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# VARIABILITY OF SEED PROTEIN CONTENT AND SEDIMENTATION VOLUME IN WHEAT (*Triticum aestivum* L.)

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## Abstract

The quality of wheat seed protein is determined by encoding gene alleles, but percentage content varies dependin from genotypes, environmental factors and interaction genotype/ environmental conditions. The aim of this work is analysis of seed protein content, sedimentation volume and their relationship with gliadin and high molecular weight glutenin subunits in bread wheat. The 10 wheat genotypes included in experimental field study in two vegetation seasons (2015/16 and 2016/17) with different climatic conditions. The results showed that seed protein content and sedimentation volume, varied in wheat genotypes within and between vegetation season. The highest seed protein content in both vegetation season was found in the genotype G-3617-1 (14.00% and 14.80%) and the least in genotype G-3634-2 (11.40% and 12.00%). The value of protein sedimentation volume in both vegetation season was the highest in genotype G-3622-1 (52.00 ml and 56.00 ml), while the least in genotype G-3601-4 (33.00 ml and 36.00 ml). Genotypes were grouped according to the presence of the same allele at all six loci for gliadins and three loci for high-molecular-weight glutenins, and found that with high value of seed protein content associate *Gli-B1b, Gli-D2b,* and *Glu-A1b, Glu-B1c, Glu-D1d*, allele, and that with high value of sedimentation protein volume associated *Gli-B1b, Gli-B2b* and *Glu-A1b, Glu-B1c, Glu-D1d* allele.

**Key words:** wheat, seed protein, protein sedimentation volume, allele, quality.

## Introduction

Wheat seed protein content is an indicator of quality desirable for milling and baking industry. Seed protein contetn is genetic trait which value greatly varies under influence of environmental factor (Zečević et al., 2013; Knezevic et al. 2016a; Peršić et al., 2023). Variation of wheat seed protein content depens from genotype, environmental growing condition, regime of nutrition by applied fertilizers, precipitation, temperature, as well as interaction of genotypes and environments (Djokić et al., 1998; Würschum et al., 2016; Tóth et al., 2019). Seed protein content in average in bread wheat genotypes varied in ratio form 10% to15% (Shewry, 2007). In modern wheat varieties is achieved increasing of seed protein content through increased nitrogen remobilization encoded by introgressed gene *Gpc-B1*

locus at 6BS chromosome (Singh et al., 2018; Orlovskaya et al., 2022). Content of seed protein in wheat related with gluten content and both of them is indicate economic value of produced seed in trade market. Nitrogen ferzilization (amount and timing) influence to increase protein content (Annavarapu et al. 2021) and also, influence to proportion content of gliadin and glutenins (Bėkės, 2012). Gliadins are encoded by gene alleles at *Gli-1* and *Gli-2* loci on the short arms of 1. and 6. homologous chromosomes of A, B and D genome (Sozinov and Poperelya, 1980) while high molecular weight glutenins encoded by gene alleles at *Glu-1* loci on the long arm of 1. homologous chromosomes of A, B and D genome. The presence of some allele or combination of alleles encoding gliadin and glutenins associate with gluten quality, sedimentation volume, dough and loaf traits (Payne et al.,1987; Dimitrijević et al., 1998; Djukić et al., 2007; 2008; Knezevic et al., 2017; 2018). The gluten protein composition, protein content and sedimentation volume and their encoding gene alleles are useful markers for breeding proces (This et al., 2001; Djukić and Knežević, 2013). Sedimentation of protein indicate gluten quality on the base ability of swell and settle of protein molecules in acidic medium (Lookhart et al., 2001). Sedimentaton protein volume change, in dependance of time and storage conditions moisture and temperature and that that elevated storage temperature accelerated protein sedimentation (Yun et al., 2021).
The aim of this work was study variability of technological quality properties of wheat genotypes grown on the base of variation (i) protein content, (ii) sedimentation protein volume, (iii) identification of relationship between protein content and sedimentation protein volume studied traits with encoding alleles for gliadin and glutenins.

