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MODIFICATION OF CYTOGENETIC AND PHYSIOLOGICAL EFFECTS OF SPACE FLIGHT FACTORS BY BIOLOGICALLY ACTIVE COMPOUNDS

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Translation of "Modifikatsiya tsitogeneticheskikh i fiziologicheskikh effektov deystviya faktorov kosmicheskogo poleta biologicheski aktivnymi soyedineniyami". Zhurnal obshchey biologii (Journal of General Biology), Vol. 47, No. 2, March-April, 1986, pp 246-251 (UDC 576.3+575: 577.462)

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16. Abstract

Physiological and cytogentic changes in the Welsh onion plants induced by a short (82 days) and long term (522 days) space flight are expressed in decrease of seed germination inhibition of stem growth, depression of cell division in root meristem and increase in the number of structural chromosome rearrangements. The treatment of such plants with solutions of α-tocopherol, auxin, and kinetin decreased the level of chromosome aberrations to the control one and normalized cell divisions and growth partly or completely.

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MODIFICATION OF CYTOGENETIC AND PHYSIOLOGICAL EFFECTS OF SPACE FLIGHT FACTORS BY BIOLOGICALLY ACTIVE COMPOUNDS

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Using the example of Welsh onion plants. the effect of α-tocopherol, auxin and kinetin was demonstrated on the frequency of aberrations in chromosomes, the mitotic cell division, the growth of the roots and stem, and the seed germination after being in space flight or after storage under earth conditions. The effect of the space flight factors or prolonged storage of the seeds under earth conditions lead to a reduction in their germination, inhibition of division and growth of root cells, reduction in stem growth. and increase in structural disruptions in the genetic apparatus. The defiations from the norm of the cytogenetic and physiological parameters of the plant organism are restored with treatment of the seeds with biologically active compounds of a vitamin and hormonal nature. /246**

INTRODUCTION

A large number of studies have been devoted to the problem of the effect of space flight factors (SFF) on the plant organism. According to the literary data, SFF effect the seed germination and cause disruptions in the genetic apparatus of the plants (Antipov et al., 1969; Glembotskiy et al., 1962; Nuzhdin et al., 1965, 1970, 1975; Nuzhdin, Pastushenko-Strelets, 1968; Platonova et al., 1977). In seeking means of increasing the resistance of biological systems to SFF, a number of studies made efforts to utilize classif specific protectors--cistein, AET, 5-MOT, and MEA (Nuzhdin et al., 1972, 1975). We know that in the cells of plants and animals these protectors reduce chromosome aberrations induced by ionizing radiation (Nuzhdin et al., 1972; Ganassi, 1977; Veksler et al., 1977; Pomerantseva, 1980, Vladimirov, Smirnov, 1981; Antoku, 1982), ultraviolet and fluorescent light (Bianchi et al., 1982). However, in tests with one- and two-time effect of SFF on barley seeds, we were unable to determina a protective or modifying effect of the protectors indicated above (Nuzhdin et al., 1972, 1975).

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** Numbers in margins indicate foreign pagination.

Evidently, due to the complexity of the factors effecting the conditions of space flight on the plant organism, for neutralization of the negative aftereffects which they cause, we must use biologically active compounds whose protective and regulatory properties bear a universal character. The works which we have published demonstrate the universality of the protective effect on the genetic apparatus of various test subjects by vitamins and phytohormones against the mutagenic effect of factors of physico-chemical and biological nature (Alekperov, Akhundova, 1974; Alekperov, 1979; Aliyev, Babayev, 1981; Alekperov et al., 1981; Aliyev et al., 1982).

Thus, the study of the possibilities of modification of effects caused by the action of a complex of SFF on the mutation and growth processes in plants after their seeds have been in space flight condition through the use of vitamin E and phytogormones is of great interest.

