Biol. Stud. 2024; 18(1): 83–98 • doi: https://doi.org/10.30970/sbi.1801.762
www.http://publications.lnu.edu.ua/journals/index.php/biology

UDC: 631.523:623.111

APPLICATION OF SSR MARKERS FOR ASSESSMENT OF GENETIC SIMILARITY AND GENOTYPE IDENTIFICATION IN LOCAL WINTER WHEAT BREEDING PROGRAM

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Batashova, M., Kryvoruchko, L., Makaova-Melamud, B., Tyshchenko, V., & Spanoghe, M. (2024). Application of SSR markers for assessment of genetic similarity and genotype identification in local winter wheat breeding program. *Studia Biologica*, 18(1), 83–98. doi:10.30970/sbi.1801.762

Background. Simple sequence repeat (SSR) markers are widely used for genetic analysis in plant breeding, allowing for the investigation of genetic divergence and similarity of genotypes, identification of unique alleles and determination of levels of genetic diversity.

Materials and Methods. Analysis of 42 wheat cultivars and lines from the breeding program of Poltava State Agrarian University was carried out using 11 SSR markers located on different chromosomes. A set of 11 microsatellite single locus primer pairs was used in this study (Xgwm 11, Xgwm 44, Xgwm 46, Xgwm 135, Xgwm 174, Xgwm 186, Xgwm 194, Xgwm 219, Xgwm 312, Xgwm 372, Xgwm 389). Amplification of 11 loci was performed using the Kapa2G FastHotStart PCR Kit (Kapa Biosystems, Boston, USA). The mixture for PCR amplification contained 1.5 x Kapa2G buffer, 0.5 mM dNTP mix, 0.5 μM of each primer (Sigma-Aldrich), 1 unit of Kapa2G FastHotStart DNA Polymerase and 11.8 ng of template DNA in a volume of 25 μl. Fragment lengths were determined using GeneMapper 4.0 software (Applied Biosystems). Dendrogram was constructed using UPGMA (unweighted pair-group method with arithmetic average) in DarWin 6.0 software (Perrier and Jacquemoud-Collet 2006) for clustering analysis.

Results and Discussion. The number of alleles detected per locus varied from 5 (Xgwm 11, Xgwm 135, Xgwm 219) to 12 (Xgwm 174). A total of 80 alleles were identi-

fied for the 11 loci studied. Among these, 25 unique alleles were found, each of which was present in only one genotype. The polimorphism information content (PIC) values ranged from 0.48 to 0.87. The markers Xgwm 174 (PIC = 0.87), Xgwm 389 (PIC = 0.84) and Xgwm 372 (PIC = 0.83) were the most polymorphic in our study. We obtained a distribution of cultivars and lines by genetic similarity into five clusters.

Conclusion. The use of SSR markers made it possible to identify rare alleles within the varieties presented. The study of the genetic similarity of the presented genotypes showed their relationship according to their origin. It was shown that unique alleles tended to occur in certain local breeding genotypes. This study has shown that genotypes representing the local Ukrainian breeding program often have the same allelic variants and at the same time some genotypes have unique allelic variants. The results obtained from the study of 42 winter wheat genotypes based on 11 SSR markers showed that molecular markers can be very useful in assessing genetic similarity and identifying genotypes in the local breeding program.

Keywords: alleles, polymorphic information content, clusters, *Triticum aestivum* L.

INTRODUCTION

Common wheat, *Triticum aestivum* L. (2n = 42 ABD), is a crucial crop for the global economy. Ukraine holds a stable position in the top 10 wheat grain exporters (Lagodiienko *et al.*, 2019). The 2021-2030 OECD-FAO Agricultural Outlook reports that an increased cereal production worldwide depends on improved varieties and better agricultural practices leading to higher yields (OECD/FAO, 2021). Accelerating the process of plant breeding by utilising advanced techniques and tools, including genotyping, marker assisted selection, high-throughput phenotyping, genomic selection, and genome editing, plays a vital role in meeting the future food demand by 2050 (Dobrova *et al*., 2014; Lenaerts *et al.*, 2019; Hickey *et al.*, 2019). For the past 50 years, wheat breeding in Western Europe has achieved significant yield and quality increase. This happened due to the accumulation of favorable genetic variants through a consistent selection of better individuals. The identification of genetic variability linked to molecular markers may aid breeders in achieving genetic gain and improving their breeding strategies (Voss-Fels *et al.*, 2019; Singh *et al.*, 2020). Molecular markers enable breeders to address diverse pragmatic goals, including enhancing agronomic features, developing resistance to diseases and pests, as well as elevating crop yields and their adaptation performance (Kong *et al.*, 2005; Guo *et al.*, 2015; Abdur Rehman Arif *et al.*, 2023). Wheat genetic maps have been developed and expanded using various molecular markers, such as RFLP, AFLP, SSR, ISSR, and SNP (Röder *et al.*, 1998; Bohn *et al.*, 1999; Somers *et al.*, 2004; Rimbert *et al.*, 2018). DNA markers are characterized by a highly polymorphic nature, codominant inheritance, a high degree of presence in the genome, neutrality to environmental conditions and can be studied at any stage of plant growth and development (Cobb *et al.*, 2019; Pandurangan *et al.*, 2022).

