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Original Article

The Effect of Genotype, Climatic Conditions and Nitrogen Fertilization on Yield and Grain Protein Content of Spring Wheat (*Triticum aestivum* L.)

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Abstract

Cultivation of spring wheat varieties has expanded into areas with abundant winters where winter wheat is not suitable. Due to lack of research in Romania regarding the influence of different factors on hard red spring wheat, the present study aimed at a better understanding of the influence of genotype, climatic conditions and nitrogen fertilization on the spring wheat yield and quality, and to analyse the correlations between grain yield and grain protein content. Experiences were conducted from 2015 to 2018 on two levels of N fertilization (50 and 100 kg ha⁻¹) at Agricultural Research and Development Station Turda. Biological material consisted of 19 genotypes, four of local origin and fifteen of foreign origin, from three different varieties (*ferrugineum, lutescens, erythrospermum*). The results indicate that the three experimental years were more important in the interactions with the genotypes than was the N fertilization, for both yield and protein content. The most productive cultivars with good stability were 'Feeling' and 'SG 5-01', and the most valuable varieties regarding the protein content were 'Pădureni', 'Corso' and 'GK Tavasz'. A high dose of N assured a high yield and good quality for all cultivars. Although negative correlations were found between production and protein content in HRSW, there were found cultivars that show positive regressions of protein content, such as 'Pădureni', 'Eeeling' and 'Lona'.

Keywords: correlation; erythrospermum; ferrugineum; hard red spring wheat; lutescens; stability

Introduction

Wheat is one of the most important food crops in the world (Gyarmati, 2017); about 21% of the world's food depends on wheat crops, which grow on 220 million hectares worldwide, representing about 30% of the global harvested cereal area (FAO, 2018). Out of this surface 70% is represented by winter wheat, and the rest of it by spring wheat.

Classification into spring or winter wheat is common, and traditionally refers to the season during which the crop is grown (Wang *et al.*, 2018). Winter wheat is sown in autumn and requires a period of cold winter temperatures (0 °C to 5 °C) for ear formation. Spring wheat is usually sown in the spring and matures in late summer, but can be sown in autumn in countries that experience mild winters.

Due to the introduction of spring wheat varieties, the

wheat cultivated area has been extended to the north, in areas with heavy winters, where winter wheat is not suitable. Thus, very early spring wheat varieties have been created in Canada with a vegetation period of less than 100 days, suitable for northern culture areas at latitude above 60° (Clarke *et al.*, 1997; DePauw *et al.*, 2000; Fox *et al.*, 2013).

Although world food needs are increasing, climate changes, such as rise in temperature and decline in rainfall may negatively affect crop yields in some major production regions of the world (Licker, 2013). Few studies have been conducted to assess the long-term impact of climate change on spring wheat production. A better understanding of how spring wheat responds to regional climatic conditions is essential for adapting the farming practices to take advantage of local conditions (Hoffmann and Sgrò, 2011; Hawkesford *et al.*, 2013).

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To cope with climate change, The Global Wheat Breeding Program at CIMMYT was founded to explore strategies for breeding widely adapted and highly stable wheat cultivars (Braun *et al.*, 1996; Singh and Trethowan, 2007). Farmers need wheat varieties that can cope with a highly variable climate, such as varieties with high yield potential to take advantage of rain and irrigation when available and also with heat and drought tolerance in hot years (Abayomi and Wright, 1999).

Temperature, solar radiation and relative air humidity were major climatic factors that affected the yields of spring wheat (Zhao *et al.*, 2017). Furthermore, the effect of the average minimum temperature on yield was greater than that of the average maximum temperature. According to Hatfield *et al.* (2011), temperature rising above 25 to 35 °C would shorten the grain-filling period and reduce wheat yield.

Nitrogen fertilization is the main factor and an indicator of the effectiveness of agricultural production. Spring wheat N requirements are high during tillering, stem elongation, booting, heading, and grain filling, for reproductive organ development and for increased protein accumulation in kernel (Delogu *et al.*, 1998).

The importance of efficient N fertilization for yield and quality of spring wheat has been highlighted by many researchers. Among the factors that have a significant impact are the dose of N fertilization (Jaćimović *et al.*, 2008; Staugaitis *et al.*, 2017) source of N fertilizer (Jarecki *et al.*, 2017), timing of N application (Rawluk *et al.*, 2000; Woolfolk *et al.*, 2002; Karamanos *et al.*, 2005), culture rotation (Kaerner, 1999; Halvorson *et al.*, 2000), frequency of rainfall and temperature during the growing season (Zhang and Oweis, 1999). A recommended technique for synchronizing the N supply with the wheat N demand is split application of the N fertilizer (Nyiraneza *et al.*, 2012).