MATERIAL AND METHODOLOGY

Studies were conducted on Welsh onion plants. The control batch of seeds was stored in a laboratory at room temperature and humidity under earth conditions for a period of 82 and 522 days. During this same time, the test batch of /247 seeds was in space flight on the "Salyut-7" space station. The effect of the storage times of the seeds and the SFF on the organism was judged by the seed germination, the mitotic index, and the frequency of chromomal aberrations in the cells of the apical root meristem, as well as the rate of their growth and stem height on the 10th day after seed germination. In order to study the modifying effect of biologically active compounds of vitamin and hormonal nature on the plant organism, the control and test batches of seeds were germinated on solutions of α-tocopherol ($1 \cdot 10^{-4}$ and $1 \cdot 10^{-2}$ μg/ml), auxin ($1 \cdot 10^{-1}$ μg/ml) and kinetin ($1 \cdot 10^{-1}$ μg/ml). The chromosome restructuring and mitotic index were determined on temporary pressed preparations, the rate of root growth was computed by means of differentiation of the kinetic curves according to the final differences.

Table 1. Effect of α-tocopherol, auxine and kinetine on the frequency of chromosomal aberrations in control seeds after 82- and 522-day storage in the laboratory and seeds found under space flight conditions

a - № п.п.	b - Вариант опыта	c - Количество просмотренных анафаз	d - Количество нормальных анафаз	e - Количество измененных анафаз		t_d
				f - число	%	
1	Контроль (начало эксперимента)	1786	1684	102	5,71±0,549	—
2	Контроль (82 дня)	2141	1992	149	6,96±0,550	1,61
3	Контроль (522 дня)	880	780	100	11,36±1,068	4,71
4	Контроль (82 дня): α-токоферол ($1\cdot10^{-4}$ мкг/мл)	821	788	33	4,00±0,680	3,40
5	Контроль (82 дня): α-токоферол ($1\cdot10^{-2}$ мкг/мл)	846	820	26	3,07±0,590	4,86
6	Контроль (82 дня): ауксин ($1\cdot10^{-1}$ мкг/мл)	730	705	25	3,42±0,673	4,07
7	Контроль (522 дня): α-токоферол ($1\cdot10^{-2}$ мкг/мл)	903	852	51	5,65±0,770	4,34
8	Контроль (522 дня): кинетин ($1\cdot10^{-1}$ мкг/мл)	855	802	53	6,20±0,825	3,82
9	ФКП (82 дня)	1985	1792	193	9,72±0,665	3,20
10	ФКП (522 дня)	1327	1137	190	14,32±0,962	2,06
11	ФКП (82 дня) + α-токоферол ($1\cdot10^{-4}$ мкг/мл)	818	776	42	5,13±0,770	3,92
12	ФКП (82 дня) + α-токоферол ($1\cdot10^{-2}$ мкг/мл)	812	783	29	3,57±0,630	5,64
13	ФКП (82 дня) + ауксин ($1\cdot10^{-1}$ мкг/мл)	637	603	34	5,34±0,890	3,94
14	ФКП (522 дня) + α-токоферол ($1\cdot10^{-2}$ мкг/мл)	875	817	58	6,63±0,840	6,85
15	ФКП (522 дня) + кинетин ($1\cdot10^{-1}$ мкг/мл)	864	800	64	7,41±0,891	5,27

a - Pp No; b - Test variant; c - Number of examined anaphases; d - Number of normal anaphases; e - Number of changed anaphases; f - number; 1 - Control (start of experiment); 2 - Control 82 days); 3 - Control (522 days); 4 - Control (82 days): α-tocopherol ($1 \cdot 10^{-4}$ μg/ml); 5 - Control (82 days): α-tocopherol ($1 \cdot 10^{-2}$ μg/ml); 6 - Control (82 days): auxin ($1 \cdot 10^{-1}$ μg/ml): 7 - Control (522 days): α-tocopherol ($1 \cdot 10^{-2}$ μg/ml); 8 - Control (522 days): kinetin ($1 \cdot 10^{-1}$ μg/ml); 9 - SFF (82 days); 10 - SFF (522 days); 11 - SFF (82 days) + α-tocopherol ($1 \cdot 10^{-4}$ μg/ml); 12 - SFF (82 days) + α-tocopherol ($1 \cdot 10^{-2}$ μg/ml). 13 - SFF (82 days) + auxin ($1 \cdot 10^{-1}$ μg/ml); 14 - SFF (522 days) + α-tocopherol ($1 \cdot 10^{-2}$ μg/ml); 15 - SFF (522 days) + kinetin ($1 \cdot 10^{-1}$ μg/ml).

