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Full Length Research Paper

Toward a molecular evaluation of grain quality using glutenin subunits in *Triticum carthlicum*

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The grain quality of tetraploid wheat, Triticum *carthlicum* was analyzed by examining seed storage glutenin (HMW) markers at Glu-A1, Glu-B1 and Glu-D1 loci by SDS-PAGE method, as well as confirmation by Zeleny and Farinograph tests. 15 populations were collected from Iran, some areas of the Middle East and the North of Africa with this assumption that this species has originated from the Middle East region. A high rate of electrophoretic polymorphism and a close relationship between molecular markers, protein content and quality were detected at the studied loci. Presence of 5+10 bands indicate high protein content and excellent quality, while the null band shows low protein and bakery quality. Populations were classified into four groups from excellent (4th Class) to low (1st Class) in the quality status. A total value of classes (TVC) for each country was calculated by adding up values of each country as a main index for quality comparisons. Iranian populations of *T. carthlicum* showed the highest value of quality (TVC= 9) between all populations. All of the Egyptian, Syrian and Iraqi accessions were classified in the second rank (TVC= 6) and the Turkish samples the lowest (TVC= 3). This study suggests that the glutenin (HMW) markers can still be used as a powerful and reliable tool for identification and prediction of important traits like grain quality.

Key words: Alleles, glutenin, markers, SDS-PAGE, variation, wheat.

INTRODUCTION

Although in the recent years the world yield of wheat has increased remarkably, it is necessary to continue genetics and plant breeding programs to raise yield because of the growing population. Moreover, grain quality (bakery quality) improvement is currently being targeted by breeders. Exploration of new alleles and the genes which may affect bakery quality are necessary to prevent genetic erosion. The study of seed storage proteins in the various crops and particularly in wheat has been utilized as powerful markers (Gepts, 1990).

Gluten composition is the main factor that determines the quality characteristics of wheat cultivars (Du Cros, 1987; Kovacs et al., 1993). Two types of gluten protein, gliadin and glutenin (prolamins), have been studied to establish their relationships with pasta quality (Carrillo et al., 2004). Although the relationships between seed storage proteins and important traits in the crops has not been still verified clearly, there are some distinct evidences of linkage between glutenin alleles and quantitative traits like grain quality (Felsnburg et al., 1991; Ciaffi et al., 1993; Levy and Feldman, 1988; Nevo and Payne, 1987).This study, attempts to assess and classify various populations of a *Triticum* wild species based on seed storage proteins by electrophoresis method. Polymorphisms in the glutenin subunits then will be used to determine grain quality**.**

Abbreviations: HMW, High molecular weight; TVC, Total value of classes for each country.

MATERIALS AND METHODS

Seeds of fifteen populations of *T. carthlicum* were collected from some regions of Iran, Middle East countries and North of Africa

Table 1. Correlation between distinguishable bands of glutenin subunits and quality status.

Class	Quality status	Subunits number reffered to Chromosomes		
		1A	1B	1D
4	Excellent	------	------	5+10
3	High	1 and 2	17+18 and 7+8	------
2	Medium	------	7+9	2+12 & 3+12
1	Low	Null	7 and 6+8	4+12

Table 2. Geographical situation and collecting zones of *T. carthlicum.*

Population Number	Country	Zone (City)	Longitude	Latitude	Altitude	Quality Class	Total Value
1	Iran	Noorabad	48.30	33.42	1246	4	Iran
2	Iran	Marivan	46.16	34.50	1200	3	9
3	Iran	Dehloran	42.6	34.5	1850	2	
4	Turkey	20KmN	27.12	38.22	30	1	Turkey
5	Turkey	28KmN	38.20	38.27	650	1	3
6	Turkey	9KmSE	35.59	38.37	1200	1	
7	Egypt	Cairo	31.23	30.22	158	2	Egypt
8	Egypt	Cairo	31.32	30.26	150	2	6
9	Egypt	Aswan	32.54	23.38	212	2	
10	Syria	Deiraljamal	37.00	36.25	580	1	Syria
11	Syria	Rawada	36.00	33.36	1320	4	6
12	Syria	Ratieyeh	36.02	33.34	1302	1	
13	Iraq	20KmE	43.25	36.23	470	1	Iraq
14	Iraq	Jabelsingar	41.47	36.20	1200	1	6
15	Iraq	Imadira	41.51	36.21	1000	4	

(Table1). The grain quality and bakery ranking of these populations were assessed using polymorphisms of high molecular weight (HMW) components such as glutenin subunits (Abdemishani and Najafian 1994). The procedure was conducted in three steps:

A) Discarding gliadin subunits: *S*eeds without embryo were ground in the mortar and then the flour immersed in 1.5 M dimethylformamide (DMF) for about 1 h at room temperature and then centrifuged for 10 min. All gliadin subunits were removed from the upper section of tube.

