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IDENTIFICATION AND CHARACTERIZATION OF MUTANTS INDUCED BY GAMMA RADIATION IN WINTER WHEAT (*Triticum aestivum* L.)

Mykola NAZARENKO

Dnipropetrovsk State Agrarian and Economic University, 25a Voroshilova str., 49600, Dnipropetrovsk, Dnipropetrovsk region, Ukraine

Corresponding author email: nik_nazarenko@ukr.net

Abstract

Mutation breeding is one of the breeding methods used successfully in durum wheat for selecting lines with increased agronomic values. This study was carried out to determine gamma rays effect and interaction between gamma-rays and the genotype of treated variety of winter wheat in mutations induction. We used physical mutagens: gamma-rays in doses 100, 150, 200, 250 Gy.
Seeds of eight winter wheat varieties were exposed with gamma-rays. Insensitive and most sensitive for mutagen actions varieties have been developed. Thirty-six different types mutated traits were observed. Among them there were seven valuable for breeding process. Mutations rate was waved from 0,4 to 30 %.
Medium dose of gamma rays (100 Gy) is recommended for winter wheat mutation breeding and high dose (200 Gy) for obtaining genetic-value mutants (parent components for crossbreeding with some valuable traits like as semi-dwarf, early-heading and other). For high-productivity lines were identified. Positive desirable mutants will be selected and be incorporated in future breeding programs.
We have shown that varieties obtained by gamma radiation are less sensitive to this mutagen. Their re-exposure is inappropriate by same mutagen. However, the varieties which obtained by field hybridization or treatment by mutagens of another nature (chemical agents, temperature) indicate a higher level of mutation rate and variability of traits.

Key words: *gamma-rays, winter wheat, mutation breeding, mutation rate.*

INTRODUCTION

Induce of mutations in crop plants contribute by increasing genetic variability and enrich plants germplasm for direct selection and cross-breeding. In wheat, chemical and ionizing radiation mutagenesis have been universally used to generate genetic variations for breeding researches and genetic studies (Cheng et al., 2015). In total, 274 mutant varieties of wheat were developed by physical or chemical mutagens from 1930 to 2014 (IAEA, 2015).
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 (Si1ngh, Balyan, 2009). Mutation breeding gave a initial material for green revolution (Ahloowalia et al., 2004).
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 mil. ha with actual productivity of 24 mln tons and average capacity of 2.8 t/ha (Morgun, Logvinenko, 1995; Nazarenko, 2012).
High doses are more successful in obtaining large quantity and a wide range of mutations. Gamma rays is the most important and frequently used mutagens well known for their effect on the plant growth and development and the appearance of morphological, cytological and physiological changes in cells and tissues; they are also traditional in breeding (van Harten; 1998; Nazarenko, 2015). Most commercial varieties are obtained by means of gamma irradiation (Ahloowalia et al., 2004).
The development of direct mutants into commercial varieties is still a common practice in seed propagated crops (Shu et al., 2011).

MATERIALS AND METHODS

Seeds (approx. 14% moisture content) of Favoritka, Lasunya, Hurtovina, Kolos Mironovschiny Sonechko, Kalinova, Voloshkova varieties and line 418 of winter wheat (Triticum aestivum L.) were subjected to 100,150, 200, 250 Gy gamma irradiation. Each treatment was comprised of 1000 wheat seeds. These doses are trivial for the breeding process that has been repeatedly established earlier (Ukai, 2006). Non-treated varieties were used as a check.

M_1 plants grown after mutagenic treatments were propagated based on the spike progeny method. The M_2 seeds obtained from each spike were sown to rows. The M_1 and M_2 generations were grown in DSAEU science-educational center, Aleksandrovka, Dnepripetrovsk region, Ukraine in 2010 and 2011. Selection of mutants was carried out in the M_2 and M_3 generations. M_2 plants showing a difference from the check (untreated mother varieties were also planted after every twenty rows for comparison with the M_2) and plants with desired phenotypes were harvested individually. Then M_3 progeny from selected M_2 plants according to the pedigree selection procedure were grown (Morgun, Logvinenko, 1995). The mutants were identified by visual screening for differences and were confirmed at M_3 generations by measuring for single spike yield and single plant grain yield in the M_2 and M_3 generations. Population sizes of M_1 was 500 in each case (total extent of examinations was 19 300 families in M_2), excepted varieties Lasunya and Kalinova (200 and 250 Gy)/

In 2013 – 2015 M_4 – M_6 generations genetic-valuable mutations were hand sown using a randomized complete block design in two-row plots of 1,5 m long row with 0,3 m spacing between the rows with tree replicates as a breeding collection (Albokari, 2014). Breeding-value lines were sown using a randomized complete block design in ten-row plots of 10 m^2 square size with 0.15 m spacing between the rows for 2-3 replications. After every 10 plots national standard Podolyanka was sown.