Note to Table 1: t_d is the reliability of the difference in computation between the variants of tests: 1 and 2, 3; 2 and 4, 5, 6, 9; 3 and 7, 8, 10; 9 and 11, 12, 13; 10 and 14, 15.

RESULTS AND DISCUSSION

From the data presented in Table 1 is it evident that 82 days after storage of the control seeds, the frequency of chromosomal aberrations increases insignificantly as compared with the initial level. The reliability of the difference between the comparable values of the frequency of structural reorganization of the chromosomes below the limit for the Student criterion has a 5% level of significance. However, the storage of control seeds for a period of 522 days leads to a significant increase in the level of disruptions in hereditary structures of the cells. The reliability of the difference between the initial frequency of chromosome aberrations and its value on the 522nd day exceeds the limit for the Student criterion with a 0.1% level of significance. The increase in mutability of the chromosomes with natural ageing of the seeds over a period of 522 days of storage in the laboratory comprised 98.8% of the initial level. These data correlate with the generally accepted conception on the accumulation of genetic load in biological objects /248 during their natural storage (D'Amato, 1965; O'Neill, 1974; Kagramanyan, 1974; Shapiro et al., 1975; Fiziologiya semyan [Physiology of Seeds], 1982).

In treating control seeds stored for 82 days with solutions of -tocopherol and auxin, the absence of an increase in the frequency of chromosomal aberrations was established. Moreover, there was a reliable reduction in the level of spontaneous mutation. The modifying effect of α-tocopherol and kinetine on the mutation process induced by natural ageing is manifested in the reduction in frequency of chromosome restructuring to values close to the initial level (Table 1). The obtained result testifies to the possibility of reversing the genetic effect of ageing with the effect of biologically active compounds of a vitamin and hormonal nature.

The effect of SFF on the genetic apparatus of plants is manifested in an increase in the frequency of structural changes in the chromosomes. Thus, after 82 days of SFF effect, the increase in mutability of the chromosomes over the initial level comprised 48.3%. In 440 days of space flight the increase in mutability of the chromosomes comprised 80.56%. At first glance it might seem that such a high negative genetic effect is the result of the prolonged effect of SFF on the organism. However, in the same period of time, with storage of the control seeds the growth in chromosome aberrations comprised 77.06% of the initial level. Consequently, in 440 days of space flight, as a result of the effect of SFF alone, the increase in mutability of the chromosomes comprised 3.5%. Thus, the total genetic effect of prolonged space flight is determined by the natural ageing of the seeds and the effect of SFF on them.

The treatment of seeds which are found for prolonged periods under space flight conditions with solutions of α-tocopherol and kinetine facilitates the reduction in the frequency of structural changes in the chromosomes to their initial level of mutability.

An analysis of the obtained results shows that the effect of modification of the mutation process by biologically active compounds of a vitamin and hormonal nature, regardless of the nature and peculiarities of joint effect of the induction factors on the genetic apparatus, is synonymous. Such universality of the antimutagenic effect of vitamin E, auxin and kinetine testify to the possibility of their application for protecting the genetic apparatus against mutation damage and reliability of its functioning under various extremal conditions.

It was noted that the treatment of the control batch of seeds with vitamin E solutions in concentrations of $1 \cdot 10^{-4}$ and $1 \cdot 10^{-2}$ μg/ml leads to an increase in their germination from 75.14 ± 0.73 to 85.67 ± 1.02 and 84.67 ± 1.03%

respectively. After the seeds had been under space flight conditions for 82 days, their germination rate drops to 40.00±0.0%. Subsequent treatment of the test batch of seeds with vitamin E in both studied concentrations practically restores their viability. The germination of the seeds increases to 66.33±0.34 and 65.0±0.67%. However, the effect of the vitamin on the activity of cell division in the root system and the growth of the above-ground part of the plants varies. Thus, with treatment of the control seeds with tocopherol in both of the studied concentrations there is observed a mitodepressive effect. This testifies to the inhibition of the growth processes in the root system. At the same time, judging by the stem height, the growth of the above-ground part of the plants is accelerated (Table 2). The treatment of the control seeds with auxin stimulates the proliferative process in the cells of the apical root meristem. This correlates with the literary data on the mitostimulating effect of auxin at the first stage of organogenesis with soaking of dry seeds (Gamburg, 1976; Araratyan, 1972; Tarasenko, Kiseleva, 1974). At later stages of organogenesis, judging by the stem growth, auxin has no effect.

