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Original Article

Physical and mechanical properties of hard seed coat on the example of *Gleditsia triacanthos* L.

Propriedades físicas e mecânicas da casca dura de sementes exemplificadas pela *Gleditsia triacanthos* L.

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Abstract

The microhardness of individual morphological structures of the hard coat of the seed of *Gleditsia triacanthos* L. was measured. Measurements were made on the transverse and frontal planes. Based on the differences in the hardness of the two planes, the anisotropy of the seed coat was revealed. The entire seed coat has a special hardness which can be compared with the hardness of hardwood like oat wood. An interesting feature was the hardness of the endosperm, comparable to the hardness of the epidermis. Further study of the processes that occur in seeds during imbibition is based on the obtained data. Mathematical modeling methods are the most promising for these tasks; they will help to identify the points of fragility and the points of the greatest tension in the seed coat. These results will allow us to find the best ways to destroy the seed coat and to accelerate the germination. Research of physical properties of the seed coat is of practical importance in the fact that in the future it will allow reducing of the hard-seeding and increasing the germination of seeds. The obtained data allows us to represent the initial hardness of the seed coat.

Keywords: *Gleditsia triacanthos* L., hard seed coat, microhardness, epidermis, parenchyma, endosperm, embryo.

Resumo

A microdureza de estruturas morfológicas individuais da casca dura da semente de *Gleditsia triacanthos* L. foi medida. As medições foram feitas nos planos transversal e frontal. Com base nas diferenças na dureza dos dois planos, a anisotropia do tegumento da semente foi revelada. Toda a casca da semente tem uma dureza especial que pode ser comparada à dureza de madeira dura, como a madeira de aveia. Uma característica interessante foi a dureza do endosperma, comparável à dureza da epiderme. Estudos posteriores dos processos que ocorrem nas sementes durante a embebição são baseados nos dados obtidos. Os métodos de modelagem matemática são os mais promissores para essas tarefas; eles ajudarão a identificar os pontos de fragilidade e os pontos de maior tensão na casca da semente. Esses resultados nos permitirão encontrar as melhores maneiras de destruir a casca da semente e acelerar a germinação. A pesquisa das propriedades físicas da casca da semente tem importância prática, pois no futuro permitirá reduzir a dureza das sementes e aumentar a taxa de germinação. Os dados obtidos permitem representar a dureza inicial da casca da semente.

Palavras-chave: *Gleditsia triacanthos* L., casca dura da semente, microdureza, epiderme, parênquima, endosperma, embrião.

1. Introduction

The seed coat is the outer shell of each mature seed. Its numerous functions include preserving the integrity of seed parts, protecting the embryo from mechanical damage, diseases and pest attacks, regulation of gas exchange between the embryo and the external environment, and, in many species, regulating the process of seed germination (Singh et al., 2020). Another important function of the seed coat is the regulation of imbibition of seed. In some plants, it is represented by a temporarily impenetrable barrier that controls the imbibition and, as a result, germination; a seed coat with the function of control of imbibition is called solid coat (Zhu et al., 2021). In this article, special attention will be paid to the structure of the seed coat of the Fabaceae family by example of *Gleditsia triacanthos* L.

A comprehensive research of the structure of the seed coat of Fabaceae can be very important for seed specialists,

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biologists and plant breeders. Much attention is paid to the study of anatomical traits of the structure of seeds, while the physical properties need additional research.

The general structure of the seed coat of Fabaceae is shown in Figure 1 (Takhtadzhyan, 1996). The outer layer is a wax shell, of varying thickness, which is the first barrier to water penetration. The epidermis, a layer of thick-walled, oblong palisade cells called macrosclereids, with a long axis oriented perpendicular to the surface, forms the next layer. The apparently denser area that refracts light is called the light line. One layer of cells forms the hypoderm; these cells are also called both hourglass cells, columnar cells, osteoscleroids, depending on their structure, thickness and shape. They are usually larger than the cells of neighboring layers and are separated by wide intercellular spaces.

The fourth layer of the seed coat is the inner parenchyma, formed by layers of thin-walled parenchyma cells evenly spaced throughout the seed surface. In the area of the hilum, the seed coat consists of epidermis and parenchyma; there is no hypodermis. Conductive bundles enter the seed coat through the center of the hilum (Rowson, 1952; Scott et al., 1962; Steiner and Jancke, 1955).

