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EFFICIENCY OF TRANSGENERATIONAL TRANSFER OF THE DAMAGES FROM M_1 TO M_2 GENERATIONS OF BARLEY UNDER THERMAL NEUTRON IRRADIATION

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Abstract: To determine the transmission of mutations in plants, it was studied how barley seeds irradiated with thermal neutrons and gamma radiation in M_1 generation affect the yield of chlorophyll mutations in M_2 ones. Experimentally and theoretically, the transmissions of damages between plant generations were evaluated.

It is known that reparation processes are strongly suppressed in barley seeds irradiated with thermal neutrons. This indicates that microdose fields in seeds under the action of thermal neutrons are 10^3 times more effective have been damage living cells than affects rarely ionizing γ - radiation.

Keywords: thermal neutrons, gamma radiation, transgenerational damage transfer, barley, damage efficiency, mutations

1. Introduction

A deep and complete analysis of the effect of high density ionizing radiation on biota remains relevant due to their ability to cause high radiobiological effects and their worldwide use in experimental mutagenesis. It is known that under the influence of densely ionizing radiation, the possibility of modifying the radio resistance of somatic cells and the effect on genetic variability by various abiotic factors is significantly narrowed and insignificant. In this respect, approach such as high density ionizing radiation as influence thermal neutrons on plants stands apart.

When plants are irradiated with thermal neutrons, the total capture dose is formed due to the nuclear reaction $^{14}N(n,p)^{14}C$ and $^{1}H(n,\gamma)^{2}H$, with the help of the nuclei ^{14}N and ^{1}H, after that capturing thermal neutrons, instantly emit proton and γ-ray from compound nuclei, respectively. Therefore as result due to internal proton irradiation in a biological material the local capture dose from thermal neutrons on the nitrogen nucleus is formed with high LET radiation.

Generally in radiobiology, the question of the relationship between the yield of genetic mutations that are preserved in vegetative cells in M_1 and transmission through generative cells in M_2 in the form of chlorophyll mutations has been discussed for a long time. In the case of the barley seeds are irradiated with thermal neutrons and gamma radiation in M_1, the qualitative and quantitative dependence of the appearance of mutations in M_2 generations is relevant [1].

It is known that barley is a self-pollinating plant and the yield of chlorophyll mutations for it is well studied and classified [2].

Goals and objectives. The purpose of this work is to study the depends on the radiation resistance of plant somatic cells and their genetic variability during the formation of capture dose from thermal neutron irradiation and evaluation the efficiency of transgenerational transfer of the damages from M_1 to M_2 generations of the barley. To mathematical predict effectiveness, the thermal neutron application and possibilities of practical implementation of the data in radiation mutagenesis were investigated.

2. Materials and methods

The seeds of barley *Hordeum vulgare L.* cultivar Romantic were irradiated with a thermal neutron within fluence from 10^{15} neutron/m^2 to 10^{17} neutron/m^2 was varied. The thermal neutron flux rate at the bottom of the TK-3 channel was $(3.6 \pm 0.3)10^{14}$ neutron$\cdot$ m$^{-2\cdot}$ $\cdot$s^{-1}. Seeds were irradiated with γ-rays using the γ-device “Researcher” from a ^{60}Co source at doses since 50 until 400 Gy at a dose rate of 0.16 Gy/s.

For the process of the multivariate experiment, a special program was developed that estimates the smallest difference between the data variants SDV_{05} and the mutual influence of the factors [3]. The barley seeds were sown at a plot of the Hlevakha experimental field (Kiev region). Seeds in randomized plots in 3 fold repeats were sown. At least 3000 seeds were taken for each experiment variant. In M_1 generation damages at the level of the whole plant organism with its quantitative and quality indicators of the growth and development of seedling germination within 2 weeks and survival of the plants to span-life of the ontogenesis was determined. For determination of chlorophyll mutations in seedlings in M_2 generation unwhipped spikes were gathered in M_1 generation were placed in wet sand. This part of the experiment was carried in January in a greenhouse, where the lighting was natural, diffused and the temperature was about 10°C. Taking into account the recommendations of other authors the analysis of chlorophyll mutations according to the classification by Hensel and Orav was evaluated [4].

In the case applying of analysis of variance, the frequency of chlorophyll mutations was expressed by the formula;

$$\varphi = 2\mathrm{arcSin}\sqrt{\mathrm{p}}$$

where p is the frequency of mutation yield in shares (on relative units).

