

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Morphological and anatomical changes of Soybean under different conditions of cultivation

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ABSTRACT

The project work has the task of considering the impact of tobacco smoke on growth and development of soybean. As a result of the analysis of enhanced studying of morphological and anatomical structures of soybean, for the first time there have been determined changes in parameters of morphological and anatomical structures. Long-term experience and researches of anatomical structures of soybean show, that anatomic features, particularly the size of a xylem and phloem, are influenced by various conditions of cultivation. Based on the analysis, it is claimed that with size decreasing of the conducting bundle, a phloem ratio to a xylem changes, in control options, the xylem is usually bigger than a phloem, and under the influence of a tobacco smoke, the phloem exceeded a xylem. Special attention is paid on xylem ratios to a phloem. The article is devoted to concerns about influence of a tobacco smoke on morphometric characteristics of soy, which had been studied for a long time, and on the basis of the conducted research, the conclusion is that if you add various chemical compounds, the size of the conducting bundle decreases, but at the same time the size of a phloem increases.

Keywords: Soybean, morphology, anatomy, tobacco smoke, morphometry.

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INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is important leguminous crops in the world, continuous increase of its value in economy is caused by a complex of valuable properties and multi-purpose use [Vishnyakova M. A., 2004]. Due to a rich and various chemical composition, soy is widely used as a food, fodder and commercial crop. Soy is the most widespread, leguminous and oil-bearing crop cultivated in more than 60 countries on five continents in temperate, subtropical and tropical zones. The main world production is carried out by the USA, Brazil, Argentina, China [Kozyrenko M. M., 2007]

The genetic center of an origin of the culture is Northeast China. However, the width of its adaptation caused three centers of shaping: east African, Australian and South-East Asian [Kobyzeva L.N., 2009]. Types of the east African center are of value as ultraflorous condition source (up to 170 flowers), resistance to a drought, salinization of the soil, diseases of a bacterial and fungal origin. Selection value of types of the Australian center is not fully studied because of the difficulties during crossing them with cultural soy. However, they have in the genotype resistance to a drought, fungal and viral diseases, many-seeded features (up to 8 seeds in a bean) and low activity of inhibitors of trypsin in seeds. In the South-East Asian center, there is a considerable variety of soy starting from wild forms to sorts with a cultural morphotype is [Kobyzeva L.N., 2009].

It is considered that the ancestor of cultural *G. max* soy. (L.) Merr. was a wild-growing Ussuriisk soy - *G. soja* Siebold et Zucc., which was during many millennia exposed to both natural and artificial selection, mainly on selection-valuable features. Domestication of *G. max* from a wild-growing type of *Glycine soja* Sieb. et Zucc. occurred in China. Cultivation of soy extended from China to Korea, then to Japan about 2000 years ago, and further in other parts of Asia. Since 17th century, soy moves ahead to Europe, America. [Glazko V. Yu., 1999]

Modern selection sorts are presented by *G. max* (L.) Merr., which is subdivided into 4 subspecies: *gracilis* (Skvortsov) Teplyak., *max* C.O. Lem., *manshurica* (Enken) Teplyak. and *ligutata* (Skvortsov) Teplyak [Kobyzeva L.N., 2009]. These subspecies differ by the size and a form of leaflets, beans, thickness and branchiness of a stem. One of the main characteristics is fineness and color of seeds. Subspecies unite 64 versions, and also 86 approbatory groups [Kobyzeva L.N., 2009].

Extensive collections of genetic resources of the wild-growing and cultivated soy gather together, remain and get studied in many genetic banks of the world [Qiu L.J., 2013].

Studying of a genetic variety of soy is carried out with use of various classes of molecular markers – spare proteins [5], RAPD [4-5], ISSR [6-9], AFLP [10] and others.

Today soy is raised in many areas of Kazakhstan. Soy is a perspective product No. 1 in the southeast of Kazakhstan, therefore, profound studying of growth and development of soy is important.

The last 5 years we have been deeply studying morphology and anatomy of soy in cultivation under different conditions, soy undoubtedly remains the interesting object of our research, therefore, we carry out various experiments on influence of different substances on soy and we have decided to reveal changes of anatomo-morphological features of soy under the influence of a tobacco smoke and to define characteristics of a perspective sort of soy.

