A COMPARISON OF *Triticum spelta* GENOTYPES USING SSR GENOTYPING

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Introduction. The development of the theory and practice of improving agricultural plants is related to expanding the gene pool of cultivated plants at the expense of foreign genetic material [1]. *Triticum spelta* L. is used as a donor of valuable economic traits in crossing with bread wheat [2, 3]. Compared to durum wheat, spelt is characterized by a lower insoluble content of polymeric proteins, but a higher content of gliadins and soluble polymeric proteins, due to which it has a softer and less elastic gluten. *T. spelta* varieties also differ in the individual essential amino acids content, containing a number of micro- and macroelements, unsaturated fatty acids and other useful substances. In addition, spelt's flour has unique taste qualities and high content of vitamins of group B and is also suitable for the best quality confectionery products production [4, 5, 6, 7]. However, the spread of this culture is hindered by its low yield and a number of morphological characteristics that lower production [7,8].

Molecular markers are a valuable tool in the study of plant genetic material. A special place belongs to SSR markers (microsatellite sequences), which are repeats of 1-5 pairs of nucleotides in the genomes of eukaryotes. The comparative analysis of DNA loci allows researchers to check close varieties for their purity and compatibility, as well as to establish phylogenetic relationships [9]. The purpose of current study was the selection of SSR markers, which can be used to differentiate genotypes of *T. spelta* to determine the relationship among them.

Materials and methods. As research material, 27 genotypes of *T. spelta* and 2 genotypes of *T. aestivum* cv. Zymoyarka and Podolianka, reproduction of 2015-2022 from NCPGRU and IPPG NASU were used. To carry out the polymerase chain reaction, total DNA was extracted by CTAB method [10]. Reaction mixtures for PCR in a volume of 20 µl included: 9.3 µl of purified Millipore Milli-Q H_2O, 0.5 µl each of forward and reverse primers (10 mM), 2 µl 10x Reaction Buffer B (Solis BioDyne), 2 µl 1 mM Cresol reagent, 1.6 µl of 2 mM $MgCl_2$, 2 µl dNTP (Thermo Fisher Scientific), 1 unit of FIREPol® DNA Polymerase (Solis BioDyne), 30 ng total plant DNA.

Amplification program: denaturation – 94°C for 4 min and 34 cycles: denaturation – 94°C for 30 s; annealing – for primers *Xgwm219*, *261* and *508* [11] was 62, 59 and 50°C, respectively, for 30 s; elongation – 72°C for 30 s, final elongation – 72°C for 5 min. Amplification products were visualized after their electrophoretic separation in a 3 % agarose gel under ultraviolet light using ethidium bromide as a staining reagent [12]. The amplicon size identification was conducted using GelAnalyzer 19.1 software.

Results and discussion. Total DNA was extracted from 30 samples of *T. spelta*, *T. aestivum* cv. Zymoyarka and Podolyanka, and evaluated by gel electrophoresis (Fig. 1). The polymerase chain reactions were performed to determine the polymorphism of microsatellite loci. At this stage of the research, the results of primer analysis for the microsatellite loci *Xgwm219*, *Xgwm261* and *Xgwm508* were obtained (Table 1, Fig. 2).