Molecular markers serve as a valuable supplement to the morphological and physiological traits of winter wheat varieties and breeding lines (Kota *et al.*, 2014; Christov *et al.*, 2022). Their usage enables precise wheat variety identification and selection, which are critical factors in achieving high-quality wheat production. Simple sequence repeat (SSR) markers, in particular, are assumed to be more informative being multi-allelic,

chromosomally specific, and genome-wide in distribution (Tams *et al.*, 2005; Kuleung *et al.*, 2006; Pandurangan *et al.*, 2022). SSR markers are extensively employed in wheat genetics for the selection of particular genes (Błaszczyk *et al.*, 2005). Moreover, SSR-based linkage maps can be applied to pinpoint QTL loci (Zhai *et al.*, 2016). Additionally, they aid in screening hard-to-phenotype traits and in the introgression of one or more desirable genes from a donor into the genome of an elite variety (Zhang *et al.*, 2007). Successful breeding of any crop relies on genetic analysis of traits and their hereditary nature. The selection of genotypes with the required gene combinations and desired traits is made possible by identifying closely linked markers, and this, in turn, enhances the efficiency of breeding programs (Korzun *et al.*, 2002). The application of Marker-Assisted Selection (MAS) could potentially decrease the expenses involved in producing novel wheat strains and enhance the precision and efficiency of the breeding process (Pandurangan *et al.*, 2022).

Modern crop cultivars released in specific areas often have a similar appearance, making it challenging to distinguish between cultivars, lines and their offspring due to insignificant morphological differences, level of adaptation, quality and productivity. Nonetheless, the use of molecular markers enables the assessment of population structure at the local level (Kormoczi *et al.*, 2020; Christov *et al.*, 2022). The utilization of these markers extends to discerning parent-offspring relationships, revealing off-types and self-crossed lines of diverse crops (Spanoghe *et al.*, 2015), and partitioning the indigenous populace into subgroups (Kumar *et al.*, 2016). Ukraine has a long history of wheat breeding (more than 100 years), which resulted in the establishment of a specific alleles combination in Ukrainian cultivars from a few breeding programs in different climatic conditions (Litvinenko *et al.*, 2001; Chebotar *et al.*, 2016). The study of genetic diversity of wheat germplasm from the Black Sea region by microsatellite markers showed a high level of diversity and a high number of unique alleles among Ukrainian cultivars (Landjeva *et al.*, 2015). Adaptation to specific local conditions plays the main role of the breeding program with the aim of providing a stable level of yield potential and quality parameters (Tishchenko *et al.*, 2014). Long-term selection for a typical local environment ensures the appearance of unique alleles and the development of unique allele combinations (Greveniotis *et al.*, 2020).

Breeding novel cultivars with high yield potential, good quality, improved agronomic traits and disease resistance without experiencing a diversity bottleneck is crucial for ensuring a steady supply of wheat grain for Ukrainian farmers. The aim of this study is to assess the genetic diversity of the genotypes presented in a local Ukrainian breeding program (Poltava State Agrarian University) and distinguish closely related materials using highly informative SSR markers.

MATERIALS AND METHODS

Plant material and DNA extraction. Forty-two winter wheat (*Triticum aestivum* L.) genotypes were used in this study and are detailed in **Table 1.** The genotype panel primarily consisted of breeding material sourced from the breeding programme of Poltava State Agrarian University (PSAU) in Ukraine, including 23 cultivars released by PSAU, 19 late-breeding lines from PSAU and 6 cultivars from other Ukrainian breeding institutions.

