Localization of the genes for high gluten content in grain in chromosomes of the second homoeologous group of bread wheat

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<u>Background</u>: GLUTEN – is an elastic protein complex formed during mixing of flour

<u>Practical importance:</u>

High gluten content in grain and flour correlates with high bread-making quality (Fig.1). Gluten content strongly correlates with grain protein content (GPC) (Kozmina, 1969; Kulkarni et al. 1987; Li et al. 2009). GPC is a classifying trait in grain trading.

<u>Scientific importance:</u>

The gene *Gpc-B1*, responsible for protein content level in wheat grain was proved to be an important transcriptional factor (NAM-B1) governing physiological processes in plant.

Alien hybridization is used for extracting the useful genes from a wild gene pool.

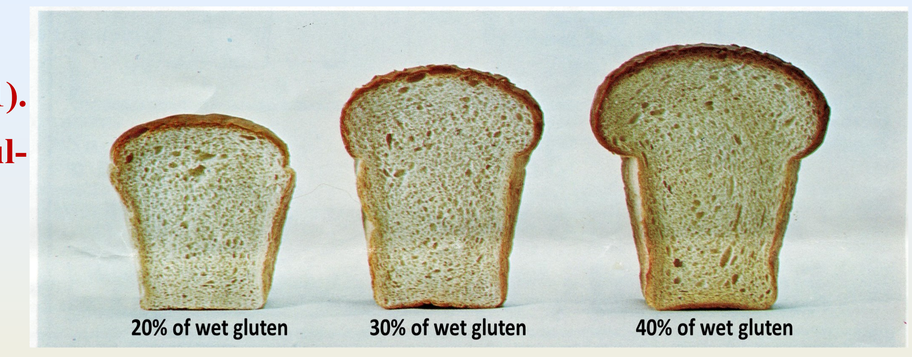
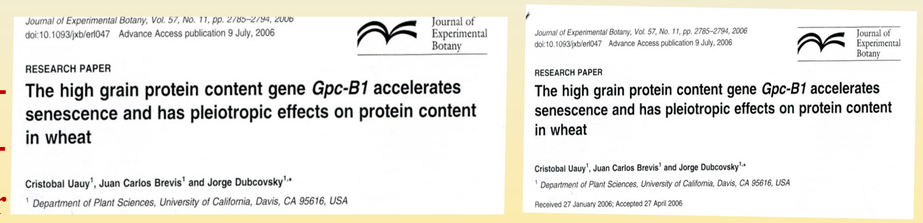


Fig.1. Dependence of bread wheat volume from gluten content (according the data of Sweden Grain Association, Svalef, Sweden (Falling number, now Perten Instruments, Sweden, 1984)

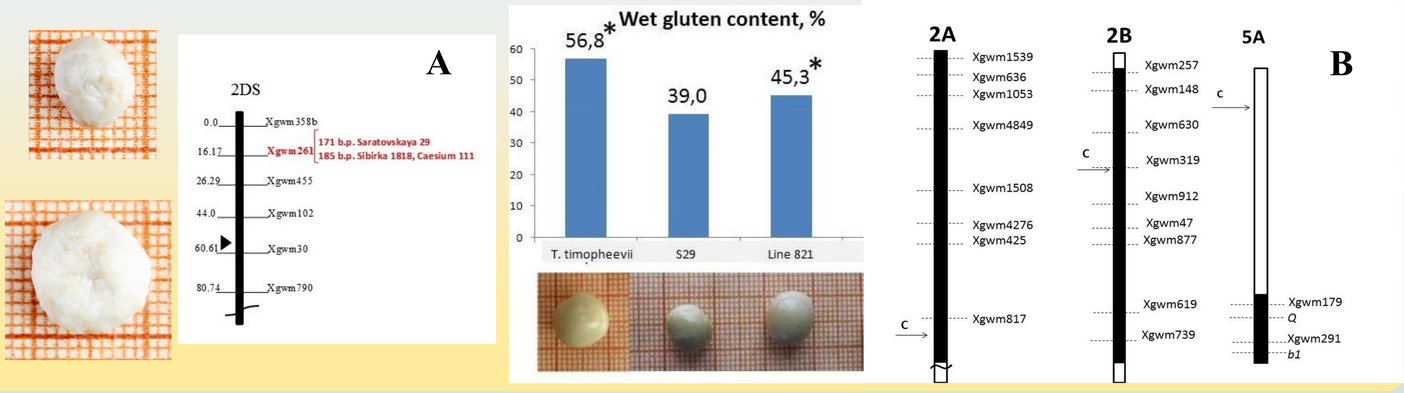


Earlier, we showed that tetraploid species *Triticum timopheevii* may be the donor of high gluten content in grain as well as the line 821 (L821) with introgressions in 2A, 2B and 5A chromosomes (Pshenichnikova et al. 2015). QTLs for grain protein content were detected by other authors in 2A and 2B chromosomes in bread wheat mapping populations (Groos et al. 2003; Li et al. 2009). Additionally, it was found that high gluten content in grain is associated with allelism of *Xgwm261* marker in 2D chromosome of old Russian cultivars Tsezium 111 and Sibirka 1818 (Morozova et al, 2016).

