Determination of variations of protein banding patterns of high molecular weight-glutenin's subunits in wheat after chromosomal substitution

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***Abstract*—** Substitution lines use for chromosomal location of genes. A storage protein of wheat seed contains gliadins and Glutenins. Glutenins is consisted of high molecular weight and low molecular weight. We extracted proteins from 27 substitution lines related to kapla – shayen substitution lines and Chinese Spring as control samples using laemmli *et al* (1970) protocol. After protein extraction, the samples were electrophoresed using SDS-PAGE. We stained the gels both commassie blue R250 and silver nitrate methods. We scored the bands based on 0 for absence and 1 for presence of each band. Then similarity matrix was calculated using Jaccard coefficient. Payne scoring system was calculated for each line. We observed 11 polymorphic bands. The ranges of coefficients of similarity were 0.55 -1. The coefficients of similarity were 1 for the most of lines. The coefficients of similarity of substitution lines of kapla and parent were 1 but about kapla 3A was 0.736. The coefficient of similarity of kapla 3A and other lines was low. These lines and control were clustered to 3 groups that kapla 3A and shayen 4B were in 2 separate and single groups. Based on silver staining, the range of coefficients of similarity were from 0.7 - 1. The coefficients of similarity of almost all lines were 1. Kapla 3A, kapla 4B and shayen 7B had lowest similarity. According cluster analysis, these lines and control were clustered to 2 groups that kapla 3A was in the separate group. Based on Payne scoring system, only kapla 3A had 2* and 10 bands that can be main reason of difference to other lines and control. According to these scoring the bands of all lines (except kapla 3A) were similar to parent kapla and Chinese Spring .But some of lines of shayen and control were different and these lines were clustered to 3 groups that shayen 7A , shayen 4B and kapla 3A were in the separate group. In the kapla cultivar, 3A substitution chromosome and in the shayen cultivar 7A and 4B chromosomes had high variation in the band pattern.

***Keyword s*—** Commassie Blue Staining, Glutenin, HMW-GS, Silver Staining, Substitution Lines.

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I. INTRODUCTION

SUBSTITUTION lines are produced using chromosomal substitution between two cultivars (without changing in chromosome number) [1]. These lines are produced using aneuploid lines [9]. All differences between two cultivars is related to substituted chromosomes [2].These lines were used for determination of chromosomal location of genes related to environmental stresses [14].

Substitution lines are produced for different cultivars of wheat, because aneuploidy can tolerate by wheat [7]. Different alleles may produce proteins with different combinations of amino acids that can be segregate well using SDS-PAGE method [11]. Gluten (proteins of wheat seed) is effective on bread making quality; therefore determination of chromosomal location of these alleles is useful for genetic improvement of bread making quality of wheat [10]. Determination of glutenin's alleles is used highly in projects of wheat breeding [6]. Studying of storage proteins has been reported in many researches such as phylogenetic relationships, genomic homologies and genetic diversity [8].

The first chromosomal experiment using substitution lines was accomplished in cultivars of tacher and hoop (*triticum aestivum*). Dashti *et al* (1998) determined chromosomal location of heading date alleles in bread wheat. They showed that location of these genes are on chromosome 3A, 3D, and 1D in vichita cultivar and on 3A,3D,1D,4D,6A and 1B in shayen cultivar [4]; Then 1A,1D and 6D chromosomes are effective on volume of bread [2]. Payne *et al* (1980) compared migration of high molecular weight glutenins in 7 cultivars of wheat using SDS-PAGE. They observed 12 subunits with molecular weight between 95-140 KD. All of these subunits are control using group 1 chromosomes [11].

Bahraii *et al* (2001) observed 21 alleles combinations in Iranian wheat cultivars.

The aim of this study was determination of effects of chromosomal substitution on glutenins' protein pattern especially high molecular weight subunits.

II. MATERIALS AND METHODS

In this research, 27 substitution lines related to Kapla and Shayen cultivars were used. We also used cultivar of Kapla and Chinese spring as control (Table I); Then we extracted total protein using Lammli protocol that modified by Flacte & Uhlen (2003) [6]. Electrophoresis of total protein was accomplished using 1- D SDS-PAGE method. Extraction buffer was consisted of 38 ml Tris 0.6M pH=6.8, 12gr SDS, 50mg commassie blue R250, 75ml H_2O, 60ml glycerol (Table II). 0.4ml mercaptoethanol and 4ml H_2O were added per 1.7ml extraction buffer. After centrifugation, supernatant was transferred to other tube for loading in gel. SDS-PAGE gels had 2 layers (Stacking gel 4%, pH=8.8 and resolving gel 10%, pH=6.8). We loaded 10µl protein's sample in each well. 30µl weight protein marker also loaded in first well.

