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GENOTYPE AND NITROGEN FERTILIZATION INFLUENCE ON THE GRAIN PROTEIN CONTENT IN SOME BARLEY VARIETIES AND LINES

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ABSTRACT

Grain protein content (GPC) is the main quality trait of barley (*Hordeum vulgare* L.) because this parameter is a decisive component of seed quality for malt industry. Reaching the level of protein concentration required by the raw industry is very difficult, due to the negative correlation with the yield level.

Seventeen winter barley genotypes (varieties and breeding lines), created at NARDI Fundulea during 2003-2017, known as having different grain protein contents, were tested with and without nitrogen fertilization in yield trials at NARDI Fundulea in 2014, 2015 and 2016.

Weather conditions during the testing period was reflected by the variation of average yield of the trial from 3861 to 7722 kg/ha (unfertilized) and 4748 kg/ha to 9492 kg/ha (fertilized) and of the average grain protein content from 9.6% to 14.8% (unfertilized) and 10.3% to 15.4% (fertilized).

Correlation between grain protein content and yield was significantly negative under unfertilized condition in two years (2014 and 2016) and under nitrogen fertilization in 2016 only.

The genotypes with higher GPC in the grains and low yield under nitrogen fertilization are not adequate for use in breeding for improved protein content. Positive and negative deviations from the regression of grain protein content on grain yield were found, several varieties and lines combining high yields and high grain protein content and few varieties and lines combining high yields and low grain protein content.

Differences between protein contents with and without nitrogen fertilization were smaller in some varieties and new lines and also presented negative deviations. The information obtained in this study might be useful in breeding new varieties combining low grain protein content and high grain yield.

Keywords: grain protein content (GPC), grain yield (GY), nitrogen supply, winter barley.

INTRODUCTION

Grain protein content (GPC) is an important characteristic for the barley quality which is used malted or unmalted either for food or feed. GPC differs greatly among barley genotypes and across different environments. According with Cai et al. (2013), it is imperative to understand the genetic control of GPC for barley and identify the genotypes with less variation under different environments.

Studies by Emebiri et al. (2003; 2004; 2007) illustrate that there is a genetic basis for variation in GPC for malting barley and is polygenic controlled by quantitative trait loci (QTLs) that had been mapped on all seven barley chromosomes, mainly on *2H, 4H, 5H* and *6H* (Emebiri et al., 2005).

A negative correlation between GPC and grain yield (GY) was registered. Distelfeld et al. (2014) gave two hypotheses to explain the negative correlation between GPC and grain yield (GY), namely delayed senescing plants or a certain pathway during the synthesis of the main compounds of the barley grain. In the first case, it is considered that delayed senescing plants with larger grains led to the diluted contents of both proteins and minerals. The second hypothesis refers to the fact that when the barley plant directs more of its energy to protein synthesis, less will be directed towards starch synthesis, and vice versa.

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For example, Ullrich (2002) showed that GPC in barley ranged from 7 to 25% in a large USDA study involving over 10000 barley genotypes. Majority of maltsters from USA tolerate protein content between 9.5-12.0% for barley used in the raw industry (Oser, 2015). According the American Malting Barley Association (AMBA, 2014) a low grain protein concentration (GPC≤13%) for malting barley is desirable, but the standards stipulate values of 11.5-13.5% for six-rowed barley varieties and 11.5-13.0% for two-rowed varieties (Hochhalter, 2015).

In Australia, the current standards are different for grain protein content: 9.5-12.5% for the Malt1 category and 9.0-12.8% for Malt2 category (Malik and Paynter, 2014).

In Canada the standard values for GPC vary from 10.5% to 13.0% for six-row types and 10.5% to 12.5% for two-row varieties (Gupta et al., 2010; Asaree, 2011).

For European malting, barley is accepted with grain protein content between 9.5-11.5%. Generally speaking, when GPC is higher, the extract yield will be low, the beer would not be clear and the germination may be slow.

When GPC is under 9.5%, it is considered that enzyme and yeast activity decreased in the process of brewing (Pettersson, 2006).

