GRAIN PROTEIN CONTENT AND YIELD IN CHROMOSOME 7B RECOMBINANT SUBSTITUTION LINES OF WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

Increasing wheat grain concentration is desirable in order to meet requirements of bread-making industry and wheat markets, but protein concentration tends to be negatively correlated with yield. High protein line F26-70, created at NARDI Fundulea, was identified as a promising parent in breeding, having acceptable yield. We studied the effect of chromosome 7B from F26-70 on protein content, yield, and yield components, by testing 45 recombinant substitution lines derived from the cross Favorit//Favorit/F26-70 (7B) in 2006 and 2007, in field trials, with and without nitrogen fertilization. Genes located on chromosome 7B of line F26-70 had significant effect on grain protein concentration and protein content per kernel of recombinant substitution lines, but not on grain yield, weight of 1,000 grains and number of grains per square meter. In the conditions of our trials, protein content was negatively associated with grain yield only in a dry year without nitrogen fertilization. Association of high protein content with earliness was significant in three out of four conditions. The interaction between RSLs and nitrogen fertilization was not significant, suggesting that the high protein gene(s) on chromosome 7B might be useful at a wide range of nitrogen availability. Our results preliminarily suggest potential usefulness of 7B chromosome genes of F26-70 in breeding for increased grain protein concentration, without significantly reducing grain yield. Further studies to estimate the effects of F26-70 7B chromosome in a modern genetic background and in more high yielding environments are necessary to confirm the real breeding value of this chromosome.

Key words: wheat, chromosome 7B, grain yield, grain protein concentration.

INTRODUCTION

Grain protein concentration directly influences water absorption of the flour and loaf volume (Finney et al., 1987), as well as mixing properties of the dough, and this explains why bread-making industry and wheat markets have definite requirements for this trait.

The largest part of wheat grain consists of carbohydrates (mostly starch) and this makes variation of grain yield, as well as variation of other grain components concentration, strongly associated with carbohydrates accumulation. Because of this association, grain protein concentration tends to be negatively correlated with yield and breaking this correlation has been a continuous challenge for wheat breeding. Breeding for increased protein concentration is becoming increasingly difficult because of limitation of nitrogen fertilizer use, for ecological and economical reasons (Triboi et al., 1990).

High protein line F26-70, created at the Fundulea Institute, was identified as a promising parent (Ceapoiu et al., 1974). Brunori et al. (1980) stated that F26-70 could contribute high protein genes without having the relatively small grain of Atlas 66. In their studies, F26-70 was among the entries for which the high contents of nitrogen per seed was considered to have resulted from high rates and/or long duration of nitrogen deposition, or a combination of the two processes (Brunori et al., 1980).

Genetic control of grain protein concentration proved to be complex, major and minor genes on many chromosomes affecting this trait. Giura and Ittu (1986), and Giura et al. (1986) found a significant effect of chromosome 7B of F26-70 on grain protein concentration.

This paper presents data on grain protein concentration, yield and yield related traits in chromosome 7B recombinant substitution lines (RSLs), tested in yield trials at two levels of nitrogen availability.

MATERIAL AND METHODS

Forty five recombinant substitution lines (RSLs), derived from the cross between the intervarietal substitution line for chromosome 7B (Favorit/F26-70 7B) and the recipient parent Favorit,

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were planted in yield trials organized by the Wheat Breeding laboratory of the National Agricultural Research and Development Institute (NARDI) at Fundulea, Romania, on chernozem soil, in 2006 and 2007.

The RSLs were obtained and kindly provided by Dr. Aurel Giura from the Genetics and Cytogenetics Laboratory of NARDI. A randomized blocks design with 3 reps was used, with harvested plots of 5 m^2. Double plots were used for each line, one with usual nitrogen fertilization (200 kg/ha NH_4NO_3) and the other without nitrogen fertilization.

Climatic conditions were very different during the two testing years and according to nitrogen availability, as reflected by the grain yield and protein concentration, averaged over all RSLs (Table 1).

Table 1. General characterization of the testing conditions

Specification	Average grain yield	Average protein concentration	Characteristic of the year
2006 – with N fertilizer	3.85	13.94	Normal growing conditions
2006 – without N fertilizer	3.58	13.03	
2007 – with N fertilizer	2.07	13.86	Very dry
2007 – without N fertilizer	1.93	12.56	

Heading date, grain yield and thousand kernels weight (TKW) were recorded, and grain protein concentration was determined using a Perten Inframatic infrared analyzer. Protein content per kernel was computed from protein concentration and TKW data. Number of kernels per square meter was estimated from yield and TKW data.

