Federal Research Center
N. I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR)

Effectivity of molecular markers in the identification of *SKr* gene – suppressor of crossability of bread wheat with rye

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SBB-2021
13th International young scientists school
Systems Biology and Bioinformatics
4-8 October 2021, Novosibirsk, Russia

PlantGen School 2021
Young scientists school
Plant genetics, genomics, bioinformatics and biotechnology
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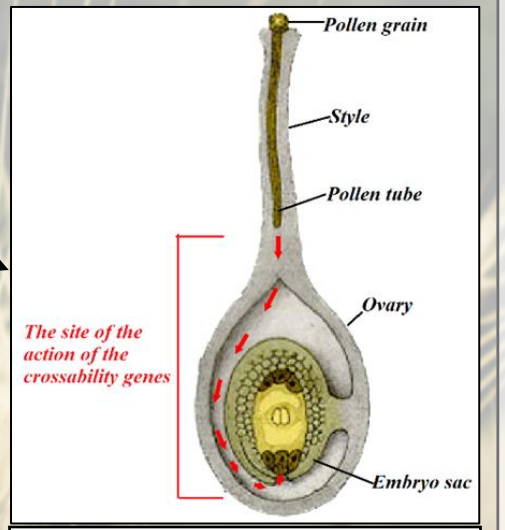
The dominant suppressor *SKr* (Suppressor of crossability), which was located on a distal region of 5BS, had the major effect on **inhibiting crossability (tube grow)** with rye



Effect of *SKr* gene on crossability
Alfares, 2009

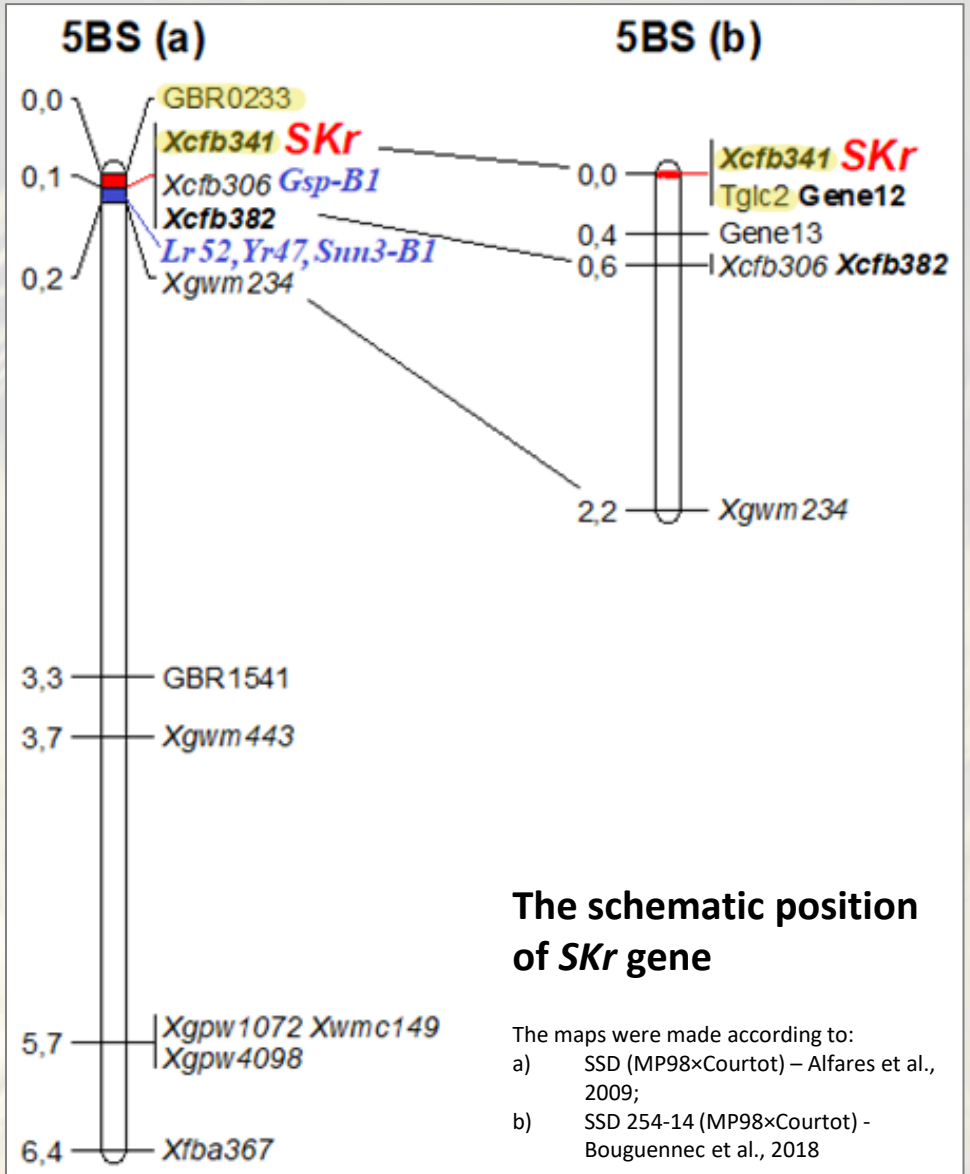
Purpose of the research:

