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THE EFFECTS OF NITROGEN NUTRITION AND GLUTENIN COMPOSITION ON THE GLUTEN QUALITY IN WHEAT GENOTYPES

The effect of nitrogen nutrition treatments on the gluten content and some quality parameters of eight winter wheat cultivars has been studied. Six different nitrogen rates were applied (0, 60, 90, 120, 150 and 180 kg N ha^{-1}) to wheat cultivars chosen according to the structure of their high molecular weight glutenin subunits (HMW-GS) at the Glu-D1 locus. Four genotypes with HMW-GS 2 + 12 and another four with HMW-GS 5 + 10 were used in the study. The analysis of gluten quality involved the wet gluten content and rheological properties determined by the sensory and instrumental methods ("Instron 4301"). It was determined that in all the cultivars the wet gluten content increased significantly ($P < 0.05$) in parallel with N rate increase. The cultivars reacted differently regarding their wet gluten rheological properties. Libellula, a cultivar with poor bread making quality (HMW-GS 2 + 12), did not react to different N rates. Sremica, a cultivar with excellent bread making quality (HMW-GS 5 + 10), reduced its gluten quality as the N rate increased. The values obtained by the instrumental method "Instron 4301" at 90% wet gluten compression varied widely (from 0.002 to 0.041 kN). The increase of N fertilizer rate was significantly positively correlated ($r_2 = 0.811$) with the wet gluten content and strength in the cultivars with HMW-GS 5+10.

Key words: wheat; N-nutrition; wet gluten; rheological properties; "Instron 4301".

Wheat flour proteins interact in the presence of water by forming gluten which provides the unique dough viscoelastic properties needed for bread-making [1]. However, its properties are influenced by various factors observed both during plant growth and grain processing [2,3].

Gluten proteins are particularly important for bread-making quality and consist of two major fractions: the monomeric gliadins and the polymeric glutenins [4]. Gliadins are responsible for dough extensibility and viscosity while glutenins are responsible for strength and dough elasticity [5]. Gluten quality of wheat is affected by genotype, environment and their interaction. Many factors can produce environmental modification of the gluten quality, including fertilizer levels [6,7] and precipitation during the grain filling period [8]. N fertilization increases the total quantity of flour proteins, resulting in an increase in both gliadins and glutenins [9-11].

Gliadins increase preferentially over glutenins as N accumulation in the grain increases [12]. Consequently, the ratio of gliadin to glutenin proteins was positively correlated with the grain N content, even when both gliadins and glutenins increased. Those variations may lead to a decrease in gluten strength and mixing properties [13]. Other authors [14,15] have reported that the relative amount of gliadins and glutenins was not affected by the increase in N fertilization. The inconsistency in responses could be a consequence of genotypic differences and variation in the allocation of N to protein subunits.

The glutenins are divided into two groups: high molecular weight (HMW-GS, 80-120 kD) and low molecular weight glutenin subunits (LMW-GS, 30-50 kD). The HMW-GS, encoded by Glu-A1, Glu-B1 and Glu-D1 loci from the long arm of chromosome 1, were found to be major protein components the properties of which are highly responsible for variations in the wheat quality. In the case of the Glu-D1 locus, good quality is specifically associated with a pair of subunits 5 + 10,

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unlike 2 + 12 [16–18]. Subunit Glu D1 5 + 10 was associated with higher dough strength in genotypes as compared to those with subunit Glu-D1 2 + 12 [19]. Genotypes of similar protein quality and the composition responded similarly to the N treatments [20].

One of the issues relating to the above problem is the research on the effects of an increasing nitrogen fertilization of wheat plants on the wet gluten strength. It is known that higher N-fertilization causes a higher gluten content. However, it is unknown whether gluten strength changes as well and, especially, to what extent the genetic background of cultivars is involved in the change.

