CULTIVAR EFFECTS ON THE RELATIONSHIP BETWEEN GRAIN PROTEIN CONCENTRATION AND YIELD IN WINTER WHEAT

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ABSTRACT

Combining high wheat grain yields (GY) with grain protein concentration (GPC) required by the bread-making industry has proved to be difficult, because of the negative correlation between the two traits. We used data from two sets of cultivar yield trials to analyze the relationship between GPC and GY in several cultivars, tested in contrasting environmental conditions and at different levels of N availability. GPC was negatively associated with grain yield, in most analyzed yield trials, regardless of nitrogen applied, and in 40% of both fertilized trials and trials which received no nitrogen fertilizer the negative correlation was significant. We estimated deviations from regression of GPC on GY using (1) the averages of deviations calculated for each individual trial and (2) the deviations from the regression of across trials average GPC on average GY. The two estimations gave similar results. ANOVA for cultivar deviations from GPC/GY regression in each trial shows very significant genotypic effects and not significant influence of N fertilizer and G*N interaction. Our results confirm that the deviations from the GPC/GY regression are under genetic control. However, deviations do not only reflect a characteristic of a particular cultivar, but are influenced by all cultivars used for estimating the regression. To eliminate this influence, we described the relationship between GPC and GY in each individual cultivar, using negative asymptotic protein response curves (APRC), described by the equation GPC = a + b/GY^2. ANOVA showed that differences between the shapes of individual cultivar regression curves (non parallelism of linear regressions on 1/GY^2) were significant in most situations. The constant and the regression coefficient of APRC were negatively correlated (r between -0.72 and -0.89 in our trials) and the constant was strongly correlated with deviations from linear GPC/GY regression (r between +0.78 and +0.94), while the *x* coefficient was generally not significantly correlated with deviations from linear regression. This suggests that APRC parameters could provide not only similar, but also additional information about genotypic effects on GPC-GY relationship.

Key words: winter wheat, grain protein, yield.

INTRODUCTION

Plant breeding is often confronted with the requirement to combine traits that are negatively correlated. The negative correlation between grain yield and grain protein concentration exemplifies an undesirable relationship in bread wheat quality types, in which protein concentration is positively correlated with bread loaf volume (DePauw et al., 2007).

The analysis of the evolution of crop yield reveals a change in grain composition: increases in yield have led to a decrease in the protein to starch ratio (Triboi et al., 2006). Simmonds (1995), Feil (1997) and Oury et al. (2003) presented comprehensive reviews of the relationship between grain yield (GY) and grain protein concentration (GPC). Data from controlled environment and field experiments, at canopy and plant level, and at different sink: source ratios confirmed the strong negative relationship between GY and GPC, or N concentration (Triboi et al., 2006). Knowing that grain mainly consists of carbohydrates, and assuming relative independence of N and C metabolism, dilution of the limited amount of available N by increased amounts of carbohydrates will automatically lead to reduced GPC.

Several selection criteria, such as protein yield or protein per grain (Brunori et al., 1980) have been suggested in breeding wheat for increased GPC without reducing GY, but they have not been extensively used. More recently, Monaghan et al. (2001) showed that analysis of residuals from regression of grain protein concentration on grain yield (grain protein deviation, GPD) can identify cultivars having a higher grain protein concentration than was predicted from grain yield alone. They deduced that the capacity to accumulate a higher grain protein concentration than predicted from grain yield is under genetic control and thus may be improved through breeding. Oury and Godin (2007) used grain protein deviations, defined as the standardized residuals of the regression of GPC on GY, and concluded that these deviations appeared to have a partly genetic basis.

This paper analyzes the relationship between GPC and GY in contrasting environmental conditions and at different levels of N availability, and provides evidence of genotypic differences both in deviations from the general GPC/GY regression and in the shape of individual cultivar response curves of GPC to GY, suggesting the latter as an additional approach in detecting possibilities of progress in breeding for higher GPC, without reducing yield.

