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YIELD EXTRACT ESTIMATION OF ROMANIAN WINTER BARLEY GENOTYPES

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Abstract

This paper aimed at estimating the yield extract of some Romanian winter barley genotypes (six and two-row varieties and breeding lines), using different growing conditions (nitrogen fertilizer rate). All the experiments were performed at NARDI Fundulea and during the 2013-2016 period, winter six-row barley and two-row barley genotypes were tested under different N rates (NR0, NR1 and NR2). The obtained results were used to determine yield extract of each genotype (using Bishop's formula), perform the variance analysis, and asses the correlations of the studied parameters (grain weight, protein content, and yield extract). ANOVA showed a genotype and N fertilizer rate significantly influenced on grain weight, protein content, and yield extract for both six and two-row genotypes and for all nine tested conditions. For all winter six-row barley quality parameters under two years an insignificant influence of genotype x N rate interaction on these was showed while winter two-row barley revealed a different behavior. This source of variation was significant only one experimental year for protein content, two years for yield extract and one year for grain weight. The yield extract, grain weight, and protein content were assessed separately among winter six-row and two-row barley. Under all N rate (except NR2 for winter six-row barley and NR0 for winter two-row barley) there was a significant negative correlation between yield extract and protein content (from -0.54 to -0.89 and -0.61 to -0.89 respectively). The same comparison showed that the grain weight was not correlated with protein content for winter two-row barley under any of the experimental year conditions; while this was positive in two years for winter six-row barley (from 0.42 to 0.77). During the 2013-2016 period, the yearly average yield extract has varied due to change of the protein content and grain weight, namely was noticed an increasing tendency from NR0 to NR1 and a decrease one from NR1 to NR2.

Key words*: six and two - row winter barley genotype, nitrogen rate, grain weight, protein content, yield extract.*

INTRODUCTION

Micro-malting techniques had begun with small barley samples at a small scale in the past, about 125 years ago (Meredith et al., 1962). The raw industry has evaluated the malting barley quality by "micro malting" (small scale grain malt producing) and then the malt is analyzed for desired traits (Haslemore et al., 1985). The pioneer of barley yield extract estimation was L. R. Bishop, which began to study barley protein content in different barley varieties in 1930 and continued in other papers in 1933 and 1934 years. In this work, he stated that protein content is negatively correlated with yield extract due to the fractions of protein named hordeins.

The extract can be defined as the percentage of dry matter, which is solubilized from the grist during the mashing process (Kunze, 1999).
Yield/malt extract is very important as quality parameter for maltsters and brewers when selecting malting barley (Dráb et al., 2014), because the amount of extract obtained from a variety is economically appreciated and also determines the amount of produced beer (Schwarz et al., 2007). So, the breeders are focused on bred new barley varieties with a high yield and high yield/malt extract for maltsters and brewers (Li et al., 2008). The quality of the extract is influenced by the environment (fertilizer, temperature, and rainfall) affecting the varieties traits which influence the protein and starch content (Fox et

al., 2003). Other traits are the type of barley (six/two-row), the fines of husk, and also the grain weight. In the latest, there is a huge interest to do varieties selection on the molecular assisted markers (MAS) so, two QTLs had been found that accounting for 35.7-53.6% of the yield extract variance (Zhou et al., 2012) and a genetic base of breeding barley with malting quality was provided by Fang et al. (2019), which identified controlling malting quality QTLs or genes located on 1, 2, 4, 5 and 7 barley chromosomes.

Nowadays, in the Czech Republic, barley malting quality is assessed on the basis of malting quality index (MQI) according to the index from the 2002 year (Psota and Kosař, 2002; Psota et al., 2019). They evaluated the quality of some spring barley varieties (Cosmopolitan, Ismena, Klarinette, Laureate, LG Aurus, Runner) and also some six-row winter barley varieties (Azrah, Impala, Journey, Laurin, SU Jule and the hybrid variety SU Hylona).

