Scientific Papers. Series A. Agronomy, Vol. LXIII, No. 1, 2020
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

WINTER WHEAT MUTATIONS BY PLANT HEIGHT AND STRUCTURE CAUSED BY CHEMICAL SUPERMUTAGENS

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Abstract

The objectives of our investigations are to describe the variation by mutations of stem architecture of the modern Ukrainian winter wheat varieties due to their interactions with mutagen concentration and genotype-mutagen interaction specific. Agronomic-value mutations like as short stem, dwarfs and semi-dwarfs have been investigated too. New perspective mutant lines have been obtained in terms of mutation breeding program. Main components for mutation breeding successful was genotype-mutagen interaction (due to factor analyses). By mutation occurs (in sense of mutation rate and spectra) genotypes can be subdivided on two groups. At the first group only varieties, which obtained with nitrosoalkylurea were observed. At second group other six varieties. Nitrosoalkylurea were less useful for obtaining mutations by plant architecture for any varieties. Higher level of short-stem and semi-dwarfs mutation were inducted by NMU 0.025%. Semi-dwarfs as mutations significance responded to nitrosoalkylurea action by genotype-mutagen interaction.

Key words: *winter wheat, chemical mutagenesis, plant height and structure, nitrosoalkylureas.*

INTRODUCTION

Experimental mutagenesis has been used successful in main crops for obtaining agronomical important traits. Induced mutations in wheat have been obtained for morphological and quantitative characters by treatment with different mutagens (Nazarenko et al., 2018). The main purpose of using mutagens has been to induce genetic variation of agronomic-value traits. Grain yield, a complex polygenic trait is highly affected due to complex of difference traits of plants architecture (Nazarenko, 2017). More than 3500 varieties of plants obtained either as direct mutants or derived from their crosses and 2700 mutant varieties of different plants including cereal crops have been released throughout the world through direct or indirect use of mutation breeding (IAEA, 2018).

Bread wheat (*Triticum aestivum* L.) with the annual production of about 757 million tons (in 2017) (USDA, 2018), is one of the world's most important cereal crops. Winter wheat is the world's leading cereal grain and the most important food crop, occupying first position in Ukraine. Ukrainian agriculture takes about 48% area under cereals and contributing 38% of the total food grain production in the country (Nazarenko, 2016).

The improvement of grain yield and yield components of wheat through application of mutagens leads towards improvement of new cultivars with improved traits. The use of induced mutations has become an important approach to optimize plant structure for bioproductivity (Naveed et al., 2015).

The present studies were therefore undertaken to investigate the effects of chemical supermutagens (nitrosoalkylureas) on so yield associated trait as plant height and structure of stem.

Plant height is important agronomic traits related to plant architecture and grain yield in wheat. Tiller number and plant height are pointed out as two major agronomic traits in cereal crops affecting plant architecture and grain yield (Ellis et al., 2004). In investigation of chines researches of NAUH167, a new mutant of common wheat landrace induced by ethylmethyl sulfide treatment, exhibits higher tiller number and reduced plant height was attributed to the decrease in the number of cells and their length. Genetic analysis showed that the high-tillering number and dwarf phenotype were related and controlled by a partial recessive gene (Xu et al., 2017).

Dwarfing and semi-dwarfing mutations have a mutual effects. As for example, dwarfing gene Rht-5 was associated with a plant height reduction, delaying heading date by 1 day, increasing the number of fertile tillers plant^{-1}, while reducing the number of spikelets spike^{-1} and number of grains spike^{-1}. The results of this study could be useful for proper use of Rht-5 dwarfing gene in breeding programs to improve lodging tolerance, yield potential in wheat and increase efficiency of marker assisted selection for agronomic traits (Daoura et al., 2014).

One strategy to meet this challenge is to raise wheat productivity by optimizing plant stature. As a sample of this investigation, the reduced height 8 (Rht8) semi-dwarfing gene is one of the few, together with the Green Revolution genes, to reduce stature of wheat (*Triticum aestivum* L.), and improve lodging resistance, without compromising grain yield. Rht8 is widely used in dry environments where it increases plant adaptability. Morphological analyses show that the semi-dwarf phenotype of Rht8 lines is due to shorter internodal segments along the wheat culm, achieved through reduced cell elongation (Gasperini et al., 2012).