Dormant seeds of *G. triacanthos* have a hard, impenetrable seed coat, which is presumably a part of an adaptive mechanism for the survival of the species. For uniform and fast germination, pre-sowing seed treatment such as mechanical scarification, chemical treatment, hot water treatment, boiling, *etc* is necessary. These methods can improve the germination of seeds with a hard seed coat as a result of damage to the integrity of the seed coat (Zimmermann, 1937).

G. triacanthos is a valuable plant. It is a fast growing tree with a short lifespan (about 125 years). It is shade-tolerant, resistant to drought, heat, pollution, compacted soil and salt. *G. triacanthos* has a number of applications: its fruits are used in agriculture to feed livestock; its dense wood is used to make furniture; and its unique compounds can be used in medicine. A water-soluble depolymerized galactomannan polysaccharide was obtained from *G. triacanthos* seeds. Pharmacological studies showed that the galactomannan was a constituent of the polyfunctional hemodynamic blood substitute rheoambrasol with hemodynamic, antiacid, and antioxidant activity for hemorrhagic shock (Rakhmanberdyeva et al., 2022; Sun et al., 2018). The fibers composed of multiple microfibers form a network construction by entangling with each other (Leboukh et al., 2020).

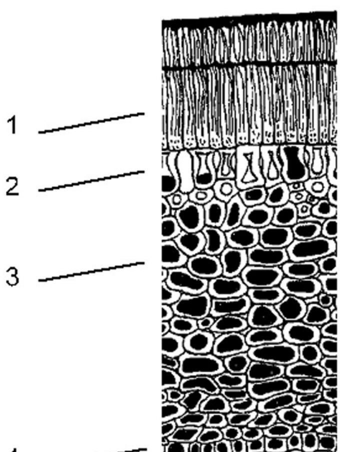

Figure 1. Cross section of the lateral side of the seed coat: 1 – ectoderm, 2 – hypoderm, 3 - mesoderm, 4 – endoderm (Takhtadzhyan, 1996).

G. triacanthos is a popular ornamental in Europe and has been reported to escape cultivated areas (Horvat & Sajna, 2021). The exotic tree is often planted as an ornamental tree in urban parks. In some European countries it has already become invasive, and thus, further spread cannot be ruled out. The production of copious long-lived seeds may contribute to its invasiveness. Larger trees tended to form better-developed soil seed bank than smaller ones; trees in neglected parks were produced five times higher density on average than those in perfectly managed parks (Csontos et al., 2020).

G. triacanthos is planted to create shade they are and hedges; they are used as protective belts along highways and streets in large cities. Forms and varieties without thorns were bred for cultivation (Zhu et al., 2021).

The seeds of *G. triacanthos* germinate slowly and not at the same time, which is associated with the phenomenon of hard-seeding. The success of honey locust imbibition and germination are known to be related to *M. dorsalis* larva acting as a scarifying agent and thus breaking the seed dormancy, delaying germination, and enabling asynchronous germination. However, the beneficial effect of larvae on germination is possible only if the larva drills the entrance hole, but dies before the seed germinates. Here we show that novel interaction established can occasionally act as mutual facilitation in which each species aids the other to exist and even to increase their invasion success (Horvat & Sajna, 2021).

2. Material and Methods

The study is aimed at identification of the traits of the physical properties of the seed coat of hard seeds of *Gleditsia triacanthos*. It will help to identify the points of fragility and the greatest tension of the coat. So, it will be possible to find the best ways to destroy the seed coat.