A typical yield is quantified under laboratory conditions after barley malting. Other mash methods or correlations between different qualitative parameters have been taken into account in order to predict the malt fermentability or extract yield because its variability is a concern for both brewers and barley breeders (Kunze, 1999). The most variation in yield extract (74.3%) has been explained on the three quality barley parameters by Li et al. in 2008 which used a predicted extract equation based on protein content (Pr), 1000 kernel weight (1000 KW) and diastatic power (DP).

The form of the equation is:

Extract = 89.3 − 1.64 Pr + 0.16 KW + 0.019 DP and here we can notice that we need diastatic power value of barley variety which only can be obtained after the grain sample micro malting.

According to the European Brewery Convention (EBC) for a Lager malt, the minimum value for yield extract has to be >80.5% (O'Rourke, 2002).

For a barley breeding programme the micro malting method is expensive and meanwhile, the behavior of barley varieties and lines (F_7 and F_8 generations) have to be made fast, before the next cropping year.

This study aims at evaluating the potential yield extract of some varieties and breeding lines of Romanian winter barley, based on a calculation formula, in order to determine the yield extract without malting under specific growing conditions.

MATERIALS AND METHODS

Seventeen winter barley genotypes (11 six-row and 6 two-row winter barley varieties and breeding lines in 2013-2014) and fifty winter barley genotypes (25 six-row and 25 two-row winter barley varieties and lines in 2015 and 2016), developed at NARDI Fundulea, were tested during 2013-2014, 2014-2015, 2015-2016 years under three experimental conditions (without applied nitrogen rate - NR0, 100 kg/ha - NR1 and 200 kg/ha - NR2, the nitrogen doses were applied every year in March).

After the harvest and seeds conditioning, the protein content (P%) was determined by Infratech 1241 (NIR method), the grain weight (GW) with Contador seed, and all three replications (1000 seeds each) were weighted for each barley samples (500 g per sample).

On the basis of the modified Bishop's mathematical formula (Gregor et al., 2011), the yield extract of each sample was calculated accordingly to the following formula:

E = K - (0.85*B) + (0.15*G), where:

E - extractivity of barley grain;

K - a constant value equal with 83;

B - protein content (P%);

G - grain weight (GW).

The experimental data have been assessed through statistical analysis (ANOVA) and the obtained results were the subject of correlation analysis and expressed as a minimum, mean and maximum values. Shares of genotype (G), nitrogen rate (NR0, NR1, NR2), and genotype x nitrogen rate interaction (G x NR) in the phenotypic expression of grain weight (GW), protein content (P) and yield extract (EXT) were performed in Microsoft Excel Software.

The yield extract, grain weight and protein content were assessed and analyzed separately among six-row and two-row winter barley, due to the phenotypic differences among them and also the nitrogen utilization efficiency.

RESULTS AND DISCUSSIONS

ANOVA showed that genotype (G) and N fertilizer rate (NR) significantly influenced the grain weight (GW), protein content (P), and yield extract (EXT) for both six and two-row genotypes and for all nine tested conditions. For all six-row winter barley quality parameters under two years (2015 and 2016) an insignificant influence of genotype x nitrogen rate interaction (G x NR) on these was showed while two-row winter barley revealed a different behavior. This source of variation was significant for only one experimental year for protein content (2014), two years for yield extract (2014 and 2016) and one year (2016) for grain weight (Table 1). The differences between six and two-row winter barley were due to the different years' climatic conditions and probably nitrogen utilization efficiency.

Share of factors (%) in achieving yield extract in six and two-row winter barley (Fundulea, 2014-2016), indicated that these components are strongly influenced by nitrogen rate (Figures 1, 3 and 5) in the case of six-row winter barley (72% in 2014, 97% in 2015, and 89% in 2016) and a little bit less in the case of two-row winter barley (Figures 2, 4 and 6), 66% in 2014, 48% in 2015, and 68% in 2016. Also, the genotype as a factor (%) describes a different influence on both, this was not so close comparing with nitrogen dose and their interaction, which means that in variable climatic conditions and management practices the yield extract presents a high degree of vulnerability (16% in 2014, 2% in 2015 and 41% in 2016 for six-row barley and 20% in 2014, 10% in 2015, and 27% in 2016 for two-row barley).