The QuickPickPlant DNA kit and Pickpen 1-M (Bio-Nobile, Finland) were used to extract DNA from 3–4-day-old seedlings of each wheat cultivar. The concentration of the DNA solution was determined with an ND-3300 NanoDrop spectrofluorometer (Thermo Scientific; Waltham, MA, USA). Each concentrated DNA solution was diluted to 2 ng/μL in order to acquire the optimal concentration for multiplexed-PCR and then stored at -20 °C.

SSR analyses. Eleven microsatellite primer pairs, previously developed and mapped by Röder (Röder *et al.*, 1998), were used in this study and listed in **Table 2**. Each pair of primers enables the examination of a single Xgwm-locus. A selection of primer pairs was made based on previous studies (Song *et al.*, 2005; Bányai *et al.*, 2006; Kuleung *et al.*, 2006; Stępień *et al.*, 2007; Schuster *et al.*, 2009). The selection criteria for primers included the level of polymorphism, the length of the obtained fragment, and similarity in annealing temperature.

Amplification of 11 loci was performed using the Kapa2G FastHotStart PCR Kit (Kapa Biosystems, Boston USA). The 5′-end of the forward primer of each pair was labeled with a fluorescent dye (D3 and D4). The mixture for PCR amplification in a volume of 25 μL contained 1.5 × Kapa2G Buffer, 0.5 mM dNTP Mix, 0.5 μM of each primer (Sigma-Aldrich), 1 unit Kapa2G Fast Hotstart DNA Polymerase and 11.8 ng of template DNA. Each amplification was carried out under the same conditions in the 9800 Fast Thermal Cycler (Applied Biosystems). The PCR profile was as follows: initial denaturation of 2 min at 95 °C, followed by 29 cycles of 15 s at 95 °C, 30 s at 58 °C (Ta), 12 s at 72 °C, and one cycle of 2 min at 72 °C. The resulting amplification products were stored at 4 °C until electrophoresis. PCR products were diluted in water at a ratio of 1:15. A volume of 2 μL of the diluted solution was added to 38 μL of denaturing mix (1% of ROX-labeled molecular weight marker GS-400HD Rox and 99% of formamide). Analysis of DNA fragments after multiplexed-PCR was carried out in an 8-capillary electrophoresis Genetic Analysis SystemGenomeLabGeXP (Beckman Coulter). Fragment lengths were determined using GeneMapper Software 4.0 (Applied Biosystems).

Data analysis. The polymorphic information content (PIC) was calculated for each marker as PIC = 1−ΣPi2, where Pi is the frequency of the allele detection in the subset (Anderson *et al.*, 1993). Microsatellites that differed in length were considered as alleles for the given locus. Distinct fragments were scored as either 1 or 0 (i.e., for presence or absence) for each accession and for all the 11 SSR loci. Genetic similarity was calculated according to M. Nei and W. Li (1979). Dendrogram was constructed using UPGMA (unweighted pair-group method with arithmetic average) in the DarWin 6.0 software (Perrierand Jacquemoud-Collet 2006) for clustering analysis.

RESULTS AND DISCUSSION

SSR polymorphism. The allele size range, total number of alleles, number of unique alleles and PIC values are presented in Table 2 for each SSR locus. All loci were multiallelic, with the number of detected alleles per locus ranging from 5 (Xgwm 11, Xgwm 135, and Xgwm 219) to 12 (Xgwm 174). A total of 80 alleles were identified across the 11 studied loci, with 19 unique alleles present in only one genotype. The Xgwm 174 locus had the highest number of distinct alleles, with a count of 5. The PIC-value ranged from 0.48 (Xgwm 312) to 0.87 (Xgwm 174).

Table 1. **List of wheat cultivars and lines included in the study, their pedigree year of registration**