Further analysis of the data showed that the effect of SFF on the seeds leads to more than a 3-time reduction in the activity of cell division in the root meristem and to an inhibition in the growth of the above-ground part of the plants. With effect of SFF, the plant stem height turned out to be 36.8% /249 less than in the control. Auxin fully reverses the inhibition of cell proliferation in the root meristem caused by SFF, and restores and even stimulates the growth of the above-ground part of the plants. The established facts of auxin modified growth function in the norm and after the effect of SFF give reason to believe that the given phytohormone is but an initial stimulus to the restoration of hormonal status of the organism and fulfills a signal function, setting into motion the mechanism of self-regulation of cell division and plant growth.

TABLE 2. Effect of α-tocopherol and auxin on the mitotic activity of root meristem cells and on stem growth after 82 days of SFF effect on Welsh onion seeds

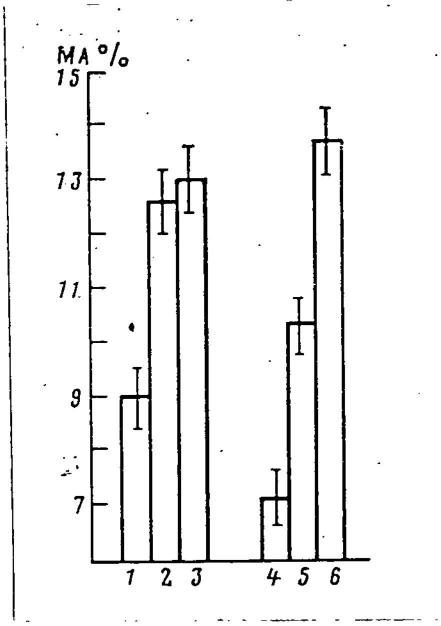
a- № п.п.	b- Вариант опыта	c- Делящиеся клетки		t_d^*	d- Высота стебля, мм	t_d^{**}
		e- число	%			
1	Контроль	692	13,44±0,48	—	47,50±1,54	—
2	Контроль: α-токоферол (1·10⁻⁴ мкг/мл)	218	7,27±0,45	9,38	59,60±2,88	3,70
3	Контроль: α-токоферол (1·10⁻² мкг/мл)	228	9,50±0,60	5,13	74,60±4,91	5,36
4	Контроль: ауксин (1·10⁻¹ мкг/мл)	253	17,94±1,02	3,99	41,40±2,88	1,87
5	ФКП	118	3,93±0,36	16,04	30,00±3,22	4,90
6	ФКП + α-токоферол (1·10⁻⁴ мкг/мл)	215	8,95±0,61	7,09	34,80±2,50	1,18
7	ФКП + α-токоферол (1·10⁻² мкг/мл)	291	9,70±0,54	8,89	35,80±5,77	0,88
8	ФКП + ауксин (1·10⁻¹ мкг/мл)	266	13,30±0,76	11,18	58,83±4,83	4,97

a - No pp; b - Test variant; c - Dividing cells; d - Stem height, mm; e - Number; 1 - Control; 2 - Control: α-tocopherol ($1 \cdot 10^{-4}$ μg/ml); 3 - Control: α-tocopherol ($1 \cdot 10^{-2}$ μg/ml); 4 - Control: auxin ($1 \cdot 10^{-1}$ μg/ml); 5 - SFF; 6 - SFF + α-tocopherol ($1 \cdot 10^{-4}$ μg/ml); 7 - SFF + α-tocopherol ($1 \cdot 10^{-2}$ μg/ml): 8 - SFF + auxin ($1 \cdot 10^{-1}$ μg/ml).

Note. t_d^*, t_d^{**} --reliability of difference computed between test variants: 1 and 2, 3, 4, 5; 5 and 6, 7, 8.

Unlike the hormonal factor, the modifying effect of vitamin E is manifested in the form of compensation of the negative effect of SFF on the growth processes. This is evidenced by the fact that the positive effect of impulse treatment of the test batch of seeds with the vitamin, judging by the mitotic index and the stem height, gradually decreases in the course of development of the plants (Table 2).