Electrophoresis buffer consisted of 50gr glycine, 10gr Tris, 3.5gr SDS and 3500ml H_2O. We adjusted the voltage on 18mA. Two staining method was used: commassie blue and silver nitrate staining [13]. Then we scored banding pattern using Payne catalogue [15]. Softwares of NTSYSPC 2.0 and SPSS 15.0 were used for data analysis and calculation of similarity matrix. Molecular weights of bands were calculated using semi-logarithmic regression model.

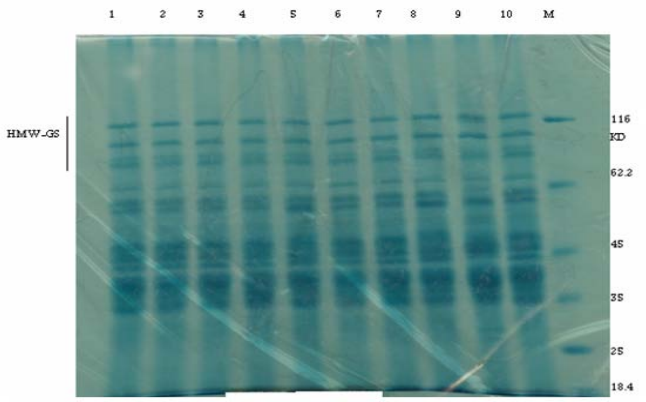

Fig. 1 A sample related to Protein gel electrophoresis (Commassie blue staining)

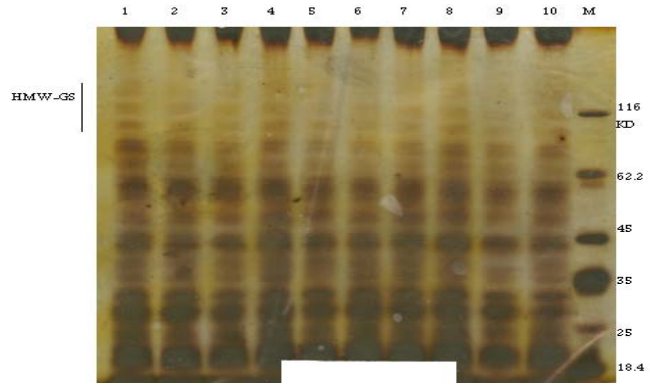

Fig. 2 A sample related to Protein gel electrophoresis (Silver nitrate staining)

III. RESULTS

We can summarize our outputs from gel electrophoresis (Fig.1 & Fig.2) in these notes:

A. Bands number

In commassie blue staining, 22 bands were observed that 10 bands were polymorphic. The lowest banding number related to shayen 4B, Kapla 3A with 17 and 18 bands; other studied lines had 19 bands.

In silver staining, 20 bands were observed that 8 bands were polymorphic. The highest banding number related to Kapla 3D, Kapla 4D and Shayen 1B (19 bands) and the lowest number related to Kapla 3A, 7A, 1B, 4B and Shayen 7B and 4D with 17 bands. The others had 18 bands.

B. κ^2 Test

The results of this test indicated that significant differences between genotypes in 5% level of significancey.Then we can accomplished cluster analysis.

C. Calculation of similarity coefficients

In commassie blue staining, range of similarity coefficients were between 0.55-1.

The lowest similarity coefficient was 0.55 between Shayen 4B and Kapla 3A.

The highest similarity coefficient was 1 between all of substitution lines and Kapla (control). Kapla 3A had the lowest coefficient with other lines.

In silver staining method, ranges of similarity coefficients were between 0.7-1.

The lowest similarity coefficient was related to Kapla 3A with Kapla 4B and Shayen 7B.

D. Calculation of cophenetic coefficients

Cophenetic's correlation coefficients were calculated for each method of staining (Table III & Table IV); then we selected Jacquard's coefficient and algorithm of UPGMA.

Ultimately, two dendrograms were drawn for each method of staining (Fig.3 & Fig.4).

