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Polymorphism of HMW-GS in Collection of Wheat Genotypes

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***Abstract*—**Processes of plant breeding, testing and licensing of new varieties, patent protection in seed production, relations in trade and protection of copyright are dependent on identification, differentiation and characterization of plant genotypes. Therefore, we focused our research on utilization of wheat storage proteins as genetic markers suitable not only for differentiation of individual genotypes, but also for identification and characterization of their considerable properties. We analyzed a collection of 102 genotypes of bread wheat (*Triticum aestivum* L.), 41 genotypes of spelt wheat (*Triticum spelta* L.), and 35 genotypes of durum wheat (*Triticum durum* Desf.), in this study. Our results show, that genotypes of bread wheat and durum wheat were homogenous and single line, but spelt wheat genotypes were heterogenous. We observed variability of HMW-GS composition according to environmental factors and level of breeding and predict technological quality on the basis of Glu-score calculation.

***Keywords*—**Genotype identification, HMW-GS, wheat quality.

I. Introduction

CEREALS are the most important source of the food all over the world. They are also important as a basic product for animal nutrition, oil production, beverages etc. Wheat has the major position among many cultivated cereal species (rice, barley, maize, oat etc.).

Plant samples stored as genetic resources in specialized facilities - gene banks, belong to the cultural richness and heritage of each nation and all mankind. Biological diversity represented by genetic resources, is and will be the starting point for the creation of improved plant genotypes and also for the production of sufficient amount of food and other raw materials for the rapidly growing human population.

Identification and verification of genetic resources are essential tools when working with plant genetic resources. The main and basic means of identification and verification of plant genotypes are genetic markers that offer the ability to identify and verify the sample based on characters derived from their genetic basis. The most commonly used markers are fractions of cereal seed storage proteins (prolamins and glutelins), which are suitable not only for the identification and differentiation between the genotypes, but also for the prediction of some economically important characteristics and properties.

The glutenin fraction is formed by highly polymerized protein molecules and can be classified according to molecular weight to two groups: high and low molecular weight glutenin subunits (HMW-GS and LMW-GS). HMW-GS represent about 10 % of glutenin fraction and these subunits are encoded by Glu-A1, Glu-B1 and Glu-D1 loci [1], [2]. Quantity and type of HMW-GS has strong influence on dough properties [1].

II. Material and Methods

A. Plant Material

102 genotypes of bread wheat (*Triticum aestivum* L.), 41 genotypes of spelt wheat (*Triticum spelta* L.) and 35 genotypes of durum wheat (*Triticum durum* Desf.) were obtained from the Gene Bank of the Slovak Republic Piešťany. Detailed genotypes with origin are described in Tables I-III in chapter Results and Discussion.

B. Sample Preparation

Proteins were extracted from individual grains according to standard ISTA method [3]. Seed storage proteins were isolated from whole, dry and mature grains. There were analyzed 20 individual grains from each genotype. Each grain was measured and mechanical homogenized. After homogenization was added 8 μL of extraction solution per 1 mg of grain.

Composition of extraction solution: 12.5 ml 1 mol.dm^{-3}Tris-HCl pH 6.8, 20 ml glycerol, 24.1 ml water, 4 g SDS, 20 mg Pyronin Y.

Extraction of storage proteins was performed by 30 min. shaking in 100°C following by 10 min. centrifugation (15000 rpm). Supernatant was transferred to a new tube.

C. Electrophoretic Separation of Protein

Seed storage proteins electrophoretic separation was realized in vertical discontinual electrophoretic system Hoefer SE 600 DeLuxe. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used as a separation medium according to standard ISTA method [3]. 5μl of each sample was loaded into gel. Seed storage proteins separation was running for 20 hours with constant current 10 mA.

D. Gel Staining and Image Analysis

Fractions of separated seed storage proteins were stained in solution of Commasie Brilliant Blue R 250 in ethanol and 10 % TCA. Image analysis of SDS-PAGE gels were carried out using DocIt-LS software (Ultraviolet Products) using an automated process supplemented with occasional manual

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adjustments. Each lane was first defined where the average intensity across the width of these lanes was depicted as a function of the distance in pixels from the top of the image. The background was subtracted from each profile (parameters adjusted on a case-by-case basis), after which the bands were identified. The DocIt-LS software was also used for statistical interpretation of the eletrophoreograms.

