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Optimal doses and concentrations of mutagens for winter wheat breeding purposes. Part I. Grain productivity

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Abstract

Evaluation of winter wheat mutant families (2011 – 2012, second – third generations, exclude a little number of dominant mutations at first generation) and lines (2013 – 2016, forth and next generations) has been carried out. Six bread winter wheat lines have been identified as high-grain productivity mutants (prevalent on national standard variety for agriculture zone by summarized three-year's results). For these lines higher value of two components of yield structure (1,000 grain weight and grain weight per plant) was typical during field estimation. Other indexes didn't get significant influences on yield performance. Gamma-rays dose 100 Gy as a mutagen factor was the most successful in induction productive mutations. Medium dose of gamma-rays (100 Gy) and concentrations of nitrosoalkylureas (0.01 – 0.0125%) are recommended for winter wheat mutation breeding on grain productivity. Four lines were recommended for state varieties exam. Two earliness, one semi- and one short-stem lines have been determined for using directly as future varieties or components of breeding crosses. Varieties obtained by gamma radiation are less sensitive to same mutagen (in terms of mutation induction). Their re-exposure is inappropriate by same mutagen. The same situation was observed for chemical mutagens. However, the varieties which obtained with field hybridization or after treated by other types of mutagens (for example exploited chemical mutagens for radiomutants) were more successful as initial material for obtaining new high-productive lines.

Keywords: chemical mutagens, gamma-rays, grain productivity, mutation breeding, winter wheat

Introduction

More than 3,200 mutant varieties have been directly or indirectly derived through mutation induction, including 256 bread wheat varieties (International Atomic Energy Agency, IAEA, 2018). Induced mutations have been applied to produce mutant varieties by changing the plant characteristic for a significant increase in production and improve quality (Ahloowalia et al., 2004; Shu et al., 2013).

Much excitement was generated as novel mutants overcame major obstacles in crop improvement and/or produced new and valuable variants. New forms such as semi-dwarfism, early maturity, disease resistance, etc. met immediate market demands and were often released directly as commercial varieties without recourse to refinement through cross breeding. The development of direct mutants into commercial varieties is still a common practice in seed propagated crops. Mutation breeding gave an initial material for green revolution (Ahloowalia et al., 2004).

Mutation induction activities had peaks in the 1950s - 1980s and enjoyed major successes in terms of mutant variety releases (Micke et al., 1990), but now this classical approaches for visual direct mutants selection at second –third generations after mutagen treatment provide new high productivity and high quality (by nutrient components) varieties (Lee et al., 2003; Singh and Balyan, 2009; Albokari, 2014). The early years of the 21st century witnessed a resurgence in mutation technologies due to a rapid and greater understanding of mutagenesis and related disciplines, which led to more applications (Henry et al., 2014; Jankowicz-Cieslak et al., 2017). New species are emerging as being important in agriculture, but also to the environment, medicine, and energy production (Ahloowalia et al., 2004). Induced mutation, become an enormous tool in plant breeding to improve genotypes in particular traits. Large number of improved varieties of many crop species has been released, revealing the economic value of the technology (Micke et al., 1990; Jankowicz-Cieslak et al., 2017). Therefore, the current experiment was designed to assess the performance of advanced mutant lines and also to know the interrelationship between grain yield and its associated traits.

The most part of mutant varieties have been obtained by using gamma-irradiation (about 85 percent from general number) (IAEA, 2018). Mutants generated through induced mutagenesis have been also used in genetics studies (van Harten, 1998; Waugh et al., 2006). Before the turn of the 21st century, experiments in plant mutagenesis were driven by the potential use of mutants in plant improvement. During the past ten years, genomics and molecular techniques have become part of plant mutagenesis research and induced mutants have become an established resource in genomics studies. Although plant induced mutagenesis has been used widely as a tool in basic studies and practical breeding programs, it is seldom considered to be an independent subject by plant scientists or plant breeders (Ukai, 2006; Henry et al., 2014; Jankowicz-Cieslak et al., 2017). Improvements in plant breeding can only be made when sufficient variation for a given trait is available to the breeder (Cheng et al., 2015). In the best case scenario the required variation (e.g. a disease resistant trait) is available within the elite gene pool of the crop. In many cases however, the desired variation may exist but only present in material distantly related to elite lines, i.e. in outdated varieties, old landraces or wild relatives. Retrieving such variation and developing it into a genetically finely tuned commercial variety is a protracted breeding process which has little appeal to plant breeders (Shu et al., 2013; Cheng et al., 2015). The prospect of delivering such variation directly into elite material without recourse to extensive upgrading (crossing and selection) was immediately ceased upon once methods of induced mutation were discovered.

