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GRAIN PROTEIN CONCENTRATION AND ITS STABILITY IN A SET OF WINTER WHEAT CULTIVARS, GROWN IN DIVERSE ENVIRONMENTS AND MANAGEMENT PRACTICES

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ABSTRACT

Bread making industry and wheat markets have strict requirements for grain protein concentration (GPC), as there is a strong positive relationship between grain protein concentration and bread volume and texture. The objective of this paper was to identify genotypic differences between 23 Romanian and foreign winter wheat cultivars in GPC, as related with grain yield, and in GPC stability across environments. Samples from yield trials performed during 30 environments (4 locations, 3 years and different management systems), widely different in nitrogen availability and yield potential, were analyzed for grain protein content.

Grain protein concentration was mainly determined by the environment, but average differences between cultivars were significant when tested against the genotype*environment interaction. More than 40% of the GPC variation between cultivars could be explained by the negative correlation with grain yield, but several cultivars had a higher GPC than expected based on their average yield. This suggests some possibilities in breeding for both grain yield and protein concentration.

Large differences were found among tested cultivars for GPC stability, as expressed by the amplitude and the coefficient of variation. Romanian cultivars showed better GPC stability than the tested foreign cultivars. Neither the amplitude of variation, nor the coefficient of variation was correlated with average GPC, indicating possibilities in breeding for both higher and more stable GPC. Based on regressions of each cultivar GPC on average GPC of all cultivars, cultivars could be classified as more responsive to environments that were favorable to higher protein concentration in the grains (such as Capo, Exotic etc), or more able to maintain GPC in less favorable environments (such as Şimnic 50, Dropia, Flamura 85, Izvor, Glosa and Lovrin 34).

Frequency of GPC value below an accepted minimum, which is important from farmer's point of view, closely correlated with the average GPC ($r = -0.847^{ooo}$), but some cultivars with similar averages had different number of cases below the required level, indicating additional possibilities of progress.

Key words: grain protein concentration (GPC), stability, wheat.

INTRODUCTION

Protein concentration is one of the main quality traits in wheat, and particularly in bread wheat, as there is a strong positive relationship between grain protein concentration and bread volume and texture (Finney and Barmore, 1948).

The considerable amount of variation in the protein content of wheat kernels may be induced by environmental factors, but can also be attributed to genetic differences (Kramer, 1979). Meeting the quality requirements of bread making industry and of wheat markets is becoming increasingly difficult because grain protein concentration is negatively correlated with grain yields and because limitation of nitrogen fertilizer use, for ecological and economical reasons, negatively affects grain protein concentration (Triboi et al., 1990; Săulescu et al., 2005).

Simmonds (1995), Feil (1997) and Oury et al. (2003) discussed the main aspects of the relationship between grain yield and grain protein concentration (GPC), and many other studies presented results on this inverse relationship (Triboi et al., 2000; Triboi and Triboi-Blondel, 2001; Mustăţea et al., 2005; Marinciu and Săulescu, 2008 etc.). Previous studies in Romania confirmed that protein content, one of the main determinants of wheat bread making quality is affected by genetics, environmental conditions and crop management (Marinciu, 2007; Stanciu and Neacşu, 2009; Neacşu et al., 2010). However, information about the

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stability of grain protein concentration is scarce.

This paper presents data on variation of protein concentration in several Romanian and foreign winter wheat cultivars grown under a wide range of environments (including organic, conventional and low input systems), during three years, at four locations in Romania. The objective was to evaluate the genotypic differences between cultivars in grain protein concentration and its stability across environments, as well as the relationship between grain protein concentration and grain yield.

