UDC 633.111.1:575.2

Use of induced mutagenesis for creation of soft spring wheat with starch of waxy type

Didenko S.[1], **Holik O.**[2], **Relina L.**[3], **Vecherska L.**[4]

[1 – 4]Plant Production Institute n.a. V. Ya. Yuriev of NAAS, 142 Moskovskyi Ave. Kharkiv, Ukraine, 61060; e-mail: [1]sydidenko1976@gmail.com, [2]golik.oleg.v.@gmail.com, [3]lianaisaakovna@gmail.com, [4]ludmila_vecherska@gmail.com

The purpose. To establish efficient doses of g-irradiation of grain of wheat for formation of its amylose-free genotypes, and to create on this basis a line for the further selection of grades of wheat with starch of waxy type. **Methods.** Biophysical, biochemical, computing-analytical, morphometric. As mutagen factor they used g-irradiation of 60Co in different doses (100; 150 and 200 Gr (Gray)). Lines of wheat irradiated with the purpose of improvement of their adaptive potential to natural settings of Ukraine. Well adapted grade of wheat Kharkivska 30 was irradiated with the purpose of induction of waxy mutation. **Results.** Effective doses of g-irradiation for creation of amylose-free lines of soft spring wheat were established. They selected specific primers to waxy genes in all three genomes of hexaploid wheat. For each pair of primers the comfort temperature of amplification was specified. By means of molecular markers mutant genotypes of wheat with amylose-free type of starch were selected. On the basis of mutant genotypes and local grades they created and transmitted in laboratory of selection of spring wheat 2 lines with waxy starch. **Conclusions.** The carried out researches have confirmed efficiency of use of the induced mutagenesis for creation of genotypes of soft spring wheat with amylose-free type of starch. On the basis of mutant samples with the changed distillation characteristic of starch new lines of soft spring wheat with a complex of valuable economic attributes — Liutescens 12/16 and Liutescens 30/16 are created. The first line is characterized by early growth, duration of vegetative period — 90 – 93 days, durability to lodging, average productivity — 2.2 – 3 t/hectare, protein content — 13.1 – 14.2%, amylopectin in starch — 99.8 – 99.9%. At the second line vegetative period — 89 – 91 days, resistant to lodging, height of plants — 50 – 52 cm, average productivity — 2.3 – 3.1 t/hectare, protein content — 13.3 – 14.4%, amylopectin in starch — 99.8 – 99.9%.

Key words: *irradiation, amylopectin, genotype, selection, genes, primer, temperature of amplification.*

DOI: https://doi.org/10.31073/agrovisnyk201909-06

Waxy wheat flour (the amylose content starch does not exceed 1%) is thoroughly studied worldwide [1–4]. Due to its unique qualities (increased water absorption capacity, small starch granules facilitating digestibility of starch in the digestive tract of animals and humans, etc.), it can be used in many branches of food, pharmaceutical and other industries [5-7].

Despite entrepreneurs' interest in such raw material, there is only one Ukrainian winter waxy wheat variety (Sofiika or Sakura Waxy bred by O.I. Rubalka) [8]. There is no Ukrainian spring waxy wheat yet.

It is well-known that gamma irradiation allows us to expand the genetic diversity of organisms and to obtain plant genotypes with the grain quality (first of all, starch quality), which is of interest to breeders and technologists.

Review of Recent Studies and Publications. Starch is a basic component (70%) of wheat endosperm. The food quality depends on its quantity and composition. There is a huge global market of waxy starch from different plants, which is used as a gel-forming agent, thickener, stabilizer, etc. [9]. Physical and chemical aspects of the synthesis of starch and its constituents, amylose and amylopectin, are described in detail in many reviews [10-12].

The term "mutagenesis" in plant breeding is a process of induction of mutations in the genome. Due to mutagenesis, about 3,000 varieties of different crops have been improved or created. In the last century, mutational breeding and its methods are seen as powerful tools for achieving many goals, including expansion of the genetic diversity by many parameters (quality of products, resistance to diseases and pests, salt tolerance, etc.), possibility of mutations in discrete genes, for example, in those that determine the starch composition, without pleiotropic effects on the entire genotype [13]. However, this trend is little pursued in Ukraine, which made our work relevant.

Our objectives were to determine the effective doses of gamma irradiation for development amylose-free wheat genotypes and to create, on this basis, lines for further breeding of waxy wheat varieties.

Materials and Methods. To generate mutant spring bread wheat genotypes with a changed amylose/amylopectin ratio, we used spring bread wheat variety Kharkivska 30 (bred at the Plant Production nd. a. VYa Yuriev), which is characterized by high yield capacity, resistance to diseases and pests as well as by plasticity, and standard variety Kharkivska 18 as starting material. Lines 99ID529 var. erythrospermum (UA0106163); 99ID536, var. lutescens (UA0106479), and 99ID546 var. lutescens (UA0106480), which are provided courtesy of the University of Nebraska (the US) for the collection of the National Center for Genetic Resources of Plants of Ukraine, and 98-134 var. lutescens kindly furnished by Laboratory of Spring Wheat Breeding of the Plant production Institute n.a. V. Ya. Yuriev served as donors of the *waxy* genes.