To study the efficiency of molecular markers for the identification of bread wheat genotypes by alleles of the *SKr* gene

DNA-markers of *SKr* gene and their localization

Marker name	Type	Genetic distance to *SKr* (cM) on 5BS	Association of alleles with *SKr/skr*	Literature source	The results of localization of markers in genome assembly of Chinese Spring (CS) IWGSC RefSeq v2.1
gene12	genomic	0	391 bp- *skr*; 342 bp- *SKr*	Bouguennec et al., 2018	5BS: 1 mln bp to distal end of 5BS
gene13	genomic	0,4	419, 456 bp – skr; 419, 451 bp - SKr	Bouguennec et al., 2018	5BS: 6,4 mln bp to distal
TGlc2	ISBP	0,0	270, 291 bp - skr 270, 279 - SKr	Bouguennec et al., 2018	5DS: 4,1 mln bp to distal end of 5DS
Xcfb306	SSR	0,6 (proximal)	No data	Alfares et al, 2009	5BS: 6,4 mln bp to distal end
Xcfb341	*SSR*	0 (proximal)	176 bp – *skr* 163 bp – *SKr*;	Bouguennec et al., 2018	5DS: 4,1 mln bp to distal end
Xcfb382	*SSR*	0,6 (proximal)	196 bp – skr; 194 bp – SKr	Alfares, 2009	5BS: 4,7 mln bp to distal end
Xgwm234	*SSR*	2,2 (proximal)	No data	Alfares, 2009	5BS: 8,2 mln bp to distal end
Xgwm443	*SSR*	3,7 (proximal)	No data	Mishina et al., 2009	5BL: 440 mln bp to the distal end of 5B

- Markers which highlighted in yellow, have another location in the IWGSC RefSeq v2.1 assembly than from the literature cited
- Markers which highlighted in bold were used in this research



The schematic position of *SKr* gene

The maps were made according to:
a) SSD (MP98×Courtot) – Alfares et al., 2009;
b) SSD 254-14 (MP98×Courtot) - Bouguennec et al., 2018