Selected mutant lines were evaluated for plant height, spike length, the number of grains per spike, single main spike yield, plant yield and 1000 grains weight (were recorded using 30 plants (10 random plants from each replication) before harvest) and general grain yield by comparison with national standard Podolyanka.

The level of variability was calculated using the formula: $P\nu = \alpha \times \gamma$

Where Pν - level of the variant variability;

α - the ratio of the total number of mutations to the total number of families in the variant;

γ – the number of modified traits types in the variant (Nazarenko, 2012; Nazarenko, 2015).

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. Factor analyses were conducted by module ANOVA. In all cases standard tools of the program Statistica 6.0 were used.

RESULTS AND DISCUSSIONS

As we can see from the table 1, the highest rate of mutations is observed in variety Kolos Mironovschiny (30%) at a dose of 250 Gr. However, for the most varieties the reducing of the mutation rate at a given dose and a higher value of rate at a dose of 200 Gr is observed. The greatest level of variability was also in the variety Kolos Mironovschiny. And we can see again that the highest dose of 250 Gr causes more narrow variability for the most varieties. That limits our ability for the selection of genetically-valuable mutations. The usage of the 200 Gr dose was more optimal. The less stable among the represented varieties Voloshova was the one obtained by exposure of low temperatures during vernalization. It is generally characteristic of such varieties.

Varieties Favoritka, Lasunya, Hurtovina (obtained by gamma rays) showed a significantly lower mutations rate and variability.

Mutations rate and variability increases linearly at doses of 100, 150 and 200 Gr, at a dose of 250 Gr. in most cases, on the contrary, there is a decrease to the level of 150 Gr dose or lower. However, we can not say that the use of this dose does not make sense. For two varieties mutations rate was highest at this dose.

Thirty-six traits were totally sorted out on which the change occurred under the influence of the mutagen. For analysis, they were classified in the following groups (Morgun, Logvinenko, 1995):

1. Mutations in the stem and leaf structures (all types of mutants by stem height and thickness were presented, mutants by waxy bloom);

2. Mutations of color and ear structures (only nine types by structure);
3. Mutations by grains color and structure (large in dimensions grain);
4. By physiological traits of growth and development of mutations (sterility, earliness, lateness, resistance to main pathogens);
5. Systemic mutations (like wild wheat relatives by spike shape);
6. Productivity and high quality mutations.

Table 1. Winter wheat mutations rates

Variant	Number of mutants	Rate, %	Variability level
Check (Kolos Mironivschini, water)	2	0,4	0,01
Kolos Mironivschini, gamma-rays, 100 Gy	46	9,2*	1,10
Kolos Mironivschini, gamma-rays, 150 Gy	67	13,4*	3,22
Kolos Mironivschini, gamma-rays, 200 Gy	83	16,6*	4,48
Kolos Mironivschini, gamma-rays, 250 Gy	90	30,0*	7,50
Check (Kalinova, water)	6	1,2	0,05
Kalinova, gamma-rays, 100 Gy.	39	7,8*	1,01
Kalinova, gamma-rays, 150 Gy	84	16,8*	3,53
Kalinova, gamma-rays, 200 Gy	83	23,7*	5,93
Kalinova, gamma-rays, 250 Gy	50	14,3*	2,43
Check (Voloshkova, water)	9	1,8	0,07
Voloshkova, gamma-rays, 100 Gy.	32	6,4*	0,90
Voloshkova, gamma-rays, 150 Gy	51	10,2*	2,14
Voloshkova, gamma-rays, 200 Gy	79	15,8*	3,63
Voloshkova, gamma-rays, 250 Gy	104	20,8*	4,99
Check (Sonechko, water)	4	0,8	0,02
Sonechko, gamma-rays, 100 Gy.	59	11,8*	1,89
Sonechko, gamma-rays, 150 Gy	90	22,5*	4,28
Sonechko, gamma-rays, 200 Gy	84	33,6*	6,38
Sonechko, gamma-rays, 250 Gy	23	23,0*	3,68
Check (Favoritka, water)	3	0,6	0,01
Favoritka, gamma-rays, 100 Gy.	28	5,6*	0,50
Favoritka, gamma-rays, 150 Gy	38	7,6*	1,06
Favoritka, gamma-rays, 200 Gy	43	9,6*	1,82
Favoritka, gamma-rays, 250 Gy	45	11,3*	2,03
Check (Hurtovina, water)	4	0,8*	0,02
Hurtovina, gamma-rays, 100 Gy.	34	6,8*	1,09
Hurtovina, gamma-rays, 150 Gy	40	8,0*	1,44
Hurtovina, gamma-rays, 200 Gy	51	10,2*	2,14
Hurtovina, gamma-rays, 250 Gy	50	12,5*	2,38
Check (Lasunya, water)	7	1,4	0,07
Lasunya, gamma-rays, 100 Gy.	26	5,2*	0,57
Lasunya, gamma-rays, 150 Gy	27	5,4*	0,86
Lasunya, gamma-rays, 200 Gy	43	9,6*	1,73
Lasunya, gamma-rays, 250 Gy	40	11,4*	1,94
Check (Line 418, water)	4	0,8	0,02
Line 418, gamma-rays, 100 Gy.	57	11,4*	2,05
Line 418, gamma-rays, 150 Gy	78	15,6*	3,28
Line 418, gamma-rays, 200 Gy	102	25,5*	4,59
Line 418, gamma-rays, 250 Gy	84	21,0*	4,20