Wheat is the top food crop in Ukraine as well as in the whole world and the biggest part of grain is obtained primarily from winter wheat. Wheat is the stable food of millions of people globally. This crop is widely adapted to wide range of climatic conditions. The total area for winter wheat cultivation in Ukraine covers 6.8 million hectares with actual total productivity of 24 million tons and average yield of 3.8 t/ha (Nazarenko, 2016a). Most modern wheat mutant varieties are not direct mutants, but a product of additional breeding (Shu et al., 2013). For the improvements of wheat crops, the assessment and isolation of promising breeding materials from introduced crop materials is the primarily step. The success of such breeding programs primarily depends on the assessment of the important characters and pattern of genetic variability of the existing germplasm (Laghari et al., 2012). One of the controversial questions of wheat mutation breeding is the problem of mutagens doses and/or concentrations suitable for creation new value genotypes (contradictory between high (Laghari et al., 2012; Mangi et al., 2016) and medium dose usage (Shu et al., 2013; Nazarenko, 2016b).

The aim of investigation is to determine optimal dose and concentrations of mutagens for mutation breeding practice and obtained new mutants with agronomic-value traits.

Materials and methods

Dried wheat grains (approx. 14% moisture content, in brackets method of obtaining varieties or used mutagens) of ‘Favoritka’, ‘Lasunya’, ‘Hurtovina’ (irradiation of initial material by gamma rays), line 418, ‘Kolos Mironovschiny’ (field hybridization), ‘Sonechko’ (chemical mutagenesis, nitrosodimethilurea (NDMU) 0.005%) and ‘Kalinova’ (chemical mutagenesis,1.4-bisdiazoatsetilbutan DAB 0.1%), ‘Voloshkova’ (termomutagenesis – low plus temperature at plant development stage of vernalizaion has been used as mutagen factor) of winter wheat (*Triticum aestivum L.*) were subjected to 100, 150, 200, 250 Gy gamma irradiation (cute dose, Co^{60}, 0.048 Gy/s) and treated with solutions of chemical mutagens – nitrosomethilurea (NMU) 0.0125 and 0.025%, nitrosoethilurea (NEU) – 0.01 and 0.025%, dimethylsulfate (DMS) – 0.0125, 0.025 and 0.05%, 1.4-bisdiazoatsetilbutan (DAB) – 0.1 and 0.2% (grains were soaked in mutagen solution). Each treatment was comprised of 1,000 wheat seeds. Exposition of chemicals mutagens was 18 hours. These concentrations and exposure are trivial for the breeding process that has been repeatedly established earlier (Nazarenko, 2016a). Non-treated varieties were used as a control for mutation identified purpose.

Treated seeds were grown in rows with inter and intra-row spacing of 50 and 30 cm, respectively, to raise the M1 population. The untreated seeds of mother varieties (parental line/variety) were also planted after every ten rows as control for comparison with the M1 population. M1 plant rows were grown in three replications with check-rows of untreated varieties in every ten-row interval (Nazarenko, 2017a).

In M2 – M3 generations productive and other value families have been selected via visual estimation. The sowing was done by hand, at the end of September, at a depth of 4-5 cm and with a rate of 100 viable seeds to a row (length 1.5 m), interrow was 15 cm, between samples 30 cm, 1 - 2 rows for sample with control-rows of untreated varieties in every twenty-sample interval.

Estimation of total yield per plot and its components was conducted from 2014 to 2016 years (M4 – M6 generations). The controls were national standard by productivity 'Podolyanka' and initial variety. The working-methods in the breeding trials are satisfied to state variety exam requests. The trial was set up as a randomized block design method with three replications and with a plot size of from 5 to 20 m^2 in 2 – 3 replications. Data on yield structure components (plant height, general number of culms, number of productive culms, spike length, spikelets per spike, number of grain per spike, grain weight per spike and plant, 1,000 grains weight) were taken from 50 randomly selected plants of each line representing properly morphological traits for this line (Shu et al., 2013).