Genetic material

Figure. 2. Parental genotypes and their bread-making characteristics, washed gluten balls and gluten content in grain. Molecular maps of 2D chromosome (A) and molecular map of L821 (B) (partially from Leonova et al. 2001)

Genotypes	Quality traits	
	Bread making quality	Gluten content in grain
Recipient - S29	high	low
Donors: Line 821 with introgressions from *T. timopheevii*	high	high
Old cultivars Tsezium 111, Sibirka 1818	mediocre	high



Aim of the work:

<u>a) to introduce 2A and 2B chromosomes from L821 and 2D from old cultivars Tsezium 111 and Sibirka 1818 into cv. Saratovskya 29; b) to develop corresponding single chromosome substitution lines with subsequent analysis for gluten content in grain; c) to obtain two groups of recombinant lines contrasting for allelic composition of molecular markers and to fulfil a comparative analysis of gluten content.</u>

Obtaining single chromosome substitution lines

I. Transfer of chromosomes from the introgression lines and cultivars (donors) into cv. S29

F1 monosomic line for 2A, 2B or 2D chromosome of cv. S29 × donor genotype

↓

BC1F1 monosomic line for 2A, 2B or 2D chromosome of cv. S29 × F1

↓

BC2F1 monosomic line for 2A, 2B or 2D chromosome of cv. S29 × BC1F1

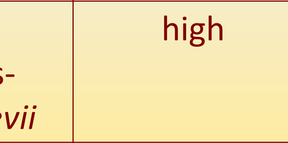
↓

Required number of backcrosses (from 3 to 8)

↓

Isolation of disomic progenitors of the lines – donors of genes for grain quality, Testing under green-house and field conditions

Only monosomics (20" + 1' in MI of meiosis) were selected as paternal component in crosses



Spikes of the parental cultivars

Obtaining recombinant substitution lines with molecular marked introgressions

II. Development of recombinant lines for 2A chromosome with introgressions from *T. timopheevii* accompanying by molecular and gluten content analyses

F1 single chromosome substitution line S29 (821 2A) × Recipient genotype (S29)

↓

F_2- F_3 Segregation for gluten content in grain among separate plants

↓

Selection two groups of plants contrasting for gluten content and allelic composition of microsatellite markers

↓

Additional cross to the recipient S29 and further selection of genotypes with high gluten content in grain, testing under green-house and field conditions

To reduce the size of the introgression in chromosome 2A, a crossing was performed between the substitution line and the recipient S29 (Fig.4). Among the hybrid plants F_3, variability for gluten content was observed. They also differed in the allelic composition of microsatellite markers. The lines with an introgression in the telomeric region of the short arm of chromosome 2A flanked with markers *Xgwm1539* and *Xgwm1508* had a high level. This region carries the gene for this trait. The two developed contrasting groups of lines consistently showed a low and high level of gluten in field and greenhouse conditions. Additional cross was made with the recipient to further reduce the introgression from *T. timopheevii*.

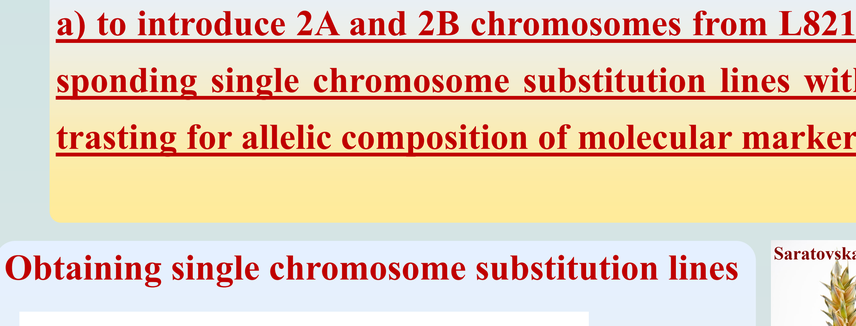


Fig. 3. Gluten content in grain of single chromosomes substitution lines for chromosomes of second homoeological group.

S29 (821 2A), BC2 F3, field; S29 (821 2B) , BC1 F2, green-house; S29 (Tsezium 111 2D) and S29 (Sibirka 18181 2D), BC4 F2, green-house

The development of single chromosome substitution lines for the chromosomes of the second homoeological group does not require a large number of backcrosses, since the line L821 was also obtained on the genetic background of S29. With the help of both microsatellite markers and cytological analysis of hybrids the plants - progenitors of single chromosome substitution lines were isolated. The lines showed a consistently high content of gluten in the grain compared to C29 (Fig. 3).

