AGROLOGY

ISSN 2617-6106 (print)
ISSN 2617-6114 (online)
Agrology, 2022, 5(3), 75–80
doi: 10.32819/021111

Original researches

Received: 17.06.2022
Revised: 08.07.2022
Accepted: 09.07.2022

Inhibition of mutagenic effect in winter wheat as a result of ethylmethansulfonat action

V. Horshchar, M.Nazarenko
Dnipro State Agrarian and Economic University, Dnipro, Ukraine

Dnipro State Agrarian and Economic University, Serhii Efremov st., 25, Dnipro, 49600, Ukraine.
Tel.: +38-095-848-53-86.
E-mail: nik_nazarenko@ukr.net

Cite this article: Horshchar, V., & Nazarenko, M. (2022). Inhibition of mutagenic effect in winter wheat as a result of ethylmethansulfonat action. Agrology, 5(3), 75–80. doi: 10.32819/021111

Abstract. Chemical genetically-active substances can significantly increase the rate of mutations. The evidence of inhibition of mutagenic effect in the first generation of wheat under the action of water solution of EMS (ethylmethansulfonate) in the concentrations of 0.025%, 0.050%, 0.100% were examined in terms of germination, survival, overwintering characteristics, photosynthetic activity parameters of eight winter wheat varieties. We took into account different concentrations, the genotype of the object of mutagenic action, the ecological adaptability of a variety, the influence of the genotype-mutagenic interaction in order to find evidence of inhibition of the mutagenic effect. Therefore, the varieties Balaton and Zoloto Ukrainy are suitable for the Forest-Steppe zone, the variety Zeleny Hai was created for the droughts of the Steppe, the varieties Borovytsia, Kalancha, Nyva Odeska, Polianka, Pochaina are suitable for growing in all the zones of Ukraine. The experiments were carried out in unfavorable climatic conditions of the North Steppe (a semi-arid region with harsh winters and maximum climatic contrasts). To one degree or another, all the analyzed parameters were subjected to a clear and significant inhibiting effect by all the mutagen concentrations. Each indicator decreased in at least three gradations, sometimes there were no differences between the control and the first concentration, the first and second concentration, which depended on the genotype of the object of action. Death of some varieties after germination was observed at a rather low, not always significant, level. The death of plants in the autumn period was only typical for genotypes that are unadapted to the conditions of the region. The highest variability of the variance, due to the increase in mutagen concentrations, was seen in the indicators of germination and survival, the most genetically determined was the indicator of photosynthetic activity. There was no stimulating effect of the mutagen action, only inhibiting effects were observed. In the future, it is planned both to conduct studies of other physiological parameters of the impact of mutagenic inhibition in the first generation, and to relate the information obtained with variability, especially hereditary in subsequent generations, to monitor the genotype-mutagenic component under the action of this factor (supermutagen).

Keywords: bread wheat; firs generation; chemical mutagen; mutation breeding; supermutagen; surviving; concentration; mutations; cereals; winter resistance.

Introduction

Significant increase in the rates of genetic improvement of the main agricultural crops is achieved by the use of chemical mutagenesis, in particular supermutagens, similarly to to the effect of ionizing radiation (Xicun et al., 2016). At the same time, supermutagens act mainly for site-specific changes, having no continuous action. This leads to several major consequences in terms of plant genetics and physiology (Mamenko & Yakymchuk, 2019): firstly, a significant increase in the frequencies of certain types of hereditary changes (Hong, et al., 2022); secondly, the possibility of the emergence of fundamentally new types of mutations, primarily complex ones (Essam et al., 2019); thirdly, specific evidence in mutagenic inhibition in the first generation, primarily in terms of fertility, depending on plant genotype (in our case, the variety) (Mangi et al., 2021); and, finally, to a much higher genotype mutagenic interaction, primarily due to plant's inherent structure of the mechanism of genetic resistance to a certain effect (Jaradat, 2018; Nazarenko & Izhboldin, 2017).

At the first stage, main interest is the identification of various forms of evidence of mutagenic effect inhibition in the first generation after action of a certain ecogenetic factor (Beiko & Nazarenko, 2022a). As known, the most informative indicators are the ability of an organism to germinate, the assessment of growth energy, the possibility of forming a mature plant (Liu et al., 2017), the possibility of forming fertile generative organs in respective development stage of (Juhi, et al., 2019), obtaining seeds for further cultivation (Lykhovyd, 2021). Particularly important is determining lethality, sublethality, criticality and semi-lethality of individual concentrations (Hiroyasu, 2018; Nazarenko et al., 2019; Nazarenko & Izhboldin, 2017). Also, photosynthetic activity is one of key importance for determining mutagenic effect inhibition, and for winter crops, the ability to survive under the adverse winter conditions is especially important (Prabhu, 2019).

The objective of the conducted cycle of investigations was determining the effect of one of the most common types of chemical site-specific substances on genotypes created in the domestic process of genetic improvement, seeking for evidences of various effects of mutagenesis inhibition in the first generation according to the ontogenetic parameters of plant development. We intended to show the possible genotype-mutagenic interaction specificity, the importance of the genotype-conditioned component of an impact on the plant, depending on ecological-genetic characteristics.