Gamma irradiation of Co^{60} in different doses (100, 150 and 200 Gy) was a mutagenic factor. The lines were irradiated to improve their adaptivity to the conditions of Ukraine. Well-adapted to Ukrainian conditions variety Kharkivska 30 was irradiated to induce a *waxy* mutation. Air-dried seeds of variety Kharkivska 30 were taken as control. DNA was extracted from three-day sprouts using BioSprint workstation for purification of total DNA from plant tissue. Polymerase chain reaction (PCR) with specifically selected primers was performed according to the routine technique [14].

The starch composition of individual kernels was qualitatively investigated by a modified method using 1 N NaOH, 1 N CH_3COOH and 0.2% J_2 in KJ. Two crushed kernels from each individual spike were used for analysis.

Kernels of control (non-irradiated) plants were stained in blue-violet color, confirming the normal for wheat amylose/amylopectin ratio (approximately 30:70).

Results. Using this method, we identified several unique plants in F_3 of irradiated seeds. Their kernels were of different color, in particular, brown-reddish, which is typical for waxy starch (it is known that J_2 in KJ is adsorbed on amylopectin molecules, staining them brown-reddish).

These unique plants were used for further research. After DNA extraction from three-day sprouts, a series of PCRs with specially selected primers was performed using BioSprint workstation for purification of total DNA from plant tissue [14]. For each of the primer pairs, the optimal amplification temperature was determined (Table 1).

1. Primers to the Waxy Genes and Optimal Temperatures for Amplification, 2012

Locus	Primer	Optimal temperature for amplification, °C
WX A1	Wx 7A2	76
	Wx7A3	80
	Wx7A4	76
WX D1	Wx7D2	80
	Wx7D3	76, 80
WX B1	Wx 4A6	76
	Wx 4A8	72, 76

The amplicons were used for further sequencing and decoding of the data using the CLC Main Workannch software.

The sequencing data and further processing of these data allowed us to reveal several substitutions in the nucleotide sequence of mutant plants in comparison with control plants and with the database. These nucleotide substitutions arrested the amylose synthesis and, consequently, gave a brown-reddish color to hydrolyzates. These substitutions were caused by 150 Gy gamma irradiation. Figure 1 illustrates one of these mutations (thymine is replaced with adenin), which changed the synthesis of starch constituents.

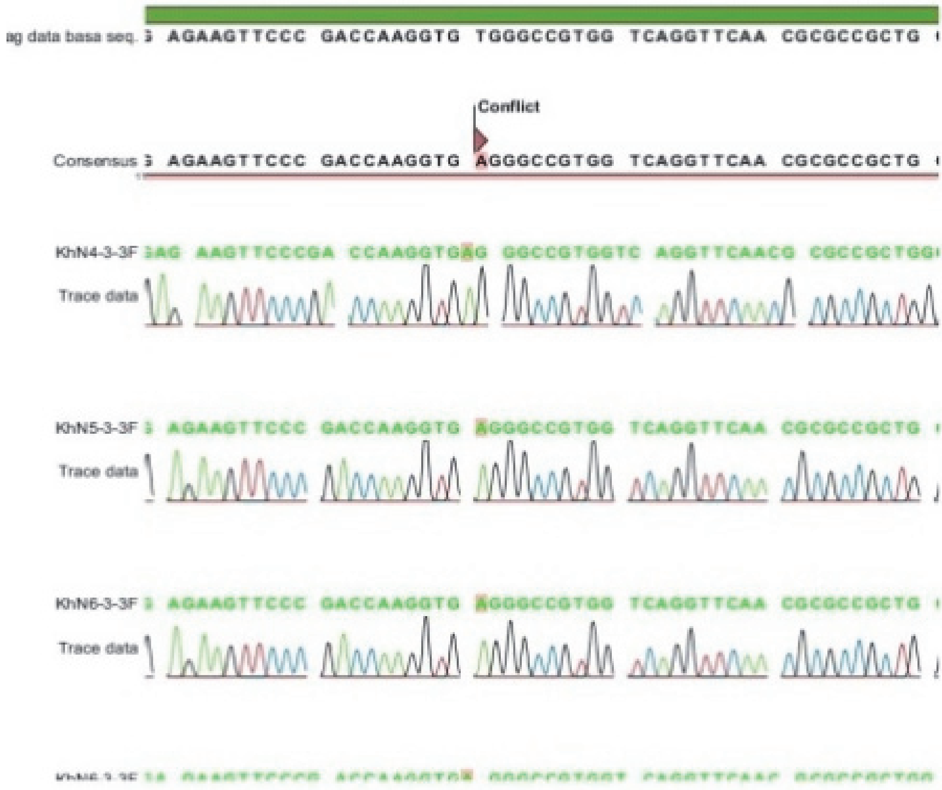

Fig. 1. Decoding the Nucleotide Sequences of Wx7A4 Amplicons of Mutant Plant DNA (F_3, 150 Gy irradiation)