Data were analyzed using three-way ANOVA mixed model with RSLs and nitrogen fertilization as fixed and Years as a random factor (Snedecor and Cochran, 1965). Accordingly, F tests for significance were made against the interaction with years for RLSs and nitrogen fertilization effects, against the triple interaction for the interaction RLSs x nitrogen fertilization and against error for the effect of Years and the rest of interactions. Correlation analysis was used to estimate relationship between traits.

RESULTS AND DISCUSSION

Recombination for chromosome 7B genes induced significant variation of grain protein concentration among RSLs, as shown by ANOVA results in Table 2. The effect of nitrogen fertilization on protein concentration was not significant, probably because of contrasting weather conditions in the two years of testing. Year's effect on grain protein concentration was close to significance, while all interactions were small and not significant.

Table 2. ANOVA for grain protein concentration in 7B recombinant substitution lines

Source of variation	SS	df	MS	F	P
RSLs	55.59	44	1.26	**6.88**	<0.05%
N fertilization	55.14	1	55.14	31.14	>10%
RSLs*N	12.82	44	0.29	0.76	>50%
Years	3.61	1	3.61	3.76	>5%
RSLs*Y	8.08	44	0.18	0.19	>50%
N*Y	1.77	1	1.77	1.84	>10%
RSLs*N*Y	16.82	44	0.38	0.40	>50%
Error	345.60	360	0.96		

F-values in bold are significant at P<5%

The effect of the 7B chromosome on grain protein concentration has been previously described by Konzak (1977), Giura and Ittu (1986), Giura et al. (1986) and Giura (2003). Based on analysis carried out using ditelosomic lines of Chinese Spring, Barneix et al. (1998) and Fatta et al. (2000) suggested that a major gene, important for N translocation into grains, is present on chromosome arm 7BS.

Lack of significant interaction between RSLs and both years and nitrogen fertilization suggests that the effect of 7B chromosome genes on grain protein concentration can be detected under a wide range of weather conditions and nitrogen availability.

Variation for grain yield among 7B recombinant substitution lines was small and not significant (Table 3). Only the effect of the years was highly significant, the effect of nitrogen fertilization and all interactions being not significant.

This suggests that, in the conditions of our tests, genes on chromosome 7B of cultivar F26-70 have a strong influence on protein concentration, more or less independently of environment, while their effect on yield is negligible.

The variation of grain size, as described by TKW, was not significantly influenced by segregation of genes on chromosome 7B or by nitrogen fertilization (Table 4). Significant TKW variation was only produced by the weather conditions of the testing years and by the differential response of the RILs to these conditions.

Table 3. ANOVA for grain yield in 7B recombinant substitution lines

Source of variation	SS	df	MS	F	P
RSLs	3.68	44	0.08	0.88	>50%
N fertilization	1.90	1	1.90	11.30	>25%
RSLs*N	2.23	44	0.05	1.13	>25%
Years	132.66	1	132.66	**829.15**	<0.05%
RSLs*Y	4.16	44	0.09	0.59	>50%
N*Y	0.17	1	0.17	1.05	>25%
RSLs*N*Y	1.97	44	0.04	0.28	>50%
Error	57.60	360	0.16		

F-values in bold are significant at P<5%

Table 4. ANOVA for TKW in 7B recombinant substitution lines

Source of variation	SS	df	MS	F	P
RSLs	238.57	44	5.42	0.17	>50%
N fertilization	26.14	1	26.14	0.82	>25%
RSLs*N	25.44	44	0.58	0.02	>50%
Years	2675.8	1	2675.8	**5445.98**	<0.05%
RSLs*Y	175.21	44	3.98	**8.10**	<0.05%
N*Y	0.56	1	0.56	1.13	>25%
RSLs*N*Y	31.24	44	0.71	1.45	>10%
Error	176.88	360	0.49		

F-values in bold are significant at P<5%

Protein content per kernel was significantly influenced by segregation of genes on 7B chromosome, by N fertilization and by the years (Table 5). None of the interactions were significant.

The variation of the number of kernels per square meter among 7B recombinant substitution lines was not significant, while the effects of both N fertilization and weather conditions were highly significant (Table 6).