Materials and methods

The experiment was conducted during 2017–2021in the conditions of the experimental field station of the Science-Education Center of the Dnipro State Agrarian Economic University.

Winter wheat seeds (1,000 grains for each concentration and water) were coated by EMS (ethylmethansulfonate) in 0.025%, 0.05%, 0.10% (Sigma-Aldrich, Germany) concentrations. The exposure of seeds lasted for 24 hours, according to the generally recommended method for chemical mutagens. These concentrations are trivial for mutagens (chemical supermutagens) of this group. The control was soaked in water (Shu et al., 2013; Spencer-Lopes et al., 2018).

Seeds samples were sown in 32 variants (in total) (10-rows plots for every variant, in water as control, interrow-spacing was 0.15 m, length of row was 1.5 m). Ecotypes in brackets: FS – forest-steppe, all - all the zones, S – steppe) Balaton (FS), Borovytsia (all), Zeleny Hai (S), Zoloto Ukrainy (FS), Kalancha (all), Nyva Odeska (all), Polianka (all), Pochaina (all). The genotypes were identified in order to characterize variability of winter wheat varieties for the North Steppe subzone (Dnipro region) (Shu et al., 2013; Spencer-Lopes et al., 2018).

The agrotechnology of crop cultivation is trivial for the Steppe zone (semi-arid area).

We analyzed phenology of each variant, three times performing evaluation of seedlings during winter period according to the overall sugars concentrations at the tillering node, evaluating the seed germination and plant survival of all the variants, and plants' photosynthetic activity in the period of spiking using an SPAD-502 device. The concentrations of chlorophyll (Chl) (a+b) were summarizes according to the generally accepted methodology using Chl = 10M^0.265 formula, where M is the value of SPAD (Soil Plant Analysis Development units) (Vesali et al., 2017). Statistic analyze of data was performed by ANOVA-analysis with Bonferroni correction, grouping and estimation of data was provided by discriminant and cluster analysis (Euclidian distanca, single linkage) (Statistic 10.0, multivariant module, TIBCO, Palo Alto, USA). The normality of the data distribution was examined using the Shapiro–Wilk W-test. Differences between samples were assessed by the Tukey HSD test.

Results

First of all, the adverse effects of the action of supermutagens were evidence in the first generation manifested in decrease in germination, and, for winter crops, also in plant survival. It is also important to assess the viability of crops before winter. Table 1 shows varieties with different ecological adaptability, demonstrating quite striking differences in resistance to a selected mutagen. It was statistically significant both for the variance in the change in mutagen concentration ($F = 98.23$; $F_{0.05} = 3.86$; $P = 3.39*10^{-7}$) and for individual genotypes ($F = 7.75$; $F_{0.05} = 3.86$; $P = 0.007$) for the germination parameter, for the parameter of the number of living plants before the winter period by the change in mutagen concentration ($F = 93.80$; $F_{0.05} = 3.86$; $P = 4.15*10^{-7}$), for individual genotypes ($F = 6.41$; $F_{0.05} = 3.86$; $P = 0.012$), for the survival rate after the winter period according to change in mutagen concentration ($F = 53.58$; $F_{0.05} = 3.86$; $P = 4.60*10^{-6}$) and for individual genotypes ($F = 8.44$; $F_{0.05} = 3.86$; $P = 0.005$).

Therefore, the selected material demonstrates a very high degree of genotype-mutagenic interaction according to the analyzed indicators of germination and survival, which were characterized by statistically significant differences depending on concentration. At the same time, we saw that the survival indicator was somewhat different from the first two, and later, when analyzing according to the varieties, it may have been because of strong response of one of the genotypes (Zeleny Hai) due to its high ecoadaptation. The mutagen had a particularly strong effect on the Balaton and Nyva Odeska varieties, to which 0.1% EMS concentration was semi-lethal.

It should be noted that, in general, the death of plants after germination prior to winter period was not always significant, especially for 0.1% EMS concentration; apparently, under its action, the death was predominantly one-stage ($F = 4.22$; $F_{0.05} = 5.16$; $P = 0.09$), however, the negative impact of the winter period was always significant, except for the steppe ecotype variety Zeleny Hai ($F = 2.94$; $F_{0.05} = 3.86$; $P = 0.11$), which shows the significance of not only the concentration, but also how adaptive is the plant. However, the variety Nyva Odeska showed high death and low adaptability ($F = 17.29$; $F_{0.05} = 3.86$; $P = 1.32*10^{-5}$) under the action of this factor. Perhaps, the reason for this reaction was its individual sensitivity to this type of site-specific EMS effect. The obtained data were confirmed by the Tukey's posthoc multitest.

According to germination and survival, the second group of genotypes, which were noted as capable to stable high productivity under the conditions of all zones of Ukraine, exhibited responses to the action of EMS which were generally similar to such of the Zeleny Hai variety (Table 2). In general, according to the results of the factor analysis, a large uniformity in reaction of this group was seen for the germination parameter. The variability in concentrations was quite high ($F = 585.95$; $F_{0.05} = 3.86$; $P = 1.24*10^{-11}$), but the genotype effect was statistically insignificant ($F = 3.84$; $F_{0.05} = 3.86$; $P = 0.054$). With the death rate after the winter period, the same pattern was again high in terms of concentrations ($F = 359.02$; $F_{0.05} = 3.86$; $P = 1.11*10^{-9}$), but for genotype is also statistically insignificant ($F = 1.62$; $F_{0.05} = 3.86$; $P = 0.25$). Thus, the second group was much more uniform in its reaction and is not of particular interest in terms of studying the mechanism of genotype-mutagenic interaction.

