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Analysis of Endosperm Proteins and Genetic Diversity in Pakistani Wheat Varieties

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ABSTRACT

The present study was undertaken to evaluate the genetic diversity in gluten subunits and protein content in seed endosperm of wheat for nutritional grading of 60 registered Pakistani wheat varieties using SDS-PAGE and this grading is useful for agricultural development regarding food stuffs and breeding purposes. After calculating the standard factor in spectrophotometer for unit absorbance at 540 nm and the amount of protein were calculated. The wheat varieties GA-2002, C-250 and Chakwal-86 contains maximum amount of HMW-Gs proteins as compared to rest of the varieties. By comparing with the thirteen bands of the standard marker band numbers 5, 9, 12 and 13 were common in all sixty wheat varieties but the other bands show variation. Most frequent HMW protein bands were with mol. Wt. 110 *KDa* marker band, while in LMW protein bands most frequent bands were among the 14 *KDa* marker band. In cluster analysis the above mentioned three wheat varieties grouped in the same cluster showing that they originate from the same parental line and genetically less diverged. However, the only reason for producing variable amounts of HMW-Gs was due to the varying combinations and expression of genes in these lines. © 2010 Friends Science Publishers

Key Words: Genetic diversity; HMW-Gs; Nutritional grading; Wheat varieties

INTRODUCTION

Wheat flour applications are innumerable being used for bread making and bakery products including fermented drinks, also fed to livestock in many ways. Bread making quality is an important and complex character of Bread wheat (*T. aestivum* L.) (Pomeranz, 1988). The most common cultivated wheat nowadays is hexaploid *T. aestivum*, AABBDD, (2n= 6X= 42) (Zohary & Hopf, 1993). The A, B and D genomes have sets of storage proteins that are electro-phoretically distinguishable from each other (Payne *et al*., 1984). Bread making quality correlates with the presence or absence of specific proteins and protein subunits (Gupta *et al*., 1989). Storage proteins accounts for about 50% of total protein in mature cereal grains and have important nutritional value for humans (Friedman & Brandon, 2001).

Wheat quality depends upon the grain hardness and its protein content. Quality is basically determined by the molecular structure of the storage protein of wheat, which in turn controls the interaction of the protein during bread making process (Bushuk, 1998; Shewry *et al*., 1999). SDS-PAGE could be conveniently used for identification of wheat varieties suitable for chapatti making (Prabhasankar & Rao, 2001). The endosperm proteins of Japanese "*Udon*" wheat lines were fractionated by SDS-PAGE to determine protein–composition differences in two soil environment (Nakamura, 2001). A number of molecular studies started from specific protein that determines the particular phenotype of an organism and go back to clone, identify and characterization of the specific gene expression of the protein of interest (Davis, 1986). The gliadins and glutenins constitute up to 80-85%, where as albumins and globulins constitute 22% of total flour protein (Singh & MacRitchie, 2001) but do not play a critical role in flour quality despite their minor importance (Schofield & Booth, 1983).

Glutenins consisted of the high-molecular-weight (HMW) and low-molecular-weight (LMW) multimeric aggregatives. The high molecular weight glutenin subunits are encoded by Glu–A1, Glu–B1 and Glu–D1 loci on the long arm of chromosome 1A, 1B and 1D, respectively (Payne *et al.*, 1980), where as low molecular weight glutenin subunits are encoded by Glu–A3, Glu–B3 and Glu–D3 loci on the short arm of these chromosomes (Gupta & MacRitch, 1994). Specific pattern of interaction between low molecular weight glutenin (<90 *KDa*) and high molecular weight glutenin (>90 *KDa*) is important for the development of a visco-elastic gluten (MacRitchie, 1994).

The ability of wheat flour to be processed into different kind of foods is largely determined by the gluten proteins, because of their unusual structures and properties (Tatham & Shewry, 2000). The Basic storage protein gluten is visco-elastic protein complex responsible for the physical properties of wheat dough such as elasticity and resistance.

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The gluten proteins form a continuous matrix in the mature dry endosperm cells, when flour is mixed with water to form dough. The protein matrices in the individual cells, are brought together to form a continuous net work. This confers visco-elastic properties that allow the dough to be expanded by fermentation and baked into leavened bread or pasta, noodles and a range of other food stuffs (Shewry & Halford, 2002). High molecular weight glutenin subunits of 10 South African wheat cultivars by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) were investigated by Manley *et al*. (1992) and positive correlation was found with technological parameters and Glu-1 quality was assessed for each cultivar that relate to dough strength.