B) Removing interfering subunits with glutenins: Remainder of samples were suspended in 0.125 M Tris-HCl buffer (pH 6.8) which contained 1% SDS and after centrifuging, the interfering subunits were discarded out of the upper part of tube.

C) Extraction of glutenin subunits and separation on the SDS-PAGE: Sediments from previous stage which contained glutenin subunits were suspended in 0.5 M Tris-HCl buffer (pH 6.8) comprising 2.75% SDS and 2% DTT (dithioteritol) for about 1 h at 60°C on water bath and after centrifugation the upper liquid containing glutenin subunits (HMW) were separated on SDS-PAGE electrophoresis according to Payne method with slight modification and then analyzed (Payne et al., 1981, 1987). Some standard cultivars (Marquis and Chinese Spring) and catalogue of alleles for the complex gene loci Glu-A1, Glu-B1 and Glu-D1 (Payne et al., 1983) were applied to identify unknown loci bands and further comparisons. The total number of distinguishable bands at each lane were counted and numbered from loading place (wells). The examination was completed with the measurement of protein percentage (%) and then bakery quality was double checked by Zeleny test and Farinograph examination to distinguish the relationship between the number of bands and the bakery ranking (Abdemeshani and Najafian, 1994).

RESULTS AND DISCUSSION

Study showed high rate of polymorphism in high molecular weight (HMW) components of glutenin subunits on Glu-A1, Glu-B1 and presumably Glu-D1 loci as well as correlation between presence of some bands and protein percentage (%). All samples with 5+10 bands have highest content of proteins and classified as an excellent (4^{th} Class) status. Although we suggest that the locus of these bands exist in the wheat D genome and is so called Glu-D1.

Strong positive correlations between 1+2, 17+18, and

Table 3. Percentage of protein content (%) and classification based on Zeleny and Farinograph tests.

Status	Percentage of protein amount %
Excellent	20 and up
High	16-20
Medium	12-16
Low	8-12

7+8 bands, and high content of proteins also, were observed (3rd Class). All accessions with 7+9, 2+12 and 3+12 were ranked as medium quality (2nd Class) and finally, samples with Null or 7 alone or 6+8 and 4+12 bands were classified as low as protein content populations (1st Class) (Table 2). First Iranian population showed 5+10 bands and therefore was ranked 4th with the excellent quality. But 2 and 3 Iranian populations were valued in the 3rd and 2nd class with high and medium quality, respectively. Population number 11 from Syria and 15 from Iraq, also showed 5+10 bands and were classified in the 4th class. All of Egyptian populations (7, 8, 9) were classified in the 2nd class medium quality. All other samples were categorized at 1st class with low protein content and bakery value (Table 2). Zeleny and Farinograph tests also confirmed the electrophoretic profiles. The percentage of protein content and their classification are shown in Table 3.

A general comparison between countries was made by a total value of quality classes TVC which was carried out by adding up quality values of each country separately and then comparing with one another. The total TVC value for Iranian populations of T. carthlicum was nine. All Egyptian samples showed medium quality and a total value of 6 (see the right hand column of Table 2). The Turkish populations were classified as low quality samples and total value of three. The genes responsible for bands 5 and 10 have been likely situated on the D1 chromosome and therefore the locus should be named Glu-D1. Although, we suppose which this species is a tetraploid plant and it should not possess the D genome. But it may be possible that the selected samples may be hexaploid and they should be comprised of the D genome and Glu-D1 locus or there is a new locus in this species which will be able to control the glutenin level. Thus, it is necessary to do cytogenetics and more molecular studies to confirm or reject this hypothesis. There are also other loci which may control and affect the total protein percentage such as low M_r glutenin subunits at the Glu-A3, Glu-B2 and Glu-B3 loci (Carrillo et al., 2004; Jackson et al., 1983; Singh and Shephered, 1988; Singh et al., 1991). It is also possible that low molecular weight subunits which have been detected are more responsible for difference in quality of wheat grain (Payne et al.,1984; Ponga et al., 1988; Ruiz and Carrillo, 1995a, b).

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