All experiments were carried out with three fold repeats, and the experimental results were subjected to statistical analysis according to generally accepted methods [5-8].

3. Results and discussion

To determine the transmission of mutations in plants, it was studied how barley seeds irradiated with thermal neutrons and gamma radiation in M_1 generation affect the yield of chlorophyll mutations in M_2 ones. Experimentally and theoretically, the transmission of damages between plant generations were evaluated.

Dependence of the dose-effect for irradiation with thermal neutrons and gamma rays of barley plants is shown in Figure 1 and Figure 2.

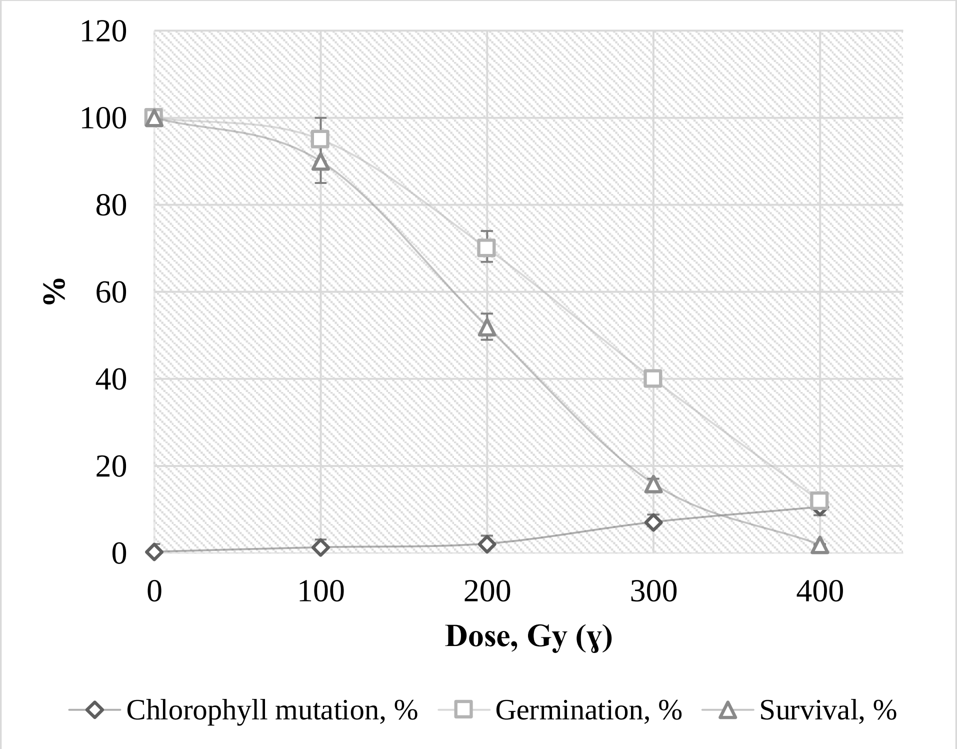

Fig. 1. Dose-effect dependence of thermal neutrons (TN) for barley plants on test-systems: Chlorophyll mutation, Germination, Survival.

