COMPARATIVE INVESTIGATIONS OF SEED STORAGE PROTEINS FROM NEW WHEAT LINES

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ABSTRACT

The objective of the present study was to determine the wheat grain protein fractions of twelve wheat lines, in characterizing their breadmaking quality. For this purpose we follow protein and starch content, sedimentation test Zeleny, selective extraction of gliadine and glutenine and electrophoretic preotein pattern. The high molecular weight glutenin subunits was identified by SDS-PAGE The obtained results recommend all analyzed wheat new lines as having a good breadmaking quality.

INTRODUCTION

Wheat has a nutritional value of particular importance and a special place in crops used as source of food to the most of the world population. The most important nutritional constituents of wheat are proteins and starch (Šramkováa et al., 2009).

The protein content of wheat grains may vary between 10%-18% of the total dry matter. Wheat proteins are classified in four fractions, according to their solubility in various solvents: albumins, which are soluble in water; globulins, which are insoluble in pure water, but soluble in dilute NaCl solutions, and insoluble at high NaCl concentrations; gliadins, which are soluble in 70% ethanol, and; glutenins, which are soluble in dilute acid or sodium hydroxide solutions (Osborne, 1907). Albumins and globulins are mostly biologically-active, performing catalytic and regulating functions. They are constitutional proteins with enzymatic activity (Singh and MacRitchie, 2001). Wheat's gliadins and glutenins, also called prolamins, function primarily as storage proteins (Waga, 2004). Gliadins constitute about 40% of the total endosperm protein and a heterogenous mixture of monomeric polypeptides. Glutenins consist of polypeptides aggregated by disulphide bonds. Both types of wheat storage proteins, the gliadins and glutenins, are the main components, building the gluten polymer and determine bread-making properties

The gliadins are divided into four groups, alpha- (α-), beta- (β-), gamma- (γ-), and omega- (ω-) gliadins, based on their electrophoretic mobility at low pH . The amino acid compositions of the α- , β- , γ- and ω- gliadins are similar to each other, although, the ω-gliadins contain little or no cysteine or methionine and only small amounts of basic amino acids (Singh and MacRitchie, 2001). All gliadins are monomers with either no disulphide bonds (ω-gliadins) or intra- chain disulphide bonds (α-, β-, and γ- gliadins). The molecular weights of ω-gliadins are between 46 and 74 KDa, and the α-, β- and γ-gliadins ranging from 30 to 45 Kda (Müller and Wieser, 1997). Genes controlling gliadins proteins are located on the short arms of chromosome groups 1 and 6 and their expression and accumulation of gliadin is often affected by the environment. (Triboi et al., 2000). In dough formation, the gliadins act as a 'plasticiser', promoting viscous flow and extensibility which are important rheological characteristics of dough. They may interact through hydrophobic interactions and hydrogen bonds.

Glutenin subunits bind to each other forming polymers linked by disulphide bonds. After reduction of disulfide bonds, glutenin subunits can be divided in two main groups: high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin

subunits (LMW-GS). The HMW-GS have molecular weight ranging from 80- 160KDa and the LMW-GS weights are 30-51 KDa. The HMW-GS account for about 5-10% of the total protein. The LMW-GS comprise about 20-30% of the total protein. HMW subunits are encoded at the Glu-1 loci of the group 1 chromosomes 1A, 1B, 1D, and each locus consists of two genes encoding an x-type and a y-type subunit. Because some genes are silent, wheat cultivars contain three, four or five subunits (Payne, 1987). Based on the studies of Payne (Payne *et al.*, 1982), most investigations were focused on the correlations between the presence or absence of subunit alleles and wheat quality, in particular, breadmaking quality. The unique breadmaking properties of wheat are generally ascribed to the visco-elastic properties of its gluten proteins. While monomeric gluten proteins (gliadin) show viscous behavior, polymeric gluten proteins (glutenin) are elastic.

Another major constituent of wheat grain is starch. The amount of starch contained in a wheat grain may vary between 60% and 75% of the total dry weight of the grain (Šramkováa et al, 2009). Starch occur in seed in the form of granules. Wheat has two types of starch granules: large (25-40 um) lenticular and small (5-10um) spherical ones. The lenticular granules are formed during the first 15 days after pollination. The small granules, representating about 88% of the total of granules, appear 10-30 days after pollination. (Belderok et al., 2000).

In the present, the increase of the nutritional quality of wheat is a goal in breeding and selection programs.

The purpose of this study is to determine the composition of the grain of ten new wheat lines, characterizing their breadmaking quality for testing and selection in comparison with two most cultivated genotypes, Dropia and Boema. The obtained data for chemical composition have been correlated with the production level.