During the washing out [21], the gluten absorbs and stores different quantities of water which can also affect its strength. However, the moisture content did not depend on the level of N-fertilization [19]. Physical properties of wet gluten can also be examined by high temperature which is an essential factor inseparably associated with bread baking [22]. Although sensory tests [23] are irreplaceable in the understanding of the rheological properties of a product, they are increasingly being substituted with modern instruments providing more objective results. One of these instruments is UTM "Instron Model 4301", which is used for measuring rheological characteristics such as hardness, cohesiveness, adhesiveness, compressive stress, force decay parameter and apparent biaxial extensional viscosity [24]. Determination of gluten strength by this instrument was not usual in estimation of wheat qaulity characteristics.

The aim of the present study was to investigate the effect of different rates of nitrogen fertilization and glutenin composition at the Glu-D1 locus on wheat cultivars gluten characteristics. The present paper discusses the possibility of instrumental measurement of the wet gluten strength and compares these (objective) results with those obtained by (subjective) sensory testing.

EXPERIMENTAL

The material comprised eight cultivars chosen from a wheat collection. Seven of these originated from Serbia and one, Libellula, from Italy. Four cultivars, NSR-2, Lasta, Libellula and Drina contain HMW-GS 2+12, while the cultivars Jugoslavija, Sremica, Somborka and Pobeda contain HMW-GS 5+10.

The analyzed samples belonged to the Internationale Stickstoff-Dauer-Versuche (ISDV) stationary field trial, established at Rimski Šančevi experimental field of the Institute of Field and Vegetable Crops in Novi Sad, Serbia, in 1971. The tested materials were taken from the growing season 2006/07. Six different nitrogen rates were used (Table 1).

Table 1. Nitrogen rates (kg N ha^{-1}) and times of application

Total amount	Before planting	1st Top dressing	2nd Top dressing
0	-	-	-
60	20	40	-
90	30	60	-
120	40	60	20
150	50	60	40
180	60	60	60

Quality parameters were measured in three replicates. Quality analyses such as wet gluten content, extensibility and adhesiveness of gluten were done using two methods [21,23]. Wet gluten contents (WG) were rated as follows: below 21.0% – low (1); 21.1–24.0% – satisfactory (2); 24.1–27.0% – good (3); 27.1–30.0% – very good (4); over 30.1% – excellent (5).

Dough was made from 25 g of flour and left to sit for 20 min at 18±2 °C before washing. After that, 4 g of wet gluten were shaped into a ball and immersed in water for 15 min at 18±2 °C [23]. The gluten samples were then extended on a ruler for 10 s and graded descriptively (sensory evaluated) as follows. Extensibility (E): (+) extensible, up to 10 cm (strong); (±) moderately extensible, 10–20 cm (medium strong); and (–) very extensible, over 20 cm (weak). Adhesiveness (A): (+) poorly adhesive; (±) adhesive and (–) very adhesive.

The instrumental determination of the wet gluten strength (WGS), which was obtained by applying the Standard method [21] after a period of rest [23], was performed on the Instron Model 4301, a universal testing machine [25]. The operating conditions were as follows: wet gluten sample weight – 1.5 g; working temperature, 18–20 °C; compression force, 0.25 kN; speed, 100 mm/min.

All quantitative results were processed in the computer program Statistika 7 (StatSoft, Inc. Corporation, Tulsa, OK, USA). Before applying the analysis of variance, the appropriate data transformations and adequate variance analysis models were applied. Mean comparisons were done on the basis of LSD values. Correlation coefficients among the studied traits were calculated in the same statistical program.

RESULTS AND DISCUSSION

The main effect of the applied N treatments was a significant increase of the wet gluten content in all cultivars. The wet gluten content at different N rates ranged between 14.3 and 32.0% (Table 2). Statistically significant (P<0.05) differences in the wet gluten content were found between the N rates of 0, 90 and 180 kg N ha^{-1} in the group of cultivars with HMW-GS 2+12 and the N rates of 0, 90 and 150 kg N ha^{-1} in the group of cultivars with HMW-GS 5+10. Significantly lower the wet gluten content was produced only by the cultivar Lasta relative to Sremica at 90, 150 and 180 kg N ha^{-1}. The highest average wet gluten content was found in the cultivar Sremica (27.7%) while the lowest WG content was in cv. Lasta (19.3%).

