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ORIGINAL PAPER

# SNP-based genetic diversity assessment among hungarian bread wheat (*Triticum aestivum* L.) genotypes

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**Abstract**
World's food supply will be a great challenge due to the rapid growth of human population. Therefore, wheat breeders are facing a great task to improve further the crop quality and quantity. Genetic improvement could be the key in this progression, which is based on the exploitation of genetic resources. So it is important to hold exact information about the wheat population structure, especially the genetic diversity of elite crop germplasm. Here, we provide the characterisation of 85 Hungarian wheat varieties from 3 different Hungarian breeding programmes (Szeged, Martonvásár and Karcag) trough Competitive Allele-Specific PCR (KASP) technique. Population structure of these accessions were examined by STRUCTURE software and the ΔK values were calculating by the STRUCTURE HARVESTER. These investigations revealed 3 subgroups in our wheat population with 21, 19 and 45 genotypes in GrI, GrII and GrIII, respectively. We could conclude that the GrI and GrIII subgroups contained genotypes from all three origins while GrII contained genotypes only from Szeged. We also examined the allele distribution of the *Xgwm261* locus since the 192 bp allele is reported to be linked to semi-dwarfing gene *Rht8*, which has important role in the breeding process. Altogether we could observe 5 size variant products, but the 174 bp (22.35%), 192 bp (55.29%), and 198 bp (12.94%) long fragments could be found the most frequently. The present study confirms that population structure and genotype relatedness, based on molecular data, are consistent with the geography origin and available pedigree data. Moreover, this study could be the starting point of a following association mapping work.

**Keywords** Wheat · Population structure · KASP · *Xgwm261*

## Introduction

Some estimates suggest that the World population will be reach the ten billion people to 2050. This is a great challenge for the future to solve the problem of food supply. Wheat (*Triticum aestivum* L.) is one of the most important crop plant and staple food source, so breeders are also facing a great task to improve the crop quality and quantity. According to the 2017′s data of the Food and Agricultural Organization of the United Nation, wheat is grown on about 218 million hectares. In Hungary this area covers one million hectares with a total grain production of five million tons (http://www.fao.org/faostat/en/#data/QC). However, further increase in yields is essential and the genetic improvement which is based on the exploitation of genetic resources could be the key for this [19].

In the recent decades, due to the strong efforts of the breeders, grain yield, quality, agronomic traits and disease resistance has improved successfully. The appropriate usage of elite germplasm as parental stocks also contributed this [10]. But the continuous application of this elite germplasm lines has led to the decrease of genetic diversity and narrowed the genetic background of wheat improvement [8, 9, 15, 23]. Roussel et al. [23] proved an increase in the genetic similarity of European varieties. They also demonstrated that the qualitative variation of allelic composition increased as well. These changes could be related with the different geographic regions and the different practices that were used by the breeding programmes. Intensive selection pressure is

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appeared earlier in Northern and Western Europe and it has also an effect of winter wheat breeding [23].

In earlier genetic investigations the locus of *Xgwm261* had special respect because of the 192 bp allele [1]. This allele is reported to be linked to semi-dwarfing gene *Rht8*, which was used in Southern European cultivars in order to reduce the plant height without any adverse effect on yield [30–32]. It has other two alleles (174 bp and 165 bp) which are also reported to be frequent but did not bring about height reduction as much as the 192 bp allele [28, 29]. As a marker of *Rht8* gene the 192 bp allele has been widely used [18, 35]. The investigation of *Rht8* gene could provide information about genetic structure of wheat population because it appears that *Rht8* contributed to subgroup separation [19].

Assessing the relationships between genotypes is an important prerequisite for the identification of core population, which are suitable for the optimization of association research [13]. Several studies investigating the population structure, especially that focused the genetic diversity of elite crop germplasm [2, 14]. For the exact interpretation of the association between functional and molecular diversity, it is important to hold sufficient information about the population structure within the germplasm collections [6, 20].

Because of its clear importance, there are a lot of different molecular techniques available to examine genetic diversity. In the recent past, the examination of the morphological and physiological characters formed to the basis of diversity studies. But these qualities were suitable for only approximate estimation, because they forced by the environment [10]. Nowadays the usage of DNA-based molecular markers has become popular, because they could eliminate the environmental impact.