MATERIAL AND METHODS

The wheat lines used in this study were produced at Research and Agricultural Development Station from Simnic. They are as follows: 1= DROPIA; 2= BOEMA; 3=S 9917-6; 4=S 9913-15; 5=S 9916-12; 6=S 0444; 7=S 0449; 8=S 072; 9=S 0718; 10= S 07116; 11=S 07167; 12=S 07170.

The protein content of samples analyzed was determined by the Kjeldhal method and sedimentation value according to Zeleny. Starch content was measured polarimetrically by the Ewers method. (Babeanu et al., 2003). Sample for SDS PAGE electrophoresis was prepared according to Naeem and Sapirstain (Naeem and Sapirstain, 2007). Flour was initially extracted twice with 50% 1-propanol for 15 min at room temperature, followed by centrifugation. The supernatant containing mainly gliadins was retained for subsequent use. The residue was then washed with 50% 1-propanol to remove any remaining soluble protein and extracted in extraction buffer (0.08M Tris–HCl containing 50% 1-propanol, pH 7.5) in the presence of 1% (w/v) DTT. Proteins were extracted for 30 min at 60 1C. The extract was then alkylated with extraction buffer containing 4-vinylpyridine (4%) at 60°C for 30 min. This brought the final concentration of DTT and 4-vinylpyridine in the sample to 0.5% and 2%, respectively. After centrifugation reduced and alkylated glutenin extracts were used for selective precipitation of HMW-GS with acetone (Melas et al, 1994) The precipitate (mainly HMW-GS) and supernatant (mainly LMW-GS) were used to separate protein fraction by SDS PAGE electrophoresis. The electrophoretic separations were achieved in a vertical electrophoresis unit coupled with a Consort E215 electric source at 25mA. The electrophoretic analyses were carried out according to the procedure of Laemmli in SDS-tris-glycine buffer (Laemmli et al., 1970). Separation gels (1.5 mm thick) consisted of a 4% polyacrylamide stacking gel and a 15% polyacrylamide resolving gel. During separation, the electrophoresis system was kept at 20°C. Gels were stained with 0,7% Coomassie Brilliant Blue R250 in 40 % methanol

overnight. After staining the gels were rinsed with 7,5 % acetic acid. Quantitative evaluation of HMW-GS was performed with a Karl Zeiss K1 40 densitometer.

From the two separate fractions, gliadins and glutenins, the protein content was determined after mineralization, by the Kjeldhal method.

All assay were performed in triplicate and the results presented here are the mean values.

RESULTS AND DISCUSSION

In the present the increase of the nutritional quality of wheat is a goal in breeding and selection programs. This study aims to characterize the new wheat lines in order to point out the most high performance variety. Having this purpose, several biochemical investigations have been made: protein and starch content, sedimentation test Zeleny, selective extraction of gliadine and glutenine and electrophoretic preotein pattern.

The protein content is one of the most important quality indices for grain wheat. For the investigated varieties, the protein content varies between 11,8% and 12,8% (Figure 1). These values are in good agreement with those reported by Başlar and Ertugayin , 2011.

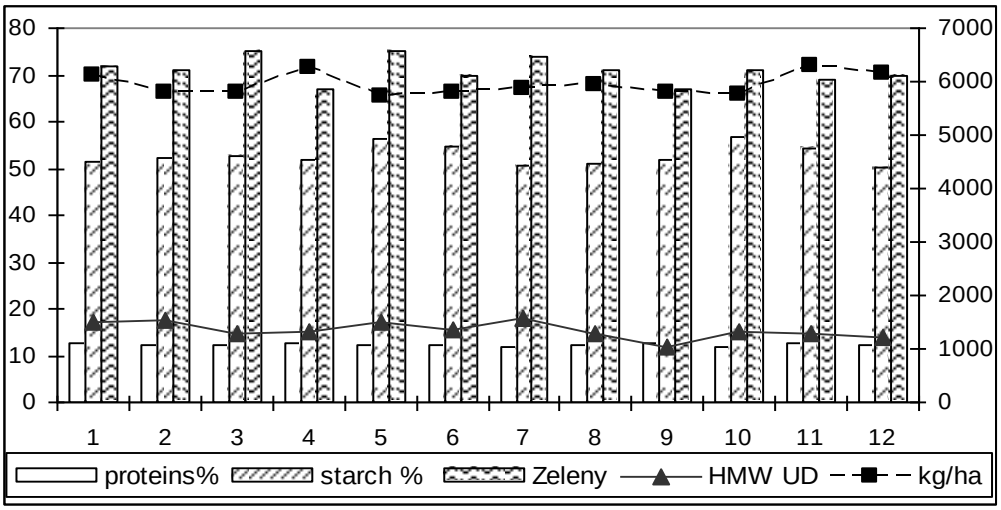

Figure 1. Chemical characterization and production level of investigated wheat genotypes (proteine, starch, Zeleny value-left axe; production kg/ha, HMW-right axe)

