

# From cytogenetic to molecular approach and backwards: investigations of grain quality in bread wheat

Ermakova MF<sup>1</sup>, Pshenichnikova TA<sup>1</sup>, Chistyakova AK<sup>1</sup>, Shchukina LV<sup>1</sup>, Osipova SV<sup>2</sup>, Röder M<sup>3</sup>, Börner A<sup>3</sup>  
<sup>1</sup>*Institute of Cytology and Genetics SB RAS, Novosibirsk, 630090 Russia, <sup>2</sup>Siberian Institute of Plant Physiology and Biochemistry SB RAS, 664033 Irkutsk, P.B. 317, Russia, <sup>3</sup>Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), D-06466 Gatersleben, Germany)*

## INTRODUCTION

Precise genetic stocks, both aneuploids and single chromosome substitution lines of bread wheat have played the key role in studying the genetic basis of variation in the technological quality in common wheat. With the aid of these stocks the chromosome assignment of genes for storage proteins composing wheat gluten was established<sup>1</sup> as well as considerable positive or negative effects of chromosome substitution on separate technological parameters<sup>2,3</sup>. The use of modern mapping techniques contributes greatly to our knowledge of the genetic control of wheat gluten quality. In particular, the results of QTL analysis have enlarged the number of chromosomes and loci involved in the genetic control of this character<sup>4,5</sup>. Investigations of genetics basis of technological properties of grain were begun at the end of 60<sup>th</sup> in Novosibirsk Institute of Cytology and Genetics (Russia). Several sets of precise genetic stocks were developed by Olga Maystrenko with co-workers with the initial aim of studying the genetics of quality. The choice of the two parental cultivars was conditioned by their contrast bread-making qualities with S29 having a good quality but rather low gluten content in grain and Dm2 having very poor quality but gluten content about 40%. The aim of this paper is to describe the main investigations fulfilled in the framework of the problem of quality during the development of wheat cytogenetic collection and to discuss the possibilities of using this genetic material for study of common wheat technological properties.

## MATERIALS AND METHODS

The results of investigations of technological parameters of grain in aneuploids, intervarietal single and double substitution lines and ITMI recombinant inbred lines are presented. The scheme of analysis corresponds to the Method of State Variety Testing of Crops accepted in Russia<sup>6</sup>. Physical properties of dough using Chopin alveograph and Brabender farinograph and bread-making properties of monosomic lines were determined.

## RESULTS AND DISCUSSION

For the first time O. Maystrenko with colleagues investigated grain quality directly in ditelosomic lines of CS and during three-year experiment the special important role of 1D chromosome was showed. These results were published in the Proceedings of 4<sup>th</sup>

International Wheat Genetics Symposium<sup>7</sup>. The role of this chromosome was studied during the analysis of grain quality in subsequent backcrosses in the process of the development of monosomic lines. Was it the effect of the early generation or the chromosome dose? Studies of mixing properties with Brabender farinograph have shown that dose is the main reason of deterioration (Fig.1). It was found that both in the second and in the fourth backcrosses just the hemizygous dose of 1D chromosome showed a significant effect on mixing parameters of high quality cultivar S29<sup>8</sup>. Further investigation of the developed monosomic lines demonstrated that the lack of one of the two homologous chromosomes 1D has a pronounced negative effect on loaf volume in S29 (Fig. 2).

The work was continued using intervarietal substitution sets of different kinds. One of them was obtained specially for the investigation of grain quality. In this set the each chromosome of 1 and 6 homoeological groups of the low quality cultivar Dm2 were substituted for the homologue from the high quality cultivar Novosibirskaya 67 (N67) as it has become clear that the genes for biosynthesis of endosperm storage proteins participating in gluten formation are located in these chromosomes. It was found that the role of 1A chromosome is important; this substitution significantly increased dough strength and tenacity (Table 1) measured by alveograph. This data coincided with the earlier results obtained with the use of substitution line CS/Hope 1A<sup>2</sup>. At the same time this substitution did not affected any mixing parameters. The most striking effect was found after the substitution of two chromosomes of the donor, 1A and 6D, in the genotype of the low quality recipient. In this case not only the alveograph parameters were significantly improved but the mixing parameters also. Further investigations were fulfilled using a full set of substitution lines S29/Janetzki Probat (S29/JP) where both the recipient and the donor have a good grain quality. This work is being continued now but preliminary results have showed that the intervarietal substitution of 4D chromosome profoundly decreased the separate physical properties of dough (Table 1).

In the last years a new genetic material presented by wheat recombinant inbred lines (ITMI population) was involved in the investigations of grain quality. The new technique of searching of associations between phenotypic variability of quantitative trait and molecular markers was used for investigations. We were able to

identify several QTLs both connected with loci for glutenin and gliadin biosynthesis in chromosomes of 1 homoeological group and located on the other chromosomes where no special genes relevant to grain quality have been detected<sup>9</sup>.