By the explosive development of DNA-based molecular techniques, we have the opportunity to use cost-effective and high-throughput systems. Single nucleotide polymorphisms (SNP) could be an example as a molecular marker for these assays [26, 27]. In their advantages are included the high abundance in the genome, the low cost/sample ratio, the locus specificity and the co-dominant inheritance. In parallel with these properties it has the potential for high-throughput analysis with relatively low error rates [22, 24]. Because of these advantages, the SNPs emerged as powerful tools for numerous genetic application. Many companies offer SNPs genotyping platforms in their portfolio. KBioscience or LGC Genomics (http://www.lgcgenomics.com) is one of these companies. In this case, the basis of the genotyping is the competitive allele-specific PCR (KASP™). KASP is a fluorescent-based technology, where the signal detection based on the allele-specific oligo extension and the following fluorescence resonance energy transfer (FRET) [17, 26].

In this study we compiled Hungarian bread wheat and durum wheat accessions in collaboration with wheat breeders of Cereal Research Non-Profit Ltd. 85 accessions were genotyped with 1920 KASP markers in order to obtain adequate information about the population structure. Our goal was to provide a molecular based information for the breeders which will help them to find the appropriate crossing combinations. Moreover, this study could be the starting point of a following association mapping work.

## Materials and methods

The wheat collections were compiled with the breeders of Cereal Research Non-Profit Ltd. Company. It numbered one durum wheat and 84 bread wheat genotypes. All of these cultivars deriving from Hungary, 55 originated from Szeged, 27 from Martonvásár and 3 from Karcag breeding programmes. All these genotypes were obtained from the Small Grain Cereal Genebank, Szeged, Hungary. Table 1 shows the list of the examined genotypes.

In order to analyse SNPs in the experimental collection, firstly we planted the wheat seeds in the greenhouse and waited for tiller formation. After that, we cut appropriate number of leaf discs which have been sent to the LGC genomics company. They performed the DNA extraction from the leaf discs and then they completed the genotyping of the samples. They used altogether 1920 kinds of KASP marker to genotype the wheats which are evenly distributed on the A, B and D genome.

The resulted raw data were edited by Microsoft Excel 2017 software to be suitable for structure analysis. For the estimation of the number of hypothetical subpopulations (K) in our collections STRUCTURE (v2.3.4) software was used. Via the usage of this program we could estimate the membership probability of each genotypes to the subpopulation as well [21]. We performed a model-based (Bayesian) clustering approach, where the number of the hypothetical subpopulation was set to 1 to 10. The Markov chain Monte Carlo (MCMC) of 10.000 burn-in phases followed by 10.000 iterations were run independently 10 times using an admixture model. For the detection of the most probable value of K which is ΔK we used the STRUCTURE HARVESTER website (http://taylor0.biology.ucla.edu/structureHarvester/) [12].

We examined the allele variation of *Xgwm261* locus in our wheat collection with PCR reaction as well. For this analysis we made DNA extractions from wheat seedlings with the aid of Wizard® Genomic DNA Purification Kit (Promega) according to manufacturer instruction. The PCR reaction volumes were 20 µl and each reaction contained 50 ng template DNA, 10 µl DreamTaq™ PCR Master Mix (2x) and 1-1 µl from the 10 µM primers stocks of the *Xgwm*-F (5′-GGTCACTGGTGGTCTGTG) and *Xgwm*-R (5′-CTC GCGCTACTAGCCATT). The PCR program started with 3 min denaturation on 94 °C and consisted of 45 cycles of

