

ISSN 2224-526X

ҚАЗАҚСТАН РЕСПУБЛИКАСЫ
ҰЛТТЫҚ ҒЫЛЫМ АКАДЕМИЯСЫНЫҢ

ҚАЗАҚ ҰЛТТЫҚ АГРАРЛЫҚ УНИВЕРСИТЕТИ

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ИЗВЕСТИЯ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН

КАЗАХСКИЙ НАЦИОНАЛЬНЫЙ
АГРАРНЫЙ УНИВЕРСИТЕТ

NEWS

OF THE NATIONAL ACADEMY OF SCIENCES
OF THE REPUBLIC OF KAZAKHSTAN

KAZAKH NATIONAL
AGRARIAN UNIVERSITY

АГРАРЛЫҚ ҒЫЛЫМДАР СЕРИЯСЫ



СЕРИЯ АГРАРНЫХ НАУК



SERIES OF AGRICULTURAL SCIENCES

4 (46)

ШІЛДЕ – ТАМЫЗ 2018 ж.

ИЮЛЬ – АВГУСТ 2018 г.

JULY – AUGUST 2018

2011 ЖЫЛДЫҢ ҚАҢТАР АЙЫНАН ШЫҒА БАСТАҒАН

ИЗДАЕТСЯ С ЯНВАРЯ 2011 ГОДА

PUBLISHED SINCE JANUARY 2011

ЖЫЛЫНА 6 РЕТ ШЫҒАДЫ

ВЫХОДИТ 6 РАЗ В ГОД

PUBLISHED 6 TIMES A YEAR

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Известия Национальной академии наук Республики Казахстан. Серия аграрных наук.

ISSN 2224-526X

Собственник: ООО «Национальная академия наук Республики Казахстан» (г. Алматы)

Свидетельство о постановке на учет периодического печатного издания в Комитете информации и архивов Министерства культуры и информации Республики Казахстан № 10895-Ж, выданное 30.04.2010 г.

Периодичность 6 раз в год

Тираж: 300 экземпляров

Адрес редакции: 050010, г. Алматы, ул. Шевченко, 28, ком. 219-220, тел. 272-13-19, 272-13-18

<http://nauka-nanrk.kz/agricultural.kz>

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News of the National Academy of Sciences of the Republic of Kazakhstan. Series of Agrarian Sciences.

ISSN 2224-526X

Owner: RPA "National Academy of Sciences of the Republic of Kazakhstan" (Almaty)

The certificate of registration of a periodic printed publication in the Committee of Information and Archives of the Ministry of Culture and Information of the Republic of Kazakhstan N 10895-Ж, issued 30.04.2010

Periodicity: 6 times a year

Circulation: 300 copies

Editorial address: 28, Shevchenko str., of.219-220, Almaty, 050010, tel. 272-13-19, 272-13-18,
<http://nauka-nanrk.kz/> agricultural.kz

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Address of printing house: ST "Aruna", 75, Muratbayev str, Almaty

NEWS

OF THE NATIONAL ACADEMY OF SCIENCES OF THE REPUBLIC OF KAZAKHSTAN

SERIES OF AGRICULTURAL SCIENCES

ISSN 2224-526X

Volume 4, Number 46 (2018), 5 – 12

UDC 577.175.12: 633.366

MRNTI 68.03.03

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**THE INFLUENCE OF GROWTH REGULATORS
ON ANATOMICAL STRUCTURE
OF SWEET CLOVER VEGETATIVE ORGANS
IN THE CONDITIONS OF AKMOLA REGION**

Abstract. The influence of growth regulators on vegetative organs anatomy structure of sweet clover, cultivated in the Akmola region has been studied.

The results showed that growth regulators (seeds treatment by: Lignohumate B super Bio; Hanse Plant Seedspor-C and Lignohumate BM potassium) increased the thickness of epidermis and primary cortex, the area of the xylem vessels and the size of parenchymal cells in the stem anatomical structure. The use of growth regulators changed the leaf anatomical structure. The vascular bundle area, the depth of sinuosity and the cells volumes of the upper and lower leaves epidermis increased.