## Material and methods

The 10 genetically divergent wheat genotypes (G-3623-1, G-3644-4, G-3619-3, G-3601-4, G-3636-2, G-3622-1, G-3617-1, G-3611-2, G-3634-2, G-3637-1) were included for analysis, protein content, sedimentation protein volume and their relationship with encoding gliadin and glutenins gene alleles. Protein content was computed on the base of obtained values of the nitrogen content determined by micro Kjeldahl method.(ICCC standard method 105/2). The established nitrogen content was multiplied by using 5.7 as the conversion factor for expression value of protein content as percentage of dry weight (d.w.). The analysis of sedimentation protein volume conducted by Zeleny method according ICC No. 116/1 (1972). This method based on the principle that the degree of sedimentation of the gluten fraction of a flour suspended in a lactic acid solution during a standard time interval which indicate baking quality. The slower sedimentation and higher sedimentation volume indicate better gluten quality.
For this ten genotypes were identified alleles at three *Gli-1* loci, three *Gli-2* loci and at three *Glu-1* loci, in previous study (Knežević et al., 2022). Gliadin proteins separated by using acid PAG electrophoresis method developed by Novoselskaya et al. (1983), and for determination of gliadin blocks alleles at *Gli-1* and *Gli-2* loci used method developed by Metakovsky (1991). The high molecular weight glutenins separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) Laemmli, (1970) and determining HMW-GS and identification of *Glu-1* alleles conducted by method Payne and Lawrence (1983). On the base determined of gliadin and glutenin compositin, were analyzed association each alleles with value of protein content, sedimentation volume in wheat genotypes.

### Weather conditions in the vegetation period

The analysis of weather condition showed that average temperature and amoun of precipitation in first vegetation season (9.96 °C, 651.0 mm) was higherthan in second vegetation season (8.74 °C, 523.1 mm). In both vegtation season (2015/16 and 2016/17) the amoun of precipitation

was higher (651.0 mm and 523.1 mm) than in long term period (2000-2010) average amount of precipitation (417.8 mm), while average temperature was higher only in first vegetation season (9.96 °C) than in logn term period (8.50 °C. According to data, in first 2015/16 (8.8 °C and 14.1 °C) temperature in February and April was significantly higher than in second vegetation season 2016/17 (5.2 °C and 11.1 °C) and than in long term period (2.6 °C and 11.1 °C). However in second vegetation season temperature in March 2016/17 (10.8 °C) was significantly higher than in first vegetation season 2015/16 (7.8 °C) and in long term period (5.9 °C). The average temperature in other mounth suding both vegetation season seasons were similar (table 1).

Table 1. Average monthly temperatures and total monthly precipitation in Kraljevo

| Parameter | Period | Oct | Nov | Dec | Jan | Feb | March | April | May | June | Xm | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Temperature $^0$C | 2015/16 | 11,6 | 7,3 | 3,3 | -0,1 | 8,8 | 7,8 | 14,1 | 15,5 | 21,3 | **9,96** | 89,64 |
| Temperature $^0$C | 2016/17 | 10,6 | 6,8 | 0,0 | -4,7 | 5,2 | 10,8 | 11,1 | 16,8 | 22,1 | **8,74** | 78,66 |
| Temperature $^0$C | 2000-2010 | 11,8 | 6,4 | 1,7 | -0,1 | 2,6 | 5,9 | 11,6 | 16,4 | 20,4 | **8,5** | 76,5 |
| Precipitation (mm) | 2015/16 | 56,8 | 64,0 | 9,0 | 86,2 | 52,7 | 157,9 | 39,9 | 135,9 | 48,6 | 72,3 | **651,0** |
| Precipitation (mm) | 2016/17 | 84,1 | 77,6 | 9,4 | 22,0 | 35,0 | 57,0 | 82,0 | 100,0 | 56,0 | 41,1 | **523,1** |
| Precipitation (mm) | 2000-2010 | 61,0 | 44,3 | 44,6 | 30,0 | 29,9 | 33,2 | 52,9 | 52,6 | 69,3 | 46,4 | **417,8** |

*(*source: Republic Hydrometeorological service of Serbia* )

## Results and discussion

In this study found differences among genotypes within and between first and second vegetation season. The average value of seed protein content varied from the least 11.7% in genotypes G-3634-2 (in first vegetation season 11.4% and in second vegetation season 12.0%) and the highest 14.4% in genotypes G-3617-1 (in first vegetation season 14.0% and in second vegetation season 14.8%). The seed protein content in all ten wheat genotypes in second vegetation season was greater than in first vegetation season. The difference in protein content of genotypes between growing seasons was in the range of 0.6% to 1.0%. On average for all genotypes, the value of protein content was in first vegetation season 12.62% and in second vegetation season 13.5% what indicate that protein content was higher in second vegetation season for 0.6% than in first vegetation season (table 2).