The deviation from the regression line between GY and GPC, namely grain protein deviation, were proposed by Monaghan et al., (2001) in order to identify genotypes having a lower or higher GPC than expected from their GY. Other authors (Oury and Godin, 2007) found that with mean deviation values obtained from trials across a wide range of environments one could identify varieties that deviated positively or negatively from the regression line, regardless of the growing environment, showing GPD has a genetic basis.

Data are limited about the relative GPC and GY responses for barley varieties registered at NARDI Fundulea during 2003-2017.

This paper aims to investigate if genotypic differences in GPC are attributable to the yield dilution effect or genetic factors and to determine if genotypes with the low protein characteristic will have better agronomic performance and grain quality under fertilized and unfertilized condition.

MATERIAL AND METHODS

Seventeen genotypes were tested in 2014, 2015 and 2016 in yield trials at NARDI Fundulea. The protein content was determined by Infratech 1224 Analyzer (NIR instrument). The following varieties and lines were tested in all three years: Dana, Cardinal FD, Univers, Ametist, Smarald, Simbol, Onix, F 8-20-10, F 8-19-10, F 8-3-01, F 8-10-12, Andreea, Artemis, Gabriela, DH 267-66, DH 314-1 and DH 315-10.

Two trials were performed at two levels of nitrogen supply in three replications, with nitrogen fertilization and without nitrogen fertilization. A dose of 100 kg urea (46 kg N/ha) was used in one application, in spring (March-April). A randomised blocks design was used, the plot size being 4.5 m^2 harvested.

Relationship between protein content and grain yield was analysed using ANOVA and linear regression. To establish if genotypes differed in their relationship between GY and GPC, residuals were derived from the linear regression of GPC on GY for each 6 conditions using Excel for Windows (2010).

The data regarding temperature and rainfall registered during the years of testing, delivered by the Weather station of NARDI Fundulea, are presented in Figures 1 and 2.

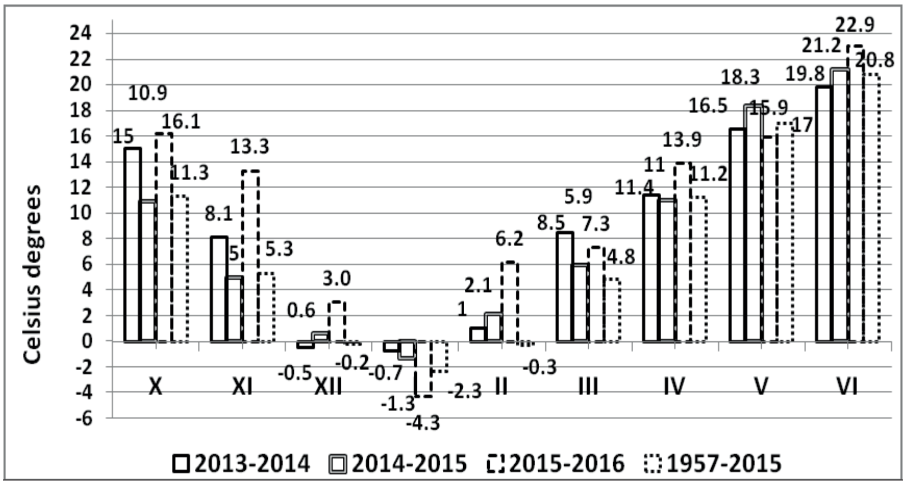

*Figure 1***.** Average temperatures registered during October - June (2013-2016)

Large differences between multiannual average temperature values and the average monthly values of the testing years were observed. Higher values were registered in December 2015 when the monthly average was positive (+3.0°C), while the multiannual average was -0.2°C and in February 2016 temperature was higher (+6.0°C) than the average (-0.3°C). Also, in the same year, the average monthly values were higher with 2.7°C and 2.1°C in April and June comparing with multiannual average temperature values.

Rainfall was variable during the testing years. The rainfall was very high (over 100 mm/m^2) in May, June (2014), in December (2014) and April and May (2016). Rainfall under average was registered in November and December 2013, May 2015 (minus 31.5 mm compared with multiannual value) and February 2016.