It is possible to single out two varieties Balaton and Nyva Odeska, which were highly sensitive to the action of EMS. The variety Zoloto Ukrainy has an intermediate significant position, but statistically significantly differs from the rest in its reaction. The varieties Zeleny Hai, Kalancha, Borovytsia, Polianka, and Pochaina were, on the whole, highly resistant and of the same type in response to all the EMS concentrations. However, the differences within each parameter are statistically significant. In some cases, for some concentrations (EMS 0.05%, EMS 0.1%), the difference between the parameters was statistically unreliable for an ecologically adapted variety ($F = 12.43$; $F_{0.05} = 5.16$; $P = 0.008$). Individual variety differences and comparison of pairs are confirmed by Tukey's test.

Table 1
Inhibition after mutagen action at the first generation by the germination and survival first group varieties (x ± SD, n = 10)

Variety	Variant	Germination, %	Beforewinter, %	Survival, %
Balaton	water	98.14±1.02 [a]	97.98±0.79 [a]	95.99±0.92 [a]
Balaton	EMS 0.025%	84.17±0.99 [b]	80.17±0.99 [b]	72.09±0.96 [b]
Balaton	EMS 0.05%	76.11±0.89 [c]	74.22±0.74 [c]	64.11±1.17 [c]
Balaton	EMS 0.1%	68.17±1.09 [d]	64.13±1.19 [d]	53.34±2.19 [d]
Zeleny Hai	water	99.22±1.12 [a]	98.46±0.82 [a]	98.17±0.84 [a]
Zeleny Hai	EMS 0.025%	90.32±1.01 [b]	87.16±0.67 [b]	84.98±0.87 [b]
Zeleny Hai	EMS 0.05%	86.14±1.09 [c]	82.98±1.16 [c]	81.01±1.17 [c]
Zeleny Hai	EMS 0.1%	78.14±1.11 [d]	76.03±1.02 [d]	74.04±1.09 [d]
Zoloto Ukrainy	water	98.98±1.06 [a]	97.99±0.88 [a]	97.01±0.98 [a]
Zoloto Ukrainy	EMS 0.025%	87.14±0.68 [b]	82.36±0.55 [b]	74.19±0.82 [b]
Zoloto Ukrainy	EMS 0.05%	80.92 ±0.87 [c]	78.11±0.92 [c]	73.01±1.00 [c]
Zoloto Ukrainy	EMS 0.1%	73.07±1.00 [d]	71.19±1.16 [d]	62.13±1.38 [d]
Nyva Odeska	water	97.45±1.17 [a]	97.06±0.86 [a]	96.19±0.99 [a]
Nyva Odeska	EMS 0.025%	85.33±0.78 [b]	81.96±0.89 [b]	74.17±0.83 [b]
Nyva Odeska	EMS 0.05%	79.16±1.11 [c]	76.97±0.81 [c]	65.92±1.23 [c]
Nyva Odeska	EMS 0.1%	65.34±1.23 [d]	63.17±1.03 [d]	51.17±2.32 [d]

Note: differences were considered significant at P < 0.05 by ANOVA-analyse with Bonferroni correction. Comparison in terms of one variety for one parameter.

Table 2
Inhibition after mutagen action at the first generation according to the germination and survival second group varieties (x ± SD, n = 10)

Variety	Variant	Germination, %	Beforewinter, %	Surviving, %
Borovytsia	water	98.92±1.02[a]	98.53±0.80[a]	97.62±0.80[a]
Borovytsia	EMS 0.025%	88.14±1.19[b]	86.22±0.81[b]	80.01±0.79[b]
Borovytsia	EMS 0.05%	84.01 ±1.01[c]	82.12±1.06[c]	79.12±1.00[c]
Borovytsia	EMS 0.1%	74.13±0.91[d]	73.42±1.12[d]	70.01±0.49[d]
Kalancha	water	99.14±1.22[a]	98.92±0.92[a]	97.16±0.75[a]
Kalancha	EMS 0.025%	87.99±1.01[b]	85.13±0.84[b]	81.69±0.99[b]
Kalancha	EMS 0.05%	83.12 ±1.00[c]	81.56±0.67[c]	78.44±0.92[c]
Kalancha	EMS 0.1%	73.22±0.90[d]	70.93±1.02[d]	68.17±0.66[d]
Polianka	water	98.43±1.11[a]	98.14±0.76[a]	96.65±0.93[a]
Polianka	EMS 0.025%	89.17±1.01[b]	86.90±0.71[b]	83.01±0.67[b]
Polianka	EMS 0.05%	85.98±1.07[c]	81.17±1.06[c]	79.23±1.07[c]
Polianka	EMS 0.1%	76.01±1.01[d]	75.08±1.01[d]	72.17±1.00[d]
Pochaina	water	99.14±1.11[a]	98.17±0.84[a]	96.33±0.92[a]
Pochaina	EMS 0.025%	90.14±1.03[b]	86.92±0.71[b]	82.99±0.85[b]
Pochaina	EMS 0.05%	84.46±0.97[c]	81.14±0.88[c]	80.00±0.97[c]
Pochaina	EMS 0.1%	76.12±0.93[d]	74.45±0.92[d]	71.90±0.57[d]

Note: see Table 1.