In commassie blue and silver staining methods, dendrograms were divided to 3 and 2 clusters, respectively.

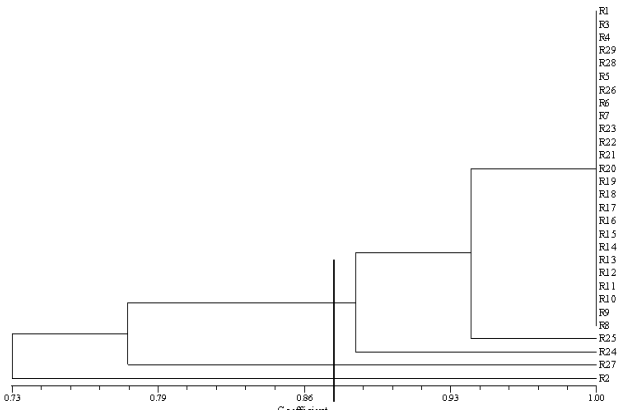

Fig. 3 Dendrogram of Substitution lines based on Commassie blue staining

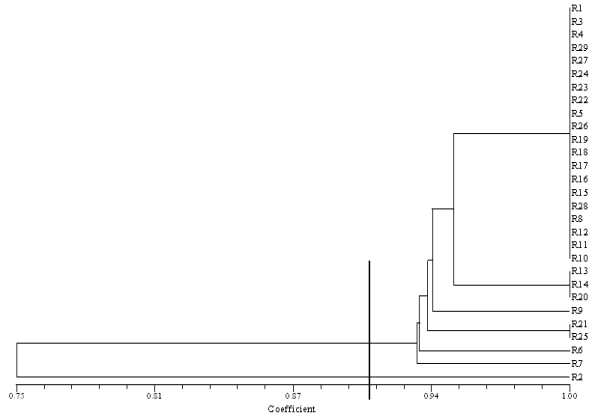

Fig. 4 Dendrogram of Substitution lines based on Silver nitrate staining

Table I- Names of used substitution lines

No	Name	No	Name	No	Name
1	Kapla 1A	**11**	Kapla 7B	**21**	Shayen 7B
2	Kapla 3A	**12**	Kapla 2D	**22**	Shayen 2B
3	Kapla 4A	**13**	Kapla 3D	**23**	Shayen 5B
4	Kapla 5A	**14**	Kapla 4D	**24**	Shayen 7A
5	Kapla 6B	**15**	Kapla 5D	**25**	Shayen 4D
6	Kapla 7A	**16**	Kapla 6D	**26**	Shayen 3A
7	Kapla 1B	**17**	Kapla 7D	**27**	Shayen 4B
8	Kapla 3B	**18**	Kapla (Parent)	**28**	Shayen 6A
9	Kapla 4B	**19**	Chinese spring	**29**	Shayen 3D
10	Kapla 5B	**20**	Shayen 1B		

Table II- Components of stacking gel 4% (Right) and resolving gel 10% (Left)

Volume	Solution	Volume	Solution
5.5 ml	Acrylamide	38 ml	Acrylamide
0.2 ml	SDS 10%	0.7 ml	SDS 10%
2.6 ml	Tris-Hcl, pH=6.8	25 ml	Tris-Hcl, pH=8.8
1.5 ml	Ammonium persulphate	1.6 ml	Ammonium persulphate
20 µl	TEMED	30 µl	TEMED
15 ml	dd H_2O	15 ml	dd H_2O

Table III- Cophenetic's correlation coefficient for Commassie blue staining method

	UPGMA	SINGLE	WPGMA
Jaccard	0.992	0.986	0.983
DICE	0.988	0.982	0.976
SM	0.990	0.982	0.979

Table IV- Cophenetic's correlation coefficient for Silver nitrate staining method

	UPGMA	SINGLE	WPGMA
Jaccard	0.979	0.976	0.966
DICE	0.978	0.975	0.967
SM	0.978	0.977	0.958