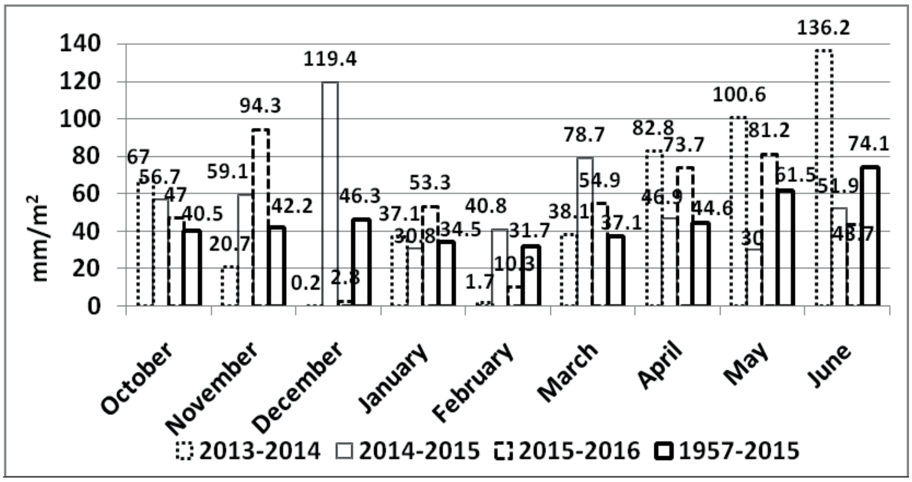

Figure 2. Monthly rainfall during October - June (2013-2016)

RESULTS AND DISCUSSION

The protein content of barley grains varies with the growth conditions particularly with the rate and timing of nitrogen fertilization and is significantly affected by cultivar (Qi et al., 2006). The level of yield and protein content with and without nitrogen (Table 1) show the different testing conditions.

The most favourable year was 2014 when the average yield with nitrogen fertilization was 8616 kg/ha and the highest yield 9492 kg/ha. The average grain protein content reaching maxim12.3% and minim 11.3%.

The year 2015 was also favourable for the yield, when we observed that under fertilized conditions the average yield was 4748 kg/ha and the highest yield 8698 kg/ha.

The GPC was minim 10.3% and average 11.5% (Table 1). Under unfertilized conditions the value of grain protein content was smaller (between 9.6% and 11.6%) than under fertilised conditions (between 10.3% and 12.2%).

Table 1. Average and extreme value of yield and grain 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	2014	8616	9492	6684	12.3	14.2	11.3
Fertilized	2015	6827	8698	4748	11.5	13.4	10.3
Fertilized	2016	6021	7392	5207	14.4	15.4	12.2
Unfertilized	2014	6248	7694	4695	12.6	13.8	10.9
Unfertilized	2015	5582	6607	3861	11.1	13.1	9.6
Unfertilized	2016	5845	7722	4051	13.2	14.8	11.6

1. Protein content of varieties and lines

The protein content of varieties and lines were analysed three years, under fertilised and unfertilized conditions. According to the obtained results, under fertilised conditions in 2014, the varieties Cardinal FD, Smarald and the lines F 8-20-2010, F 8-19-2010 and DH 267-66 were noticed for the smaller protein content (between 11.0% and 12.4%). Under unfertilized conditions the same genotypes had the smallest protein content, the largest difference regarding the protein content with and without nitrogen fertilization being registered by Gabriela variety (-1.4%). The smallest were found in Cardinal FD (0.2%) and Onix variety (0.0%), but this genotype registered 0.8% over maximum standard value accepted for malt and beer industry (Table 2).

Table 2. Average values of protein content, in 2014, under fertilized and unfertilized condition

Variety/line	Unfertilized	Fertilized	Difference
Dana	12.9	12.4	-0.5
Cardinal FD	**11.8**	**11.6**	**-0.2**
Univers	12.9	12.2	-0.7
Ametist	13.6	13.0	-0.7
Smarald	**12.4**	**12.0**	**-0.4**
Simbol	13.2	12.8	-0.5
F 8-20-2010	**11.0**	**11.3**	**0.3**
F 8-19-2010	**11.4**	**11.5**	**0.1**
F 8-3-2001	12.8	12.0	-0.8
Onix	**12.3**	**12.3**	**0.0**
F 8-10-2012	12.3	12.3	0.1
Andreea	12.8	12.4	-0.4
Artemis	12.5	12.3	-0.2
DH 267-66	**11.9**	**11.6**	**-0.3**
Gabriela	**13.6**	**12.1**	**-1.4**
DH 314-1	13.3	13.7	0.4
DH 315-10	13.8	14.2	0.4