III. Results and Discussion

Cereals are the most important source of food protein. Quality and quantity of cereal seed storage proteins are the major factor, which influenced technological quality of wheat flour [4]. Quality of cereal seed is influenced in positive and negative way by presence of individual high molecular weight glutenin subunits (HMW-GS). HMW-GS named 5+10 localized on Glu-D1 loci influenced technological quality in positive way and presence of subunit named 2+12 has a negative effect on technological quality of wheat grain [5].

Electrophoretic analysis of wheat grain glutenin proteins is utilized for identification of phenotypic effect of individual alleles of each gene. Separation of storage protein by electrophoresis is suitable for genetic analysis, which can be applicable in genetic studies and breeding.

We focused on detection of genotypes and variability of electrophoretic spectra of individual HMW-GS in relationship to technological quality of bread wheat (*Triticum aestivum* L.), spelt wheat (*Triticum spelta* L.) and durum wheat (*Triticum durum* Desf.).

Genotypes of bread wheat and durum wheat which we analyzed were homogenous and single line, in contrast with genotypes of spelt wheat, which were heterogeneous and multiline. We detected from one to five lines in collection of spelt wheat genotypes. The highest number of lines we observed in genotype H-92-20 (5 lines) and H-92-27 (3 lines). Heterogenity and higher number of lines per genotypes is the intrinsic property of cereals, which provide them better adaptability to gown conditions. Based on the above, we can conclude that genotypes of spelt wheat have better adaptability.

We observed 16 electrophoretic profiles in analysis of 102 genotypes of bread wheat (Table I). The dominant composition of HMW-GS was 0, 7+9, 5+10 (35.3%) and 1, 7+9, 5+10 (16.7%), respectively. Electrophoretic analysis of 51 lines of spelt wheat showed presence of 14 electrophoretic profiles (Table II) and we detected major composition of HMW-GS 1, 6+8, 2+12 (49%).We also observed 6 profiles in analysis of 35 genotypes of durum wheat (Table III) and HMW-GS composition 0, 7+8 (54.3%) was the dominating one. Our results show that the lowest level of breeding we detected by analysis of spelt wheat cultivars were we observed approximately 3.6 line per one electrophoretic profile which indicates the highest resistance to environmental conditions. Higher level of breeding we noticed in bread wheat genotypes and durum wheat genotypes which showed average number of genotypes per electrophoretic profile 6.4 and 5, respectively. On the basis of this, we can assumed that bread wheat and durum wheat are on the one hand less adaptable to environmental condition, but on the other hand provide better technological parameters.

Gene's analysis of bread wheat genotypes collection, which we performed on HMW-GS coding loci showed similar results between 85 genotypes originated from the former Czechoslovakia and 15 genotypes originated from Europe (Tables I-III).

We determined 3 HMW-GS named 0, 1, and 2* in whole collection on the loci Glu-A1. The most frequent HMW-GS in collection of bread wheat was allele 0, which we identified in 60% of genotypes originated from former Czechoslovakia and in 53% of genotypes originated from Europe. Analysis of spelt wheat genotypes originated from Western Europe showed us the highest frequency of allele 1 (78%) and analysis of durum wheat cultivars originated mainly from Eastern Europe provide us similar results as in bread wheat collection. We observed allele 0 as the most frequent HMW-GS allele (94.3%) coding by locus Glu-A1 in collection of durum wheat.

We detected presence of 7 HMW-GS named 20, 6+8, 7+8, 7+9, 13+16, 14+15, and 17+18 on Glu-B1 loci in all wheat genotypes collection. Our results showed that variability on Glu-B1 loci was the highest. We investigated that HMW-GS allele 7+9 was the most frequent in collection of bread wheat. 71.7% of genotypes originated from former Czechoslovakia and 80% of genotypes originated from Europe which we analyzed showed presence of this allele. The highest occurrence of HMW-GS allele 6+8 we observed in 80.3% of spelt wheat genotypes. Analysis of durum wheat genotypes indicated that 54.3% of genotypes showed presence of HMW-GS allele 7+8.