- Markers which highlighted in yellow, have another location in the IWGSC RefSeq v2.1 assembly than from the literature cited
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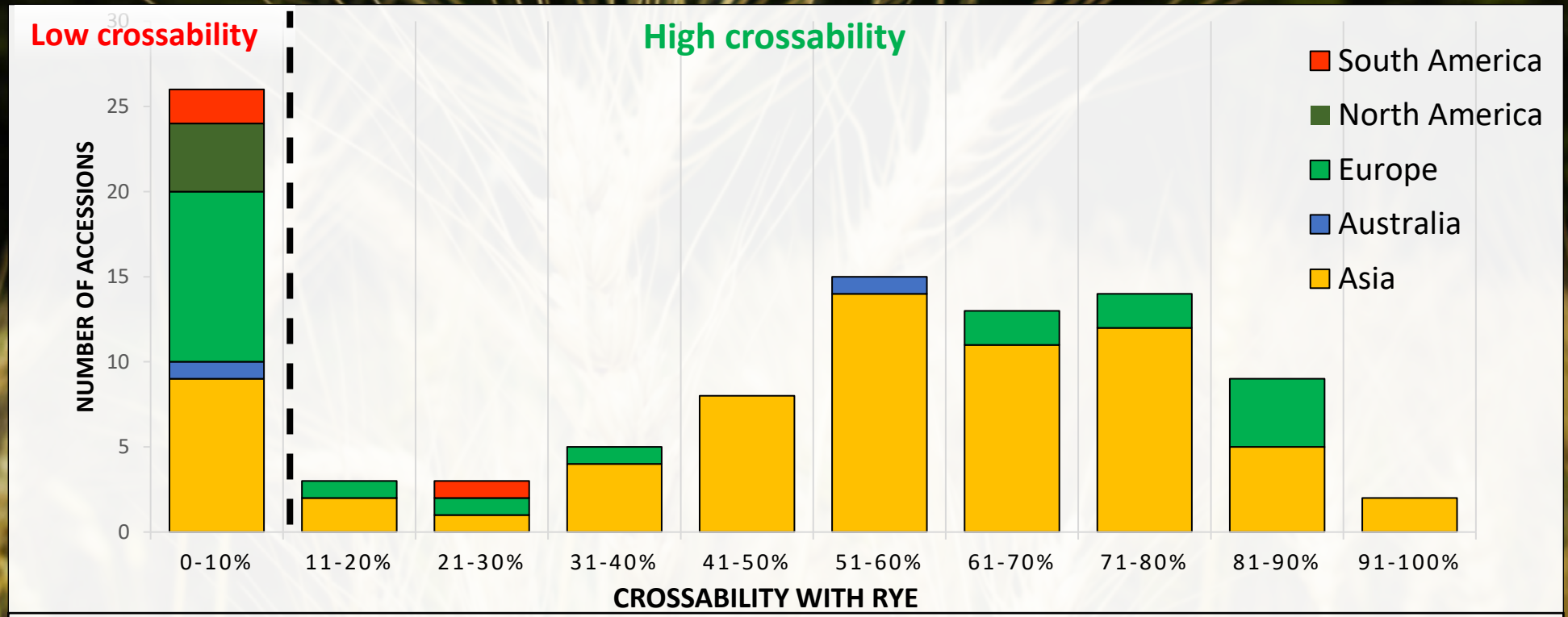
Plant material:

108 genotypes (98 accessions) of common wheat from 21 countries and with different habits were studied



Distribution 80 genotypes with high (14,5-93%) & 28 with low (0-11,3%) crossability by continents

Crossable forms were included:

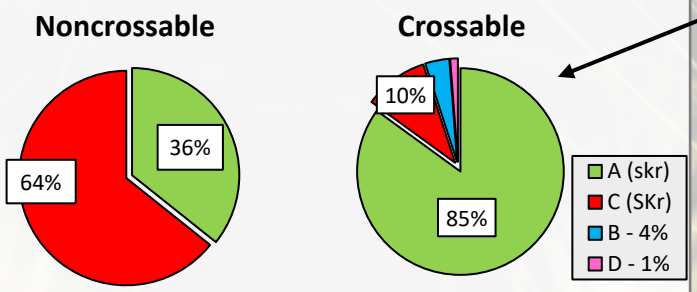
- 45 lines were created by VIR employees in different times;
- 26 accessions from VIR collection

Markers *Xcfb341* and gene12 showed high effectivity (81,0% и 82,3%) for identification of crossable with rye genotypes – 85% and 87,5% of them had associated fragment with this trait, respectively

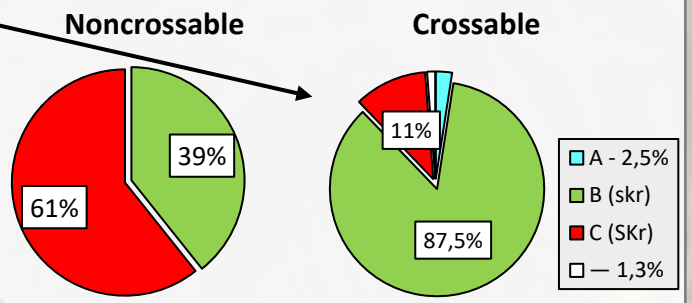
Markers *Xcfb341* and gene12 showed not good enough effectivity for identification noncrossable genotypes – 1/3 of them had the diagnostic fragment of high crossability

