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PINE SEED GERMINATION UNDER WEIGHTLESSNESS (A STUDY OF THE KOSMOS 782 SATELLITE)

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16. Abstract Orientation of the above- and underground organs of pine plants, grown from seeds under weightlessness, was found to be determined by seed position on the substrate. Normal plant growth was observed only if the seed embryos were oriented toward the substrate. Some differences were noted between the experimental and control plants concerning the amount of nucleoli in the root meristematic cells and the cell shape in cotyledonous leaves. No complete similarity was found in experimental results obtained with plants under weightlessness and under compensated gravity (in horizontal rotation on a clinostat). The seeds were obtained from Pinus silvestris, considered to be particularly suitable for this experiment.

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PINE SEED GERMINATION UNDER WEIGHTLESSNESS (A STUDY OF THE KOSMOS 782 SATELLITE)

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One main and interesting problem in the growing of plants in weightlessness is ascertaining their spatial orientation, under growth and development conditions unusual for them. /770*

In study of this question aboard Biosatellite-2, on one year old pepper plants and wheat seedlings, variability of geotropic reactions was noted under 10^{-5} gravity.[1,2]. Weightlessness caused downward bending of the pepper leaf stalks (epinasty) and the spatial orientation of the roots and sprouts of wheat seedlings grown in a moist atmosphere and not in contact with a solid substrate changed. Moreover, the statolith starch granules in the cells of the test plants were distributed randomly, in contrast to their location on the lower cell walls of vertical control group plants [3]. In study of the effect of brief space flights (33-50 hours) on the germination, sprout formation and the first phases of growth of lettuce and peas, it was determined that the sprouts lose spatial orientation. However, this did not lead to a change in the mutual order and shape of the organs, tissues and cells, characteristic of seedlings grown in constant gravity.[4]. The tests conducted aboard Biosatellite-2 and aboard other space vehicles do not give a complete ideas of the initial geotropic reactions of the roots and shoots, or of the nature of plant growth in weightlessness. The objects used in these tests (tilted seeds, plant seedlings installed in the vertical position and intact plants) received the initial stimuli on the earth, and the briefness of the flight did not permit the plants to be formed in weightlessness.

We determined in 1974, in 21 day cultivation of pine plants from seeds oriented parallel to the substrate surface, in weightlessness aboard a satellite (Kosmos-690) that, for manifestation of the normal geotropic response, exogenous stimuli are required, i.e., this process does not occur in the absence of gravity [5]. The obligatory different directions of the roots and sprouts also was established. Moreover, it was shown in this experiment that the somatic cells in the test plant leaflets had a rounder shape than those of the ground controls. The conduct of additional tests was required, to establish the spatial corientation of the underground and aboveground organs of plants grown in weightlessness,

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*Numbers in the margin indicate pagination in the foreign text.

the anatomical characteristics of their support tissues and the conduct of cytological analysis of the nuclear structures of the meristem cells of the growing plants. This task was set up in the /771 present study aboard the Kosmos-782 satellite, with the cultivation of pine seeds in weightlessness, with different, but fixed locations of the embryos relative to the substrate surface.

Material and Method

Pine seeds (Pinus silvestris) were used as the object of study. Its selection was based on the fact that pine seeds germinate in only 5-7 days at a temperature of 20°, and the sprouts and leaflets grow slowly. Pine seedlings have a straight standing stem and main root stem, which permits their mutual location to be clearly ascertained. These qualities are extremely important for the conduct of this experiment. Slow root growth permits timely placement of the material aboard the satellite. Slow stem growth and the presence of chlorophyll in the seeds makes it possible to use this object for long-term experiments (1-1.5 months) in the absence of illumination.

The substrate for cultivation of the pine plants from the seed was agar (1.8%), wetted with distilled water. The seeds were treated with a 3% hydrogen peroxide solution and were placed in the agar, at a depth of 1.5 mm, in four positions: embryo up, down, horizontally to the left and right, 70 seeds each. Simultaneously with the test version, pine seeds were similarily planted in agar and used as a synchronous control.

The synchronous control was installed in a biosatellite mock-up, and it was for the purpose of differentiation of the effect of weightlessness from the possible effect of specific plant maintenance conditions aboard the biosatellite.