The level of mutagenic effect inhibition manifested in resistance to adverse winter conditions (Tables 3 and 4). Both visual scoring and laboratory analysis of sugar content in the tillering node were carried out for the three most contrasting periods. As a result of the visual evaluation, the varieties Zeleny Hai, Kalancha, Borovytsia, Polianka, and Pochaina showed the highest resistance, Balaton and Nyva Odeska were theleast tolerant, the variety Zoloto Ukrainy exhibited average tolerance.

Sugar content significantly decreased during the winter period in all the varieties. In the varieties Zeleny Hai, Kalancha, Borovytsia, Polianka, during the first period, there were no difference between the control and the first concentration ($F = 3.01$; $F_{0.05} = 3.86$; $P = 0.011$). For the Pochaina variety, there were no differences in the effect of 0.025% and 0.05% concentrations ($F = 4.17$; $F_{0.05} = 5.16$; $P = 0.09$). For the second period, no differences were seen between the Balaton and Nyva Odeska varieties regarding the effect of 0.05% and 0.1% ($F = 3.09$; $F_{0.05} = 4.82$; $P = 0.12$) EMS concentrations. In all the other varieties, the difference was significant ($F = 46.17$; $F_{0.05} = 3.86$; $P = 4.11*10^{-5}$). For the third sampling period, there was no difference between the effect of 0.05% and 0.1% EMS between the Balaton, Zeleny Hai, Polianka, Pochaina ($F = 1.98$; $F_{0.05} = 3.86$; $P = 0.19$), as well as in the effect of 0.025% and 0.05% concentrations in the variety Kalancha ($F = 3.92$; $F_{0.05} = 5.16$; $P = 0.11$). Corresponding results were confirmed by the Tukey's test.

In the first group of the varieties (Table 3), for all the sampling periods, there were significant differences both in EMS concentrations ($F = 37.03$; $F_{0.05} = 3.86$; $P = 2.16*10^{-5}$) and by genotype ($F = 62.44$; $F_{0.05} = 3.86$; $P = 2.40*10^{-6}$). For the second group, the differences in EMS ($F = 308.15$; $F_{0.05} = 3.86$; $P = 2.19*10^{-9}$) concentrations were significant, but there were no more differences in genotypes, again demonstrating a homogeneous reaction $F = 1.34$; $F_{0.05} = 3.86$; $P = 0.32$). In general, it can be stated that this parameter is less reliable than the previous indicators of similarity and survival, but in general, it reflects the variability of the material both in concentrations and depending on the object of mutagenic action. An important parameter evidencing the negative consequences of the impact of the mutagenic factor is the assimilation activity of the plant. In this case, it was represented through the photosynthetic activity of plants (Tables 5 and 6). This parameter turned out to be high in the Balaton variety, which is generally typical for varieties of foreign selection breeding. In this way, it was extremely different from all the other varieties, even well the adapted genotypes ($F = 7.98$; $F_{0.05} = 5.16$; $P = 0.01$).

In the first group of cultivars (Table 5), for all the sampling periods, there were significant differences both in EMS concentrations (F = 78.60; F0.05 = 3.86; P = 8.92*10-7) and in genotype (F = 185.77; F0.05 = 3.86; P = 2.07*10-9). For the second group (Table 6), there were also significant differences for concentrations (F = 71.01; F0.05 = 3.86; P = 1.38*10-6) and genotypes (F = 22.74; F0.05 = 3.86; P = 0.0001), but to a much lower degree. It can be noted that the same group of five varieties again stands out for its uniformity. The lowest variability was in the Zoloto Ukrainy and Nyva Odeska varieties, in which control and 0.025%, 0.05% and 0.1% concentrations did not differ equally in pairs. Varieties of a homogeneous group, as a rule, do not differ in one of the concentrations (although between different variants), however, in general, they were more variable. In the Balaton variety, which showed the highest activity, there were no differences when exposed to 0.025% and 0.05% concentrations. In general, the variability indicator is approximately at the level of sugar content indices, but more dependent on the genotype.

Table 3
Winter tolerance parameters during winter period (2019/2022 periods of vegetation) (x ± SD, n = 5). First group