Another important observation is that one of the most relevant quality traits in spring wheat, grain protein concentration, which affects the milling and baking quality of the grain, is also affected by N levels (Gauer *et al.*, 1992; Lopes-Bellido *et al.*, 2001; McKenzie *et al.*, 2006). Previous researches have reported that the maximum protein content is generally attained at N levels much higher than those required to reach maximum yield (Fowler, 2003).

In order to optimize spring wheat production on different cultivation condition, the identification of stable genotypes, with high and good quality yield, in various environments has been a continuous challenge for plant breeders. The response of each genotype to environmental variations results in different patterns of genotype-by-environment interaction (Săulescu and Handrea, 1986; Benin *et al.*, 2014; Crespo-Herrera *et al.*, 2017).

Due to the lack of research in Romania regarding the previously presented aspects, our study aimed at a better understanding of the influence of genotype, climatic conditions and nitrogen fertilization on the spring wheat yield and quality, having as objectives (i) to analyses the effect of genotype, year and N fertilization on spring wheat yield and (ii) to analyses the effect of genotype, year and N fertilization on spring wheat grain protein content and (iii) to analyses the correlations between the grain yield and the grain protein content in spring wheat.

Materials and Methods

Climatic conditions

In 2015, during the spring months, the thermal regime was warm and a little rainy in March, and drought in April (Fig. 1). Seeding the experimental fields in the third decade of March caused rapid and uniform emergence of all experimental variants. Further, the warm heat regime and rainfall in May and June created very favorable conditions for HRSW, with the highest yields.

Although in 2017 no dry periods were recorded during the entire HRSW vegetation period, the obtained yields were lower compared to the previous year, due to the minimum temperatures of 5-6 °C which coincided with differentiation phenophases of floral primordia.

Year 2018 was the most special in terms of climatic conditions and that is why a more detailed presentation is necessary. January was excessively dry and February normal in terms of precipitation. March was warm and excessively rainy so that it was possible to complete the water deficit in the soil, which in combination with favourable temperatures led to favourable conditions for the sowing of HRSW on March 20th. In March and April, alternate periods of higher and lower temperatures, in the range of 11-20, the minimum temperatures being very low, caused stagnation in the development of the phenophases of HRSW. On April 20th, a 3 cm thick layer of snow covered the soil, and it persisted until the next day. Ear emergence of HRSW began on May 28th and continued, depending on the genotype, until June 8th when the ear of 'T. 265-01' line (alternate) emergenced the last.

Experimental procedures

Between 2015-2018, a three factor experiment including two levels of N (50 and 100 kg N ha⁻¹) and 19 cultivars of hard red spring wheat (HRSW) was conducted in the conditions of the Agricultural Research and Development Station (ARDS) Turda (46°35' N; 23°47'E; 345 m above Adriatic Sea), which is located in the Transylvanian Plain, Romania.

The experiment was established on a typical clay Chernozem soil, typical for the forest steppe encountered over half of the Transylvanian Plain. The agrochemical indexes for this soil type had the following average values: the soil reaction is neutral (pH 6.9-7.1) and the humus content is 3.56-3.92% in the arable layer. The soil is rich in total nitrogen (0.183-0.196%) and potassium content (249 ppm in Amp), and poor in mobile phosphorus (15 ppm in Amp).

The biological material consisted of 19 genotypes, four of local origin and 15 of foreign origin, from three varieties (Table 1).

Nitrogen fertilization was carried out in one or two stages: the variant N_{50} and half of N_{100} was applied in early spring (before straw elongation) and the other half (50 kg ha⁻¹N) before heading.

The plot surface area was 10 m². Sowing was made in every year during the last decade of March. Plots were harvested individually by combine and grain yield reported at uniform moisture content (14%). Determination of protein content was performed on the whole grain with Infragrain 9500 analyser.