Table 1. P-value for six and two-row winter barley genotypes, 2014-2016 period (grain weight, protein content and yield extract)

2014 year		**Grain weight**		**Protein content**		**Yield extract**	
		P-value		P-value		P-value	
Source of variation	**df***	**six-row**	**two-row**	**six-row**	**two-row**	**six-row**	**two-row**
Nitrogen rate (NR)	2	0.011	0.001	0.000	0.000	0.000	0.000
Genotype (G)	10 (5)	0.000	0.000	0.000	0.000	0.000	0.001
G x NR	20 (10)	0.000	0.413	0.000	0.000	0.000	0.007
2015 year		**Grain weight**		**Protein content**		**Yield extract**	
		P-value		P-value		P-value	
Source of variation	**df**	**six-row**	**two-row**	**six-row**	**two-row**	**six-row**	**two-row**
Nitrogen rate (NR)	2	0.000	0.007	0.000	0.000	0.000	0.000
Genotype (G)	24	0.000	0.000	0.000	0.000	0.000	0.000
G x NR	48	0.163	0.980	0.947	0.258	0.624	0.757
2016 year		**Grain weight**		**Protein content**		**Yield extract**	
		P-value		P-value		P-value	
Source of variation	**df**	**six-row**	**two-row**	**six-row**	**two-row**	**six-row**	**two-row**
Nitrogen rate (NR)	2	0.000	0.000	0.003	0.000	0.001	0.000
Genotype (G)	24	0.000	0.000	0.000	0.000	0.000	0.000
G x NR	48	0.595	0.000	0.317	0.986	0.954	0.000

* 11 six-row genotypes and 6 two-row genotypes

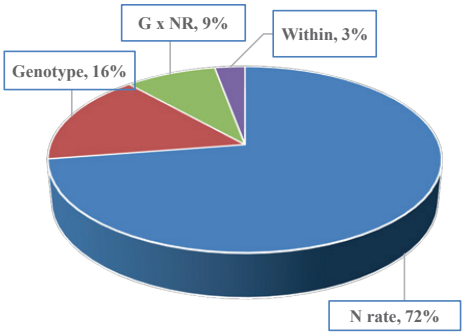

Figure 1. The influence of genotype, nitrogen rate and their interaction on yield extract, 2014 (six-row barley)

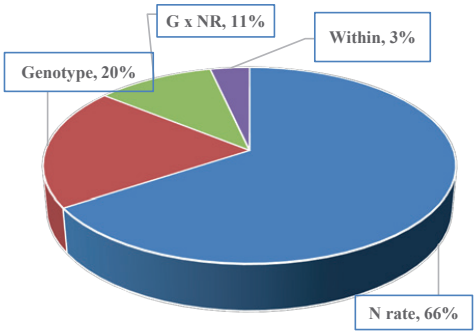

Figure 2. The influence of genotype, nitrogen rate and their interaction on yield extract, 2014 (two-row barley)

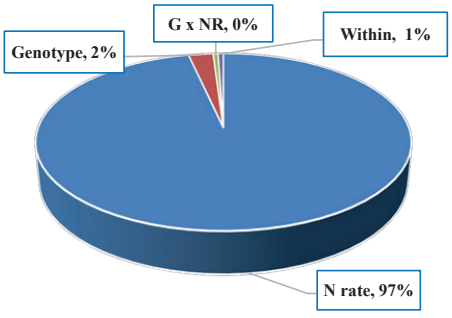

Figure 3. The influence of genotype, nitrogen rate and their interaction on protein content, 2015 (six-row barley)

Regarding the interaction of genotype and nitrogen rate (G x NR) was noticed a different yearly and row barley type contribution in achieving yield extract. This ranged from 0% (2015) to 4% (2016) and 9% (2015) for six-row barley. Comparing the two-row with six-row barley type in all tested years, the interaction had the same percentage contribution in 2015 (0%), higher with 2% in 2014 (11%), and lower with just 1% in 2016 (3%). The share of factors (%) showed that always the extract yield will be different among the six and two-row barley under different climatic and technological sequences (nitrogen rate).

