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# Analysis of SSR Markers Associated with Yield Components of Soft Winter Wheat

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**Abstract** In this article 18 local and foreign varieties of soft winter wheat were used in the study to analyze the SSR markers associated with yield components. It was observed that 5 out of 10 pairs of SSR microsatellite markers showed polymorphism, while the remaining 5 SSR markers were monomorphic. Winter soft wheat varieties showing polymorphism were involved as donors in selection work in order to create new varieties with high productivity and grain quality.

**Keywords** Winter soft wheat, SSR markers, Yield components, DNA extraction, PCR and gel electrophoresis

## 1. Introduction

Winter soft wheat (*Triticum aestivum* L.) is one of the food crops widely planted in the world and related to food security and social stability. However, its yield and quality are constantly challenged by various biotic and abiotic stresses [5]. Systematically evaluating the adaptability, disease resistance and stress tolerance of released wheat lines at molecular and genetic level is momentous to rationally distribute and apply them.

Wheat production is strongly influenced by the size of cultivated land, climate change, agronomic methods, and different wheat varieties. The global cultivated land accounts for 14.2 million km$^2$ or almost 10% of the Earth's land area. However, factors such as aridity, soil erosion, and vegetation decline will degrade cultivated lands [7].

The shrinking wheat cultivated area and the considerably negative influence of climate change may lead to decreased wheat production. However, global wheat production per annum increased by 1.0% [10]. The optimal crop management practices (including irrigation, fertilizing, pest/disease management, and weed control) [2] and the improvement of wheat varieties [1], have significant contributions in maintaining or enhancing wheat grain yield under adverse conditions.

Yield stability under different environments relies on the compensatory of the main yield components, such as thousand-grain weight, kernel number per spike, and spike number per square meter. During the process of yield formation, the yield components perform differently under various environments. The adverse environments may hamper the specific yield components, and others may compensate for the loss of poor performance of specific components [12].

A comprehensive understanding of the genetic gain in yield and yield-relevant traits is important for breeders and farmers to refine the directions of breeding programs and cropping cultivation. Three different approaches, the process-based models, statistical data, and field experiments, were applied to assess the effects of variety replacement on grain yield. All those approaches have specific advantages and limitations [6].

The results from the field experiments are often more reliable and straightforward although more time and labor force are required [4]. In a previous study, the data from 2–3 year field experiments were used for analyzing the genetic gain in wheat yield using historical varieties in specific environmental conditions [13]. However, due to the limited number of experiments and varieties used, the results may not be robust [8].

The use of yield as a selection criterion for the development of stress tolerant varieties is prohibitive in early generations. As well, drought stress does not happen predictably and evenly in the field. Therefore, plant breeders look for alternative methods such as marker assisted selection (MAS). Rapid advances in genome research and molecular technology have led to the use of DNA marker-assisted selection which holds promise in improving selection efficiency and expediting the development of new cultivars with higher yield potential [11].

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## 2. Materials and Methods

**Plant materials:** 18 domestic and foreign varieties of winter soft wheat: 1-Chillaki, 2-Yaksart, 3-Bo‘zqal’a, 4-Yonbosh, 5-Do‘stlik, 6-Turkiston, 7-Polovchanka, 8-Vostorg, 9-Tobor, 10-Bezostaya-1, 11-Fortuna, 12-Kroshka, 13-Krasnodarskaya-99, 14-Pamyat, 15-Retezat, 16-MV-Nemere, 17-Kate-A1, 18-Konya 2002 varieties were used in the research.

**DNA Extraction and SSR marker assay:** Varieties were grown under climate control at 24°C for 15 days to isolate DNA. The CTAB method was used to isolate genomic DNA from research samples. SSR markers specific for wheat were used to determine mutual genetic polymorphism between wheat varieties.

PCR (hot-start wheat program) working mixture was prepared in a volume of 10 µl. The DNA concentrations of the samples were determined by gel electrophoresis by visual comparison with lambda (λ) phage DNA at a specific concentration (25 ng/µl) on a 0.9% agarose gel. Then, PCR amplification products were subjected to horizontal electrophoresis in 2.5% agarose gel at 100 V voltage for 90 minutes. Molecular weight marker is Invitrogen 50 bp DNA ladder (Cat. No. 10416014).