**Table 1** List of the wheat genotypes

| ID | Genotype | Origine | Year | Xgwm 261 alleles | POP | ID | Genotype | Origine | Year | Xgwm 261 alleles | POP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | GK Julidur | Szeged | 2012 | 165 | 1 | 44 | GK Tisza | Szeged | 2003 | 192 | 3 |
| 2 | Ati/Capo | Szeged | – | 198 | 3 | 45 | GK Verecke | Szeged | 1999 | 174 | 3 |
| 3 | GK 05.12 | Szeged | – | 174 | 1 | 46 | GK Vitorlás | Szeged | 2010 | 198 | 3 |
| 4 | GK 06.14 | Szeged | – | 192 | 1 | 47 | GK Zombor | Szeged | 1985 | 192 | 3 |
| 5 | GK 09.15 | Szeged | – | 192 | 3 | 48 | GK Zugoly | Szeged | 1993 | 192 | 2 |
| 6 | GK 16.15 | Szeged | – | 174 | 1 | 49 | PR E | Szeged | – | 192 | 2 |
| 7 | GK 18.14 | Szeged | – | 192 | 3 | 50 | PR163 | Szeged | – | 198 | 3 |
| 8 | GK 20.15 | Szeged | – | 174 | 3 | 51 | PR17 | Szeged | – | 198 | 3 |
| 9 | GK 22.14 | Szeged | – | 174 | 1 | 52 | PR19 | Szeged | – | 198 | 3 |
| 10 | GK 27.15 | Szeged | – | 192 | 3 | 53 | PR223 | Szeged | – | 198 | 3 |
| 11 | GK 28.15 | Szeged | – | 192 | 1 | 54 | Szín2 | Szeged | – | 200 | 3 |
| 12 | GK 35.14 | Szeged | – | 174 | 1 | 55 | Szín6 | Szeged | – | 198 | 3 |
| 13 | GK 41.15 | Szeged | – | 200 | 3 | 56 | Bánkúti 1201 | Martonvásár | 1931 | 192 | 3 |
| 14 | GK 51.15 | Szeged | – | 174 | 2 | 57 | Mv 07.13 | Martonvásár | – | 192 | 3 |
| 15 | GK Arató | Szeged | 2016 | 165 | 1 | 58 | Mv 08.13 | Martonvásár | – | 174 | 3 |
| 16 | GK Ati | Szeged | 2001 | 192 | 2 | 59 | Mv 14.13 | Martonvásár | – | 174 | 3 |
| 17 | GK Bagó | Szeged | 2016 | 200 | 2 | 60 | Mv Béres | Martonvásár | 2003 | 192 | 3 |
| 18 | GK Bakony | Szeged | 2015 | 192 | 2 | 61 | Mv Bodri | Martonvásár | 2008 | 192 | 3 |
| 19 | GK Békés | Szeged | 2005 | 192 | 2 | 62 | Mv Csárdás | Martonvásár | 1999 | 192 | 3 |
| 20 | GK Berény | Szeged | 2010 | 174 | 2 | 63 | Mv Emese | Martonvásár | 2000 | 192 | 3 |
| 21 | GK Csillag | Szeged | 2005 | 192 | 2 | 64 | Mv Ikva | Martonvásár | 2015 | 174 | 3 |
| 22 | GK Élet | Szeged | 1996 | 192 | 1 | 65 | MV Ispán | Martonvásár | 2015 | 174 | 1 |
| 23 | GK Fény | Szeged | 2006 | 192 | 2 | 66 | MV Karéj | Martonvásár | 2011 | 192 | 3 |
| 24 | GK Futár | Szeged | 2011 | 192 | 2 | 67 | Mv Karizma | Martonvásár | 2009 | 192 | 3 |
| 25 | GK Garaboly | Szeged | 1998 | 192 | 2 | 68 | Mv Kikelet | Martonvásár | 2010 | 192 | 3 |
| 26 | GK Göncöl | Szeged | 2009 | 192 | 2 | 69 | MV Kokárda | Martonvásár | 2012 | 192 | 3 |
| 27 | GK Hajnal | Szeged | 2010 | 192 | 2 | 70 | Mv Kolo | Martonvásár | 2006 | 174 | 3 |
| 28 | GK Hattyú | Szeged | 2002 | 174 | 1 | 71 | MV Kolompos | Martonvásár | 2009 | 174 | 1 |
| 29 | GK Holló | Szeged | 2001 | 192 | 2 | 72 | MV Krajcár | Martonvásár | 2013 | 174 | 1 |
| 30 | GK Hunyad | Szeged | 2005 | 174 | 1 | 73 | MV Lepény | Martonvásár | 2011 | 200 | 1 |
| 31 | GK Kalász | Szeged | 1996 | 192 | 2 | 74 | MV Lucilla | Martonvásár | 2007 | 198 | 3 |
| 32 | GK Kapos | Szeged | 2003 | 174 | 3 | 75 | Mv Magdaléna | Martonvásár | 1996 | 198 | 3 |
| 33 | GK Kincső | Szeged | 1984 | 192 | 1 | 76 | Mv Mente | Martonvásár | 2015 | 165 | 1 |
| 34 | GK Körös | Szeged | 2010 | 192 | 3 | 77 | Mv Nádor | Martonvásár | 2012 | 192 | 3 |
| 35 | GK Március | Szeged | 2008 | 198 | 3 | 78 | Mv Pántlika | Martonvásár | 2012 | 192 | 3 |
| 36 | GK Mini Manó | Szeged | – | 192 | 2 | 79 | Mv Pengő | Martonvásár | 2012 | 192 | 3 |
| 37 | GK Petur | Szeged | 1999 | 192 | 1 | 80 | MV Suba | Martonvásár | 2002 | 198 | 3 |
| 38 | GK Pilis | Szeged | 2013 | 192 | 2 | 81 | Mv Tallér | Martonvásár | 2010 | 200 | 3 |
| 39 | GK Rába | Szeged | 2000 | 192 | 1 | 82 | Mv Toldi | Martonvásár | 2008 | 192 | 3 |
| 40 | GK Rozi | Szeged | 2010 | 192 | 2 | 83 | KG Kunglória | Karcag | 2005 | 174 | 1 |
| 41 | GK Ságvári | Szeged | 1982 | 192 | 3 | 84 | KG Kunhalom | Karcag | 2002 | 192 | 3 |
| 42 | GK Szilárd | Szeged | 2013 | 192 | 1 | 85 | KG Széphalom | Karcag | 2004 | 192 | 3 |
| 43 | GK Tavasz | Szeged | 1996 | 192 | 3 | | | | | 192 | 3 |