The development of wheat mutants not only provided new genetic resources for wheat improvement, but also facilitated our understanding of the regulation of these traits at the molecular level. Identification of a dwarf mutant with a compact spike, NAUH164, produced from ethyl methyl sulfonate treatment of wheat variety Sumai 3, has reduced plant height and shortened spike length. Dwarfness and compact spike were controlled by a single dominant gene that was designated Rht23 (Chen et al., 2015).

Regarding 47 wheat cultivars carrying different Rht alleles screening for their ability to emerge from deep sowing, and for detailed physiological characterization in the field the modern wheat lines have been shown differences in early developmental stages were associated with grain yield, as indicated by a reduction of 37.3% in the modern cultivars (Amram et al., 2015). But reducing by grain productivity at modern investigations not always characteristic for dwarf winter wheat varieties with typical gibberellin-responsive (GAR) dwarfing genes, such as Rht12. In investigations of chines sciences (Chen et al., 2014) plant height of the tall lines was not affected significantly by GA3 treatment. Plant biomass and seed size of the GA3-treated dwarf lines was significantly increased compared with untreated dwarf plants while there was no such difference in the tall lines. This effect has addictive value effect Rht12 dwarf plants developed faster than control plants and reached double ridge stage 57 days, 11 days and 50 days earlier and finally flowered earlier by almost 7 days while the tall lines. Both possibilities are confirmed by several investigations (Bachir et al., 2014; Fellahi et al., 2018; Hans et al., 2019; Lingling et al., 2019).

The objectives of our investigations are to describe the genotypic variation of new mutant winter wheat lines by plant height and structure, investigation of role genotype-mutagen interactions at formation of new trait. The most target objects are developing relations between genotype and nature of chemical mutagen, mutagen concentration. Second our purpose to estimate new lines and their suitability as direct new varieties or components for future breeding crosses.

MATERIALS AND METHODS

Dried seeds (approx. 14% moisture content, in brackets method of obtaining varieties or used mutagens) of 'Favoritka', 'Lasunya', 'Hurtovina' (mutation and mutation-recombination varieties regarding IAEA classification, radiomutans), line 418, 'Kolos Mironovschiny' (hybrid varieties), 'Sonechko' and 'Kalinova' (mutation varieties, chemomutant), 'Voloshkova' (mutation variety, termomutagenesis - low plus temperature at plant development stage of vernalizaion) of winter wheat (*Triticum aestivum* L.) were treated with solutions of chemical mutagens - nitrosomethilurea (NMU) 0.0125 and 0.025%, nitrosoethilurea (NEU) - 0.01 and 0.025%. 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 and national standard by grain yield Podolyanka were used as a control for mutation identified purpose by all traits changes.
Treated seeds were grown in rows with inter and intra-row spacing of 50 and 30 cm, respectively, to raise the M_1 population. The untreated seeds of mother varieties and standard (parental line/variety) were also planted after every ten rows as control for comparison with the M_1 population. M_1 plant rows were grown in three replications with check-rows of untreated varieties in every ten-row interval (Nazarenko, 2017).
In M_2-M_3 generations mutation 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 and standard in every twenty-sample interval.
Estimation of total characteristics and heritability of changed traits was conducted from 2014 to 2018 years (M_4-M_8 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 (Shu et al., 2013).
Experiments were conducted on the experiment field of Dnipro State Agrarian-Economic University (village Oleksandrovka, Dnepropetrovsk district, Dnipro region, Ukraine). Normal cultural practices including fertilization were done whenever it is necessary. Weeds were manually removed where necessary, and fungicides and insecticides were applied to prevent diseases and insect damage. Evolution was conducted during 2011-2018 years.
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 mutants cases was performed by cluster and discriminant analysis, factor analyses was conducted by module ANOVA. In all cases standard tools of the program Statistica 8.0 were used.