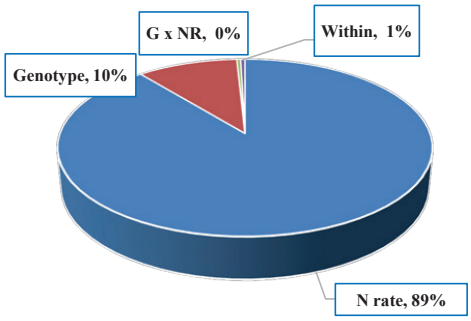

Figure 4. The influence of genotype, nitrogen rate and their interaction on yield extract, 2015 (two-row barley)

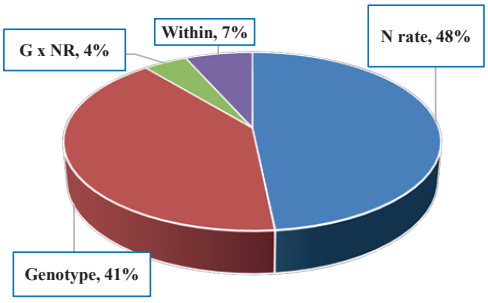

Figure 5. The influence of genotype, nitrogen rate and their interaction on protein content, 2016 (six-row barley)

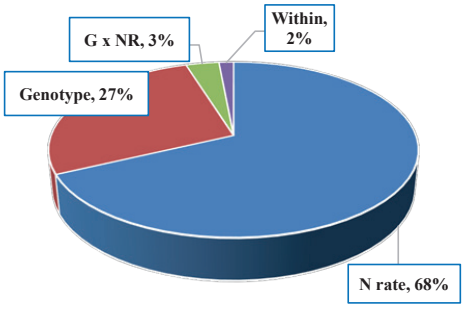

Figure 6. The influence of genotype, nitrogen rate and their interaction on yield extract, 2016 (two-row barley)

Depending on genotypes and growing conditions (nitrogen rate) in the 2014 year (Table 2), six-row barley grain weight (GW) ranged from 33.0 g (NR0) to 42.4 g (NR2), protein content from 11.0% (NR0) to 14.1% (NR2) and yield extract decreased from 77.3% (without nitrogen) to 79.2% (NR1).

The grain weight values of two-row barley ranged from 38.3 g (NR0) to 48.5 g (NR1 and NR2), whilst the protein content varied between 11.6% (NR1) to 14.7% (NR2). Regarding two-row barley yield extract, the minim values were higher than six-row barley genotypes value (for all three cultivation conditions) and the higher value was equal just under the NR2.

Table 2. The minimum, mean and maximum value of grain weight, protein content and yield extract, 2013-2014 cropping year

Grain weight (g)						
N dose	**NR0**		**NR1**		**NR2**	
No. of row	six row	two row	six row	two row	six row	two row
Minim	33.0	38.3	34.3	42.0	33.2	40.0
Mean	37.3	43.1	38.8	45.5	37.9	45.1
Maxim	41.2	46.0	41.5	48.5	42.4	48.5
Protein content (%)						
N dose	**NR0**		**NR1**		**NR2**	
No. of row	six row	two row	six row	two row	six row	two row
Minim	11.0	11.9	11.3	11.6	12.0	13.2
Mean	12.4	13.0	12.1	12.7	13.1	14.0
Maxim	13.6	13.8	13.0	14.2	14.1	14.7
Yield extract (%)						
N dose	**NR0**		**NR1**		**NR2**	
No. of row	six row	two row	six row	two row	six row	two row
Minim	**77.3**	**78.0**	**77.9**	**78.1**	**76.2**	**76.8**
Mean	78.0	78.4	78.5	79.0	77.5	77.9
Maxim	**79.0**	**79.3**	**79.2**	**79.8**	**78.8**	**78.8**

