# GRAIN YIELD AND PROTEIN CONCENTRATION IN WINTER WHEAT CULTIVARS TESTED WITH AND WITHOUT NITROGEN FERTILIZER

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## ABSTRACT

Market prices and environmental concerns favor low-input wheat production systems and lower N fertilizer doses. To detect possible genetic differences regarding the adaptation to N deficiency conditions, we analyzed grain yield (GY) and grain protein concentration (GPC) data from yield tests with winter wheat cultivars, organized by NARDI Fundulea in Romania, in 40 very diverse environments (locations x years), with and without N fertilizer application in spring.

Correlation between cultivar performance with and without N fertilizer for both GY and GPC was highly variable, depending on the environment. The strength of the correlation between N fertilized and not fertilized plots decreased with increasing N stress, estimated by the difference due to fertilization, for both GY and GPC. The cultivar effect on differences between plots with and without N fertilizer was significant for both yield and protein concentration, when tested against G x E interaction and only a small part of this effect was associated with the potential expressed under N fertilization conditions. Significant deviations from the general regression of performance under N deficiency stress against performance in N fertilized plots were detected in some cultivars for both GY and GPC. These deviations were too small to be of immediate practical importance, but might be useful in breeding for cumulating genes favorable in low N conditions.

The relationship between GPC and GY was negative and significant under N fertilization, but not significant under N deficiency. This suggests that, under N deficiency stress, differences in dilution of proteins by carbohydrates accumulation play a less important role than differences in other mechanisms (like N absorption or translocation) in determining genotypic variation of grain composition.

Our results suggest that, in breeding for low input conditions, direct selection in N deficiency conditions could be more effective than selection in non-stress conditions for GPC, but probably not for GY.

Key words: grain yield, grain protein concentration, nitrogen deficiency, wheat.

## INTRODUCTION

Modern wheat cultivars were generally bred under intensive crop management, including recommended doses of N fertilizer. Presently, market prices and environmental concerns favor low-input wheat (*Triticum aestivum* L.) production systems and lower N doses. In some cases farmers cannot afford fertilizers or fertilizers may not be available.

This raises the question of breeding cultivars adapted for conditions where less or no N fertilizer is applied.

Adaptation to both high and low fertility situations is considered a key component of breeding at CIMMYT (Van Ginkel et al., 2001), as well as at many other wheat breeding programs. Ortiz-Monasterio et al. (1997) found better expression of N uptake efficiency under low N conditions and better N utilization efficiency under high N conditions. Therefore, alternating selection in high and low N conditions was considered the best route to producing wheats adapted to a range of conditions (Van Ginkel et al., 2001).

Low N conditions negatively affect both grain yield and grain protein concentration. Grain protein concentration is one of the main factors determining differences in baking quality, as this trait directly influences water absorption of the flour and loaf volume. Because of that, bread-making industry and wheat markets have definite grain protein concentration requirements. Meeting these requirements is becoming increasingly difficult, because limitation of nitrogen fertilizer use, for ecological and economical reasons, reduces grain protein concentration.

To address these problems, yield tests with wheat cultivars and new lines, organized by NARDI Fundulea in several locations in Romania, have been performed during the last years both with and without N fertilizer application in spring. This paper analyzes some of the results obtained.

### MATERIAL AND METHODS

Grain yield (GY) data from winter wheat cultivar yield trials, performed from 2002 to 2007 in 12 locations situated from 40° 59' to 47° 51' latitude and 20° 44' to 28° 29' longitude, totaling 40 environments (site x year combinations), each with and without N fertilizer, were used in this study.

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Location included fertile soils and favorable average weather conditions for wheat growing (Teleorman, Caracal, Valu lu Traian, Fundulea – irrigated and non irrigated – Oradea and Tg. Mureş – non irrigated), as well as less fertile soils and less favorable average weather conditions (Albota, Livada, Braşov, Perieni, Secuieni, Suceava).

Preceding crops (peas, soybeans, and potatoes) favored good fertility of the soil and crop management was according to recommendations.

Weather conditions were very diverse, from the very dry year 2003, to favorable ones (2002 and 2007).

Nine cultivars were tested in all trials, and were used in this study.

Grain samples were collected and grain protein concentration (GPC) was determined in the NARDI Fundulea laboratory using a Perten Inframatic 9100 infrared analyzer.

GY and GPC data were analyzed using ANOVA, correlation and regression analyses.

### RESULTS

Environmental conditions varied very much among trials, as shown by amplitude of GY trials average from 2402 to 9633 kg/ha in fertilized trials and from 2068 to 7121 kg/ha 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 (Table 1).