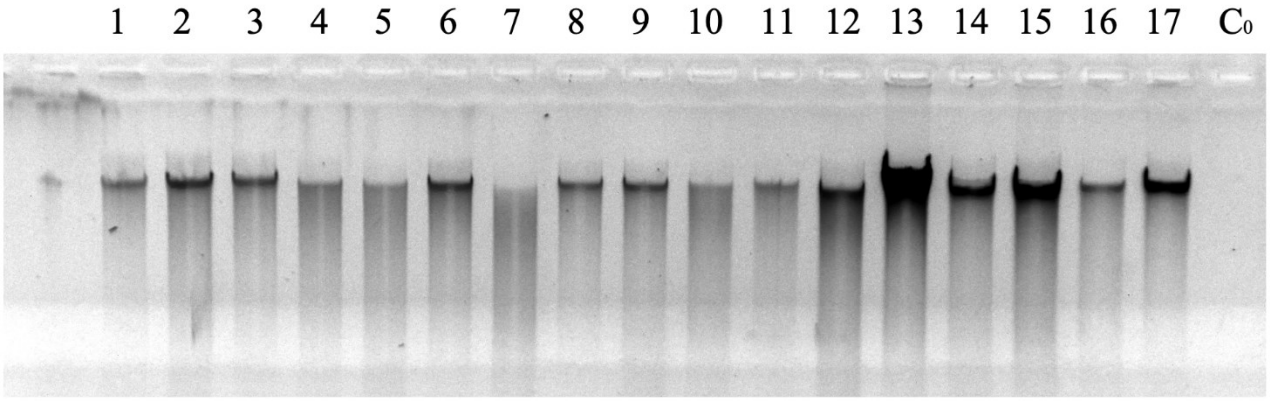

Fig. 1. Electrophoregram of total DNA extracted from seeds by CTAB method. Lane 1, *T. spelta,* var. *duhamelianum,* Poland,; 2, *T. spelta*, var. *duhamelianum,* Baulaender, Germany (Austria),; 3, *T. spelta,* var. *duhamelianum,* Frankenkorn, Germany,*; 4, T. spelta* var. *duhamelianum*, Baulaender (Bauländer); 5, *T. spelta* var. *duhamelianum*, Frankenkorn; 6, *T. spelta* var. *duhamelianum*, *T.spelta* Zorya Ukrainy 7, *T. spelta* var. *arduini*, Europe; 8, *T. spelta* Europe; 9, *T. spelta* № 851*; 10, T. spelta* № 853*; 11, T. spelta* № 845*; 12, T. spelta* Zorya Ukrainy; 13, *T. spelta*, Zuricher Rotkorn; 14, *T. spelta*, Filderweiss; 15, *T. spelta*, Oberkulmer Rothkorn; 16, *T. spelta* № 3995; 17, *T.*, Filderstolz.

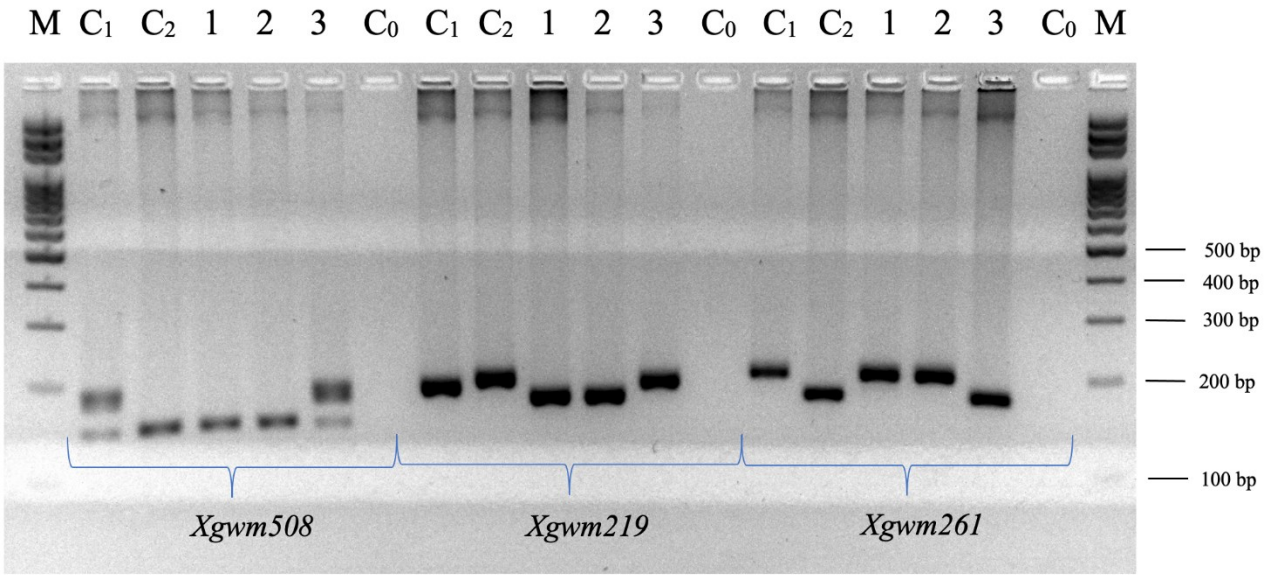

Fig. 2. Electrophoregram of DNA amplification products of *T. aestivum* and *T. spelta* for determination of loci *Xgwm508*, *Xgwm219* and *Xgwm261.* Lane 1, *T. spelta,* var. *duhamelianum,* Poland,; 2, *T. spelta*, var. *duhamelianum,* Baulaender, Germany (Austria),; 3, *T. spelta,* var. *duhamelianum,* Frankenkorn, Germany,*;* C_1, *T. aestivum,* cv. Podolianka; C_2, *T. aestivum,* cv. Zymoyarka; C_0, no DNA control.