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MATERIAL AND METHODS

GY data from winter wheat cultivar yield trials, with and without nitrogen fertilizer, performed from 2003 to 2007 in 12 locations situated from 40°.59' to 47°.51' latitude and from 20°.44' to 28°.29' longitude, were grouped in two sets. Set 1 included trials performed during 2003-2007 in 12 locations, totaling 40 environments (site x year combinations) where the recommended nitrogen fertilizer rate was applied and 40 trials where no N fertilizer was applied. Nine cultivars were common to all trials in set 1. Set 2 included trials from 9 locations during 2003-2006 (23 site x year combinations), each with and without N fertilizer, and had 13 cultivars common to all trials.

Grain samples were collected and GPC was determined in the NARDI Fundulea laboratory using a Perten Inframatic Infrared Analyzer.

Environmental conditions varied very much among trials, as shown by amplitude of GY trials average from 2402 to 9600 kg ha^{-1} in fertilized trials and 2068 to 7121 kg ha^{-1} in non-fertilized trials, and variation of GPC of 10.7 to 17.4% in fertilized trials and 10.0 to 16.9% in non-fertilized trials.

Correlations, linear regressions and residuals from GPC on GY regressions were computed for each trial, including all 25 entries. Regression analysis was also performed using the averages for common cultivars (9 in set 1 and 13 in set 2).

ANOVA was used to estimate significance of residuals for cultivars that were common to all environments for trials set 1 and set 2.

Relationship between GPC and GY was also estimated by fitting asymptotic response curves for each of the cultivars common to set 1 or set 2 respectively.

RESULTS

Relationship between GPC and GY in winter wheat yield trials performed in contrasting environments

Grain protein concentration was negatively associated with grain yield, in most analyzed yield trials, regardless of nitrogen applied (Figure 1). GPC-GY correlations varied from +0.2 to -0. 95, and in 40% of both fertilized trials and trials which received no nitrogen fertilizer the negative correlation was significant. Triboi et al. (2006), working in controlled environment, observed that under limiting N conditions, grain N concentration is more sensitive to yield variation than under non-limiting N conditions. Similar correlations between GPC and GY in trials with and without nitrogen fertilizer, found in our trials, suggest that in field conditions, the effect of N availability on the GPC-GY relationship is more complex, as it depends on the timing of limitations in N supply to plants.

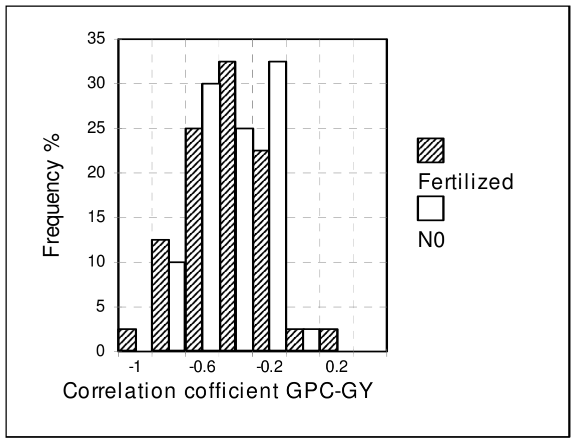


Figure 1. Histogram of correlation coefficients between GPC and GY in 80 yield trials

Excluding the long term historical check Bezostaya 1, which generally had the lowest yield and high protein concentration, and therefore tended to increase the GPC-GY correlation, slightly reduced the percentage of significant correlations (to about 32% in both fertilized and not fertilized trials), but did not change the general trend of negative association between GPC and GY (data not shown).

Oury and Godin (2007) also found that the correlations between GPC and GY, calculated environment by environment, were highly variable due to high „genotype × environment” interactions for both characters, but, working in much higher yielding environments (yields in the range of 8 to 10 t ha^{-1}) and using a trimming algorithm to neutralize the effect of outliers, generally found larger percentages of significant correlations. Our results demonstrate that even at lower yields, frequent in continental climate, and without any correction of original data, the GPC-GY correlations are important.

Values of correlation coefficients were not associated with average yield level (r = -0.17 n.s.) or GPC (r = 0.10 n.s.), suggesting that the dilution effects, which are the main cause of the negative association between GPC and GY, can be present in very different conditions.

