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The Characterization of Anatomical Indices of Seed Coat Formation of Diploid and Tetraploid Representatives of the Genus Gossypium L

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Abstract: The biomorphological and genetic diversity of cotton that exists in nature includes about 50 species belonging to the genus Gossypium L. of the family Malvaceae Juss. Many of its representatives, and especially wild ones, are carriers of valuable traits and biological properties, but due to a number of objective reasons, they are little used in practical breeding. Of all the diversity, only some representatives of diploid species - G. herbaceum L., G. arboreum L. and tetraploid - G. hirsutum L., G. barbadense L. are used in culture and have become widespread. In modern breeding programs, much attention is paid to a comprehensive study and selection of source material. A large and valuable contribution, along with others, is made by morphological and anatomical methods for studying the vegetative and generative organs of cotton. The seed coat plays an important biological role during its germination, performs the functions of protecting the embryo, maintaining its viability, and also contains a number of valuable chemicals used in the national economy. The structure of the peel of the mature seed of wild-growing species, varieties and cultivated representatives has been studied quite fully, but its development in the process of seed maturation has been studied very little. The article presents the results of anatomical studies of the structure of the peel of ovules of different ages and mature seeds of wild and cultivated representatives of the cotton plant Gossypium L. A total of 16 diploid subspecies were studied. A comparative analysis of indicators of the structure and rate of formation of the integumentary layers of the seed coat was carried out to identify both distinctive and common features in wild and cultivated representatives of the genus Gossypium L. Diagnostically significant features were also identified. in the structure of the seed coat. In particular, the structural features of the hardness (strength) of the seed coat of the studied representatives were determined, which correlate with precocity and contamination of the fiber, which are of interest for genetic breeding work. And the level of development and systematic position of individual representatives of the genus Gossypium L.

Keywords: Cotton, Species, Anatomy, Seed, Spermoderm, Diploid

1. Introduction

As of today, ripening of cotton breeds and infestation of its fiber is one of the global problems on a world scale, causing great harm to the branches of economics. «At present, in the world the most widely used is textile fiber. At present its share in market is 56 percent, fiber infestation problem is one of the main».

In agriculture of our country during the years of independence wide-ranging reforms are held, meanwhile special attention paid to the gaining of early ripening and less infested breeds of cotton plant. On the ground of realized program measures in this area certain results are obtained, including the use of some representatives not by a long shot of the genetic potential of a genus, having useful economic

valuable features, as well as the preservation of the global gene pool of cotton for its further use in genetic and breeding works.

Morphological features of seeds useful for humans are not only scientific, but also of great agrotechnical and economic importance. Along with anatomical, they are widely used by botanists as taxonomic, in solving controversial issues of taxonomy, evolution and phylogeny of representatives of various taxa [4, 5, 12].

One of the actual problems is determination of predisposition to fiber defect-formation based on anatomical traits of species Gossypium L. genus and their introduction into practice. Determination of the anatomical features of generative organs of Gossypium L. genus for determination of fiber contamination and their use in practice is as follows: classification of systematics and phylogeny of species of Gossypium L. genus, study of the structural features of the generative organs development with the help of anatomical methods, identification of biometrical readings, rate of growth and development integumental layers; identification of diagnostically significant features in the structure of the seed coat [18].

Foreign scientists carried out research in the field of micro- and macro morphology of seeds as well as layered surface structure of a peel of a mature seed from various representatives of cotton, it was found that the successful use of LM/SEM technique assists in solution of taxonomy problems as well as genetic resources management of a genus [14, 3, 6]. In particular, it was determined the dependence of seed and gin spindle diameter, as well as amount of turns and the effect of these parameters on the fiber infections. T. R. Johnson, et al. studied the interaction of light, nutrients and carbohydrates on seed germination and early seedling development of Bletia purpurea (Orchidaceae) [16]. Ioelovich M. the structural indicators of cellulose were considered [8, 9]. Shahbandeh M. cited the statistics of various countries of the world on the production of cotton [15]. The biochemistry of the cell wall of cotton fiber was studied [7, 11]. Ioelovich M. established a correlation between cotton fiber performance and yarn quality [10].

