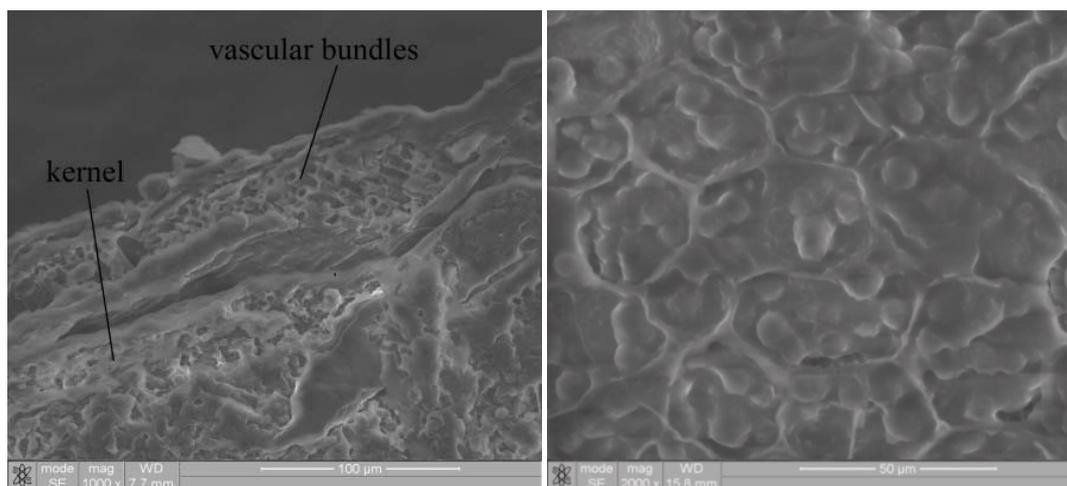


Fig 7: Transverse section of the seed of the genus *Juglans Mandshurica*.; **Fig 8:** Longitudinal section of the seed of the genus *Juglans Mandshurica*

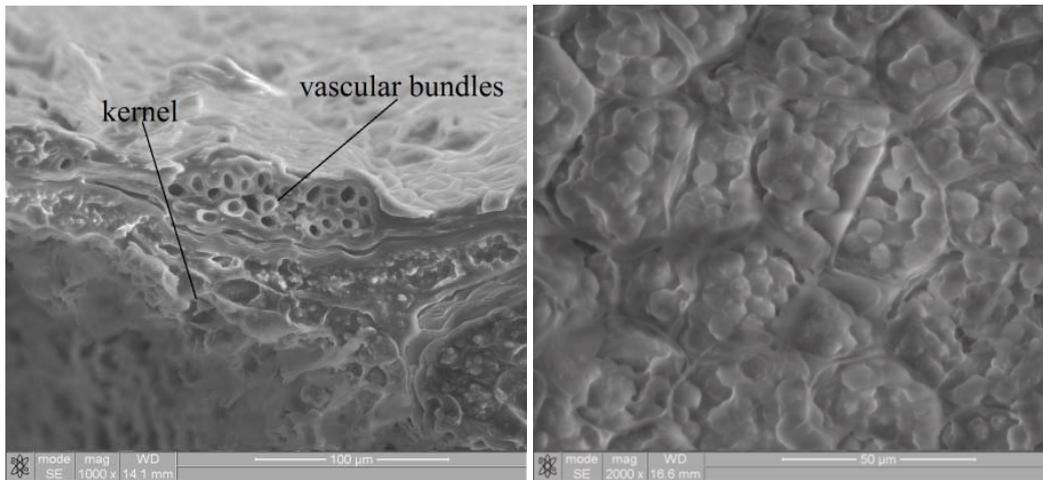


Fig 9: Transverse section of the seed of the genus *Juglans cinerea* **Fig 10:** Longitudinal section of the seed of the genus *Juglans cinerea*

Table 1 shows a comparison of several morphological parameters of the epidermal cells of the seed coat between the genera *Juglans regia*, *Juglans mandshurica* and *Juglans cinerea*. Epidermal cells of *Juglans regia* are quadrangular and polygonal shaped. Average size of the epidermal cells is 19.83 µm. Cell area is 245.0 µm². Epidermal cells of *Juglans*

cinerea is the largest one – 24.48 µm (p < 0.05). The form of these epidermal cells is polygonal. Its cells have the largest round factor – 0.76 (p < 0.05). The size of epidermal cells of the walnut *Mandshurica* is 21.98 µm (p < 0.05). Its form is polygonal.

Table 1: Morphological parameters of the epidermal cells of the seed coat of the genus *Juglans* L.