The cultivars with HMW-GS 2+12 had a lower average wet gluten content (23.6%) than the cultivars with HMW-GS 5+10 (25.7%) but the difference was not significant. The wet gluten content was found to vary significantly ($P<0.05$) due to a cultivar, a fertilizer rate and the interaction fertilizer rate x cultivar (Table 2).

All cultivars exhibited the same reaction to the increased N-rate, *i.e.* they increased their wet gluten content (Table 2). Pepo *et al.* [26] have arrived at the same conclusion in their 18-year trial with dominant Hungarian cultivars. The cultivar Lasta had the lowest wet gluten contents at all levels of N fertilization, while most of the other cultivars showed no significant differences (Table 2).

Changes in the wet gluten strength expressed by the rheological properties of the cultivars are presented in Table 3. All the cultivars had the highest level of wet gluten at 180 kg N ha^{-1}, but some of the cultivars achieved the same level at 150 kg N ha^{-1} (NSR-2, Lasta, Sremica, Somborka and Pobeda). Wet gluten of cv. Libellula could be described as very weak. Immediately after washing, the gluten becomes compact, sticky and viscous. In the cultivar Drina, mineral nutrition had more effect on rheological properties than on the wet gluten content. Even at 60 kg N ha^{-1}, wet gluten became medium strong, sufficiently elastic, moderately extensible and adhesive. The cultivar NSR-2 had the most favorable rheological properties at 150 and 180 kg N ha^{-1}. Already at 90 kg N ha^{-1}, the medium strong gluten that had moderate extensibility was formed. At the N-rates below 150 kg ha^{-1}, wet gluten of the cultivar Lasta changed a little. Adding 150 and 180 kg N ha^{-1} increased the level of wet gluten (2), which became firmer (medium strong).

The cultivar Sremica from the group with genetically strong gluten (HMW-GS 5+10) had better rheological properties of gluten at lower N-rates (Table 3). With increased N-rates (above 90 kg ha^{-1}), the gluten became weaker and attained greater extensibility and adhesiveness, meaning that its quality was reduced. Abad *et al.* [27] also suggest that even using high quality wheat varieties, the quality can still be increased by nitrogen fertilization, even at low nitrogen rates. Gluten of the cultivar Somborka became progressively firmer and more elastic with the increase of the N-rate above 90 kg N ha^{-1}. Wet gluten rheological properties of the cultivar Jugoslavija were distinctive. Right after washing, with all N-rates, the gluten was adhesive and weak. When more than 120 kg N ha^{-1} was used, the gluten became medium strong. The cultivar Jugoslavija is quite specific, since it takes up more nitrogen from the soil than from N-fertilizers. Because of that, it needs higher amounts of *N* for achieving the

Table 2. Wet gluten content (%) of wheat cultivars with different HMW-GS at increasing N fertilization rates (LSD (0.05) - N-treatment 2.9; cultivar 4.0; interact. N/C 8.7)

N-rate, kg N ha^{-1}	Cultivars with HMW-GS 2+12				
	Libellula	Drina	NSR-2	Lasta	Aver.
0	20.3	18.7	21.0	14.3	18.6
60	22.3	20.0	22.0	16.7	20.3
90	25.0	23.3	25.7	18.3	23.1
120	26.7	25.0	28.0	21.0	25.2
150	27.0	26.7	30.3	22.0	26.5
180	30.0	28.8	30.3	23.3	28.1
Average	25.2	23.8	26.2	19.3	23.6
	Cultivars with HMW-GS 5+10				
	Sremica	Somborka	Jugoslav.	Pobeda	Aver.
0	22.0	18.7	20.3	20.0	20.3
60	24.7	20.0	21.3	20.7	21.7
90	27.3	23.7	25.7	25.0	25.4
120	30.0	25.0	27.3	27.7	27.5
150	30.3	27.7	29.7	28.0	28.9
180	32.0	30.0	31.7	28.7	30.6
Average	27.7	24.2	26.0	25.0	25.7

