GENOTYPE AND NITROGEN FERTILIZATION INFLUENCE ON PROTEIN CONCENTRATION IN OLD AND NEW WHEAT CULTIVARS

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ABSTRACT

Protein concentration is a decisive component of bread-making quality in wheat. Reaching the protein concentration required by the industry is becoming an increasingly difficult task, because of the negative correlation with the yield level, and because of reduced nitrogen availability in organic or low-input agriculture systems. Thirteen winter wheat cultivars, including old and new cultivars and breeding lines, known as having different grain protein contents, were tested, with and without nitrogen fertilization in yield trials at NARDI Fundulea in 2004, 2005 and 2006. Weather conditions during the testing period were very diverse, as reflected in the variation of average yield of the trial from 2586 to 5737 kg/ha, and of average grain protein concentration from 11.70 to 14.87%. Correlation between protein concentration and yield was significantly negative only under nitrogen fertilization. This suggests that physiological mechanisms causing genotypic differences in protein content with and without nitrogen fertilization might be different. Old varieties had higher protein concentration in the grains, mostly under nitrogen fertilization, but yields were low. This makes them uninteresting for use in breeding for improved protein content. Positive deviations from the regression of protein concentration on yield were found, several lines combining relatively high yields and protein concentrations. Differences between protein contents with and without nitrogen fertilization were smaller in some new lines than in old cultivars. These lines might have a better nitrogen absorption ability and/or a better nitrogen use efficiency. The information obtained in this study might be useful in breeding new cultivars combining high protein concentration and high yield.

Key words: protein content, yield, nitrogen supply, wheat

INTRODUCTION

Grain protein content in wheat can vary from 6 to 25%, depending on growth conditions. Genetic variation of protein content is much smaller than that caused by differences in growing conditions, and this makes increasing protein concentration a difficult breeding goal (Blackman and Payne, 1987).

The major fact that determines the grain protein content is nitrogen availability. Higher protein content, in a crop with high yield, can be obtained just by application higher nitrogen quantities (Blackman and Payne, 1987).

On the other side, Pepó (2000) considers that it is important to identify the demand for nutritive elements and the genotypic responses in order to increase economic and agrochemical efficiency and to decrease the environmental damage.

Ittu (1982) estimates that for improving the protein content, selection should be made for high nitrogen uptake ability until anthesis and for extension of nitrogen uptake during the grain filling period. There is a strong negative relationship between grain protein content and grain yield. Breeders try to compensate this relationship by selecting combinations having both protein content and grain yield higher than the average.

Saulescu (1984) considers that the negative correlation yield - protein concentration can be broken by gradual accumulation of genes that contribute to higher nitrogen use efficiency. Transferring genes from *Triticum turgidum* ssp. *dicoccoides* could produce deeper changes in nitrogen metabolism efficiency.

Ginkel et al. (2000) suggested a methodology for selecting populations with improved nitrogen-use efficiency. They crossed lines with high nitrogen-uptake efficiency with lines with high nitrogen-translocation efficiency. The progenies were tested under different regimes of nitrogen supply. They concluded that nitrogen-uptake efficiency is much more correlated with yield and total biomass than nitrogen-translocation efficiency, at all levels of nitrogen supply. Nitrogen-uptake efficiency could better predict the total biomass, and there is probably more variability or genetic diversity for it.

According to Oury (2000), combining high yield with high protein content might be possible through the following ways: by selection programs based, mainly, on productivity, using agricultural practices which allow at the same time to com-

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pensate the reduction of protein content (a possibility can be the fertilization with nitrogen in the late vegetation stage) and to look for genotypes that deviate favorably from the general negative relationship between yield and protein concentration.