The seeds of *G. triacanthos* were used as the research material. The research and seed selection were carried out in Volgograd, Russia. A territory of the Volgograd region is a part of a Lower Volga region. It is located in the southeast of the Russian Plain. It is mainly located in steppe and partly in semi-desert zones. The climate is sharply continental, and the amplitude of the annual air temperature is 33–35 °C. In summer, the air and soil temperature is high, air humidity suffers shortages, and evaporation dominates. Total solar radiation rate is approximately 113 kcal·cm^{-2} and adversely affects a soil biological process. Sunshine lasts for 2265 h a year. The period with air temperature above 0 °C is circa 235–260 days and up to 300 days in the southern part of the Volgograd region. The sum of the air temperature above

10 °C is 3400–3600 °C. Due to high air temperature and lack of precipitation, the moisturizing coefficient of the territory is 0.6–0.7. The weather regime is anticyclonic. The annual precipitation is low and varies from 270 mm or even less to 450 mm, decreasing in the northwest–southeast direction. The hot weather lasts approximately 4–5 months over the year. The drought comes every 2–3 years. The aridity of the climate and the complexity of terrain relief affected the soil water regime, flux and accumulation of nutrients, accumulation and mineralization of the soil organic matter, development of the soil microbiome, and organogenesis of the plant and animal communities. Consequently, the soils of the region have a short depth profile and a depleted humus layer in comparison with the soils of a similar type in other regions of the world. The soil organic matter content is low. The soil types and subtypes change from the Haplic Chernozem to the Endosalic Calcisols in the northwest–southeast direction. The soils predominantly have a slightly alkaline environment (pH 7.5-8.5). In terms of organic carbon content, the soils are low in humus, the share of organic carbon in the upper horizon is 0.77%, CEC 23.87 mEq/100 g soil (Okolelova et al., 2022). The experiment was carried out on light chestnut heavy loamy soils. The environmental heterogeneity of the black locust seed origin within different provenances is known to finally result in different germination performances (Roman et al., 2022).

There are 5 main type of hardness measuring methods : ball hardness, cone hardness, needle hardness, wedge hardness and cylindrical hardness. Industrially the mainly used test methods are Janka-hardness and Brinell-Mörath hardness, in French the method of Monnin is the standard. All researchers bear a purpose, to work out a method, that comply with the criterion, to rid of mistakes arised from wood anatomy as well as anisotropy (Vörös and Németh, 2020).

Microhardness was determined with a microscopic microhardness meter PMT-3M. The device was designed to assess the microhardness of the structure of opaque objects by pressing of diamond pyramidal tip into the tested material. Observation of the image is carried out under illumination by methods of bright and dark fields and in polarized light. The measurement of the lengths of the diagonals (or sides) of the imprints to determine the microhardness is carried out with a screw ocular micrometer or a photoelectric ocular micrometer with automatic processing of the measurement results.

To determine the microhardness of seeds, 20 imprints were measured, each of which was measured 5 times. In total, there were 100 measurements per each anatomical structure.

The results were processed according to the following methodology.

- For each imprint, the measurement difference $d1-d2, \mu m$, was determined. Then the value $(d1-d2)\cdot 0.3$, which considers the magnification of the microscope was calculated.
- For a tetrahedral pyramid with a square base, the microhardness value, expressed in MPa, was calculated by the following formula:

$$H=\frac{F}{S}=\frac{2F\cdot \sin\frac{d}{2}}{d^2}=0.189\frac{F}{d^2} \quad (1)$$

where the mass of the load is 10 g, the angle at the sine is 137 degrees, and *d* is the length of the diagonal. Finally, the formula takes the following form

$$H=\frac{18900\cdot 1\cdot 0,98}{d^2} \quad (2)$$

• Average microhardness:

$$H_{ave}=\frac{1}{n}\sum_{i=2}^{n}\left(\frac{18900\cdot 1\cdot 0,98}{d_n^2}\right) \quad (3)$$

• Standard deviation:

$$\sigma=\sqrt[2]{\frac{\sum_{i=1}^{n}\left(H_i-H_{cp}\right)^2}{n}} \quad (4)$$

The measurement results are shown in Table 1.

Electron micrographs were obtained with an electron scanning microscope «FEI Versa 3D LoVac».

3. Results and Discussion

Microhardness was determined in different parts of the seed, such as seed coat, endosperm, cotyledons (Figure 2), embryo (Balakina et al., 2021a).

To assess the normality of the distribution, the Anderson-Darling test was used; it has a power for small samples (n ~ 100) either greater or similar to the χ^2 criterium (Lemeshko and Ogurtsov, 2007). Further, in parentheses, the p-value, which was obtained by calculating the significance of the Anderson-Darling test will be indicated.

Table 1. Microhardness of parts of native seeds, Mpa.