Table 3. Sensory evaluation of wet gluten rheological properties of wheat cultivars with different HMW-GS at increasing N fertilization rates (wet gluten contents (WG) were graded as follows: below 21.0% – low (1), 21.1-24.0% – satisfactory (2); 24.1-27.0% – good (3); 27.1-30.0% – very good (4), over 30.1% – excellent (5); E: Extensibility: (+) up to 10 cm - inextensible (short); (±) 10-20 cm – moderately extensible; (-) over 20 cm – highly extensible (weak); A: Adhesiveness: (+) poorly adhesive, (±) adhesive; (-) very adhesive)

N-rate, kg N ha^{-1}	Wheat cultivars (HMW-GS 2+12)											
	Libellula			Drina			NSR-2			Lasta		
	WG (%)	E (cm)	A	WG (%)	E (cm)	A	WG (%)	E (cm)	A	WG (%)	E (cm)	A
0	1	-	-	1	–	–	1	-	±	1	+	±
60	2	-	-	1	±	±	2	-	±	1	+	±
90	3	-	-	2	±	±	3	±	±	1	±	±
120	3	-	-	3	±	±	4	±	-	1	+	±
150	3	-	-	3	+	-	5	+	±	2	+	±
180	4	-	-	4	±	±	5	+	±	2	+	+
	Wheat cultivars (HMW-GS 5+10)											
	Sremica			Somborka			Jugoslavija			Pobeda		
0	2	+	+	1	–	–	1	±	–	1	±	±
60	3	+	+	1	±	±	2	±	–	1	±	±
90	4	+	+	2	+	±	3	±	–	3	±	±
120	4	±	±	3	+	±	4	±	±	4	±	±
150	5	–	±	4	+	+	4	±	±	4	+	+
180	5	–	–	4	+	+	5	±	±	4	+	+

maximum wet gluten content than for the maximum protein content [6]. The cultivar Pobeda was the only one that achieved the highest level of wet gluten (4) at 120 kg N ha^{-1}. Up to 120 kg N ha^{-1}, the gluten had little extensibility and was less adhesive (medium strong). At 150 and 180 kg N ha^{-1}, it turned less extensible and adhesive and became elastic (strong). The different properties of the gluten samples indicate that the gluten proteins differed in quality [19,28,29].

The sensory analysis of the gluten extensibility and adhesiveness showed that the cultivars reacted highly specifically, regardless of the HMW-GS content. Unlike the total wet gluten content, the cultivar Libellula showed no reaction to different N rates, *i.e.*, it had weak and adhesive gluten at all levels of N fertilization. This Italian cultivar is known for its poor bread making quality [6]. In the case of Sremica, the only cultivar from the group of excellent quality cultivars, the increased N rate tended to reduce the gluten quality (Table 3).

The same pattern has been reported by Johansson *et al.* [10,11] for Swedish cultivars. In our investigation, all cultivars tended to increase the gluten content as the N-rate increased, *i.e.*, the gluten became stronger and less adhesive (Table 3). It is of interest to mention that the cultivars Lasta and Pobeda had strong and non-adhesive gluten with all N-rates.

Changes in wet gluten strength (WGS) expressed by the compression of the studied cultivars in relation to N-fertilization were influenced mainly by the cultivar (Table 4). The values for WGS obtained at 90% gluten compression ranged between 0.002 and 0.041 kN. The cultivars with HMW-GS 2+12 had different values of WGS (0.003, 0.009 and 0.008 kN) in the control treatment (0 kg N ha^{-1}), while the cultivars with HMW-GS 5+10 had smaller variation with the values of 0.007 and 0.008 kN (Table 4). Libelulla had extremely low WGS at 90% gluten compression in all N treatments with the average value of 0.003 kN, while the cultivar Pobeda had the highest average value (0.024 kN). Sensory and instrumentally determined gluten strength of these extreme cultivars was comparable at different N-rates as well as on the average. The WGS was found to vary significantly ($P<0.05$) due to the cultivar, the fertilizer rate and the interaction fertilizer rate x cultivar.