The aim of the study is determination of signs of formation and rate of development of the seed coat of breeds for clarification the systematic position and evolution of *Gossypium L.* genus and the possibility of practical use of individual representatives in selection.

2. Materials and Methods

Plant Materials: The objects and methods of the study are wild subspecies of cultivated species (2n = 26), cultivars and breed-samples created on the basis of their subtropical subspecies: *G. herbaceum* (subsp. africanum, subsp. pseudoarboreum, subsp. frutescens, A-738 (90 days), A-833 (105 days), A-739 (135 days), A-184 (149 days)); *G. arboreum* (subsp. obtusifolium, subsp. perenne, subsp. neglectum, subsp. nanking, A-352 (85 days), A-361 (115 days), A-2802 (147 days), A-2845 (161 days)).

The experiments were carried out in 2005-2015. Seed material was obtained from the collection of cotton gene pool of Laboratory of Systematic and introduction of cotton IG & EBP Uzbek Academy of Sciences. We studied 50 plants of each breed and breed-sample grown on allotment areas of laboratory. Temporal fixation was performed for anatomical analysis (in 50% of ethanol) of ovules uneven-aged ovaries (1, 2, 3, 4-week-old and mature seeds). Anatomical investigations were performed according to accepted methods of R. P. Barikina et al. [1, 2], Grabovets N. V. [13], Zaitsev GN [17]. At conduction of research microscopes Avicon Tex and Leica ES3.

3. Results and Discussion

3.1. Cultivated Representatives of G. herbaceum

The genus Gossypium L. (family Malvaceae) includes about 50 species growing on different continents in subtropical and tropical zones. Most of the representatives are wild-growing diploid representatives (45), the rest are tetraploid. Of all the diversity, only 4 of its representatives are used in culture and are distributed - tetraploid G. hirsutum, G. barbadense and diploid G. herbaceum and G. arboreum, the so-called cultivated species.

The Republic of Uzbekistan is located in the northernmost area of observation of cotton growing (430 north latitude) and is not the birthplace of cotton, and therefore the most intensive and comprehensive research of this crop is observed here to create natural local varieties.

Great importance in systematics is given to the size, shape, surface and color of the seed, as well as various kinds of appendages. When studying the morphology of cotton seeds, special attention was paid to the shape, degree and nature of pubescence, the absence or presence of down, color, and the structure of the hairline, since these features, according to many scientists, are considered more conservative than size. In the literature on cotton, when characterizing seeds, the terms are more often used - large, medium, small, sometimes the sizes of mature seeds are indicated, which, as studies have shown, also play an important role in the diagnosis of taxa of the genus Gossypium. In the practice of cotton growing and selection work, the main attention is paid to the signs of size and weight of seeds, pubescence, color and length of the fiber. There is no information about the change in the size of the ovaries and ovules that occurs during the development of the seed.

An active development and growth of ovule integuments begin from fertilization moment, and differentiation into separate layers marked by us in all 2 weeks-old age (from blossoming). Each integument - outer (OI) and inner (II) consists of 3 outer layers- (OE) and inner (IE) layer epidermis and parenchyma (P) located between them in all studied representatives (see Figure 1). Breed-samples of G. *herbaceum* species, possessing the general plan of structure of seed peel differ from each other in terms of quantitative readings of structural signs, rate and duration of growth of integumental layers, the percentage of tissues in the ovules of

different ages in all stages of development. The epidermal layers of the outer integument (OEOI, IEII) and internal (IEII) are monostichous. OEOI cells increase in size, their walls are thickened and lignified. Parenchymal layer of outer integument (POI) 3-4 or 4-6-rowes depending on the sample, a great number of rows (6) in late-ripening breed-sample A-184. The numbers of rows in the parenchyma in the process of development of integuments in this layer do not change. The cells of all layers of the outer integument are developing at unequal speeds and thus reach a maximum value at different times. Cells of parenchymal layers differ with higher rate of development. In mature peel they become elongated in the tangential direction of the form in all studied representatives. The walls of the cells of all integumental layers are thickened and lignified. The outer integument at the initial stage of development thicker in late breed-sample A-184, and in the peel of the mature seeds in early-ripening breed-sample A-738.