A special type of gluten protein agglomeration takes place under dough making condition (high energy input, relatively low water levels). During dough making, gluten proteins are stretched and bonds are broken and subsequently new bonds are formed during dough resting. The result is gluten matrix that is much stronger than gluten protein agglomerates formed under more dispersed conditions in flour suspension and with lower energy in put. The strong agglomeration during dough making is referred to as "gluten development" (Goesaert *et al*., 2005).

The present study was undertaken to evaluate the genetic diversity in gluten subunits and protein content in endosperm of wheat seed for nutritional grading of these newly inbred wheat lines in set of 60 wheat lines using SDS-PAGE and this grading/selection is useful for agricultural development regarding food stuffs and breeding purposes.

MATERIALS AND METHODS

Plant material. Seeds of sixty wheat varieties were taken from the gene bank of plant genetic resource programme (PGRP) at NARC, Islamabad (Table I) and used for the study of high and low molecular weight glutenins (HMW-Gs & LMW-Gs).

Protein estimation. The endosperm of well dried healthy seeds was separated with sand paper and ground to powdered form. Biuret assay (Gornall *et al*., 1949) was made to determine the wheat endosperm proteins. Proteins were extracted by adding 400 µL protein extraction buffer (0.05 M Tris-HCL, pH 8.0, 2% SDS, 5 M Urea, 1% β-Mercaptoethanol, 0.2 g Bromophenol blue) in about 10 mg powdered endosperm in 1.5 mL eppendorf, vortex to mix thoroughly and spun at 13000 rpm for 15 min at room temperature and supernatant was taken into new eppendorf to store at -20°C. The standard factor was calculated in spectrophotometer for unit absorbance at 540 nm and the amount of protein were calculated using the following formula:

Amount of protein mg/mL = absorbance of sample x standard factor.

For standard factor:

Table I. Wheat germplasm used for SDS-PAGE analysis

Varieties	Varieties
C-217	4112
C-228	SHAFAQ-2006
C-250	BAKHAR-2002
C-271	PIRSABAK-2004
C-518	PIRSABAK-2005
C-591	SH-2002
SA-42	SH-88
PAK-81	SH-02
SINDH-81	FAREED-2006
BARANI-83	SAHER-2006
FAISALABAD-83	TJ-83
FAISALABAD-85	WAFAQ-2002
PB-96	IMDAD-2005
CHAKWAL-86	T- 96775
BAHAWALPUR-79	KIRAN-95
POTOHAR	KAGHAN-93
MEXIPAK	AUQAB-2000
DIRK	IQBAL-2000
PARI-73	GA-2002
MEHRAN-89	U- 4057
ANMOL-91	MARGALLA-99
PARWAZ-94	ZARLASHTA-99
BAKHTAWER-93	BLUE SILVER
D- 06623	NOWSHERA-96
MH-97	V 03138
MARGALLA-99	KOHISTAN-97
CHENAB-2000	BHITTAI
MARVI-2000	TD-1
IMDAD-2005	SKD-1
PARULA	SALEEM- 2000

Table II. Protein Estimation by spectrophotometer (Absorbance x Stand Factor)