MATERIAL AND METHODS

Grain samples of 18 Romanian (Flamura 85, Dropia, Boema 1, Crina, Dor, Delabrad 2, Faur F, Glosa, Gruia, Izvor, Litera bred at NARDI Fundulea and Lovrin 34, Alex, Ciprian, Şimnic 30, Şimnic 50, Albota 69, Trivale, bred at other Romanian breeding centers) and 5 foreign varieties (Capo and Josef from Austria, Apache and Exotic from France, Serina from Hungary) from harvest years 2008, 2009 and 2010, tested in yield trials in environments that were widely different in nitrogen availability and yield potential, were analyzed for grain protein content. Environments included:

- NARDI Fundulea and ARDS Şimnic, in three technological systems: organic, conventional, fertilized with recommended doses of nitrogen and conventional without additional nitrogen. Details about crop management in these three technological systems were presented by Neacşu et al. (2010).

- ARDS Piteşti-Albota: fertilized with recommended doses of nitrogen and without additional nitrogen and

- ARDS Valu lui Traian: fertilized with recommended doses of nitrogen.

Protein concentration was determined using a Perten infrared analyzer.

Grain yield data from all environments were also available and were used to study the correlation between yield and protein concentration[1].

ANOVA was used to analyze the results obtained for protein concentration, significance being tested against the interaction between cultivars and environments (years*locations*crop management).

Stability of protein concentration across environments was analyzed using:

- amplitude of variation:

  Ax = xMax - xMin

- coefficient of variation:

  $$CV_i = s_i / \bar{x}_i * 100$$

- regression coefficient (b) and the constant (a) of the regression line protein describing the realationship between protein concentration of each cultivar and the average protein concentration of all cultivars in each environment (as suggested by Finlay and Wilkinson 1963) were computed using the "regression analysis" tool in Excel software, which calculates:

$$b_i = \frac{\sum (x_{ij} - \bar{x}_i.)(y_{ij} - \bar{y}_i.)}{\sum (\bar{x}._j - \bar{x}..)^2}$$

$$a_i = y_i. + b_i * x_i.$$

- number of cases when grain protein concentration was below the 12% minimum level, generally required by bread-making industry.

Correlation analysis was used to study the relationship between average protein concentration and stability parameters, as well as between studied stability parameters.

RESULTS AND DISCUSSION

The Grain protein concentration (GPC) varied from 8.5% in cultivar Apache grown at Fundulea under organic agriculture system in

[1] The author is grateful to Pompiliu Mustăţea and Ion Toncea (NARDI Fundulea), Gabriela Păunescu and Gabriel Păunescu (ARDS Simnic), Maria Voica (ARDS Albota) and Ioana Stere (ARDS Dobrogea) for providing yield data and samples for protein analyses.

2008 to 19.0% at Fundulea in cultivar Exotic grown at Fundulea in 2009 under conventional system with adequate N fertilization.

ANOVA, showed that, when tested against the interaction between cultivars and environments, both the effect of environments and the effect of cultivars were significant (Table 1). Environments had the largest effect, as the location averages of all tested cultivars varied from 9.9% at Fundulea under organic management in 2008 to 16.7% at Fundulea under conventional management with adequate N fertilization in 2009. The cultivars protein concentration averaged over all locations only varied from 11.2% in Trivale to 13.1% in Şimnic 50 (Figure 1).

Table 1. ANOVA for grain protein concentration of 23 wheat cultivars in 30 environments

Source of variation	SS	df	MS	F	F crit	P-value
Cultivars	131.7	22	5.99	10.64	1.56	<0.001
Environments	1990.2	29	68.63	121.93	1.49	<0.001
Interaction Cultivars*Environments	359.1	638	0.56			
Total	2481.0	689				

