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OPTIMAL DOSES AND CONCENTRATIONS OF MUTAGENS FOR WINTER WHEAT BREEDING PURPOSES. GRAIN QUALITY

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Abstract

In addition to increasing grain yield, a promising direction in the use of mutagens is to obtain complex changes that increase the content and quality of grain protein. The main purpose of investigation is to develop possibilities of difference mutagen agents in induction mutants by protein content and quality, bread-making technological qualities and pathways for obtaining forms with combination of good grain production and qualities parameters. From 13 perspective high grain productive mutant lines 3 were separated out by combination of good grain quality and grain productivity on the level no less than national standard. Medium dose of gamma-rays (100 Gy) and concentrations of nitrosomethilureas (0.0125%) are effectively for mutation breeding on grain productivity and quality in complex. It is planned to conduct research for the optimal doses and concentrations of mutagens to obtain forms which are tolerant to abiotic stresses.

Key words*: grain quality, mutagenesis, mutation breeding, protein content, winter wheat.*

INTRODUCTION

Induced mutagenesis is well recognized as one of the key technology for the development of new varieties by all agronomic-value traits. Induced mutations have been applied to produce mutant varieties by changing the plant characteristic for a significant increase in production and improve quality (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 (Per et al., 2016). 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).

The most part of mutant varieties have been obtained by using gamma-irradiation (about 85 percent from general number) (Ahloowalia et al., 2004). Mutants generated through induced mutagenesis have been also used in genetics studies (van Harten, 1998; Waugh et al., 2006). 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 (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).

Wheat is the top food crop in Ukraine. 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 (Chazav et al., 2010). 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 (Mangi et al., 2016) and medium dose usage (Shu et al., 2013; Nazarenko, 2016b).

The increase of overall collection of grain is, obviously, one of the priorities, but the grain must also be of quality. Wheat grain is still the main source of protein and minerals; it has an optimal balance of protein and starch. That is, the grain quality combined with other properties of economic value is the required characteristic (Kenzhebayeva et al., 2018).

Grain quality character polygenic nature and high modification variability typical of it provide additional difficulties at selection by

quality of created mutant lines (Taulemesse et al., 2016).

The selection by grain quality characteristic is also complicated by the great dependency of functional properties of grains on the conditions of their handling, which becomes a significant problem when creating a genotype that combines high quality and high-yielding ability (Albokari, 2014; Aamir et al., 2016; Jaradat, 2018).

Mainly, the proteins of protein fractions, which are difficult to dilute (globulins, prolamins), are involved in the process of creating a high-quality grain. Such fractions of proteins are synthesised by membrane-bound ribosomes. The hydrophobic (membrane) environment promotes the synthesis of protein within scaled-up hydrophobic properties, namely the high quality proteins. A temperature drop and treatment with sodium chloride result in the destruction of hydrophobic bonds and synthesis of highly soluble proteins of lower quality. The varieties, which are characterised by a steady state of the protein synthesis systems under all conditions, synthesise mainly the protein fractions that are difficult to dilute (Kang and Banga, 2013).

The aim of the investigation is to determine optimal dose and concentrations of mutagens for mutation breeding practice and obtain new mutants with combinations of high-grain yield and quality, methods of identification of new mutants among the mutant population.

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 (acid dose, Co60, 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 (Nazarenko, 2017). These concentrations and exposure are trivial for the breeding process that has been repeatedly established earlier. Non-treated varieties were used as a control for mutation identified purpose.

In M2 - M3 generations agronomic-value families (by protein content) have been selected. Estimation of protein and its components was conducted from 2014 to 2018 years (M4 - M8 generations). The controls were national standard 'Podolyanka' and initial varieties. 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. Total size of field trial at M2 - M3 generations was 53,450 families (include controls).

The mutant lines were processed during the period of grain filling (X-XI stages of organogenesis) with the substances that change the state of protein synthesizing systems as a method for protein quality identification (sodium chloride in concentration of 5 g/l). The bread-making characteristics were identified for lines too (2016-2017 fields seasons). Total size of field trial was 148 lines.

Wheat samples were held at room condition at 18 - 20 for several days before grinding. Each sample of 30 g weigh was separately ground on a laboratory cyclone grinder (LMT-1, PLAUN LLC, Russia). Protein, gluten and water content of the samples were measured by Near-infrared Reflectance Spectroscopy (Spectran-IT, CJSC, Russia). Triplicate data of each sample were averaged. Contents of gliadin and glutenin were identified on CNS Model Flash EA 1112 (for protein content) and RP-HPLS (for gliadins and glutenins).

Experiments were carried out on the experimental fields of Dnipro State Agrarian and Economic University. The field`s geographic coordinates are: 48°30'N lat. and 35°15' E long. The experimental field is lied on 245 meters above the sea level. The air temperature during winter wheat growing

season (September - July) is 8-11°C, the average rainfall is about 350-550 mm in similar vegetation season.