In the second year of testing (2015), two-row barley genotypes had higher GW (minimum value from 48.3 to 48.9 g and maximum from 63.6 to 64.5 g) for all experimental conditions comparing six-row barley (Table 3) and higher protein content (minimum 11.6% under NR0 and maximum 16.6% under NR2). The yield extract ranged from 76.7 to 80.1% under NR2, and from 78.2 to 81.8% under NR0. The type six-row achieved lower values of GW and protein content under all nitrogen rates but a maximum extract value was registered for six-row barley without nitrogen addition at cultivation with a similar behavior under NR1 and NR2 (from 82.2% under NR0 to 81.9% under NR1 and 80.6% under NR2).

Table 3. The minimum, mean and maximum value of grain weight, protein content and yield extract, 2014-2015 cropping year

Grain weight (g)						
N dose	**NR0**		**NR1**		**NR2**	
No. of row	six row	two row	six row	two row	six row	two row
Minim	38.2	48.3	40.0	48.5	40.3	48.9
Mean	49.2	56.4	49.6	55.5	50.9	56.5
Maxim	53.5	63.8	54.7	64.5	56.1	63.6
Protein content (%)						
N dose	**NR0**		**NR1**		**NR2**	
No. of row	six row	two row	six row	two row	six row	two row
Minim	9.4	11.6	10.3	11.9	11.7	13.5
Mean	10.7	12.9	11.2	13.3	13.1	14.8
Maxim	11.5	14.2	12.3	14.6	14.6	16.6
Yield extract (%)						
N dose	**NR0**		**NR1**		**NR2**	
No. of row	six row	two row	six row	two row	six row	two row
Minim	**80.5**	**78.2**	**79.9**	**77.9**	**78.6**	**76.7**
Mean	81.3	80.5	80.9	80.0	79.5	78.9
Maxim	**82.2**	**81.8**	**81.9**	**81.5**	**80.6**	**80.1**

Table 4. The minimum, mean and maximum value of grain weight, protein content and yield extract, 2015-2016 cropping year

Grain weight (g)						
N dose	**NR0**		**NR1**		**NR2**	
No. of row	six row	two row	six row	two row	six row	two row
Minim	36.3	30.4	39.3	34.6	36.1	33.2
Mean	41.2	37.0	44.6	41.9	44.3	40.2
Maxim	46.6	47.6	50.5	49.1	51.8	46.8
Protein content (%)						
N dose	**NR0**		**NR1**		**NR2**	
No. of row	six row	two row	six row	two row	six row	two row
Minim	11.7	12.8	12.2	13.3	12.9	14.1
Mean	14.3	14.5	14.5	15.0	15.1	15.7
Maxim	15.9	16.5	16.1	17.1	16.2	17.9
Yield extract (%)						
N dose	**NR0**		**NR1**		**NR2**	
No. of row	six row	two row	six row	two row	six row	two row
Minim	**76.0**	**74.1**	**76.3**	**74.2**	**75.9**	**73.2**
Mean	77.0	76.1	77.3	76.5	76.9	75.7
Maxim	**78.6**	**78.1**	**79.0**	**78.2**	**78.0**	**76.9**

As is show in Table 4, the third year was characterized by an increase of minimum GW for six-row barley comparing with the two-row barley (36.1-39.3 g for six-row and 30.4-34.6 g

for two-row) and also of maximum value under NR1 and NR2 (50.5-51.8 g for six-row and 46.8 g at NR2 and 49.1 g at NR1 for two-row). Grain protein content was higher under all growing conditions for both type of row with a maximum of 16.2% for six-row (NR2) and 17.9% for two-row (NR2). The minimum and maximum values of yield extract were lowest than 2014 and 2015 due to considerable increased protein content. Also, Molina-Cano et al. (2000) performed a large experiment (346 trials, with many barley varieties harvested across all the EBC countries during 1980-1990 and 1993-1995 periods) and had concluded that the negative correlation between these two variables (extract yield and barley protein content) has been more or less significant, the trends being affected by regional peculiarities (2000 year). A common point of these trends was registered, namely that yield extract decreased when barley protein content increased with some variations.