In 2015, the smallest values of the studied parameter in both conditions of testing were observed. A number of eight genotypes registered grain protein contents higher than 11.5% under fertilised condition and the same varieties (Cardinal FD, Smarald) and lines (F 8-20-2010 and F 8-19-2010) had a low protein content. In this year other varieties (Dana, Univers, Ametist, Simbol and Onix) which presented low protein content values, between 10.3% and 11.4% were noticed (Table 3). Under unfertilized conditions, the lowest values of this parameter was 9.6% (Smarald), 9.8% (Cardinal FD) and 9.9% (Simbol). Eleven genotypes from seventeen, registered values under 11.5% level (unfertilized condition).

Table 3. Average values of protein content, in 2015, under fertilized and unfertilized condition

Variety/line	Unfertilized	Fertilized	Difference
Dana	**10.5**	**11.3**	**0.8**
Cardinal FD	**9.8**	**10.4**	**0.6**
Univers	**9.9**	**10.9**	**1.0**
Ametist	**11.3**	**11.4**	**0.1**
Smarald	**9.6**	**10.3**	**0.7**
Simbol	**9.9**	**10.3**	**0.4**
F 8-20-2010	**10.3**	**10.3**	**0.0**
F 8-19-2010	**10.3**	**10.3**	**0.0**
F 8-3-2001	11.0	11.8	0.8
Onix	**10.9**	**11.3**	**0.4**
F 8-10-2012	11.2	11.8	0.6
Andreea	11.6	12.4	0.8
Artemis	12.4	12.1	-0.3
DH 267-66	11.9	11.9	0.0
Gabriela	12.4	12.1	-0.3
DH 314-1	12.8	13.3	0.5
DH 315-10	13.1	13.4	0.3

Table 4. Average values of protein content, in 2016, under fertilized and unfertilized condition

Variety/line	Unfertilized	Fertilized	Difference
Dana	14.0	14.6	0.6
Cardinal FD	**12.2**	**12.2**	**0.0**
Univers	14.4	14.4	0.0
Ametist	14.4	15.0	0.6
Smarald	**11.6**	**12.3**	**0.7**
Simbol	13.1	13.2	0.1
F 8-20-2010	14.8	15.4	0.6
F 8-19-2010	13.0	13.7	0.7
F 8-3-2001	14.3	15.3	1.0
Onix	14.7	14.6	-0.1
F 8-10-2012	14.6	14.8	0.2
Andreea	14.6	15.1	0.5
Artemis	14.2	14.4	0.2
DH 267-66	14.1	14.8	0.7
Gabriela	14.0	14.7	0.7
DH 314-1	14.0	14.7	0.7
DH 315-10	14.4	15.0	0.6

In 2016 year, the highest values of GPC under the studied conditions and small differences between them were registered. Even so, the varieties Cardinal FD and Smarald presented the lowest values (Table 4) with and without nitrogen (12.2% and 12.3%, as compared with 12.2% and 11.6% respectively).

The studied varieties and lines had a different behaviour during the testing period, the GPC value being strongly influenced by the environment. Under nitrogen fertilization conditions, in the three years, the varieties Cardinal FD and Smarald registered the smallest protein content and the lines F 8-20-2010 and F 8-19-2010 in two from years of testing. These varieties and lines are interesting in the barley breeding for GPC stability, due to low protein content under unfertilized conditions.

2. Relationship between yield and protein content

In order to illustrate the relationship between yield and grain protein content, the three experimental years and the two conditions of nitrogen supply were analysed (Figure 4). In 2014 and 2016 grain yield and protein content were significantly correlated in unfertilized conditions (-0.68 and -0.69 respectively) and under fertilized conditions only in 2016 (-0.64). In 2015 year, the relationship was not significant, but the protein content reached the lowest values, both with and without nitrogen fertilization, between 9.8-11.3% (10 genotypes) and 10.3-11.4% respectively (9 genotypes).