In contrast with results of Oury and Godin (2007), we did not find significant association between GPC-GY correlation coefficients and the variation amplitude of GY or GP (r = -0.21 and r = -0.18 n.s.). Slopes of regression varied considerably among trials, but distributions of b values were similar for trials with and without nitrogen fertilization (Figure 2).

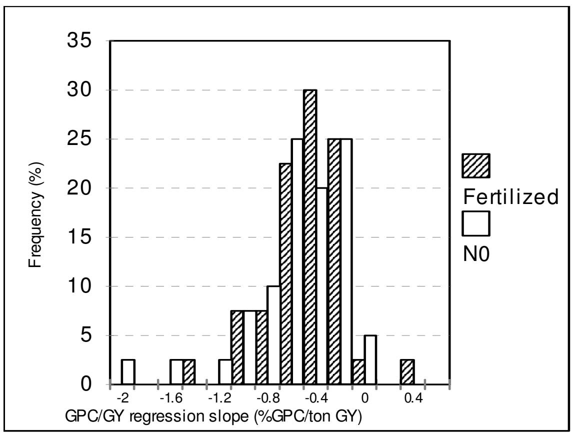


Figure 2. Histogram of slope coefficients of GPC on GY regressions in 80 yield trials

In 25 and 35% of the trials, with and without additional nitrogen respectively, GPC decreased by more than 0.5% and up to more than 1% units for yield differences of 1000 kg ha^{-1} among cultivar.

Deviations from the GPC/GY regression

Deviations from regression of GPC on GY have been suggested as a way of identifying genotypic differences in nitrogen storage efficiency (Monaghan et al., 2001; Oury and Godin, 2007).

We estimated these deviations using: (1) the averages of deviations calculated for each individual trial and (2) the deviations from the regression of across trials average GPC on average GY.

First approach allowed a straightforward test of significance of cultivar and nitrogen effects on protein deviations from the regression. ANOVA for cultivar deviations from GPC/GY regression in each trial for the nine cultivars which were tested in all 80 trials (trial set 1) shows very significant genotypic effects and not significant influence of N fertilizer and G*N interaction. Similar results of ANOVA were also obtained for trial set 2, including 13 common cultivars tested in 46 environments (Table 1).

Table 1. ANOVA for grain protein deviations from GPC/GY regression in winter wheat yield trials

Source of variation	Set 1 (80 trials)		Set 2 (46 trials)	
	df	MS	df	MS
Cultivar	8	2.802***	12	3.658***
Nitrogen fertilization	1	0.023 n.s.	1	0.020 n.s.
Cultivar * Nitrogen	8	0.126 n.s.	12	0.237 n.s.
Rest	702	0.227	572	0.277
Total	719		597	

***) significant at P<0.001.
n.s.) not significant (P>0.05).

The two estimations of protein concentration deviations gave similar results (R^2 = of 0.64 and 0.80, for trials with and without N fertilizer, respectively), with several exceptions, mainly in the case of historical check Bezostaya 1, which had positive values when deviations were averaged across all trials, but a small negative deviation from the average regression.

Average protein concentration deviations from regressions on grain yield varied among cultivars from -0.260% to +0.208% with N fertilization, and from -0.328% to +0.384% without N fertilization (Table 2).

In the second set of trials, average deviations were larger, with cultivar Holda having more than 0.5% positive deviation and cultivar Fundulea 4 about 0.5% negative deviation (Table 3). The two estimations of GPC deviations gave similar results (R^2 = of 0.68 and 0.72, for trials with and without N fertilizer, respectively). The historical check Bezostaya 1, showed again the largest difference between the two estimations of deviations from GPC/GY regression. For the cultivars that were common to the two sets of trials, correlation between average deviations was highly significant ($r > 0.80$), suggesting a good repeatability of the estimations.