To detect possible differences between genotypes which had the same PCR fragments but with the different crossability - the restriction analysis for these fragments were performed (11 endonucleases in total)

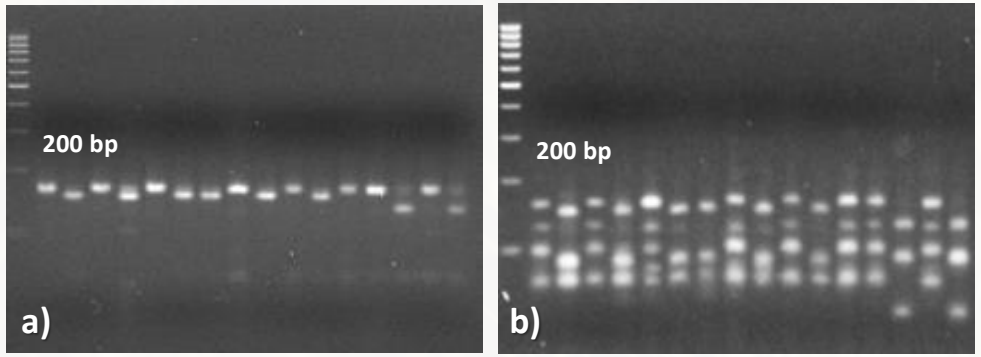
Frequency of diagnostic fragments of *Xcfb341* in crossable and noncrossable wheat genotypes



Frequency of diagnostic fragments of gene12 in crossable and noncrossable wheat genotypes

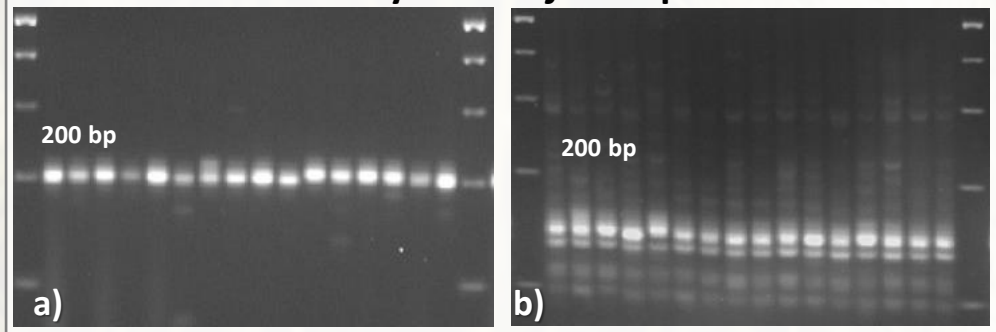


Restriction analysis of *Xcfb341* products



Amplification products of *Xcfb341* on agarose (3%) gel*:*
***a*) without restriction; b) after subjected to Sse9I**

Restriction analysis of *Xcfb382* products

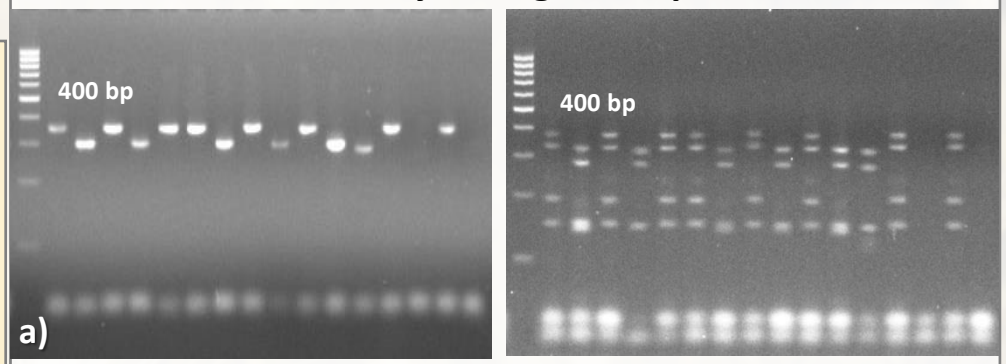


Amplification products of *Xcfb382* on agarose (3%) gel*:*
a) without restriction; b*)* after subjected to MnlI

