The studying of hybrid line with spherical grains and reduced height obtained by crossing triticale and synthetic hexaploid wheat

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Plant material

The hybrid wheat line 1102 (Fig. 1) that is featured by spherical grain and short stem obtained from crossing of a wheat-rye amphiploid (triticale) BBAARR (2n = 6x = 42) and synthetic wheat (2n=6x=42, BBAADD) (the whole scheme of hybridization is showed below).
Bread wheat varieties Kinelskaya 40 and Lutescens 85.

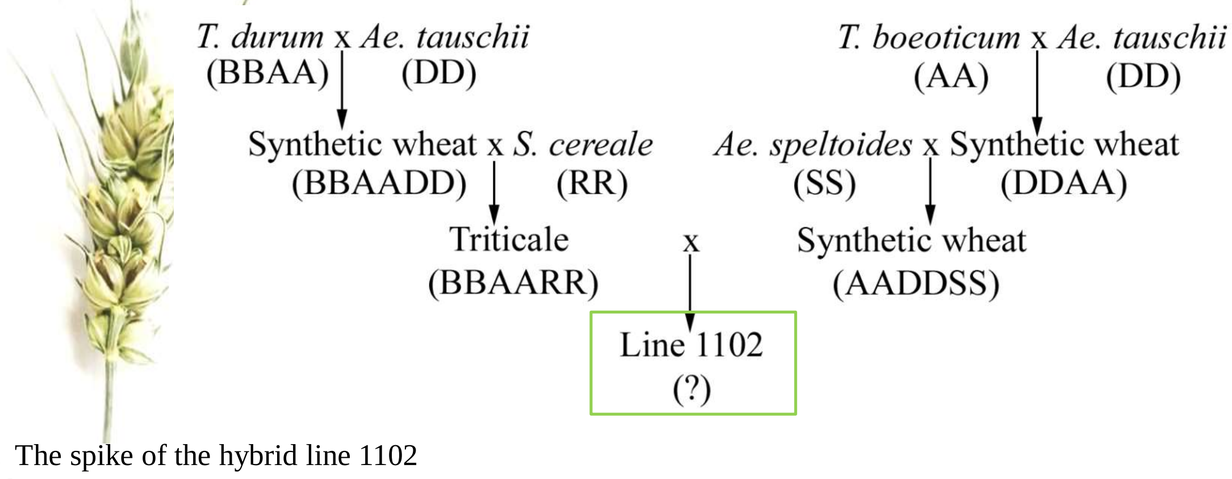


Fig. 1. The spike of the hybrid line 1102 with sphaerococcum grain.

Purpose

The aim of the research is identification of genes (loci) determining spherical grain and short stem in the studying hybrid line

Methods

Cytological analysis:

Genome *in situ* hybridization (GISH) with *S. cereale* genomic DNA;

Fluorescent *in situ* hybridization (FISH) with probes that allow the identification of wheat, rye and *Ae. speltoides* chromosomes;

-pAs1 (Rayburn and Gill, 1986b);
-pSc119.2 (Bedbrook et al., 1980);
-Spelt1 (Salina et al., 1997, 1998);
-Spelt52 (Salina et al., 2004).

Molecular-genetic analysis:

PCR with specific markers to *Rht-B1a* (Ellis et al., 2002), *Rht-B1b* (Ellis et al., 2002), *Rht-D1a* (Ellis et al., 2002), *Rht-D1b* (Ellis et al., 2002) and microsatellite markers to *Rht4* (Ellis et al., 2005), *Rht5* (Konzak et al., 1987; Ellis et al., 2005), *Rht8* (Korzun et al, 1998) and *Rht9* (Elis et al., 2005).

Phenotype analysis:

The program SeedCounter.2.3 was used to estimate grain form of the studied line and local bread wheat varieties.

Results and Discussion

The karyotype of the line 1102 was determined by FISH and GISH and don't differ from the karyotype of bread wheat as analysis with pSc119.2 and pAs1 showed (Fig. 2a). We supposed that the line could have translocations from rye or *Ae. speltoides* so we conducted hybridization with rye DNA and pSc119.2, Spelt1 and pSc119.2 (Fig. 2b) and Spelt52 and pSc119.2. Rye DNA and Spelt52 signals were not detected but we found Spelt1 signals on the long arm of the chromosome 3B (Fig. 2b). To know if Spelt1 signals are translocations from *Ae. speltoides* genome or it is inherited from *T. durum* we tested ancestral triticale with probes Spelt1 and pSc119.2 (Fig. 2c). The analysis showed that triticale also has Spelt1 signals on the long arm of the chromosome 3B that indicates that Spelt1 was inherited from *T.durum.*