ID refers to ID in PCA-plot (Figs. 1, 2). Year refers to the year of registration. POP refers to the subgroups according to Structure results

94 °C for 1 min, 55 °C 1 min and 72 °C for 1 min and a final extension step of 72 °C for 10 min. The PCR products were separated by QIAxcel Advanced capillary electrophoresis system with the QIAxcel DNA High Resolution Cartridge (Qiagen).

Furthermore, we conducted a Principal Component Analysis (PCA) with the aid of TASSEL (v5.2.51) software in

order to compare the population structure outcome, with the one, which resulted by STRUCTURE analyses [4]. Comparison on the basis of *Xgwm261* locus was also performed. For the visualization of these results we used the GraphPad Prism (v8.1.2) software.

## Results

Population structure of the 85 genotypes was examined by STRUCTURE (v2.3.4) software, utilizing data deriving from LGC Genomics, using 1920 KASP markers. The number of subpopulations (K) were determined by calculating the ΔK values by STRUCTURE HARVESTER. These analyses showed 3 subgroups in our wheat population so these 3 subgroups were further analysed by Bayesian clustering, calculating the Q matrix in Structure v2.3.4. These Q-values showed the level of relatedness in case of these genotypes to the 3 defined subgroups. Most of the genotypes (45) belonged to the subgroup 3 (GrIII) and the other 2 subgroups were almost equal in number, having 21 and 19 genotypes, named subgroup 1 (GrI) and 2 (GrII) respectively (Table 1.). Considering the composition of the 3 subgroups we could conclude that the GrI and GrIII subgroups contained genotypes from all three origins. Genotypes, originated from Martonvásár and Karcag, mostly belonged to the GrIII, and genotypes from Szeged could be found also, in equal number to samples from Martonvásár. In the GrII only genotypes from Szeged were presented. After assigning the origins and the resulted subgroups to the results of the PCA analysis, we could see the correlation (Fig. 1).