No.	Breeding line / cultivar	Pedigree	Breeding institution	Year of registration / patent
1	Kolomak-3	Myronivska 808 / Pliska // Albatros odeskyi	PSAU[1], Ukraine	1997
2	Kolomak-5	Dniprovska-782 / Priboy		1997
3	Ukrainka poltavska	Dniprovska-782 / Priboy		2002
4	Dykanka	Yuzhnaya Zarya / Chaika		2005
5	Levada	Albatros odeskyi / Pliska // Myronivska 808		2005
6	Vilshana	Hadmerslebener-12174-85 / Myronivska 61		2010
7	Sahaidak	Lutescens-89 / POLT-50144		2010
8	Sydir Kovpak	Yuzhnaya Zarya / Chaika		2011
9	Orzhytcia	Lelya / Donetska-46		2013
10	Tsarychanka	Donskaya polukarlikovaya / Kolomak-3		2013
11	Karmeliuk	Kolomak-5 / Partyzanka // Skifyanka		2015
12	Poltavchanka	Kolomak-3 / Skifyanka		2015
13	Zelenyi hai	Myronivska 808 / Skifyanka		2016
14	Ariivka	Donskaya polukarlikovaya / Unknown		2017
15	Orzhytcia nova	6687-7 / Donetska-46 // Lelia		2020
16	Radyvonivka	Donetska-4 / Lelia		2018
17	Sanzhara	Donetska-4 / Lelia / Lutescens-89		2018
18	Sonata Poltavska	Kolomak-3 / Skifyanka		2018
19	Samara	Peremoha-2 / Kolomak-5		2019
20	Ivanivska ostysta	Unknown	IRS[2], Ukraine	1997
21	Soniachna	Unknown		–
22	Vira	Unknown		–

End of the Table 1

23	Kryzhynka	Myronivska-27 / Myronivska-28	MIP / IFRG[3], Ukraine	2004
24	Prestyzh	KS 54104-1764 / Sava // Severodonskaya // Urozhaynaya / Albatros odeskyi	DRC[4], Ukraine	2007
25	Kiriia	Obrii / Yuzhnaya Zarya // Yan / 3 / Yuvileina-75	PBGI[5], Ukraine	2010
26	POLT-1P	Vilshana / Yermak	PSAU, Ukraine	–
27	POLT-2P (Tahamlyk)	Erythrospermum / Nahodka-4 // Stanichnaya		–
28	POLT-7P	Donskaya polukarlikovaya / Yermak		–
29	POLT-8P	Peremoha-2 / Kolomak-3 // Zernogradskaya-11		–
30	POLT-9P	Yuzhnaya Zarya / Chaika // Chervona		–
31	POLT-13P	Vilshana / Manzheliia		–
32	POLT-15P	Ukrainka Poltavska / Stanichna		–
33	POLT-7-32	Vilshana / Manzheliia		–
34	POLT-7-35	Vilshana / Manzheliia		–
35	POLT-7-44	Ukrainka Poltavska / Yermak		–
36	POLT-7-54	Vilshana / Levada		–
37	POLT-7-55	Vilshana / Manzheliia		–
38	POLT-7-71	Manzheliia / Sahaidak		–
39	POLT-7-77	Donskaya polukarlikovaya / Yermak		–
40	POLT-8-4	Vilshana / Manzheliia		–
41	POLT-8-8	Ukrainka Poltavska / Yermak		–
42	POLT-8-10	Yuzhnaya Zarya / Chaika // Kryzhynka		–

Comments: [1] – PSAU – Poltava State Agrarian University; [2] – IRS – Ivanivka Research and Breeding Station of the Institute of Bioenergy Crops and Sugar Beet of the NAAS of Ukraine; [3] – MIP / IFRG – the V. M. Remeslo Myronivka Institute of Wheat of the NAAS of Ukraine / Institute of Plant Physiology and Genetics of the NAS of Ukraine; [4] – DRC – Donetsk Research Centre; [5] – PBGI – Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation of the NAAS of Ukraine

Table 2. **Characteristic of the 11 Multiplexed SSR markers used in the assessment of the genetic similarity of winter wheat cultivars and breeding lines. Locus, Chromosome location, primer sequences (5′–3′) with fluorochrome label, allele size range, number of alleles, and PIC are presented for each Locus**