RESULTS AND DISCUSSIONS

Total size of population 20000 families at second-third generation (include controls) and represented by variants of mutagen treatment at Table 1. Investigators are conducted with trivial mutagen concentrations for breeding purposes (Nazarenko, 2016a; Nazarenko, 2017b).
From M_2-M_3 generations (from all experiments, included all variants with other mutagens) 1,482 potential productivity winter wheat mutation lines and 5,862 lines with mutation changes were determined overall. In all variants 500 families have been investigated, all concentrations are optimal for plant surviving. General rate of mutations was up to 14.2% under NEU action (Sonechko) and to 15% under NMU action (Voloshkova) (Table 1).
The lowest general mutation rate was 3.6% (NEU) and 4.2% (NMU) (at both cases for chemomutant Sonechko, which obtained with nitrosoalkylureas action). NMU is more active as mutagen by general rate of mutations, but its depends on genotype-mutagen interaction and can be changed according to initial variety.
Regarding rate of plant structure mutations action of both mutagens was equal at average and depended on initial genotype only.
From these investigations fact of decreasing general mutation rates and number of mutation traits (level of changeability) for chemomutants after nitrosoalkylureas action has been developed.
Regarding dates of Tables 2-4 any statistically reliable difference between rates in this group between three types of genotypes has been observed for varieties Sonechko and Kalinova, but line 418 is close to these genotypes by this parameter and cluster and discriminant analysis has been used for more precision classification of material.

Table 1. General rate of mutations at second – third generations (rate of mutations by plant structure in brackets)

Trial	Kolos Mironivschini	Kalinova	Voloshkova	Sonechko	Favoritka	Hurtovina	Lasunya	Line 418
Control	0.4(0.4)	1.2(1)	1.8(1.4)	0.8(0)	0.6(0.2)	0.8(0)	1.4(0.8)	0.8(0.2)
NEU 0.01%	9.6(1)	6.4(0.6)	8.8(1.4)	4.2(1.4)	8.8(1.6)	7.0(0.6)	8.49(1.8)	9.4(0.8)
NEU 0.025%	13.4(1.8)	10.8(0.6)	15.0(2.6)	7.8(0.6)	12.4(2.2)	11.2(1)	13.8(2.6)	12.0(2.6)
NMU 0.0125%.	8.8(1.4)	5.0(1)	7.8(1.6)	3.6(0.6)	7.6(1.8)	8.0(1.6)	8.0(1.2)	7.6(1.2)
NMU 0.025%	13.6(2)	7.0(1.6)	15.8(2.2)	4.8(1.8)	12.0(2.8)	10.2(2.6)	12.0(2)	14.2(1.4)

Table 2. Spectrum of mutations under nitrosoalkylureas action (radiomutants), %

N	Trait	Check	NEU 0.01%	NEU 0.025 %	NMU 0.012 5%.	NMU 0.025%
	variety Favoritka					
1	high stem	0.0	0.6	0.8	0.6	1
2	short stem	0.2	0	0.2	1	0.8
3	semi-dwarf	0.0	0	0	0.2	0.6
4	dwarf	0.0	0	0	0	0.2
5	thick stem	0.0	0	0	0	0.2
6	thin stem	0.0	1	1.2	0	0
	variety Hurtovina					
1	high stem	0	0.4	0.6	1	1.2
2	short stem	0	0,2	0.2	0.6	0.6
3	semi-dwarf	0	0	0.2	0	0.4
4	thin stem	0	0	0	0	0.4
	variety Lasunya					
1	high stem	0.4	1	1.6	0.4	0.4
2	short stem	0.4	0,8	0.6	0.6	0.8
3	semi-dwarf	0.0	0	0.2	0.2	0.6
4	dwarf	0.0	0	0	0	0.2
5	thin stem	0.0	0	0.2	0	0