On the basis of the examination the *Xgwm261* locus, we could observe 5 size variant products. Among these 5 alleles, the 174 bp (22.35%), 192 bp (55.29%), and 198 bp (12.94%) long ones were found to be the most frequently presented in the investigated population. The 165 bp and 200 bp alleles were less numerous, with 3.52% and 5.88% occurrence respectively (Table 1.). We examined the distribution of these alleles within the 3 subgroups resulted by STRUCTURE analysis, by assigning the allele-types to subgroup on the PCA output (Fig. 2). These results showed that 16 of the 19 genotypes displaying the 192 bp band belonged to GrII. The most abundant allele was the 174 bp in the case of GrI while in the GrIII it was the 192 bp similar to GrII.

## Discussion

In the recent years several studies investigated the genetic diversity and the population structure of wheat [3, 5, 7, 10, 16, 19, 34]. Despite these investigations we still have poor knowledge about this field [5]. These investigations focused on bread wheat originated from the United States, Europe, India, Turkey and China, but the Hungarian genotypes have very low significance in these approaches. The growing number of germplasm exchange between breeding centres could affect the historical structure of genetic diversity. Development of SNP arrays was the major breakthrough in wheat genotyping, which were aid to modern genomics approaches that has the ability towards the high-throughput and high-density genotyping platforms [33]. LGC Genomics offer one of these SNP array genotyping platforms, which

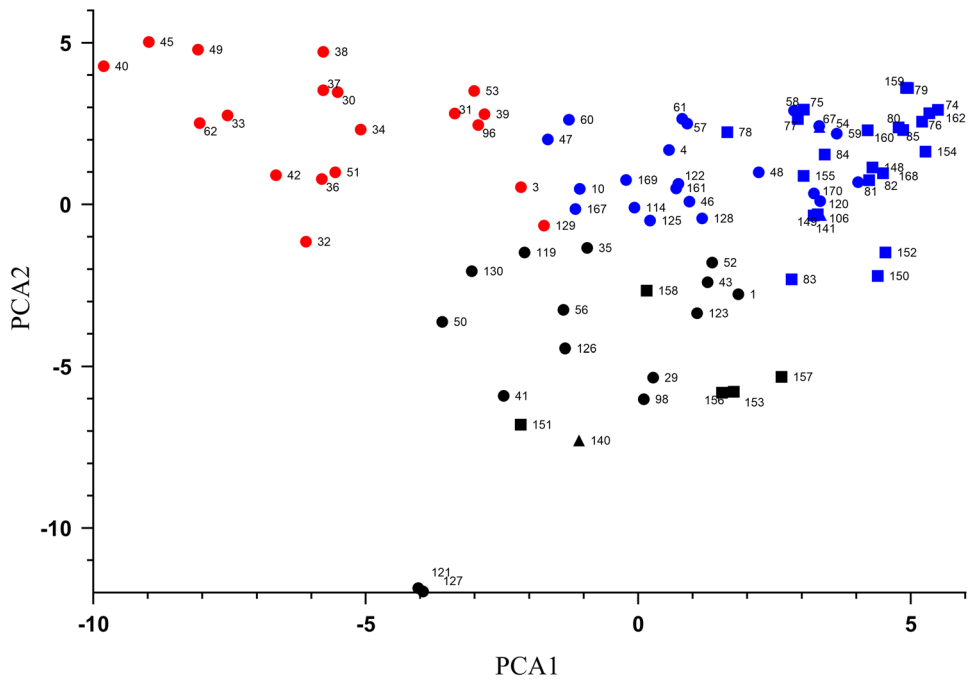


**Fig. 1** Structure analysis of the examined genotypes based on SNP data, visualised by PCA. Numbers refer to the genotypes described in Table 1. Coloured according to geographical origin