Research methods:

For the research we used soy seeds of the Dikovik variety. The experiment had begun on 10.02.2015. For the research we used 120 seeds and 24 plastic dishes. In each of them we placed 5 seeds and in four replications. Thereby we studied two options: control and under the influence of a tobacco smoke. Daily phenoobservations were carried out according to the standard technique. We had taken photos and measurements, defined viability and growth rate, watered the control option with usual water from the crane, placed the experimental option into the rooms for smokers and watered with wash of tobacco ashes. For research, we measured seeds of soy which average length was $1,4 \pm 0,6$; and width $0,9 \pm 0,8$. The first sprouts appeared on the 4th day after landing, and 100% germinability was showed only on the 7th day. The option under the influence of a tobacco smoke had grown the first. On the 18th day the plants were taken for research of anatomy.

For anatomic researches we did fixations in the ratio 1:1:1 glycerin: alcohol: water (according to the method of Strasburger - Flemming). For anatomic researches temporary preparations by the standard technique were prepared. Anatomic cuts of elevated bodies of plants were done by means of the MZP-01 microtome "Technom". Temporary preparations were put into glycerin. Thickness of anatomic cuts reached 10-15 microns. More than 100 temporary preparations for microphotography and for the morphometric analysis had been prepared. Morphometric measurements were taken by MCX100 microscope with a camera 519CU 5.0M CMOS Camera.

Review of literature

Tobacco smoke is heterogeneous aerosol which is formed as a result of incomplete combustion of a tobacco leaf. The tobacco smoke has a bad influence not only on humans, but also on plants. It influences cells of leaf scapes, causing premature formation of a separating layer and subsidence of leaves. Results of studying of influence of gaseous allocations of fruits and a tobacco smoke show, that the lower leaves fall down the first and after that the older leaves. It is connected with a big amount of auxin in young organs, leaves tightly hold onto spears and cells of separating tissues of scapes have a more slow reaction on increase of ethylene content [Light, Marnie E, 2015].

The phytogenesis smoke received from burning of plant material, as it was shown to stimulate germination of seeds and increases energy of a number of species of plants from fire, depends on the Mediterranean type of climate of the district. Water smoke consequences on germinations of seeds of 13 plant species from the southern tropical and subtropical monsoons of the climate of South China as it is reported, for the first time in this research were carried out in pots under laboratorial conditions. Among 13 types only *Aristolochia debilis* showed a considerable positive reaction on a water smoke at dilution 1:10 [to Zhou, JF., 2014].

The tobacco smoke was a target of numerous researches because of its harmful consequences for a human body. It was necessary to prove that there are both useful and harmful effects when the tobacco smoke influences plants [Zhao, Y., 2014].

Just like when plants are air purifiers, removers of carbon dioxide and eliminators of oxygen, they can also be affected by polluting substances, such as tobacco smoke [Bargmann, T., 2014].

Phytogenesis of smoke and some connections of smoke improve germination of seeds and raises growth of plants of many types. Thus, smoke in water and active connections of smoke have potential for use in various agricultural and gardening spheres. However, despite these interesting and potentially - practical properties, it is also established that such connections can represent risk for health if they are used in production of food or forage crops [Wang, Y; 2014].

Efficiency methods of smoke, the charred wood, ashes and heat in advance of germination were registered in the wide range of types and systems, but little was made on the fire signals under northern European anthropogenous fire mode conditions. We studied fire by checking the influence of water solution of smoke, ashes and a combination of two processings on germination of cereals. Greenhouse germination research compared banks of seeds from an old heathland (28 from the moment of the last fire) with seed banks from fresh heathland (burned down in previous year), as we expected, after fire there were germinations in lawns [De Micco, V; 2008].

Research was conducted for an assessment of ecological influence of polycyclic aromatic hydrocarbons in the subjects living in the field of utilization of electronic garbage in the Southern China and to investigate influence of a tobacco smoke on patients by means of active and passive smoking [Thomas, R; 2007].

The tobacco smoke unpleasantly affects not only plants. It contains more than 90 organic substances, many of which have stronger negative impact on a human body than nicotine. For example, the content of acetone in a tobacco smoke exceeds doses, admissible for a person by 74 times, the content of such active carcinogens as benzopyrene, styrene, exceed admissible doses by 1037 times, 2-methylpropene – by 513

times. There is also an ethylene among substances which are allocated at tobacco combustion. It influences cages of scapes of leaves, causing premature formation of a separating layer and subsidence of leaves.