* - difference is statistically significance from preliminary variant at $t_{0.05}$

Rates of genetically (short-stem plants, semi-dwarf plants, a large spike, large grain, earliness) and breeding-value mutations are included (productive mutants) were low (0.2-0.6% for the variant) and usually derived forms carried the additional negative qualities. Totally it was received (selected as genetically-valuable): short-stem plants 35 (especially a lot of forms from variety Voloshkova), semi-dwarf plants 11, with a large spike 41, with a large grain 14, earliness 40.

Mutant lines with the high grains productivity were induced primarily at the dose of 100 Gr. Their number is varied from 0 to 0.4 % in the variant (as an exception in the Hurtovyna variety), preferably – 0.2-0.4%. However, almost all these mutations have negative traits such as high stem or little resistance to pathogens, low drought resistance and winter hardiness. They represented a very limited interest.

After three year field estimation 4 lines with the productivity above national standard were allocated. All these lines were obtained by radiation of 100 Gy dose (Tables 2).

These three lines are derived from Sonechko variety, one line from the Kalinova variety. These varieties also showed a higher sensitivity to gamma-rays compared with others in the first generation (died of plants was high at 200 and 250 Gy doses (Nazarenko, 2015).

The usage of gamma rays in a 100 Gy dose was the most effective for the mutation breeding on the varieties obtained by chemical mutagenesis. Varieties, which showed even greater mutation rate and the level of variability in this dose, do not give anything.

Table 2. Grain productivity of winter wheat mutants lines after three years of estimation (2013 -2015)

Number of mutant line	Yield 2013	Yield 2014	Yield 2015	average yield	+/- to standard
			t./he		
Podolyanka	5,772	10,878	9,761	8,804	--
130	8,436	11,766	11,39	10,531*	1,727
133	5,195	11,033	10,71	8,979*	0,176
157	6,078	17,316	10,27	11,221*	2,418
157-1	6,078	11,5	11,12	9,566*	0,762
$LSD_{0,05}$			0,14		

* - difference is statistically value at $t_{0.05}$

Higher productivity of these lines was formed due to the increasing (in comparison with the standard) of the weight of thousand grains, weight of grain per spike and plant, more number of grains in the spike.

CONCLUSIONS

Thus, the most optimal for obtaining of breeding-value mutations is the usage of 100 Gy dose. For the most promising genetically-value mutations it makes a sense to use higher doses - 200-250 Gr.

We have shown that varieties obtained by gamma radiation are less sensitive to this mutagen. Their re-exposure is inappropriate by same mutagen. However, the varieties which obtained by field hybridization or treatment by mutagens of another nature (chemical agents, temperature) indicate a higher level of mutation rate and variability of traits.

REFERENCES

Ahloowalia B.S., Maluszynski M., Nicjterlein K., 2004. Global impact of mutation-derived varieties. Euphytica, vol. 135: 187-204.

Albokari M., 2014. Induction of mutants in durum wheat using gamma irradiation. Pakistan Journal of Botany, vol.46: 317-324.

Cheng X., Chai L., Chen Z., 2015. Identification and characterization ofa high kernel weight mutant induced by gamma-radiation in wheat (Triticum aestivum L.). BMC Genetics, vol. 17: 112-118.

Harten van A.M., 1998. Mutation breeding. Theory and practical applications. Cambridge University Press.

International Atomic Energy Agency, 2015. Mutant varieties database. WEB: (http://www-naweb.iaea.org/nafa/pbg/)

Morgun V.V., Logvinenko V.F., 1995. Wheat mutation breeding. Kyiv, Agroosvita.

Nazarenko M., 2015. Peculiarities of negative consequences of mutagen action, Ecological Genetics, 4: 25-26.

Nazarenko M., 2012, Increasing biodiversity of source material for breeding of winter wheat", Plant genetic sources, vol. 9: 147-154.

Si1ngh N.K., Balyan H.S., 2009. Induced Mutations in Bread Wheat (*Triticum aestivum* L.) CV. 'Kharchia 65' for Reduced Plant Height and Improve Grain Quality Traits. Advances in Biological Research, vol.3: 215-221.

Ukai Y., 2006. Effectiveness and efficiency of mutagenic treatments. Gamma Field Symposia, 45: 1-4.

Shu Q.Y., Forster B.P., Nakagava H., 2011. Plant Mutation breeding and Biotechnology.