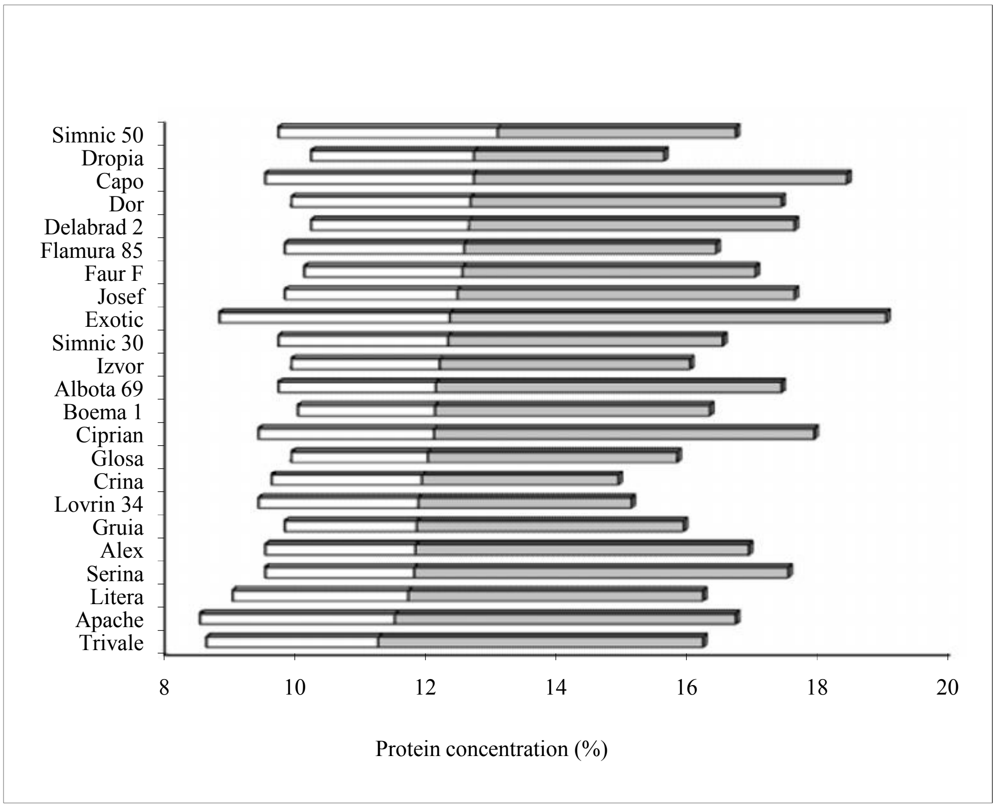


Figure 1. Protein concentration average and amplitude in 23 winter wheat cultivars

Part of the differences between cultivars in GPC was due to the well known negative correlation with grain yield. This correlation was significant in 15 out of 30 environments included in this study and on average over all environments (Figure 2). The highest GPC was found in the cultivar Şimnic 50, which had the lowest average yield, and the highest yielding cultivar (Apache) had the second lowest GPC. Only Exotic and Faur F had higher than average both GPC and yield. However, several cultivars (including Glosa, Izvor, Delabrad 2 etc.) were placed above the regression line, indicating a higher GPC than expected based on their average yield. Oury et al. (2007) recommended the use of such deviations from the regression line to identify favourable genotypes in breeding.

The amplitude of GPC variation was very large, but varied among cultivars, from under 6% in the Romanian cultivars Crina, Dropia, Lovrin 34 and Glosa to over 10% in the French cultivar Exotic. This suggests that there are large differences between cultivars in the environmental stability of grain protein concentration (Figure 1 and Table 2).