Similar variation between vegetation season (12.4% and 12.8%) in study different wheat genotypes, reported Khan et al. (2013). In study of 50 varieties Zečević et al. (2001) found differences of protein content which varied from 13.6% to 18.2% in first year, and from 13.9% to 18.1% in second year, depends of variety . Considering that protein content is in negatively correlation with grain yield it means that breeders effort for increasing yield in new creating variety, could resulted on reduction in quality (Knezevic et al., 2016b).

The average value of sedimentation volume varied from the least 34.5 ml in genotypes G-3601-4 (in first vegetation season 33.0 ml and in second vegetation season 36. ml) and the highest 54. ml in genotypes G-3622-1 (in first vegetation season 52. ml and in second vegetation season 56.0%). The sedimentation protein volume was different between in analyzed genotypes and between two vegetation seasons, and in in all 10 genotypes sedimentation volume in second vegetation season was higher than in first vegetation season. The difference in sedimentation volume of genotypes between growing seasons was in the range of 2.0 *ml* (G-3634-2) to 6.0 *ml* (G-3636-2). On average for all 10 genotypes, the value of sedimentation protein volume was 42.3 *ml* in the first vegetation season, and 46.0 *ml* in the second vegetation season, what indicate that sedimentation volume was higher in second vegetation season for 3.7 *ml* than in first vegetation season (table 2).

Table 2. Variation of seed protein content (%) and sedimentation volume (*ml*) in wheat genotypes grown in two vegetation season (2015/16 and 2016/17)

| Trait | Year | G-3623-1 | G-3644-4 | G-3619-3 | G-3601-4 | G-3636-2 | G-3622-1 | G-3617-1 | G-3611-2 | G-3634-2 | G-3637-1 | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Protein content (%) | 2015/16 | 12.60 | 12.80 | 12.00 | 11.60 | 13.20 | 13.60 | **14.00** | 12.40 | **11.40** | 12.60 | 12.62 |
| | 2016/17 | 13.20 | 13.60 | 12.80 | 12.60 | 14.20 | 14.40 | **14.80** | 13.20 | **12.00** | 13.40 | 13.50 |
| | Average | 13.40 | 13.20 | 12.40 | 12.10 | 13.70 | 14.00 | **14.40** | 12.80 | **11.70** | 12.00 | 13.06 |
| | | | | | | | | | | | | |
| Sedimentation volume (*ml*) | 2015/16 | 44.0 | 42.0 | 36.0 | **33.0** | 46.0 | 52.0 | **50.0** | 42.0 | **38.0** | 40.0 | 42.3 |
| | 2016/17 | 48.0 | 46.0 | 40.0 | **36.0** | 52.0 | 56.0 | **52.0** | 46.0 | 40.0 | 44.0 | **46.0** |
| | **Average** | **46.0** | **44.0** | **38.0** | **34.5** | **49.0** | **54.0** | **51.0** | **44.0** | **39.0** | **42.0** | **44.15** |
| Chroms./ | | **A B D** | **A B D** | **A B D** | **A B D** | **A B D** | **A B D** | **A B D** | **A B D** | **A B D** | **A B D** | **A B D** |
| Locus | Gli-1 | ***b. l. a.*** | ***f. b. b.*** | ***h. d. b.*** | ***m. l. b.*** | ***a. b. a.*** | ***b/c b. b.*** | ***b. g. a.*** | ***f. l. k.*** | ***b. g. a/b*** | ***m. k. k.*** | ***b. b. b.*** |
| | Gli-2 | ***f. b. b.*** | ***e. o. j.*** | ***b. a. k.*** | ***b/j. e. b.*** | ***k. b. a.*** | ***a. v. b.*** | ***e. b. m.*** | ***b. p. b.*** | ***b. o. m/b*** | ***g. b. j.*** | ***b. b. b.*** |
| | Glu-1 | ***b. c . d.*** | ***b. c . a.*** | ***c. i . a.*** | ***c. d . a.*** | ***b. c . d.*** | ***b. c . d.*** | ***b. c . d.*** | ***b. u . a.*** | ***c. d . a.*** | ***a. c . a.*** | ***b. c . a.*** |

Similar variation between vegetation season (55.0 *ml* and 52.0 *ml*) in study different wheat genotypes, reported Khan et al. (2013). In ealier study of 10 Serbian wheat varieties sedimetation volume varied from 33.0 *ml* and 64.0 *ml*, depend of genotype and year of study wheareas the average sedimentation volume for all 10 varieties in four year was 45.6 *ml*, 45.8 *ml*, 50.4 *ml* and 42.2 *ml* (Zečević et al., 2007). The sedimentation volume for seven genotypes had value on level of the first quality class or at the level of enhancing class, and three genotypes G-3619-3, G-3601-4 and G-3634-2 had sedimentation volume on the level of second quality class.