Experiments were conducted on the experiment field of Dnipropetrovsk State Agrarian-Economic University (village Aleksandrovka, Dnepropetrovsk district, Dnepropetrovsk region, Ukraine). Black soil of the center is characterized by clay loam containing high organic matters. The fields are lying 245 m above the sea level with 8 - 11 °C temperature during wheat growing season (September/July) and the average rainfall is about 400 - 550 mm in vegetation season respectively. Normal cultural practices including fertilization were done whenever it is necessary. Industrial evolution was conducted for Dnepropetrovsk region farms too during 2016 – 2017 years (fields about 2 – 3 hectares in three farms in different agroecology districts of region).

Mathematical processing of the results was performed by the method of analysis of variance, the variability of the mean difference was evaluated by Student's t-test, the grouping of lines by grain productivity was performed by cluster analysis, factor analyses was conducted by module ANOVA. In all cases standard tools of the program Statistica 6.0 were used.

Results and discussion

Total size of field trial at M2 – M3 generations was 53,450 families (include controls) and represented by variants of mutagen treatment at Table 1. Investigators ran on with trivial problem for high doses limited number of material for next stapes of breeding process (Nazarenko, 2016a; Nazarenko, 2017b).

From M2 – M3 generations 1,482 potential productivity winter wheat mutation lines and 5,862 lines with mutation changes were determined overall. Results of three years of estimation were 13 lines (Table 2). Lines, with statistically lower than standard in grain productivity, were sowing in genetically-value mutant's collection.

Generally, all lines at Table 2 can be subdivided into two groups. That's first group (lines number 133, 142-1, 156, 172, 174, 179 and 186), which productivity is on standard level and second one (130, 157, 157-1, 185, 211 and 213) with grain productivity statistically more than standard. Every year with help of cluster analysis all material were classified on this two groups and third one which yield was statistically lower than standard (that's why this lines are not representing at this table and establish for breeders only interest as source for other value morphological traits. The increase in yield varied from 44.9 (line 185) to 266.3 (line 213) gram per m^2.

Table 1. Number of mutant families at second – third generations

Trial	Kolos Mironivschini	Kalinova	Voloshkova	Sonechko	Favoritka	Hurtovina	Lasunya	Line 418
Control	500	500	500	500	500	500	500	500
Gamma-rays, 100 Gy	500	500	500	500	500	500	500	500
Gamma-rays, 150 Gy	500	500	500	400	500	500	500	500
Gamma-rays, 200 Gy	500	350	500	250	450	500	450	400
Gamma-rays, 250 Gy	300	350	500	100	400	400	350	400
NMU 0.0125%	500	500	500	500	500	500	500	500
NMU 0.025%	500	500	500	500	500	500	500	500
NEU 0.01%	500	500	500	500	500	500	500	500
NEU 0.025%	500	500	500	500	500	500	500	500
DAB 0.1%	500	500	500	500	500	500	500	500
DAB 0.2%	500	500	500	500	500	500	500	500
DMS 0.0125%	500	500	500	500	500	500	500	500
DMS 0.025%	500	500	500	500	500	500	500	500
DMS 0.05%	400	400	350	400	500	500	400	400

In industrial estimation lines 130, 157, 211, 213 were identified too as stable in their high grain productivity (about 0.8 – 1.5 t/ha more than 'Podolyanka') and hand to state variety exam as candidate varieties ‘Giant’, ‘Leroy’, ‘Deada’, ‘Leana’ (correspondently).

All three years parameters of yield structure have been investigated. Components such as plant height, 1,000 grain weight, grain weight per plant, number of grains per spike, number of yield culms have been developed. These dates are represented at Table 3. Asterisks mean that parameters are statistically discrepancy from standard ‘Podolyanka’.

Table 2. Grain productivity of winter wheat mutant’s lines

Number of mutant line	Years of field experiment			Average yield	+/- to standard
	2014	2015	2016		
	g/m^2				
Podolyanka (standard)	577.2	1,087.8	976.1	880.4	--
130	843.6	1,176.6	1,139	1,053.1*	172.7
133	519.5	1,103.3	1,071	897.9	17.6
142-1	752.6	908	1,050	903.5	23.2
156	446.4	1,103.3	1,187.8	912.5	32.1
157	607.8	1,731.6	1,027	1,122.1*	241.8
157-1	607.8	1,150	1,112	956.6*	76.2
172	414.4	1,051.8	1,217	894.4	14
174	512.9	1,099	1,039	883.6	3.3
179	574.5	996.8	1,078.9	883.4	3
185	414.3	1,205.5	1,156	925.2*	44.9
186	276.2	1,280.9	1,099	885.4	5
211	813	1,216.6	1,015	1,014.8*	134.5
213	781	1,505.2	1,153.9	1,146.7*	266.3
$LSD_{0.05}$			41.3		