Key words: sweet clover, anatomical structure, growth stimulators, stem, leaf, epidermis.

Introduction. The use of plant growth regulators could stabilize a high level of yield and quality of crop production. Determination of the application time and the correct concentration for treatment with growth regulators allow regulation of plant growth and development, improving resistance to unfavorable environmental factors, and, as a result could increase the yield and product quality.

The use of growth regulators is focused on the solution of specific tasks for the production of agricultural products, obtaining a given quality and quantity of products. In some agricultural sectors, as vegetable growing, fruit growing, ornamental gardening, the use of plant growth regulators has become a necessary agrotechnical practice.

The application of biological products leads to increased seed quality, activation of physiological and biochemical processes at first germination stages; stimulation of root formation and increased root system capacity. The observation showed increased the assimilation apparatus efficiency, the leaf surface area, and biomass growth, the photosynthetic activity of agrocenosis. The increase in growth rates under the influence of growth regulators improves the use of nutrients and increases the NPK content in the plant green mass. The combined use of bacterial preparations and growth regulators for seed treatment provides an increase in yield and biological resistance of plants [1, 2].

Humic acids are high-molecular substances with a different composition. The essential functions of humic substances in the biosphere are: accumulative; transporting; regulatory; protective; physiological [3]. When humic acids are used as growth stimulators the plant's physiological activity develops not because of humic acids, but because of salts with monovalent alkali metals and ammonium. This is because humic acids are insoluble and plants can not absorb the water. The salts of monovalent alkali metals, as well as ammonium and humic acids, are soluble in water and become available to plants [4,5]. The mechanism of stimulating by humic substances and their physiological effect connected with the

influence of humates on the energy metabolism of the cell, that leads to the activation of oxidative and photosynthetic phosphorylation processes and the enhancement of the protein-synthesizing system [6,7].

Researchers in Akmola region studied the effect of sodium humate on the yield and quality of spring wheat seeds (S.K. Memeshov, 2005) [8, 9], oil flax (A.A. Begalin, 2007) [10], buckwheat (A.A. Tlep-paeva, 2009) [11]. Based on the results of these researchers, it was established that sodium humate has a positive effect on plant growth and development, the photosynthetic potential of the plant, and a decrease in the water-use ratio. Under the influence of sodium humate, the number and dimensions of the conducting bundles in the anatomical structure of the stem and leaf, the thickness of the mechanical tissue, the size of the parenchymal cells and the number of their layers were increased. The yield and quality of grain are increased, the content of heavy metals in wheat grains is reduced [8-11].

The studies on the effect of growth regulators with microelements on the anatomical features of the sweet clover cultivated in Akmola region have not been carried out.

The working goal. To study the influence of growth regulators on the vegetative organs anatomical structure of sweet clover cultivated in Akmola region.

Methods of research. The object of research was sweet yellow clover variety Kokshetau 10. It was developed by the North Kazakhstan Research Institute of Agriculture and authors: U.M. Sagalbekov, S.Zh. Onalov, M.E. Kusainova, E.U. Sagalbekov. A complex hybrid variety created by the polycross method from the limited open pollination of biotypes from Alsheevsky, Kokshetau, Omsk early ripening and Siberian two varieties. The selection goal was the maximum yield of the vegetative mass, taking into account the growth power, tilling capacity, seed productivity, winter hardiness, drought resistance and quality of the forage weight [12].

The plant has a tap-root system, well developed with an expressed main root. Stems are upright, tall, roundish, with a height of 90-125 cm. Branchiness is good and even. The form of the bush is vertical. The tilling capacity is above average - 10-14 stems per bush. Leaf coverage is 42-48%, which is above the average. Egg-shaped leaves, large, green; stipules are filiform-subulate, widened at the bottom. The inflorescence is truss, fusiform, medium density. The color of the corolla is yellow. Pods are small, single-seeded, dark gray with a transversely wrinkled surface. Seeds are small, kidney-shaped, greenish-yellow. The weight of 1000 seeds is 2.1-2.6 g. [12].

Field experiments conducted in 2015-2017 in "North Kazakhstan Scientific Research Institute" LLP, Akmola region, Zerendinsky district.