*Table 1*. Average values and amplitude of GY and GPC in 40 yield trials with and without N fertilizer

	Fertilized with N	Without N fertilization	Difference
Average yield	5398	4244	973
(kg/ha)			
Maximum yield	9633	7121	2512
(kg/ha)			
Minimum yield	2402	2068	334
(kg/ha)			
Average protein	13.45	12.27	1.18
concentration (%)			
Maximum protein	17.37	16.90	0.47
concentration (%)			
Minimum protein	10.72	10.03	0.69
concentration (%)			

Difference between N fertilized and non fertilized plots averaged 973 kg/ha (18.0% from fertilized plots) for GY, and 1.18% (8.8% from fertilized plots) for GPC, but differences due to N fertilization varied considerably among environments, from -1238 to 3599 kg/ha for grain yield and from -0.4 to 3.6% for GPC. The few trials where yield was higher without N fertilizer were affected by lodging and/or diseases, which affected more the N fertilized plots.

Average yield differences between plots with and without N fertilizer were higher in favorable environments, correlation between average yield differences and average yield in fertilized plots being significant (r = 0.57\*\*). In contrast, average GPC differences between N fertilized and not fertilized plots were not correlated with the GPC average of plots neither with nor without N fertilizer.

Correlation coefficients between yields of the nine cultivars tested with and without N fertilizer varied considerably among environments, from -0.11 to 0.92, only half of coefficients being significant (Figure 1). However, the correlation between yields of cultivars with and without N fertilizer averaged across environments, was very high and highly significant (0.89\*\*\*).

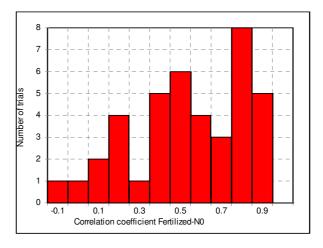
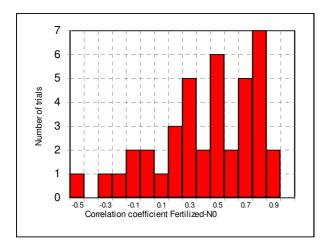


Figure 1. Variation of correlation coefficients between grain yields with and without N fertilization in nine wheat cultivars (40 environments)

Correlation coefficients between GPC of the nine cultivars tested with and without N fertilizer also varied considerably among environments, from -0.44 to 0.92, less than half of

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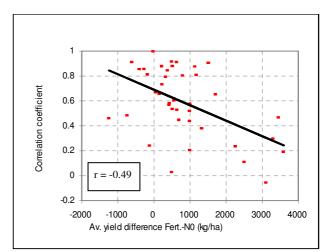
coefficients being significant (Figure 2). Correlation between GPC of the nine cultivars averaged across all environments however was highly significant (0.74\*\*\*).



*Figure 2.* Variation of correlation coefficients between grain protein concentrations with and without N fertilization, in nine wheat cultivars (40 environments)

Brancourt-Hulmel et al. (2005) found that correlations between N fertilized and N deficient plots in 10 experiments were always positive ranging from 0.10 to 0.95 for grain yield and from 0.78 to 0.98 for grain N concentration. The larger variation of the correlation between averages of plots with and without N fertilizer, found in our study, suggests that, in many environments, both GY and GPC were affected more by other factors than N availability.

As seen in figures 3 and 4, the strength of the correlation between N fertilized and not fertilized plots was negatively associated with the difference due to fertilization ( $r = -0.49^{**}$ for GY and  $r = -0.43^{**}$  for GPC). When the soil provided sufficient nitrogen or when other factors limited crop requirements for N, yield differences between fertilized and not fertilized plots were smaller and correlation between plots with and without N fertilizer was higher. Similar results were obtained by Bänziger et al. (1997) in maize (Zea mays L.), who found that the genetic correlation between GY under low and high N levels decreased with increasing N stress, estimated by the relative yield reduction.



*Figure 3.* Relationship between average yield differences between N fertilized and not fertilized plots and correlation coefficients between yields of nine wheat cultivars, with and without N fertilization

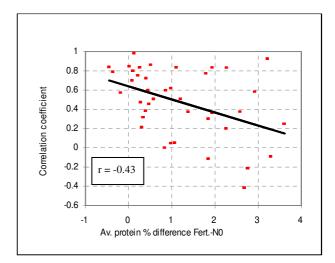


Figure 4. Relationship between average grain protein concentration difference between N fertilized and not fertilized plots and correlation coefficients between GPC of nine wheat cultivars, with and without N fertilization

In order to detect differential genotypic responses to lack of N fertilization, it is necessary to reduce the influence of other factors which can mask the response to N fertilization. To obtain this, we selected for further analysis 20 environments, where average yield difference due to fertilization was positive and significant and correlation between fertilized and not fertilized plots was not significant.