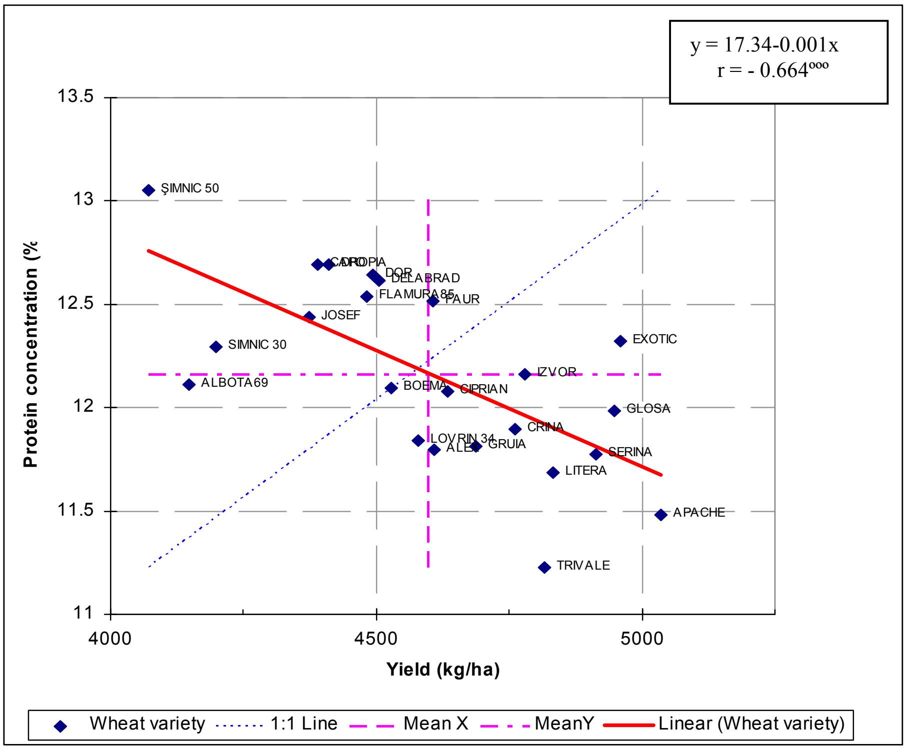


Figure 2. Relationship between grain yield and grain protein concentration averaged over 30 environments, in 23 winter wheat cultivars

The coefficient of variation for protein concentration of the cultivars across environments, also varied largely, from 12.4% in the cultivar Crina to 20.1% in the cultivar Exotic. The coefficient of variation was not correlated with the average protein concentration, and several cultivars (such as Şimnic 50, Dropia, Delabrad 2 etc.) combined above average protein concentration and good stability, expressed as below average coefficient of variation (Figure 3). It is interesting to mention that all studied foreign cultivars had above average coefficients of variation. This suggests that these cultivars have lower adaptability to the environments where they were tested in this study.

Regression of the values of each cultivar vs. the average value of all cultivars in the trial was suggested as an index of stability (Finlay and Wilkinson, 1963). Keim and Kronstad (1979) described a genotype as adapted to unfavorable environments when the regression coefficient b <1 and a (the regression intercept) is large, as adapted to favorable conditions when b >1, and as widely adapted when b ≥1 and a is large. Mustăţea et al. (2009) also found that regressions according to Finlay and Wilkinson were more useful in detecting differences in response to environment favorability than in estimating the stability of cultivars.

The cultivars included in this study varied widely both in the regression coefficients b and in the regression line intercept a (Table 2).

Table 2. Average grain protein concentration and some indices of its stability in 23 winter wheat cultivars