Table 2. Protein concentration deviations from GPC/GY regression (yield trials set 1)

Cultivar	Estimated as					
	Average of deviations in each trial			Deviation from average regression		
	Fertilized	N_0	Average	Fertilized	N_0	Average
Flamura 85	0.208	0.384	0.293 a	0.195	0.421	0.308
Bezostaya 1	0.225	0.208	0.217 ab	-0.029	-0.011	-0.020
Faur	0.127	0.074	0.100 abc	0.195	0.112	0.154
Delabrad	0.094	0.027	0.061 cd	0.156	0.045	0.101
Crina	-0.055	-0.054	-0.055 cd	-0.014	-0.067	-0.041
Boema	-0.107	-0.098	-0.103 cde	-0.073	-0.049	-0.061
Gruia	-0.145	-0.097	-0.121 cde	-0.061	-0.085	-0.073
Glosa	-0.169	-0.106	-0.138 de	-0.124	0.013	-0.056
Fundulea 4	-0.260	-0.328	-0.294 e	-0.265	-0.311	-0.288

Averages followed by the same letter are not significantly different (P>0.05) according to Duncan test.

Table 3. Average protein concentration deviations from GPC/GY regression (yield trials set 2)

Cultivar	Estimated as					
	Average of deviations in each trial			Deviation from average regression		
	Fertilized	N_0	Average	Fertilized	N_0	Average
Holda	0.743	0.516	0.630a	0.470	0.274	0.372
Flamura 85	0.192	0.385	0.289 b	0.164	0.361	0.263
Bezostaya 1	0.253	0.282	0.268 bc	-0.181	-0.121	-0.151
Faur	0.151	0.086	0.118 bc	0.217	0.163	0.190
Dropia	0.022	0.203	0.112 bcd	-0.108	0.114	0.003
Jiana	0.151	0.020	0.086 bcd	0.270	0.141	0.206
Delabrad	0.097	0.075	0.086 cd	0.146	0.152	0.149
Gruia	-0.054	-0.117	-0.086 de	0.125	0.016	0.071
Glosa	-0.123	-0.077	-0.100 de	0.020	-0.057	-0.019
Crina	-0.154	-0.102	-0.128 de	-0.131	-0.098	-0.115
Boema	-0.159	-0.102	-0.130 de	-0.079	-0.127	-0.103
Izvor	-0.406	-0.147	-0.277 ef	-0.468	-0.201	-0.335
Fundulea 4	-0.414	-0.575	-0.495 f	-0.446	-0.619	-0.533

Averages followed by the same letter are not significantly different (P>0.05), according to Duncan test.

Figures 3, 4 and 5 exemplify distributions of protein deviations from the GPC/GY regression for several cultivar pairs, suggesting that despite large variation among trials, genetic differences were important.

The regressions of average GPC on average Y of the common cultivars in sets 1 (Figure 6a) and 2 (Figure 6b) show a significant associations, both in fertilized ($R^2 = 0.71$ and 0.54) and not fertilized ($R^2 = 0.47$and 0.52) trials.

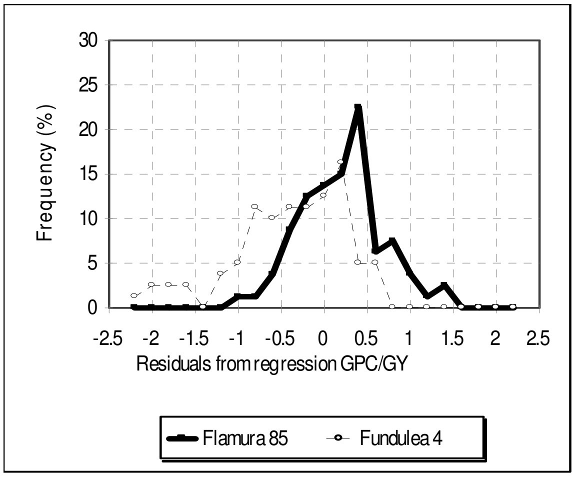


Figure 3. Distribution of deviations from GPC/GY regression in cultivars Flamura 85 and Fundulea 4, tested in 80 trials