Meteorological conditions of overwintering, growth, and development of perennial grasses for 2014-2015 should be considered as arid. During the vegetation period, there were 268.4 mm of precipitation at an average long-term norm of 327.2 mm. In 2015-2016 the total precipitation was 446.5 mm, which had a favorable effect on the growth of perennial grasses. This year, during the vegetation period there were 338.7 mm of precipitation, which should be considered at the level of the average long-term rate. Over the three study years (2015-2017), the first year is arid, the second year is favorable, and the third one at the level of the average annual data.

The soil is represented by chernozem with medium humus, with a depth of humus horizon of 25-27 cm and average humus content of 4.01%. There is 3.21 mg of nitrate nitrogen and 35.0 mg of potassium per 100 g of topsoil. Consequently, the content of nitrogen is high; the content of phosphorus is average; the content of potassium is high. The soil texture is heavy loam, the bulk weight in plough layer is 1.19 g/cm³, and about 1.30 g/cm³ in a meter-deep layer. Permanent wilting moisture is 12-13%.

Field experiments were in three replications. The applied agricultural technology was zonal. The area of the pilot plot was 15 m², the location of the plots was randomized. Forecrop is fallow tillage. Seeding date was 16th of May. Seeding was carried out by selective seeder; the seeding depth was 2-3 cm.

Method of seeding was in rows and wide-rows; row spacing was 75 cm. Seeding rate for the wide-row method was 8.0 kg/ha. The following three growth stimulators were used for seed treatment: Lignohumate B super Bio (2.5 ml/l), Hanse Plant Seedspor-C (1.0 ml/l), Lignohumate BM potassium (2.5 ml/l). Seed treatment by growth stimulators was carried out 12 hours before seeding, followed by drying.

Growth regulators were used for the preseeding treatment of yellow sweet clover seeds.

The experimental design included the following variants:

- 1 – control (water seed treatment);
- 2 – seed treatment by Lignohumate B super Bio;

- 3 – seed treatment by Hanse Plant Seedspor-C;
 4 – seed treatment by Lignohumate BM potassium.

Anatomical studies were carried out in the flowering phase according to the accepted method. To study the features of the anatomical structure, the works of M.L. Prozina and W. Braune were used. [13; 14]. Statistical processing of morphometric indicators was carried out by the method of G.F. Lakina (1990) [15].

Results of the research and discussions. In these experiments, the authors compared the vegetative organs anatomical structure of variety Kokshetau 10 sweet clover, selected from the control variant without growth regulators, with anatomical structures of the vegetative organs selected from the variants where growth regulators have been used.

Transversal section of Kokshetau 10 sweet clover stem consists of the epidermis, primary cortex, and central cylinder.

The anatomical structure of the stem associated with those essential functions that it performs. The stem connects such important vegetative organs as roots and leaves. It provides movement of water and minerals from roots to leaves and the movement of organic matter from leaves to roots [16].

The movement of these two mutually opposite currents of fluid is facilitated by well-developed conductive tissues in the stem. Strengthening of the stem is carried out by various mechanical tissues and their particular arrangement. Exodermis protect the plant from the adverse effects of the environment. The stem may have well-developed parenchyma tissue, where nutrients can be deposited. In addition to permanent tissues, the stems have formative tissues that ensure the growth of stem in length and thickness [16].

The anatomical structure of sweet clover stem has the following structure: three types of tissues - exodermis, parenchyma and conductive. The epidermis of the sweet clover stem has a relatively small number of stomata. The primary cortex is located directly under the epidermis. The outer layer of the primary cortex is the mechanical tissue of collenchyma; its cells contain chloroplasts. Chlorophyll-bearing parenchyma of the primary cortex lies under the collenchyma. The innermost layer of the primary cortex is endoderm. It is weakly expressed in the stems; its cells are filled with starch grains, which is why this layer was called starch sheath.