MATERIAL AND METHODS

Twelve cultivars were tested in 2004 and thirteen in 2005 and 2006 in yield trials at NARDI Fundulea, and protein content was determined. The following cultivars: Dropia, Fundulea 4, Odvos 241, Dacia, Delabrad, Izvor, 96869 G1-108, 93122 G6-201, 96257 G5-12 were tested in all three years. In addition to these, the cultivars: 98140 G1-1, 96831 G6-201, 00143 GP1 were tested in 2004; F26-70, 00368 GP3 (Junona), 00099 GP2, 00143 GP1 in 2005 and F26-70, Junona, 00099 GP2, 00356G3-2, in 2006. The trial was made at two levels of nitrogen supply: two replications with nitrogen fertilization and two replications without nitrogen fertilization in 2004, 2005 and four replications with nitrogen fertilization and four replications without nitrogen fertilization in 2006. A dose of 200 kg urea (93 kg N/ha) was divided in two applications: in winter on snow and in spring when vegetation began (March - April).

A randomized blocks design was used, the plot size being 5 square meters harvested.

The protein content was determined with the Perten Inframatic 9100 Analyzer.

Relationship between protein percentage and grain yield was analyzed using linear regression.

The data concerning temperature and rainfall regime during the years of testing, delivered by the Weather station of NARDI Fundulea, are presented in figures 1 and 2.

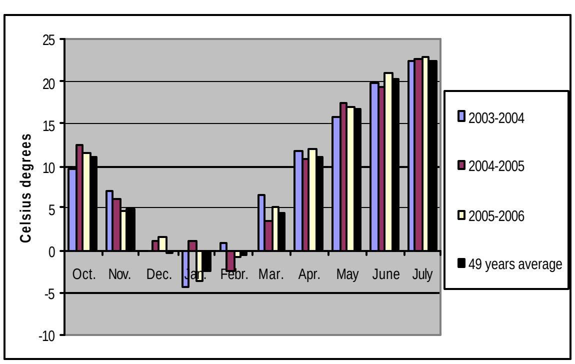


Figure 1. Average temperatures registered during October - July (2003-2006)

Generally, there are not large differences between multiannual average temperature values and the average monthly values of the testing years. Higher values were registered in December 2004 and 2005; a special case was observed in January 2005, when the monthly average was positive (+1.1°C), while the multiannual average was only -2.5°C, but the February 2005 temperature was much lower than the average.

Rainfall was much variable during the testing years. The rainfall was high in November (2004), May, June and July (2005), and very high in October 2005. Rainfall much under average was registered in February 2004 and 2006, March and April 2004 and 2005.

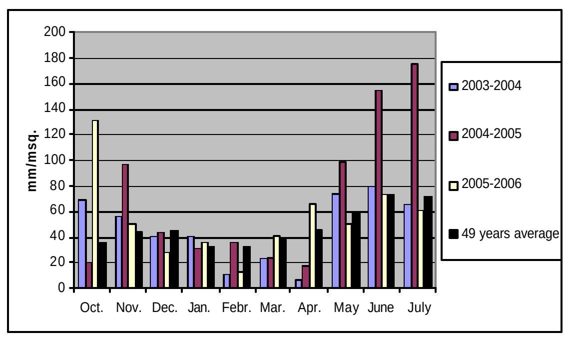


Figure 2. Monthly rainfall during October - July (2003-2006)

RESULTS AND DISCUSSION

The mechanisms that regulate grain yield and protein concentration are the availability of nitrogen, followed by the redistribution of nitrogen within the plant (Del Molino, 1992, cited by Bramble et al., 2002).

The conditions of testing are illustrated by level of yield and protein content with and without nitrogen (Table 1).

The year 2004 was unfavorable for the yield, the average yield with nitrogen fertilization being 3051 kg/ha and the highest yield 3672 kg/ha. Nitrogen was relatively accessible for protein storage within the kernel, the average protein content reaching 14.34%.

The most favorable year was 2006 when we observed that under fertilized conditions the average yield was 5737 kg/ha and the highest yield 7003 kg/ha. Also, the protein percentage was high. However, under unfertilized conditions we observed much smaller values of yield and protein percentages. That is due to a big level of nitrogen leaching due to high rainfall in the summer and the autumn of 2005.