The proteine content of genotypes Dropia (12.8%) is higher than the values determined for all new wheat lines. The lines 7=S 0449; 10= S 07116 presents the lowest proteins content (11.8%). The protein content is negatively correlated with the production level. The existence of this inverse relationship has been established in a range of wheat-growing areas for a number of wheat varieties (Dupont and Altenbach, 2003; Johansson et al., 2003)

Another major constituent of wheat grain is starch. The obtained results showed that starch composition varied with studied wheat line (figure 1). The values ranged from 50.18 to 56.72%. The starch content of line 5=S 9916-12; 6=S 0444; 10= S 07116; 11=S 07167; is higher than the values determined for varieties Dropia and Boema, used as comparison.

The sedimentation value according to Zeleny (Zeleny value) describes the degree of sedimentation of flour suspended in a lactic acid solution during a standard time interval and this is taken as a measure of the baking quality. Swelling of the gluten fraction of flour in lactic acid solution affects the rate of sedimentation of a flour suspension. Both a higher gluten content and a better gluten quality give rise to slower sedimentation and higher Zeleny test values (Hruskova and Famera, 2003). The sedimentation value of flour

depends on the wheat protein composition and is mostly correlated to the protein content, the wheat hardness, and the volume of bread.

The obtained results for Zeleny value, for the studied lines are presented in figure 1. The values ranged from 67 to 75. The Zeleny values of line 3=S 9917-6; 5=S 9916-12; 7=S 0449 are higher than the values determined for varieties Dropia and Boema. The lines S 9913-15 and S 0718 have the lowest sedimentation value (Zeleny=67). Similar results are shown in other studies (Curic et al., 2001)

Wheat's breadmaking potential is derived largely from the quantity and quality of its protein. Protein quantity is influenced by environmental factors, while the quality of the protein is genetically determined. In wheat varieties that are grown under comparable environmental conditions, a high quality wheat will produce good bread over a fairly broad range of protein levels. A poor quality wheat will yield relatively low quality bread even at high protein contents.

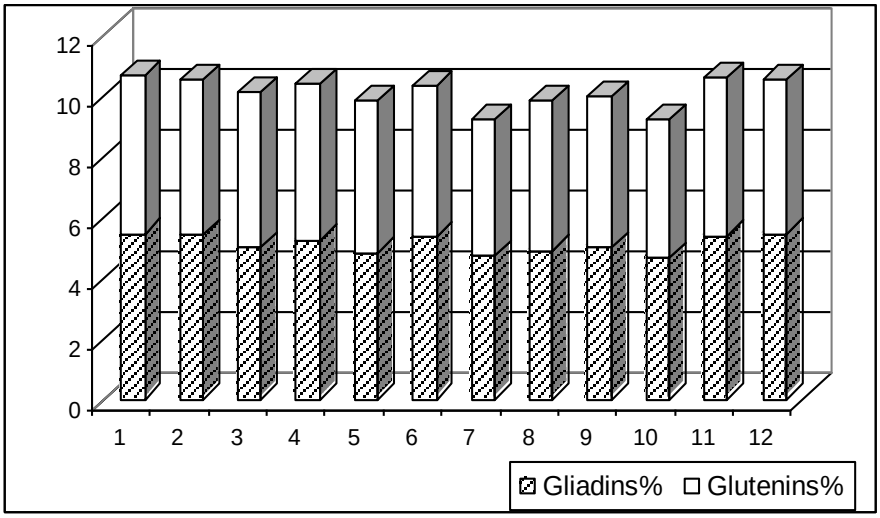

Figure 2. Gliadins and glutenins content of investigated wheat genotypes

The results for glutenins and gliadins contents are presented in figure 2. The glutenins content ranged from 4.5 (S 0449) to 5.23% (Dropia). The gliadins content ranged from 4.67 to 5.44% (Dropia and line 12=S 07170). The gliadin content for the new lines is lower than the gliadins content of variety Dropia, except line 12=S 07170. The line 10= S 07116 has the lowest gliadins content (4.67%). Same variation is observed from the glutenins content. The ratio glutenine/gliadine range from 0.93 to 1.03, signifying a good quality gluten.