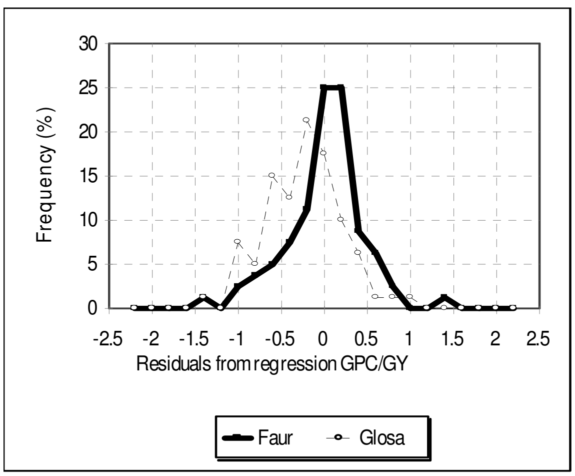


Figure 4. Distribution of deviations from GPC/GY regression in cultivars Faur and Glosa, tested in 80 trials

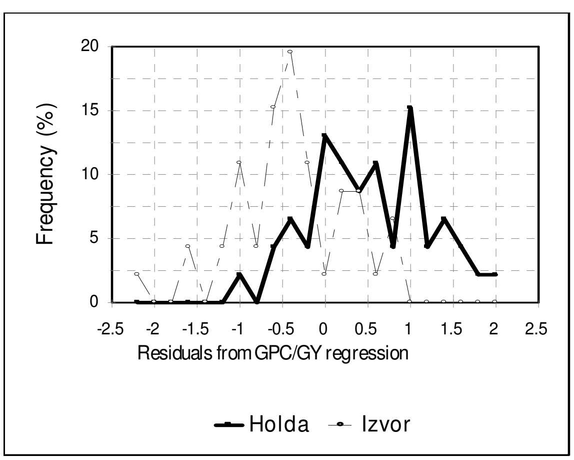
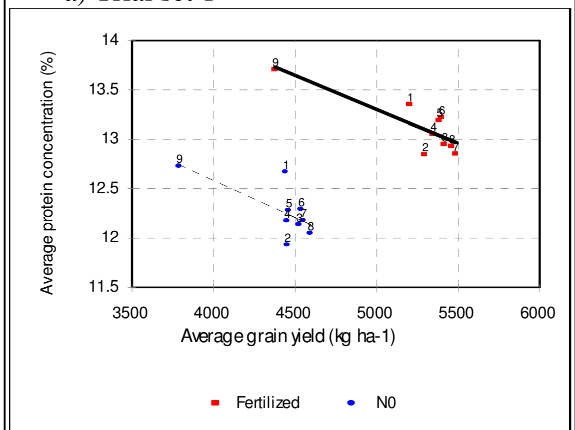


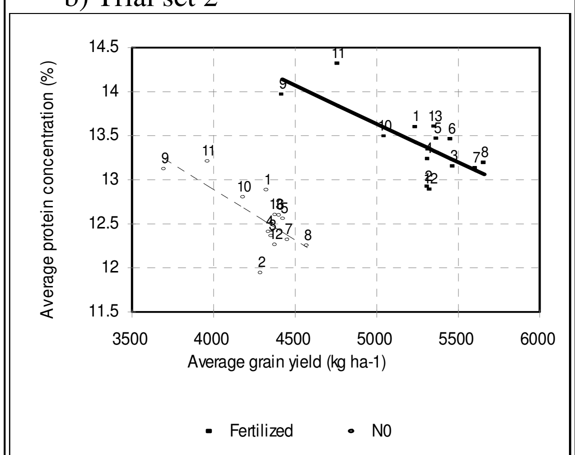
Figure 5. Distribution of deviations from GPC/GY regression in cultivars Holda and Izvor, tested in 46 trials

a) Trial set 1



Fertilized: GCP = 17.17 - 0.00069*GY $R^2 = 0.71$
Without N fertilization: GCP = 15.50 - 0.00075*GY $R^2 = 0.47$

b) Trial set 2



Fertilized: GCP = 17.99 - 0.00087*GY $R^2 = 0.54$
Without N fertilization: GCP = 17.48 - 0.00115*GY $R^2 = 0.52$

1 •Flamura85	2 •Fundulea4	3 •Boema	4 •Crina
5 •Delabrad	6 •Faur	7 •Glosa	8 •Gruia
9 •Bezostaya 1	10 •Dropia	11 •Holda	12 •Izvor
13 •Jiana			

Figure 6. Regression of average GPC on average GY for nine wheat cultivars tested in trial set 1(a) and for thirteen cultivars tested in trials set 2 (b), with and without N fertilization

On average, for set 1, GPC was reduced by 0.69 and 0.75% for each ton ha^{-1} yield difference among cultivars, in fertilized and not fertilized trials respectively, while in set 2 the reductions were 0.87 and 1.15%. On the other hand, at similar average yields, cultivars had GPC that differed by more than 0.5%.