The outer layer of the central axial cylinder is a pericycle, which is often expressed by one or more layers of sclerenchyma of pericyclic origin. The rest of the central axial cylinder is filled with parenchyma, and fibrovascular bundles are located in one circle. Conducting bundle is open. In the transverse section, the presence of large fan-shaped conductive bundles surrounded by sclerenchyma was noted (figure 1). The bundles located close to each other, provide significant strength.

In the internal structure of the sweet clover stem selected from the control variant without growth regulators, the thickness of the epidermis is $7.3 \pm 0.66 \mu\text{m}$, the thickness of the primary cortex is $22.66 \pm 1.08 \mu\text{m}$, the area of the xylem vessels is $4.65 \pm 0.6 \times 10^{-3} \text{ mm}^2$, the dimensions of parenchymal cells of the core is $37.00 \pm 2.14 / 35.65 \pm 1.65 \mu\text{m}$ (table).

There is an increase in the anatomical structure of plants selected from variants using growth regulators. In the variant with the treatment by Lignohumate of Brand B, super Bio, the thickness of epidermis and primary cortex, the area of xylem vessels and the size of parenchymal cells of the core were increased, as well as the number of their layers.

Table 1 – The effect of growth regulators on the stem anatomical structure of sweet clover Kokshetau 10

№	Variants	The thickness of epidermis, μm	The thickness of primary cortex, μm	Area of xylem vessels 10^{-3} mm^2	Dimensions of parenchymal cells of the core
1	Control (water seed treatment)	7.3 ± 0.66	22.66 ± 1.08	4.65 ± 0.6	$37.00 \pm 2.14 / 35.65 \pm 1.65$
2	Seed treatment by Lignohumate B super Bio	8.66 ± 1.01	31.33 ± 1.01	5.11 ± 0.40	$37.01 \pm 2.36 / 37.96 \pm 3.15$
3	Seed treatment by Hanse Plant Seedspor-C	8.0 ± 0.88	27.33 ± 1.19	6.53 ± 0.98	$38.61 \pm 1.94 / 37.18 \pm 2.07$
4	Seed treatment by Lignohumate BM potassium	9.33 ± 1.07	32.0 ± 0.88	6.81 ± 0.96	$40.06 \pm 1.87 / 39.26 \pm 1.69$

An increase in anatomical parameters of the stem is observed on the plants selected from variants with Hanse Plant Seedspor-C seed treatment and Lignohumate BM potassium seed treatment (table, figure 1).