	<i>Juglans regia</i>	<i>Juglans mandshurica</i>	<i>Juglans cinerea</i>
Area (µm²)	245.0 ± 64.59	312.48 ± 78.35 *	415.59 ± 137.64 *
Perimeter (µm)	65.22 ± 10.17	71.58 ± 9.23 *	81.94 ± 12.22 *
Length (µm)	25.13 ± 5.34	26.83 ± 4.49	27.45 ± 4.65
Width (µm)	14.54 ± 2.57	17.14 ± 3.14 *	21.51 ± 4.32 *
Round factor (relative units)	0.72 ± 0.08	0.76 ± 0.1	0.76 ± 0.08
Ellipse factor (relative units)	0.97 ± 0.01	0.98 ± 0.01	0.97 ± 0.02
Average size (µm)	19.83 ± 2.93	21.98 ± 2.6 *	24.48 ± 3.94 *
Elongation (relative units)	1.79 ± 0.49	1.62 ± 0.42	1.31 ± 0.25 *
Roundness (relative units)	0.49 ± 0.11	0.55 ± 0.12	0.61 ± 0.1 *

* Accuracy in comparison with the walnut regia (by Student's test (p < 0.05).

3.2 Elemental and chemical composition of seed kernel of species of the genus *Juglans* L. Carbon and oxygen take the most of the elemental composition. Other elements such as

aluminum, magnesium silicon, phosphor, sulfur, and potassium, calcium together with chlorine, copper and manganese were found in various mass fractions.

Table 2: Elemental compositions of seeds of some species of *Juglans* L

Element	Seed coat				Kernel			
	1*	2	3	4	1	2	3	4
C	64.68	64.13	64.93	63.16	76.75	75.74	81.45	80.07
O	33.50	32.98	32.57	33.10	16.91	21.42	14.11	15.47
Mg	0.12	0.11	-	0.23	0.27	0.22	0.25	0.38
Al	0.88	0.60	1.11	1.32	3.00	0.75	2.13	1.82
P	-	0.19	1.40	-	0.78	0.59	0.78	0.83
S	0.11	0.22	-	0.21	0.42	0.24	0.40	0.29
Cl	-	-	-	-	-	0.03	-	-
K	0.40	1.42	-	1.62	1.48	0.92	0.69	0.67
Ca	0.25	0.34	-	0.35	0.38	0.09	0.07	0.06
Si	0.06	-	-	-	-	-	0.11	0.09
Cu	-	-	-	-	-	-	-	0.21
Mn	-	-	-	-	-	-	-	0.11
Total	100	100	100	100	100	100	100	100

Conditional symbols: 1- *Juglans regia*, 2- *Juglans nigra*, 3- *Juglans mandshurica*, 4- *Juglans cinerea*

Analysis of the seeds showed that the heavy metal copper (0.21%) was found in the kernel of *Juglans cinerea*. This testifies to the fact that they grow in the vicinity of roadway. Manganese (0.11%) were also found in these kernels. The research of the impact of chlorine on nitrogen metabolism revealed that chlorides stimulate the activity of asparagine synthetase, which is involved in the transfer of amino groups on asparagine [6, 7, 8]. Chlorine is found only in the seed coat of the black walnut (0.03%). This may explain the fact that black walnut kernel has a specific smell. In the seed coat of *Juglans mandshurica* besides basic elements (carbon and oxygen) only aluminum and phosphorus were observed.

Table 3: Chemical compositions of seeds of some species of *Juglans* L.

Species	Fat	Protein	Dry matter	Moisture
<i>J. nigra</i>	54.58	25.26	94.91	5.09
<i>J. mandshurica</i>	60.57	25.98	94.87	5.13
<i>J. cinerea</i>	58.25	26.12	95.74	4.73

As can be seen (table 2 and 3) all species of the genus *Juglans* contain high percentages of fat and protein. The seeds also contain aluminum, potassium, calcium, phosphorus and other chemical elements (table 2). The rather high amount of potassium and aluminum increases value of the seeds.

4. Conclusion

1. Using the ion-electron scanning microscope Quanta 200 3D, 12 chemical elements in different parts of seeds of some species of the genus *Juglans* were detected. The obtained data on the elemental composition of seeds of species of the genus *Juglans* determine their prospects of further experimental research in the food and pharmaceutical industries.

2. Anatomical features of the structure of the seed coat and the presence of vascular bundles and stomata were revealed. The differences in the size of the epidermal cells of the seed coat were also determined. The data of the anatomy of the seed coat can be successfully used for taxonomy and phylogeny of the studied species of the genus *Juglans* L.

3. Representatives of the genus *Juglans* contain high nutrients and can be widely used in the food and pharmaceutical industry.

5. Acknowledgement

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