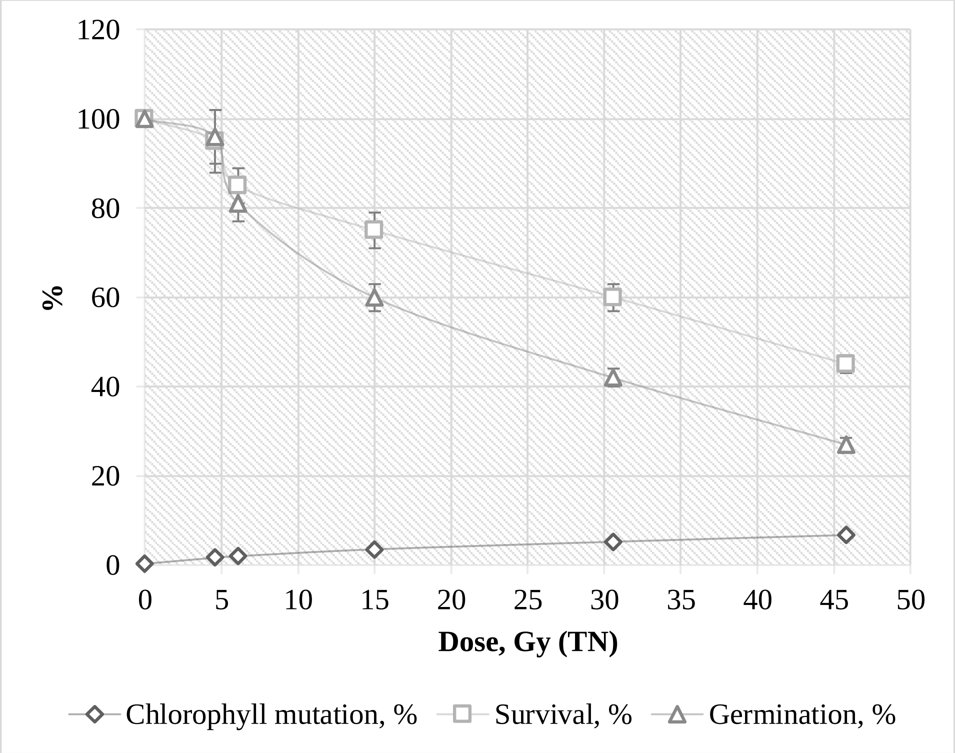

Fig. 2. Dose-effect dependence of Gamma irradiation for barley plants on test-systems: Chlorophyll mutation, Germination, Survival.

In regarding to this hypothesis we tried to find a mathematical relationship between the amount or level of damages in M_1 and the yield of chlorophyll mutations in M_2 generation.

The idea is that having dose-effect (Fig. 1 and Fig. 2) curves according to the germination within 2 weeks-old seedlings or survival criterion [2] by the end of the growing season M_1 of matured barley plants can be described using an equation with parameters m, and n (Table 1):

$$\ln[1-(1-\eta)^{1/m}]= - nD,$$

where η - characterizes the degree of damage of barley seedlings in M_1.

Table 1. Parameters m and n, half-lethal dose (LD_{50}, Gy) and relative biological efficiency (RBE) of thermal neutrons in according to test criteria, germination and survival rate for barley seeds in M_1 generation

Test-system	n	m	LD_{50}, Gy	RBE
Thermal neutron irradiation				
Germination	0.025±0.002	0.89±0.07	30	8.7
Survival	0.017±0.008	0.94±0.38	38	7.1
γ - irradiation				
Germination	0.0086±0.0002	5.7±2.7	262	1.0
Survival	0.013±0.002	10.2±4.7	268	1.0

On the other hand, the dose curves for the yield of chlorophyll mutations in M_2 are described analytically by the formula:

$$Y=Y_0+kD^N,$$

where Y and Y_0 are the proportions of the yield of chlorophyll mutations in M_2 generation (Fig. 1 and Fig.2) and are characterized by parameters (k, N) (Table 2).

Table 2. The appearance of families with chlorophyll mutations in M_2 in barley seedlings irradiated with thermal neutrons and γ – radiation

Radiation dose, Gy	Mutant family, %	The yield of the mutant family, %	
		Monochromatic, (%)*	Bicolor, (%)**
Thermal neutron irradiation			
0	0.24 ±0.09	0.24 (100)	0 (0)
4.6	1.64±0.23	1.03 (63)	0.61 (37)
6.1	1.96±0.31	0.91 (46)	1.05 (54)
15.1	3.5 ± 0.22		
30.6	5.20±0.45	3.02 (58)	2.18 (42)
45.8	6.72±0.56	3.47 (52)	3.25 (48)
SDV_{05} =1.29			

γ - irradiation			
0	0.30±0.17	0.30 (100)	0 (0)
100	1.35±0.27	1.35 (100)	0 (0)
200	2.17±0.23	2.17 (100)	0 (0)
300	7.14±0.90	6.35 (88)	0.79 (12)
400	10.60±1.80	9.00 (85)	1.60 (15)
$SDV_{05}=1.08$			

* - Monochromatic chlorophyll mutations are *albina, xantha* and *viridis.*

** - Bicolor chlorophyll mutations are *alboviridis, xanthaviridis, alboxantha, tigrina, striata, maculata* etc.

We aim is to find such a relationship between (m, n) and (k, N) that would allow us to pass from the parameters (m, n) of the dose-effect curves in M_1 to the parameters (k, N) of the dose-effect curves in M_2, that is, to predict the yield of chlorophyll mutations in M_2.

