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Relationships between HMW-GS *GluA1*, *GluB1* alleles and agronomic traits

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A correlation analysis including alleles of high molecular weight glutenin subunits (HMW-GS) *GluA1, GluB1* and agronomic characteristics of winter wheat cultivars was made at the Lithuanian Institute of Agriculture in 2006 and 2007. A total of 24 characteristics were analysed. A strong correlation was found between *GluB1* alleles and overwintering (r = 0.71**), medium correlations were determined between *GluA1* alleles and a visual thriftiness of plants (r = 0.63**), autumn growth habit (r = 0.61**) and resistance to powdery mildew (0.58**). The lines containing allele *c* in *GluB1* had a significantly higher value in the yield comparison test (0.88 t ha^{-1}) than those with allele *d*. The mean overwintering score was higher for lines possessing alleles at *GluB1d* (1.18). Protein content and sedimentation value were higher in the group of lines possessing *GluB1d* (0.78% and 5.84 ml). Hectolitre weight was also found to be higher (31.2 g) for the lines possessing *GluB1d* alleles. Lines with *GluB1c* alleles were more resistant to take-all (by 1.06 score). The locus *GluA1* had a lesser effect on the agronomic traits. The highest effect was determined for autumnal growth habit, plant visual thriftiness and sedimentation value (1.30 score, 1.05 score and 6.31 ml, respectively). All these traits were positively influenced by allele *GluA1a*. The alleles studied did not correlate with common bunt incidence, starch content, heading date and plant lodging.

Key words: winter wheat, *GluA1*, *GluB1*, agronomic traits, correlation

INTRODUCTION

Glutenins are important components of gluten which is the main contributor to the rheological and bread-making properties of wheat flour. High molecular weight glutenin subunits (HMW-GS) are inherited at several loci on each genome A, B and D. HMW-GS genes are located on the long arm of chromosomes 1A, 1B and 1D at loci *GluA1*, *GluB1* and *GluD1*, respectively [1].

Different combinations of HMW-GS alleles influence the baking quality of wheat cultivars in a different way. It has been proven that subunits designated as *GluA1* 1, *GluA1* 2*, *GluB1* 7+9, *GluB1* 17+18 or *GluD1* 5+10 are related to a high technological quality, whereas their allelic variants such as *GluA1* null, *GluB1* 6+8, *GluD1* 2-12 are related to a lower baking quality [2]. The above dependencies are of a statistical character, and among breeding materials inverse relations can sometimes be observed [3].

Nevertheless, wheat breeders are interested in the introduction of beneficial HMW-GS alleles into new genotypes. The main reason is that many factors affect wheat technological traits. HMW-GS coding by different alleles is the only factor that does not change under the influence of environmental conditions. Very few studies analysed associations of HMW-GS alleles with agronomic traits. Węgrzyn and Waga [4] found relations of HMW-GS alleles at locus *GluA1* with 1000 kernel weight, number of kernels per ear and resistance to leaf diseases. The recent studies of Witkowski et al. [5] revealed a new influence of locus *GluA1* on frost tolerance and resistance to leaf blotch.

The distribution of alleles within different *Glu-1* loci has been investigated in many countries. Allele *GluA1a* dominates in the Lithuanian cultivars due to selection for quality traits. However, allele *GluA1c* dominates in the breeding lines with a lower selection level [6]. Among the Polish wheat cultivars, the distribution of the most important alleles within loci on chromosome 1B and 1D is normal, which is not the case on chromosome 1A [4]. At locus *GluA1*, a significant quantitative dominance of the *null* allele over the coding alleles was detected. A similar distribution was found in a few other European countries [7, 8]. Theoretically, such a linkage disproportion could be a consequence of a strong relation between *GluA1* and some loci responsible for important agronomic traits, such as yield potential or resistance to biotic or abiotic stresses. Our study was performed to verify the hypothesis of a linkage between *GluA1* alleles and important agronomic traits not investigated in the above-mentioned researches. Also, we expanded the analysis and involved alleles *GluB1c, d* into the study.

MATERIALS AND METHODS

The data used were from experiments conducted at the Lithuanian Institute of Agriculture (LIA) during 2006/2007 in the breeding nurseries with a natural and artificial infection.