The cultivar Sremica has strong gluten with good bread-baking qualities. It had higher gluten strength at 0 and 60 kg N ha^{-1} (0.007 kN) compared with the other N-rates. Gluten of the cultivars with HMW-GS 2+12, Drina, NSR-2 and Lasta (0.012, 0.01 and 0.01 kN, respectively) as well as Somborka (0.01 kN) and Pobeda (0.041 kN) with HMW-GS 5+10 had the highest strength at 150 kg N ha^{-1}. With the cultivar Jugoslavija, the situation was reverse, as greater WGS was observed at lower N-rates (below 150 kg N ha^{-1}). Three cultivars differing in the gluten quality, Libellula, Sremica and Jugoslavija, had relatively small WGS values at the highest N-rates of 150 and 180 kg ha^{-1}.

Correlations (including rang correlations) between studied quality parameters and applied N doses are presented in Table 5. Increasing N-rates had a

Table 4. Wet gluten strength (kN), under different nitrogen nutrition conditions, determined on the „Instron Model 430" (LSD (0.05) - - N-treatment 0.00413; cultivar 0.00854; interact. N/C 0.005103)

N-rate, kg N ha^{-1}	HMW-GS 2+12					HMW-GS 5+10				
	LI	DR	NSR-2	LA	Average	SR	SO	JU	PO	Average
0	0.003	0.009	0.008	0.008	0.007	0.007	0.008	0.007	0.008	0.007
60	0.003	0.008	0.006	0.008	0.006	0.007	0.009	0.008	0.007	0.007
90	0.004	0.01	0.007	0.04	0.015	0.006	0.009	0.008	0.009	0.008
120	0.005	0.009	0.009	0.04	0.016	0.005	0.009	0.008	0.04	0.016
150	0.003	0.012	0.01	0.01	0.009	0.006	0.01	0.005	0.041	0.016
180	0.002	0.01	0.009	0.01	0.008	0.004	0.01	0.004	0.041	0.014
Average	0.003	0.010	0.008	0.019	0.010	0.006	0.009	0.007	0.024	0.012

highly significant effect on the wet gluten content of both HMW-GS cultivar groups. N treatments had a significant effect (r_1 = 0.817) on the gluten strength only in the cultivars with HMW-GS 5+10. The significant correlations (r_2 = 0.811) between the gluten strength and the content found in the cultivars with HMW-GS 5+10 are an indication of greater N utilization efficiency for the quality improvement. The lack of significance in rang correlations (r_3), on the other hand, indicates that increased N-rates did not have the same effect on the gluten content and gluten strength, suggesting that the two traits are controlled by different genetic mechanisms regardless of the HMW-GS composition. It is also known that the total amount of HMW-GS is more important than HMW-GS composition in determining the flour quality. Thus, the accumulation of HMW-GS in grain could be a very important aspect of the wheat quality development [19].

Table 5. Correlation coefficients between studied wheat quality characteristics and the increasing N-rates (r_1 - correlation coefficients between the increasing N-rates and quality characteristics; r_2 - correlation coefficients between quality characterristics; r_3 - spearmans coefficients of rang correlation)

N-rate kg N ha^{-1}	HMW-GS 2+12		HMW-GS 5+10	
	WG	WGS	WG	WGS
0	18.6	0.007	20.3	0.007
60	20.3	0.006	21.7	0.007
90	23.1	0.015	25.4	0.008
120	25.2	0.016	27.5	0.016
150	26.5	0.009	28.5	0.016
180	28.1	0.008	30.6	0.014
r_1	0.987	0.232	0.978	0.817
r_2	0.299		0.811	
r_3	0.428		0.794	