Technological quality of wheat grain flour is the most influenced by alleles localized on Glu-D1 loci [6]. We detected 3 HMW-GS alleles named 5+10, 2+12, and 3+12. Positive affected HMW-GS 5+10 we detected in 77.6% of bread wheat genotypes originated from former Czechoslovakia and in 86.6% of genotypes originated from Europe. Detection of HMW-GS alleles, which we performed in collection of spelt wheat genotypes on Glu-D1 loci provide different results compared to analysis of bread wheat genotypes collection. We observed the most frequent presence of HMW-GS allele 2+12 with negative effect on technological quality of wheat flour, which we detected in 82% of spelt wheat genotypes. Collection of durum wheat genotypes does not contain genes on Glu-D1 loci, because of tetraploidy.

Our results are consistent with research of other authors, who confirmed, that variability of HMW-GS coding by individual Glu-loci is based mainly from geographical attributes. Reference [7] analyzes collection of 200 genotypes of bread wheat originated from France and proved majority (69.5%) of allele named 0 coded on Glu-A1 loci. Dominancy of 7+9 allele coded by Glu-B1 loci demonstrated by our research was not in conformity with analysis of French wheat. Reference [7] identifies flatter distribution of individual HMW-GS alleles encoded by Glu-B1 loci, with highest incidence of HMW-GS allele 7+8 only at the level of 30.5%. High polymorphism of French wheat confirmed occurrence of rare HMW-GS allele named 18 (0.5% of genotypes). Research

realized on French wheat also indicated other results on alleles coded by Glu-D1 loci. Positive affected HMW-GS allele 5+10 which we recognized in 79.4% of the entire collection of bread wheat was not in agreement of HMW-GS composition coded by Glu-D1 loci in the collection of French wheat, where the HMW-GS allele 2+12 was the dominating one (53%).