Locus	Chromosome	Primer	Multiplex	Allele size range (bp)	Number of alleles	Number of unique alleles	PIC*
Xgwm 11	1B	GGATAGTCAGACAATTCTTGTG **D3** GTGAATTGTGTCTTGTATGCTTCC	1	184–194	5	1	0.78
Xgwm 44	7DS	GTTGAGCTTTTCAGTTCGGC **D4** ACTGGCATCCACTGAGCTG	4	173–184	8	3	0.79
Xgwm 46	7BS	GCACGTGAATGGATTGGAC **D3** TGACCCAATAGTGGTGGTCA	4	144–177	7	2	0.77
Xgwm 135	1A	TGTCAACATCGTTTTGAAAAGG **D3** ACACTGTCAACCTGGCAATG	2	136–195	5	1	0.54
Xgwm 174	5DL	GGGTTCCTATCTGGTAAATCCC **D3** GACACACATGTTCCTGCCAC	3	183–226	12	4	0.87
Xgwm 186	5A	GCAGAGCCTGGTTCAAAAAG **D3** CGCCTCTAGCGAGAGCTATG	3, 4	91–132	7	1	0.64
Xgwm 194	4DL	GATCTGCTCTACTCTCCTCC **D4** CGACGCAGAACTTAAACAAG	1	129–137	6	1	0.77
Xgwm 219	6B	GATGAGCGACACCTAGCCTC **D4** GGGGTCCGAGTCCACAAC	1	163–189	6	1	0.75
Xgwm 312	2A	ATCGCATGATGCACGTAGAG **D4** ACATGCATGCCTACCTAATGG	3	189–218	5	2	0.48
Xgwm 372	2A	AATAGAGCCCTGGGACTGGG **D3** GAAGGACGACATTCCACCTG	2	284–320	9	1	0.83
Xgwm 389	3B	ATCATGTCGATCTCCTTGACG **D4** TGCCATGCACATTAGCAGAT	2	115–142	10	2	0.84

Comment: PIC* – polymorphic information content

The distribution of alleles among genotypes is shown in **Figure 1.** The less polymorphic locus, i.e., Xgwm 312, presented the SSR fragment with the size of 191 bp in most genotypes. The loci Xgwm 186 and Xgwm 135 also presented one size of allele in more than twenty genotypes. For the loci Xgwm 11, Xgwm 44, Xgwm 194, Xgwm 219 and Xgwm 372, the genotypes were distributed in 3–4 main groups according to allele size and had unique alleles in certain cultivars.

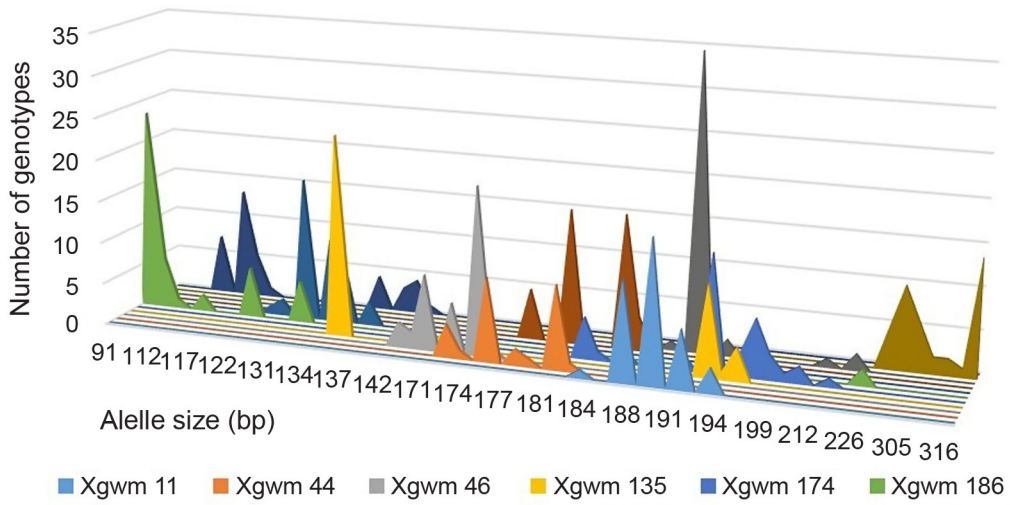

Fig.1. Allelic polymorphism of 11 SSR loci among 42 winter wheat genotypes

Cluster analysis. Genetic relationships among the varieties and breeding lines were estimated through a dissimilarity matrix according to the hierarchical unweighted pair-group method with arithmetic means analysis (UPGMA). The clustering analysis shows the genetic diversity within the 42 wheat genotypes based on the genetic distances revealed by 11 SSR markers (**Fig. 2**). Five major clusters were distinguished with different numbers of genotypes within each cluster.