Cultivar	Average protein concentration	Amplitude	Coefficient of variation %	Finlay-Wilkinson regression		Number of cases below 12% protein
				Intercept *a*	*b*	
Şimnic 50	13.05a	7.0	13.02	3.27±1.31	0.804±0.11	8
Dropia	12.69ab	5.4	12.91	2.31±0.96	0.853±0.08	13
Capo	12.69ab	8.9	19.86	-3.20±1.51	1.306±0.12	14
Dor	12.64 bc	7.5	14.48	0.33±0.73	1.012±0.06	12
Delabrad 2	12.62 bc	7.4	14.40	0.34±0.69	1.010±0.06	14
Flamura85	12.54 bc	6.6	12.56	2.43±0.87	0.831±0.07	10
Faur F	12.52 bcd	6.9	15.23	-0.27±0.78	1.051±0.06	13
Josef	12.47 bcde	7.8	15.71	-0.68±0.80	1.081±0.07	17
Exotic	12.32 bcdef	10.2	20.10	-4.30±1.01	1.366±0.08	16
Şimnic 30	12.29 cdef	6.8	16.01	-0.82±0.98	1.063±0.08	16
Izvor	12.16 cdefg	6.1	13.32	2.06±1.01	0.831±0.08	16
Albota69	12.11 defgh	7.7	16.18	-0.19±0.92	0.985±0.07	19
Boema 1	12.09 defgh	6.3	13.44	0.97±0.51	0.915±0.04	17
Ciprian	12.08 fgh	8.5	16.82	-1.06±1.08	1.080±0.09	18
Glosa	11.98 fghi	5.9	13.43	1.34±0.74	0.875±0.06	18
Crina	11.89 ghi	5.3	12.43	2.41±0.82	0.780±0.06	17
Lovrin 34	11.84 ghij	5.7	13.79	1.41±0.93	0.857±0.08	18
Gruia	11.81 ghij	6.1	14.19	0.66±0.74	0.917±0.06	17
Alex	11.80 ghij	7.4	15.12	-0.57±0.72	1.058±0.06	20
Serina	11.78 hij	8.0	17.21	-1.92±0.77	1.126±0.06	20
Litera	11.69 ij	7.2	15.43	0.04±0.97	0.957±0.08	19
Apache	11.48 jk	8.2	18.21	-2.69±0.76	1.165±0.06	19
Trivale	11.23 k	7.6	17.96	-1.88±1.04	1.077±0.09	19

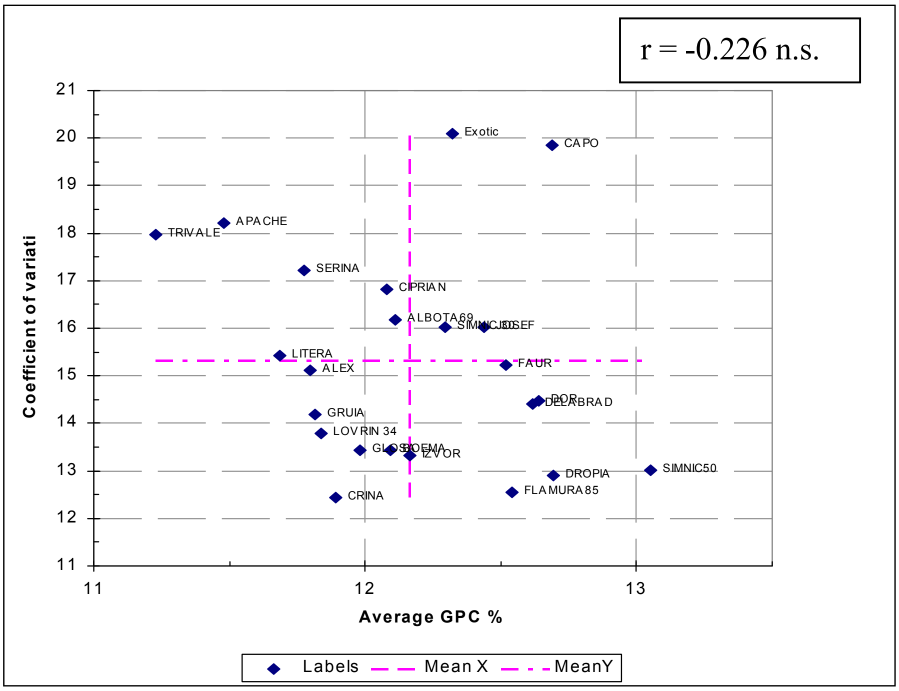
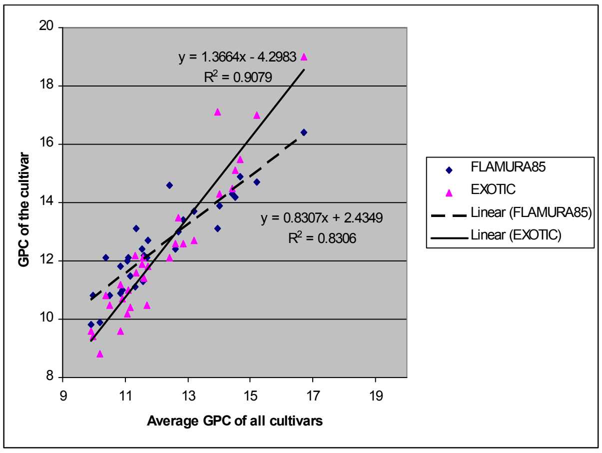


