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CHARACTERIZATION OF GLIADIN AND HMW GLUTENIN PROTEIN COMPOSITION IN COLOURED WHEAT (*TRITICUM AESTIVUM* L.) VARIETIES

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ABSTRACT

Wheat is one of the most important grains in our daily diet. Coloured wheat contains natural anthocyanin compounds. Bioactive compounds in wheat have attracted increasingly more interest from breeders because of their benefits. It is important to fully understand protein properties of red, blue, purple, and yellow-coloured wheat in order to predict their potential uses for culturing new varieties. All 21 accessions originating from different geographical areas of world were evaluated for high molecular weight glutenin subunit (HMW-GS) and T1BL.1RS wheat-rye translocation using SDS-PAGE and A-PAGE. The data indicated the prevalence of the allele 1 (36%), allele 0 (30%) and allele 2* (34%) at the Glu-1A and five alleles, namely 7+8 (36%), 7+9 (29%), 20 (21%), 7 (12%) and 17+18 (2%) represented the Glu-1B. Existence of 2 alleles at the locus Glu-1D was revealed, in fact 21% of them showed the subunit pairs Glu-1D 5+10 correlated with good bread making properties. Protein subunit Glu-1A1 and Glu-1A2* were correlated positively with improved dough strength as compared to subunit null. On the chromosome Glu-1B subunit 17+18 and 7+8 were associated with slightly stronger gluten type than 7+9, whereas subunit 20 and 7 were associated with weak gluten properties. On the basis of electrophoretic separation of gliadin fraction it was found that only one genotype contained T1BL.1RS wheat-rye translocation. The Glu-1 quality score ranged from 4 to 10. Suitable accessions can be used for the crossing programs to improve colour and good technological quality of bread wheat.

Keywords: coloured wheat grain, glutenin, gliadin

INTRODUCTION

Wheat kernels, also called wheat grains, have three main parts: the endosperm, the germ, and the brain. While whole wheat flour contains all three parts of the kernel, usually white flour is milled from the endosperm. Coloured grain wheat is also recognized as a significant source of antioxidants which promote health and reduce the risk of disease. Wheat naturally contains numerous classes of antioxidant compounds such as flavonoids, carotenoids, alkaloids, and others **(Qin et al. 2010; Gilchrist & Sorrells 1982)**. Basic wheat pigments include xanthophylls, carotenoids, anthyocyanins, anthocyanins and are known for exhibiting good antioxidant activity **(Humphries et al. 2004; Knievel et al. 2009)**. Wheat grain colour was controlled by genes, but also by the environment conditions such as light, temperature and fertilization, etc. For blue wheat grain the colour is due to the blue aleurone layer **(Knott 1958)**. The colouration of a purple wheat grain is located in the pericarp and testa. In New Zealand the purple wheat Konini as released in 1981 used as specialty bred making wheat for the whole meal wheat loaf market. Purple grain colour controlled by two complementary dominant genes on chromosomes 3A and 7B **(Piech & Evans, 1979)**. These unique phenomena may be due to the influences of environmental factors as well as genetic factors. Wheat gluten proteins are classified into two groups on the basis of their aggregation and functional properties. These are the gliadins which are present as monomers which interact by mono-covalent forces and the glutenins which form polymers stabilized by interchain disulphide bonds.

MATERIAL AND METHODOLOGY

In this study we analyzed seed storage proteins extracted from hexaploid wheat (*Triticum aestivum* L.) grain. All samples were obtained from the collection of genetic wheat sources of Gene Bank of the Slovak Republic in Piešťany and from Agrotest Kroměřiž of the Czech Republic. Seed storage proteins were isolated from the endosperm of intact, dry and mature single seeds. Seed homogenization was carried out by grinding. Glutenins were extracted by standard referee method ISTA **(Wrigley 1992)**. Gliadins were obtained using standard referee method ISTA in the presence of acid solution **(Draper 1987)**. The glutenin separation was performed by discontinuous PAGE based on ISTA methodology **(Wrigley 1992)** and using the electroseparatic unit Protean II (Biorad). The electrophoretic separation of gliadins was followed by referee method ISTA **(Draper 1987)** using mixture of glycine and acetic acid as electrolyte at pH 3.2. They were separated in continuous polyacrylamide gels at acid environment **(Qi et al. 2006)**. Protein fractions were stained by Coomassie Brilliant Blue R-250. The separate gluten subunits were identified by the nomenclature of **Payne and Lawrence (1983)**. According to the specific protein profile of the HMW alleles in each cultivar its quality score (Glu-score) was calculated by **Payne et al. (1987)**.

RESULTS AND DISCUSION

Twenty-one hexaploid wheat accessions originating from different geographical areas of the world were evaluated for high molecular weight glutenin subunit (HMW-GS) and gliadins composition using SDS-PAGE and A-PAGE.