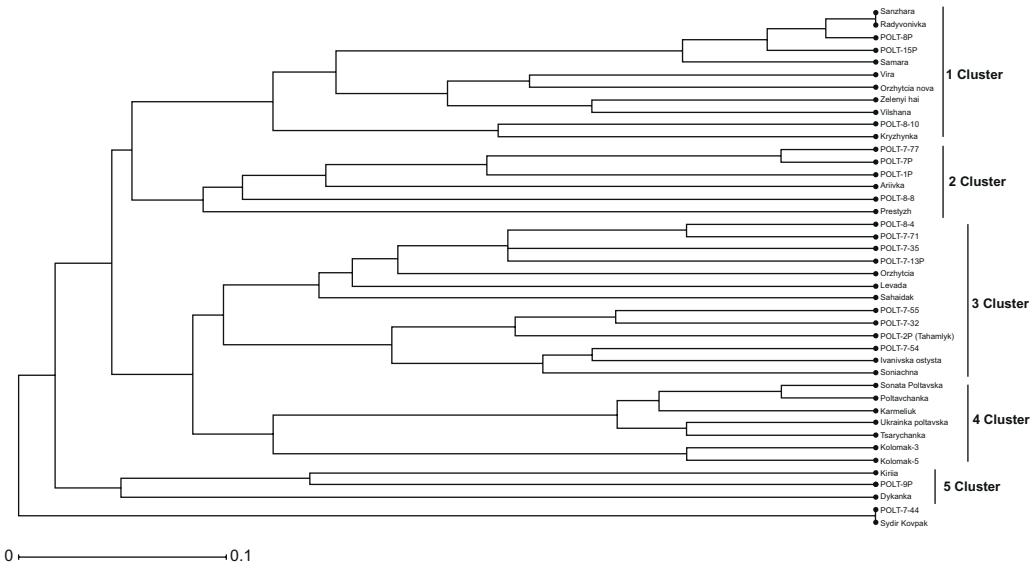

Fig. 2. Hierarchical clustering dendrogram based on the genetic distances among 42 wheat cultivars and lines from breeding program of Poltava State Agrarian University, Ukraine. The genetic distance coefficients were calculated from the data of 11 SSR loci

The first cluster (**1**) consists of 11 genotypes which include local cultivars (Radyvonivka, Sanzhara, Samara, Zelenyi hai, Vilshana) and breeding lines (POLT-8P, POLT-15P, POLT-6P, POLT-8-10) and others (Vira, Kryzhynka). Although not verified by the cluster analysis, Sanzhara and Radyvonivka were distinguishable as two of the genotypes in the group. The second cluster (**2**) consists of six genotypes; five of them are from local breeding program and the cultivar Prestyzh. The origin of Donskaya polukarlikovaya and Yermak is confirmed by the pedigree information of these genotypes (**Table 1**). Furthermore, both of these cultivars as well as Prestyzh were created by the Donetsk Research Centre, demonstrating their similarity. The third cluster (**3**) consists of mostly local cultivars and lines (total number is 13); including two cultivars Ivanivska ostysta and Soniachna from Bilotserkivska research station. The lines in this cluster have been created by crossing local cultivars, including Vilshana and Manzheliia.

The fourth cluster (**4**) consists of seven local cultivars which present the first released cultivars from the local breeding program Kolomak-3 and Kolomak-5, and Ukrainka Poltavska (**Table 1**). The other cultivars developed and released from their crosses are included in this cluster as well. The smallest fifth cluster (**5**) consists of three genotypes (Kiriia, Dykanka and POLT-9P) which are derived from crosses with Yuzhnaya Zarya and Chaika. There were two separated genotypes which showed one SSR profile at a time – Sydir Kovpak and POLT-7-44. The organization of varieties and lines on the UPGMA dendrogram clusters them according to their origin. The use of SSR markers allows for the differentiation of lines from the same cross combination. These data demonstrate the genetic similarity of the presented genotypes and may serve as a significant criterion in the final breeder's decision-making process.

Microsatellite diversity. Molecular markers are useful tools for assessing diversity and similarity within the population and provide an insight into the breeding progress. Additionally, they increase selection intensity and accuracy. Long-term breeding under certain conditions, with the aim of increasing adaptation, leads to the concentration of specific alleles in the local genetic pool (Landjeva *et al.*; 2015; Toth *et al.*, 2019; Körmöczi *et al.*, 2020). G. A. Chebotar *et al.* (2016) used 24 microsatellite markers to evaluate wheat germplasm from the Black Sea region. In their study, 50% of the alleles were regionally specific. On the other hand, this could lead to a decrease in genetic variability, loss of valuable traits and a bottleneck. Roussel (Roussel *et al.*, 2005) studied 41 wheat microsatellite markers in 559 French wheat accessions from different historical periods. A decrease in allelic richness of about 25% was found between landraces and modern varieties.