Results:

- ✓ There were restriction sites in the amplification products for the following restrictions:
  Xcfb341 - AuI; BstMBI; Sse9I; HaeIII
  Gene12 - BstMBI; RsaI; Sse9I
  Xcfb382 - HaeIII, HinfI, MnlI
- ✓ There is no significant difference between genotypes after using these restrictions for *Xcfb341* and gene12

Restriction analysis of gene12 products



Amplification products of gene12 on agarose (3%) gel*:*
***a)* without restriction; b) after subjected to Sse9I**

- ✓ Restriction analysis of *Xcfb382* products, which couldn't detect differences between crossable and noncrossable forms for except of Sibirka Yarcevskaya. There is no significant difference between genotypes after using these restrictions for these markers
- ✓ Moreover there, are no significant differences between genotypes after using the restrictions enzymes for fragments of these markers

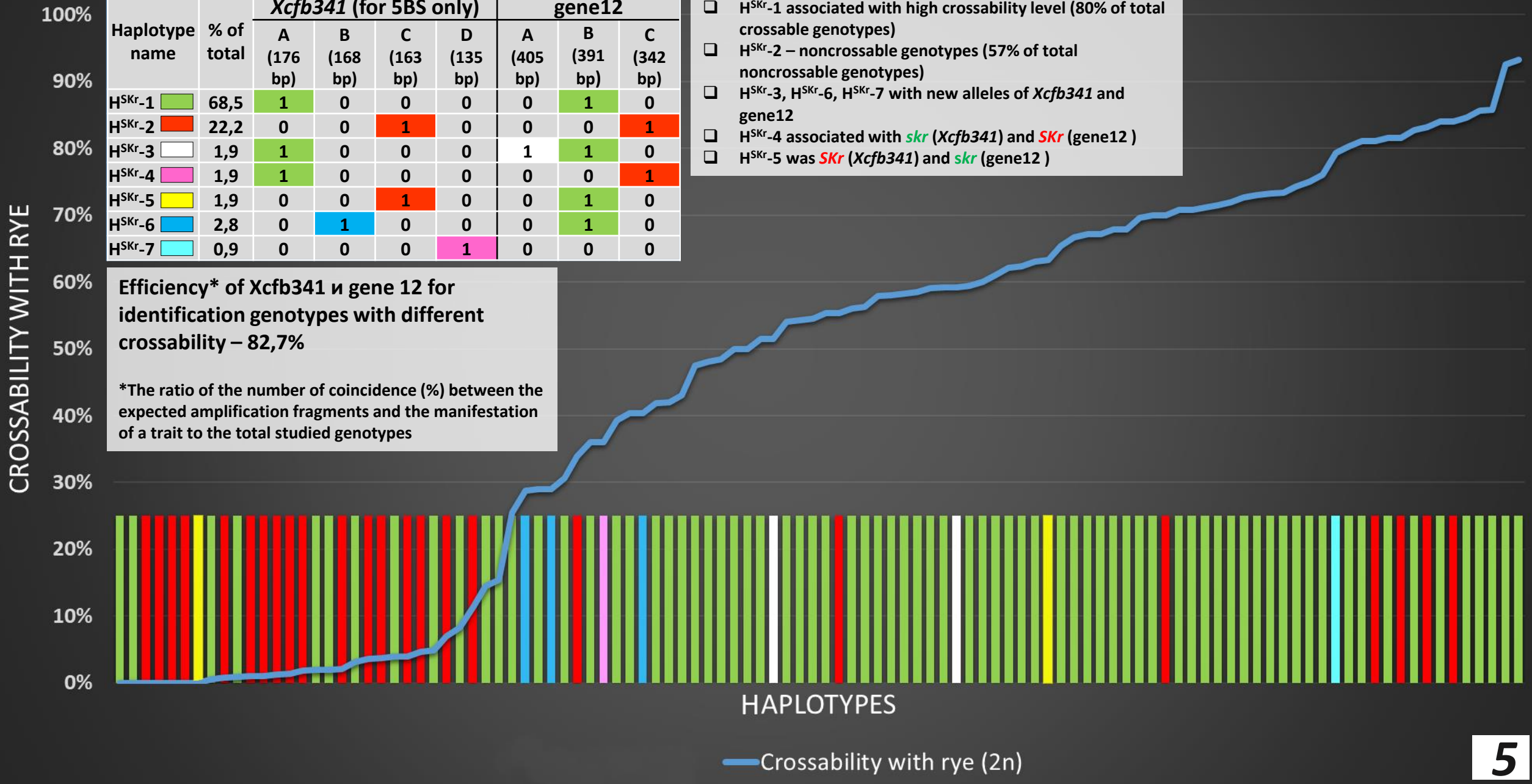
Haplotypes of *Xcfb341* & gene12

Haplotype name	% of total	*Xcfb341* (for 5BS only)				gene12		
		A (176 bp)	B (168 bp)	C (163 bp)	D (135 bp)	A (405 bp)	B (391 bp)	C (342 bp)
H^{SKr}-1	68,5	1	0	0	0	0	1	0
H^{SKr}-2	22,2	0	0	1	0	0	0	1
H^{SKr}-3	1,9	1	0	0	0	1	1	0
H^{SKr}-4	1,9	1	0	0	0	0	0	1
H^{SKr}-5	1,9	0	0	1	0	0	1	0
H^{SKr}-6	2,8	0	1	0	0	0	1	0
H^{SKr}-7	0,9	0	0	0	1	0	0	0

- H^{SKr}-1 associated with high crossability level (80% of total crossable genotypes)
- H^{SKr}-2 – noncrossable genotypes (57% of total noncrossable genotypes)
- H^{SKr}-3, H^{SKr}-6, H^{SKr}-7 with new alleles of *Xcfb341* and gene12
- H^{SKr}-4 associated with *skr* (*Xcfb341*) and *SKr* (gene12)
- H^{SKr}-5 was *SKr* (*Xcfb341*) and *skr* (gene12)

Efficiency* of Xcfb341 и gene 12 for identification genotypes with different crossability – 82,7%

*The ratio of the number of coincidence (%) between the expected amplification fragments and the manifestation of a trait to the total studied genotypes



