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POPULATION STRUCTURE AND GENETIC ASSOCIATION STUDIES IN WHEAT

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ABSTRACT
To define genetic diversity and population structure among a collection of wheat cultivars and lines of mainly European origin, Kompetitive Allele Specific PCR (KASP) technology was used to characterize a population of 95 bread wheat genotypes. In total, 860 of 960 tested markers were polymorphic and could be used for further analysis. Four subgroups of wheat genotypes were identified using Neighbor Joining (NJ) cluster analysis. Two of this subgroups comprised mainly varieties from Hungarian breeding programs (GrI, GrII); one subgroup contained varieties from Western Europe (GrIII) and one contained varieties with various origin (GrIV). GrI mainly contained genotypes originated from crosses including GK Kincső (Arthur 71/Sava) as one of the parents, or derivatives of this genotype. The results of this study should provide valuable information for future association mapping studies using this wheat collection. Furthermore, the genetic diversity and distance data combined with specific genotype data can be used by breeders to guide selection of crossing parents.

Keywords: wheat (*Triticum aestivum* L.), population structure, KASP, GK Kincső

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one the most important cereal crops worldwide. The genetic diversity conserved in wheat genebanks represents the genetic heritage of this crop. Knowledge of genetic diversity is important for understanding the extent of genetic variability in existing plant material and assessing relatedness among accessions is an important prerequisite for optimizing association studies (GARRIS ET AL., 2003).
Various types of molecular markers can be used for wheat genetic studies. They can be used for marker-assisted selection when tightly linked to target genes, and can also be employed to genetic diversity estimation in wheat germplasm. The evolution of cost efficient DNA marker platforms enable effective utilization of high density SNP (Single-nucleotide polymorphism) markers in research and breeding applications. KASP (Kompetitive Allele Specific PCR) is a uniplex PCR-based technology which is based on allele-specific oligo extension and fluorescence resonance energy transfer for signal generation. Against multiplex methods KASP technology has many advantages: shorter turnaround time, lower genotyping error rate (0.7-1.6%) and more flexibility. It can be used when there are many SNP markers in a few samples or when there are few SNP markers in many samples to analyze (SEMAGN ET AL., 2014).
Reasons for the presence of subgroups within larger germplasm populations can include differences in geographical origin, human or environmentally driven selection or genetic drift. Differences in allelic composition could be caused by different breeding practices and requirements (ROUSSEL ET AL., 2005). Additionally, differences along chromosomes can be caused by the introduction of certain germplasm in specific geographical regions. One example is *Sr36* stem rust resistance gene which was derived from *Triticum timopheevii* (MCINTOSH AND GYÁRFÁS, 1971) and was originally transferred into two

spring wheat lines and then into derivatives of these lines, e.g. Arthur 71 and TP-114-1965-A. Arthur 71 is an improved version of the high-yielding American soft red winter wheat Arthur (PATTERSON ET AL., 1975), which along with *Sr36/Pm6* stem rust and powdery mildew resistance genes carries many other disease resistance genes: *Sr2, Sr6, Sr8, SrTt1, Sr5, Sr8a, Lr9 , Lr14a , Pm2*. In Hungary, one of the first stem rust resistant cultivars was GK Kincső (Arthur 71/Sava) registered in 1980, which carries the *Sr36* gene (PURNHAUSER ET AL., 2011).

MATERIAL AND METHOD

Plant materials

In this study 95 hexaploid wheat varieties and lines were studied. 37 of this genotypes originate from the breeding program of Cereal Research Non-profit Ltd., Szeged, Hungary. The other 58 genotypes which were obtained from the Small Grain Cereal Genebank, Szeged, Hungary, are from different geographical regions, carrying traits important from breeding viewpoint. The distribution of the genotypes of the examined population by origin is presented in *Figure 1*.