As a result of the analysis, 11 amplicons of 141-188 bp sizes were detected in *T. spelta* and *T. aestivum* samples using all three markers. Using *Xgwm219* locus *5* amplicones were identified. *Xgwm261 - 3* amplicones were determined; *Xgwm508 – 3* amplicones were identified. The analysis results are summarized in table 1.

Table 1. The found amplicons in *T. spelta and T. aestivum samples for SSR markers.*

	Amplicons										
	Xgwm508			*Xgwm219*					*Xgwm261*		
Samples, genbank number	*141*	*144*	*171*	*158*	*163*	*173*	*179*	*188*	*166*	*171*	*181*
T. aestivum cv. Podolianka	+	-	+	-	-	+	-	-	Undefined		
T. aestivum cv. Zymoyarka	-	+	-	-	-	-	+	-	Undefined		
T. spelta var. *duhamelianum*	-	+	+	+	-	-	-	-	-	-	+
T. spelta var. *duhamelianum*, Baulaender (Bauländer)	-	+	+	-	-	-	+	-	Undefined		
T. spelta var. *duhamelianum*, Frankenkorn	-	+	-	-	-	+	-	-	-	+	-
T. spelta var. *duhamelianum*, *T.spelta* Zorya Ukrainy	-	+	-	+	-	-	-	-	-	-	+
T. spelta var. *arduini*, Europe	-	+	-	-	+	-	-	-	Undefined		
T. spelta Europe	-	+	-	-	+	-	-	-	Undefined		
T. spelta, № 851	-	+	-	-	-	+	-	-	+	-	-
T. spelta, № 853	-	+	+	-	-	-	+	-	-	+	-
T. spelta, № 845	-	+	-	-	+	-	-	-	-	+	-
T. spelta, Zorya Ukrainy	-	+	-	-	+	-	-	-	-	-	+
T. spelta, Zuricher Rotkorn	-	+	-	-	-	-	-	+	-	+	-
T. spelta, Filderweiss	+	-	+	-	-	-	-	+	Undefined		
T. spelta, Oberkulmer Rothkorn	-	+	-	+	-	-	-	-	-	+	-
T. spelta, № 3995	-	+	-	-	+	-	-	-	-	+	-
T. spelta, Filderstolz	-	+	-	-	+	-	-	-	-	-	+
T. spelta, Rouquin	-	+	-	-	+	-	-	-	+	+	-
T. spelta, Zollernspelz	-	+	-	-	+	+	-	-	+	-	-
T. spelta, Alkor	-	+	-	-	+	-	-	-	-	+	-
T. spelta, Badengold	-	+	-	-	+	-	-	-	+	-	-
T. spelta, Badenstern	-	+	-	-	-	-	+	-	+	-	-
T. spelta, Frankenkorn	-	+	-	-	-	-	-	+	+	-	-
T. spelta, № 3897	-	+	-	-	+	-	-	-	-	+	-
T. spelta, № 3939	-	+	-	-	+	-	-	-	-	-	+
T. spelta var. *griseoturanorecens*	-	+	+	-	+	-	-	-	+	-	-
T. spelta var. *album*	-	+	+	+	-	-	-	-	-	+	-
T. spelta var. *caeruleum*	-	+	-	-	+	-	-	-	-	+	-
T. spelta var. *caeruleum*, Tridentina	-	+	+	-	+	-	-	-	-	-	+
T. spelta var. *album*	-	+	+	-	+	-	-	-	-	+	-
T. spelta var. *caeruleum*, CDC Zorba	-	+	-	-	+	-	-	-	-	+	-
T. spelta var. *arduini*	-	+	+	-	-	-	+	-	-	-	+

Conclusions. Molecular markers analysis showed differences among spelt genotypes. The markers of microsatellite loci *Xgwm219, Xgwm261* and *Xgwm508* are suitable to analyze both *T. aestivum* and *T. spelta* genotypes by PCR. Based upon the obtained polymorphic amplicons, the phylogenetic relationships can be easily established among different genotypes.

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