Varieties	Absorb.	Protein mg/ml	Varieties	Absorb	Protein mg/ml
C-217	180	1.12	4112	150	0.93
C-228	161	1.00	SHAFAQ-2006	185	1.15
C-250	231	1.44	BAKHAR-2002	161	1.00
C-271	211	1.31	PIRSABAK-2004	150	0.93
C-518	141	0.87	PIRSABAK-2005	222	1.38
C-591	137	0.85	SH-2002	183	1.14
SA-42	196	1.22	SH-88	162	1.01
PAK-81	195	1.21	SH-02	193	1.20
SINDH-81	184	1.14	FAREED-2006	133	0.82
BARANI-83	177	1.10	SAHER-2006	187	1.16
FAISALABAD-83	204	1.26	TJ-83	205	1.27
FAISALABAD-85	192	1.19	WAFAQ-2002	139	0.85
PB-96	146	0.91	IMDAD-2005	181	1.12
CHAKWAL-86	241	1.50	T- 96775	195	1.21
BAHAWALPUR-79	136	0.84	KIRAN-95	149	0.92
POTOHAR	218	1.36	KAGHAN-93	188	1.17
MEXIPAK	183	1.14	AUQAB-2000	145	0.90
DIRK	178	1.11	IQBAL-2000	206	1.28
PARI-73	211	1.03	GA-2002	247	1.54
MEHRAN-89	198	1.23	U- 4057	198	1.23
ANMOL-91	168	1.04	MARGALLA-99	187	1.16
PARWAZ-94	211	1.31	ZARLASHTA-99	167	1.04
BAKHTAWER-93	190	1.18	BLUE SILVER	109	0.74
D- 06623	197	1.22	NOWSHERA-96	201	1.25
MH-97	187	1.16	V 03138	165	1.02
MARGALLA-99	172	1.07	KOHISTAN-97	175	1.09
CHENAB-2000	196	1.22	BHITTAI	207	1.29
MARVI-2000	137	0.85	TD-1	199	1.24
IMDAD-2005	163	1.01	SKD-1	121	0.75
PARULA	208	1.29	SALEEM- 2000	202	1.26

Table III. Molecular weight analysis of proteins in the seeds of wheat varieties

Protein Type	Mol. Wt. (kDa)	Wheat Varieties																													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
HMW-GS	120	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1
	110	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1
	100	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1
	90	1	1	1	1	1	1	0	0	0	1	1	0	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1
	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LMW-GS	65	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1	1	1
	60	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1
	55	1	1	1	0	1	1	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	0
	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1	1	1
	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Protein Type	**Mol. Wt. (kDa)**	**Wheat Varieties**																													
		31	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**
HMW-GS	120	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	110	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	100	0	0	0	1	0	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	0	1	1	1	0	1	1	1	1
	90	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	1	1	0	0
	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LMW-GS	65	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0
	60	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	55	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	0
	25	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

1=C-217, 2=C-228, 3=C-250, 4=C-271, 5=C-518, 6=C-591, 7=SA-42, 8=PAK-81, 9=SINDH-81, 10=BARANI-83, 11=FAISALABAD-83; 12=FAISALABAD-85, 13=PB-96, 14=CHAKWAL-86, 15=BAHAWALPUR-79, 16=POTOHAR, 17=MEXIPAK, 18=DIRK, 19=PARI-73, 20=MEHRAN-89, 21=ANMOL-91, 22=PARWAZ-94, 23=BAKHTAWER-93, 24=D- 06623, 25=MH-97, 26=MARGALLA-99, 27=CHENAB-2000, 28=MARVI-2000, 29IMDAD-2005, 30=PARULA, 31=4112, 32=SHAFAQ-2006, 33=AKHAR-2002,34=PIRSABAK-2004, 35=PIRSABAK-2005, 36=SH-2002, 37=SH-88, 38=SH-02, 39=FAREED-2006, 40=SAHER-2006, 41=TJ-83, 42=WAFAQ-2002, 43=IMDAD-2005, 44=T- 96775, 45=KIRAN-95, 46=KAGHAN-93, 47=AUQAB-2000, 48=IQBAL-2000, 49=GA-2002, 50= U- 4057, 51=MARGALLA-99, 52=ZARLASHTA-99, 53=BLUE SILVER, 54=NOWSHERA-96, 55=V 03138, 56=KOHISTAN-97, 57=BHITTAI, 58=TD-1, 59=SKD-1, 60=SALEEM- 2000

Standard Factor = concentration of BSA mg per mL/Absorbance.

SDS-PAGE analysis. Profiling of proteins were made by one dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (10% separating gel & 4.5% stacking) following Laemmli (1970) in a mini gel apparatus (Bio-Rad) according to the method of Hames (1990). The gels containing protein samples with protein molecular weight markers allowed to run on 90 V until blue line of BPB came at the bottom of the gel plates. The separating gel was shaken gently for 1 h in the staining solution and then destain until bands sharpened. Finally the gel was analyzed in gel documentation system (Bio Rad Instruments, Italy).