Plane	Epidermis		Parenchyma	Endosperm	Cotyledon	Embryo
	upper layer	lower layer				
transverse	42.1 (39.8-44.3)	35.3 (34.2-36.5)	32.6 (29.2-34.7)	34.9 (31.3-38.5)	14.0 (13.1-14.9)	15.4 (13.7-17.1)
frontal	48.8 (47.7-49.9)	38.9 (37.9-39.8)	35.6 (34.9-36.7)	33.2 (31.8-34.6)	20.1 (18.7-21.6)	–

The graphs below are a distribution density histogram superimposed on a probability density curve.

End hardness for the upper layer of the epidermis M = 42.1 (39.8-44.3) MPa w as shown in Table 1. Kurtosis coefficient = 2.3, asymmetry = −0.3, p-value = $2.07 \cdot 10^{-7}$ according to the Anderson-Darling test. This layer is highly durable as compared to other anatomical and morphological layers; and it is much harder than any wood. In particular, the values are comparable with the end hardness of wood species such as birch (40.1) and larch (37.7) (Papulova 2014).

Natural structures are often known to be heterogeneous. Previous studies (Balakina et al., 2021b; Balakina et al., 2021c) demonstrate that the outer layer of epidermal cells has cracks. Water enters through these cracks, and it causes imbibition of the seed. Pressure in the seed increases, and the cracks become even wider. The external appearance of the surface of the seed coat of *G. triacanthos* is cracked (Figures 3, 4). It is possible that we measured the area including a crack.

Radial hardness for the upper layer of the epidermis is slightly higher than the end, it was M = 48.8 (47.7-49.9) MPa as shown in Table 1; p-value = 0.7. According to microhardness (Green et al.,1999), hardwoods are divided into hard (hardness 50 N/mm^2 equal to 50 MPa, or more) and soft (hardness 49 N/mm^2 or less) (Papulova 2014). Thus, the hardness of the seed coat approaches the values of hardwood.

Similar values for structures that are much smaller than the whole tree are significant. Of course, in this case, the germination of seeds will not only be difficult if the measured structures do not have special adaptations such as seed hilum that allow water to imbibe into the seed. In dormant seeds, the lignino- and suberinified epidermis, apparently, can undergo natural aging, which also has a positive effect on overcoming the seed hardness.

The obtained values of microhardness can be partly explained by the shape of the cells: elongated cells towards the surface makes resistance to the load. It can also be partially explained by the presence of complex polymeric substances such lignin, which is responsible for lignification, and suberin, which prevents imbibition of water in seed. Lignin is assumed to be located in the cell wall in rings and spirals along the long walls of the cells in two layers of epidermis (Balakina et al., 2021c). These traits can have a positive effect on microhardness.

The seed coat of *G. triacanthos* includes lignified elements and suberin (Balakina et al., 2021c). The seed

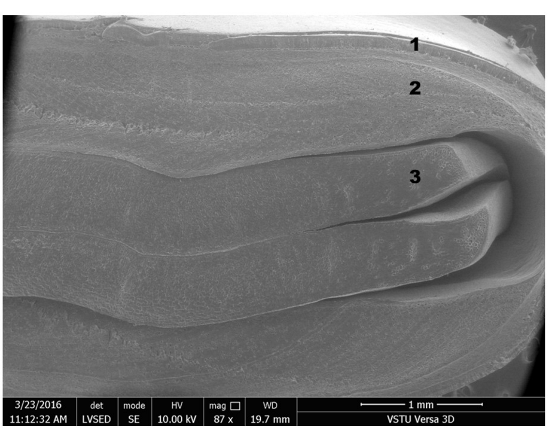

Figure 2. Cross section of seed of *G. triacanthos*. Electron scanning microscope «FEI Versa 3D LoVac» (Balakina et al., 2021a, c) *1 – seed coat; 2 – endosperm; 3 – cotyledons*.