A mathematical search for the form of the dependence (m, n) on (k, N) showed that it has a rather complex functional form for various micro-doses fields formed under the action of thermal neutrons and gamma radiation. In general, the display of parameters can be done as follows:

$$Y=Y_0+[f(m,n)]D^{g(m,n)},$$

where f (m, n) and g (m, n) are functions of two variables. Mathematical processing of the data shows that the functions f (m, n) and g (m, n) are extremely difficult to analytically express and it is difficult to calculate these functional dependences, therefore, these functions can be transformed with one variable:

$$f(m,n)=f_1(m)+Ff_2(n)$$

and

$$g(m,n)=g_1(m)+Fg_2(n),$$

where the functions f_1 (m), g_1 (m), f_2 (n), and g_2 (n) can be easily calculated on the basis of experimental data by computer processing of the parameters of the dose-effect curves by the method of least squares.

The value of these partial functions for fixed values of m, n, and F according to the criteria of survival and germination of barley seedlings for irradiation with thermal neutrons and gamma radiation are given in Table 3.

Table 3. Selection of F values for matching the theoretical and experimental data of the parameters of the equation $Y=Y_0+kD^N$

Criteria	m	n	k	N	F
For γ-irradiated barley seeds Experimental data					
Survival	10	0,01	$3{,}63\cdot10^{-4}$	1,72	-
Germination	10	0,01	$1{,}60\cdot10^{-8}$	3,34	-

Theoretical data					
Survival	10	0,01	$3,83\cdot10^{-4}$	1,75	10^{-3}
Germination	10	0,01	$6,90\cdot10^{-9}$	3,00	10^{-4}
Seeds irradiated with thermal neutrons					
Experimental data					
Survival	1	0,1	1,40	0,70	-
Germination	1	0,1	0,52	0,71	-
Theoretical data					
Survival	1	0,1	1,30	0,70	1
Germination	1	0,1	0,95	0,75	1

Comparison of the data in Tables 1 and 2 indicates that the theoretical and experimental data are in fairly good agreement for seeds irradiated with thermal neutrons and gamma radiation.

In the case of γ-irradiation of barley seeds, for to the theoretical and experimental data to coincide, it is necessary to multiply the partial function $f_2(n)$ by the factor F.

As mentioned above, the private function $f_2(n)$ depends on the dose parameter (n). This means that during ontogenesis of γ- irradiate barley plants, most of the dose is dissipate in M_1 and the final stage is not realized damages in the create seeds and output of chlorophyll mutations in M_2. This is because during γ - irradiation of barley seeds in seedlings during growth and development, recovery processes are intensively occurring, which also reduces the effectiveness of γ-radiation [7, 8].

In the case of irradiation of barley seeds with thermal neutrons, the reduction processes in M_1 for seedlings are not essential, therefore the coefficients k and n correspond to each other unambiguously. For γ - irradiation of barley seeds for the calculated data to correspond to the experimental data, it is necessary to multiply the dose proportionality coefficient by $F=10^3$.

It is known that reparation processes are strongly suppressed in barley seeds irradiated with thermal neutrons. This indicates that microdose fields in seeds under the action of thermal neutrons are 10^3 times more effective than with rarely ionizing radiation from a γ-source.

Even with a wide range of dose variation in M_1, the correspondence between the yield of chlorophyll mutations in M_2 is preserved in barley seeds irradiated in M_1 with thermal neutrons.

For example, similar facts were discovered by A. Pryadchenka and K. Greenwald in their experiments with barley seeds irradiated with thermal neutrons [9]. By repeated selection over several years, they managed to obtain lines of winter barley with valuable economic properties caused by irradiation with thermal neutrons ($7.5\cdot10^{12} - 10^{13}$), which remained constant for 4 - 5 years.