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The plot size in the competitive trial nursery was 17 m^2 and in the take-all nursery 2 m^2. The row length in the common bunt nursery was 2.5 m. All plots were replicated four times. Experimental trials were sown with chemically treated seeds in the main breeding nursery. Chemically non-treated seeds were used in the take-all nursery. Seeds inoculated with *Tilletia tritici* were sown in the common bunt nursery.

The weather conditions during the experimental period were favourable for an adequate evaluation of the traits under study. The autumnal growth habit was evaluated after cessation of active vegetation. Overwinter survival was recorded in spring after resumption of vegetation. Spring re-growth was scored until the pseudo-stem erection stage. The angle and colour of leaves, visual soil cover and plant thriftiness were evaluated at the flowering stage. Leaf diseases were assessed throughout the growing season every 2 weeks from the pseudo-stem elongation stage. These traits were evaluated in scores on a 1–9 score scale, 1 being the lowest value. Leaf area in m^2 was determined using a Delta-T SunScan device at the flowering stage. Plant height was measured in cm at the end of flowering. Heading was evaluated as a number of days from the 1st day of January. Lodging was scored before harvesting. Eyespot was scored after harvesting. Take-all was estimated at the milk development stage on a 1–9 point scale. Common bunt was counted at the milk development stage as the percentage of infected heads. The plots were harvested at the full ripening stage. The 1000 kernel and hectolitre weight (g) was measured after drying and cleaning the harvested grain. Pre-harvest sprouting was evaluated in 1–9 scores for ears stored for 6 months. The percentage of protein and starch, sedimentation value were determined at the LIA laboratory of chemical analyses.

The electrophoretic analysis of HMW-GS alleles of each genotype was made by SDS-PAGE using 12% (w/v) acrylamide gels as described by Kraic [9]. Proteins were extracted from individually ground grains. The electrophoresis was carried out in a Multigel Long system (Biometra), gels were stained using Coomasie Brilliant Blue R250. To establish the presence or absence of subunit 2* in genotypes with subunit 2 + 12, a further analysis was carried out by SDS-PAGE using 5% (w/v) acrylamide gels [10]. The nomenclature system used for the HMW-GS was as in [11]. To determine the electrophoretic mobility of each HMW-GS by SDS-PAGE, standards (Bezostaya-1, Bredo, Camp Remy, Chinese Spring, Gabo, Kosack, Lona and Talent) that included spectra of the expected subunits were used. 8–10 kernels were used for electrophoretic analysis.

There were selected 28 breeding lines and cultivars possessing different *GluA1* and *GluB1* alleles, of which 13 genotypes possessed subunit *GluA1* null (allele *GluA1c*) and 15 genotypes had subunit *GluA1* 1 (allele *GluA1a*); 10 genotypes with subunit *GluB1* 6+8 (allele *GluB1d*) and 18 genotypes with subunit *GluB1* 7+9 (allele *GluB1c*).

The calculation of the correlation between *GluA1, GluB1* alleles and agronomic traits was done as follows: allele *GluA1c* was denoted as 0 and allele *GluA1a* as 1; allele *GluB1d* as 1 and allele *GluB1c* as 0. The obtained correlation coefficients were compared for significance levels at p = 0.05 and p = 0.01. The least significant difference (LSD) was calculated at the probability levels p = 0.05 and p = 0.01.

RESULTS AND DISCUSSION

The genotypes selected for analysis were Lithuania-registered varieties and advanced breeding lines. The correlations between alleles *GluA1, GluB1* and agronomic characteristics are shown in Table 1. The correlation included 24 agronomic characteristics, and highly varying correlation coefficients were obtained. A strong correlation was found between *GluB1* alleles and overwinter survival (r = 0.71**), medium correlations were obtained between *GluA1* alleles and visual thriftiness of plants (r = 0.63**), autumnal growth habit (r = 0.61**) and resistance to powdery mildew (0.58**). *GluB1* alleles were found to correlate moderately with the yield (r = –0.58**). The relations of *Glu1* alleles with other agronomic traits were weaker.