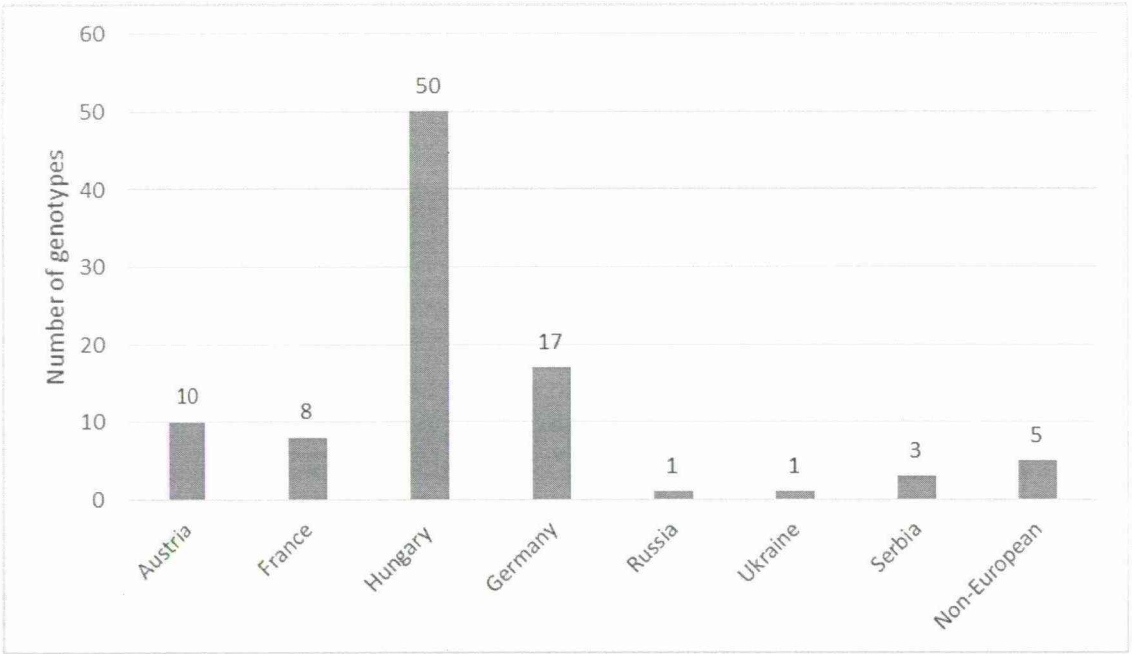

Figure 1. Number of genotypes of the examined population originated from different countries

DNA extraction and genotyping by Kompetitive Allele Specific PCR (KASP)

Genomic DNA from the 95 different genotypes was extracted using Wizard® Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions. The quality and quantity of DNA were measured with NanoDrop 1000 Spectrophotometer (Thermo Scientific Company). The DNA was sent to LGC Ltd. (United Kingdom). The samples were examined using KASP technology (SEMAGN ET AL., 2014) with a set of 960 pre-validated SNP assays that are evenly distributed at 10 cM intervals throughout the wheat A, B and D genomes.

PCR conditions and primers

The 95 genotypes were evaluated with *Xgwm271* and *Xgwm477* SSR primer pairs associated with the presence of *Sr36* stem resistance gene (PURNHAUSER ET AL., 2011). The PCR products were separated using QIAxcel Advanced capillary electrophoresis system (Qiagen) according to the manufacturer's instructions (*Figure 2*).