Cultivar deviations from the average GPC/GY regression were similar at both levels of N availability with large positive deviations for cultivars Flamura 85, Faur and Delabrad and negative deviation for Fundulea 4.

Our results confirm previous results of Monaghan et al. (2001), Oury and Godin (2007) and others, on the usefulness of deviations from the GPC/GY regression and provide new evidence that these deviations are under genetic control. An obvious disadvantage of this approach is however the fact that deviations do not only reflect a characteristic of a particular cultivar, but are influenced by all cultivars used for estimating the regression. To reduce the impact of outliers on regression, Oury and Godin (2007) used repeated iterations applying a specially constructed algorithm to assess the „true" position of the regression line. Another possibility might be to describe the behavior of individual cultivars in a series of trials, independently on the other tested cultivars.

Cultivar specific curves of GPC response to GY variation

Săulescu et al. (2005) suggested that GPC/GY regressions computed using data of each cultivar in many environments can provide useful information about genetic influence on the relationship between protein concentration and yield.

In our sets of trials, linear regression on GY only accounted for a small and not significant part of overall GPC variation (R^2 between 10 and 11% for fertilized trials in sets 1 and 2, and between 4 and 7% for non-fertilized trials respectively). A negative **asymptotic protein response curve** (APRC), described by the equation $GPC = a + b/GY^2$, accounted for a larger and significant part of overall protein variation (R^2 between 23 and 25% for fertilized trials in sets 1 and 2, and between 7 and 12% for non-fertilized trials respectively).

ANOVA shows that not only the average asymptotic regression had a significant effect, but also differences between the shapes of individual cultivar regression curves (non parallelism of linear regressions on $1/GY^2$) were significant in all situations, except the unfertilized trials of set 1 (Table 4).

The same type of equation was identified by Rharrabti et al. (2001) as giving the best fit to the relationship between yield and protein content in durum wheat under Mediterranean conditions, but they did not used it for characterizing individual cultivar response of GPC to GY variation.

Possible explanation of the departure from linearity and of negative asymptotic response can be the fact that fertile environments, inducing high yields, are also able to provide enough Nitrogen during grain filling, to reduce the linear dilution effect.

Table 4. ANOVA for testing significance of GPC regressions on GY, in two sets of winter wheat trials

Source of variation	Trial set 1			Trial set 2		
	df	MS		df	MS	
		With N fertilizer	Without N fertilizer		With N fertilizer	Without N fertilizer
Average GPC/GY asymptotic regression	1	241.88***	79.43***	1	211.40***	122.55***
Shape differences of individual cultivar regressions curves	8	6.20**	4.00 n.s	12	19.14***	8.59**
Residual	342	1.95	2.88	273	1.71	2.82
Total	351			286		

**) significant at P<0.01
***) significant at P<0.001; n.s) not significant

The part of GPC variation explained by individual asymptotic regressions varied widely between cultivars, from only 0.03% in cultivars Delabrad, Faur and Gruia tested in unfertilized trials of set 1, to more than 60% in cultivar Fundulea 4, tested in fertilized trials of set 2 (Table 5). The fact that in all situations and cultivars regressions on GY explained a smaller parte of GPC in unfertilized trials than in the fertilized ones might be due to a relatively large number of trials on poor soils, which could not provide enough nitrogen for a higher grain protein concentration, even at very low yields (data not shown).