* statistically significant differences from standard at $t_{0.05}$

Six lines are shorter by steam than ‘Podolyanka’. From these 130, 185 and 211 lines are in productive group. Other tree lines (133, 174, 186) are going to be used as a component in breeding crosses for improvement this impotent trait. High grain productive lines 130, 157, 157-1, 185, 211, 213 also are dominated under standard by such parameters as 1000 grain weight, grain weight per plant. Selection by these two indexes always led to high grain yield. Correlation between this two parameters and yield was on 0.88 – 0.95 level in the investigations.

Table 3. Main components of estimation winter wheat mutant lines yield structure

Line	Plant height	Number of yield culms	Number grains per spike	Grain weight per spike	Grain weight per plant	1,000 grain weight
	cm	pcs			g	
Podolyanka (standard)	106.2±4.3	4±0.1	29.6±4.2	1.3±0.4	4.4±0.4	44.6±1.3
130	101.8±4.3*	4.8±0.4*	47.4±6.8*	2.3±0.1*	5.9±0.4*	47.8±0.9*
133	99±2.7*	4±0.7	31.8±2.4	1.3±0.2	4.2±0.2	44.2±1
142-1	108±4.8	3.8±0.4	37.2±9*	2.4±0.2*	4.4±0.3	44.7±1
156	105.8±4	3.8±0.4	36.4±2.3*	1.6±0.2	4.9±0.3*	46.2±1.1*
157	103.4±3	4.9±0.4*	46.4±2.1*	2.3±0.1*	5.8±0.2*	51.7±1.6*
157-1	111±3.7*	4.6±0.7*	34.4±8.3*	1.2±0.3	5.2±0.4*	47.8±1*
172	109.4±3.5	3.8±0.6	30.4±5.8	1.4±0.3	4.2±0.4	43.9±0.9
174	98.8±3.4*	3.8±0.6	30.2±5.8	1.3±0.2	3.7±0.3*	45.1±0.7
179	115.2±3.7*	4.4±0.5	29.2±5	1.4±0.2	3.9±0.5	39.6±1.4*
185	101.2±3	4.5±0.5	34.6±3.2*	1.7±0.4	5.4±0.5*	48.3±0.9*
186	78.7±3.7*	3.9±0.3	30.3±4	1.3±0.3	4±0.4	45.0±1
211	88.2±3.2*	4.9±0.7*	39.7±4.8*	2.1±0.2*	5.7±0.3*	48.8±1.1*
213	103±4.1	5.2±0.6*	49.2±7*	2.4±0.2*	5.5±0.2*	54.8±1.5*

* statistically significant differences from standard at $t_{0.05}$

Other parameters, which can be influenced on yield, number of productive culms, grain weight per spike and number of grains per spike are not so informative. Sometimes, when one of this indexes is on standard level or higher than standard it doesn't speak anything about real grain productivity of line.

Initial variety of mutant lines, mutagen dose or concentration (in case of chemical mutagens), characteristic of main morphological traits (via phenological field observations) are represented at Table 4 (productive forms are underlined).

For obtaining productive forms next initial varieties are preferable 'Sonechko' (2 forms), 'Kolos Mironovschiny' (2), 'Kalinova' (1), 'Favoritka' (1).

High-yield lines have been derived by using gamma-rays in 100 Gy dose (3 lines), chemical mutagens NMU 0.0125% (2) and NEU 0.01% (1). Effectiveness of mutagens are ranked in following orders (from higher to lower) gamma-rays → NMU → NEU. Medium dose and concentrations are more properly for induction this type of mutations.

Only one from 13 lines at Table 4 doesn't have waxy bloom. May be, it relates with resistance for drought, which is very impotent trait under Ukrainian climatic conditions. One short-steam and one semi-dwarf lines have been obtained and in future would be used as a component at breeding crosses. Also, two earliness lines have been identified.