Still another version of the test was set up under earth conditions, in a horizontal clinostat, rotating at a speed of 2 rpm. This experiment was for the purpose of determining the extent of similarity of data obtained in weightlessness and in the clinostat. The vertically rotating container with the pine seeds in the clinostat was a control for horizontal rotation.

At the end of the flight, the plants grown in weightlessness were photographed at the landing site in a field laboratory. The germination and survival of the plants, morphological characteristics and spatial orientation of the aboveground and underground organs were recorded. The plants were then fixed in acetic acid-alcohol (3:1) and delivered to a fixed laboratory for further work. Acetocarmine "squash" preparations were prepared from the leaflets and root tips, for cytological and anatomical analysis. The synchronous control was processed according to the same scheme. A morphological analysis was conducted of the plants grown in the clinostat.

Results and Discussion

The plants grew normally (with roots in the substrate, and sprouts and leaflets in the atmosphere, only in the group where the seeds were oriented with the embryo vertically downward. The stems here were light pink, and they had 5-6 green cotyledonous leaflets each. The tiny roots (1-3 mm) were white, flexible and viable, but the longer roots (6-10 mm) turned brown and rotted. The angle of inclination to the surface of the substrate of both the aboveground and underground organs was 85-90°.

With the seeds located horizontally in the substrate, the plants grew parallel to its surface, but the roots and sprouts were in different directions. Some plants climbed into the atmosphere by the roots or the sprouts, at an angle of 30-35°. In distinction from the plants of the other two test groups, the sprouts here were completely white, and the leaflets had a less intense coloring.

In the synchronous control, with any orientation of the seeds, the plants grew with the shoots vertically upward into the atmosphere (they climbed above the substrate) and with the roots vertically downward (into the substrate). Positive and negative geotropism were distinctly expressed here. Geotropic bending of roots, which grew from horizontally oriented seeds and seeds located with the embryos at the top of the substrate, was noticeable in 2-3 mm long roots. Immediately after bending, they assumed the vertical position. In the group of seeds oriented with the embryo upward, some plants climbed into the atmosphere by the roots; but, in distinction from the test version with the same seed orientation, at various distances from the surface of the substrate, they bent downward and grew into the agar at a right angle. The shoot and cotyledon color did not differ in the test and the control. Long roots (6-10 mm) turned brown and rotted, but the very small roots (2-3 mm), as in the test material, were viable. The growth of pine plants in weightlessness and on the earth is presented in Fig. 1a and b, and it is represented schematically in Fig. 2.

Data on the number of growing plants in all test and control versions are presented in the Table.

The fact attracts attention that the number of growing plants is the greatest (almost 100%) in those test and control versions, in which the seed were oriented with the embryo upward relative to the surface of the substrate, despite the fact that it would seem, from the earth point of view, that this position is the most inconvenient for germination. It is possible that this circumstance is connected with better access of oxygen in this position to the roots, grown together with the growing shoots and, /772 consequently, with an improvement in respiration. It is not excluded that it is difficult for the roots to grow into the agar, because of its elasticity.