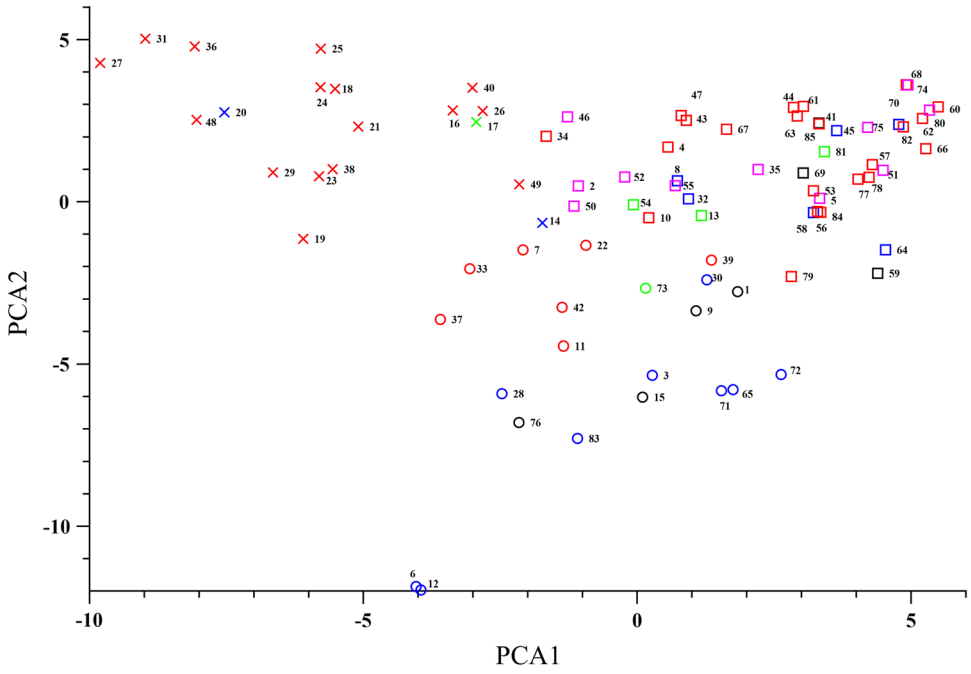


**Fig. 2** Allele distribution of the Xgwm261 locus, based on Principal Component Analyses. Numbers refer to the genotypes described in Table 1. Coloured by the band size of the Rht8 marker Xgwm261

was used for our studies in order to determine the population structure of 85 Hungarian wheat varieties from 3 different Hungarian breeding programmes (Szeged, Martonvásár and Karcag).

Data analysis, which were conducted with the STRU CTURE, STRUCTURE HARVESTER and the TASSEL software's we could conclude that the GrI and GrII contained mostly genotypes that has been breed by the breeders of Cereal Research Non-Profit Ltd. company. Almost all of the genotypes, that originated from Martonvásár belonged to the GrIII subgroup. As we also saw this phenomenon in case of the Karcag breeding programme. Based on the available pedigree data we could conclude that the GrI subgroup contained genotypes from West-European progenitors. These genotypes for instance GK Arató, GK Petur or GK Kincső. It is interesting, that in spite of GK Kincső belonged to the GrI, the descendants consist the main part of GrII (Fig. 3.). The most dominant genotypes of this subgroup were GK Kalász, GK Mini Manó and GK Garaboly.

GrIII subgroup consisted different origin genotypes from the Szeged breeding programme. GK Március, GK Tavasz, GK Körös, Szín2, Szín6 and all the genotypes from the prebreeding programme (PR) mainly originated from the western hemisphere (Brazil, Mexico, US, Chile). Old varieties, like GK Verecke, GK Zombor, GK Tisza and GK Ságvári have East European (Soviet Union) progenitors.

We also performed the detection of dwarfing gene *Rht8* with the microsatellite marker *Xgwm261* which has been widely used for this purpose [1, 11, 25, 28, 36]. It has been described previously, that it has 3 major alleles that generates of 165, 174 or 192 bp products after PCR [25, 29]. These band sizes partially correspond with those in our study (Fig. 1.), except for the 165 bp band, which appearance is relatively low, only 3.52%. But in our case the 198 bp allele has higher abundance than the 165 bp allele. The *Rht8* associated 192 bp band is widespread among wheat varieties from South-East Europe. This is corresponding well with the results of Nielsen et al. [19] who also found the majority of this band size in case of Hungarian genotypes. Furthermore, their result showed that most of the varieties with 174 bp alleles originated from Western Europe and formed a separate subgroup [29]. In our case we found that the majority of 174 bp allele in GrI, which group contains certain genotypes with Western European progenitors according the pedigree data. All these results are supporting the idea, that breeding for specific *Rht8* alleles has contributed the genetic structure observed within our population [19].