Table 1. Average and extreme values of yield and protein content in three years of testing, in two conditions of nitrogen supply

Nitrogen fertilization	Years	Average yield (kg/ha)	Maximum yield (kg/ha)	Minimum yield (kg/ha)	Average % proteins	Maximum% proteins	Minimum% proteins
Fertilized	2004	3051	3672	1652	14.33	16.20	12.70
Fertilized	2005	3946	4700	1890	14.87	16.80	13.10
Fertilized	2006	5737	7003	3789	14.83	17.97	13.52
Unfertilized	2004	2586	3747	1606	12.15	13.30	10.95
Unfertilized	2005	3526	4370	2420	13.31	15.90	11.80
Unfertilized	2006	2725	3481	2095	11.70	14.20	10.62

In the field differences in color between fertilized and unfertilized plots were easily noticed.

1. Protein content of cultivars

According to Collaku et al. (2002), genotype influences protein content on average with 20% of total variability, varying from 8.6% to 10.6%, the environment influences with 54% and the interaction genotype-location influences phenotypic variation of protein content with 6%.

We analyzed protein content of cultivars tested in the three years, under two conditions of nitrogen supply.

Under fertilized conditions, in 2004, the old cultivars Odvos 241 and Dacia were noticed for the high protein content (significantly superior to the average value of the trial). The cultivars Izvor (95948 G1-4), Fundulea 4 and 96257 G5-12 had the lowest protein content (significantly lower than the average value of the trial).

Under unfertilized conditions the cultivars Dropia and 00143 GP1 had significantly higher protein content. The cultivar Delabrad had the smallest protein content significantly inferior the trial average (Figure 3).

We observed a variation of differences between protein content with and without nitrogen fertilization. Cultivars Odvos 241, Dacia and Delabrad had the largest differences, while the smallest differences of protein content between fertilized and unfertilized plots were found in cultivars Dropia and 00143 GP1. These two cultivars had a protein content of more than 13% even without nitrogen fertilization (Table 2).

Table 2. Average values of protein content, in 2004, under fertilized and unfertilized condition and the effects of nitrogen fertilization

Cultivar	Unfertil-ized	Fertilized	Differ-ence
Dropia	13.30	14.15	0.85
Fundulea 4	11.80	13.25	1.45
Odvos 241	12.45	16.15	3.70
Dacia	12.60	16.20	3.60
Delabrad	10.95	14.70	3.75
96869 G1-108	11.30	14.70	3.40
93122 G6-201	11.70	14.70	3.00
95948 G1-4	11.45	12.70	1.25
96257 G5-12	12.15	13.60	1.45
98140 G1-1	12.45	14.10	1.65
96831 G6-201	12.55	13.85	1.30
00143 GP1	13.15	13.95	0.80

In 2005, under nitrogen fertilization, cultivars Odvos 241, Dacia and F26-70 had significantly higher protein content, while the protein concentration of cultivars 00143 GP1, F4 and Izvor (95948 G1-4) was significantly lower. Under unfertilized conditions the cultivar F26-70 had the highest protein content and cultivar Fundulea 4 had significantly lower protein content (Table 3).

Differences between protein contents with and without nitrogen fertilization in cultivars Odvos 241 (by 2.95%) and Dacia (by 2.80%) were the largest.

Table 3. Average values of protein content, in 2005, under fertilized and unfertilized conditions and the effect of nitrogen fertilization

Cultivar	Unfertil-ized	Fertilized	Differ-ence
Dropia	12.80	14.35	1.55
Fundulea 4	11.80	13.70	1.90
Odvos 241	13.85	16.80	2.95
Dacia	13.45	16.25	2.80
Delabrad	12.65	14.50	1.85
96869 G1-108	13.40	14.95	1.55
93122 G6-201	13.30	15.25	1.95
95948 G1-4	12.50	13.10	0.60
96257 G5-12	13.10	14.75	1.65
00143 GP1	14.20	13.95	-0.25
00368 GP3	13.40	14.60	1.20
00099 GP2	12.70	14.35	1.65
F26-70	15.90	16.70	0.80

The cultivar F26-70 had a high protein percent in both conditions of nitrogen supply. Similar to the results obtained in 2004, the cultivar 00143 GP1 had almost the same protein content in the both nitrogen supply conditions (Figure 3).