Table 5. ANOVA for protein content per kernel in 7B recombinant substitution lines

Source of variation	SS	df	MS	F	P
RSLs	12.95	44	0.29	**3.45**	<0.05%
N fertilization	10.02	1	10.02	**332.67**	<5%
RSLs*N	1.61	44	0.04	0.82	>50%
Years	54.38	1	54.38	**727.80**	<0.05%
RSLs*Y	3.75	44	0.09	1.14	>25%
N*Y	0.03	1	0.03	0.40	>50%
RSLs*N*Y	1.97	44	0.04	0.60	>50%
Error	26.90	360	0.07		

F-values in bold are significant at P<5%

Table 6. ANOVA for number of kernels per square meter in 7B recombinant substitution lines

Source of variation	SS	df	MS	F	P
RSLs	31873942.9	44	724407.8	0.93	>50%
N fertilization	6269685.5	1	6269685.5	**23.97**	<0.05%
RSLs*N	15848643.6	44	360196.4	1.36	>10%
Years	413131010	1	413131010	**634.32**	<0.05%
RSLs*Y	34320074.6	44	780001.7	1.19	>10%
N*Y	261519.6	1	261519.6	0.40	>50%
RSLs*N*Y	11633553.8	44	264398.9	0.41	>50%
Error	234467065.7	360	651297.4		

F-values in bold are significant at P<5%

Significant correlations among protein concentrations recorded in the contrasting environmental conditions of the two years of study and two levels of nitrogen availability, also illustrate the low G x E interaction. In contrast, correlation coefficients among environments for yield were not significant, with the exception of the year 2007, when the correlation between yields obtained with and without fertilization was significant (Table 7).

Table 7. Correlations among environments for grain yield (above the diagonal) and for grain protein content (below the diagonal)

Specification	2006 with N	2006 without N	2007 with N	2007 without N
2006 - with N	1	0.28	-0.10	-0.06
2006 - without N	**0.72**	1	-0.14	-0.02
2007 - with N	**0.57**	**0.67**	1	**0.47**
2007 - without N	**0.54**	**0.58**	**0.35**	1

The relationship yield – protein concentration is particularly interesting (Figure 1 and Table 8). Contrary to the known strong negative relationship found in most studies (Triboi et al., 1990; Triboi and Triboi-Blondel, 2002), segregation for genes on chromosome 7B of F26-70 produced variation of grain protein concentration that was correlated with grain yield variation only in 2007 at low nitrogen availability. One possible explanation of this exception is that in a very dry year and without fertilization, nitrogen availability was limiting both for yield and protein accumulation in the grain.

Lack of the usual negative correlation between grain yield and protein concentration in the grains might be due to relatively low yields recorded in our trials, caused by the low yielding potential of the recurrent parent Favorit, by lodging and by unfavourable climatic conditions. However, in the same conditions, the usual negative correlation yield - % protein was present in other trials, where cultivars or breeding lines were compared (data not shown).