Figure 3. Relationship between average protein concentration and the coefficient of variation of protein concentration in 23 wheat cultivars

Several cultivars, such as Şimnic 50, Dropia, Flamura 85, Izvor, Glosa and Lovrin 34, had *b* values below 0.9 and positive intercepts, suggesting a better response in environments conducive to low GPC. In contrast, cultivars such as Capo, Exotic, Apache and Serina, had *b* values above 1.1 and negative intercepts, suggesting that they responded better to environments that were favorable to higher protein concentration in the grains. This favorability could have been due to better nitrogen availability, but also to less favorable conditions for grain filling.

Figure 4 illustrates the different response of two pairs of cultivars to the favorability of the environment from the point of view of grain protein concentration.



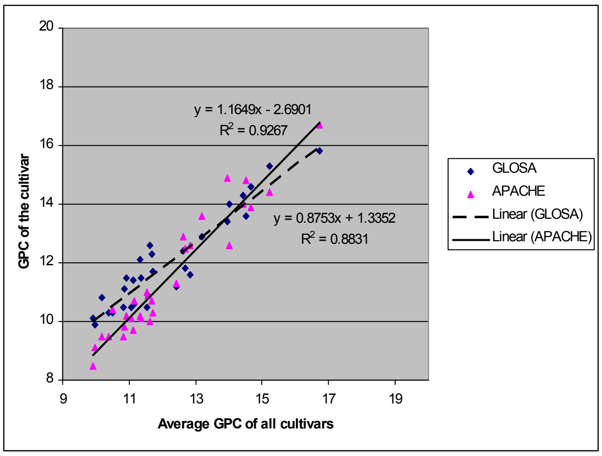


Figure 4. Comparison between regression lines of protein concentration vs. average protein content of the trial in two pairs of cultivars

Only few cultivars, such as Dor and Delabrad, with *b* close to 1 and positive intercepts, seem to corespond to Keim and Kronstad definition of wide adaptation.

From the point of view of a farmer, GPC stability would mean, first of all, to have as few as possible situations when the grain protein concentration falls below the limit required by the market. We took into consideration a limit of 12%, as desirable for bread making, and found that the number of cases below this limit varied from 8 to 20 (Table 2). The number of cases with GPC below the 12% limit closely correlated with the average GPC (r = -0.847^{oo}), but some cultivars with similar averages had different number of cases below the required level (Figure 5). For example the cultivar Flamura 85, with an average GPC of 12.54% had only 10 cases below 12%, while cultivar Capo, with a higher average GPC (12.69%), had 14 results below the same limit.

Among stability indices, only the frequency of cases with GPC below 12% was significantly correlated with the average GPC, the frequency of cases below a certain limit decreasing as the average GPC increases (Table 3). Lack of correlation of average GPC with other measures of stability suggests that breeding for increased GPC could be combined with breeding for increased GPC stability, as expressed by the amplitude of variation and the coefficient of variation, or with a specific response of GPC to favorability of the environment, as expressed by the regression parameters.

The amplitude of variation was positively correlated with the coefficient of variation. Both were positively correlated with *b*- the slope of regression, and negatively with *a*- the intercept of regression (Table 3). The intercept was negatively correlated with the slope of the regression line. A weaker negative correlation was found between the intercept of the regression line and the number of cases below the limit of 12% GPC.