Table 5. Percentage of total GPC variation explained by negative asymptotic regressions (R^2 values) in several wheat cultivars

Cultivar	Trial set 1		Trial set 2	
	Fertilized	Not fertilized	Fertilized	Not fertilized
Flamura 85	0.22	0.07	0.28	0.17
Fundulea 4	0.36	0.19	0.61	0.36
Boema	0.24	0.11	0.50	0.24
Crina	0.38	0.14	0.60	0.32
Delabrad	0.30	0.03	0.60	0.09
Faur	0.38	0.03	0.54	0.09
Glosa	0.24	0.05	0.37	0.21
Gruia	0.23	0.03	0.49	0.08
Bezostaya 1	0.18	0.06	0.51	0.15
Holda			0.19	0.13
Izvor			0.27	0.16
Dropia			0.44	0.26
Jiana			0.33	0.21

Considerable variation was found between coefficients of asymptotic regression in tested cultivars (Table 6). Cultivars like Flamura 85 and Holda consistently had higher constant and lower *x* coefficient than other cultivars.

Figures 7 and 8 exemplify cultivar differences in the shape of the curves describing GPC response to GY variation. Despite large deviations from regressions, due to the „noise” produced by the large genotype x environment interactions present in both GPC and GY, cultivar differences can be observed both in the level at which the curve becomes asymptotic and in the slope of the initial part of the curve.

The constant and the regression coefficient were negatively correlated (r between -0.72 and -0.89 in our trials) (Table 7). On the other hand, the constant of the asymptotic regression was strongly correlated with deviations from linear GPC/GY regression (r between +0.78 and +0.94), while asymptotic regression *x* coefficient was not significantly correlated with deviations from linear regression in all situation, except unfertilized trials of set 2. This suggests that asymptotic regression parameters could provide not only similar, but also additional information about genotypic effects on GPC-GY relationship.

Table 6. Coefficients of regression equations describing the negative asymptotic relationship between GPC and GY in several winter wheat cultivars tested in fertilized trials

Cultivar	Trial set 1				Trial set 2			
	With N fertilizer		Without N fertilizer		With N fertilizer		Without N fertilizer	
	Constant	x coefficient	Constant	x coefficient	Constant	x coefficient	Constant	x coefficient
Flamura 85	13.08	9.42	12.21	5.61	12.91	10.13	12.15	8.04
Fundulea 4	11.98	20.98	10.87	13.33	11.06	34.35	10.32	18.18
Boema	12.41	17.79	11.63	6.23	11.95	23.16	11.59	8.32
Crina	12.20	23.15	11.50	8.17	11.66	28.95	11.29	12.11
Delabrad	12.39	26.45	11.83	6.40	11.56	39.96	11.68	12.26
Faur	12.30	26.17	11.90	5.54	11.90	32.17	11.73	11.70
Glosa	12.47	13.90	11.86	3.82	12.29	16.11	11.67	7.06
Gruia	12.18	23.78	11.71	4.85	11.64	35.86	11.62	8.53
Bezostaya 1	12.94	16.56	11.96	8.47	11.93	31.61	11.83	13.31
Holda					14.02	2.76	12.87	2.42
Izvor					12.19	12.31	11.64	7.25
Dropia					12.22	22.18	12.15	6.06
Jiana					12.91	10.49	12.23	2.44

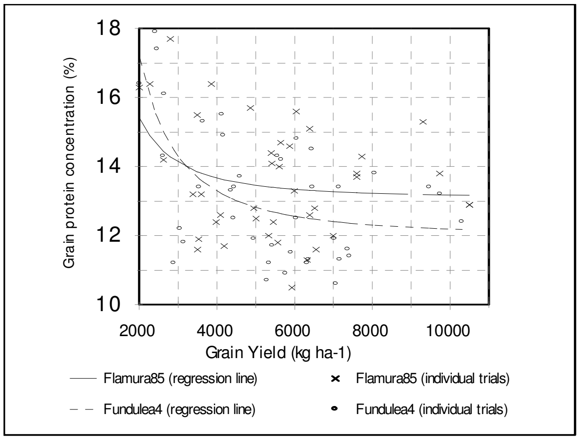


Figure 7. The negative asymptotic relationship between grain protein concentration and grain yield in winter wheat cultivars Flamura 85 and Fundulea 4