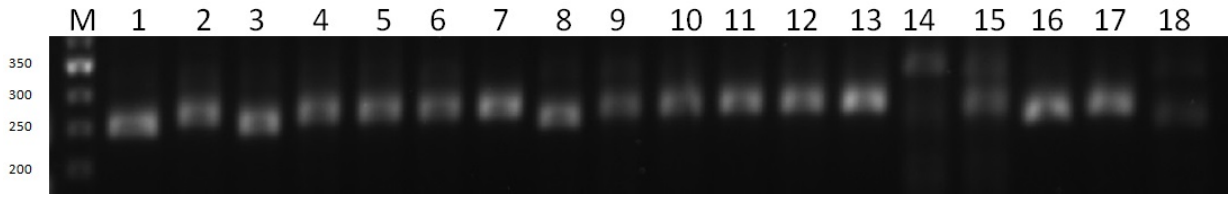
## 3. Results

It was observed that 5 out of 10 pairs of SSR microsatellite markers showed polymorphism, while the remaining 5 SSR markers were monomorphic. Winter soft wheat varieties showing polymorphism were involved as donors in selection work in order to create new varieties with high productivity and grain quality.

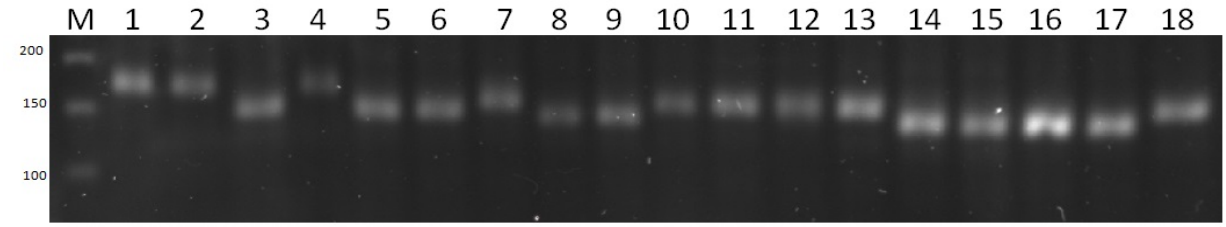
Research samples were screened with microsatellite DNA markers using the polymerase chain reaction (PZP) method.

**Table 1.** A set of primers for SSR microsatellite markers used to analysis yield components

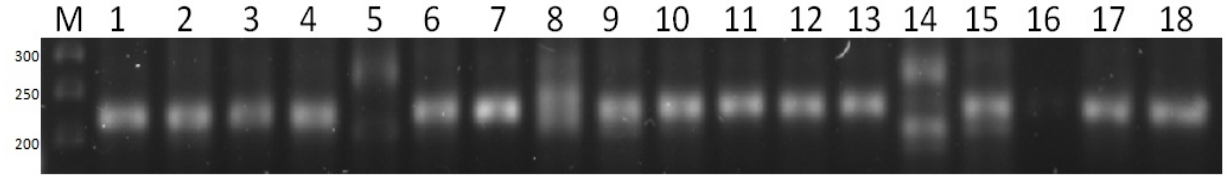
| T/r | Primer | Forward | Reverse |
|---|---|---|---|
| 1 | GDM 93 | AAAAGCTGCTGGAGCATACA | GGAGCATGGCTACATCCTTC |
| 2 | BARC 126 | GCG CCG TGT AAA TAG TTT TGT TTA | CTTGCACAGCCAAATAGTGTGGATAA |
| 3 | BARC 251 | 5' CAATTACGCCAAAAACAAGTGC 3' | 5' GTT TGT TTC GTG ATG TTA AGT TCC A 3' |
| 4 | BARC 121 | 5' ACTGATCAGCAATGTCAACTGAA 3 | 5' CCGGTGTCTTTCCTAACGCTATG 3' |
| 5 | Xwmc 396 | TGCACTGTTTTACCTTCACGGA | CAAAGCAAGAACCAGAGCCACT |
| 6 | BARC 11 | GCGATGCGTGTAAAGTCTGAAGATGA | GCGTCCATGGAGCTCTGTTTTATCTGA |
| 7 | Xwmc 525 | GTTTGACGTGTTTGCTGCTTAC | CTACGGATAATGATTGCTGGCT |
| 8 | BARC 74 | 5' GCGCTTGCCCCTTCAGGCGAG 3' | 5' CGCGGGAGAACCACCAGTGACAGAGC 3' |
| 9 | Xgwm577 | ATG GCA TAA TTT GGT GAA ATT G | TGT TTC AAG CCC AAC TTC TAT T |
| 10 | Xwmc 44 | GGT CTT CTG GGC TTT GAT CCT G | GTT GCT AGG GAC CCG TAG TGG |