Table 3. Spectrum of mutations under nitrosoalkylureas action (chemomutants), %

N	Trait	Check	NEU 0.01%	NEU 0.025 %	NMU 0.0125 %	NMU 0.025 %
	variety Kalinova					
1	high stem	0.8	0.6	0.6	0.6	1
2	short stem	0.2	0	0	0.4	0.4
3	semi-dwarf	0.0	0	0	0	0.2
	variety Sonechko					
1	high stem	0.0	0.6	0.2	0.4	1
2	short stem	0.0	0.4	0.2	0.2	0.4
3	semi-dwarf	0.0	0.2	0.2	0	0.4
4	dwarf	0.0	0.2	0	0	0

Rate of this type of mutations varied from 0.6 (Kalinova, Sonechko, Hurtovina) to 2.6% (Voloshkova, line 418) for NEU and from 0.6 (Sonechko) to 1.4% (line 418) for NMU. As we can see from the tables, higher rates and more types of this group mutations were characterized for recombination and radiomutant varieties, chemomutants like Kalinova and Sonechko (at higher level) were less sensitive to this type of mutagen action.

Table 4. Spectrum of mutations under nitrosoalkylureas action (hybrid varieties), %

N	Trait	Check	NEU 0.01%	NEU 0.025 %	NMU 0.012 5%.	NMU 0.025%
	variety Kolos Mironivschini					
1	high stem	0.2	0.8	0.6	0.8	0.6
2	short stem	0,2	0.2	0.2	0.4	1
3	semi-dwarf	0.0	0	0.2	0	0.2
4	thin stem	0.0	0	0.8	0.2	0.2
	variety Voloshkova					
1	high stem	0.0	0.4	0.2	0.8	0.4
3	short stem	0.0	1	1.6	0.8	1.6
4	semi-dwarf	0.6	0	0.6	0	0
5	dwarf	0.8	0	0.2	0	0.2
	line 418					
1	high stem	0.2	0.8	1.8	1	0.6
2	short stem	0.0	0	0.6	0.2	0.6
3	semi-dwarf	0.0	0	0.2	0	0.2

Cluster analyses (Figure 1) confirmed complicated and complex character of mutagen-genotype interaction. Only one group has been identified with statistically reliability.

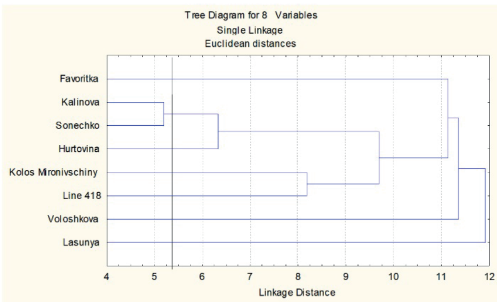

Figure 1. Results of cluster analyze

General mutation rate to all types mutation has been increased concentration growth. High level of changeability was corresponded to higher concentrations of NEU and NMU. Rate of mutations by plants structure obeys this tendency (excluded varieties Sonechko (decreasing of rate) and Kaliniova (rate is