This means that wet gluten rheological properties could not be distinguished between the groups of cultivars containing HMW-GS 5+10 and 2+12. The nature of the cultivar-by-fertilizer interaction for the gluten strength suggested that the conventional strength cultivars would benefit more from the nitrogen fertilizer than the cultivars with extra-strong gluten, which showed no change or slight decreases in the gluten strength with the nitrogen fertilizer, despite the increase in total gluten [11,27]. The incongruity of the mutually variable relations during the wet gluten compression and dough making, fermentation and baking can be explained after studying the links between the reserve proteins glutenin HMW and gliadin and bread volume [30].

CONCLUSION

The increasing of N-rates significantly increased the wet gluten content of the wheat grain. The differences in the wet gluten strength and content between cultivars with HMW-GS 2+12 and those with HMW-GS 5+10 were not significant. The increasing of N-rates reduced the wet gluten quality as assessed by the sensory and instrumental methods in the highest quality cultivar Sremica with HMW-GS 5+10 and the poorest quality cultivar Libellula with HMW-GS 2+12.

Sensory and instrumental methods used in this study for gluten strength determination are not absolutely equivalent. However, they are in good agreement when analyzing cultivars with extremely different gluten quality and strength as in the case of the cultivars Libellula (HMW-GS 2+12), Sremica and Pobeda (HMW--GS 5+10). Wet gluten physical properties tested by the sensory method depended on the cultivar, which is the most important factor in determining the wheat quality. A genotype selection for high strength seems to be the best strategy for avoiding an undesirable worsening of the gluten quality

The increase of N-fertilizer rate was significantly positively correlated with the wet gluten content and strength in the cultivars with HMW-GS 5+10.

Most of the tested cultivars achieved a high gluten level with 120 kg N ha^{-1}. It appears that further increases in N rate are not cost-effective in commercial production from the aspect of bread making quality. This should be taken into account especially for the cv. Pobeda, which is one of the widely grown cultivars in Serbia.

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NAUČNI RAD

UTICAJ AZOTNE ISHRANE I GLUTENINSKE STRUKTURE NA KVALITET GLUTENA PŠENICE

Ispitivan je uticaj azotne ishrane na sadržaj i kvalitativna svojstva glutena osam sorti ozime pšenice. Primenjene doze azota su bile: 0, 60, 90, 120, 150 i 180 kg N ha^{-1}. Sorte su odabrane u skladu sa strukturom njihovih visokomolekularnih gluteninskih subjedinica (HMW-GS) na Glu-D1 lokusu. Odabrane su četiri sorte sa HMW-GS 2+12 i četiri sorte sa HMW-GS 5+10. Analize su obuhvatale sadržaj i reološka svojstva glutena određena senzornom i instrumntalnom ("Instron 4301") metodom. Utvrđeno je da se paralelno, sa povećavanjem nivoa N-ishrane značajno (P < 0.05), povećavao i sadržaj vlažnog glutena kod svih sorti. Sorte su različito reagovale u pogledu njihovih reoloških svojastava. Sorta Libelula, sa slabim pekarskim kvalitetom i strukturom (HMW-GS 2+12), nije reagovala na različite doze azota. Kod sorte Sremica, odličnog pekarskog kvaliteta i strukturom (HMW-GS 5+10), sa povećavanjem doza azota pogoršana su reoloških svojstava glutena. Rezultati dobijeni instrumentalnom "Instron 4301" metodom, pri (90%) kompresiji vlažnog glutena varirali su od (0,002 do 0,041 kN). Povećavanje primenjenih doza azota bilo je u značajnoj pozitivnoj korelaciji (r_2 = 0.811) sa sadržajem i jačinom glutena kod sorti sa HMW-GS 5+10 strukturom.

Ključne reči: pšenica; N-ishrana; vlažni gluten; reološka svojstva; Instron 4301.