Results of research of influence of gaseous allocations of fruits and tobacco smoke show that the lower, older leaves are the first to fall down. It is because young bodies have a big content of auxin, leaves strongly hang on sprouts and cells of separated fabric of scapes react more slowly to increase in the content of ethylene. It is established that transition of wood plants from active vegetation to winter rest is regulated by change of duration of the light period within a day. If in July in middle latitudes day length is 16–17 h, in September it decreases to 11–14 h, and in October – to 9–11 h [Hilaire, E; 1996, Xu D.H., 2002]. So far tobacco products contain about 4000 chemical compounds, and a tobacco smoke - about 5000 chemical compounds from which about 60 cause cancer.

Leaves from 181 soy variety comes from the fifth knot of an inoculated fungus and various plant extracts, waters and alcohol at 70%. For comparison, anatomy of the healthy and struck leaves is subjected to the analysis and leaf designs which were measured. In infected leaves, there was a destruction of epidermis and a lacunary parenchyma, distribution by the trich and denser cuticle, especially on an abaxial surface. There were also phenolic connections in the damaged cells of epidermis, because of growth of a mycelium. In the struck leaves processed by Nim, *Maytenus ilicifolia* and *Allium sowing* extracts, vegetable fabrics there was increase in thickness due to lengthening of cages [Koti, S; 2004, Mudibu J., 2011]

Soy is the universal and important agronomical culture which is grown up around the world. Every year millions of dollars of the potential income of a crop are lost because of an illness of the root decay caused by

Oomycota of *sojae* phytophthora. As a root of the main infection of the organism we undertook studying of physical and chemical barriers in soy roots, namely, suberization of epidermis walls and an endoderma, to establish whether the suberina can play a part in partial resistance to *P. sojae*. Here we will describe anatomic distribution and a chemical composition of a soy root of a suberin, and also its relation to partial resistance to *P. sojae*. Chemically, soy root of suberin reminds typical suberin, and consists of waxes, fatty acids, omega-hydroxy acids, an alpha, omega - bibasic acids, primary alcohols and guaiacyl- and syringyl-replaced phenols. [Feng, J; 2007]

Conditions of a microgravity of increase of space flight influences on morphology and metabolism in an etiolated soy growth to define, whether the clinostatting will similarly influence these processes, there were conducted land researches in combination with two opportunities of space experiment. Seeds of soy were put in BRIC (biological researches in a cylinder), canisters and grown up within seven days in 20C degrees under clinostatting conditions or in the stationary vertical mode. There were selected tests of gas per day, and reaped a crop of plants after seven days for measurement of growth and morphology. In comparison with stationary vertical elements of control, plants under clinostatting conditions are exposed to increase in length root (125%) and a bigger weight (higher than 42%) while length and crude weight decreased by 33% and 16% accordingly. The plants which are grown up under clinostatting conditions have twice more ethylene as stationary elements of control. The saplings processed by Triyod of benzole acid (TBA), Transport inhibitor auxin under clinostatting made 50% less than ethylene of the raw control with the same treatment of weight while treatment by means of 2,4-D is increased ethylene on five times in clinorotated plant. These data allow to assume that the slow clinostatting influences on division of biomass and production of ethylene in the etiolated plants of soy. [Merrifield, DL; 2009, Feng C., 2008]

Soy is important culture in the world, continuous increase of its value in economy is caused by a complex of valuable properties and multi-purpose use. Because of a rich and various chemical composition, soy is widely used as a food, fodder and industrial crop. [Abugaliyeva S. And., 2010]

Soy is the most widespread, leguminous and oil-bearing crop cultivated in more than 60 countries on five continents in moderate, subtropical and tropical zones. The main world production is carried out by the USA, Brazil, Argentina, China. [Xu D.H., 2003]

Today soy is grown up in many areas of Kazakhstan. Soy is a perspective product in the southeast of Kazakhstan, therefore, studying of growth and development of soy is actual.

RESULTS OF RESEARCH

The aim of the research is to study anatomo-morphological features of soy under the influence of a tobacco smoke and to define characteristics of a perspective soy cultivar.