The data on the effect of phytohormones and vitamin E on the growth function after prolonged presence of the seeds under space flight conditions and with their prolonged storage on Earth are also of interest. It was noted that the germination of control seeds stored for 522 days is delayed by 24 hrs and their sprouting rate is reduced to $49.5 \pm 0.5\%$ as compared with seeds analyzed after 82 days. When this batch of seeds was treated with kinetine, the germination time did not change, but the sprouting rate increased to $53.3 \pm 0.3\%$. After the seeds had spend 522 days under space flight conditions, their sprouting rate is reduced to $38.0 \pm 1.0\%$. Treatment with kinetine of this batch of seeds increases the sprouting rate to $45.3 \pm 1.0\%$. From here we see that the drop in the level of viability of the seeds as a result of their natural ageing and with prolonged effect of SFF is partially restored under the influence of kinetine. The effect of neutralization by kinetine of the negative consequences of prolonged SFF effect and natural ageing of the seeds is most apparently manifested in analysis of the data on its effect on the mitotic activity of the cells and the rate of root growth. Kinetine not only fully eliminates the depression in cell division in the root meristem caused by natural ageing of the seeds as well as by prolonged effect of SFF on them. It also significantly intensifies the root growth as a whole (Fig. 1 and 2). The modifying effect of tocopherol in studying the natural ageing of seeds is manifested by the increase in the mitotic activity of the cells /250 to the control level. The positive effect of tocopherol is noted in the variant with prolonged effect of SFF. However, unlike the phytohormone, under the influence of the vitamin there is no complete restoration in the activity of the cell division (Fig. 1). This fact is one more piece of evidence regarding the compensatory character of tocopherol regulation of the organism's growth function.



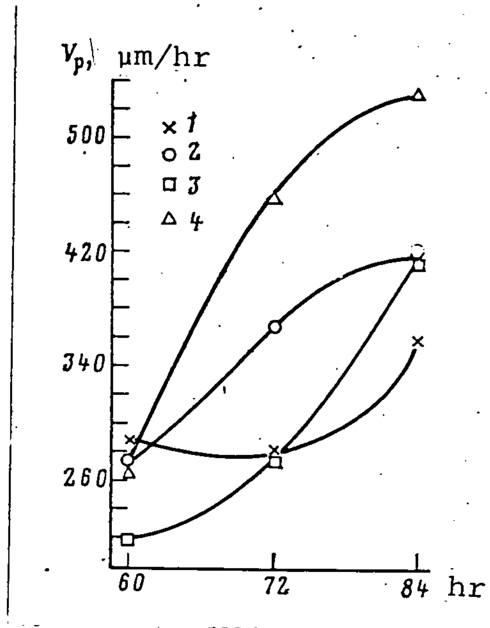


Fig. 1

Fig. 2

Fig. 1. Effect of α-tocopherol and kinetine on the mitotic activity of root meristem cells of Welsh onion after 522 days of storage of the seeds under earth conditions or their presence in space flight. 1 - control --batch of seeds stored under earth conditions; 2 - control: α-tocopherol ($1 \cdot 10^{-2}$ µg/ml); 3 - control: kinetine ($1 \cdot 10^{-1}$ µg/ml); 4 - SFF- batch of seeds subjected to the effect of space flight factors; 5 - SFF + α-tocopherol ($1 \cdot 10^{-2}$ µg/ml); 6 - SFF + kinetine ($1 \cdot 10^{-1}$ µg/ml); MA - mitotic activity of the root meristem cells.

Fig. 2. Effect of kinetine on the growth rate of Welsh onion roots after 522 days of storing seeds under earth conditions or of being in space flight. 1 - control--batch of seeds stored under earth conditions 2 - control: kinetine ($1 \cdot 10^{-1}$ µg/ml); 3 - SFF--batch of seeds subjected to the effect of space flight factors; 4 - SFF + kinetine ($1 \cdot 10^{-1}$ µg/ml). V_p - rate of growth (µm/hr).

Thus, modification of the negative consequences of SFF effect and natural ageing on the growth activity through the use of biologically active compounds testifies to the compensatory effect of vitamin E and a signal of the character of regulation of physiological functions by phytohormones in plants in the direction of increasing their nonspecific resistance to a complex of unfavorable factors of varying nature.

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