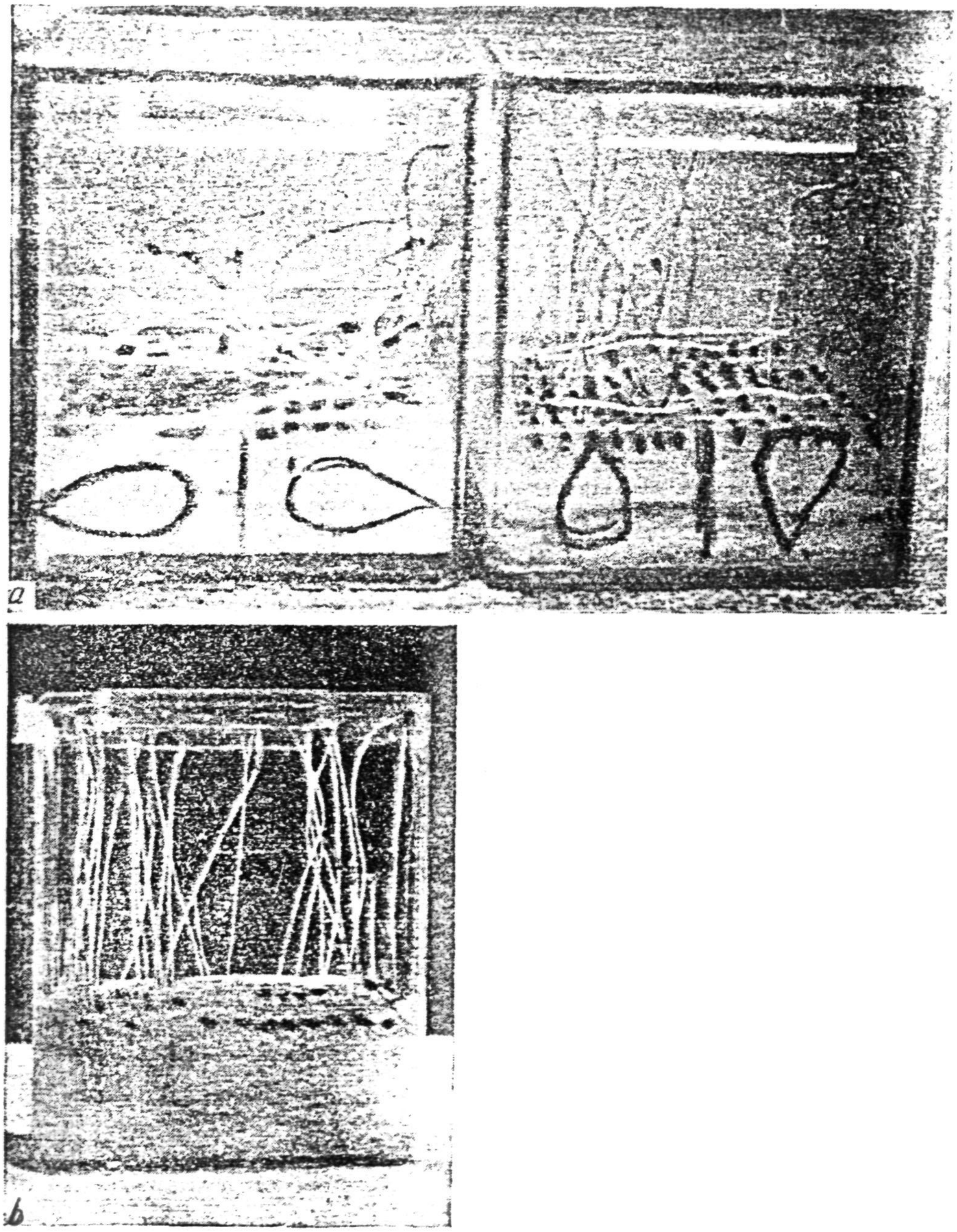

Fig. 1. Growth of pine plants in weightlessness (a) and on the earth (b), with various seed orientations relative to substrate surface.

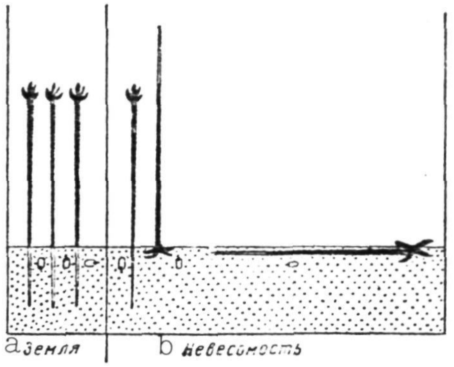

Fig. 2. Schematic representation of orientation of underground and aboveground plant organs in growing pine plants in weightlessness and on the earth.

Key: a. Earth
b. Weightlessness

PLANT SURVIVAL IN WEIGHTLESSNESS AND IN SYNCHRONOUS CONTROL

a Варианты (направление зародыша по отношению поверхности субстрата)	b Опыт		c Контроль	
	d Количество	$M \pm m$, %	d Количество	$M \pm m$, %
e Вверх	68	97,2±2.0	69	98.6±1.4
f Вниз	23	32.8±9.8	21	30,0±10.0
g Параллельно влево	27	38.5±9.3	23	32.8±9.7
h Параллельно вправо	27	38.5±9.3	32	45.8±8.0

Key: a. Version (direction of embryo relative to substrate surface)
b. Test
c. Control
d. Number
e. Upward
f. Downward
g. Parallel to the left
h. Parallel to the right