Characterization of protein profiles. In the data matrix, the presence of a band was scored as 1, whereas the absence of the band was coded as 0 in the electrophorograms. The basic data structure finally consisted of a binary (0/1) data was analyzed and genetic diversity was measured. Heirarchical cluster analysis was made by the complete linkage method with Euclidean distance measure for evaluating the relative presence of stored endosperm wheat proteins in different wheat lines. The data was analyzed using STATISTICA computer software (Shuaib *et al*., 2007).

RESULTS

The amount of proteins was estimated in 10 mg of wheat seed sample. Results revealed a wide variation in seed storage proteins in 60 wheat lines (Table II) and in HMW-Gs subunits between different wheat varieties (Fig. 1). Protein content of these wheat varieties ranged from 0.74 mg/mL to 1.5 mg/mL. The minimum seed protein content was shown in wheat variety Blue Silver, while the maximum seed protein content was observed in wheat variety GA-2002. The seed proteins contents for the rest of the wheat varieties were intermediary of these two wheat varieties.

The seed storage protein patterns of each sample were determined by examining the molecular weight of the bands and the intensity of staining (Table III). Total band intensity of each lane was taken to represent 100% protein of the lane. The relative proportion of each band was calculated on the basis of standard bands of marker (Bonfil *et al.,* 1997).

In order to better study the observed variation for the high protein contents in wheat varieties, cluster analysis was performed (Fig. 2). At the Eucladian distance of 2.75 all the varieties show similarity but at Eucladian distance of 2.5 all the wheat varieties split into two major groups that were further divide into sub-groups and a mixed trend was seen in

Fig. 1. Electrophorogram showing banding pattern of wheat proteins and molecular weight marker of sixty wheat varieties, the arrows indicate the proteins of biological importance

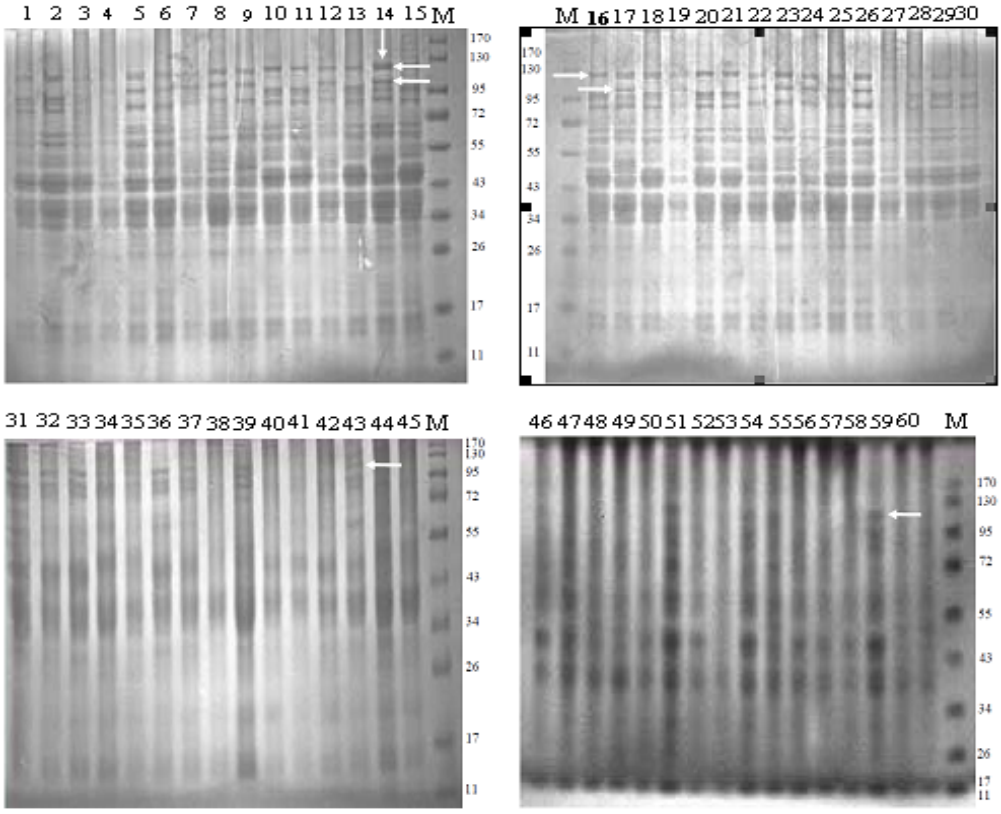
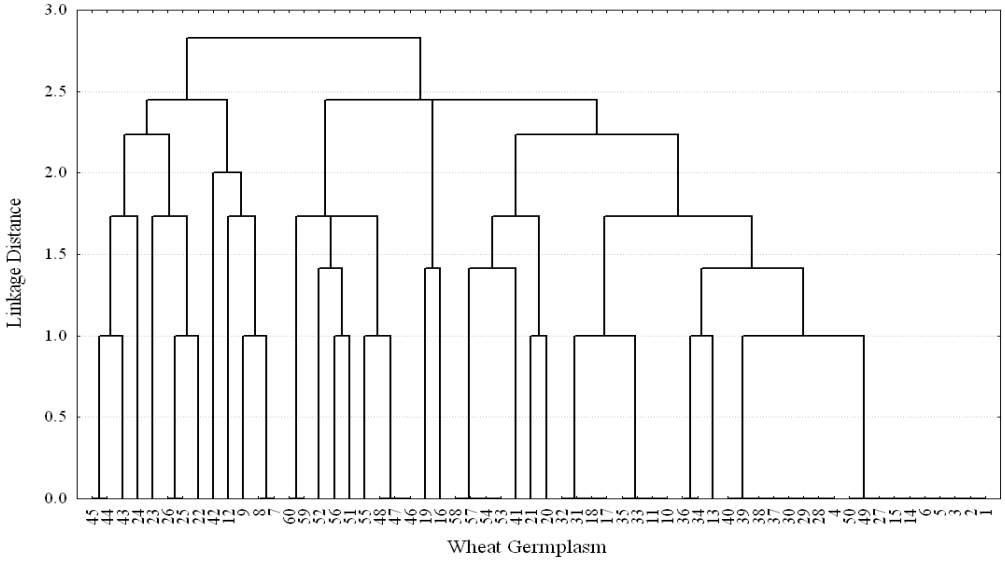