Table 1. **Correlation between *GluA1, GluB1* alleles and agronomic traits of winter wheat**

Trait	*GluA1*	*GluB1*	Trait	*GluA1*	*GluB1*
Coleoptile length, cm	+0.31**	+0.26*	Protein content, %	+0.18	+0.32**
Autumn growth habit, scores	+0.61**	–0.35**	Sedimentation value, ml	+0.39*	+0.43**
Overwintering, scores	–0.35**	0.71**	Starch content, %	–0.04	–0.13
Spring re-growth, scores	+0.34**	+0.15	Preharvest sprouting, scores	–0.44*	–0.14
Plant height, cm	–0.05	+0.41**	1000 kernels weight, g	+0.46**	+0.03
Lodging, scores	+0.21	–0.24	Hectolitre weight, g	–0.47**	–0.57**
Heading, days	–0.20	+0.15	Powdery mildew, scores	+0.58**	+0.43
Leaf area index, m^2	–0.37*	+0.41*	Septoria leaf blotch, scores	–0.29*	+0.37**
Angle of top leaves, scores	+0.42**	+0.13	Common bunt, %	+0.04	+0.09
Colour of leaves, scores	+0.35**	–0.38**	Take-all, scores	+0.10	+0.43**
Visual soil cover, scores	+0.38**	+0.16*	Yield in take-all nursery, t ha^{-1}	+0.10	+0.43**
Visual thriftiness of plants, scores	+0.63**	+0.09	Eyespot, scores	+0.40**	–0.17
Yield, t ha^{-1}	+0.17	–0.58**			

* Significant at p = 0.05. ** Significant at p = 0.01.

Data presented in Table 2 reveal the effect of each allele on the agronomic characteristics under study, selected after correlation analysis, provided the correlation coefficients were equal or above r = –0.30, r = 0.30. The *Glu1* alleles had the highest impact (significant at p = 0.01) on the following traits: in the yield comparison test, the lines with alleles at *GluB1c* had a significantly higher value (0.88 t ha^{-1}) than those with the *GluB1d* allele. The mean score of overwinter survival was higher for the lines possessing alleles at *GluB1d* (1.18 score). Protein content and sedimentation value were higher for the group of lines possessing *GluB1d*, 0.78% and 5.84 ml, respectively. Hectolitre weight was also higher (31.2 g) for the lines possessing alleles *GluB1d*.

Table 2. **Student's t test for some agronomic traits of winter wheat genotypes grouped according to alleles at *GluA1* and *GluB1* loci**

Trait	Locus and allele	Mean	T†	Locus and allele	Mean	T
Coleoptile length, cm	*GluA1c*	5.95	0.42*	*GluB1d*	6.09	0.42*
	GluA1a	6.55	0.51**	*GluB1c*	6.59	0.51
Autumn growth habit, sores	*GluA1c*	2.23	0.12*	*GluB1d*	2.61	0.12*
	GluA1a	3.53	0.16**	*GluB1c*	3.51	0.16**
Overwintering, scores	*GluA1c*	8.15	0.23*	*GluB1d*	8.08	0.23*
	GluA1a	7.39	0.31**	*GluB1c*	6.90	0.31**
Spring regrowth, scores	*GluA1c*	2.77	0.24*	*GluB1d*	3.28	0.24*
	GluA1a	3.50	0.32**	*GluB1c*	2.95	0.32**
Plant height, cm	*GluA1c*	107.38	3.50	*GluB1d*	109.56	3.50*
	GluA1a	106.28	4.70	*GluB1c*	101.80	4.70**
Leaf area index, m^2	*GluA1c*	4.73	0.69*	*GluB1d*	4.63	0.69*
	GluA1a	3.94	1.19	*GluB1c*	3.73	1.19
Angle of top leaves, scores	*GluA1c*	2.27	0.08*	*GluB1d*	2.86	0.08*
	GluA1a	3.13	0.11**	*GluB1c*	2.70	0.11**
Leaf colour, scores	*GluA1c*	2.85	0.18*	*GluB1d*	2.86	0.18*
	GluA1a	3.25	0.24**	*GluB1c*	3.41	0.24**
Visual soil cover, scores	*GluA1c*	6.92	0.28*	*GluB1d*	7.33	0.28*
	GluA1a	7.50	0.37**	*GluB1c*	7.05	0.37
Plant visual thriftiness, scores	*GluA1c*	6.62	0.38*	*GluB1d*	7.20	0.38
	GluA1a	7.67	0.50**	*GluB1c*	7.15	0.50
Yield, t ha^{-1}	*GluA1c*	6.09	0.36	*GluB1d*	5.90	0.36*
	GluA1a	6.29	0.47	*GluB1c*	6.78	0.47 **
Protein content, %	*GluA1c*	9.52	0.30*	*GluB1d*	10.11	0.30
	GluA1a	10.10	0.40**	*GluB1c*	9.32**	0.40
Sedimentation value, ml	*GluA1c*	23.79	1.26*	*GluB1d*	29.64	1.26*
	GluA1a	30.10	1.68**	*GluB1c*	23.80	1.68**
Pre-harvest sprouting, scores	*GluA1c*	8.12	0.74*	*GluB1d*	7.77	0.74
	GluA1a	7.29	1.17	*GluB1c*	7.50	1.17
1000 kernel weight, g	*GluA1c*	39.70	2.83*	*GluB1d*	41.53	2.83
	GluA1a	43.57	3.87**	*GluB1c*	42.20	3.87
Hectolitre weight, g	*GluA1c*	822.90	19.28*	*GluB1d*	819.80	19.28*
	GluA1a	796.30	26.00**	*GluB1c*	788.60	26.00**
Powdery mildew, scores	*GluA1c*	1.17	0.29*	*GluB1d*	1.19	0.29
	GluA1a	2.03	0.39**	*GluB1c*	1.18	0.39
Septoria leaf blotch, scores	*GluA1c*	7.83	0.41*	*GluB1d*	7.80	0.41*
	GluA1a	7.37	0.53	*GluB1c*	7.21	0.53**
Yield in take-all nursery, t ha^{-1}	*GluA1c*	4.60	0.13	*GluB1d*	4.20	0.13*
	GluA1a	4.60	0.17	*GluB1c*	5.10	0.17**
Take-all, scores	*GluA1c*	5.63	0.46	*GluB1d*	6.13	0.46*
	GluA1a	5.85	0.73	*GluB1c*	5.07	0.73**
Eyespot, scores	*GluA1c*	4.45	0.36*	*GluB1d*	4.75	0.36
	GluA1a	5.19	0.51**	*GluB1c*	4.97	0.51