Inspection of the meristem cells in the pine root tips grown in all seed orientations has shown that the majority of the interphase nuclei in the control contained from 1 to 8 nucleoli. The modal class was the class with four nucleoli in the nucleus. The number of cells with one nucleolus was insignificant, 0.39± 0.17%. Interphase nuclei of the test plant cells contained 1-5 nucleoli. The modal class here was the class with 3 nucleoli. The frequency of cells with one nucleolus in the test was significantly higher ($P<0.05$) than in the control. It was 5.62±0.79%. The latter could have been connected with spatial change of the chromosomes, including the nucleolus forming chromosomes. The small number of dividing cells (2.1±0.5) hampers proof of this hypothesis. Although the mitotic activity was low in both the control and the test, it was noted that it was higher in those roots, which grew from seed located in the substrate with the embryo upward and parallel to its surface ($P<0.01$). The cause of this could be their more rapid growth. Both in the control and in the test, not a single cell with chromosome aberrations was found in /773 the dividing cells. In the majority of the plants grown in weightlessness, the cells in the leaflets had a rounder shape than cells in the leaflets of the synchronous control (Fig. 3a, b). Only 11.0 ±0.6% had corners in the test, while their occurrence rate in the control was 71.8±1.2%. A similar pattern was observed in the analogous material after the flight of the Kosmos-690 satellite [5]. Here, the frequency of cells with corners was still less. It was 3.53±0.9%. The change in cell shape found in the initial stages of development of the plants (in the cotyledenous leaflets) in weightlessness compels it to be thought that it is connected with the absence of pressure of the cells on each other. In this case, by obeying the laws of surface tension, the cells attempt to assume a spherical shape.

Analysis of the plants which grew for 21 days in the horizontally rotating clinostat showed the following. In the group in which the seeds were oriented with the embryos upward, the plants grew with the roots in the atmosphere and the shoots in the substrate. The slope of the aboveground and underground organs was ∿5-40°, and only one of the 23 growing plants grew at an angle of 90°. In the group in which the seeds were oriented with the embryo downward, 15 plants grew with the roots in the substrate and the stems in the atmosphere. The slope angle was ∿45-90° here. In the two horizontally placed seed versions (25 and 24, respectively), the plants grew partially creeping along the surface of the substrate and partially obliquely into the atmosphere ∿20-60°. Primarily the shoots grew in the atmosphere and the roots, only in two cases. Analysis of the plants which grew for 21 days in the vertical clinostat, with random seed orientation in the substrate; they grew with the roots vertically downward into the substrate and with the shoots vertically upward in the atmosphere (Fig. 4a, b). The plants grown in the clinostat in all versions had pale pink shoots, green leaflets and white roots. In proportion to root growth, they rotted.

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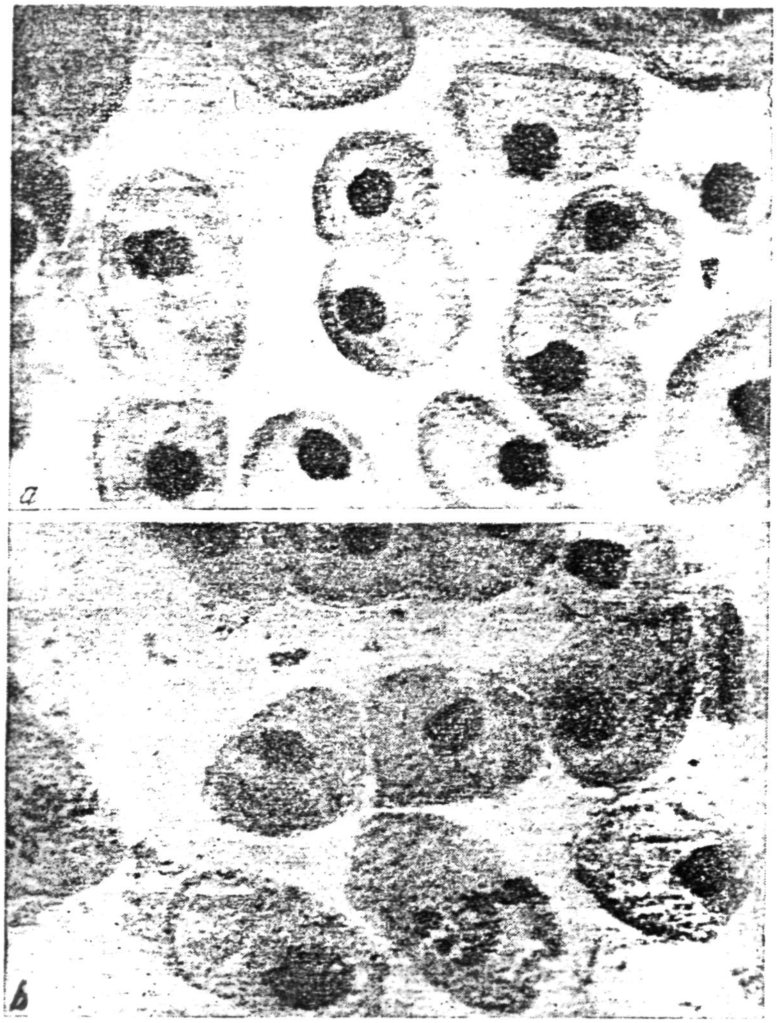