For achievement of the aim it was necessary to solve the following problems:

1. To establish stability of soy on influence of a tobacco smoke under experimental conditions;
2. To give a formation assessment of morphological and anatomic features of soy cultivar which are grown up under the influence of a tobacco smoke.
3. To define morphological and anatomic changes of soy at cultivation under various conditions.

Novelty of work is that for the first time we defined influence of a tobacco smoke on an anatomic structure of vegetative bodies of soy cultivar.

As a result of research we can say that on the 4th day of research it was possible to see the first sprouts in option under the influence of a tobacco smoke. At identification of growth rate of a plant the option under the influence of tobacco exceeded by 2-3 times, than the control option. On the seventh day germinability of soy in two options was made by 100%, figure 1.



Figure 1- Germinability of soy



Figure 2- Comparison of influence of a tobacco smoke on soy



Figure 3- Influence of a tobacco smoke on growth and development of soy (11th-17th days)



Figure 4- Photographs of the research control option (11th-17th days)



Figure 5- Comparative analysis of soy morphology on the 18th research day

During the studying of soy morphology we observed that in option with tobacco height of soy reached on average $25,9 \pm 1,03$ cm, and in control option height of the plants reached only $13,7 \pm 0,64$ cm, these are nearly two times lower in comparison with tobacco, figure 5. Also, we noticed developments of a stalk in option with tobacco which length made $13,5 \pm 0,18$ cm, while in control option only a hypocotyl and an epicotyl had sprouted, which length did not particularly differ in both options.

According to phenoobservation results it was recorded that the size of leaf lamina exceeded by nearly 2 times and it was noticeable that plants under the influence of a tobacco smoke developed better than in the control option.

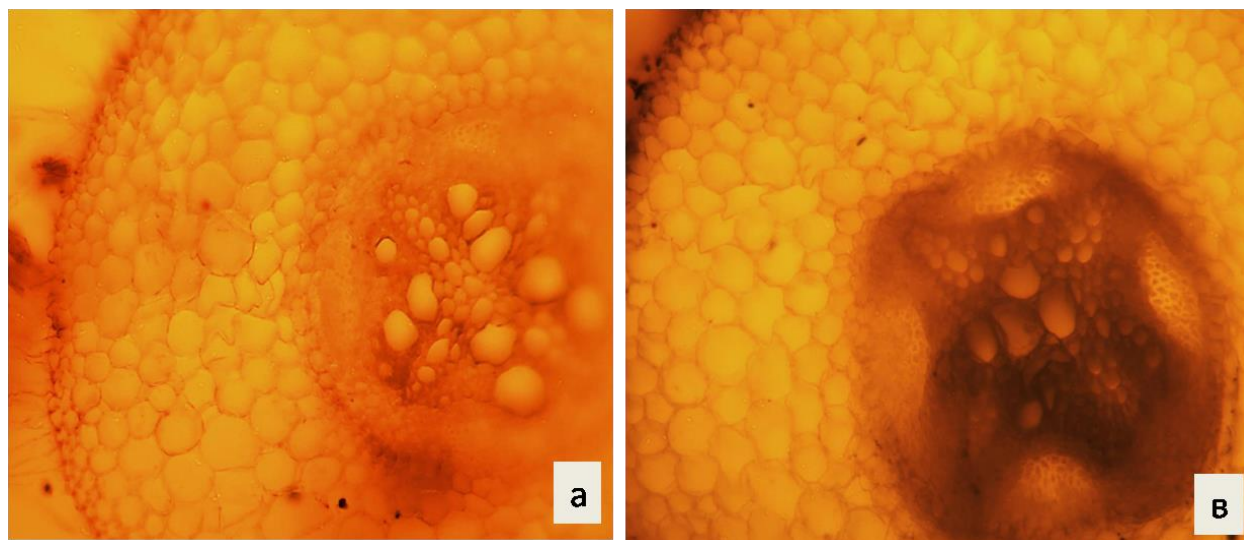


Figure 6- Anatomical structure of soy root; a-control option, b- option with tobacco

As a result of research of an anatomic structure of a root, it was revealed that root of Dikovik soy cultivar was initially tetraarchy, after some time in control option the quantity of xylary beams was 8, it is polyarch structure of a root, and in roots under the influence of tobacco it is considered tetraarch and the phloem in comparison with the control option is well developed. The root is covered with rizodermis and trichoblast, in control option diameter of a root is 1421,76 microns, primary bark - 445,44 microns, diameter of tube is 629,71 microns. Diameter of a root under influences of tobacco is 1509,78 microns, including primary bark of 440,29 microns and diameter of the tube of 637,91 microns.