lower). In spite of gamma-rays from previous investigations dates were enough clearly for this conclusion, may be due to more site-specific action of chemical mutagens.
We can subdivided initial material by the method of breeding as radiomutants (Favoritka, Hurtovina, Lasunya), chemomutants (Kalinova and Sonechko), thermomutants (low plus temperature at plant development stage of vernalizaion has been used as mutagen factor) (Voloshkova) and forms, obtained after hybridization (Kolos Mironivschini, line 418).
For first group (Table 2) similar number and types of mutations was characterized to all mutagens and concentrations, but reaction of genotypes was differing for all three genotypes. Rates of mutations are not high, variety Favoritka characterized by more types of mutations, dwarfs mutations were seldom and only for two genotypes under NMU 0.025% fction. Lower mutability was inherited for variety Hurtovina. Seldom mutations of stem thickness in spite of gamma-rays can be observed at all cases, and were appeared at all cocncentrations and mutagens, but not genotypes. Nitrosoalkylureas action are more useful for this type of mutations then gamma-rays.
For second group (Table 3) lower rate of mutations was developed for all varieties and concentrations. We observed only mutations by plant height.
Regarding Table 4 the same situation was observed as for radiomutants from table 1. At all cases for all genotypes concentration NMU 0.025% was more suitable for mutation induction by plant structure.
Thus, mutants with thick stem for NEU - for a thick stem of mutations is completely not marked; for unlikely, arose with a very high frequency for the variety Kolos Mironivshchyni - the frequency of occurrence was 0.8% and one case for the variety Lasunya, that is, the mutation is rather specific; high-stem mutants on average 0.8%, high-frequency mutation occurring in any variant with a frequency from 0.2 to 1.8%, but mainly for Favoritka, Lasunya, line 418; short stem - high probability of occurrence, but more rarely than high- frequency, an average of 0.5%, the frequency in some variants up to 1.6%, which is considerably lower than in the case of gamma rays, this mutation is completely absent for the variety Kalinova, but unlikely for the varieties Sonechko and Kolos Mironivschini; semi-dwarfs - mutation average probability, much less frequent than gamma rays, up to 0.6%, on average - 0.1%, characteristic for higher concentrations of NEU, almost absent at NEU 0.01% (except for the Sonechko) and absent from the Kalinova; dwarf - for NEU, in contrast to gamma rays almost absent, only one case for varieties Voloshkova and Sonechko.

Table 5. Results of discriminant analyze

Variables at model	Wilks Lambda λ	Partial Lambda	F-remove (4.02)	p-level
high stem	0.289232	0.783392	2.212000	0.089858
short stem	0.258270	0.877308	1.118807	0.364900
semi-dwarf	0.416547	0.634530	2.657860	0.043019
dwarf	0.277161	0.817512	1.785787	0.156017
thick stem	0.336470	0.867650	2.545489	0.132456
thin stem	0.317843	0.965437	2.097654	0.245678

For NMU for this group - only one case of a mutant with a thick stem (Favoritka, NMU 0.025%) was noted for the thickness of the stem; thin stem - very rare mutation. the frequency of occurrence from 0 to 0.2%, arose for only three variants; high-stem mutants on average 0.7% variations, high-frequency mutation occurring in any variant with a frequency from 0.4 to 1.2%, more or less evenly in all varieties, which significantly differs from NEU; short stem - high probability of occurrence, in all cases, an average of 0.7%, the frequency in individual variants up to 1.6%, which is much lower than in the case of gamma-rays, approximately uniform and on average more frequent than in NEU; semi-dwarf - mutation is also highly probable, comparable frequency to the frequency of gamma rays, up to 0.6%, on average 0.2%, characterized by a higher concentration of NMU, for the Voloshkova this mutation is absent, which is partially coincides with NEU; dwarfs occur only in three cases - for the varieties Voloshkova NMU 0.025%, Favoritka and Lasunya variety, that is, unlike NEU, isolated cases occur.
Regarding analyze of these groups it has been developed that rate of these types of mutations was significantly lower for first group, than for others. According to ANOVA analyses number

of mutations was depended on concentrations at all cases, relation with genotype and mutation rate has been identified with significance reliability for only one case semi-dwarf mutations (F 3.01, $F_{critical}$ 2.15). In spite of this fact, genotype and mutagen interaction are statistically reliable for all cases and traits, just the same for gamma-rays at previous investigations.

CONCLUSIONS

Due to results of our investigations NEU and NMU as a mutagens for creation new variation material on plant height and stem structure has been shown as less successful than gamma-rays.

Large number of material has been obtained both as for perspective new varieties and as the sources for future winter wheat breeding program for changing plant architecture. Only one trait appeared significant influence of genotype as a key component for mutation breeding success but at all times genotype-mutagen interaction regarding results of ANOVA analyze was significance in its influence on mutation rates. Genotype-mutagen interaction and classification of mutant material ia possible by rate of semi-dwarf forms

Nitrosoalkylureas were less effective to mutants, which obtained with same action, for mutations by stem high. NMU 0.025% were more preferable to short-stem and semi-dwarfs mutation obtaining, no concentrations of NEU or NMU useful of dwarfs mutations.

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