Variety	Variant	Before winter period, score	Content of sugars in tillering nod (CS), %			After winter period, score
			November (11)	February (02)	March (03)	
Balaton	water	4.5	27.3 ± 0.4[a]	23.1 ± 0.6[a]	20.8 ± 0.4[a]	4.25
Balaton	EMS 0.025%	4.25	25.1 ± 0.4[b]	20.3 ± 0.5[b]	18.3± 0.4[b]	4.0
Balaton	EMS 0.05%	4.0	24.2 ± 0.4[c]	19.2 ± 0.5[c]	17.5 ± 0.4[b]	3.75
Balaton	EMS 0.1%	3.75	22.0 ± 0.4[d]	19.0 ± 0.6[c]	16.9 ± 0.4[bc]	3.5
Zeleny Hai	water	5	34.1 ± 0.5[a]	30.8 ± 0.4[a]	23.2 ± 0.5[a]	5
Zeleny Hai	EMS 0.025%	4.75	33.2 ± 0.5[a]	29.5 ± 0.3[b]	21.4± 0.5[b]	4.75
Zeleny Hai	EMS 0.05%	4.5	30.3 ± 0.5[b]	27.1 ± 0.4[c]	20.1 ± 0.5[c]	4.5
Zeleny Hai	EMS 0.1%	4.25	26.4± 0.5[c]	25.0 ± 0.4[d]	19.5 ± 0.5[c]	4.25
Zoloto Ukrainy	water	4.75	28.8 ± 0.5[a]	24.9 ± 0.4[a]	21.4± 0.6[a]	4.5
Zoloto Ukrainy	EMS 0.025%	4.5	26.2 ± 0.5[b]	22.2 ± 0.4[b]	19.5 ± 0.5[b]	4.25
Zoloto Ukrainy	EMS 0.05%	4	24.0 ± 0.4[c]	21.1 ± 0.4[c]	18.4± 0.4[c]	4
Zoloto Ukrainy	EMS 0.1%	3.75	23.1 ± 0.4[d]	20.2 ± 0.4[d]	17.6 ± 0.3[d]	3.75
Nyva Odeska	water	4.5	26.2 ± 0.4[a]	23.7± 0.6[a]	21.3± 0.3[a]	4.25
Nyva Odeska	EMS 0.025%	4	24.3 ± 0.4[b]	22.0 ± 0.6[b]	20.1 ± 0.3[b]	4
Nyva Odeska	EMS 0.05%	3.5	22.7 ± 0.4[c]	21.2 ± 0.6[c]	19.2 ± 0.3[c]	3.5
Nyva Odeska	EMS 0.1%	3.5	21.2 ± 0.4[d]	20.5 ± 0.5[c]	17.5 ± 0.3[d]	3.5

Note: see Table 1.

Table 4
Winter tolerance parameters during winter period (2019/2022 periods of vegetation) (x ± SD, n = 5). Second group

Variety	Variant	Before winter period score	Content of sugars in tillering nod (CS), %			After winter period, scores
			November (11)	February (02)	March (03)	
Borovytsia	water	5	33.9 ± 0.5[a]	30.9 ± 0.4[a]	23.7 ± 0.5[a]	5
Borovytsia	EMS 0.025%	5	32.1 ± 0.5[a]	29.1 ± 0.3[b]	21.3 ± 0.5[b]	4.8
Borovytsia	EMS 0.05%	4.5	29.9 ± 0.5[b]	26.7 ± 0.4[c]	20.0 ± 0.4[c]	4.5
Borovytsia	EMS 0.1%	4.5	25.8 ± 0.5[c]	24.8 ± 0.4[d]	18.9 ± 0.5[d]	4.3
Kalancha	water	5	34.3 ± 0.5[a]	31.1 ± 0.4[a]	23.4 ± 0.5[a]	5
Kalancha	EMS 0.025%	5	33.5 ± 0.5[a]	29.2 ± 0.5[b]	20.9 ± 0.5[b]	4.8
Kalancha	EMS 0.05%	4.5	30.4 ± 0.5[b]	26.7 ± 0.3[c]	20.0 ± 0.5[b]	4.5
Kalancha	EMS 0.1%	4.3	25.9 ± 0.5[c]	24.8 ± 0.4[d]	18.9 ± 0.4[c]	4.3
Polianka	water	5	34.7 ± 0.5[a]	31.1 ± 0.3[a]	22.9 ± 0.5[a]	5
Polianka	EMS 0.025%	5	32.1 ± 0.5[b]	28.5 ± 0.3[b]	22.0 ± 0.5[b]	4.8
Polianka	EMS 0.05%	4.5	30.1 ± 0.5[b]	26.4 ± 0.4[c]	20.5 ± 0.5[c]	4.5
Polianka	EMS 0.1%	4.5	26.0 ± 0.5[c]	24.5 ± 0.4[d]	19.3 ± 0.5[c]	4.3
Pochaina	water	5	34.9 ± 0.5[a]	31.6 ± 0.4[a]	23.7 ± 0.5[a]	5
Pochaina	EMS 0.025%	5	33.0 ± 0.5[b]	29.1 ± 0.4[b]	21.3 ± 0.5[b]	4.8
Pochaina	EMS 0.05%	4.5	29.8 ± 0.5[b]	26.6 ± 0.4[c]	19.9 ± 0.4[c]	4.5
Pochaina	EMS 0.1%	4.3	25.5 ± 0.5[c]	24.8 ± 0.4[d]	19.2 ± 0.5[c]	4

Note: see Table 1.