† The first number in the cell denotes the least significant difference at * p = 0.05, the second number in the cell denotes the least significant difference at ** p = 0.01.

Resistance to take-all was the last trait with considerable differences among the lines tested. The lines with *GluB1c* alleles were more resistant (by 1.06 scores). The locus *GluA1* had a lesser effect on the agronomic traits. The highest effect was determined for the autumnal growth habit, plant visual thriftiness and sedimentation value (1.30 score, 1.05 score and 6.31 ml, respectively). All the three traits were positively influenced by *GluA1a* allele.

The most comprehensively investigated and described is the effect of *Glu1* alleles on sedimentation value and protein content [12]. Our research agrees with the previous findings. Very little research has been done on associations of *GluA1* alleles and frost resistance [4, 5]. Our study showed a similar relation of *GluA1* alleles with overwintering tolerance. The same but higher relation was found while analysing the effect of alleles *c* and *d* of locus *GluB1*. Cold tolerance in wheat is inherited in a polygenic manner. The main locus is situated on the long arm of chromosome 5A [13]. This study and many years of practical breeding for high grain quality and overwintering, for which cold tolerance is the key component, show a possible presence of a genetic locus for frost tolerance on chromosomes 1A and B1. These relations suggest a close genetic linkage between these loci and loci responsible for cold resistance, although this linkage appears closer in the case of alleles *GluA1c* and *GluB1d* than the other two alleles analysed.

Associations of alleles at *GluA1* and *GluB1* loci with the complex traits of winter wheat vegetative development correlated at a different strength. The correlation coefficients ranged from almost null in the case of plant height and locus *GluA1* to medium strong (r = 0.61**) in the case of autumnal growth habit and locus *GluA1*. The associations were strongest for autumnal growth in the case of both loci. The autumnal growth habit is the first trait characterizing the possible further plant growth habits. The vegetative growth peculiarities of Nordic, Western and part of Middle European winter wheat genotypes depend on the height reducing genes *RhtB1* and *RhtD1* as well as on QTLs [14]. Consequently, it is likely that some height-reducing QTLs can be related with alleles of *GluA1* and *GluB1* loci as well as in the case of overwintering.