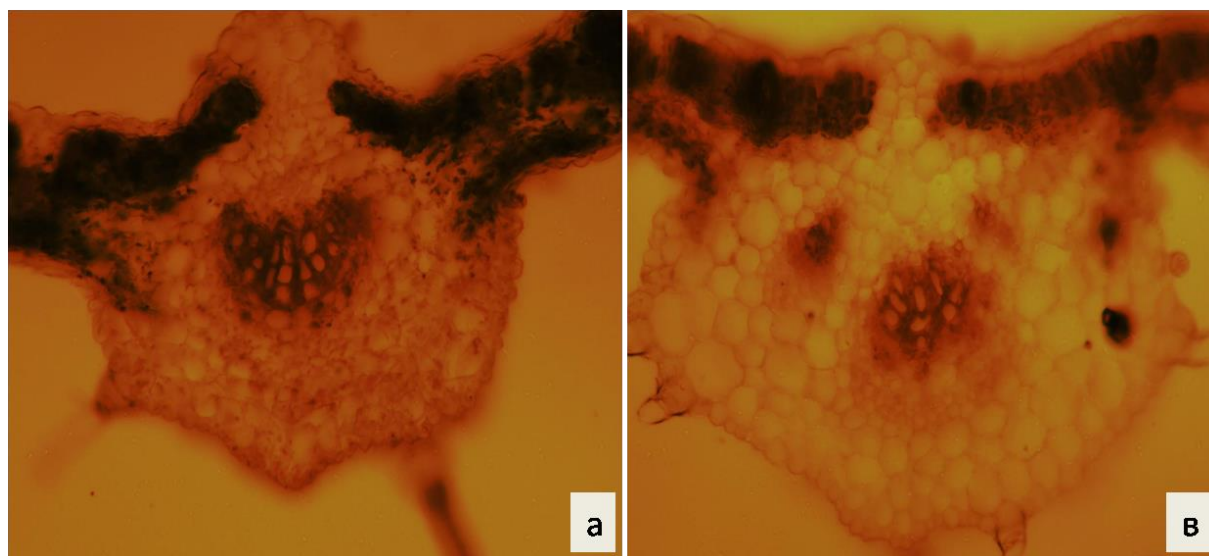


Figure7- Anatomical structure of soy leaf; a-control option, b- option with tobacco

Thickness of Lamina in the control option is 1711,47 microns, length of the average conducting bundle - 792,87microns, in control option there is one big conducting bundle in the central vein, in option with tobacco the conducting bundle is of the small size, but two more bunches were noticeable. In the central vein the size of the conducting bundle in option with tobacco is less than in control option, but a phloem ratio in comparison with a xylem significantly increased, therefore, we can claim that tobacco smoke influences the size and structure of a xylem and phloem.

The table 1-Morphometric indicators of a root and a leaf in various options, micron

Root	Control	Tobacco	Leaf	Control	Tobacco
Root diameter, micron	1421,76±0,16	1509,78±1,67	Midvein thickness	1711,47±0,03	1780,19±0,25
Cortex thickness	445,44±1,07	440,29±3,56	Length of average conducting bundle	792,87±1,27	576,06±0,97
Tube diameter	629,71±0,97	637,91±1,46	Thickness of average conducting bundle	700,29±1,23	745,85±0,95

It should be noted that the sizes of lamina in option with tobacco are bigger, but the sizes of the central conducting bundle is fewer. In option with tobacco, changes of an arrangement of xylary vessels in bunches, and their smallest quantity were also found.

Table 2-Morphometric indicators of a hypocotyl and an epicotyl in various options, micron

Hypocotyl	Control	Tobacco	Epicotyl	Control	Tobacco
Hypocotyl diameter	2099,03±1,02	2281,06±1,02	Epicotyl diameter	1978,47±2,02	2197,04±2,21
Thickness of conducting bundle	641,04±0,05	508,06±0,29	Thickness of conducting bundle	547,07±0,89	589,12±0,78

During the research, that is within 18 days, only in option with tobacco the stalk appeared unlike control option, therefore data in table 2 specifies comparative indicators of two options of a hypocotyl and an epicotyl, table 2.