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, cluster and correlation analyses was conducted by module ANOVA. In all cases standard tools of the program Statistica 8.0 were used.

RESULTS AND DISCUSSIONS

Table 1 summarises the data of investigations of the second- and third-generation mutant families on high protein content mutations rate. It can be seen from the table that the rate of this type of mutation was not as high as for changes in grain yield. For most of the variants, this mutation was rare (not more than 0.2-0.4%). For only two variants it was 1.0 % and average by frequency of occurrence. According to the previous investigations (Singh and Balyan, 2009), the rates of this type of mutants did not exceed 0.4-0.6% on average and reached 1.2- 1.4% for some variants with optimal genotype and mutagen factor com-bination. The rate generally depended on the nature of mutagen, the dose or concentration of mutagen and genotype.

Regarding ANOVA analysis, the level of factors corresponded to the nature of mutagen (F = 10.80; Fcritical = 3.22: p-level 0.01), for genotype (F = 4.34; Fcritical = 2.96: p-level 0.01), for dose or con-centration (F = 3.18; Fcritical = 3.11: p-level 0.01).

Table 1. Rates of mutations by grain quality after mutagen action

Trial	Kolos Mironivschini	Kalinova	Voloshkova	Sonechko	Favoritka	Hurtovina	Lasunya	Line 418
Control	0	0	0	0	0	0	0	0
Gamma-rays, 100 Gy	0.4	0.4	0.8*	0.4	0.6*	0.8*	0.2	0.2
Gamma-rays, 150 Gy	0.2	0.2	0.4	0.4	0.6*	0.6*	0.2	0.2
Gamma-rays, 200 Gy	0.2	0	0.2	0	0	0.2	0	0
Gamma-rays, 250 Gy	0.2	0.2	0.4	0	0.2	0.2	0	0
NMU 0.0125%	0.6	0.6*	0.6*	0.4	0.4	0.6*	0.2	0.6*
NMU 0.025%	0.4*	0.6*	0.4	0.2	0.6*	0.4	0.2	0.6*
NEU 0.01%	0.8	0.2	0.8*	0.2	0.6*	1.0*	0.4	1.0*
NEU 0.025%	0.6	0.2	0.8*	0.2	0..8*	0.6*	0.4	1.0*
DAB 0.1%	0	0.2	0	0.2	0	0	0	0.2
DAB 0.2%	0.2	0	0.2	0.2	0.2	0.2	0	0.2
DMS 0.0125%	0.4	0.4	0.2	0.4	0.4	0.2	0.2	0.4
DMS 0.025%	0.2	0	0	0.2	0	0.2	0	0.2
DMS 0.05%	0	0.2	0	0.2	0	0	0.2	0

*statistically significant differences from standard at $P_{0.95}$

As for mutagen nature, more successful for this type of mutations was the gamma-radiation of 100 Gy (less, but also suitable, 150 Gy) - 0.4-0.8%, nitrosoalkylureas (NEU more successful than NMU) induced mutations up to 1.0%, without statistically reliable difference between the concentrations for both muta-gens.

Gamma-rays irradiation was more suitable for the varieties of Voloshkova, Favoritka, Hurtovina only (0.6-0.8%), and chemical exposure was more effective for the genotypes of Kolos Mironivschini, Voloshkova, Favoritka, Hurtovina, line 418. DMS and DAB were not effective for this type of mutation induction for any concentrations and any genotypes.

Two lawlike regularities can be observed. Mutagens were more effective in induced mutation by protein content, primarily for varieties with low protein content than for high-protein varieties (e.g. Sonechko and Lasunya). Mutagens have proved more effective in genetic alterations for varieties with non-mutational origins (line 418).

It has been observed that most high-protein mutations were associated with changes in spike morphology (Nazarenko, 2016) and in grains shape, which is consistent with the previous investigations into mutation breed for crop improvement, but for this type of complex mutations, the level of grain production was

unsatisfactory and these forms were useless as a future commercial variety.

The objective of the second stage of the research was to assess the quality of grain with multiple parameters of a well-known variety (as the standard) (Kang and Banga, 2013), and then new mutant lines using the substances that destroy hydrophobic bonds. The analysis of protein-synthesising systems of 14 lines and the standard variety "Podolianka" was carried out. The content of wet gluten was determined by Kjeldahl method using Sereniev's devices.

The lines were processed during the grain-filling stage with the substances that alter protein-synthesising systems (sodium chloride in a concentration of 5 g/l).

The previous research has shown a decrease in total protein content for high-quality varieties after spike treatment during the grain-filling stage with the substances reducing the amounts of membrane-bound ribosomes.