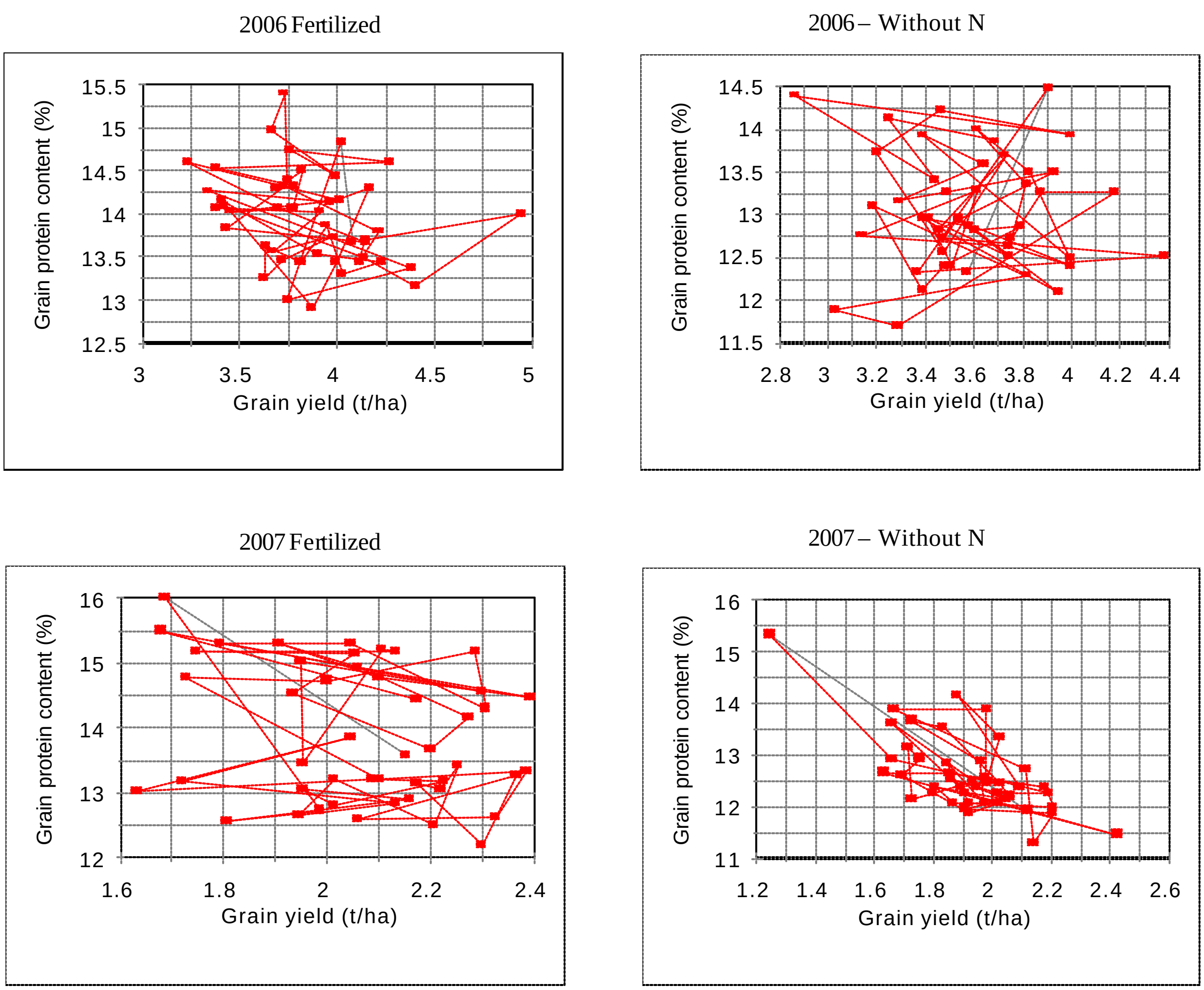

Figure 1. Relationship grain yield – grain protein concentration in recombinant substitution lines

from the cross Favorit // Favorit/F26-70(7B)

Table 8. Correlation between grain protein concentration and grain yield in 7B recombinant substitution lines

Year	With nitrogen fertilizer	Without nitrogen fertilizer
2006	-0.27	-0.03
2007	-0.26	**-0.67**

Further studies to estimate the effects of F26-70 7B chromosome in a modern lodging resistant genetic background and in more high yielding environments are necessary before the real value of this chromosome in breeding for higher protein concentrations can be established.

Relationship of grain protein concentration and yield with other measured traits is illustrated by the correlation coefficients in table 9. Heading date was significantly associated with protein concentration in the grains in three of the four environments of our study. In preliminary tests with fifteen RSLs from the same cross, Giura (2003) also found that protein content was associated with earliness. This association is interesting for breeding, because earliness is a desirable trait in dry and hot years, common in South Romania.

Table 9. Correlation between grain protein concentration, grain yield and other traits in 7B recombinant substitution lines

Specification		Grain protein concentration		Grain yield	
		With nitrogen fertilization	Without N fertilization	With nitrogen fertilization	Without N fertilization
Heading date	2006	**-0.463**	**-0.394**	**-0.320**	0.119
	2007	-0.005	**-0.364**	-0.100	-0.051
TKW	2006	0.009	-0.214	0.119	0.204
	2007	**-0.439**	-0.001	0.228	0.137
Protein content/kernel	2006	**0.653**	0.198	-0.090	-0.081
	2007	**0.899**	**0.706**	-0.180	-0.268
Number of kernels/m^2	2006	-0.253	-0.127	**0.860**	**0.847**
	2007	-0.124	**-0.665**	**0.942**	**0.935**

Yield was negatively associated with earliness only in 2007 with nitrogen fertilization, in a higher yielding environment that might have favoured later heading lines.

Weight of 1000 kernels was not correlated with yield and was significantly and negatively associated with protein concentration only in one of the four testing environments.

Protein content per kernel showed a close significant correlation with protein concentration in most conditions, but was not correlated with grain yield.

Estimated number of grains per square meter strongly correlated with grain yield in all environments, but the correlation with protein concentration was significant only in 2007 without nitrogen fertilization, the same environment that showed a negative significant correlation between protein concentration and yield.

CONCLUSIONS

Genes located on chromosome 7B of line F26-70 had significant effects on grain protein concentration of recombinant substitution lines, but not on grain yield and yield components.

In most conditions of our trials, protein content was not negatively associated with grain yield, a significant correlation being observed only in a dry year, at low nitrogen availability.

Association of high protein content with earliness was significant in three out of four conditions of our experiment.

The interaction between RSLs and nitrogen fertilization was not significant, suggesting that the high protein gene(s) on chromosome 7B might be useful at a wide range of nitrogen availability.

Results preliminarily suggest potential usefulness of 7B chromosome genes of F26-70 in breeding for increased grain protein concentration without significantly reducing grain yield. Further studies to estimate the effects of F26-70 7B chromosome in a modern genetic background and in more high yielding environments are necessary to confirm the real breeding value of this chromosome.

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