It is thought now that the key mechanism determining the rheological properties of gluten is provided by interactions between branching polymer protein chains. The most important are the interactions provided with covalent disulfide bonds<sup>10</sup> which number correlates with grain quality parameters. Their formation and decomposing are catalysed by the system of thiol-disulfide metabolism enzymes. We investigated the activity of one of such enzymes – disulfide reductase, responsible for decomposing S-S bonds in gluten in ITMI population. It was found that most of QTLs detected for this parameter form cluster with QTLs earlier found for physical parameters of gluten. Such clusters were detected in 4A, 5D, and 7D chromosomes (Fig.3). Earlier, the QTLs for some physical properties of dough were identified in the same positions<sup>9</sup>. Although the genetic control of this enzyme is still unknown it may be supposed that one of the detected QTLs is attributed to the structural gene for disulfide reductase.

The new application of precise genetic stocks consists in using them for verification of the identified QTLs. Now we are fulfilling the introducing of chromosomes carrying the QTLs positively and negatively influencing on different technological characteristics from ITMI lines into genotypes of S29 and Dm2. The material is on different stages of backcrossing. In addition, the future investigations will include the use of double haploid recombinant substitution lines S29/JP for further mapping of genes for technological properties of dough.

## REFERENCES

1 Law C.N., Young C.F., Brown J.W.S. (1978) The study of grain protein control in wheat using whole chromosome substitution lines. Seed Protein Improvement by Nuclear Techniques. Intern. Atomic Energy Agency, Vienna, Austria, p. 483-502.

2 Payne PI, Seekings JA, Worland AJ, Jarvis MI, Holt LM (1987) Allelic variation of glutenin subunits and gliadins and its effect on bread-making quality in wheat: analysis of F5 progeny from Chinese Spring x Chinese Spring (Hope 1A). *J Cereal Sci.* 6:103-118.

3 Mansur L.M., Qualset C.O., Kasarda D.D., Morris R. (1990) Effects of ‘Cheyenne’ chromosomes on milling and baking quality in ‘Chinese Spring’ wheat in relation to glutenin and gliadin storage proteins. *Crop Sci.* 1990. 30:593-602.

4 Perretant M.R., Cadalen T., Charmet G., Sourdille P., Nicolas P., et al. (2000) QTL analysis of bread-making quality in wheat using a double haploid population. *Theor. Appl. Genet.*, 100: 1167-1175.

5 Rousset M, Brabant P, Kota RS, Dubkovsky J Dvorak J (2001) Use of recombinant substitution lines for gene mapping and QTL analysis of bread-making quality in wheat. *Euphytica* 119: 81-87.

6 Anonymous (1988) *Methods of State Variety Testing of Crops.* Moscow, Gosagroprom Publisher, 122 p.

7 Maystrenko OI Troshina AV, Ermakova MF (1973) Chromosomal arm location of genes for flour quality in wheat using ditelosomic lines. *Proc. 4th Int. Wheat Genet. Symp., Missouri Agr. Exp. Sta., Columbia, Mo.* 51-56.

8 Maystrenko OI (1977) Cytogenetic studies of grain quality in bread wheat. In: *Problema povysheniya kachestva zerna.* Ed.: V.N. Remeslo & A.A. Sozinov, Kolos Publisher, Moscow, p. 79-92.

9 Pshenichnikova TA, Ermakova MF, Chistyakova AK, Shchukina LV, Berezovskaya EV, Lohwasser U., Röder M., Börner A. (2008) Mapping of QTL associated with grain quality of the bread wheat grown under different environmental conditions. *Russ. J. Genet.* 44 : 74-84.

10 Shewry PR, Tatham AS (1997) Disulfide bonds in wheat gluten proteins. *J Cereal Sci* 25: 207-227.

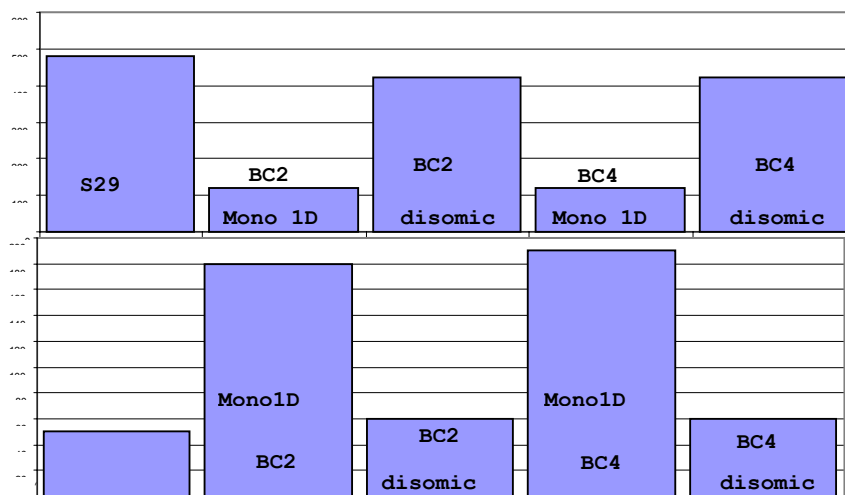


Figure 2. Dough resistance to mixing (seconds) and dough thinning (units of farinograph) in monosomic and disomic populations selected in BC2 and BC4 during the development of 1D monosomic line of S29

