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The using of speed breeding to obtain *Waxy* lines of bread wheat

M. Alkubesi[1, 2]*, A.O. Blinkov[1], A.G. Chernook[1], M.G. Divashuk[1, 2]
[1] *All-Russia Research Institute of Agricultural Biotechnology, Moscow, Russia*
[2] *Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, Moscow, Russia*
* *malak94kifah@gmail.com*

The aim: Acceleration of obtaining *Waxy* wheat lines with amylopectin-type starch.
Relevance: The quality of starch is closely related to the ratio of its two main components: amylose and amylopectin. Granule-bound starch synthase (GBSSI) is the enzyme responsible for synthesizing amylose in wheat grain. The wild-type alleles of the *Waxy* genes, referred to as "*a*" are the most common in various wheat varieties; they do not carry mutations and express the GBSSI protein at high levels. In contrast, the "*null*" allele is nonfunctional, less common, and results in a decreased amylose content in starch. *Waxy* genes are located in each of the three subgenomes of bread wheat. The presence of three null alleles for the *Wx-A1*, *Wx-B1*, and *Wx-D1* genes leads to the formation of amylopectin-type starch, which can be utilized to develop new wheat varieties suitable for producing bakery products and noodles [1]. Several factors limit the potential for rapid selection: the long growing season of plants, dependence on seasonal conditions, the necessity to reproduce the obtained forms to generate a substantial amount of grain (for various quality and selection assessments), and the requirement to conduct multiple cycles of self-pollination to develop homozygous forms. The solution to these challenges is Speed Breeding technology, which focuses on optimizing conditions for the rapid development of plants.
Materials and methods: The study focused on promising lines of winter bread wheat that carry different allelic variants of the *Waxy* genes. The plants were grown using Speed Breeding technology in phytotrons at the All-Russia Research Institute of Agricultural Biotechnology. DNA was extracted from lyophilized leaves following the CTAB protocol. The plants were genotyped using molecular markers (Wx-A1: AFC, AR2; Wx-B1: BDFL, BRC1, BFC, BRC2, and BDFL, BRD; also, WxB1L/R; Wx-D1: Wx-D1-2F/R) [1]. The work on developing wheat lines with the "*null*" alleles is conducted in our laboratory in several stages. In the first stage, a field experiment was carried out at the Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, during which parental lines with various combinations of *Waxy* gene alleles were developed. Based on the genotyping results, one crossing combination was selected that exhibited the highest number of "*null*" alleles of the *Waxy* genes, totaling 370 lines. In the second stage, only those plants that possessed a "*null*" allele in a homozygous state for three *Waxy* genes, as well as plants with two "*null*" alleles and one "*e*" allele, were selected for further work based on genotyping results. A qualitative analysis of the grain from the selected lines was also performed using the I_2/KI dye to validate the findings. Subsequently, utilizing Speed Breeding technology, we conducted several continuous cycles of growing and self-pollination of the selected lines to obtain homozygous forms, which was confirmed by genotyping results with molecular markers.
Results and conclusions: As a result of our two years of research, we have developed a set of promising homozygous lines of F_6 winter bread wheat that possess "*null*" alleles of the *Wx-A1*, *Wx-B1*, and *Wx-D1* genes. The methodology employed will enable us to rapidly develop new varieties of common wheat characterized by amylopectin-type starch. Furthermore, by utilizing the proposed Speed Breeding approach, we can efficiently replace the "*a*" alleles with "*null*" alleles in any variety, thereby altering the properties of its starch.
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Reference
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