TABLE I
ALLELIC COMPOSITION OF BREAD WHEAT GENOTYPES AT GLU-LOCI AND GLU SCORE

Genotype	Origin	HMW-GS			Glu Score
		GLU-A1	GLU-B1	GLU-D1	
Agra	SVK	1	7+9	5+10	9
Arnold	CAN	2*	7+9	5+10	9
Arida	SVK	0	7+9	5+10	7
Armelis	SVK	0	7+9	5+10	7
Cornelius	AUT	1	7+9	5+10	9
Astela	SVK	2*	7+9	5+10	9
Auburn	SVK	1	7+9	5+10	9
Axis	SVK	2*	7+8	5+10	10
Bardotka K.	SVK	0	7+8	5+10	8
Barma	SVK	0	7+9	5+10	7
Bertold	SVK	0	7+8	5+10	8
Favorit	GER	1	7+9	5+10	9
Impulsiv	AUT	1	7+9	5+10	9
Bonita	SVK	0	7+9	5+10	7
Brea	CZE	0	7+9	5+10	7
Bučianská 106	SVK	0	7+8	2+12	6
Bučianská 202	SVK	1	7+9	3+12	7
Butín	SVK	2*	7+9	5+10	9
Cálovská	CSK	1	7+9	2+12	7
Lukullus	AUT	1	7+9	5+10	9
Diana I.	CZE	0	7+9	5+10	7
Eva	SVK	0	7+9	5+10	7
Faustina	CZE	0	7+9	2+12	5
Magvas	HUN	1	7+9	5+10	9
Midas	AUT	1	7+9	5+10	9
Fulvio	SVK	1	7+9	5+10	9
Gallus	SVK	1	7+9	5+10	9
Hana	CZE	0	7+8	5+10	8
Apache	FRA	0	7+9	5+10	7
Betty	USA	0	7+9	5+10	7
Ilava	SVK	0	7+9	5+10	7
Ilias K	SVK	1	20	5+10	8
Ilona K	SVK	2*	7+9	5+10	9
Blasius	AUT	1	7+9	2+12	7
Is Apage	SVK	2*	7+8	5+10	10
Is Ezopus	SVK	1	7+9	5+10	9
Is Jarissa/1	SVK	1	7+9	5+10	9
Is Jarissa/2	SVK	1	7+9	2+12	7
Is Jarissa/3	SVK	1	14+15	5+10	8
Is Median	SVK	0	7+8	5+10	8
Is Questor	SVK	1	7+9	5+10	9
Istar (Venistar)	SVK	0	7+9	5+10	7
Istra	SVK	1	7+8	5+10	10
Klea	SVK	0	7+9	5+10	7
Košútka	SVK	0	7+9	5+10	7
Krajová Brestovec	SVK	2*	7+9	5+10	9
Krajová Chmelnica	SVK	2*	7+9	5+10	9
Livia	SVK	0	7+9	5+10	7
Favorit	RUS	0	7+9	5+10	7
Madejka	SVK	0	7+8	2+12	6
Matador	HUN	0	7+9	5+10	7
Malé Karpaty	SVK	0	6+8	5+10	6
Malyska	SVK	0	6+8	5+10	6
Pannonikus	AUT	0	7+9	5+10	7
Rába	HUN	0	7+9	5+10	7
Mila	SVK	0	7+8	5+10	8
Ms 1588	SVK	0	7+9	2+12	5
Ms 1744	SVK	0	6+8	5+10	6
Ms 1752	SVK	0	7+9	5+10	7
Niagara	CZE	0	7+9	5+10	7
Nový Život	CSK	1	7+9	2+12	7
Helmut	AUT	0	6+8	5+10	6
Petrana	SVK	0	7+9	5+10	7
Petur	HUN	0	6+8	5+10	6
Pinta	SVK	0	7+8	5+10	8
Ps 28/08	SVK	0	7+9	5+10	7
Charger	GBR	0	20	2+12	4
Rada	SVK	0	7+9	5+10	7
Radošinská Karola	SVK	1	7+9	5+10	9
Radošinská Norma	SVK	2*	7+9	5+10	9
Sana	SVK	0	7+9	2+12	5
Silvanus	SVK	0	7+9	5+10	8
Sk 47	SVK	0	7+9	2+12	5
Sk 53	SVK	0	7+9	5+10	7
Sk 60	SVK	0	7+9	5+10	7
Sk 69	SVK	0	7+8	2+12	6
Sk 75	SVK	0	7+9	5+10	9
Sk 76	SVK	1	7+9	5+10	9
Sk 77	SVK	0	7+9	5+10	7
Sk 81	SVK	0	6+8	2+12	4
Sk 87	SVK	0	7+9	5+10	7
Sk 95	SVK	0	7+9	5+10	7
Slovenská 2	SVK	0	6+8	2+12	4
Slovenská 200	SVK	1	7+9	5+10	9
Slovenská 777	SVK	1	7+9	3+12	7
Slovenská B	SVK	1	7+9	3+12	7
Slovenská Intenzívna	SVK	2*	7+9	2+12	7
Solara	SVK	1	7+8	5+10	10
Solaris	SVK	1	7+9	5+10	9
Solida	SVK	2*	7+9	5+10	9
Torysa K	SVK	0	7+8	2+12	8
Vanda	SVK	2*	7+9	5+10	9
Venistar K	SVK	0	7+9	5+10	7
Viator (Viador)	SVK	0	7+9	5+10	7
Viginta	SVK	0	7+9	5+10	7
Viglanka	SVK	0	7+9	5+10	7
Viglašská	SVK	0	7+9	5+10	7
Viglašská Červenoklasá	SVK	1	7+9	3+12	7
Viola	SVK	1	7+8	5+10	10
Vrakúnska	SVK	0	7+8	2+12	6
Zaira	SVK	0	6+8	5+10	6
Zerda	SVK	0	7+9	5+10	7

SVK–Slovakia, CZE–Czech Republic, AUT–Austria, GER–Germany, CSK–Czechoslovakia, CAN–Canada, USA–The United States of America, HUN–Hungary, FRA–France, GBR–The Great Britain, RUS-Russia