**Picture 1.** Agarose gel electrophorogram of PCR analysis with BARC 251 microsatellite marker association used in in yield research




**Picture 2.** Agarose gel electrophorogram of PCR analysis with the association Xwmc 396 microsatellite marker used in harvest index




**Picture 3.** Agarose gel electrophorogram of PCR analysis with the association Xwmc 525 microsatellite marker used in 1000 grain weight

In particular, the PCR analysis using Barc251 DNA marker, which is associated with productivity, allowed us to obtain the following results. According to this, the amplicons generated by this feature of the research samples were analyzed in the GelAnalyzer 19.1 bioinformatics program based on visual comparison with the weight marker with a molecular weight of 50 ng/µl. Based on the obtained data, 2, 4, 5, 6, 7, 9, 10, 2, 4, 5, 6, 7, 9, 10, 2, 3, 8, and 16 samples have 350, 240, and 270 nucleotide pairs, respectively, in samples 1, 3, 8, and 16. 11, 12, 13, 15 and 17 th samples are similar to each other it turned out to be (picture-1).

The results of PCR analysis using Xwmc396 DNA marker associated with harvestindex can be clearly interpreted: PCR electrophorogram supports 175 pairs of nucleotides only in 3 samples 1, 2 and 4, and in sample 7 it supports 160 pairs of nucleotide alleles, 150 pairs of nucleotide alleles, 150 pairs of nucleotides per allele, 150 pairs of nucleotides per allele. Samples 5, 6, 10, 11, 12, 13 and 18 are similar to each other, and samples 8, 9, 14, 15, 16 and 17 showed polymorphism in 140 pairs of nucleotides (picture-2).

Among the various grain related traits, grain weight (GW) is one of the more important ones, since it is phenotypically the most stable component of yield and is also positively correlated with flour yield. Grain weight has been shown to be controlled by a number of quantitative trait loci (QTLs) located on different chromosomes [3]. The 1000 grain weight was characterized by high values of the coefficient of heritability and is controlled in most cases by dominance and overdominance type of gene action, and hence, is of great interest in breeding for wheat productivity [9].

The result of PCR analysis using the Xwmc525 DNA marker associated with 1000 grain weight can be interpreted as follows: samples 1, 2, 3, 4, 6, 7, 9, 10, 11, 12, 13, 15, 17 and 18 with 220 nucleotide alleles that they are similar to each other, two alleles in samples 5, 8 and 14 it was observed that it has 210, 270, 230 pairs of nucleotide alleles as amplification, and this DNA marker was not amplified in sample 16 (picture-3).

Based on the results of electrophoresis, the obtained gel was genotyped according to the allele states of wheat genotypes of the relevant DNA markers (according to molecular mass), and the images of the electrophorogram were analyzed in the GelAnalyzer program, and the data were entered into the MS Excel program.

## 4. Conclusions

18 local and foreign varieties of soft winter wheat were used in the study to analyze the SSR markers associated with yield components. It was observed that 5 out of 10 pairs of SSR microsatellite markers showed polymorphism, while the remaining 5 SSR markers were monomorphic. Winter soft wheat varieties showing polymorphism were involved as donors in selection work in order to create new varieties with high productivity and grain quality.

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