Our study has shown that genotypes representing the local Ukrainian breeding programme often have the same allelic variants and at the same time some genotypes have unique allelic variants. The allelic variants of the earlier released cultivars were different from those of the later ones. Only the old varieties of Kolomak-3 and Kolomak-5 had an allelic variant for Xgwm 312 at 218 bp; Levada and Prestyzh had a specific allelic variant at 144 bp for Xgwm 46. Dykanka had four unique allelic variants for Xgwm 44 at 183 bp, for Xgwm 46 at 176 bp, for Xgwm 135 at 194 bp and for Xgwm 174 at 184 bp. Analysis of the distribution of unique alleles showed their presence only in earlier cultivars from local and other breeding programmes. Unique allele variants were also detected in the cultivars Prestyzh, Ivanivska ostysta and Ariivka. The newly developed lines and recently released cultivars did not have any unique alleles according to the

investigated markers. This clearly shows the tendency of breeding to discard unfavourable allelic variants during selection.

Some well-known earlier cultivars, such as Dykanka and Kryzhynka, may still be used in breeding as a source of valuable adaptability or disease resistance traits. Their allelic profile can be used not only for comparison with the profiles of lines in advanced breeding stages, but also to select parent pairs for crosses and to identify unique genotypes of value for breeding.

Genetic relationships. A combined study of phenological, physiological and molecular characteristics of the crop collection, especially the set of cultivars and lines in the breeding programme, can reveal divergence between subgroups and help classify varieties (Shuster *et al.*, 2009). Usually, cultivar ideotypes for a specific growing area can reduce the diversity of breeding material and parents for crosses. On the other hand, ideotypes help to optimise the breeding programme and achieve genetic gain. Assessing the genetic diversity of specific regions, geographical areas or countries using molecular markers provides a clear picture of allelic divergence and breeding trends according to the demands of the modern market (Stępień *et al.*, 2007; Oslovičová *et al.*, 2014; Toth *et al.*, 2018).

This study has shown that clustering of varieties and lines reflects their origin and genetic relationships. For example, all newly developed lines selected from crosses with Vilshana and Manzheliia were clustered into one group. The key message for breeders from these clustering results is to continue with higher performing lines. Otherwise, Vilshana appears in a different cluster, which could be explained by the breeder's selection strategy. Some well-known cultivars have been used as parents in different breeding programmes in Ukraine for years and are the source of many cultivars. Their specific valuable alleles are distributed in many Ukrainian cultivars and could even be the cause of clustering of genotypes from different breeding programmes. For example, genotypes derived from crosses with Donskaya polukarlikovaya have been clustered into a single group. Most of the earlier cultivars are characterised by allelic heterogeneity, which complicates the interpretation of molecular marker analysis. The cultivars Levada, Ivanivska ostysta, Soniachna, Vira, Karmeliuk, Ariivka showed one to three cases of heterogeneity per marker. Unique and some specific alleles were detected only in earlier developed cultivars. Thus, the older varieties such as Kolomak-3, Kolomak-5, Ukrainka Poltavska and some other cultivars derived from them were distinguished in a separate cluster.

Each breeding programme is based on a specific gene pool of plant material. This pool contains sources of disease resistance, sources of adaptation to specific environmental conditions and desirable agronomic parameters. Hybridization and selection over generations within a given pool, with the constant addition of new sources, creates a valuable set of alleles for adaptation to specific environmental conditions. The involvement of a large amount of diverse genetic material of different origins in hybridization over a long period of time allows for the concentration of valuable alleles that can provide a high level of adaptability to environmental conditions.

CONCLUSION

1. The results obtained from the study of 42 winter wheat genotypes based on 11 SSR markers showed that molecular markers can be very useful in the assessment of genetic similarity and identification of genotypes in the local breeding program.
2. The number of detected alleles per locus varied from 5 (Xgwm 11, Xgwm 135, Xgwm 219) to 12 (Xgwm 174).
3. A total of 80 alleles was identified for the studied 11 loci. Among them, 25 unique alleles were found, each of them being present only in one genotype.
4. The PIC values ranged from 0.48 to 0.87. The markers Xgwm 174 (PIC = 0.87), Xgwm 389 (PIC = 0.84) and Xgwm 372 (PIC = 0.83) were the most polymorphic in our study.
5. This study has shown that clustering of varieties and lines reflects their origin and genetic relationships.
6. The allelic profile can be used to select parent pairs for crosses and to identify unique genotypes of value for breeding, as well as for comparison with the profiles of lines at advanced breeding stages.