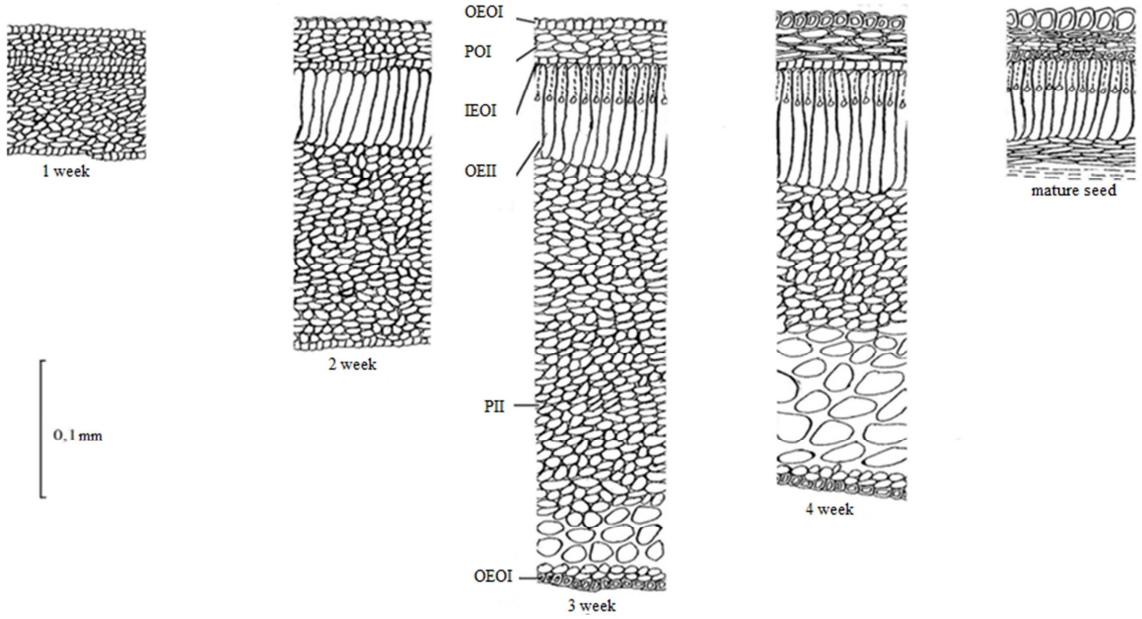

Figure 1. *A structure of integuments of uneven-aged ovules and ripe seed peel of A-738 (G. herbaceum L.).*

Age-specific alterations more intensively occur in the inner integument, whose thickness is always greater than the outside. The outer epidermis (OEII) – represented by a single-row palisade layer, which cells in the development process rapidly increased in the first 2-3 weeks in the radial direction and reach the greatest height at the time of maturity. In early-ripening and middle-maturing breeds they increase in 10-12 times, and the late-maturing A-184 in 15.5 times. The growth rate of the cell layer higher, in early-ripening breed-samples A-738, A-833, and the height (thickness) in the late-ripening breed-sample A-184.