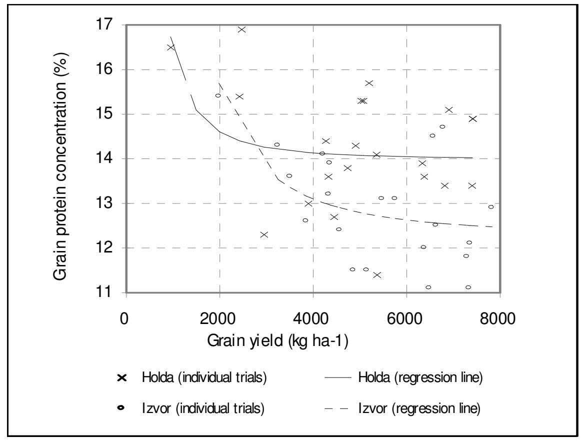


Figure 8. The negative asymptotic relationship between grain protein concentration and grain yield in winter wheat cultivars Holda and Izvor

Table 7. Correlation between coefficients of individual cultivars asymptotic regressions and deviation from linear regression

Correlations between	Trial set 1		Trial set 2	
	With N fertilizer	Without N fertilizer	With N fertilizer	Without N fertilizer
Constant and *x* coefficient of GPC/GY regression	-0.72*	-0.78*	-0.89***	-0.89***
Asymptotic regression constant and deviation from linear regression	+0.78*	+0.87*	+0.78**	+0.94***
Asymptotic regression *x* coefficient and deviation from linear regression	-0.16 n.s.	-0.40 n.s.	-0.41 n.s.	-0.70**

DISCUSSION

GPC differences among cultivars, after eliminating the GY influence, are relatively small but still deserve attention. In some cases they can contribute directly to fulfillment of market requirements, and this can make a difference in the economics of the wheat crop. On the other hand one can hope to exploit these small genetic differences in further breeding work.

Our results provide new proof that the deviations from the GPC/GY regression, either averaged across yield trials, or derived from an average regression, can identify genetic opportunities for increasing GPC without reducing GY.

On the other hand, APRC estimations, which are not influenced by the performance of other cultivars used in computing the GPC/GY regressions, can offer additional information. Both the constant and the *x* coefficient of APRC regression can be considered to have biological meanings. The constant provides information about the level at which GPC tends to stabilize at high yields, and genetic differences in this respect might suggest different degrees of „coupling" between nitrogen and carbohydrates metabolisms. On the other hand, the *x* coefficient might be interpreted as providing information about the dilution phenomenon. We therefore suggest that the parameters of the negative asymptotic regression of GPC on GY could be used as selection criteria in breeding for improved nitrogen storage efficiency.

Besides offering opportunities for using small genetic differences existing in any breeding program, both GPD and APRC should provide a better understanding of the effect of major genes, such as *Gpc-6B1* from *Triticum turgidum* ssp. *dicoccoides* (Khan et al., 2000).

One problem in determining both GPD and individual cultivar protein response curves is the need for many tests, covering sufficient variation of environmental conditions.

Oury and Godin (2007), based on simulations, showed that at least five sites per year for two consecutive years, were necessary to have a good assessment of the GY–GPC relationship, and hence reliable estimates of GPD. Such a number of trials is often available for advanced lines in most breeding programs. Estimation of APRC parameters will probably require a larger number of tests and larger variation in yield. It might however be feasible in programs with large testing facilities, if not for selection among a large number of lines, at least for selecting appropriate parents for the breeding program.

High GPD may be achieved through increased N accumulation after anthesis, combined with efficient re-translocation of vegetative N reserves (Monaghan et al., 2001). The use of GPD and/or APRC provides selection criteria in wheat breeding programs for increased grain protein concentration without a concurrent grain yield reduction.

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CONCLUSIONS

GPC was negatively associated with grain yield, in most analyzed yield trials, regardless of nitrogen applied, and in 40% of both fertilized trials and trials which received no nitrogen fertilizer the negative correlation was significant.

ANOVA for cultivar deviations from GPC/GY regression in each trial shows very significant genotypic effects and not significant influence of N fertilizer and G*N interaction. Confirming that the deviations from the GPC/GY regression are under genetic control.

Asymptotic protein response curves (APRC), described by the equation GPC = a + b/GY^2, can provide additional information about genotypic effects on GPC-GY relationship.

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