In this subset of yield trials the cultivar effect on differences between plots with and without N fertilizer was significant for both yield and protein concentration, when tested against G x E interaction (Table 2).

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*Table 2.* ANOVA for differences between plots with and without N fertilizer in 20 environments

Source of Variation	df	MS	F	P-value	
Grain yield					
Cultivars	8	624542.7	1.999	0.05	
Environments	19	23418021	74.967	< 0.001	
G x E interaction	152	312377			
Grain protein concentration					
Cultivars	8	0.6741	2.65	0.009	
Environments	19	10.5033	41.42	< 0.001	
G x E interaction	152	0.2535			

Differences between yields with and without N fertilizer varied from 1330 kg/ha in cultivar Bezostaya 1 to 1912 kg/ha in cultivar Delabrad (Table 3) and several cultivar ranks significantly changed at the two levels of N availability. For example, the highest yielding cultivar in fertilized plots (Glosa) was only on fourth position without fertilization, but Gruia, which was the fourth with N fertilization, was the highest yielding in not fertilized plots.

*Table 3.* Grain yield and rank of nine wheat cultivars tested with and without N fertilizer

	N fertilized		Without		Difference		%
			N fertilizer				with-
Cultivar	Kg/ha	Rank	Kg/ha	Rank	Kg/ha	Rank	out N
							/with
							Ν
Glosa	5932	1	4037	4	1895	-3	68.1
Faur	5916	2	4059	2	1857	0	68.6
Fundulea 4	5847	3	4032	5	1815	-2	69.0
Gruia	5833	4	4126	1	1707	+3	70.7
Boema	5825	5	4051	3	1774	+2	69.5
Delabrad	5794	6	3882	8	1912	-2	67.0
Flamura 85	5664	7	4008	6	1656	+1	70.8
Crina	5650	8	3908	7	1742	+1	69.2
Bezostaya 1	4898	9	3568	9	1330	0	72.8
Average	5706		3963		1743		69.5

Differences in GPC due to N fertilization also varied, from 1.22% in cultivar Glosa to 1.73% in cultivar Faur (Table 4). There were fewer changes in ranking: Flamura 85 and Bezostaya 1 had highest GPC and Gruia and Fundulea 4 had lowest GPC at both levels of N availability. However Glosa, which had one of the lowest GPC with N fertilizer was third when N was not applied.

*Table 4.* Grain protein concentration and rank of nine wheat cultivars tested with and without N fertilizer in 20 environments

		ilized Without N fertilizer		Difference		wittii-	
Cultivar	%	Rank	%	Rank	%	Rank	out N /with N
Bezostaya 1	13.45	1	11.72	2	1.73	-1	87.1
Flamura 85	13.25	2	11.97	1	1.28	+1	90.3
Faur	13.20	3	11.47	6-7	1.73	-3.5	86.9
Delabrad	13.00	4	11.48	5	1.62	-1	87.7
Crina	12.97	5	11.47	6-7	1.50	-1.5	88.4
Boema	12.91	6	11.55	4	1.36	+2	89.4
Glosa	12.79	7	11.57	3	1.22	+4	90.5
Gruia	12.77	8	11.34	8	1.43	0	88.8
Fundulea 4	12.69	9	11.15	9	1.54	0	87.9
Average	13.02		11.53		1.49		88.6

Smallest yield difference between fertilized and not fertilized plots were found in lower yielding cultivars (Bezostaya 1 and Flamura 85), but only 24% of the variation of differences among cultivars was explained by the variation in yielding potential.

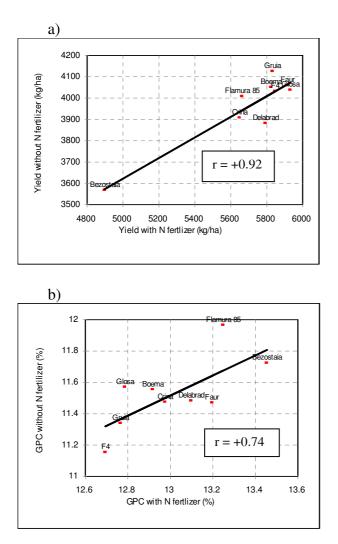
GPC differences between fertilized and not fertilized plots were not associated with GPC in either N fertilized or unfertilized plots.

Plotting grain yields under N deficiency stress against yields in N fertilized plots shows significant deviation from the general regression in some cultivars (Figure 5 a and Table 5). Largest deviations were found in Gruia (positive) and Delabrad (negative).