Parenchymal polystichous layer of inner integuments differ from thickness during in all development time as compared to the other layers and the most intense cell growth up to 2 weeks of age. The maximum thickness of this layer reaches in all samples in 3 weeks of age. In early ripening breed-sample A-738 in this age parenchymal layer thicker than late ripening breed-sample A-184, in the peel of the mature seeds- in the late ripening. The number of parenchyma cells' rows in the development process is changed. In the integuments of 2 weeks-old they form 15-20 rows at ripening (A-738, A-833) and 18-21- at late ripening (A-739, A-184) representatives. By the time of maturity, a number of rows is reduced to 3-4 times due to started with 3-week-old process of degradation of the lower layers of cells, adjacent to the inner epidermis. As a result, the thickness of the parenchymal layer decreased to 14 times in precocious breed-sample A-738 and 8.5 in late-ripening ones. The destruction and reduction processes of parenchymal layer are more active in early ripening (A-738, A-833) and in middle ripening (A-739) breed-samples. Cells of this layer are different from other by high speed of up to two weeks of age and especially in late ripening A-184, and subsequently from precocious breed-samples A-738. In general, early ripening breed sample A-738 significantly differs from the mid-season A-739 by large thickness of all integumental layers in the peel of the mature seed and has a lower palisade indicator. Late-ripening breed-sample A-184 differs from other with the greatest thickness of the peel and its palisade parenchymal layers (see the Table 1) and a high rate of development in the early stage (from one to two weeks-old). Cells of monostichous layer of the inner epidermis II breed-sample differ little on quantitative indicators of their height in the development process and as compared to cells of other layers have the lowest indicators of growth rate.

In percentage the palisade layer of integuments one-week ovules takes in all samples 4-5%, in the peel of mature seeds is much more (see Table 1). Parenchymal layer in the inner integument of week-old ovules takes 64% in early ripening breed-sample A-738, in the skin of mature seeds - 16% in late- ripening A-184 — 57% and 21%, respectively. Along with the growth of integumental layers cells sclerification

and lignification of the cell walls occurs, and the pigmentation cells of all layers and particularly the outer integument, promoting the formation of a solid and durable peel ripe seeds.

3.2. Wild Growing Representatives of G. herbaceum

Comparative analysis of wild growing representatives and breed-samples of G. *herbaceum* species revealed significant differences of quantitative readings of analyzed traits, both within each group and between them (see the Table 1). Cells of the outer epidermis of the outer integument reach the maximum height in the representatives of the two groups to 3-4 weeks (see Figure 2). In the peel of mature seeds, a height of cells of this layer varies from 33.5 to 51.5 micrometer in wild growing, from 22.5 to 44.5 micrometer for samples. The parenchyma of the outer integument (POI) in wild growing subspecies consists of 3-4 rows of cells, in breed-samples — 4-6. The greatest height of this layer reaches to 2 weeks (*subsp. africanum, subsp. pseudoarboreum*), 3-weeks in *subsp. frutescens* and increases in the first two 1.3-1.8 times, the latter is 2.3 times. In breed-samples to the 2-week (A-184), 3-week (A-738, A-739) and 4-weekly (A-833), and its thickness is increased to 1.3; 1.1-1.2 and 1.7 times, respectively. Readings of thickness of this layer is higher in breed-samples compared to the wild growing species.