Table 5a. Correlations between the six-row winter barley analyzed parameters, 2013-2014

Parameters	NR0 Ext	NR1 Ext	NR2 Ext	NR0 GW	NR1 GW	NR2 GW	NR0 Prot	NR1 Prot	NR2 Prot
NR0 Ext	1								
NR1 Ext	0.80	1							
NR2 Ext	0.14	-0.02	1						
NR0 GW	**0.17**	-0.04	-0.14	1					
NR1 GW	0.19	**0.35**	-0.35	0.75	1				
NR2 GW	0.04	-0.08	**0.41**	0.62	0.49	1			
NR0 Prot	**-0.79**	-0.74	-0.21	**0.47**	0.29	0.35	1		
NR1 Prot	-0.56	**-0.60**	-0.28	0.67	**0.54**	0.48	0.92	1	
NR2 Prot	-0.12	-0.02	**-0.80**	0.56	0.70	**0.22**	0.46	0.62	1

Table 5b. Correlations between the two-row winter barley analyzed parameters, 2013-2014

Parameters	NR0 Ext	NR1 Ext	NR2 Ext	NR0 GW	NR1 GW	NR2 GW	NR0 Prot	NR1 Prot	NR2 Prot
NR0 Ext	1.00								
NR1 Ext	0.94	1.00							
NR2 Ext	0.89	0.86	1.00						
NR0 GW	**0.70**	0.67	0.58	1.00					
NR1 GW	0.72	**0.73**	0.60	0.95	1.00				
NR2 GW	0.68	0.67	**0.62**	0.97	0.94	1.00			
NR0 Prot	**-0.61**	-0.56	-0.61	**0.13**	0.04	0.13	1.00		
NR1 Prot	-0.61	**-0.68**	-0.63	0.03	**0.00**	0.02	0.87	1.00	
NR2 Prot	-0.43	-0.40	**-0.63**	0.24	0.17	0.22	0.89	0.79	1

Table 6a. Correlations between the six-row winter barley analyzed parameters, 2014-2015

Parameters	NR0 Ext	NR1 Ext	NR2 Ext	NR0 GW	NR1 GW	NR2 GW	NR0 Prot	NR1 Prot	NR2 Prot
NR0 Ext	1.00								
NR1 Ext	0.83	1.00							
NR2 Ext	0.70	0.69	1.00						
NR0 GW	-0.23	-0.31	-0.17	1.00					
NR1 GW	-0.40	-0.31	-0.21	0.91	1.00				
NR2 GW	-0.50	-0.52	-0.19	0.87	0.88	1.00			
NR0 Prot	**-0.85**	-0.77	-0.60	**0.71**	0.78	0.83	1.00		
NR1 Prot	-0.72	**-0.89**	-0.66	0.66	**0.67**	0.77	0.88	1.00	
NR2 Prot	-0.75	-0.75	**-0.71**	0.69	0.76	**0.77**	0.92	0.90	1

Table 6b. Correlations between the two-row winter barley analyzed parameters, 2014-2015

Parameters	NR0 Ext	NR1 Ext	NR2 Ext	NR0 GW	NR1 GW	NR2 GW	NR0 Prot	NR1 Prot	NR2 Prot
NR0 Ext	1.00								
NR1 Ext	0.94	1.00							
NR2 Ext	0.89	0.86	1.00						
NR0 GW	**0.70**	0.67	0.58	1.00					
NR1 GW	0.72	**0.73**	0.60	0.95	1.00				
NR2 GW	0.68	0.67	**0.62**	0.97	0.94	1.00			
NR0 Prot	**-0.61**	-0.56	-0.61	**0.13**	0.04	0.13	1.00		
NR1 Prot	-0.61	**-0.68**	-0.63	0.03	**0.00**	0.02	0.87	1.00	
NR2 Prot	-0.43	-0.40	**-0.63**	0.24	0.17	0.22	0.89	0.79	1