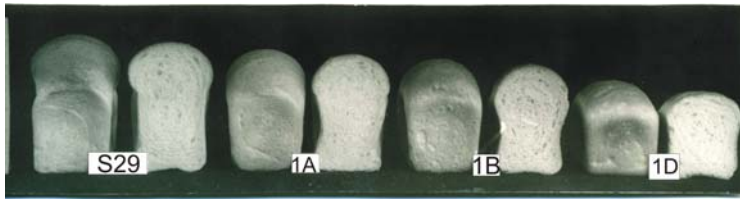


Fig. 2. Loaves baked from monosomic lines 1A, 1B and 1D of cultivar S29

Table 1. Physical properties of dough in different intervarietal substitution lines of bread wheat

Lines, cultivars	Alveograph parameters (3-year field trials)			
	Dough strength, u.a.	Tenacity, mm	Extensibility, mm	P/L
Dm2, poor quality recipient	153±47 <sup>§</sup>	66±4	84±18	0,78±0.2
N67, high quality donor	352±79*	114±17*	101±22	1,13±0.2*
Dm2/N67 1A	233±48*	74±4*	118±41	0,62±0.3
Dm2/N67 1A 6D	287±14*	76±8*	130±41*	0,58±0.2
S29, high quality recipient	513±15	156±6	108±2	1,44±0.03
JP, middle quality donor	316±17*	101±7*	103±8	0.98±0.00*
S29/JP 4D	356±32*	97±2*	153±0.3*	0,63±0.01*

§ - s\* ±m<sub>s</sub>; \* - P < 0,05 (comparing to the recipients)

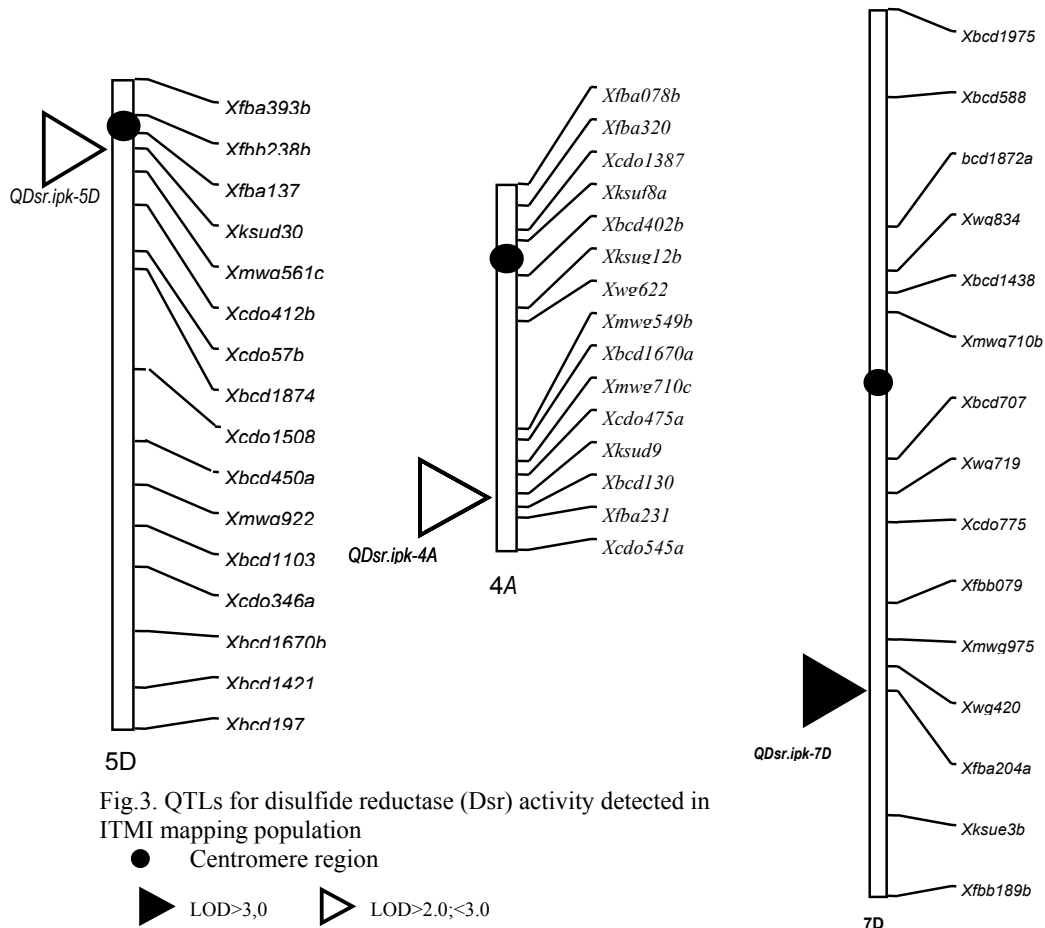


Fig. 3. QTLs for disulfide reductase (Dsr) activity detected in ITMI mapping population

● Centromere region  
▶ LOD > 3,0  
◁ LOD > 2,0; < 3,0