The selected in this way mutant plants of Kharkivska 30, lines 99ID546, 99ID529, and others were crossed. Selections from these hybrid combinations allowed us to create starting initial material for the breeding of the first Ukrainian spring bread wheat varieties with amylose-free starch. The morphological parameters of the most promising lines are given in Table 2.

2. Morphological Parameters of Spring Bread Wheat Hybrids with Waxy Starch (average for 2013-2016)

Variety (line)	Starch	Plant height, cm	Upper internode length, cm	Spike length, cm	Spike weight, g	Grain weight per spike, g
Mutant Kharkivska 30 / 99ID536	waxy	60.1 ± 2.3	9.4 ± 0.27	8.4± 0.25	1.9 ± 0.07	1.0 ± 0.03
Mutant 99ID546 / Kharkivska 18	waxy	54.3 ± 2.4	8.6 ± 0.34	7.5 ± 0.33	1.9± 0.06	0.9 ± 0.02
Mutant Kharkivska 30 / 99ID546	waxy	75.1 ± 3.4	8.9 ± 0.42	9.9 ± 0.39	1.9 ± 0.07	1.0 ± 0.04
99ID546 / Mutant Kharkivska 30	waxy	49.7 ± 2.3	7.8 ± 0.36	8.6 ± 0.35	1.7 ± 0.08	0.9 ± 0.03
Mutant 99ID529 / 98-134	waxy	49.7 ± 2.3	7.8 ± 0.36	8.6 ± 0.35	1.9 ± 0.08	0.9 ± 0.03
99ID529	waxy	49.1 ± 2.5	7.2 ± 0.36	7.1 ± 0.35	1.3 ± 0.07	0.6 ± 0.03
99ID546	waxy	74.4 ± 3.3	7.9 ± 0.41	8.8 ± 0.40	1.5 ± 0.07	0.8 ± 0.05
Kharkivska 30	**wild type**	**81.3 ± 4.0**	**13.5 ± 0.59**	**11.5± 0.56**	**2.1 ± 0.1**	**1.2 ± 0.06**

Our results demonstrated that the spring bread wheat lines with starch that contains little or no contain amylose were considerably superior to the lines-homozygous carriers of the recessive *waxy* mutations, which were the parents in crossing, in terms of the 'grain weight per spike' trait. The grain weight per spike in the experimental lines was by 0.2–0.4 g higher than that in the initial lines and comparable with the grain weight per spike in recognized variety Kharkivska 30.

The morphological parameters of these lines (plant height, upper internode length, spike length) were intermediate between these values of the lines - homozygous carriers of the recessive *waxy* mutations (99ID529, 99ID546) and variety Kharkivska 30.

At the same time, there were smaller differences between the morphological parameters of the waxy lines derived from hybridcombinations and the variety with wild type starch if the female parent of a hybrid was a wild type variety, and vice versa, if the female parent of a hybrid was a line-homozygous carrier of the recessive *waxy* mutation, its morphological parameters were closer to those of the line. A rather pronounced reciprocal effect was observed, which made it possible, through selection of parents, to develop lines and, in prospect, varieties with amylose-free starch that would not be inferior to recognized spring bread wheat varieties in terms of yield and major morphological parameters.

Assessments of several economically valuable characteristics of spring bread wheat accessions generated via induced mutagenesis distinguished lines, which were assigned numbers (Lutescens 12/16 and Lutescens 30/16) and transferred to the Laboratory of Spring Wheat Breeding of the Plant production Institute n.a. V. Ya. Yuriev NAAS for competitive trials.

Line mutant Lutescens 12/16 (mutant 99ID546 / Kharkivska 18) is characterized by early ripening (the growing period = 90–93 days vs. 100–105 days in variety Kharkivska 18). It is resistant to lodging (the plant height is 54 - 58 cm). The average yield is 2.2–3.0 t/ha. The yield of standard variety Kharkivska 18 is 2.5 – 3.4 t/ha. The protein content is 13.1–14.2%; the amylopectin content in starch is 99.8 - 99.9%. The resistance scores are as follows: to covered smut – 7, to brown leaf rust – 6, to powdery mildew – 7, to Phorbia larvae – 7, and to Hessian fly larvae – 7.