TABLE II
ALLELIC COMPOSITION OF SPELT WHEAT GENOTYPES AT GLU-LOCI AND GLU SCORE

Genotype	Origin	HMW-GS			Glu Score
		GLU-A1	GLU-B1	GLU-D1	
Altgold	CHE	1	6+8	2+12	6
Ardenne	BEL	1	6+8	5+10	8
Baetting Niederwill	GER	1	6+8	2+12	6
Bauländer Spelz	GER	1	13+16	2+12	8
Burgdorf	GER	1	6+8	2+12	6
Franckenkorn	GER	1	6+8	5+10	8
Fuggers Babenhauser	GER	1	6+8	2+12	6
H 92-20 (P. Kunz)	CHE	0	6+8	2+12	4
		0	6+8	5+10	6
		0	7+9	2+12	5
		1	6+8	2+12	6
		1	6+8	5+10	8
H 92-27 (P. Kunz)	CHE	1	6+8	2+12	6
		1	6+8	5+10	8
		1	13+16	2+12	8
Hercule	BEL	0	6+8	2+12	4
Holstenkorn	GER	1	7+8	2+12	8
Kipperhaus Weisser Spelz	GER	1	6+8	2+12	6
Kr 489-11-15 (P. Kunz)	CHE	0	6+8	5+10	6
		1	6+8	2+12	6
Lueg	CHE	1	6+8	2+12	6
		1	6+8	5+10	8
Lw 12 (Nuertingen)	GER	1	6+8	2+12	6
		1	13+16	2+12	8
Lw 13 (Nuertingen)	GER	1	6+8	2+12	6
Oberkulmer Rotkorn	CHE	1	6+8	2+12	6
Oberkulmer Schwarzer	CHE	1	6+8	2+12	6
Ostro	CHE	1	6+8	2+12	6
Ostro Schwarzer	CHE	1	6+8	2+12	6
Renval	BEL	1	20	2+12	6
Roter Kolbendinkel	AUT	1	6+8	2+12	6
Rotthweller Fruhkorn	GER	1	6+8	2+12	6
Rouquin	BEL	1	6+8	5+10	8
Schwabenkorn	GER	1	6+8	2+12	6
Schwabenkorn (Dinkel)	GER	1	6+8	2+12	6
		1	13+16	2+12	8
Landrace 1-96	-	1	6+8	2+12	6
Steiner Roter Laupheimer	AUT	0	6+8	2+12	4
T. Spelta (Uhrineves)	-	1	6+8	2+12	6
Tri 2400 (Gatersleben)	GER	1	6+8	2+12	6
Waggershauser Kolbendinkel	GER	1	6+8	2+12	6
Weisser Kolbenspelz	GER	1	6+8	2+12	6
Weisser Winter Granendinkel	AUT	1	6+8	2+12	6
Cv.Baetting Niederwill	GER	2*	7+8	2+12	8
Cv.Fuggers Babenhauser Zuchtveessen	GER	2*	6+8	2+12	6
Cv.Weisser Winter-Granendinkel Aus Hohen	AUT	2*	6+8	2+12	6
Line 3/96	SVK	0	17+18	2+12	6
Ostro	CHE	2*	6+8	2+12	6
Renval	BEL	1	7+9	2+12	7
Rotweil Fruhnkorn	GER	1	6+8	5+10	8
Schwabenkorn	GER	1	6+8	2+12	6

CHE–Switzerland, BEL-Belgium, GER–Germany, AUT-Austria, SVK-Slovakia

TABLE III
ALLELIC COMPOSITION OF SPELT WHEAT GENOTYPES AT GLU-LOCI AND GLU SCORE

Genotype	Origin	HMW-GS			Glu Score
		GLU-A1	GLU-B1	GLU-D1	
Istrodur	SVK	0	7+8	-	4
Martondur	HUN	0	7+8	-	4
Soldur	SVK	0	7+8	-	4
Ajsberg Odesskij	UKR	0	20	-	2
Armet	FRA	0	20	-	2
Capetti	ITA	0	20	-	2
Ciccio	ITA	0	7+8	-	4
Df 69680/82	ROM	0	20	-	2
Df 51/71	ROM	0	7+8	-	4
Dwh	-	0	20	-	2
Gordeiforme 1124/82	RUS	0	20	-	2
Gordeiforme 440/75	RUS	1	20	-	4
Makaronka	SVK	2*	20	-	4
Macoun	CAN	0	6+8	-	2
Platani	ITA	0	7+8	-	4
Soldur	SVK	0	7+8	-	4
Waakoma	CAN	0	6+8	-	2
Wascana	CAN	0	6+8	-	2
SO-90-D-55	SVK	0	7+9	-	3
SO-93-D-126	SVK	0	7+9	-	3
SO-94-D-166	SVK	0	7+9	-	3
SO-94-D-168	SVK	0	7+9	-	3
SO-94-D-169	SVK	0	7+8	-	4
SO-94-D-170	SVK	0	7+8	-	4
SO-94-D-201	SVK	0	6+8	-	2
SO-94-D-205	SVK	0	7+8	-	4
SO-94-D-57	SVK	0	7+8	-	4
SO-94-D-64	SVK	0	7+8	-	4
SO-94-D-66	SVK	0	7+8	-	4
N	SVK	0	7+8	-	4
SO-94-D-70	SVK	0	7+8	-	4
SO-D-90-112	SVK	0	7+8	-	4
SO-D-90-31	SVK	0	7+8	-	4
Soldur	SVK	0	7+8	-	4
Vendur	SVK	0	7+8	-	4