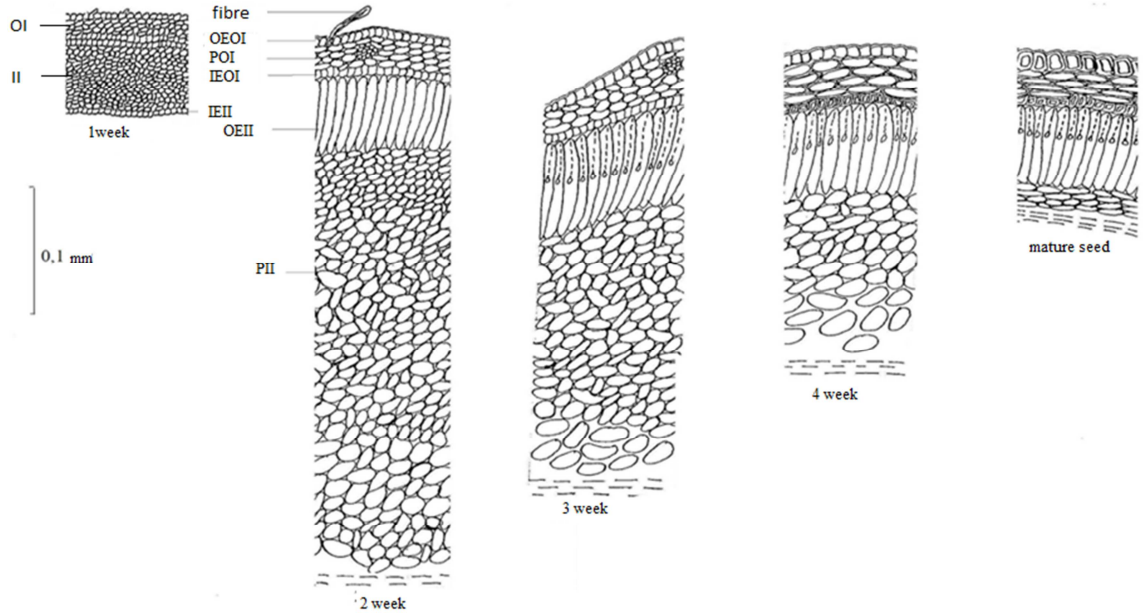

Figure 2. *A structure of integuments of uneven-aged ovules and ripe seed peel of subsp. frutescens (G. herbaceum L.).*

Wild growing representatives differ as well, by less readings of the height of cells of the inner epidermis of the outer integument (OEOI). The cells of this layer have the lowest rates of growth rate, especially in the wild growing ones. In the inner integument (II) palisade layer (OEII) is different from others high rate of its thickness in the peel of mature seeds, and especially wild species, except *subsp. frutescens*, but this indicator is higher in cultivars. Multi-row parenchymal layers characterized by the greatest thickness and the speed of development, and especially up to two weeks. In all wild subspecies the maximal quantity of rows and thus the height of this layer is marked at 2 weeks (15-20) in the breed-samples in 3-week (17-20 rows). Readings of the thickness is higher in wild representatives - *subsp. africanum, subsp. pseudoarboreum*, in *subsp. frutescens* is considerably less. Destruction process leads to a significant reduction in the number of rows and consequently the layer thickness and the overall thickness of the integuments. In the wild representatives in the mature seeds are 4-5 rows, in cultivars - 5-8 ones. Remaining parenchymal layer considerably thicker and occupies a larger percentage of the thickness of the peel of wild growing except *subsp. frutescens* (see Table 1). The cells of inner epidermis of inner integument (IEII) are larger in cultivars than those of the wild growing ones. In the peel ripe seeds this layer is absent at all.

3.3. Cultivated Growing Representatives of G. arboreum

The results are stated of the study of breed-samples of different origins and degrees of earliness: A-352 (85 days), A-361 (105 days), A-2802 (147 days) and A-2845 (151 days) and representatives of the diploid wild Indo-Chinese species *G. arboreum*. Among the samples of *G. arboreum* species higher rates of thickness of the outer integument and parenchymal layer of the inner integument ovules 1-week-old in early ripening breed-sample A-352 (see Figure 3). With increasing of age of ovules differences are getting smaller, but at the time of maturity manifest themselves more clearly and distinctly different from each other in the total thickness of the peel of ripe seeds, the height of the outer epidermal cells of the outer integument, palisade and parenchyma layers (see the Table 1). In early ripening breed-samples A-352, A-361 marked the high altitude of the palisade layer and lower of parenchymal one. Late ripening A-2845 has a thin peel of

the mature seed, the outer epidermis OE and the lower height of palisade layer. The speed of cells development of the palisade layer is higher than early ripening breed-samples A-352, A-361. Parenchymal layer of the inner integument is multi-row and at an early stage of development consists of 5-10 rows – in early ripening and late-ripening 10-13. The growth speed of these cells is different and does not depend on the earliness of breed-samples, but the process destruction is more active in early ripening ones. The height of cells of the inner epidermis IE in the development process is not significantly altered. Active growth of these cells in height occurs within the first 2 weeks in early ripening and 3 week-in late-ripening breed-samples. A layer of these cells is missed in mature peel.