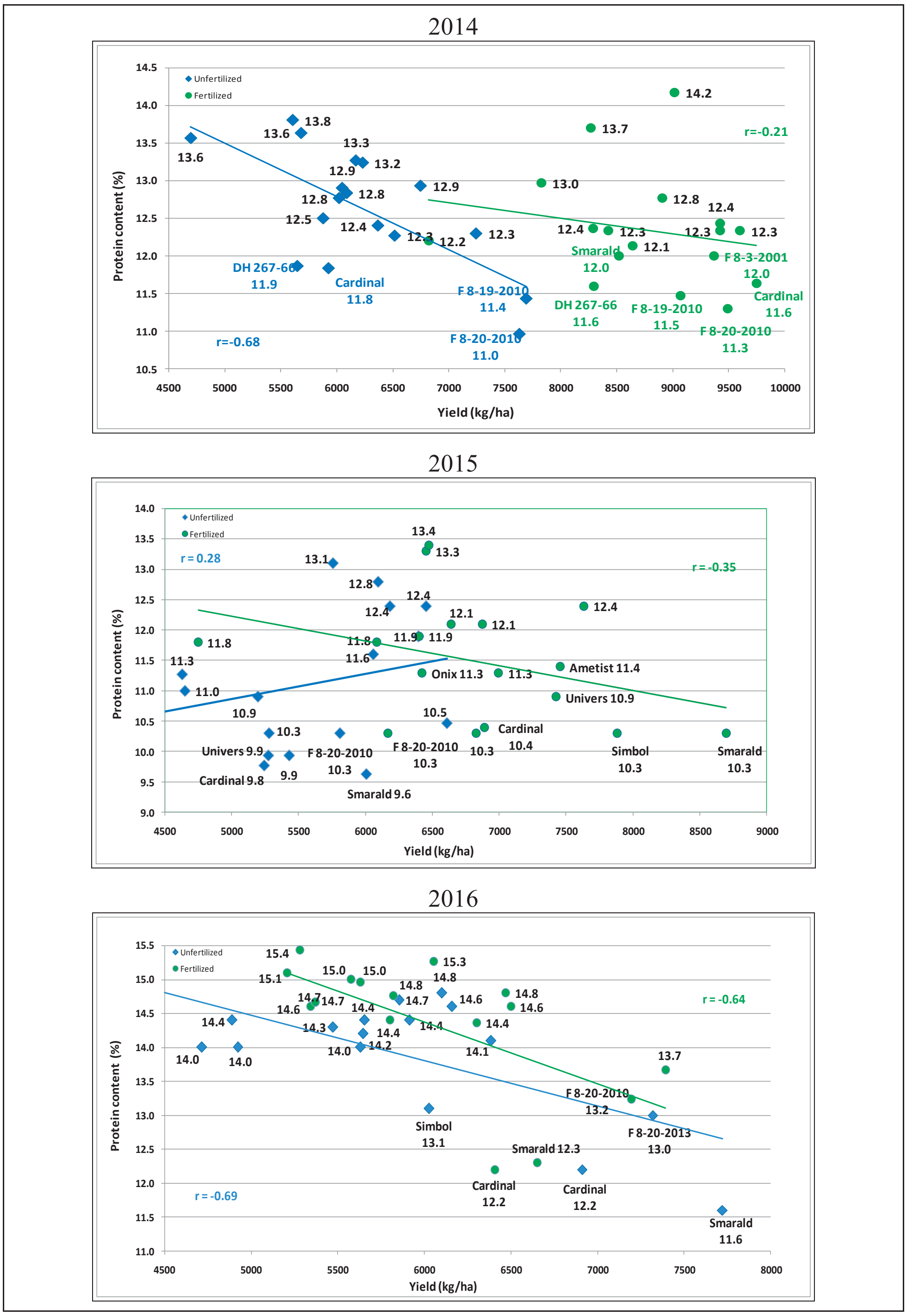

Figure 4. Relationship between grain yield and protein content in 2014, 2015 and 2016 without and with nitrogen fertilization

In all the six testing conditions we noticed deviations from regression, positive and negative (Table 5). The varieties Cardinal FD and Smarald were the most stable genotypes, presenting negative deviations from the regression protein content-yield in all conditions. The Simbol variety and perspective barley lines F 8-20-2010 and F 8-19-2010 presented negative deviations in most cases and these genotypes registered a lower protein content and a higher yield level.