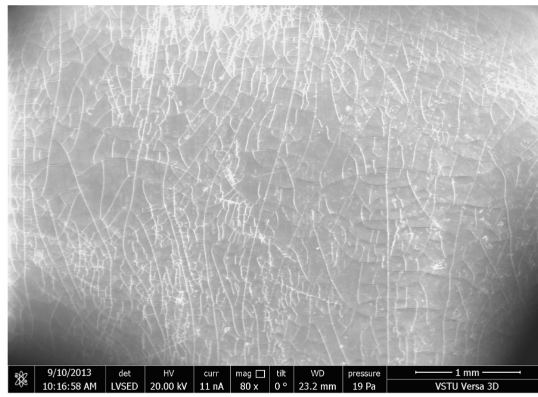

Figure 3. The surface of the seed peel of *G. triacanthos*.

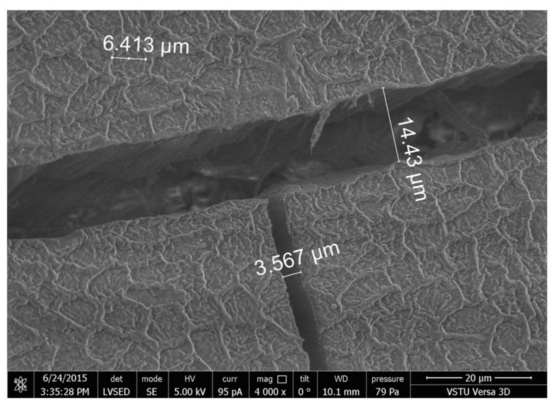

Figure 4. Cracks on the surface of the seed coat of G. triacantho.

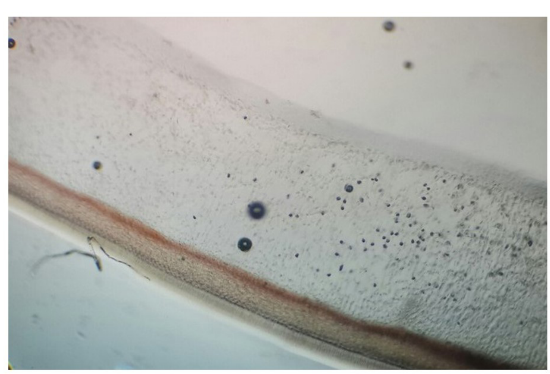

Figure 5. Seed coat of *G. triacanthos* with phloroglucinol reaction. Lignification has been identified.

coat of *G. triacanthos* includes lignified elements, since a dark crimson color appeared in the phloroglucinol reaction (Figure 5). The inner coat of the *G. triacanthos* showed an orange color after staining the seeds with sudan III, which confirms the presence of suberin in the seed coat (Figure 6).

The micrograph (Figure 7) shows the characteristic arrangement of seed coat cells. They are located perpendicular to the surface of the seed. Loosely located cells form cracks.

Despite the visual similarity of the two layers of the epidermis, an electron micrograph shows the difference between those layers, which is determined by microhardness. The underlying layer of the epidermis has a lower average hardness: M = 35.3 (34.2 – 36.5) MPa as shown in Table 1; p-value = 0.89 (horizontal (transverse) plane, lower layer of the epidermis). Cherry wood and pear wood will be similar in hardness. The processing of these materials is easy (Papulova 2014; Green et al., 1999).

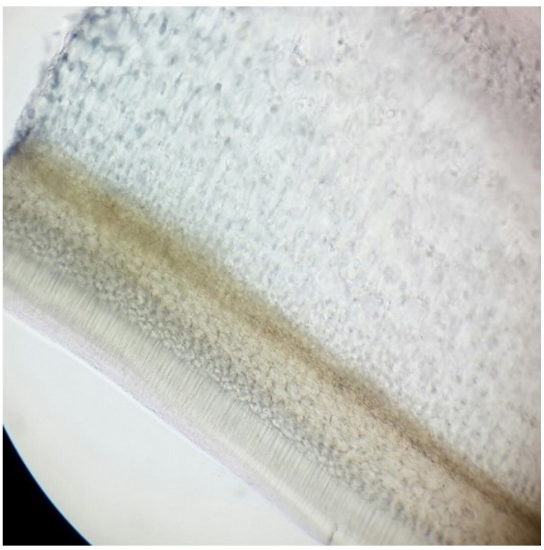

Figure 6. Seed coat of *G. triacanthos* treated with sudan III. Suberinification has been identified.