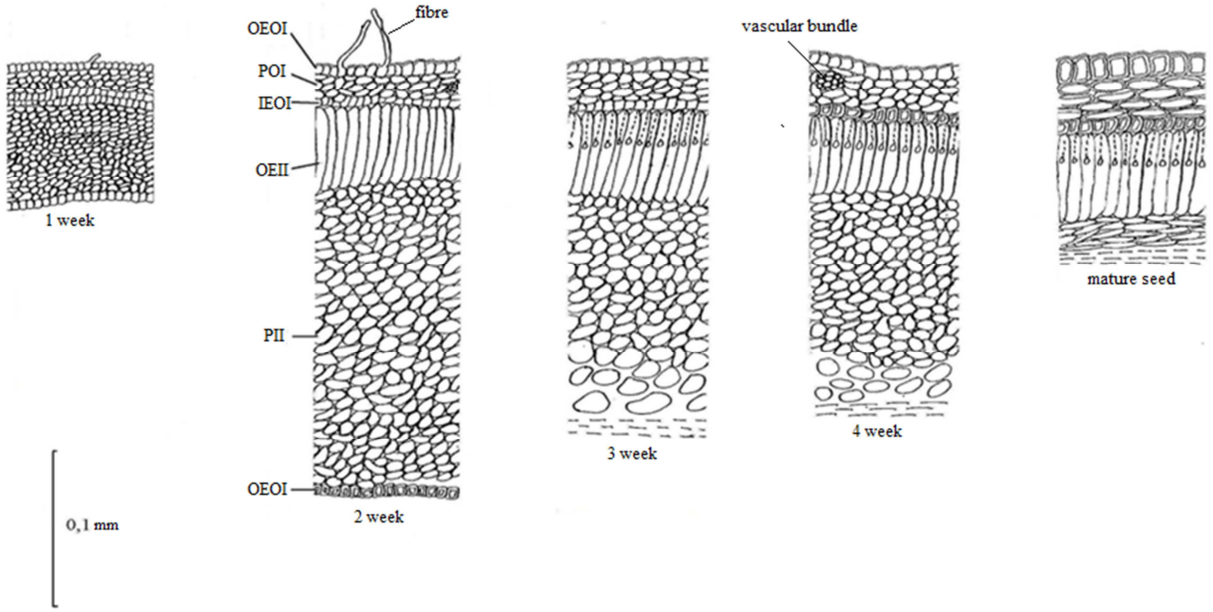

Figure 3. *A structure of integuments of uneven-aged ovules and ripe seed peel of A-361 (G. arboreum).*

3.4. Wild Growing Representatives of G. arboreum

Growth and development of integumental layers the cultural breed-samples and wild growing species of *G. arboreum* subspecies proceeds similarly. They differ from each other by biometric indicators of analyzed signs, the growth rate and development of the separate layers, ratio of tissues. In the peel of mature seed indicators of overall thickness of integuments, OI, its epidermal layer (OEOI), the height of the palisade and parenchymal layers OI higher in wild growing subspecies (see Figure 4 and the Table 1).