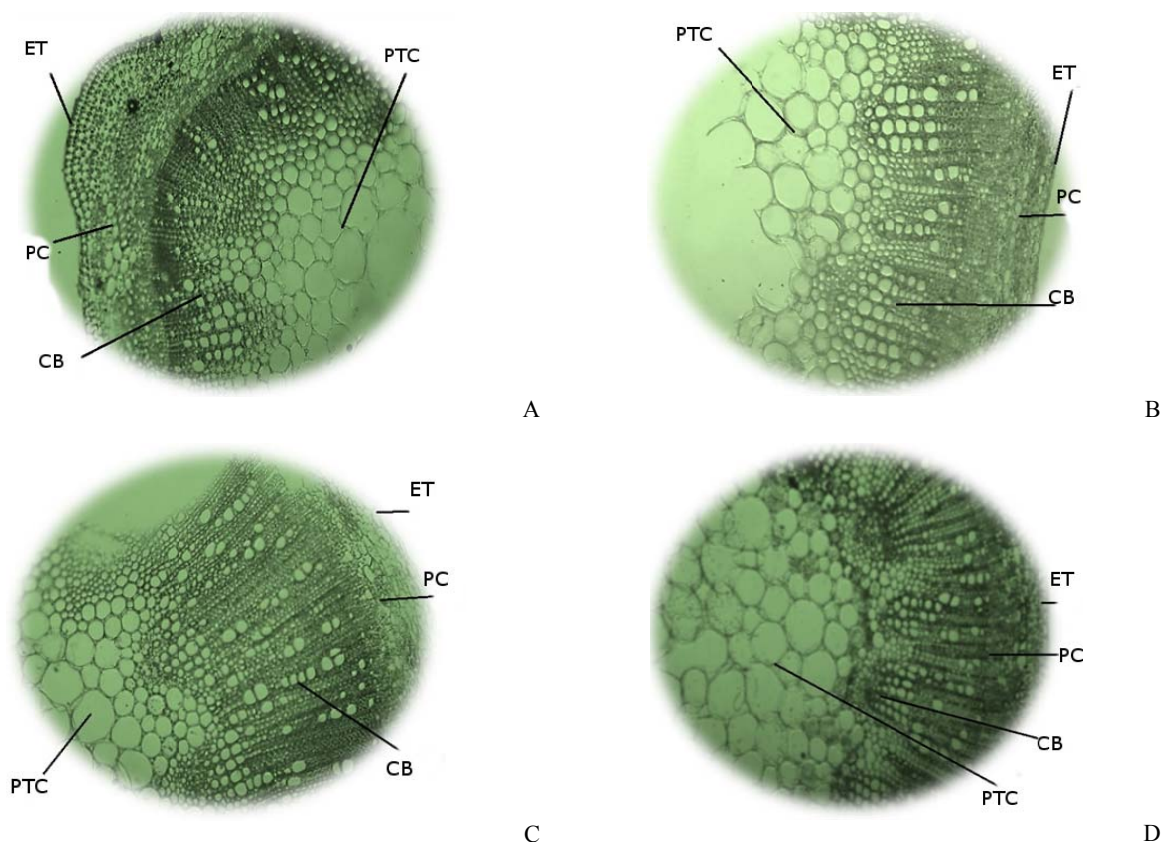


Figure 1 – The stem anatomy:

A – control (water seed treatment); B – treatment by Lignohumate (B super Bio);
C – seed treatment by Hanse Plant Seedspor-C; D – seed treatment by Lignohumate BM potassium.
et – epidermic tissue; pc – primary cortex; cb – conducting bundle; ptc – parenchyma of the core

Under the influence of growth regulators (seed treatment by Lignohumate (B super Bio); seed treatment by Hanse Plant Seedspor-C; seed treatment by Lignohumate BM potassium) the thickness of epidermis and primary cortex, the area of xylem vessels was increased; the size of parenchymal cells of the core and the number of their layers, too.

The strength of the clover stem is determined by the presence of a considerable number of collenchyma, as well as some conducting bundles. In our study, it was established that evaluation of lodging resistance, based on the study of stem anatomical structure, is useful for legumes, for which the only way to test lodging resistance was field assessment, which does not always makes it possible to determine the level of manufacturability in unregulated environmental conditions.

The anatomical structure of photosynthetic organs was considered on the example of middle leaves.

Cells of the upper and lower epidermis are round-polygonal, slightly sinuous; numerous stomata are located on both sides of the leaf blade, there are considerably more of them on the underside. The guard cells of stomata are surrounded by 3-4 perostic cells, the anomocytic type of stomatal apparatus. Along the broad veins, there is a crystal-bearing lining, which contains prismatic crystals of calcium oxalate. As a rule, small veins are not accompanied by a crystal-bearing lining. There are two types of hairs: common and 2-3 cell hairs.

The difference in form and volume of the upper and lower epidermis cells of the leaves was noted. In the variants with the use of growth regulators, the depth of sinuosity and cell volumes of the upper and lower epidermis of plant leaves were increased (figure 2).