Conclusions:

1. The statistically significant relationship (χ^2 =27,03, v=1, $p_{0,001}$=10,83) was shown between the presence of diagnostic (*SKr-skr*) fragments for *Xcfb341*, gene 12 and crossability phenotype of bread wheat with rye. The efficiency of these markers was high - 82,7%;

2. The approbation of marker *Xcfb382* didn't show differences between genotypes with different crossability with rye. Restriction analysis of amplification products didn't find differences in their nucleotide composition. The unique amplification fragment which had another molecular weight (bp) was detected for accession Sibirka Yartsevskaya (k-38587);

3. Restriction analysis for PCR fragments of markers *Xcfb341* and gene12 for genotypes with different crossability didn't find differences. Discrepancy between presence of diagnostic (*SKr-skr*) fragments and crossability phenotype may indicate to another factors, that can change this trait: weather conditions; allelic condition of another crossability genes (*Kr1-Kr4*);

4. The primers of closely linked (according to the literature cited) to *SKr* molecular markers were located in genome assembly of Chinese Spring (CS) IWGSC RefSeq v2.1. (see Table 1). The localization of markers gene12, gene13, *Xcfb306*, *Xcfb382 и Xgwm234* was confirmed on a distance from 1 mln bp to 8,2 mln bp from the distal end of 5BS chromosome. The markers TGlc2 and *Xcfb341* were located on 5DS, *Xgwm443* – 5BL;

5. It's expedient to continue search the new molecular markers for *SKr* or to develop original markers which are located in the 4,7 mln bp area between telomere of 5BS and marker *Xcfb382* which on the proximal side of *SKr* and closely linked with it (0,6 cM).

This work is fulfilled in the framework of Government Mission
№ 0481-2019-0002 & № 0662-2019-0006