Several new lines like: 96869 G1-108, 93122 G6-201, 96257 G5-12, 00368 GP3 (Junona) were able to accumulate, even under unfertilized conditions, a protein content at the level of requirements for bread-making.

In 2006, under nitrogen fertilization, the highest protein content was also found in the cultivar F26-70, (17.97% very significantly higher than the trial average). In this year the cultivars Odvos 241 and Dacia had significantly higher protein content, too. Minimum value of protein content, of 13.52%, very significantly lower than the average of the trial, was found in cultivars Fundulea 4 and Junona. Cultivars Izvor and 00099 GP2 also had low protein content (Table 4). High protein contents without nitrogen fertilization were noticed in the lines F26-70 and 96257 G5-12. The lowest level of protein concentrations were observed in cultivars Fundulea 4 and Odvos 241.

Table 4. Average values of protein content, in 2006, under fertilized and unfertilized conditions and the effect of nitrogen fertilization

Cultivar	Unfertil-ized	Fertilized	Differ-ence
Dropia	12.10	14.75	2.65
Fundulea 4	10.62	13.52	2.90
Odvos 241	10.80	15.92	5.12
Dacia	11.18	16.22	5.05
Delabrad	11.12	14.47	3.35
93122 G6-201	12.35	14.70	2.35
96869 G1-108	12.25	14.87	2.62
Izvor	11.20	13.67	2.47
Junona	10.97	13.52	2.55
00099 GP2	11.27	14.05	2.77
00356 G3-2	11.22	14.57	3.35
F26-70	14.20	17.97	3.77
96257 G5-12	12.85	14.45	1.60

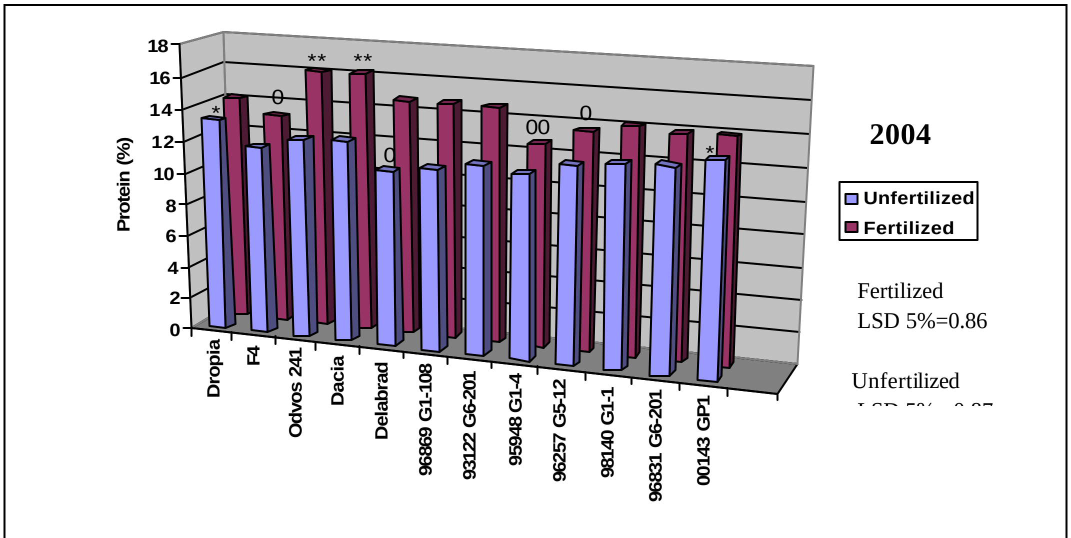
In 2006 year large differences in protein content between fertilized and unfertilized were observed in almost all cultivars, but the highest differences were found in cultivars Odvos 241 and Dacia (Figure 3).