Table 7a. Correlations between the six-row winter barley analyzed parameters, 2015-2016

Parameters	NR0 Ext	NR1 Ext	NR2 Ext	NR0 GW	NR1 GW	NR2 GW	NR0 Prot	NR1 Prot	NR2 Prot
NR0 Ext	1.00								
NR1 Ext	0.83	1.00							
NR2 Ext	0.70	0.69	1.00						
NR0 GW	-0.23	-0.31	-0.17	1.00					
NR1 GW	-0.40	-0.31	-0.21	0.91	1.00				
NR2 GW	-0.50	-0.52	-0.19	0.87	0.88	1.00			
NR0 Prot	**-0.85**	-0.77	-0.60	**0.71**	0.78	0.83	1.00		
NR1 Prot	-0.72	**-0.89**	-0.66	0.66	**0.67**	0.77	0.88	1.00	
NR2 Prot	-0.75	-0.75	**-0.71**	0.69	0.76	**0.77**	0.92	0.90	1

Table 7b. Correlations between the two-row winter barley analyzed parameters, 2015-2016

Parameters	NR0 Ext	NR1 Ext	NR2 Ext	NR0 GW	NR1 GW	NR2 GW	NR0 Prot	NR1 Prot	NR2 Prot
NR0 Ext	1								
NR1 Ext	0.78	1.00							
NR2 Ext	0.55	0.82	1.00						
NR0 GW	**0.60**	0.35	0.12	1.00					
NR1 GW	0.13	**0.44**	0.33	0.58	1.00				
NR2 GW	-0.01	0.29	**0.47**	0.44	0.82	1.00			
NR0 Prot	**-0.63**	-0.60	-0.54	**0.24**	0.40	0.44	1.00		
NR1 Prot	-0.72	**-0.72**	-0.61	0.07	**0.30**	0.33	0.95	1.00	
NR2 Prot	-0.59	-0.62	**-0.65**	0.25	0.35	**0.37**	0.95	0.93	1

The obtained results of correlation coefficients are presented separately for six and two-row barley in Tables 5a, 5b, 6a, 6b, 7a, and 7b. In all three years, for six-row barley, yield extract (Tables 5a, 6a, 7a, red cells) and grain weight were correlated neither. Meanwhile, the grain weight is strongly negatively correlated with protein content (green cells). In the case of two-row barley, the situation is changed, in all the tested years extract is positively correlated with grain weight (Table 5b, 6b and 7b, green cells) and strongly negatively correlated with protein content at all NR (green cells). In 2014, there was no correlation between grain weight and protein content at all NR for six and two-row and in 2015 and 2016 the correlations among grain weight and protein content were positively for six-row (Tables 6a and 7a) and did not correlate for two-row barley (Tables 6b and 7b). According to Mohammadi et al. (2015), protein content is strongly related to yield extract and is negatively correlated, high

protein content can lead to reduced yield extract. During the malting process is produced the hydrolysis of starch, malt protein provides a-amylase and b-amylase enzymes for starch degradation (Elía et al., 2010).

CONCLUSIONS

The potential yield extract generally shows a decreasing trend from one experimental condition to another, their variation being closely related to grain weight (GW) and protein content (P).

Protein content (P) tends to be stable for some genotypes, especially for six-row winter barley genotypes, no matter the growing conditions.

Increasing the dose of nitrogen (NR) used during cultivation leads to higher protein content (P) in two-row winter barley in comparison with the six-row winter barley, which significantly affects the extract yield (EXT).

The "Bishop's law" on yield extract decrease with barley protein increase became a general statement also in the case of Romanian six and two-row winter barley varieties and lines.

A pattern of yield extract variation with changes in barley protein content has been observed, the extract decreases progressively with increases in barley protein content more for two-row and less for six-row barley.

When the extract yield (EXT) is estimated on the grain weight and protein content basis it would be necessary to take into account the temperatures and rainfall from the growing geographical region and the negative protein content deviation from regression, not only the genotype and technology.

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