Table 1 HMW-GS composition and quality score of coloured wheat cultivars

Genotype	Color of grain	Origin	Glu-1A	Glu-1B	Glu-1D	Glu score	Rye score
48M	blue	USA	0	7	2+12	4	4
Tschermarks Blaukorniger	blue	AUT	1	7+8	2+12	8	8
UC66094 A	blue	CHL	1	7+8	5+10	10	10
UC66094 B	blue	CHL	1	7+8	2+12	8	8
Abyssinskaja arraseita A	purple	RUS	0	7	2+12	4	4
Abyssinskaja arraseita B	purple	RUS	0	7+8	2+12	6	6
ANK 28	purple	RUS	1	7+8	2+12	8	8
Konini A	purple	NZL	1	7	2+12	6	6
Konini B	purple	NZL	0	7	2+12	4	4
Laval 19	purple	CAN	1	7+9	5+10	9	9
AC Andrew	yellow	CAN	2*	20	2+12	6	6
BonaDea	yellow	SVK	0	7+9	5+10	7	7
Broom	yellow	DEU	0	7+9	5+10	7	7
Cranbrock	yellow	AUS	0	7+8	2+12	6	6
Dundee A	yellow	AUS	2*	20	2+12	6	6
Dundee B	yellow	AUS	1	17+18	2+12	8	8
Fiorina	yellow	CHE	2*	20	2+12	6	6
Glugas	yellow	AUS	1	20	2+12	6	6
Kiata	yellow	AUS	2*	7+8	2+12	8	8
Kolibri	yellow	DEU	1	7+9	5+10	9	9
Passo Fundo	yellow	BRA	2*	7+8	2+12	8	8
Pomerelle	yellow	USA	2*	7+9	2+12	7	7
Saffrasi A	yellow	SWE	2*	7+9	2+12	7	5
Saffrasi B	yellow	SWE	2*	7+9	2+12	7	7
Whitebird	yellow	USA	0	7+8	2+12	6	6
Zenith	yellow	AUS	1	20	2+12	6	6

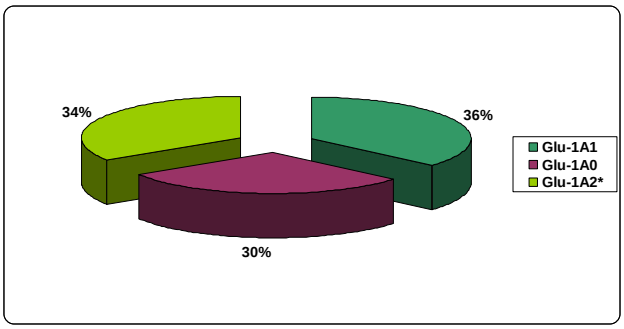

Fig. 1 Allelic frequency at *Glu-1A* locus

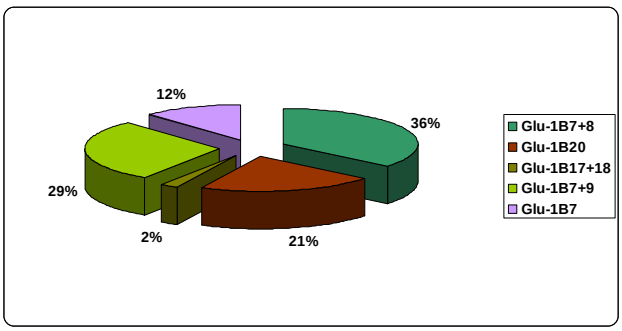

Fig. 2 Allelic frequency at *Glu-1B* locus

Sixteen that is 76% of examined accessions showed homogeneous patterns, whereas remaining 5, that is 24% were heterogeneous, containing two different glutenin phenotypes. Considering the composition of HMW-GS

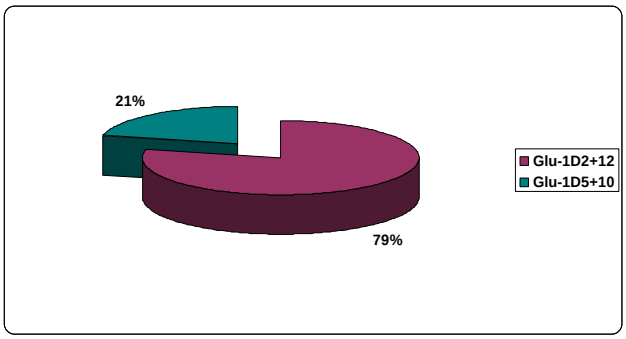

Fig. 3 Allelic frequency at *Glu-1D* locus

there were found 12 different electrophoretic protein profiles among 21 tested cultivars (Table 1). The data indicated the prevalence of the allele 1 (36%), allele 0 (30%) and allele 2* (34%) at the *Glu-1A* (Figure 1) and five alleles, namely 7+8 (36%), 7+9 (29%), 20 (21%), 7 (12%) and 17+18 (2%) represented at the *Glu-1B* (Figure 2). Ram (2003) showed that protein subunit 2* and subunit 1 were found correlated positively with improved dough strength as compared to subunit null. Combination 17+18 was relatively rare being found in genotype Dundee B only. The existence of 2 alleles at the locus *Glu-1D* was revealed, in fact 21% of them showed the subunit pairs Glu-1D 5+10 correlated with good bread making properties (Figure 3). Genotypes containing wheat-rye translocation **(Graybosch et al. 1999, Wieser et al. 2000)** were corrected in this score: 3 points less to scores 8-10, 2 points to scores 5-7 and one point to scores 3-4.

CONCLUSION

Good baking quality is strongly correlated with the presence of 1 and 5+10 or 2* and 5+10 HMW-GS and poor baking quality **(Bradová et al. 2005, Gálová et al. 1998)** usually associated with 2+12 HMW-GS, some of coloured wheat can be classified as a bread wheat **(Chňapek et al. 2010)**. The cultivar with blue grain colour UC66094 A reached the maximum *Glu-score* and *Rye-score* 10. The cultivar with yellow grain colour Kolibri possessed alleles and allelic pairs 1, 7+9, 5+10 evaluated *Rye-score* and *Glu-score* 9. The cultivar with purple grain colour Laval 19 reached *Glu-score* 9 with the same alleles and allelic pairs as the cultivar Kolibri. Each of grain colours (blue, purple, or yellow) is under simple genetic control **(Dobrovolskaja et al. 2006, Trojan et al. 2010)** and coloured wheat can be used for the crossing programs to improve colour and good technological quality of bread wheat.

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