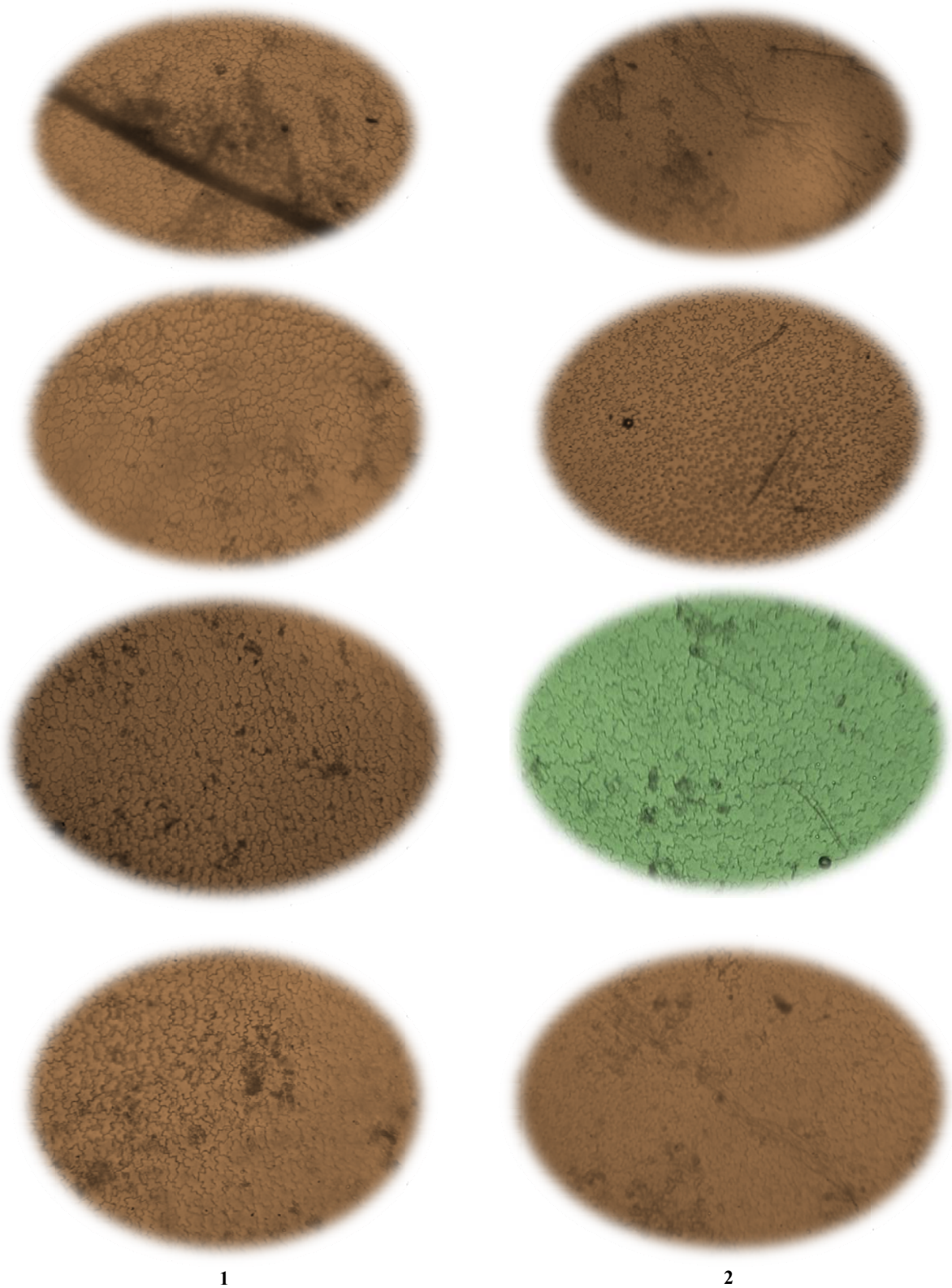


Figure 2 – Sample of Sweet clover leaf (x280):
A – control (water seed treatment); B – seed treatment by Lignohumate (B super Bio);
C – seed treatment by Hanse Plant Seedspor-C; D – seed treatment by Lignohumate BM potassium.
1 – epidermis of the leaf upper side; 2 – epidermis of the leaf bottom side

The transverse section of the leaf blade consists of exodermis, parenchyma, and conductive tissues system. The mesophyll is differentiated into the palisade and spongy parenchyma. The palisade tissue consists of cells elongated in a direction, perpendicular to the surface of the epidermis and it is represented by two rows of cells. The cells of the palisade parenchyma have a prismatic shape. The rest of the mesophyll consists of the loose spongy parenchyma. Conducting bundles are collateral; the xylem is located in the upper part of the leaf blade, phloem is located in the lower part. The mechanical tissue is located in the bottom and upper side of the conducting bundles. (figure 3).