According to doses and concentrations useful for breeding purposes there is a contradictory between two approaches. First part of breeders justifies that high doses (such as 200 – 250 Gy) is more suitable for obtaining new wheat varieties (Laghari et al., 2012; Mangi et al., 2016). Their statement is supported by dates of properly department of IAEA (by official released mutant plant variety database (IAEA, 2018). Second part writes that the minimal exchange in phenotype (for improving one-two insufficient agronomic-value traits) more fitting for breeding process. Especially it's true for low concentrations of chemical mutagens (such as NEU, NMU, DMS, DAB in investigations) (Jankowicz-Cieslak et al., 2017; Nazarenko, 2017b). But as for results and experience firstly it depends on the object of mutagen action.

Gamma-ray irradiated more fitting for varieties which have been obtained without gamma-rays. Chemical mutagens are more effective for radiomutants and hybrids varieties.

Regarding to investigations (Nazarenko, 2016b) high doses and concentrations are more suitable for obtaining genetic-value lines like as dwarfs, semidwarfs, earliness forms, forms with changes in shape of spike (for example system mutation, when mutants are similar with other species of this genus). But these forms were less in grain productivity than standard. As for traditional opinion high doses and concentrations are more suitable for obtaining high rate of mutations. But these mutations can be used only as components for field hybridization, not direct as new varieties. For breeding practice have to use average means of mutagens. This one supports to avoid mutagen depression in first generation too.

From the obtained results, such mutagens as gamma-rays, nitrisoalkylureaes proved to be more effective. Such mutagens are at the same time most widely used in worldwide practice for mutation breeding. DAB and DMS produced only one productive line each, which didn't exceed the standard in terms of yielding performance and showed no additional value traits.

Table 4. Origin and describe of main morphological traits of winter wheat mutant lines

Line	Initial variety	Mutagen	Waxy bloom	Awn	Plant height	Additional positive traits
Podolyanka (standard)	--	--	+	Awnless	High	
130	Kalinova	100 Gy	+	Awn	Medium	
133	Sonechko	100 Gy	+	Awnless	Medium	
142-1	Sonechko	NEU 0.01%	+	Awnless	High	Earliness
156	Kolos Mironovsc hiny	NMU 0.0125%	+	Awn	High	
157	Sonechko	100 Gy	+	Awn	Medium	
157-1	Sonechko	100 Gy	+	Awnless	High	
172	Hurtovina	NEU 0.01%	+	Awn	High	
174	Lasunya	DAB 0.1%	+	Awnless	Medium	
179	Hurtovina	DMS 0.0125%	+	Awn	High	
185	Kolos Mironovsc hiny	NEU 0.01%	+	Awn	Medium	
186	Kolos Mironovsc hiny	NEU 0.01%	+	Awnless	Semi-dwarf	
211	Kolos Mironovsc hiny	NMU 0.0125%	-	Awnless	Short	
213	Favoritka	NMU 0.0125%	+	Awnless	High	Earliness

In all cases the high-yielding lines were obtained as a result of combination of a mutagen and a breed only in the cases, when used a different mutagen than the mutagen used at creating the variety (in cases of ‘Sonechko’ and ‘Favorytka’), or when a variety obtained as a result of a recombination breeding with no mutagen factors used.

The chemical mutagenesis is effective for radio-mutants, gamma rays – for chemomutants, for the breeds created using the method of field hybridization it makes no difference. It is better to use the best by agronomic-value traits material for mutagenesis.

Four winter wheat mutant lines with high grain productivity have been obtained which proposed for state variety exam for Steppe region. In addition, obtained lines with other agronomic-value traits, such as two earliness (from which one 213 line with high productivity), one semi-dwarf (186, yield on standard level) and one short-stem lines (high-productivity line 211), which can be used as components for future crossbreeding program.

Conclusions

The mutagens in the investigations may be represented by their efficiency as follows (from high to low): gamma rays → NMU → NEU → DMS, DAB. Medium dose of gamma-rays (100 Gy) and medium concentration of chemical mutagens (NMU 0.0125% and NEU 0.01%) are better variant for wheat mutation breeding.

In all cases the high-yielding lines were obtained when different mutagen have been used than the mutagen used at creating the variety or when a variety obtained as a result of a recombination breeding with no mutagen factors used.

Lines 130 (‘Giant’), 157 (‘Leroy’), 211 (‘Deada’), 213 (‘Leana’) have been recommended for official released state variety exam. Only selection by high 1,000 grain weight and grain weight per plant have been guaranteed future high yield. After estimation two earliness, one semi-dwarf and one short-stem lines have been identified.

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