Line mutant Lutescens 30/16 (mutant 99ID529 / 98-134) is even more early-ripening in comparison with the standard variety (the growing period = 89-91 days). When the climate is becoming more arid, the early ripeness is increasing in relevance in the eastern forest-steppe of Ukraine. This line is resistant to lodging (the plant height is 50–52 cm). The average yield is 2.3–3.1 t/ha. The protein content is 13.3 –14.4%; the amylopectin content in starch is 99.8–99.9%. The resistance to major diseases and pests is similar to that of Kharkivska 18: to covered smut – 7, to brown leaf rust – 6, to powdery mildew – 7, to Phorbia larvae – 5, and to Hessian fly larvae – 5.

Conclusions

Our study confirmed the effectiveness of induced mutagenesis in generation of spring bread wheat genotypes with amylose-free starch. Based on mutant specimens with a modified composition of starch, new spring bread lines with several valuable economic features were developed – Lutescens 12/16 and Lutescens 30/16. The first line is characterized by early ripeness (with a growing period of 90-93 days), lodging resistance, average yield of 2.2–3.0 t/ha, protein content of 13.1–14.2%, and amylopectin content in starch of 99.8 - 99.9%. The second line has a growing period of 89-91 days and is lodging resistant (plants are 50–52 cm tall). The average yield is 2.3–3.1 t/ha; the protein content is 13.3–14.4%; the amylopectin content in starch is 99.8–99.9%.

References

1. Graybosh, R.A. (1998). Waxy wheats: origin, properties and prospect. *Food science and technology, 9,* 135-142.
2. Abdel-Aal, E.-S. M., Hucl, P., Chibbar, R.N., Han, H.L., & Demeke, T. (2002). Physicochemical and Structural Characteristics of Flours and Starches from Waxy and nonwaxy Wheats. *Cereal Chemistry, 79* (3), 458–464.
3. Bhattacharya, M., Erazo-Castrejon, S., Doehlert, D.C., & McMullen, M.S. (2002). Staling of Bread as Affected by Waxy Wheat Flour Blends. *Cereal Chemistry, 79* (2), 178 – 182.
4. Hayakawa K., Tanaka K., Nakamura T., Endo S., & Hoshino T. (2004). End Use of Waxy Wheat Flour in Various Grain-Based Foods. *Cereal Chemistry, 81* (5), 666 – 672.
5. Qin, P., Ma, C., Wu, R., Kong, Z., & Zhang, B. (2009). Effect of waxy wheat flour blends on the quality of fresh and stale bread. *Agricultural Sciences in China, 8*, 4, 401-409.

6. Kowalski, R.J., Siyuan, Wang, A.M., Joyner, Melito, H., & Ganjyal, G.M. (2017). Waxy wheat flour as a freeze-thaw stable ingredient through rheological studies. *Food and Bioprocess Technology, 10,* 7, 1281–1296.

7. Bajaj, R., Singh, N., & Kaur, A. (2019). Effect of native and gelatinized starches from various sources on sponge cake making characteristics of wheat flour. *J Food Sci Technol., 56,* 2, 1046-1055. doi:10.1007/s13197-019-03632-w.

8. Rybalka, O.I., Polishchuk S.S., & Morhun, B.V. (2018). Novi napriamy v selektsii zernovykh kultur na yakist zerna [New trends in cereal breeding for grain quality]. *Bulletin of Agricultural Science, 11*(788), 120-133. [In Ukrainian].

9. Alcazar-Alayi, S.C., & Almeida Meirelesi, M.A. (2015). Physicochemical properties, modifications and applications of starches from different botanical sources. *Food Science and Technology, 35,* 2, 215-236. dx.doi.org/10.1590/1678-457X.6749.

10. Smith, A.M. (2001). The biosynthesis of starch granules. *Biomacromolecules, 2,* 2, 335-341. http://dx.doi.org/10.1021/bm000133c.PMid:11749190

11. Tester, R.F., Karkalas, J., & Qi, X. (2004). Starch – Composition, fine structure and architecture. *Journal of Cereal Science, 39*, 2, 151-165. http://dx.doi.org/10.1016/j.jcs.2003.12.001

12. P rez, S., Bertoft, E. (2010). The molecular structures of starch components and their contribution to the architecture of starch granules: a comprehensive review. *Starch/Staerke, 62*, 8, 389-420. http://dx.doi.org/10.1002/star.201000013

13. Shu, Q.Y., Forster, B.P., Nakagawa, H. (Eds.) (2012). Plant Mutation Breeding and Biotechnology. CABI: Oxfordshire, UK.

14. Slade, A.J., Fuerstenberg, S.I., Loeffler, D., Steine, M.N., Faccciotti, D. (2005). A reverse genetic, nontransgenic approach to wheat crop improvement by TILLING. *Nature biotechnology, 23*, 75 – 81.