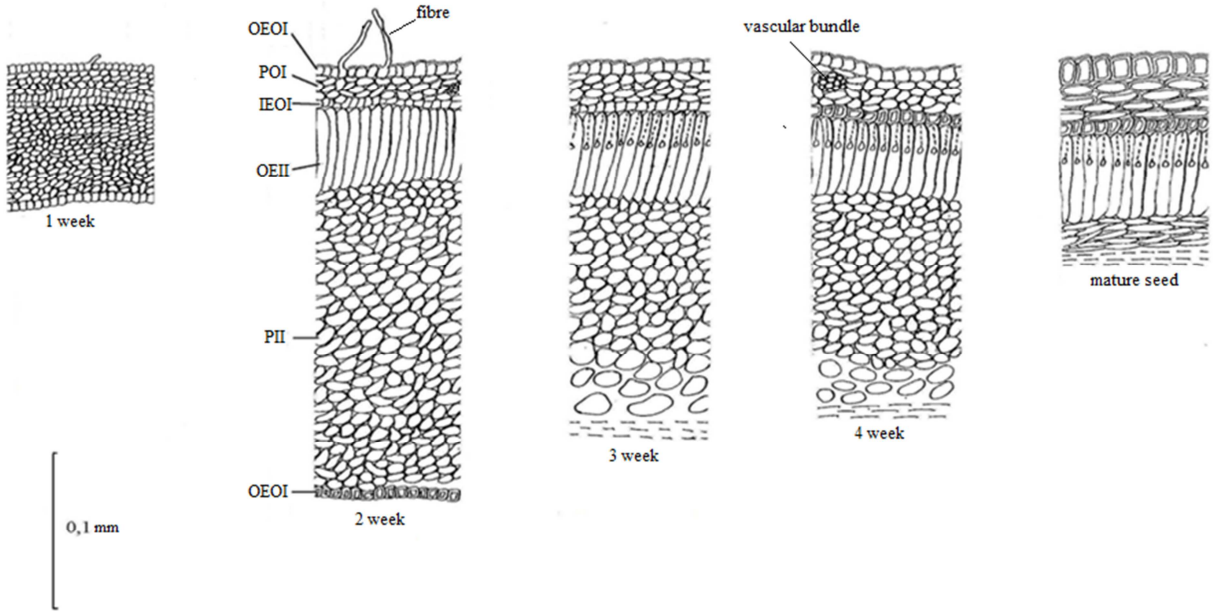

Figure 4. *A structure of integuments of uneven-aged ovules and ripe seed peel of subsp. obtusifolium (G. arboreum).*

Table 1. *Readings of structural signs of integuments of ripe seed peel of diploid representatives of Gossypium L, genus n = 10.*