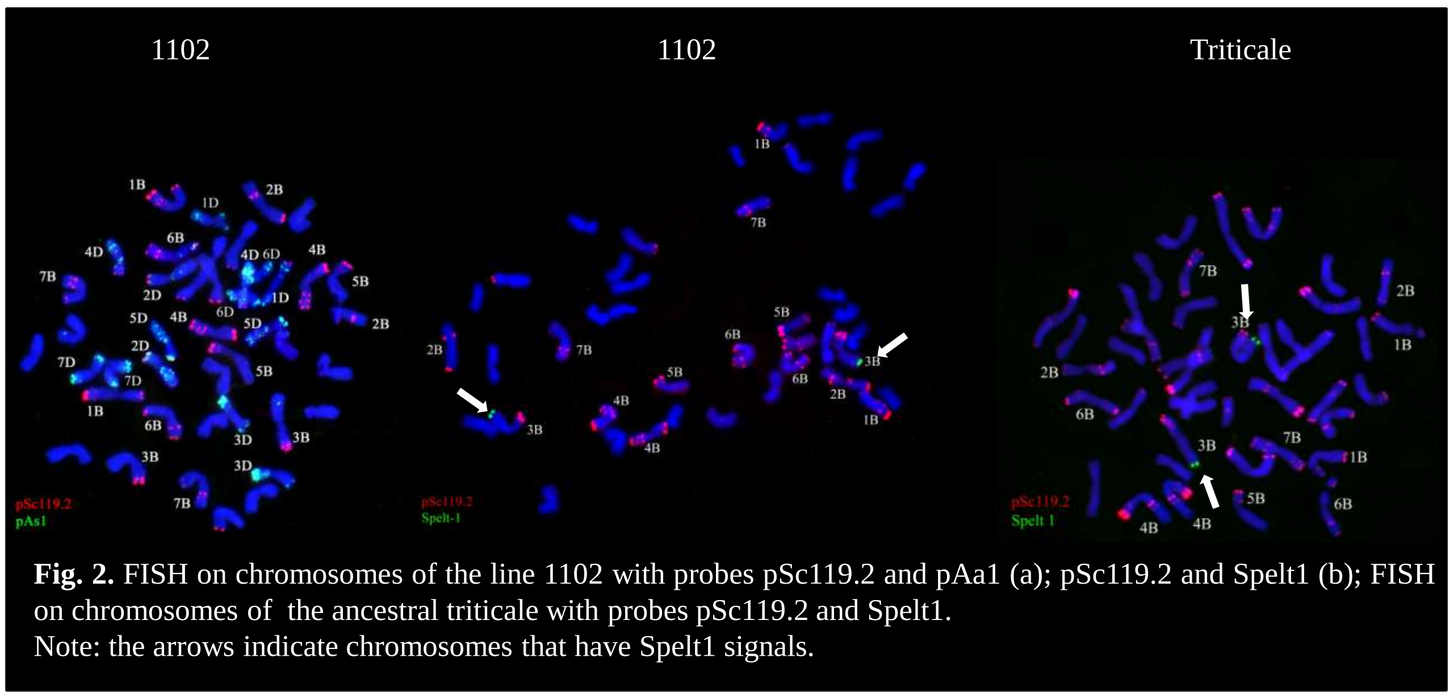


Fig. 2. FISH on chromosomes of the line 1102 with probes pSc119.2 and pAa1 (a); pSc119.2 and Spelt1 (b); FISH on chromosomes of the ancestral triticale with probes pSc119.2 and Spelt1.
Note: the arrows indicate chromosomes that have Spelt1 signals.

To establish which of already known reduced height genes are present in the 1102 line we isolated DNA from the line and conducted molecular-genetic analysis with specific markers to *Rht-B1a*, *Rht-B1b*, *Rht-D1a*, *Rht-D1b* and microsatellite markers to *Rht4*, *Rht5*, *Rht8 and Rht9*. Molecular-genetic analysis was made by polymerase chain reaction (PCR):

Marker	Gene/allele	1102	Kinelskaya 40	Lutescens 85	Chineese Spring
Specific marker	*Rht-B1a (wt)*	+	+	+	+
	Rht-B1b	-	-	-	-
	Rht-D1a (wt)	+	+	+	+
	Rht-D1b	-	-	-	-
Microsatellite marker	*Rht4*	~150 bp	~ 120 bp	~ 120 bp	~ 110 bp
	Rht5	~140 bp	~ 120 bp	~ 120 bp	~ 150 bp
	Rht8	-	-	-	-
	Rht9	~250 bp	~ 230 bp	~ 230 bp	~ 220 bp

We established that the studied line contains wild-type alleles of genes *Rht-B1* and *Rht-D1* that don't have decrease height of plant. Analysis of microsatellite locuses linked with *Rht4*, *Rht5*, *Rht8* and *Rht9* showed the difference in length between obtained and expected fragments. These difference will be considered in the future work.