Fig. 3. Shape of cells in cotyledenous leaflets of plants grown in weightlessness and on the earth: a. test; b. control

In comparing the flight data with the data obtained in the horizontal clinostat, it is evident that the nature of the orientation of the seeds grown in various positions is similar. However, the results obtained in weightlessness are "cleaner." There were not exceptions in weightlessness and, moreover, the slope angles of the roots and shoots were closer (orientation, with embryos upward and downwards) to the vertical. Horizontal rotation of the clinostat at this speed compensates the force of gravity incompletely.

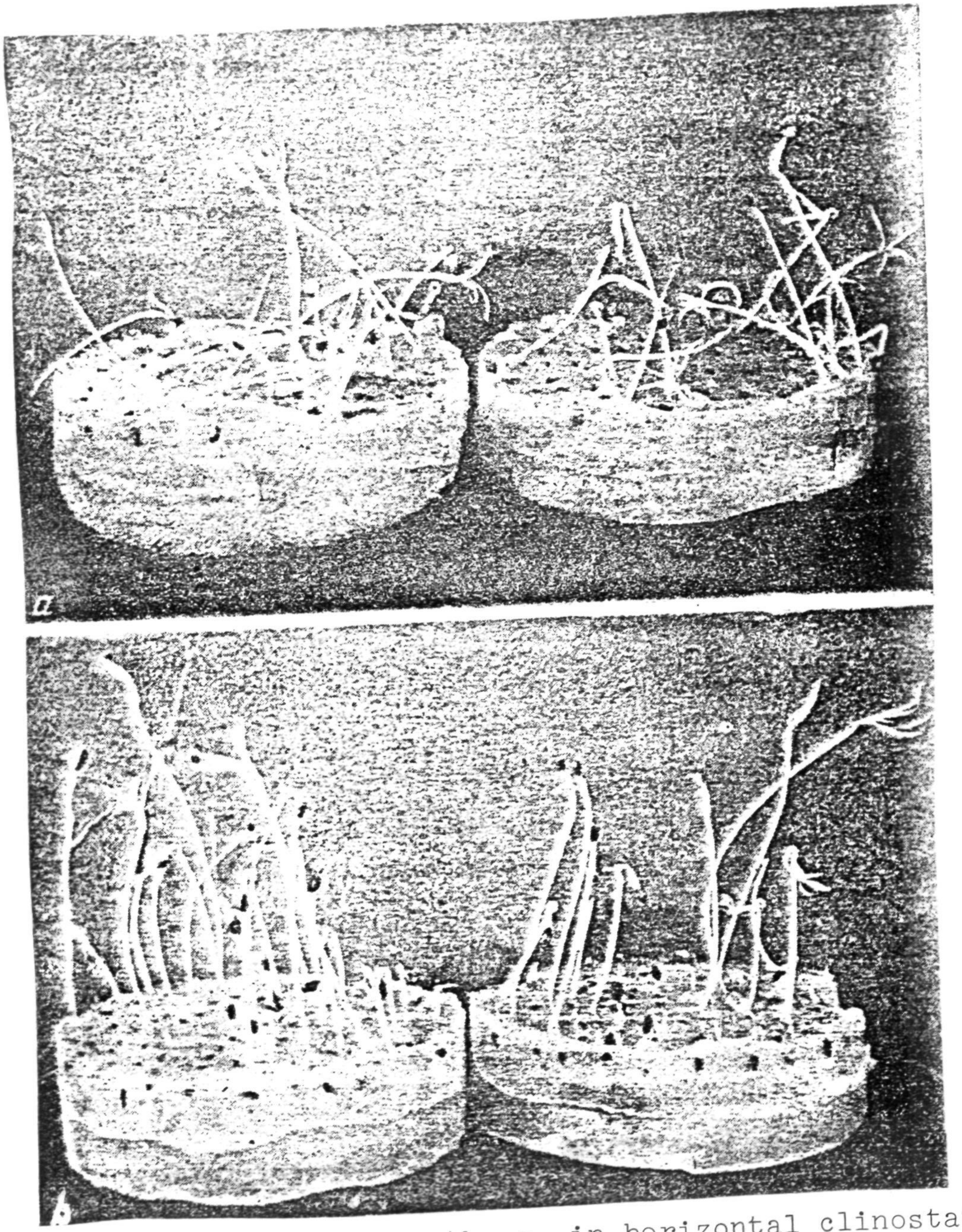

Fig. 4. Pine plant growth: a. in horizontal clinostat (test); b. in vertical clinostat (control).

The experiment showed that the orientations of the underground and aboveground organs (roots and shoots) does not occur completely like on the earth, in the absence of phototaxis. Plants can grow differently in weightlessness: with roots "down" into the substrate and shoots "upward" into the atmosphere; with the roots "upward" into the atmosphere and with the shoots "downward" into the substrate; parallel to the surface of the substrate and at various angles to it, in which both roots and shoots can climb into the atmosphere in this position. Such unusual growth and orientation in weightlessness was caused by the placement of the seeds with respect to the substrate surface. The roots grow in the direction towards which the embryo is oriented and the shoots, in the opposite direction along the embyronic axis. Under normal conditions on the earth, whatever the placement of the seeds in the substrate, the plants without fail will grow with the roots in the substrate and with the shoots in the atmosphere. Consequently, by removal of /774 gravity from the germinating seeds, the ability to display positive and negative geotropism natural to the majority of higher plant organisms disappears. On the basis of the experiments in the Kosmos-690 and Kosmos-782 satellites, it can be suggested that geotropism is not a genetically determined property, but is of an exogenous nature and can be changed with change in the living environment. Both experiments also show that the polarity of the direction of growth of the roots and shoots does