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Table 1.Biological material used in the experiment							
No.	Cultivar	Origin	var.				
1	'Pădureni'	Romania-Turda	ferrugineum				
2	ʻT. 265-01'	Romania-Turda	erythrospermum				
3	'SG 5-01'	Czech Republic	erythrospermum				
4	'SG 106-01'	Czech Republic	erythrospermum				
5	'SG U 773'	Czech Republic	erythrospermum				
6	'Corso'	Germany	lutescens				
7	'Lona'	Switzerland	lutescens				
8	'Feeling'	Lithuania	lutescens				
9	'Gk Tavasz'	Hungary	ferrugineum				
10	'Triso'	Germany	lutescens				
11	'Broma'	Poland	erythrospermum				
12	'Beloterkovskaia'	Russia	lutescens				
13	'Henica'	Poland	lutecens				
14	'Jara'	Czech Republic	lutescens				
15	'Jota'	Poland	lutescens				
16	'Prif 3'	Romania-Fundulea	erythrospermum				
17	'Prif 4'	Romania-Fundulea	erythrospermum				
18	'Sigma'	Poland	lutescens				
19	'Silva'	Poland	lutescens				



Fig. 1.Temperatures and rainfall regime within the experimental area

Statistical analyses

Analysis of variance (ANOVA) was used to estimate the main effects of genotype, year (climatic conditions) and nitrogen fertilization, and the interactions among these factors. The intensity of the link between production and content of protein in the three experimental years and fertilization variants was estimated using the correlation coefficient (r).

Results

Grain yield

Analysing Table 2 it is noticeable that for grains yield there is variance for the genotype, years, N fertilization and G×Y interaction, and no variance among G×F interaction and G×Y×F interaction.

The results of ANOVA indicate that the years were more important in the interactions with the genotypes than was the N fertilization. The interaction of genotype, year and N fertilization also showed that the effect of N fertilization was relatively unimportant for the occurrence variance; this aspect is in accord with other researches (e.g. Ayoub *et al.*, 1995; Cooper *et al.*, 2001 and Ma *et al.*, 2004). In eastern Canada, Ma *et al.* (2004) explain the lack of N fertilization main effect, and N x environment interaction by due to the high N-supply of soils, as well as the experimental conditions at ARDS Turda.

Fertilisation with N_{100} presents the highest grain yield, regardless of cultivar or year (Table 3). Our data are in the same range as those in the studies reported by CRAAQ, 2010, which recommended 90 to 120 kg N ha rate for spring wheat in Quebec.

In the climatic years favourable to the wheat crop, 2015 and 2017, the 'Feeling' cultivar provided the highest production, and in 2018 (extreme climatic conditions) the 'SG 5-01' cultivar was noted for the same character. Both varieties have good production stability, showing statistically positive difference as compared with the control (mean of the experiment) for average production over the three experimental years.

Table 2. Analysis of variance on grain yield in the experiment $(19 \times 3 \times 2)$

Source of variation	Df	MS	F	Р
Genotype (G)	18	289.580	27.165	0.0001
Year (Y)	2	6114.021	178.763	0.0001
N fertilizers (F)	1	3694.082	66.675	0.0001
G×Y interaction	36	82.792	7.767	0.0001
G×F interaction	18	12.356	1.159	NS
G×Y×F interaction	36	15.389	1.444	NS
Error A	4	34.202		
Error F	6	55.404		
Error S	216	10.660		

Note: NS - not significant at the $P \le 0.05$ level.

Table 3. Average grain yields (kg ha-1) obtained in HRSW cultivars, on two levels of nitrogen fertilization (ARDS Turda, 2015-2018)