The present study confirms that population structure and genotype relatedness with molecular markers are consistent with the geography origin and available pedigree data. All these results could help the work of wheat breeders and would also provide a stable base for a future implementation of association mapping work.

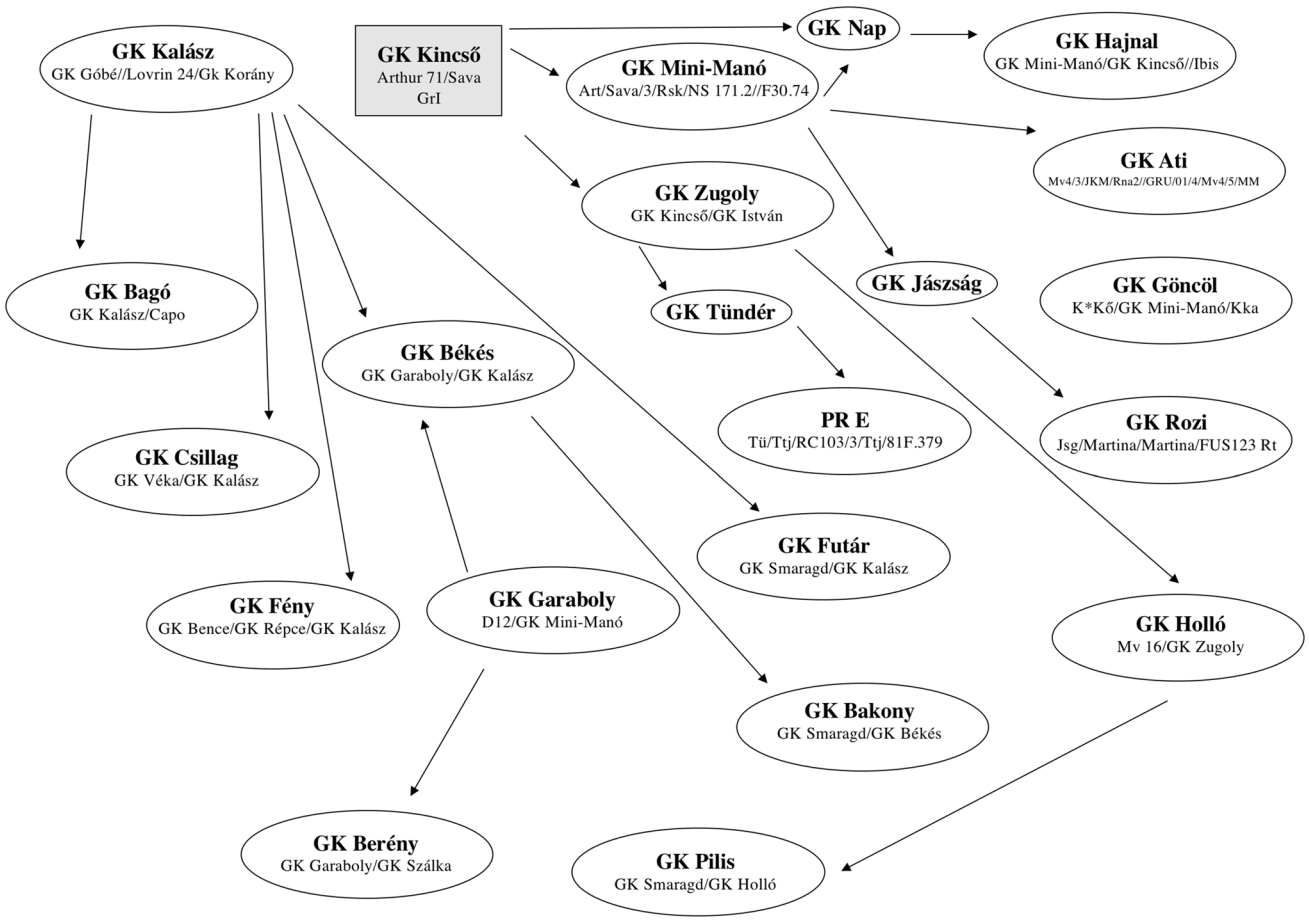


**Fig. 3** Relation between the Szeged-bred wheat cultivars and their connection to GK Kincső

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