In this study are grouped genotypes according to presence of same allele at each *Gli-1*, *Gli-2* and *Glu-1* locus and computed average values for protein content and for sedimentation protein volume. Group of genotypes with high values of seed protein content carried the same allele (oftenly the most frequent) what indicate in both vegetation season their positive relationship *Gli-A1b* (12.9%; 13.80%), *Gli-B1b* (13.2%; 14.06%), *Gli-D1a* (12.80%; 13.75%), *Gli-A2e* (13.40%; 14.20%), *Gli-B2b* (13.10%; 14.10%), *Gli-D2b* (12.55%; 13.55%), *Glu-A1b* (13.10%; 14.03%), *Glu-B1c* (13.13%; 14.06%) , *Glu-D1d* (13.35%; 14.35%) table 3.

Group of genotypes with high value of sedimentation the same allele (oftenly the most frequent) what indicate in both vegetation season their positive relationship *Gli-A1b* (46.0 *ml*; 49.0 *ml*), *Gli-B1b* (46.66 *ml*; 51.33 *ml*), *Gli-D1a* (44.50 *ml*; 48.0 *ml*), *Gli-A2e*(46.00 *ml*; 49.0 *ml*), *Gli-B2b* (45.0 *ml*; 49.0 *ml*), *Gli-D2b* (42.75 *ml*; 46.50 *ml*), *Glu-A1b* (46.0 *ml*; 50.0 *ml*), *Glu-B1c* (45.66 *ml*; 49.66 *ml*) , *Glu-D1d* (48.0 *ml*; 52.0 ml) table 3.

From this combination of nine alleles, located at the three *Gli-1*, three *Gli-2* and three *Glu-1* loci, which associated with high protein content in one genotype (G-3623-2) present combination of seven allele, in three genotypes (G-3636-2; 3622-1; G-3617-1;) present combination of diferent six alleles in one genotype (G-3644-4) present combination of four alleles, in three gentypes (G-3611-2; G-3634-2; G-3637-1) present different combination of two alleles, in one genotype (G-3601-4) present one allele. However in G-3619-3 there are none of the nine alleles associated with protein content (table 2 and 3).

Table 3. Alleles at *Gli-1, Gli-2* and *Glu-1* associated with high value of protein content (%) and sedimentation volume (*ml*) in wheat genotypes

| Locus | *Gli-A1* | *Gli-B1* | *Gli-D1* | *Gli-A2* | *Gli-B2* | *Gli-D2* | *Glu-A1* | *Glu-B1* | *Glu-D1* |
|---|---|---|---|---|---|---|---|---|---|
| Alleles assoc., with high gluten content | ***b*** | ***b*** | ***a*** | ***e*** | ***b*** | ***b*** | ***b*** | ***c*** | ***d*** |
| Group of genotypes which carry the same allele at *Gli-1, Gli-2* and *Glu-1* associated with the high value of protein content | G-3623-1 | G-3644-4 | G-3623-1 | G-3644-4 | G-3623-1 | G-3623-1 | G-3623-1 | G-3623-1 | G-3623-1 |
| | G-3622-1 | G-3636-2 | G-3636-2 | G-3617-1 | G-3636-2 | G-3601-4 | G-3644-4 | G-3644-4 | G-3636-2 |
| | G-3617-1 | G-3622-1 | G-3617-1 | | G-3617-1 | G-3622-1 | G-3636-2 | G-3636-2 | G-3622-1 |
| | G-3634-2 | | G-3634-2 | | G-3637-1 | G-3611-2 | G-3622-1 | G-3622-1 | G-3617-1 |
| | | | | | | | G-3617-1 | G-3617-1 | |
| | | | | | | | G-3611-2 | G-3637-1 | |
| Vegetation season | The highest average value of protein content (%) in genotypes which carry same allele at *Gli-1, Gli-2* and *Glu-1*loci | | | | | | | | |
| 2015/16 | 12.90 | 13.20 | 12.80 | 13.40 | 13.10 | 12.55 | 13.10 | 13.13 | 13.35 |
| 2016/17 | 13.80 | 14.06 | 13.75 | 14.20 | 14.10 | 13.55 | 14.03 | 14.06 | 14.35 |
| Average | 13.35 | 13.63 | 13.275 | 13.80 | 13.60 | 13.05 | 13.565 | 13.595 | 13.85 |
| Vegetation season | The highest average value of sedimentation volume (*ml)* in genotypes which carry same allele at *Gli-1, Gli-2* and *Glu-1*loci | | | | | | | | |
| 2015/16 | 46.00 | 46.66 | 44.50 | 46.00 | 45.00 | 42.75 | 46.00 | 45.66 | 48.00 |
| 2016/17 | 49.00 | 51.33 | 48.00 | 49.00 | 49.00 | 46.50 | 50.00 | 49.66 | 52.00 |
| Average | 47.50 | 48.995 | 46.25 | 47.50 | 47.00 | 44.625 | 48.00 | 47.66 | **50.00** |