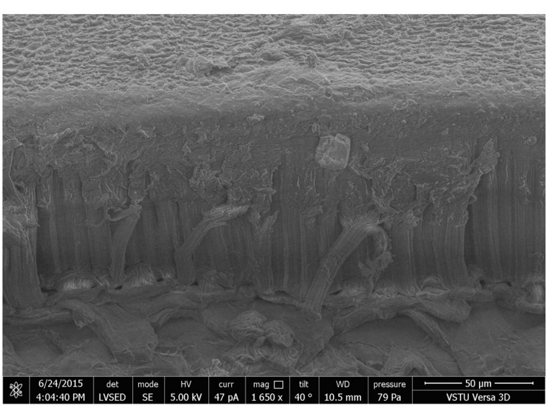

Figure 7. Two layers of palisade cells of the epidermis. Cracks are visible on the micrograph.

The differences between the hardness of the cells of the upper and lower layers of the epidermis (in the same plane) were assessed for two dependent samples by the Student's paired T-criterion. Its value was at the level of $2.2 \cdot 10^{-16}$, in the area of acceptance of an alternative hypothesis. The power of the Student's paired T-test with values Δ=95, standard deviation = 125, and a significance level = 0.05 was 99.8%; at a significance level of 0.001 with the same parameters, the power was 98.7%. The values used for the power check correspond to the values from the sample. It can be concluded that the differences in the mean values are significant between the two samples.

The microhardness of the lower layer of the epidermis in the frontal plane was M=38.9 (37.9 – 39.8) MPa as shown in Table 1; p-value = 0.38. The Student's T-test for dependent samples rejects the null hypothesis of a random difference in means. The microhardness of the upper cells M = 48.8 (47.7-49.9) MPa as shown in Table 1; p-value = 0.7; the microhardness of the lower cells M = 35.3 (34.2 – 36.5) MPa as shown in Table 1; p-value = 0.89. The score $2.07 \cdot 10^{-10}$ corresponds to the area of acceptance of the alternative hypothesis. The power test (Δ=35, Standard deviation σ = 75) at the significance level p 0.05 showed power 90.7%, and at the significance p 0.001 it showed power 48.57%. In the second case, the power of the test is small, but for a significance level of 0.05, the power is acceptable. We consider an acceptable test power equal to or exceeding 80%, which corresponds to a β-level of 20%. This level is a consequence of the so-called "one-to-four trade-off", between the levels of the α-level and β-level.

In the transverse plane, the differences in means of microhardness were up to 9.5 MPa, in the frontal plane they were up to 9.9 MPa. Perhaps this effect is due to a decrease in the amount of lignin in the cell walls while maintaining their similarity to the overlying layer.

There were no significant differences in microhardness between the epidermis and the underlying layer – parenchyma.

The measurement of the hardness of the parenchyma showed similar results to the lower layer of the epidermis: M=32.6 (29.2 – 34.7) MPa as shown in Table 1; p-value = 0.90 for the end section; M=35.6 (34.9 – 36.7) MPa as shown in Table 1; p-value = 0.89 for radial incision. In both cases, the null hypothesis of random differences in the samples was accepted.

Parenchymal cells morphologically differ from epidermal cells. They are inhomogeneously thickened, their walls consist of cellulose and pectin, located unevenly, especially at the junctions of cells. Filling with water, these cells provide a great impact from the inside on the overlying tissues: water slimes the cells, so the tissue macerates. Parenchymal cells contain a large amount of lignin (Balakina et al., 2021c).

The endosperm has a significant microhardness in the transverse plane M = 34.9 (31.3 – 38.5) MPa, p-value = 0.97. In the frontal plane, the microhardness is M = 33.2 (31.8 – 34.6) MPa as shown in Table 1; p-value = 0.95. The endosperm in *G. triacanthos* seeds is powerful, especially in the middle of the lateral side. It takes up to

1750 micrometers; the values are indicated for the seeds of plants of genera Erythrophleum and others. This is also true for *G. triacanthos* (Hegnauer 1957).

Endosperm cells are large, thin-walled, isodiametric or slightly radially elongated. The content of the cells in most species is mucilaginous by half or more, sometimes weakly. The inner rows of endosperm cells are obliterated. Aleurone layer is present. The outer surface of the endosperm is ruminated (Figure 1).