Table 5
Parameters of photosynthetic activity. First group (x ± SD, n = 5)

Variety	Variant	SPAD	Concentration of chlorophyll (a+b), μmol/m^{-2}
Balaton	water	55.78 ±0.60[a]	799.5 ±7.5[a]
Balaton	EMS 0.025%	52.99 ±0.66[b]	730.5 ±7.9[b]
Balaton	EMS 0.05%	51.34 ±0.72[b]	691.34±8.3[b]
Balaton	EMS 0.1%	48.92 ±0.43[c]	636.2 ±6.3[c]
Zeleny Hai	water	50.45 ± 1.19[a]	670.8 ±11.2[a]
Zeleny Hai	EMS 0.025%	49.16± 1.10[a]	641.5±10.6[a]
Zeleny Hai	EMS 0.05%	46.22± 1.18[b]	577.7±11.1[b]
Zeleny Hai	EMS 0.1%	43.66 ± 1.19[c]	525.2±11.2[c]
Zoloto Ukrainy	water	48.12±1.39[a]	618.5±12.3[a]
Zoloto Ukrainy	EMS 0.025%	47.01 ±1.34[a]	594.5±12.0[a]
Zoloto Ukrainy	EMS 0.05%	44.91 ±1.00[b]	550.5±10.0[b]
Zoloto Ukrainy	EMS 0.1%	43.17 ± 2.11[b]	515.5 ±16.6[b]
Nyva Odeska	water	45.17 ±1.89[a]	555.9 ±15.3[a]
Nyva Odeska	EMS 0.025%	44.21 ±1.79[a]	536.3 ±14.7[a]
Nyva Odeska	EMS 0.05%	41.01 ±1.22[b]	473.8 ±11.3[b]
Nyva Odeska	EMS 0.1%	40.32 ±1.34[b]	460.9 ±12.0[b]

Note: see Table 1.

Table 6
Parameters of photosynthetic activity. Second group (x ± SD, n =5)

Variety	Variant	Soil Plant Analysis Development (SPAD)	Concentration of chlorophyll (a+b), μmol/m^{-2}
Borovytsia	water	52.11± 1.01[a]	715.4 ±10.1[a]
Borovytsia	EMS 0.025%	49.22 ± 1.17[b]	642.9 ±11.0[b]
Borovytsia	EMS 0.05%	48.17 ± 1.23[b]	619.6 ±11.4[b]
Borovytsia	EMS 0.1%	45.01 ± 1.31[c]	552.5 ±11.9[c]
Kalancha	water	52.01± 1.45[a]	714.1 ±12.7[a]
Kalancha	EMS 0.025%	48.88 ± 1.32[b]	635.3 ±11.9[b]
Kalancha	EMS 0.05%	47.14 ± 1.24[b]	597.3 ±11.5[b]
Kalancha	EMS 0.1%	44.32 ± 1.16[c]	538.5 ±10.9[c]
Polianka	water	54.17 ± 1.32[a]	759.2 ±11.9[a]
Polianka	EMS 0.025%	52.47 ± 1.12[a]	718.0 ±10.7[a]
Polianka	EMS 0.05%	49.02± 1.09[b]	638.4 ±10.6[b]
Polianka	EMS 0.1%	47.17 ± 1.32[c]	597.9±11.9[c]
Pochaina	water	49.98 ± 0.92[a]	660.0±9.5[a]
Pochaina	EMS 0.025%	47.02 ± 0.83[b]	594.7±8.9[b]
Pochaina	EMS 0.05%	44.45 ± 0.86[c]	541.1±9.1[c]
Pochaina	EMS 0.1%	43.32 ± 0.79[c]	518.5 ±8.7[c]

Note: see table 1.

All the genotypes could be divided into three distinct groups. The first group would consist of Balaton and Nyva Odeska, which in all cases showed a uniform vulnerability to the action of EMS, differing only in a much higher photosynthetic activity in the variety Balaton. The second group would include one variety Zoloto Ukrainy, which actually occupies an intermediate position among the genotypes of the first and third groups in terms of resistance to mutagenic effect inhibition. This applies to all the parameters, except photosynthetic activity. The third group would contain varieties Zeleny Hai, Kalancha, Borovytsia, Polianka, Pochaina, which were not always completely homogeneous, some variability was manifested in the difference in the effect of individual concentrations that had decreased sugar concentration in winter; however, in general, these differences were insignificant.

Table 7
Factor Loadings (Unrotated) and Discriminant Function

Parameter	Concentration	Genotype	Wilks' - Lambda	F_{remove} (5,20)	p-value
Germination	-0.915*	-0.789*	0.019	13.64	< 0.01
Befor winter	-0.811*	0.116	0.016	10.11	< 0.01
Surviving	-0.841*	-0.917*	0.020	15.32	< 0.01
CS 11	-0.805*	0.913*	0.019	13.55	< 0.01
CS 02	-0.516	0.523	0.003	1.97	0.09
CS 03	0.892*	0.779*	0.015	11.31	< 0.01
SPAD	-0.798*	-0.614	0.015	11.20	< 0.01
Explenation variants	4.617	3.344	--	--	--
Non-explanation	0.314	0.677	--	--	--

Table 8
Results of classification of winter wheat genotypes

Genotype	Objects in model, %
Balaton	100.0
Zeleny Hai	83.3
Zoloto Ukrainy	100.0
Nyva Odeska	100.0
Borovytsia	83.3
Kalancha	83.3
Polianka	83.3
Pochaina	83.3
Total	89.6