Calaina	2015		20	2017		2018		Average of N fertilisation	
Cuitivar	N50	N100	N50	N100	N50	N100	N50	N100	
'Pădureni'	5,387	6,657	4,203 ⁰⁰⁰	5,460	3,600°°°	4,243°00	4,397 ⁰⁰	5,453	
ʻT. 265-01'	5,800	6,283	5,330	5,930	4,177	5,203	5,102	5,805	
'Sg 5-01'	6,267	7,140	6,003***	6,407*	5,213	5,923	5,828	6,490	
'Sg 106-01'	6,343	7,097	5,380	5,780	5,453 **	6,007	5,725	6,295	
'Sg U 773'	5,723	6,917	5,520	5,853	4,813	5,577	5,352	6,116	
'Corso'	4,763 ⁰⁰⁰	5,650 ⁰⁰⁰	5,137	5,513	5,107	5,447	5,002	5,537	
'Lona'	5,720	6,987	5,683	6,080	4,840	5,840	5,414	6,302	
'Feeling'	6,947 **	8,010"*	5,920	6,320	4,597	5,313	5,821**	6,548**	
'Gk Tavasz'	6,093	6,343	3,867000	5,227	4,587	4,863	4,849	5,478	
'Triso'	5,910	6,970	5,607	6,007	5,377	5,737	5,631	6,238	
'Broma'	5,763	6,260	5,440	6,120	4,910	5,210	5,371	5,863	
'Beloterkovskaia'	6,030	6,387	4,963	5,330	4,887	5,030	5,293	5,582	
'Henica'	5,530	6,450	4,647	5,047°	3,377	4,033	4,518°	5,177°	
'Jara'	5,797	6,517	4,663	5,070°	4,333	4,513	4,931	5,367	
'Jota'	6,623	7,163	5,023	5,423	3,790	4,997	5,145	5,861	
'Prif 3'	5,570	6,423	5,090	5,623	4,707	5,197	5,122	5,748	
'Prif 4'	5,363	6,423	4,870	5,273	4,177	4,937	4,803	5,544	
'Sigma'	5,967	6,607	4,620	5,023°	3,610	4,873	4,732	5,501	
'Silva'	5,933	6,517	5,117	5,517	3,913	4,800	4,988	5,611	
Mean of experience – control	5,870	6,674	5,110	5,632	4,498	5,144	5,159	5,817	
Average of year	6,2	.72	5,3	71	4,8	321			

LSD 5% - 525; LSD 1% - 693; LSD 0.1% - 890

Although fertilization with N_{100} provided the highest yields, there were also certain varieties 'SG 106-01', 'Corso' (2018 - unfavourable conditions), 'Jota' and 'Lona' (2015 - favourable conditions) that performed better under conditions of N 50 fertilization.

The Romanian 'Pădureni' variety, created at ARDS Turda, has an acceptable yield potential, assimilates nitrogen well, but under the unfavourable climatic conditions of 2018, did not performed well. The alternative 'T. 265-01' line, also created at Turda, exceeds the 'Pădureni' variety for production and stability, being a good alternative for its replacement in culture.

Protein content of grains

Analysis of variance for grain protein content showed there was variance for the same factors as in the case of the yield: genotype, years, N - fertilisation and $G \times Y$ interaction. For $G \times F$ and $G \times Y \times F$ interaction there was also no variance (Table 4). These results prove that the interaction between genotype and year is the most important in the occurrence of variance for this HRSW character.

Table 4.Analysis of variance on grain yield in the experience $(19 \times 3 \times 2)$

The low temperatures which coincided with floral primordia differentiation phenophases in 2017, influenced the grain protein content of HRSW (Koga *et al.*, 2015), because the highest values for this character were recorded in the years 2015 and 2018. As well as in the production, high nitrogen fertilization increased the protein content of the grains (Table 5). Our data are in line whit previous studies which showed that higher N rates are very important to increase grain protein concentrations for all spring wheat varieties studied by Ma *et al.* (2004). Nyiraneza (2012) prove the same fact, that the split application of N fertilizer at a rate of 120 kg N ha⁻¹ was sufficient to maximize yield and attain the grain protein content level needed for the maximum price premium.

Regardless of the climatic conditions and the N fertilization dose, 'Pădureni', 'Corso' and 'GK Tavasz' varieties performed better as compared to the other varieties in all experimental years.

A particular case for the protein content was the 'Jara' cultivar, which showed statistically negative differences compared to the experience average (control) in 2015, the favourable year for HRSW and statistically positive

Source of variation	Df	MS	F	Р
Genotype (G)	18	7.870	126.113	0.0001
Year (Y)	2	243.411	642.361	0.0001
N fertilizers (F)	1	238.334	2369.481	0.0001
G×Y interaction	36	1.283	20.561	0.0001
G×F interaction	18	0.283	4.540	NS
G×Y×F interaction	36	0.175	2.805	NS
Error A	4	0.378		
Error F	6	0.100		
Error S	216	0.062		
Note: NS not significant at the D < 0.05 level				

Note: NS - not significant at the $P \le 0.05$ level.