HUN-Hungary, UKR-Ukraine, ROM-Romania

Differences in HMW-GS composition affected by environmental factors were confirmed by analysis of 44 genotypes of bread wheat originated from Turkey and Russia [8]. Reference [8] detects the highest level of presence of HMW-GS allele 2* encoded by Glu-A1 loci, while HMW-GS allele 7+8 was the most frequent on Glu-B1 loci and loci Glu-D1 encoded mainly HMW-GS allele 5+10.

Percentages of individual alleles also differ in analysis of 97 bread wheat genotypes originated from Iberian Peninsula [9].

HMW-GS composition of individual genotypes of bread wheat is mainly affected by environmental conditions during evolution and by level of breeding, which is in accordance of others and our results. References [10], [11] monitor composition of storage proteins of bread wheat ancestors and detected new and rare combination of HMW-GS alleles, which confirmed hypothesis about close correlation of composition of HMW-GS alleles and origin of bread wheat

genotypes. Environmental influence on composition of HMW-GS allele showed investigation of old genotypes Bánkúti 1201 originated from Hungary [12]. Reference [12] recognizes heterogenity and multiline character of this genotype which is typical for landraces. Results indicate needs for detailed research of old genotypes and landraces as potential donors of specific features which can be utilized in bread wheat breeding program.

Composition of individual HMW-GS localized in wheat grain can provide possibility to predict technological quality of wheat flour by calculation of Glu-score (Table IV) [13]. The highest possible calculated Glu-score value is 10. The lowest calculated value for good technological quality is 8.

TABLE IV
PREDICTION VALUE OF INDIVIDUAL ALLELES

Loci	Allele	Points
1A	0	1
	1	3
	2*	3
1B	6+8	1
	7	1
	7+8	3
	7+9	2
	13+16	3
	14+15	1
	17+18	3
	20	1
1D	2+12	2
	3+12	2
	4+12	1
	5+10	4

We determined average Glu-score 7.5 in collection of Czechoslovakian bread wheat cultivars, with highest value 10, reached by genotypes AXIS, IS APAGE, ISTRA, SOLARA, and VIOLA, as well as 31.8% of genotypes achieved Glu-score 9. Analyzed genotypes of 15 European bread wheat cultivars provided us the same average Glu-score 7.5, and highest Glu-score value 9 reached 77.8% of genotypes.

Heterogenity of spelt wheat genotypes caused problems with Glu-score calculation, especially for multiline genotypes. Because of this, is possible to calculate Glu-score only for single line cultivar, or for individual lines. On the basis of this, we established the average calculated Glu-score for all lines of spelt wheat genotypes 6.4 and only 25.5% of spelt wheat lines reached Glu-score 8.

Durum wheat genotypes does not contain D-genome and because of this we observed the highest calculated Glu-score value 4 which was monitored in 60% of genotypes. Low Glu-score value of durum wheat genotypes indicates suitability for pasta production.

Our findings are consistent with the extensive work devoted to the impact of the storage proteins on the technological quality realized by [14], [15]. Reference [15] monitors correlation between predicted and real technological quality of bread wheat and postulated, that essential element necessary for production of optimal dough is presence of insoluble protein matrix mainly consist of glutenin proteins. Glutenin proteins are the most important part of wheat grain proteins affected dough production. Their composition mainly depends on genetic relationship [16]-[18]. References [19]-[21] investigate suitability of Glu-score as a universal tool for simple and rapid prediction of qualitative parameters of wheat genotypes.

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