not depend on gravity. The tests confirm that weightlessness affects processes which are induced upon exposure to it and for which there is a gravitational reception mechanism. It is thought that the geotropic reactions of plants are accomplished at the cell level. In all likelihood, the starch grains, amyloplasts, are gravity statoliths. The movement of the growth hormone, indoleacetic acid (IAA), which determines the direction of geotropic bending, is closely connected with their distribution in the cell. It must be thought that disorientation of plant growth in weightlessness occurs, as a consequence of the fact that the amyloplasts stop "working" as cell gravity receptors. Their delocalization can probably result in uniformity of IAA distribution, in connection with which the conditions for the perception of geotropic bending, necessary for normal plant orientation, disappear.

It can be thought that only those plants can grow and develop normally in weightlessness, the seeds of which, if they are cultivated subsequently with adequate nutrition, illumination, aeration and other conditions necessary for vital activities, are located in the substrate with the embryo downward. In all other test versions, the very young growing plants should perish, even if all the necessary conditions are observed. Whether roots, which began growing in the atmosphere bend and continue into the /775 substrate in weightlessness, if the test is conducted with illumination, i.e., whether phototaxis can replace positive geotaxis, remains to be studied. It also was found in the experiment that the gravity compensation achieved with sufficiently effective clinostats does not make it possible to display normal geotropic reactions. Whether the effectiveness by gravity compensation (with the clinostat) can be 100%, by changing the rotation rate and by producing the optimum growth conditions for the plants, remains to be studied.

The data obtained on the initial geotropic reactions of plants occurring in weightlessness are of theoretical importance for the development of gravitational biology. They are important practically, in the development of greenhouses intended for the conduct of various kinds of studies, which require the cultivation of plants in space.

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