To map genes / alleles and to find QTLs connected with dwarf phenotype 102 local varieties of bread wheat was analyzed (Komyshev et al. 2017; Fig.3) and we chose the most contrast cultivars (Kinelskaya 40 and Lutescens 85) to the line 1102. We bred chosen varieties with the studied line and for now we have seeds of F_2-population plants that will be phenotyped and genotyped in the future. The fig. 4 show plants and grain of the F_1-population compared with line 1102 and standard bread wheat. The hybrid plants have intermediate phenotype (the form of spike, the form of grain and the height)

Fig. 3 Length/Width (Y) vs Roundness (X) of bread wheat varieties

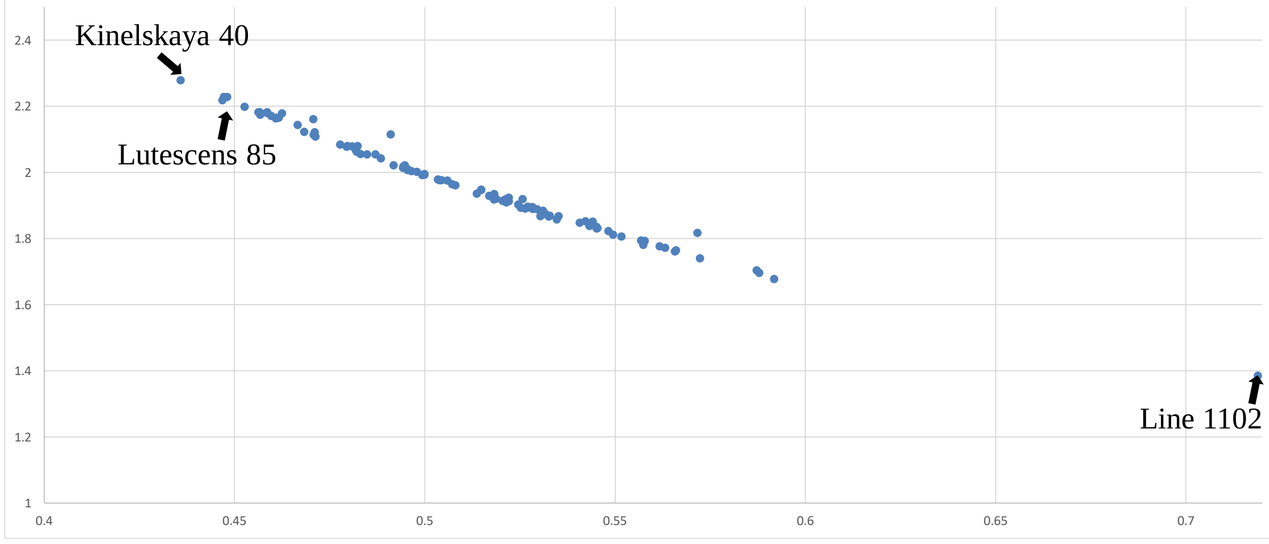
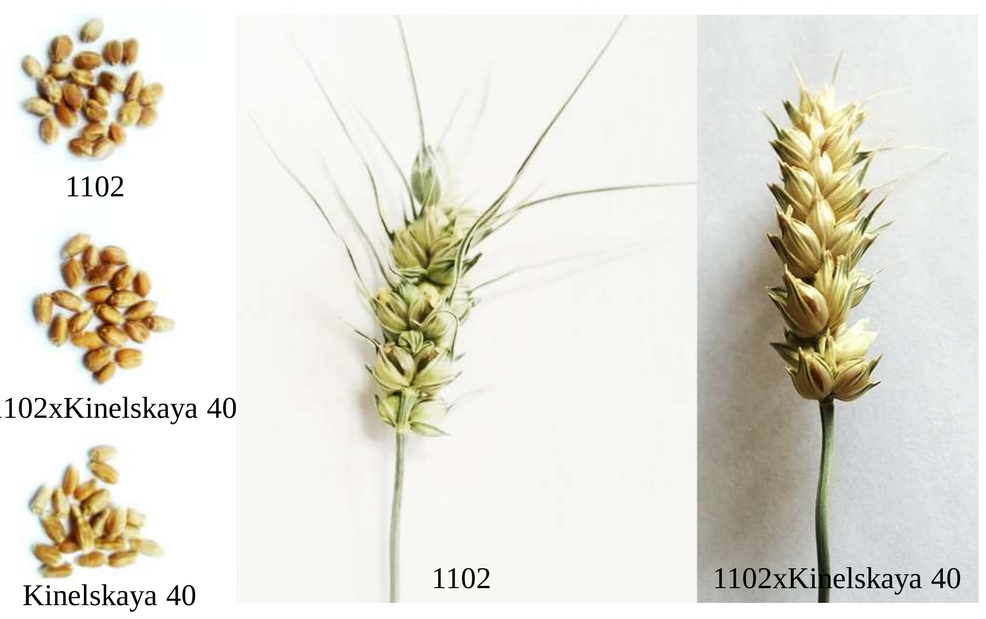


Fig. 4 . Spikes and grain of the line 1102, F_1-plant and Kynelskaya 40



Conclusions

The studying line 1102 has standard bread wheat karyotype according to hybridization with probes pSc119.2 and pAs1. Some translocations from rye or *Ae. speltoides* haven't been found despite complex origin of the line. It is also determined that the line 1102 has wild-type allele *Rht-B1a* and *Rht-D1a* with no contribution to reduction of the stem length.
In the future work we will conduct an additional molecular-genetic analysis of the line 1102, genotyping of F2-population and QTL-analysis.

Acknowledgments:

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