Table 3. Correlations between average GPC and several indices of GPC stability

Indices	Average GPC	Amplitude of variation	CV%	Regression	
				Intercept	*b*
Average GPC	1.00				
Amplitude of variation	0.03	1.00			
Coefficient of variation CV%	-0.23	**0.88**	1.00		
Finlay-Wilkinson regression: - Intercept	0.29	**-0.86**	**-0.97**	1.00	
- *b*	-0.07	**0.90**	**0.95**	**-0.97**	1.00
Frequency of cases below 12% GPC	**-0.85**	0.12	0.35	**-0.44**	0.26

Bold correlation coefficients are sigificant at P<0.05.

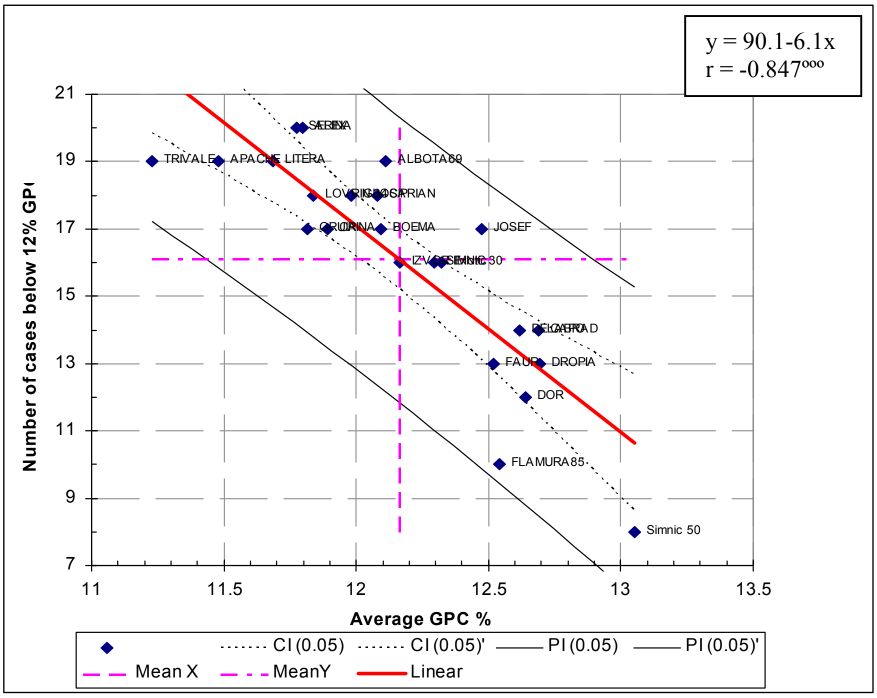


Figure 5. Relationship between average protein concentration and the number of cases with protein concentration below 12%, in 23 wheat cultivars

CONCLUSIONS

Grain protein concentration was mainly determined by the environment, but average differences between cultivars were significant when tested against the genotype*environment interaction.

More than 40% of the GPC variation between cultivars could be explained by the variation of grain yield. Two cultivars (Exotic and Faur) had both GPC and yield higher than the average, and several cultivars (including Glosa, Izvor, Delabrad etc.) had a higher GPC than expected based on their average yield. This suggests some possibilities in breeding for both grain yield and protein concentration.

The tested cultivars were different for GPC stability, as expressed by the amplitude and the coefficient of variation. Romanian cultivars showed better GPC stability than the tested foreign cultivars. Neither the amplitude of variation, nor the coefficient of variation was correlated with average GPC, indicating possibilities in breeding for both higher and more stable GPC.

Regressions of each cultivar GPC on average GPC of all cultivars showed large differences among cultivars in their response to the favorability of environments for protein accumulation in the grains. Cultivars could be classified as more responsive to environments that were favorable to higher protein concentration in the grains (such as Capo, Exotic etc), or more able to maintain GPC in less favorable environments (such as Şimnic 50, Dropia, Flamura 85, Izvor, Glosa and Lovrin 34).

Frequency of GPC value below an accepted minimum can be important from farmer's point of view. The number of cases with GPC below a 12% limit closely correlated with the average GPC (r = -0.847^{ooo}), but some cultivars with similar averages had different number of cases below the required level, indicating additional possibilities of progress.

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