*Table 5.* Deviations of GY and GPC from the regression between fertilized and not fertilized plots, in nine wheat cultivars

Cultivar	Deviations from the regression fertilized - non fertilized			
	Yield	Protein concentration		
Flamura 85	65	0.29*		
Fundulea 4	1	-0.17*		
Boema	30	0.09		
Crina	-28	-0.02		
Delabrad	-124*	-0.10		
Faur	-5	-0.17*		
Glosa	-35	0.19*		
Gruia	101*	-0.03		
Bezostaya 1	-5	-0.08		

\*) significant at P<0.05

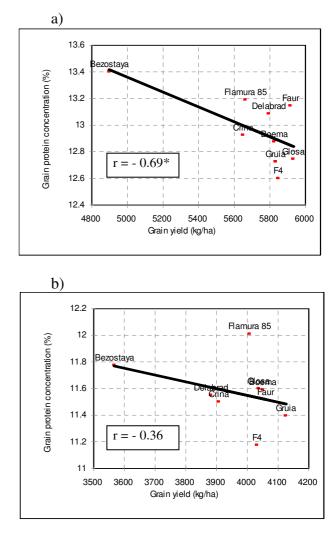


*Figure 5.* Relationship between GY (a) and GPC (b) in fertilized and not fertilized plots

Significant deviations from regression were also found for GPC, with Flamura 85 and Glosa having the largest positive deviations, while Fundulea 4 and Faur had the largest negative ones (Figure 5 b and Table 5). However, deviations from regression, are small and of little practical importance, both for GY and GPC.

Grain protein concentration is negatively correlated with grain yields (Triboi et al., 1990; Feil, 1997; Oury and Godin, 2007; Marinciu and Săulescu, 2008). Therefore, an analysis of GPC variation is only meaningful in relation with GY variation.

Figure 6 shows that the relationship between GPC and GY was negative and significant under N fertilization, but not significant under N deficiency. Flamura 85 shows large positive deviations, while Fundulea 4 shows



*Figure 6.* Relationship between GPC and GY in N fertilized (a) and not fertilized (b) plots

large negative deviations, both with and without N fertilizer.

## DISCUSSION

Both GY and GPC are complex traits, reflecting the interaction between genotype and a multitude of environmental factors acting during the vegetation period. As a result, correlation between cultivar performance with and without N fertilizer was highly variable, the effect of N fertilization being sometimes masked by other factors affecting plant N requirements or N availability from the soil.

Selecting environments with larger yield differences and lower correlation between fertilized and not fertilized plots was effective in reducing the influence of masking factors and revealing specific genotypic responses. Average GY reduction due to N deficiency was larger than the reduction in GPC (30.5% from the average of fertilized plots for GY versus 11.4% for GPC), but correlation between fertilized and not fertilized plots was higher for GY than for GPC. This suggests that, although chances of finding specific cultivar responses to N availability are higher for GPC, possibilities of significant progress are small.

The negative correlation between GY and GPC was significant in fertilized trials, but not significant under N deficiency. This suggests that, under N deficiency stress, differences in dilution of proteins by carbohydrates accumulation play a less important role than differences in other mechanisms (like N absorption or translocation) in determining genotypic variation of grain composition.

Differential cultivar responses to N deficiency, as expressed by deviations from the general regression of not fertilized on fertilized plots, although some statistically significant, were too small to be of practical interest. Obviously, wider genetic diversity should be explored to increase chances of progress in adaptation to low N conditions that might have an impact at farm level.

On the other hand, the small differences detected could be exploited for cumulating favorable genes in breeding programs for low input conditions.

Our results can be useful in assisting decisions on best strategy in breeding for low N conditions. Brancourt-Hulmel et al. (2005) found that, in breeding for adaptation to low N availability, indirect selection in high N environments was never more efficient than direct selection, and concluded that breeding programs targeting low-input environments should include low-input selection environments to maximize selection gains. On the other hand, in oats, according to Atlint and Frey (1989), indirect selection in high-N environments was predicted to be as effective as direct selection in producing yield gain in low-N environments. Our results suggest that direct selection in plots without N fertilizer could be more effective than selection in fertilized plots for GPC, but probably not for GY.

### CONCLUSIONS

Correlation between cultivar performance with and without N fertilizer for both grain yield and grain protein concentration was highly variable, depending on the environment.

The strength of the correlation between N fertilized and not fertilized plots decreased with increasing N stress, estimated by the difference due to fertilization, for both GY and GPC.

The cultivar effect on differences between plots with and without N fertilizer was significant for both yield and protein concentration, when tested against G x E interaction and only a small part of this effect was associated with the potential expressed under N fertilization conditions.

Significant deviations from the general regression of performance under N deficiency stress against performance in N fertilized plots were detected in some cultivars for both GY and GPC. These deviations were too small to be of immediate practical importance, but they might be useful in breeding for cumulating genes favorable for better response to low N conditions.

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