Discussion

It is believed that supermutagens, unlike other mutagenic factors, are more promising in the induction of germplasm diversity due to the fact that they both cause significantly weaker effects of mutagenic effect inhibition in the first generation and, as a result, the survival of the material is much higher. The site-specificity of its action (Semenov, 2020) is localized and does not have such a large-scale effect as in the case of the same ionizing radiation (Xicun et al., 2016). Of course, mutagens have downsides (Abdoun et al., 2022). First, in this case, the genetic activity of these compounds is significantly limited by the object of action, which is shown in our case by a group of five varieties with a more or less uniform response to the mutagen (Asif, 2020; Yali & Mitiku, 2022). Although it is possible to expect more diversity in parameters that are poorly reflected in the resulting factor model, such as photosynthetic activity (and, therefore, possible drought tolerance) (Ariraman et al., 2018; Li et al., 2019).

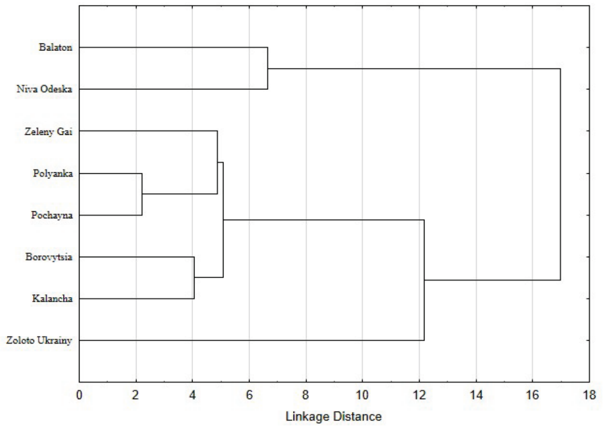

Fig. 1. Results of cluster analyzis by genotypes parameters

Thus, parameters of germination, survival, overwintering, and photosynthetic activity alone cannot be sufficiently informative. It would be bestdesirable to additionally use indicators of the yield structure, fertility, and cytogenetic activity of mutagenic factor (Oney-Birol & Balkan, 2019; Beiko & Nazarenko, 2022b). However, in general, they cover the most unfavorable and critical periods of growth and development of winter crop plants (Yakymchuk et al., 2021). If these stages were quite successful, and the classification of concentrations showed no more than semi-lethal values (in our case for two varieties), then there is no reason to believe that significant adjustments will be made to the amount of material used for further research (OlaOlorun et al., 2021). Additional parameters are needed rather to refine the model of mutagenic effect inhibition for more accurate monitoring of changes/disturbances, and not from the point of view of assessing the activity of the agent as a whole (Cann et al., 2022).

It can be concluded that the level of evidence of mutagenic effect inhibition also depends on the genetically determined potential of this trait (Nazarenko, 2020). This does not make the trait less sensitive to this type of impact. However, as in the case of photosynthetic activity, the decrease did not lead to such significant consequences for the life of the plant as a whole, and the manifestation of mutagenic effect inhibition was significantly weakened (Nazarenko, 2020). Of course, it is best to verify this position in the future by other methods of measuring the activity of the photosynthetic apparatus and plant respiration. Nonetheless, already within the framework of this experience, there are grounds for such a conclusion, given the presence of a fairly large group of genotypes that are homogeneous in reaction, which form the basis for a reliable assessment (Vesaliet al., 2017).

Conclusion

The use of even long-known and widely used for this area, i.e., genetic improvement of cereal crops by mutagenic factors can still allow the discovery of new patterns, if carefully studied. It greatly depends on the object of the mutagenic action. The results above will again and again point to the diversity of genetically determined resistance mechanisms and, as a result, to new variations of already, it would seem, finally determined patterns. The search for new parameters evidencing mutagenic effect inhibition raises to a qualitatively new level the understanding the complex nature of the processes that cause the evidence of certain consequences of exposure to DNA already at the level of the organism as a whole. In the future, it is a rather serious and important task to determine a relation between these effects and the frequency and spectrum of changes at the cell level, inherited changes in the plant itself, and the parameters of the formation of complex, polygenic mutations. Those changes are the ultimate goal of programs to improve cereal crops through the induction of biodiversity, while understanding their mechanisms makes it possible to make the process itself manageable in a certain sense and within certain limits, at least within the framework of specific ecological-genetic types.

References

Abdoun, A., Mekki, L., Hamwieh, A., & Badr, A. (2022). Effects of γ-radiation on chickpea (*Cicer arietinum*) varieties and their tolerance to salinity stress. Acta Agriculturae Slovenica, 118(2), 1–16.

Ariraman, M., Dhanavel, D., Seetharaman, N., Murugan, S., & Ramkumar, R. (2018). Gamma radiation influences on growth, biochemical and yield characters of *Cajanus cajan* (L.) Millsp. Journal of Plant Stress Physiology, 4, 38–40.

Asif, J. (2020). Effect of different pre-treatments on seed germination of *Prosopis juliflora* and *Dalbergia sissoo*: A step towards mutation breeding. Journal of Forest Science, 66, 80–88.

Beiko, V., & Nazarenko, M. (2022a). Early depressive effects of epimutagen in the first generation of winter wheat varieties. Agrology, 5(2), 43–48.