The largest negative deviations were noticed in all three years under fertilized conditions (from -0.39% to -0.89% in 2014, from -0.43% to -1.46% in 2015 and from 0.05% to -1.80% in 2016). In 2014 under fertilization we observed that the Cardinal FD and Smarald varieties had grain protein content 11.6% and 12.05 respectively and a yield level higher than 9700 kg/ha and 8500 kg/ha. Also the above mentioned lines had a yield level over 9000 kg/ha and a GPC under 11.5%. In 2015 year with nitrogen fertilization, the Cardinal FD, Univers, Smarald, Simbol varieties and the lines F 8-20-2010 and F 8-19-2010 showed yield levels from 6100 kg/ha to 8690 kg/ha with a low protein content between 10.3% and 10.9%.

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

Variety/line	2014		2015		2016	
	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized
Dana	0.23	0.67	-0.12	-1.07	-0.37	-0.66
Cardinal FD	**-0.50**	**-1.01**	**-1.06**	**-1.20**	**-1.80**	**-0.99**
Univers	**-0.54**	0.14	**-0.34**	**-1.05**	**-0.16**	**-0.15**
Ametist	0.43	0.62	0.17	0.55	0.24	0.37
Smarald	**-0.39**	**-0.13**	**-0.43**	**-1.65**	**-1.48**	**-1.05**
Simbol	0.46	0.60	**-0.76**	**-1.11**	**-0.05**	**-0.68**
F 8-20-2010	**-0.89**	**-0.67**	**-1.46**	**-0.90**	0.40	1.06
F 8-19-2010	**-0.81**	**-0.16**	**-1.19**	**-0.68**	0.56	0.08
F 8-3-2001	-0.21	0.10	0.01	0.28	0.94	0.14
Onix	0.17	0.39	-0.35	-0.05	0.68	0.80
F 8-10-2012	0.13	-0.16	-0.53	0.80	0.23	0.90
Andreea	-0.07	-0.01	1.24	0.29	0.00	-0.51
Artemis	-0.08	-0.38	0.63	1.04	0.27	0.16
DH 267-66	**-0.84**	**-1.18**	0.24	0.45	0.85	0.56
Gabriela	-0.23	-0.15	0.53	0.93	-0.28	-0.05
DH 314-1	1.26	0.60	1.66	1.48	-0.28	-0.52
DH 315-10	1.88	0.73	1.77	1.92	0.25	0.54

The above mentioned lines also had a yield level over 9000 kg/ha and a GPC under 11.5%. In 2015 year with nitrogen fertilization, the Cardinal FD, Univers, Smarald, Simbol varieties and the lines F 8-20-2010 and F 8-19-2010 showed yield levels from 6100 kg/ha to 8690 kg/ha with a low protein content between 10.3% and 10.9%.

Without nitrogen fertilization, the same genotypes registered yield levels between 5200 kg/ha and 6000 kg/ha and maintained a good GPC level (9.6-10.3%).

Under both testing conditions, the line DH 267-66 presented negative deviations only one year (2014), when it showed 11.9% GPC and over 5600 kg/ha under unfertilizd and 11.6% GPC and over 8000 kg/ha under fertilized conditions. This is one of the cases when the GPC maintained low values under both conditions, but the grain yield was significantly increased under N fertilization.

CONCLUSIONS

The variable level of protein indicated that GPC was dependent on genotypes and year.

We found two winter barley varieties and two winter barley lines, which combined high grain yields and low protein content with and without fertilization.

The deviations from the regression line between GY and GPC could be used to identify barley genotypes having a lower or higher GPC than expected from their GY and on this basis 2 varieties showed negative deviations in all six conditions (Cardinal FD and Smarald).

The analysis of relationship between grain protein content and grain yield responses for barley varieties could determine if barley genotypes with a low GPC would have better agronomic performance and grain quality under fertilized and unfertilized conditions.

The registered differences regarding the grain protein content values proved that for some genotypes these were not explained by the yield differences and had a genetic basis.

It is necessary to apply different nitrogen management strategies required for some genotypes in order to maximise their chances of meeting malt barley standards for GPC in the raw industry.

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