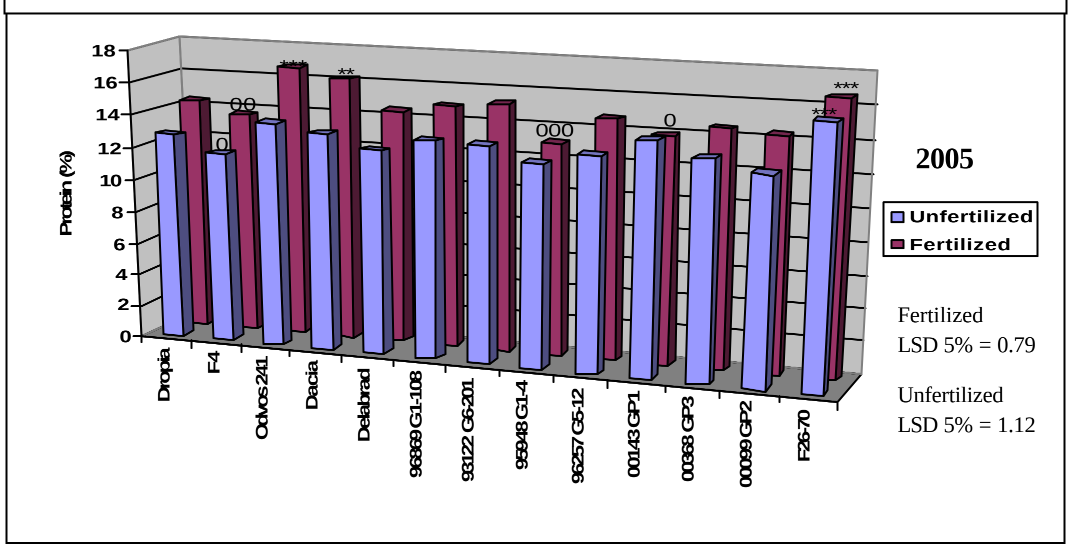
Ittu (1982) showed that the line F26-70 had the highest nitrogen concentration in the leaves among the studied lines. More over, the line F26-70 combined a high protein level with a high harvest index (ratio between grain yield and total biomass yield).

One can notice that the studied cultivars had a different behavior in different years, the protein content being strongly influenced by the environment.

However, in the three years, under nitrogen fertilization, the old cultivars Odvos 241 and Dacia, and also the line F26-70 in 2005 and 2006, had consistently shown high protein content. The cultivars Izvor and Fundulea 4 had in all years the smallest protein content under nitrogen fertilization.

The cultivars, like Dropia and 00143GP1, which also had high protein concentration without nitrogen fertilization, are interesting in breeding for grain protein content stability.