After the treatment of winter wheat at the X-XI stages of organogenesis with the substances that break the hydrophobic bonds, the Podolyanka variety in lines 157 and 213 showed an increase in protein content (Table 2). This indicates poor grain quality. In other lines, there was no response to the exposure. For these lines, the protein processing is more related to free ribosomes, and they have not reacted to the influence of the agent breaking ionic bonds, indicating their stability in terms of grain quality. Of particular note are lines 133, 156, 174, which have excellent technological characteristics of flour.

Table 2. Influence of substances, which destroyed hydrophobic relations on protein content and other technological parameters of winter wheat mutation lines grain

Line	Protein content, %		Technological parameters of quality		
	Control	Treatment	Sedimentation, ml	Gluten, %	Volume of bread for 100 g floura
Podolyanka	13.3±0.04	14.0±0.06	71	30.3	620
130	14.1±0.08*	12.6±0.08	49	23.3	660
133	13.5±0.03	13.2±0.05	54	25.1	590
142-1	13.2±0.09	13.6±0.1	36	22.2	470
156	14.3±0.04*	12.8±0.09	49	30.2	560
157	13.5±0.03	13.9±0.08	59	30.5	520
157-1	13.5±0.08	13.4±0.04	55	29	620
172	13.7±0.07	13.9 ±0.03	61	30.4	560
174	14.2±0.05*	11.3±0.05	51	27	620
179	13.0±0.06	13.2±0.07	53	26.8	640
185	13.2±0.02	13.4±0.03	76	27	610
186	14.2±0.07*	13.8±0.04	68,5	31	630
211	13.9±0.01*	13.5±0.5	64	24.5	570
213	13.9±0.10*	14.5±0.01	60	27.1	630

*statistically significant differences from standard at $P_{0.95}$

The analysis of correlations showed that the content of gluten is largely related to the flour sedimentation (0.51) and the percentage of protein in grains, obtained by plotting with the substances destroying hydrophobic bonds (0.57), and the volume of bread correlates with a total appraised value to the total score of 0.7.

The two-year analysis of the grain quality has confirmed the available data. The grain yielding capacity is backward dependent on the flour sedimentation -0.71, as well as on the increase of protein content -0.73.

The increase in protein content, with regard to the reference data, also has a positive correlation with grain swelling in water for 24 hours (0.77), and a negative correlation with yield of -0.67.

Table 3 summarises the dates by grain productivity (Nazarenko et al., 2018) and quality. Within the framework of this breeding program, 3 lines combining high yields and sufficient grain quality have been obtained. One of the lines, 156, has excellent baking qualities as does the complex. As for mutagens for two mutant lines, NMU 0.0125% has been used as a mutagen factor, for one 100 Gy. NEU, which was effective on a high level for induction of high-protein mutations in general, does not give a final result in new perspective lines obtained. Likewise for NMU, this case for this mutagen according to previous investigations. According to previous investigations, gamma-rays have to be used at low doses.

Table 3. Origin and describe of main morphological traits of winter wheat mutant lines

Line	Initial variety	Mutagen	Protein	Gliadin	Glutenin
Podolyanka (standard)	--	--	13.3	0.024	0.61
130	Kalinova	100 Gy	14.1*	0.031*	0.72*
156	Kolos Mironovschiny	NMU 0.0125%	14.3*	0.033*	0.74*
211	Kolos Mironovschiny	NMU 0.0125%	13.9*	0.024	0.71*

*statistically significant differences from standard at $P_{0.95}$

All 3 lines have a higher content of gliadins and glutenins than the standard Podolyanka and, the high content of these ingredients is thought to be indicative of the potentially high quality of grain and can be used as a complex parameter for breeding material evaluation.

CONCLUSIONS

Thus, gamma rays and nitrosolalburea can be used as mutants to induce mutation in grain quality. In all cases, DMS and DAB as mutagens are useless for these purposes. Mutants with high protein content were rare and often associated with lower grain yields due to changes in spike structure. For the gamma-rays, the more effective doses were in the interval of 100-150 Gy, while the prevalent ones were 100 Gy.

In spite of grain productivity mutations in previous studies, the frequency of high-quality grain mutations depends on the interaction between the genotype and the nature of the mutagen. The action of the corresponding mutagenic factor (gamma-rays, NMU) entirely determined the number of mutations by grain quality.

Our research has shown that the protein content in potentially high-quality lines decreases after the spike treatment while the grain is filled with the proposed solution, which lowers the number of membrane-bound ribosomes. The status of the protein-synthesising system of 13 mutant lines has been described. The dependence of hydrophobic bonds activity and protein quality allowed us to identify 3 high-quality mutant lines (133, 156, 174). In addition, 3 lines have been determined by a complex of high grain yield and good baking quality (mutant agents 100 Gy and NMU 0.0125%).

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