Alleles *c* and *d* at locus *GluB1* exerted a considerable effect on the yield. This relation was not observed in the case of locus *GluA1*. The effect was so strong that the lines with *GluB1c* alleles outyielded the lines with *GluB1d* alleles by 0.88 t ha^{-1}. Moreover, higher yielding lines were more winter-damaged. It is possible that the rest of the plants utilized resources for yield formation more efficiently, even when these lines were characterized by a significantly lower leaf area index. Also, lines with *GluB1c* alleles were significantly shorter than those with *GluB1d* alleles. Significantly more erect and darker leaves and visual soil cover prove that *GluB1c* alleles in the lines were more related with the height-reducing genes, since such genes positively influence the use of soil and light resources [15]. Significant multi-associations of both loci prove a genetic relation with some height-controlling genes.

Another object of the study was reaction to diseases. Of all the diseases screened, only resistance to common bunt did not correlate at all. The other diseases were related mostly to one of *Glu1* locis. Resistance to Septoria leaf blotch was more influenced by *GluB1* than *GluA1*. Significant differences were determined among the groups of lines with different *Glu1* alleles. However, the actual difference in resistance was too low to suggest that some QTL responsible for resistance could be genetically linked with *Glu1* loci. According to the study of Joshi and Chand [16], wheat genotypes with more erect leaves tended to be less prone to spot blotch. The same trend was observed in the study of Gasowski [17] on wheat reaction to leaf rust. A similar relation could exist with Septoria leaf blotch because of the weak correlation (r = 0.31*) with leaf angle. Resistance to powdery mildew among the groups of lines with different alleles of locus *GluA1* was significant, however, the total severity of the disease was too low for a valid analysis.

Take-all is not affected by plant architectonic type, therefore, a higher resistance of lines with *GluB1b* alleles could be genetically determined. A higher resistance was also significantly related to a higher yield (0.9 t ha^{-1}). Lines with *GluA1c* alleles were more a resistants to eyespot. A direct effect of *Glu1* loci and alleles on the resistance to take-all and eyespot was found.

Our research suggests a possibility of determining the valuable traits affecting QTLs using other techniques than genetic mapping. Further research is needed in order to apply the new findings in practical breeding.

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DIDELĖS MOLEKULINĖS MASĖS GLIUTENINŲ *GLUA1 AND GLUB1* ALELIŲ ĮTAKA ŽIEMINIŲ KVIEČIŲ AGRONOMINIAMS POŽYMIAMS

Santrauka

2006 ir 2007 metais Lietuvos žemdirbystės institute buvo tiriami žieminių kviečių didelės molekulinės masės gliuteninų alelių koreliaciniai ryšiai su 24 agronominiais požymiais. Stipri koreliacija buvo nustatyta tarp *GluB1* alelių ir atsparumo žiemojimui (r = 0,71**), vidutinio stiprumo – tarp *GluA1* alelių ir augalų vizualaus vešlumo, rudeninio augimo tipo (r = 0,61**) bei atsparumo miltligei (0,58**). Derlius linijų grupėje, turinčioje *GluB1c* alelį, buvo vidutiniškai didesnis 0,88 t/ha nei linijų grupėje su *GluB1d* aleliu. Geriau žiemojo *GluB1d* alelį turinčios linijos (1.18 balo). Baltymų kiekis ir sedimentacija buvo atitinkamai 0,78% ir 5,84 ml didesni linijų grupėje, turinčioje *GluB1d* alelį, nei *GluB1c* alelį. Natūrinis grūdų svoris taip pat buvo aukštesnis linijų grupėje su *GluB1d* aleliu. Genotipai su *GluB1c* aleliu buvo atsparesni javaklupei (1,06 balo) nei genotipai su *GluB1d* aleliu. *GluA1* lokusas turėjo mažesnę įtaką agronominėms savybėms. Labiausiai šis lokusas paveikė rudeninį augimo tipą, vizualų augalų vešlumą ir sedimentaciją – 1,30 balo, 1,05 balo ir 6,31 ml atitinkamai. Šiems požymiams teigiamą įtaką turėjo *GluA1a* alelis. Silpniausiai ir statistiškai nepatikimai tirti aleliai koreliavo su atsparumu kietosioms kūlėms, krakmolo kiekiu, išplaukimo laiku ir atsparumu išgulimui: r = – 0,24–0,21.

Raktažodžiai: *GluA1, GluB1,* agronominės savybės, koreliacija