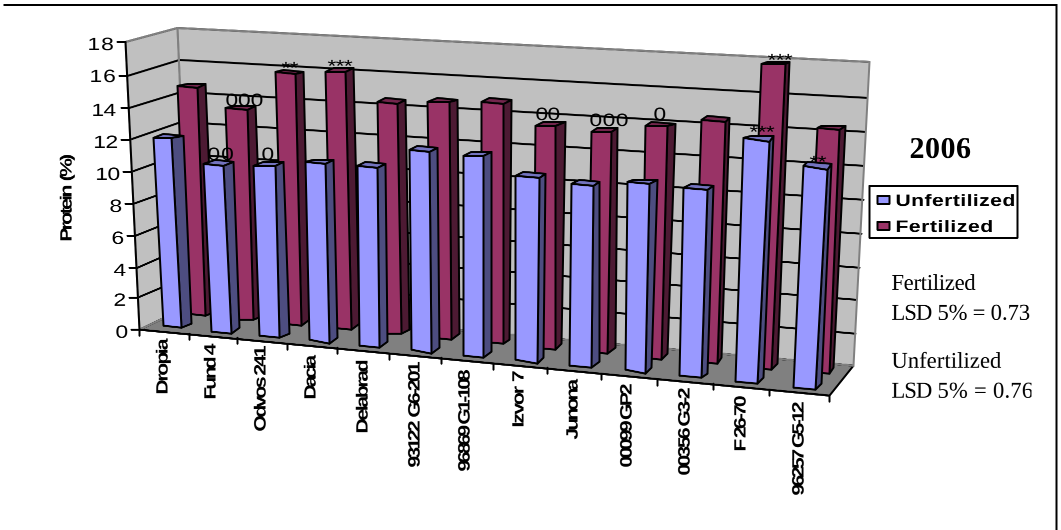


Figure 3. Protein content of cultivars tested in 2004, 2005 and 2006

2. Relationship between yield and protein percentage

The most difficult problem in breeding for high grain protein content is the correlation between grain yield and grain protein concentration. This negative correlation is caused by the fact that the most of yield is represented by carbohydrates (starch) and so the genotypes or the conditions that encourage obtaining high yields are bound to the capacity to accumulate higher amounts of starch in the grain, and that dilutes the protein content (Mustatea et al., 2005).

For this reason we analyzed the relationship between yield and grain protein content in the tested cultivars during the three experimental years, under the two conditions of nitrogen supply, and also the deviations from the general regression line (Figure 4).

In 2004 and 2006 grain yield and grain protein content were significantly correlated only under nitrogen fertilization.

In 2005, the relationship was not significant, both with and without nitrogen fertilization, but there was tendency of yield reduction with the increase of protein content.

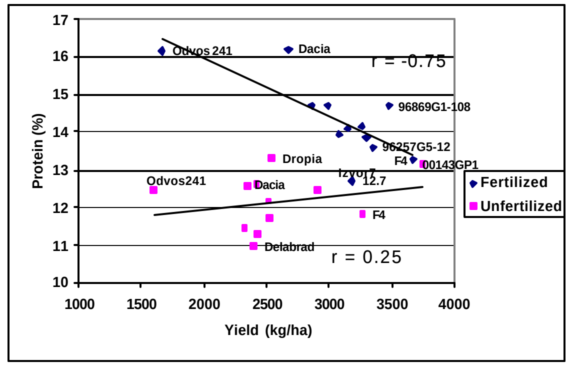
In the six testing conditions we noticed deviations from regression, both positive, and negative (Table 5).

F26-70 is the most stable cultivar in this respect, presenting positive deviations from the regression protein content-yield in all conditions.

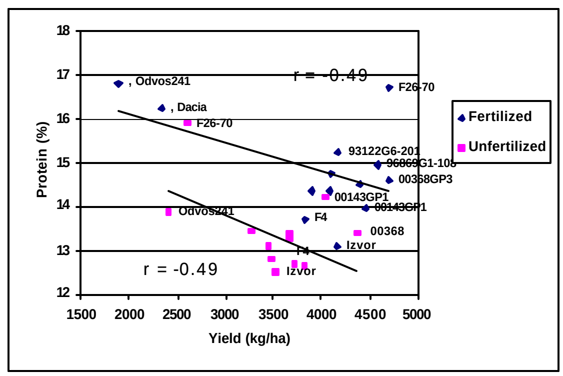
Fundulea 4 and Izvor (95948 G1-4) presented negative deviations from regression in most cases. The old cultivar Odvos 241, noticed for its high protein content, had low yield levels, being under the regression line in 4 out of 6 cases. The cultivar 96869 G1-108 had positive deviations from regression in all situations, the largest being in 2004 with nitrogen fertilization, when it showed both a high yield (about 3500 kg/ha when the average yield of the trial was by 3051 kg/ha) and a high protein percent (more than 14.5% at an average value of 14.34%).

The cultivar 00143 GP1 is remarkable for a relative stability despite the conditions of nitrogen supply. In 2004 under fertilization the cultivar 00143 GP1 is on the regression line but we notice that it had protein content higher than 14% and a yield level higher than 3000 kg/ha. In 2004 without nitrogen fertilization the same cultivar had a yield of about 4000 kg/ha and a protein content of more than 13% (the average values of the trial being 2586 kg/ha and 12.15%).