The area of the central conductive bundle of the leaf blade, selected from the control variant during the flowering phase was $47.03 \pm 1.38 \times 10^{-3} \text{ mm}^2$. In the variant with the treatment of seeds by Lignohumate B Super Bio, it was $50.82 \pm 2.12 \times 10^{-3} \text{ mm}^2$. An improvement in anatomical parameters is observed in the variants treated by Hanse Plant Seedspor-C ($52.84 \pm 2.09 \times 10^{-3} \text{ mm}^2$) and seed treatment with Lignohumate BM potassium ($53.07 \pm 2.90 \times 10^{-3} \text{ mm}^2$).

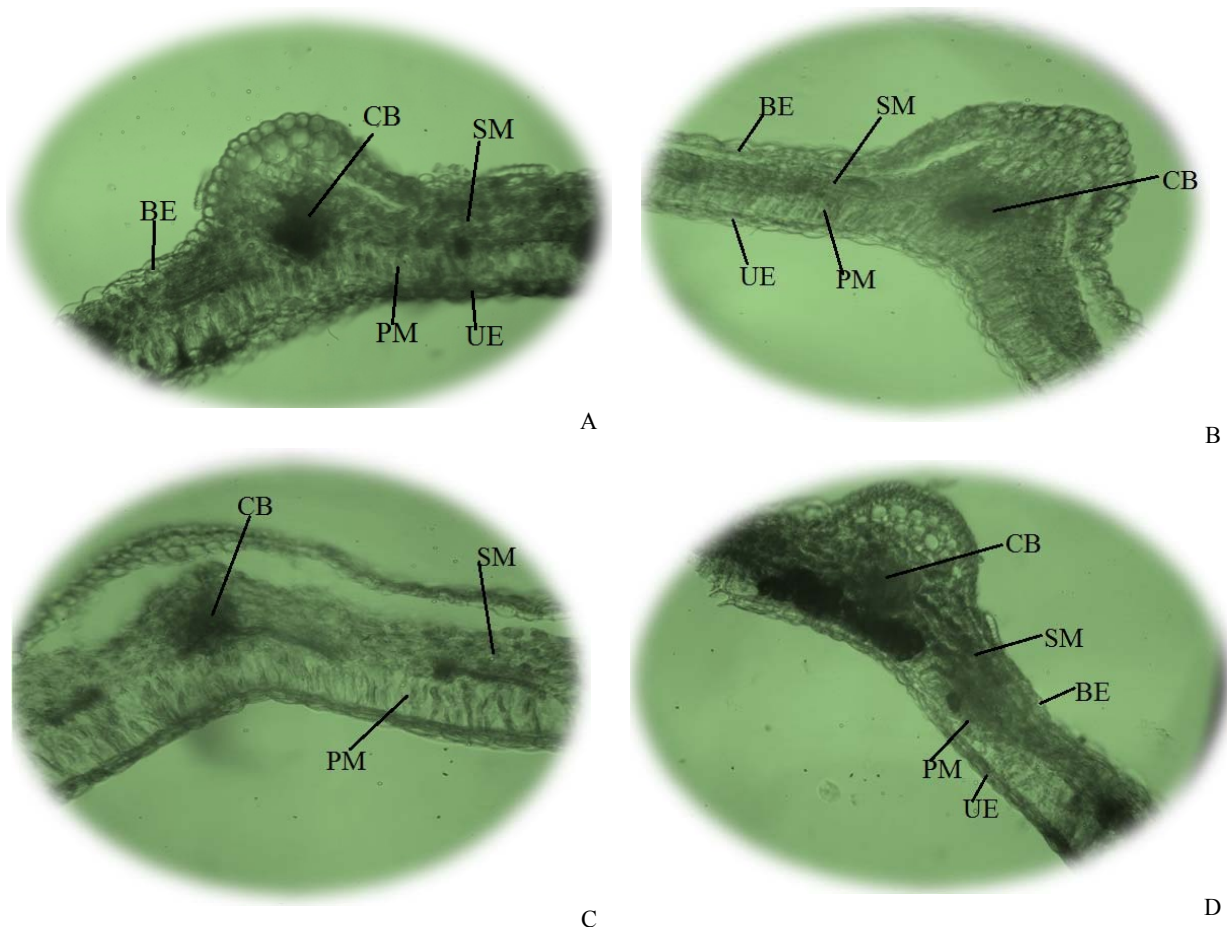


Figure 3 – The leaf anatomical structure

A – control (water seed treatment); B – seed treatment by Lignohumate (B super Bio);
C – seed treatment by Hanse Plant Seedspor-C; D – seed treatment by Lignohumate BM potassium.
use – upper epidermis; be – bottom epidermis; pm – palisade mesophyll; sm – spongy mesophyll; cb – conducting bundle

Conclusion. On the base of obtained results could be concluded that the area of the central conducting bundle increases under the influence of plant growth regulators.

The positive effect of plant growth regulators on the features of the anatomical structure of sweet yellow clover was determined. Under the influence of growth regulators the thickness of the epidermis and primary cortex, the area of the xylem vessels and the size of the parenchymal cells of the centrum, as well as the number of their layers were increased. In the anatomical structure of the leaf in variants with the growth regulators application, the conducting bundles' area, the sinuosity depth and the volumes of cells of the upper and bottom epidermis of plant leaves were increased.

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АҚМОЛА ОБЛЫСЫ ЖАҒДАЙЫНДА ТҮЙЕЖОҢЫШҚАНЫҢ ВЕГЕТАТИВТІК МҮШЕЛЕРІНІҢ АНАТОМИЯЛЫҚ ҚҰРЫЛЫСЫНА ӨСУ РЕТТЕУШТЕРІНІҢ ӘСЕРІ