Beiko, V., & Nazarenko, M. (2022b). Occurrence of cytogenetic effects under the epimutagen action for winter wheat. Regulatory Mechanisms in Biosystems, 13(3), in press.

Cann, D., Hunt, J., Rattey, A., & Porker, K. (2022). Indirect early generation selection for yield in winter wheat. Field Crops Research, 282, 108505.

Essam, F., Badrya, M., & Aya, M. (2019). Modeling and forecasting of wheat production in Egypt. Advances and Applications in Statistics, 59(1), 89–101.

Hiroyasu, Y. (2018). Mutation breeding of ornamental plants using ion beams. Breeding Science, 68(1), 71–78.

Hong, M., Kim, D., Jo, Y., Choi, H.-I., Ahn, J.-W., Kwon, S.-J., Kim, S., Seo, Y., & Kim, J.-B. (2022). Biological effect of gamma rays according to exposure time on germination and plant growth in wheat. Applied Sciences, 12, 3208.

Jaradat, A. (2018). Simulated climate change deferentially impacts phenotypic plasticity and stoichiometric homeostasis in major food crops. Emirates Journal of Food and Agriculture, 30(6), 429–442.

Juhi, C., Alisha, A., Vacha, B., Sonali, C., & Rupesh, D. (2019). Mutation breeding in tomato: Advances, applicability and challenges. Plants, 8(5), 128.

Li, H. J., Timothy, D. M., Mc Intoshc, R. A., & Zhou, Y. (2019). Wheat breeding in Northern China: Achievements and technical advances. The Crop Journal, 7(6), 718–729.

Liu, Y., Liang, X., Zhou, F., & Zhang, Z. (2017). Accessing the agronomic and photosynthesis-related traits of high-yielding winter wheat mutants induced by ultra-high pressure. Field Crops Research, 213, 165–173.

Lykhovyd, P. V. (2021). Seasonal dynamics of normalized difference vegetation index in some winter and spring crops in the South of Ukraine. Agrology, 4(4), 187–193.

Mamenko, T. P., & Yakymchuk, R. A. (2019). Regulation of physiological processes in winter wheat by growth regulators in conditions of powdery mildew infection. Regulatory Mechanisms in Biosystems, 10(3), 331–336.

Mangi, N., Baloch, A., Khaskheli, N., Ali, M., & Afzal, W. (2021). Multivariate analysis for evaluation of mutant bread wheat lines using metric traits. Integrative Plant Sciences, 1(1), 29–34.

Nazarenko, M. (2020). Induction of winter wheat plant structure mutations by chemomutagenesis. Agrology, 3(1), 57–65.

Nazarenko, M., & Izhboldin, O. (2017). Chromosomal rearrangements caused by gamma-irradiation in winter wheat cells. Biosystems Diversity, 25(1), 25–28.

Nazarenko, M., Beiko, V., & Bondarenko, M. (2019). Induced mutations of winter wheat caused by gamma-rays fixed on plant height and stem structure. Agriculture and Forestry, 65(3), 75–83.

OlaOlorun, B., Shimelis, H., Laing, M., & Mathew, I. (2021). Morphological variations of wheat (*Triticum aestivum* L. Em. Thell.) under variable ethyl methanesulphonate mutagenesis. Cereal Research Communications, 49, 301–310.

Oney-Birol, S., & Balkan, A. (2019). Detection of cytogenetic and genotoxic effects of gamma radiation on M_1 generation of three varieties of *Triticum aestivum* L. Pakistan Journal of Botany, 51(3), 887–894.

Prabhu, L. (2019). The green revolution and crop biodiversity. In: Biological extinction. New perspectives. Cambridge University Press, Cambridge.

Semenov, A., Korotkova, I., Sakhno, T., Marenych, M., Hanhur, V., Liashenko, V., & Kaminsky, V. (2020). Effect of UV-C radiation on basic indices of growth process of winter wheat (*Triticum aestivum* L.) seeds in pre-sowing treatment. Acta Agriculturae Slovenica, 116(1), 49–58.

Shu, Q., Forster, B., & Nakagava, H. (2013). Plant mutation breeding and biotechnology. CABI Publishing, Vienna.

Spencer-Lopes, M., Forster, B., & Jankuloski, L. (2018). Manual on mutation breeding. Third edition. Food and Agriculture Organization of the United Nations, Rome.

Vesali, F., Omid, M., Mobli, H., & Kaleita, A. (2017). Feasibility of using smart phones to estimate chlorophyll content in corn plants. Photosynthetica, 55, 603–610.

Xicun, D., Xia, Y., & Wenjian, L. (2016). Plant mutation breeding with heavy ion irradiation at IMP. Journal of Agricultural Science, 8(5), 34–41.

Yakymchuk, R. A., Valyuk, V. F., Sobolenko, L. Y., & Sorokina, S. I. (2021). Induction of useful mutations in *Triticum aestivum* in the conditions of the radionuclide-contaminated alienation zone of the Chornobyl Power Plant. Regulatory Mechanisms in Biosystems, 12(3), 506–512.

Yali, W., & Mitiku, T. (2022). Mutation breeding and its importance in modern plant breeding. Journal of Plant Sciences, 10(2), 64–70.