2004



2005



2006

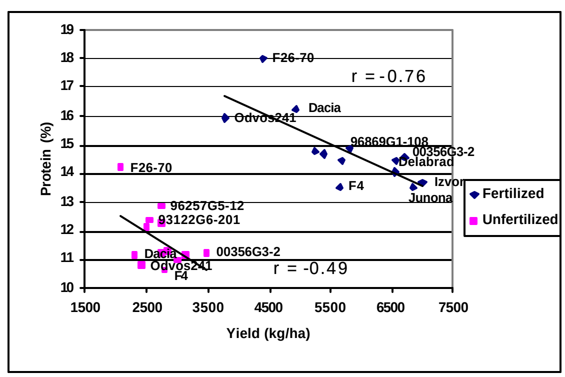


Figure 4. Relationship between grain yield and protein content in 2004, 2005 and 2006 with and without nitrogen fertilization

In 2005 with fertilization the cultivar 00143 GP1 is under the regression line, but with a protein content of more than 14% and an yield level about 4500 kg/ha. In 2005, without nitrogen fertilization, it presents obvious positive deviation from the regression, with protein content higher than 14% and a yield level of almost 4000 kg/ha.

Table 5. Deviations from the linear regression of protein content on yield

Cultivar	2004 fertilized	2005 fertilized	2005 unfertilized	2006 fertilized	2006 unfertilized
Dropia	0.12	-0.42	-0.54	-0.54	0.08
Fundulea 4	-0.13	**-1.24**	**-1.28**	**-1.38**	**-0.98**
Odvos 241	-0.32	**0.59**	**-0.49**	**-0.78**	**-1.32**
Dacia	**1.28**	0.34	-0.09	**0.62**	**-1.08**
Delabrad	0.25	-0.07	-0.37	0.45	-0.0021
96869 G1-108	**1.01**	**0.49**	0.32	0.13	**0.59**
93122 G6-201	0.06	0.52	0.11	-0.46	0.41
95948 G1-4	**-1.44**	**-1.62**	**-0.80**	0.07	**-0.48**
96257 G5-12	-0.28	-0.01	-0.28	-0.42	1.17
98140 G1-1	-0.09	-	-	-	-
96831 G6-201	-0.11	-	-	-	-
00143 GP1	-0.34	**-0.58**	**1.36**	-	-
00368 GP3 (Junona)	-	0.22	0.87	-0.23	-0.32
00099 GP2	-	-0.54	-0.43	0.0019	-0.25
F26-70	-	**2.32**	**1.72**	**1.86**	**1.64**
00356 G3-2	-	-	-	**0.67**	**0.54**

We find a similar situation in cultivar 00368 GP3 (Junona) in 2005. Under fertilization it reached a protein content of 14.6 % and a yield of almost 4700 kg/ha. Without nitrogen fertilization it maintained a high protein content of 13.4% and a grain yield of 4370 kg/ha, being above the regression line.

CONCLUSIONS

The storage of proteins and carbohydrates in the grain is influenced by environmental variation and genetic variation.

The analysis of protein content and of the relationship between protein % and yield, in the three years of study, proves that it is always necessary to analyze the protein content in relationship with grain yield.

We obtained a significant correlation between protein content and yield only under nitrogen fertilization in 2004 and 2006. In our study, yield variation caused a variation of protein content between 0.06 and 0.57%. Cultivars with high protein content had low yields, and cultivars with low protein content had, generally, high yield levels.

Several deviations from this rule were observed too. The deviations were generally variable depending on growing conditions. However, some cultivars, like F26-70, showed positive deviations in all conditions.

Several cultivars did not have significant differences in grain protein content between fertilized and unfertilized plots. It is possible that these cultivars present a superior ability for nitrogen uptake and/or a more efficient nitrogen use.

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