ACKNOWLEDGMENTS AND FUNDING SOURCES

This study was carried out due to a collaboration of Poltava State Agrarian Academy with High School of Province Hainaut CONDORCET and Centre for Agricultural research CARAH during 2005–2020.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Rights: This article does not contain any studies with animal subjects performed by any of the authors.

AUTHOR CONTRIBUTIONS

Conceptualization, [M.S., M.B, V.T]; methodology, [M.S.]; validation, [M.S.]; formal analysis, [M.B, B.M-M]; investigation, [M.B, L.K]; resources, [M.S., V.T]; writing – review and editing, [M.B, B.M-M, L.K.]; visualization, [M.B, B.M-M] supervision, [M.S., V.T]; project administration, [M.B, V.T].

All authors have read and agreed to the published version of the manuscript.

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ВИКОРИСТАННЯ SSR-МАРКЕРІВ ДЛЯ АНАЛІЗУ ГЕНЕТИЧНОЇ ПОДІБНОСТІ Й ІДЕНТИФІКАЦІЇ ГЕНОТИПІВ У ЛОКАЛЬНІЙ СЕЛЕКЦІЙНІЙ ПРОГРАМІ ПШЕНИЦІ ОЗИМОЇ

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Вступ. Для генетичного аналізу в селекції рослин широко використовують SSR-маркери, які дають змогу вивчати генетичну різноманітність і подібність генотипів, ідентифікувати унікальні алелі та визначати рівень генетичного різноманіття досліджуваного матеріалу.

Матеріали та методи. Аналіз 42 сортів і селекційних ліній пшениці зі селекційної програми Полтавського державного аграрного університету проводили з використанням 11 SSR-маркерів. У цьому дослідженні використовували набір з 11 пар мікросателітних одиничних локусних праймерів (Xgwm 11, Xgwm 44, Xgwm 46, Xgwm 135, Xgwm 174, Xgwm 186, Xgwm 194, Xgwm 219, Xgwm 312, Xgwm 372, Xgwm 389). Ампліфікацію 11 локусів проводили за допомогою Kapa2G FastHotStart PCR Kit (Kapa Biosystems, Бостон, США). Розчин для ПЛР-ампліфікації в об'ємі 25 мкл містив 1,5 × буфер Kapa2G, 0,5 мМ dNTP Mix, 0,5 мкМ кожного праймера (Sigma-Aldrich), 1 одиницю Kapa2G Fast Hotstart ДНК-полімерази та 11,8 нг матричної ДНК. Довжину фрагментів визначали за допомогою програмного забезпечення GeneMapper 4.0 (Applied Biosystems). Дендрограму було побудовано за допомогою UPGMA (метод незважених пар-груп за середнім арифметичним) у програмному забезпеченні DarWin 6.0 (Perrierand Jacquemoud-Collet 2006).

Результати. Кількість виявлених алелів на локус варіює від 5 (Xgwm 11, Xgwm 135, Xgwm 219) до 12 (Xgwm 174). Усього виявлено 80 алелів для 11 досліджуваних локусів. Серед них знайдено 25 унікальних алелів, кожен із яких наявний лише

в одному генотипі. Значення PIC коливалися від 0,48 до 0,87. Найбільш поліморфними в нашому дослідженні виявилися маркери Xgwm 174 (PIC = 0,87), Xgwm 389 (PIC = 0,84) та Xgwm 372 (PIC = 0,83). Отримано розподіл сортів і ліній за генетичною подібністю на п'ять кластерів.

Висновки. Використання SSR-маркерів дало змогу ідентифікувати рідкісні алелі у представлених сортах і лініях. Вивчення генетичної подібності представлених генотипів встановило їхню спорідненість за походженням. Було доведено, що унікальні алелі мають тенденцію траплятись у певних генотипах. У цьому дослідженні з'ясовано, що генотипи, які представляють локальну українську селекційну програму (Полтавський державний аграрний університет), часто мають однакові алельні варіанти і що водночас деякі генотипи мають унікальні алельні варіанти. Результати, отримані під час дослідження 42 генотипів озимої пшениці на основі 11 SSR-маркерів, довели, що молекулярні маркери можуть бути дуже корисними для аналізу генетичної подібності й ідентифікації генотипів у локальній селекційній програмі.

Ключові слова: алелі, індекс поліморфізму (PIC), кластери, *Triticum aestivum* L.

Received / Одержано	Revision / Доопрацьовано	Accepted / Прийнято	Published / Опубліковано
19 December, 2023	12 January, 2024	20 March, 2024	27 March, 2024