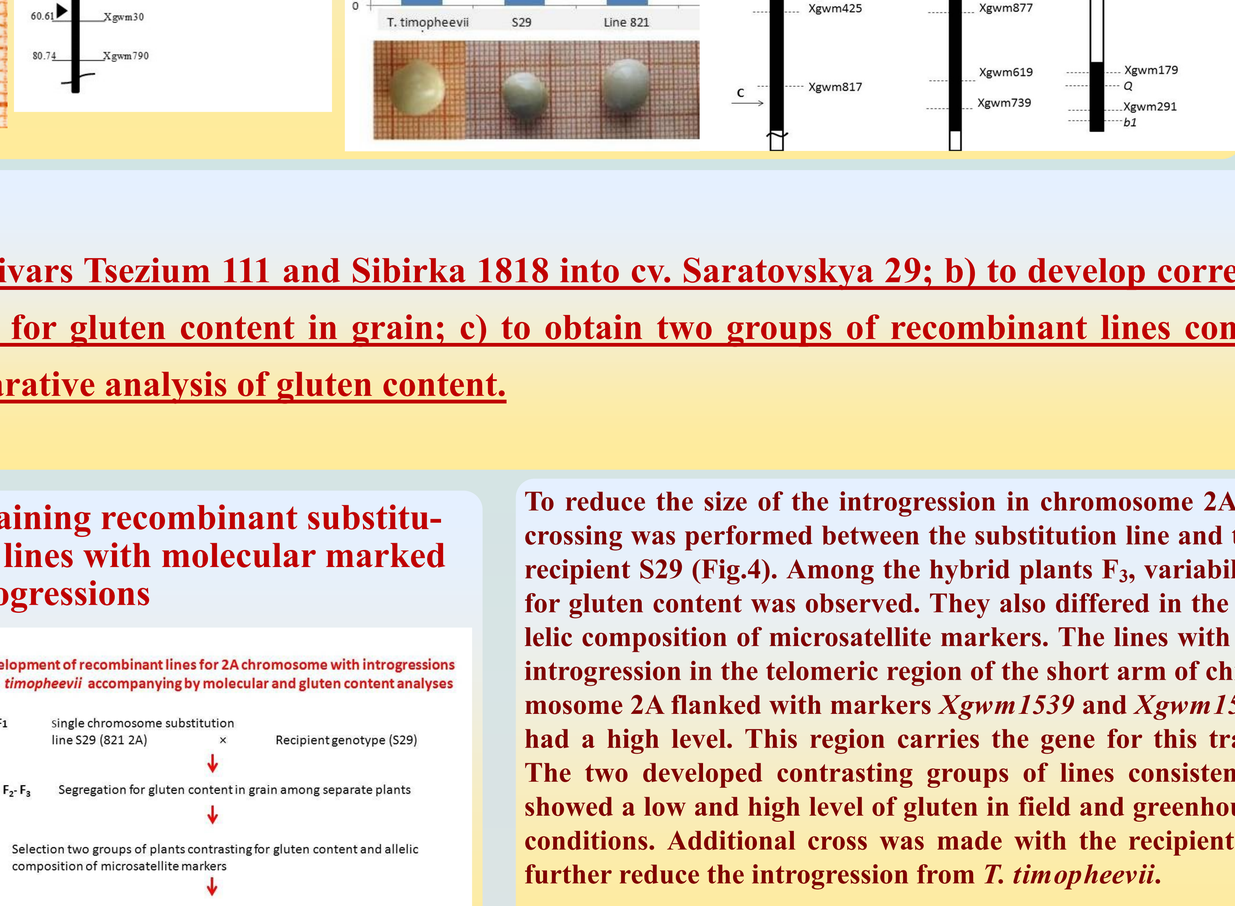


Fig. 3. Diagram of obtaining of the substitution recombinant lines for 2A chromosome. The results of analysis of gluten content in grain in lines (L) and their molecular markers composition are presented. *Xgwm*—microsatellite markers.

Conclusion: The data obtained indicate the possibility of the existence of a homoeoallelic series of genes in chromosomes of the second homoeological group of cereals responsible for gluten biosynthesis in wheat grain.

Pshenichnikova T.A., Simonov A.V., Shchukina L.V., Morozova E.V., Chistyakova A.K., Börner A. (2015) Enlargement of the genetic diversity for grain quality in bread wheat through alien introgression. Advances in Wheat Genetics: From Genome to Field. Eds.: Yasunari Ogihara, Shigeo Takumi, Hirokazu Handa, chapter 32, p.287-292.
Li Y., Song Y., Zhou R., Branlard G., Jia J. 2009. Detection of QTLs for bread-making quality in wheat using a recombinant inbred line population. Plant Breeding 128:235–243.
Groos C., Robert N., Bervas E., Charmet G. 2003. Genetic analysis of grain protein content, grain yield and thousand-kernel weight in bread wheat. Theor. Appl. Genet. 106:1032–1040.
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