In order to characterize the breadmaking quality, the glutenin fractions with a high molecular weight have been separated through electrophoresis, given that a high level in these is associated with gluten quality (figure 3) Quantitative and qualitative evaluation of HMW-GS is plotted in figure 4

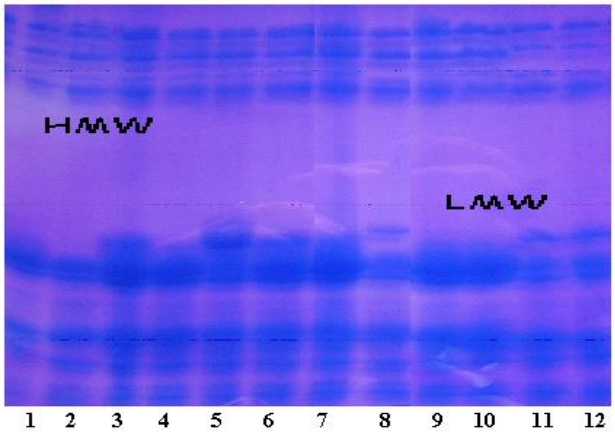

Figure 3 The image of electrophoregram of high molecular weight glutenin subunits for investigated wheat lines

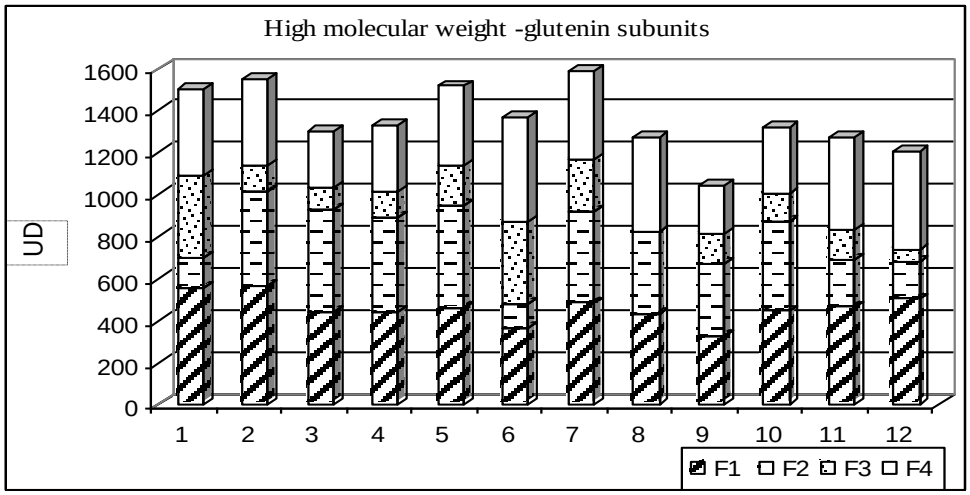

Figure 4 Electrophoretic patterns of high molecular weight glutenin subunits for investigated wheat lines

All investigated wheat lines show four fractions, except line 8=S 072 which presents three fractions. The fractions with high molecular weight indicates quantitative differences among wheat lines. The values for total HMW-GS range from 1044 UD (densitometric units) for line 9=S 0718 to 1585 UD (densitometric units) for line 7=S 0449. Dropia and Boema, the most cultivated genotypes in Romania, used as comparison in this study, present 1503 UD respectively 1546 UD. The values for total HMW-GS for the new lines is lower than the values for total HMW-GS content of varieties Dropia and Boema, except line 7=S 0449. Lines 5=S 9916-12 and 7=S 0449 are distinguished as having high content of HMW-GS and high breadmaking quality.

CONCLUSIONS

The chemical and biochemical analyses show the following outcomes:

- The protein content for all the analyzed lines is lower than for the Dropia genotype. In particular, the lowest protein content is found in lines 7=S 0449 and 11=S 07167.
- The lines investigated have a protein content level close to Boema.
- The obtained production level varies compared to the Dropia production, from -6.37% to +2,64%, and compared to the Boema production between -1.1% and +8,45%.

- Another quality indicator for the obtained production is the level of starch content. All the lines that were investigated have a level of starch close to the level of the comparison genotypes. The level of starch content compared to the one of the Dropia genotype varies between – 2.75% and +9.92%. Compared to the Boema genotype, the variance is between -4.23% and 8.24%.
- The index of sedimentation (breadmaking quality indicator) ranges between -6% and +5% compared to the genotypes utilized for comparison.
- The ratio glutenins/gliadins ranges from 0.93 to 1.03, which indicates gluten of good quality.
- In order to characterize the breadmaking quality, the glutenin fractions with a high molecular weight have been separated through electrophoresis, given that a high level in these is associated with gluten quality
- The values for total high molecular weight – glutenin fraction for the new lines is lower than the values for total high molecular weight – glutenin fraction content of varieties Dropia and Boema, exception being line 7=S 0449.
- Lines 5=S 9916-12 and 7=S 0449 are distinguished as having high content of high molecular weight – glutenin fraction and high breadmaking quality.

For the investigated lines, results show that breadmaking quality parameters indicate levels close to the ones of the genotypes used as comparison. In particular, line 11=S 07167 is distinguishable as having the highest protein content, and line 7=S 0449 which despite having a lower protein content, shows the highest HMW-GS content.

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