Figure 2: Dendrogram showing relationship of 60 wheat varieties based on total seed protein detected by SDS-PAGE



grouping of varieties. The three wheat varieties (3, 14 & 49) with maximum proteins contents (Table II) group into the same cluster but with some other varieties (Fig. 2). All these varieties possess same proteins due to which they group into same cluster but only differ in their protein concentration (mg/mL) and showed lowest dissimilarity among them. At the same time some other groups show variations among them too in the dendrogram.

DISCUSSION

In this study SDS-PAGE of grain storage proteins was performed in order to analyze molecular weight of gluten subunits and investigate genetic diversity among different newly inbred varieties. The electrophorogram showed proteins banding pattern of different wheat varieties (Fig. 1). The seed storage protein patterns of each sample were determined by examining the molecular weight of the bands and the intensity of staining. The relative proportion of each band was calculated on the basis of standard bands of marker (in the range of 10-120 kDa) and a total of thirteen bands were obtained among, which band numbers 5, 9, 12 and 13 were common in all sixty varieties but the other bands show variation.

Over all most frequent HMW protein band was with mol. Wt. 110 kDa. While in LMW protein bands most frequent bands were 14 kDa. These banding patterns were confirmed with the results by Shuaib *et al*. (2007). Same method was used by Nakamura (2001) for quality evaluation of common wheat endosperm protein finds a significant banding pattern in Kanto 107 with respect to LMW-Gs. Salcedo *et al*. (1979) also fractioned the LMW-Gs the sample bands below 19 kDa were found as in our results the bands with molecular weight 14 kDa are also present. Some other authors Ghasemzade *et al*. (2008) and Chaparzadeh *et al*. (2008) have also been reported similar findings in wheat varieties and/or lines. Wheat improvement activities for wheat-based cropping systems and also to study genetic behavior on the basis of protein profiles of the parents for development of high yielding wheat varieties is very essential for developing and releasing superior-quality wheat varieties.

CONCLUSION

In cluster analysis GA-2002, C-250 and Chakwal-86 group in the same cluster showing that they originate from the same parental line, because least difference was observed among them. However the only reason for production of variable amounts of HMW-Gs is the varying combinations and expression of genes in these lines.

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