To obtain mutant highly productive plants, the authors of [10] irradiated seeds with thermal neutrons. In the first year of irradiation, the yield of plants significantly decreased. In subsequent years, during mass selection, an increase in productivity was achieved in the offspring of plants irradiated with thermal neutrons in comparison with the initial population, especially in the second and fourth generations. Based on this fact, the authors hope that the combination of irradiation and mass selection can increase the effectiveness of these methods and will be able to obtain positive results. Similar data are reported in [11], where the authors tried to parameterize data on mutation rates in human diploid fibroblasts HF19 and Chinese hamster V79 depending on the LET, using the relationship between RBE and LET from track theory. The parameters obtained had almost the same meaning as for cell death of the same lines used. Fewer mutations than lethal lesions per track are attributed to decreased lateral mutation yield. The theory suggests that the mutation yield is proportional to the 10^4 lethal damage yield. Since the observed mutation frequency per cell surviving irradiation is an example of the probability of mutation induction on the probability of survival, and since these processes depend on the LET, there is every reason to assume that there is a window for mutation induction in the LET values. At lower LETs, the likelihood of mutations is low, while at higher LETs, the probability of cell survival tends to zero. The experiments of these authors give reason to believe that the total probability is maximum for fast α-particles. This can explain the high RBE values of thermal neutrons for seeds enriched by stable isotope ^{10}B, where α-particles are also emitted under influence of the thermal neutrons [4, 12].

In this regard, it should be noted that in recent years in practice in the field of plant mutagenesis, high doses of γ - radiation is often used. At the same time, in order to avoid a high degree of somatic damage for high doses of radiation and, accordingly, to obtain a high level of mutation yield, experimental practitioners from among the breeders often use various radioprotectors [13, 14].

Our data show that the above approach is not very promising for increasing and causing a wide range of mutations in M_2 [15]. Unfortunately, this method of using thermal neutrons is limited by the fact that such experiments require an atomic reactor with a high thermal neutron beam (fluence).

4. Conclusions

In seeds irradiated with thermal neutrons, reparation processes are strongly suppressed. This indicates that in seeds microdose fields with high LET radiation generated by the emission of protons from ^{14}N nuclei under the action of thermal neutrons are 10^3 times more effective than with rarely ionizing radiation from a γ-source. It is better to use exposition with high LET radiation than a high dose of γ- radiation in combination with various radioprotectors. All the data presented indicate the advisability of using the method of capture dose accumulation using thermal neutrons in mutagenesis and plant breeding.

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ЭФФЕКТИВНОСТЬ ТРАНСГЕНЕРАЦИОННОЙ ПЕРЕДАЧИ ПОВРЕЖДЕНИЙ ОТ M_1 К M_2 ПОКОЛЕНИЙ ЯЧМЕНЯ ПРИ ТЕПЛОВОМ НЕЙТРОННОМ ОБЛУЧЕНИИ

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Резюме: Для определения передачи мутаций между поколениями у растений было изучено на примере ячменя, как у семян облучение тепловыми нейтронами и гамма-излучением в генерации M_1, влияет на выход мутаций хлорофилла в генерации M_2. Экспериментально и теоретически оценивалась передача повреждений между поколениями растений.

Известно, что в облученных тепловыми нейтронами растениях ячменя процессы репарации сильно подавляются. Это свидетельствует о том, что микродозные поля в семенах под действием тепловых нейтронов в 103 раза эффективнее повреждают живые клетки, чем редко ионизирующее γ-излучение.

Ключевые слова: тепловые нейтроны, гамма-излучение, трансгенерационная передача повреждений, ячмень, эффективность повреждений, мутации.

TERMAL NEYTRON ŞÜALANMA ZAMANI M_1-DƏN M_2-YƏ KEÇƏN TRANSGENERASİON XƏTALARIN ARPA BİTKİSİNDƏ EFFEKTİVLİYİ

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Xülasə: Bitkilərdəki xətaların nəsillərdə ötürülməsini təyin etmək üçün, M_1 nəslində istilik neytronları və qamma şüaları ilə şüalanmış arpa toxumlarının, M_2 nəsldə xlorofil mutasiyalarının çıxışına necə təsir etdiyi öyrənilmişdir. Təcrübə və nəzəri cəhətdən bitki nəsilləri arasında xətaların ötürülməsi qiymətləndirilmişdir.

Məlumdur ki, istilik neytronları ilə şüalanmış arpa bitkisində reparasiya prosesləri güclü şəkildə zəifleyir. Bu isə canlı hüceyrələrə nadir ionlaşdırıcı şualanması olan γ-radiasiyanın təsirinin termal neytronların təsirinə nisbətən toxumdakı mikrodoz sahələrinin 103 dəfə daha zəif olduğunu göstərir.

Açar sözlər: Termal neytronlar, qamma şualanma, transgenerativ xətaların ötürülməsi, arpa, xətaların effekliyi, mutasiyalar