Table V- Payne's Scoring for HMW-GS subunits in substitution lines

Lines	Subunits	Score
Kapla 1A	Null , 7+8 , 2+12	6
Kapla 3A	2* , 7+8 , 2+10 , 2+12	9
Kapla 4A	Null , 7+8 , 2+12	6
Kapla 5A	Null , 7+8 , 2+12	6
Kapla 6B	Null , 7+8 , 2+12	6
Kapla 7A	Null , 7+8 , 2+12	6
Kapla 1B	Null , 7+8 , 2+12	6
Kapla 3B	Null , 7+8 , 2+12	6
Kapla 4B	Null , 7+8 , 2+12	6
Kapla 5B	Null , 7+8 , 2+12	6
Kapla 7B	Null , 7+8 , 2+12	6
Kapla 2D	Null , 7+8 , 2+12	6
Kapla 3D	Null , 7+8 , 2+12	6
Kapla 4D	Null , 7+8 , 2+12	6
Kapla 5D	Null , 7+8 , 2+12	6
Kapla 6D	Null , 7+8 , 2+12	6
Kapla 7D	Null , 7+8 , 2+12	6
Kapla (Parent)	Null , 7+8 , 2+12	6
Chinese spring	Null , 7+8 , 2+12	6
Shayen 1B	Null , 7+8 , 2+12	6
Shayen 7B	Null , 7+8 , 2+12	4
Shayen 2B	Null , 7+8 , 2+12	6
Shayen 5B	Null , 7+8 , 2+12	6
Shayen 7A	Null , 7+8 , 7+9 , 3+12	8
Shayen 4D	Null , 7 , 2+12	4
Shayen 3A	Null , 7+8 , 2+12	6
Shayen 4B	Null , 7+8 , 7+9 , 3+12	8
Shayen 6A	Null , 7+8 , 2+12	6
Shayen 3D	Null , 7+8 , 2+12	6

CONCLUSIONS

Seven HMW-GS subunits were determined between substitutions lines. These subunits are 2* in locus of GLU-A1, subunits of 7, 7+8, 7+9 in locus of GLU-B1 and subunits of 2+10, 2+12, 3+12 in locus of GLU-D1.

Each of lines had 2-4 different subunits. Most of lines had 2 subunits, but Kapla 3A had 4 subunits. Shayen 7A and Shayen 4B had 3 subunits (TableV). Then only Kapla 3A had

4 and 2* subunits; but other lines didn't have any subunits in chromosomes of A genome (Null).

Kapla 3A also has only 2+10 subunit and other lines have 2+12 and 3+12 subunits instead of 2+10 subunit.

2+12 and 3+12 subunits have negative effects on bread making quality of wheat, but 2* subunit has positive effect on bread making quality; Thus Kapla 3A has the highest bread making quality between these substitution lines.

Subunits of Kapla 3A differ from other substitution lines.

Kapla 3A has 2*, 7+9 and 2+10 subunits, but other substitution lines didn't have these subunits (Table V).

Then substitution of 3A chromosome is responsible of these variations between substitution lines.

In substitution lines of Shayen cultivar, Shayen 3A and Shayen 4B had 7+9 and 3+12 subunits, but other substitution lines of this cultivar didn't have these subunits.

Shayen 4D and Shayen 7B have 7 subunits, but other substitution lines of Shayen didn't have this subunit. Then substitution of 7B and 4D chromosomes is responsible for 7 subunits. Substitution of 7A and 4B had variation in 7+9 and 3+12 subunits.

We scored value of bread making quality based on Payne method [15].Only Kapla 3A was known as strong cultivar in bread making quality. Other substitution lines were recognized as poor cultivars in bread making quality except Shayen 7A and Shayen 4B (Table V).

IV. Discussions

Payne *et al* (1983) could determine 20 different subunits in 185 cultivars of wheat.

Subunits related to chromosomes of B genome had the highest diversity. They recognized 7 different subunits between lines and 2-4 subunits for each line [12].

They determined that 1A, 1B, 1D and 7B chromosomes are effective on baking quality and 3A, 7A and 4B chromosomes have important role in increasing of bread making quality [12].

Deng *et al* (2005) studied effect of HMW-GS on baking quality in Chinese spring using isogonics lines and SDS-PAGE. They find no significant differences in protein combinations of seed [5]. They studied effect of HMW-GS on baking quality of wheat in advanced lines of Chinese spring [5].

V. Propositions

A- Studying of other components of proteins effective on bread making quality of wheat.

B- Studying of other alleles effective on bread making quality using DNA markers (for example STS marker).

C- Application of advanced mutagenesis methods in order to improvement of alleles in 7B and 4D chromosomes.

D- Transfer of alleles on 3A, 7A and 4D chromosomes to other lines

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