Table 5.Grain protein content (%) in HRSW cultivars, on two levels of nitrogen fertilization (ARDS Turda, 2015-2018)

Cultinur	2015		20	2017		2018		Average of N fertilization	
Cultivar	N50	N100	N50	N100	N50	N100	N50	N100	
'Pădureni'	13.6	16.0***	10.6**	13.3	12.8	14.1	12.3	14.5	
'T. 265-01'	12.8	15.4 **	10.2	12.8	12.4	14.3	11.8	14.2	
'Sg 5-01'	12.0	13.4	9.1	11.4	12.1	13.0°	11.1	12.6	
'Sg 106-01'	12.5	13.5	9.9	12.4	11.9°	12.8 ⁰⁰	11.4	12.9	
'Sg U 773'	12.5	13.4	9.6	12.0	12.3	13.5	11.5	13.0	
'Corso'	14.2	16.2"*	10.4	13.0	13.9	15.0	12.8	14.7***	
'Lona'	12.6	14.1	9.6	12.0	12.6	13.6	11.6	13.2	
'Feeling'	12.7	13.7	9.2	11.5	12.2	12.9°	11.4	12.7	
'Gk Tavasz'	14.1	15.5"*	10.9	13.6	13.7	14.6	12.9	14.6	
'Triso'	12.2	13.9	9.1	11.4	12.8	14.4	11.4	13.2	
'Broma'	12.4	13.9	9.3	11.6	12.5	13.4	11.4	13.0	
'Beloterkovskaia'	12.3	14.2	9.1	11.4	13.2	14.7"	11.5	13.4	
'Henica'	12.4	14.0	9.8	12.3	12.7	13.8	11.6	13.4	
'Jara'	11.300	13.1 ⁰⁰	9.0	11.3	13.3 [°]	14.5	11.2	13.0	
'Jota'	11.0^{000}	13.3°	8.5 ⁰⁰	10.6°00	12.4	13.7	10.6	12.5	
'Prif 3'	12.2	13.3°	9.2	11.5	11.8°	13.0°	11.1	12.6	
'Prif 4'	12.4	13.6	9.3	11.6	12.3	13.3	11.3	12.8	
'Sigma'	11.2000	13.4	8.7°	10.9 ⁰⁰	12.2	13.8	10.7°	12.7	
'Silva'	11.300	12.9°°	9.0	11.4	11.500	12.7 ⁰⁰	10.6 ⁰⁰	12.3 ⁰⁰	
Mean of experience control	12.4	14.0	9.5	11.9	12.6	13.7	11.5	13.2	
Average of year	13	.2	10	0.7	13	3.1			

LSD 5% - 0.70; LSD 1%- 0.90; LSD 0.1% - 1.20



Fig. 2. Correlation between yield and protein content on HRSW

differences in 2018, year with extreme climatic conditions. Probably the low temperature alternation with the high temperatures of that year has positively influenced the accumulation of protein, irrespective of the fertilization dose. 'Beloterkovskaia' and 'Triso' are also two varieties that in 2018 reached high protein content in condition of N_{100} fertilization, although in the favourable years did not show statistically assured differences.

'Pădureni' variety had the high protein content regardless of the dose of N; the other variety created at ARDS Turda, 'T. 265-01', had a good response of this character only at high doses of N. Both varieties had good stability for quality, not being influenced by climatic conditions.

Although negative correlations were found between the yield and the protein content in HRSW, the regression analysis shows that only a small part of the variation in protein content can be attributed to the variation in production, which gives the possibility of overcoming this obstacle (Fig. 2).

Genotypes that show positive regressions of protein content, such as 'Pădureni', are an argument in this respect, a variety that has an acceptable production potential, being a solution for expanding the areas cultivated with HRSW in Romania, to this date. Two other genotypes: 'Feeling' and 'Lona', which are very productive and have quality indices that correspond to the bakery industry, have been noted in this study.

The 'T. 265-01' alternative wheat line, which is sown in spring (March), presents quality indices close to 'Pădureni', being more productive than it. This line can replace it in the near future, taking into consideration the growing demand for seed of HRSW varieties and ARDS Turda is the only bidder among the breeding centres in Romania for this species.

Conclusions

The present research concluded that years were more important in the interactions with genotypes than the N fertilization, for both studied characters. Higher application of N assured high yields and good protein content for all studied cultivars, but there were also certain varieties, 'SG 106-01', 'Corso', 'Jota' and 'Lona', that behaved well under conditions of N_{50} fertilization. The most productive cultivars, with good stability of yield, were 'Feeling' and 'SG 5-01'; the most valuable varieties regarding the protein content were 'Pădureni', 'Corso' and 'GK Tavasz'. Negative correlations were found between the production and the protein content in HRSW (4 out of 6 cases), but also were found some cultivars that showed positive regressions of protein content: 'Pădureni', 'Feeling' and 'Lona'.

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