Representatives	Total thickness of seed peel, µm	OI		OEOI, µm	IEOI, µm	OEII		PII	
		µm	%			µm	%	µm	%
G. herbaceum L.									
subsp. africanum	502,8 ± 37,8*	96,0 ± 8,5*	19,0	51,5 ± 2,4*	12,5 ± 1,2	236,0 ± 22,6*	48,0	170,8 ± 15,2*	34,0
subsp. pseudoarboreum	473,5 ± 36,8	111,5 ± 10,2	23,5	33,5 ± 2,9	11,7 ± 0,4	213,8 ± 19,6	45,1	148,2 ± 12,9	31,3
subsp. frutescens	284,0 ± 45,1	68,7 ± 6,3	24,2	34,3 ± 2,8	13,3 ± 1,3	175,5 ± 15,9	61,7	39,8 ± 2,9	14,1
A-738 (90 days)	364,9 ± 10,3*	104,9 ± 5,5*	28,7	44,5 ± 2,7	17,6 ± 1,1*	199,2 ± 2,9*	54,6	60,8 ± 8,7*	16,7
A-833 (105 days)	327,8 ± 6,4	91,7 ± 2,9	27,9	37,3 ± 1,8	22,2 ± 1,0	187,8 ± 4,1	57,0	48,4 ± 3,8	15,0
A-739 (135 days)	276,8 ± 3,8	57,5 ± 1,9	20,8	22,5 ± 1,3	15,6 ± 0,7	172,6 ± 2,6	62,4	46,7 ± 2,6	16,9
A-184 (149 days)	379,5 ± 10,7	81,7 ± 2,7	21,5	43,1 ± 1,4	15,1 ± 0,5	216,1 ± 4,0	57,0	81,8 ± 7,9	21,5
G. arboreum L.									
subsp. obtusifolium	415,1 ± 37,5	97,4 ± 9,1	23,5	42,1 ± 3,6*	14,0 ± 1,1*	243,6 ± 22,6	58,7	74,1 ± 6,9*	17,9
subsp. perenne	378,5 ± 34,3	84,4 ± 8,1	22,3	39,0 ± 3,5	12,5 ± 1,2	223,1 ± 18,4	59,0	71,0 ± 6,5	18,7
subsp. neglectum	450,9 ± 21,9	95,2 ± 9,1	21,1	36,7 ± 2,3	10,9 ± 1,0	209,8 ± 20,3	46,5	145,9 ± 11,8	32,4
subsp. nanking	413,6 ± 38,4	87,5 ± 6,3	21,1	51,3 ± 4,2	12,0 ± 1,0	227,0 ± 20,7	54,9	99,1 ± 10,2	24,0
A-352 (85 days)	303,4 ± 15,2	62,3 ± 5,0	20,5	25,4 ± 2,1	12,4 ± 0,8	198,0 ± 14,7	65,3	43,1 ± 4,4*	14,2
A-361 (115 days)	321,1 ± 28,5	64,9 ± 5,0	20,2	32,1 ± 2,5	10,2 ± 1,3	216,0 ± 20,3	67,3	40,2 ± 3,1	12,5
A-2802 (147 days)	314,0 ± 7,2	61,1 ± 4,0	19,5	29,9 ± 1,6	12,2 ± 1,2	194,9 ± 6,5	62,1	57,7 ± 3,5	18,4
A-2845 (161 days)	300,3 ± 10,1	60,6 ± 4,0	20,1	24,8 ± 1,5	11,7 ± 1,0	185,0 ± 7,2	61,6	54,7 ± 4,7	18,2

Note: ∑- total peel thickness;
Underlined representatives compared with each other on studied marks
*- existence of significant differences between readings of the compared representatives.

4. Conclusions

The integuments of uneven-aged ovules and a peel of ripe seed in all studied representatives have a common plan of structure that shows the phylogenetic relationship and monophyletic origin of species of Gossypium L. genus; differences of quantitative readings of structural signs, different speed and duration of growth of integumental layers indicate membership of a particular taxonomic group.

Common marks of formation and development of the seed peel for wild species and cultivars of different ploidy are: differentiation of structure of one-week ovules on integumental layers; active growth and development in the first two weeks; different growth rate of integumental cell layers; large thickness of the inner integument cells compared with an external, due to the palisade layer, reaching the highest value to ripening; Multiserial of parenchymal layer II and high-rate of growth of its cells in the first few weeks; destruction of inner rows of parenchymal layer, resulting in a significant reduction in its thickness and its number of rows; different levels of thickening of the walls of integumental layers.

Diagnostically significant marks in the structure of a peel of wild growing diploid species are: the peel thickness, external integuments, crystal- bearing cells (IEOI), the palisade and parenchyma layers of the inner integument. Thickness of a peel of ripe seed correlates with the duration of period of germination in representatives of G. herbaceum L. species.

Firmness (strength) of the seed peel due to cell size and wall thickness of the outer epidermis, height of palisade layer, ratio of mechanical parenchymal tissues, the degree of pigmentation of integumental layers, sclerification and lignification of cell walls.

The thickness of the mature seed peel is not always an indicator of early ripening breeds and more dependent on the rate and duration of cell growth of integumental layers.

It was specified a degree of evolutionary advancement and systematic position of individual taxa. The readings of primitiveness are small-seediness, high thickness and density of the peel, conservativeness of structure, expressed in general plan of the structure of the seed peel.

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