The cells of the aleurone layer consist mainly of fiber; cells are connected to each other by a substance, apparently not associated with fiber. Some authors who used a special technique to research the fine structure of the aleuron layer note the presence of small tubules or the thinnest protoplasmic strands between adjacent cells of the aleuron layer, as well as between the cells of the aleuron layer and adjacent cells of the starchy endosperm (Kobylyansky et al., 2021).

The microhardness of the cotyledon was M = 14.0 (13.1 – 14.9) MPa as shown in Table 1; p-value = 0.55 in the horizontal plane. Microhardness in the frontal plane was more than that one in the horizontal plane: M = 20.1 (18.7 – 21.6) MPa, at p-value = 0.85.

The germinal leaf also did not differ in high microhardness: M =15.4 (13.7–17.1) MPa as shown in Table 1; p-value = 0.36.

The microhardness as shown in Table 1 decreases from the upper layer of the seed coat to the lower one; it is very high and approaches the values of the microhardness of hardwood. Anisotropic properties are expressed slightly. The microhardness of the parenchyma and endosperm decreases, it is comparable to the microhardness of softwoods like cherry and pear wood. The microhardness of the embryo and cotyledons is lower than the microhardness of linden. Cotyledons have obvious anisotropic properties associated with the distribution of biopolymers and the orientation of the cell wall.

4. Conclusion

The upper cells of the epidermis in the transverse section are very heterogeneous. The average microhardness was 42.1 MPa. Most likely, the fluctuation in microhardness in the region of 27 MPa reflects the microhardness of the cracks on the surface of seeds, which forms the net on the surface of the seed coat, and which form a kind of deep cuts.

The upper cells of the epidermis in the frontal plane are well described by the normal distribution (0.7), and the average microhardness is 48.8 MPa. In both cases, such a significant microhardness can be partly explained by the deposition of lignin in spirals along the tall cell walls of the epidermis, and by the shape and arrangement of the cells.

The Student's T-test confirmed the non-randomness of the differences between the two planes (3.94 · 10-6).

The microhardness of the underlying epidermal cells corresponds to a normal distribution, with rather high p-value = 0.89 and 0.38, with average values of 35.3 MPa and 38.9 MPa in the transverse plane and the frontal plane, respectively. The T-test rejected the null hypothesis about the randomness of the mean differences between the upper and lower layers of cells in the corresponding planes. The average differences of means were 9.5 MPa for the transverse plane and 9.9 MPa for the frontal plane. It is possible that such a change occurred due to a decrease in the amount of lignin in the cell walls while maintaining their similarity to the overlying layer and vertical shape, but the reason for this phenomenon is not reliably known.

Parenchyma has microhardness 32.6 and 35.6 MPa in transverse and frontal planes, respectively. The values are close to the overlying layer. Both distributions are described by the normal distribution (p-value = 0.9 for both planes). Recent studies using the reaction with phloroglucinol have shown that these cells contain a large amount of lignin (Balakina et al.,2021b; Takhtadzhyan 1996).

5. Practical Recommendations

A comprehensive research of the structure of the seed coat of Fabaceae can be very important for the seed treatment. Dormant seeds of *G. triacanthos* have a hard seed coat. Pre-sowing seed treatment such a mechanical scarification is necessary for fast germination.

End microhardness for the upper layer of the epidermis is M = 42.1 (39.8-44.3) MPa, and radial microhardness for that layer is M = 48.8 (47.7-49.9) MPa. The values are more than the hardness of wood species such as birch (40.1) and larch (37.7). The microhardness of the upper layer of seeds of *G. triacanthos* is compared with the microhardness of wood like ash (*Fraxinus excelsior*), wenge (*Milletia laurentii*), pear tree (*Pyrus communis*) with hardness 40-45 MPa on the one hand, and maple (*Acer saccharum*), mahogany (*Swietenia macrophylla*), and walnut (*Juglans regia*) with hardness 50 MPa, or more, on the other hand.

As the hardness of the seed increases, the energy expended on effective mechanical action increases. The data of the hardness of the seed coat allows us to evaluate the behavior of seeds under force loading. Hardness characterizes the possibility of destruction of the material of the anatomical parts of seeds at the point of application of force. Equipment for mechanical scarification should be chosen based on the data provided.

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