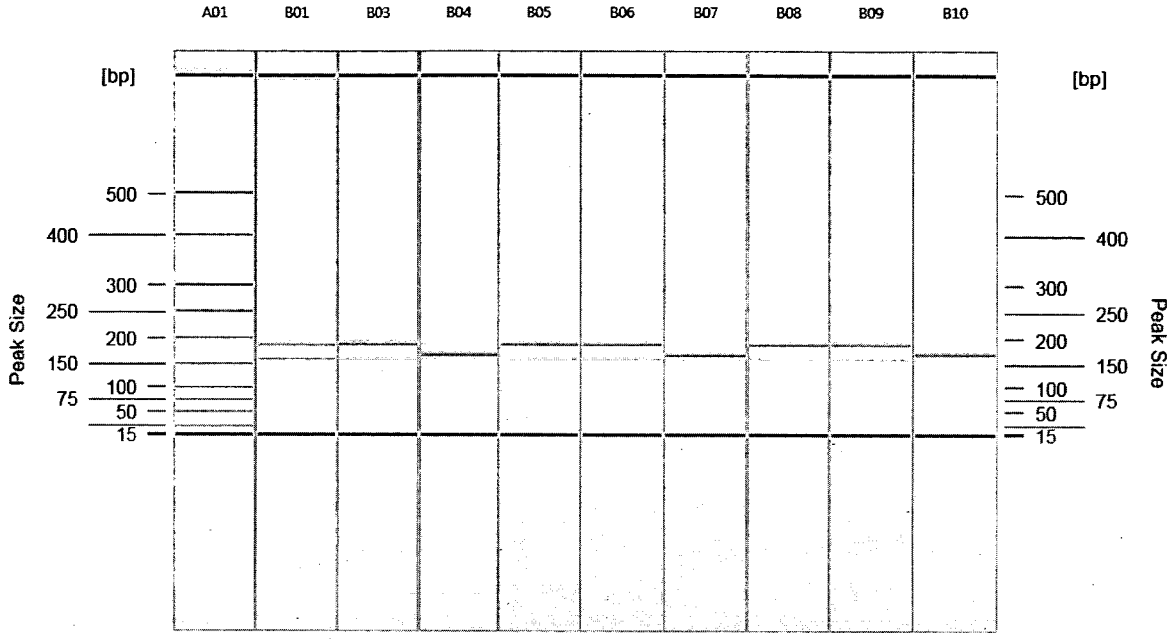

Figure 2. Amplification products obtained after PCR analysis with the *Xgwm477* SSR primer pairs, separated with the use of QIAxcel Advanced capillary electrophoresis (A01: Size marker 15bp-3kb; B01, B02, B05, B06, B08, B09: specific fragments associated with the presence of *Sr36* gene)

Cluster analysis

After the aligning of the marker data, mapped polymorphic marker data were used for performing Neighbor Joining (NJ) cluster analysis, which was carried out using TASSEL (v5.2) software.

RESULTS AND CONCLUSIONS

As a result of the survey of 960 SNP markers, 860 markers were polymorphic, which could be used for further analysis. 303 of these markers were distributed on the A genome, 319 on the B and 238 on the D genome.

NJ cluster analysis identified four subgroups within the test population. Two of the subgroups comprised mainly varieties from Hungarian breeding programs (GrI, GrII); one subgroup contained varieties from Western Europe (GrIII) and one contained varieties with various origin (GrIV). Comparing the segregation of the subgroups with pedigree data, it was found that one of the subgroups containing mainly Hungarian genotypes (GrI) contained principally genotypes which are derived from Arthur 71. Most of these genotypes are originated from crosses including GK Kincső (Arthur 71/Sava) as one of the parents, or derivatives of this genotype.

As GK Kincső was one of the first stem rust resistant cultivars in Hungary, and carries *Sr36* stem rust resistance gene, the whole population was genotyped with molecular

markers (*Xgwm271* and *Xgwm477*) associated with the presence of *Sr36* stem resistance gene. The marker data showed that 16 of the 95 genotypes carries the *Sr36* gene. All of this genotypes were placed in the GrI subgroup by the cluster analysis and the pedigree data show that all of them are derivatives of GK Kincső.

The result of this study agrees with the study of ROUSSEL ET AL. (2005) that the separation of the subgroups is affected by the geographical origin, and the introduction of a certain germplasm into a breeding program may have significant impact. It gives a confirmation by genotyping data about the defining role of GK Kincső in the breeding program of Cereal Research Non-profit Ltd., Szeged, Hungary.

These results should provide valuable information for future association mapping studies using this wheat collection. Furthermore, these data combined with specific genotype information can be used by breeders in selection of crossing parents based on their genetic distance.

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