From the combination of nine alleles, located at the three *Gli-1*, three *Gli-2* and three *Glu-1* loci, which associated with high sedimentation volume of protein in two genotype (G-3623-2; G-3617-1) present different combination of seven allele, in two genotypes (G-3636-2; 3622-1) present different combination of six alleles, in one genotype (G-3644-4) present combination of four alleles, in three gentypes (G-3611-2; G-3634-2; G-3637-1) present different combination of two alleles, in one genotype (G-3601-4) present one allele. However in G-3619-3 there are none of the nine alleles associated with high sedimentation volume (table 2 and 3).

This combination of nine alleles was not found in the analyzed genotypes. The highest value of sedimentation protein volume (52 *ml* and 56 *ml*) had genotype G-3622-1 which had possess six allele combination (*Gli-A1b, Gli-B1b, Gli-D2b, Glu-A1b, Glu-B1c, Glu-D1d*), while genotype G-3636-2 had less but high sedimentation volume possess different combination of six allele (*Gli-B1b, Gli-D1a, Gli-B2b, Glu-A1b, Glu-B1c, Glu-D1d*). On the other side, in two genotypes which had combination of seven alleles found lesser value of sedimentation volume G-3617-1 (50.0 ml and 52.0 ml) and G-3623-1 (44.0 ml and 48.0 ml). Genotypes G-3601-4 which had the lowest sedimentation volume (33 *ml* and 36 *ml*) possess only one alleles from nine alleles (*Gli-D2b*) which associated with high value of sedimentation protein volume.

In this study found that genotypes which carried Glu-B1c allele have high sedimentation volumes, what is in agrement with investigation (Gao et al., 2016) which found that the highest frequency of Glu-B1c and its corellation with high sedimentation value.

Based on this result, it is not possible to determine the reliable regularity of the association between protein content and alleles encoding gliadin and glutenin proteins, as well as between sedimentation volume and *Gli-* and *Glu-1* alleles, although the grouped genotypes were carriers of alleles with the calculated average value of protein content and sedimentation volume. The differences between genotypes which had the same number of alleles associated with high protein content and high sedimentation volume can be results of differeces of combination of alleles encoding gliadin and high molecular weight glutenin as well as effect alleles at *Glu-3* loci which was not analzed in this investigation.

## Conclusion

On the base of results established differences in wheat genotypes for protein content and sedimentation protein volume, within each vegetation season and between vegetation season. The highest grain protein content in G-36217-1 (14.00% and 14.80%) and the highest protein sedimentation volume in G-3622-1 (52.0 ml and 56.0 ml) in both vegetation season.
Genotypes which carried allele ***b.*** at *Gli-B2,* ***b.*** at *Glu-A1* (encoded 2*), allele ***c.*** at *Glu-B1* (encoded 7+9) and ***d.*** at *Glu-D1* (encoded 5+10 subunit) had the high value of protein content (G-3617-1─14.10% and 14.40%; G-3636-2, 13.20% and 14.20%). Genotypes which carried allele ***b.*** at *Gli-A1*, ***b.*** at *Gli-B,* ***b*** at *Gli-D1,* ***b.*** at *Glu-A1* (encoded 2*), allele ***c.*** at *Glu-B1* (encoded 7+9) and ***d.*** at *Glu-D1* (encoded 5+10 subunit) had the high values of sedimentation volume G-3622-1 (52.0 ml and 56.0 ml), G-3617-1 (50.0 ml and 52.0 ml). Genotype ***b.*** at *Gli-B2,* ***c.*** at *Glu-A1* (none subunits), allele ***d.*** at *Glu-B1* (encoded 6+8) and ***a.*** at *Glu-D1* (encoded 2+12 subunit) had the least protein content (G-3617-1─11.40% and 12.00%; G-3601-4 - 11.60% and 12.60%) and the least sedimentation volume (G-3601-4 ─33.00 *ml* and 36.00 *ml*; G-3634-2─38.00 *ml* and 40.00 *ml*).

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