Аннотация. Ақмола облысы жағдайында түйежоңышқаның вегетативтік мүшелерінің анатомиялық құрылысына өсу реттеуіштерінің әсері зерттелді.

Өсу реттеуіштерінің әсерінен (тұқымды Марка Б супер Био Лигногуматымен өңдеу; тұқымды Hanse Plant Seedspog-C өңдеу; тұқымды БМ калийлі Лигногуматымен өңдеу) өсімдік сабағының анатомиялық құрылысында эпидерма және алғашқы қабық қалыңдығы, ксилема түтіктерінің ауданы және өзектің паренхима клеткаларының мөлшері артады. Өсу реттеуіштері қолданылған варианттарда жапырақтың анатомиялық құрылысында өткізгіш шоқтардың ауданы, жоғарғы және төменгі эпидермис клеткаларының мөлшері мен клетка қабықшаларының иректілігі артады.

Түйін сөздер: түйежоңышқа, анатомиялық құрылысы, өсу реттеуіштері, сабақ, жапырақ, эпидермис.

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ВЛИЯНИЕ РЕГУЛЯТОРОВ РОСТА НА АНАТОМИЧЕСКОЕ СТРОЕНИЕ ВЕГЕТАТИВНЫХ ОРГАНОВ ДОННИКА В УСЛОВИЯХ АКМОЛИНСКОЙ ОБЛАСТИ

Аннотация. Изучено влияние регуляторов роста на анатомическое строение вегетативных органов донника в условиях Акмолинской области.

Под влиянием регуляторов роста (обработка семян Лигногуматом Марка Б супер Био; обработка семян Hanse Plant Seedspro-C; обработка семян Лигногуматом БМ калийным) в анатомическом строении стебля увеличиваются толщина эпидермы и первичной коры, площадь ксилемных сосудов и размеры паренхимных клеток сердцевины. В анатомическом строении листа на вариантах с применением регуляторов роста увеличены площадь проводящих пучков, глубина извилистости и объемы клеток верхнего и нижнего эпидермиса листьев растений.

Ключевые слова: донник, анатомическое строение, стимуляторы роста, стебель, лист, эпидермис.

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Редактор *М. С. Ахметова, Т. М. Апендиев, Д. С. Аленов*
Верстка на компьютере *Д. Н. Калкабековой*

Подписано в печать 22.05.2018.
Формат 60x881/8. Бумага офсетная. Печать – ризограф.
5,4 п.л. Тираж 300. Заказ 4.