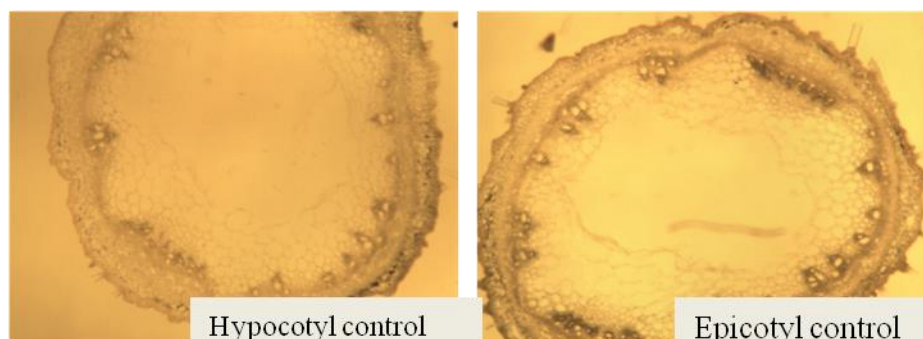


Figure 8- microscopic structure of soy stalk in control option

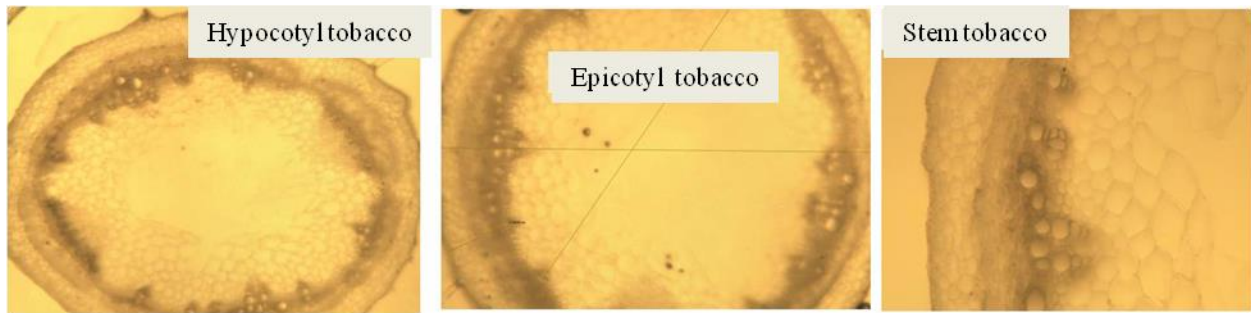


Figure 8- Microscopic structure of soy stalk under influence of tobacco

At morphometric measurement of a structure of a stem under the influence of tobacco the diameter was 2444,12 microns, width of the conducting bundle was 650,06 microns. Small bundles were seen on a cross-section of a stem, and also there were trichomes.

CONCLUSION

We can conclude that a tobacco smoke has an influence on soy and its development as there was established impact of a tobacco smoke in the growth increase than the control option. Studying morphology of soy, it is possible to note that emergence of the first shoots happened on the 4th day of research in option with tobacco. In both options the speed and viability of seeds were made by 100%. But in respect of growth and development the soy growing under the influence of a tobacco smoke exceeded on 2-3 times, than in control option. The soy grown under the influence of a tobacco smoke was the steadiest in relation to water, than control option. And also leaf plates under the influence of tobacco had the large sizes, thereby we can draw a conclusion that the tobacco smoke favorably influenced growth and development of soy.

The comparative analysis of an anatomic structure of a root of soy showed that morphometric measurements considerably exceed in option with tobacco, also leaf plate is bigger than in control option, but the sizes of the conducting bundles in the control option is bigger than with tobacco. In option with tobacco changes of an arrangement of xylary vessels in bunches and their smallest quantity were also found. Morphometric measurements of a phloem in leaves exceed in option with tobacco as the sizes of a phloem increase with various chemical connections in plants. During stalk research, it was revealed that the stalk was shown only in option with tobacco, and in both options there were a hypocotyl and an epicotyl.

According to this research it is possible to give an assessment that the tobacco smoke favorably influences on formations of morphological and anatomical structures of soy under laboratory conditions.

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