*Quest Journals*
*Journal of Research in Agriculture and Animal Science*
*Volume 4 ~ Issue 10 (2016) pp: 10-13*
*ISSN(Online) : 2321-9459*
www.questjournals.org

**Research Paper**

# Variability of HMW And LMW Glutenin Subunits in Durum Wheat (*Triticum Durum* Desf.)

Ayed Sourour[1], Bechrif Salah[2], Othmani Afef[1], Chamekh Zoubeir[3], Ben Younes Mongi[1]

[1]*University Of Carthage, Regional Research Development Office Of Agriculture In Semi Arid North West Of Kef, Boulifa 7100-Kef, Tunisia*
[2]*University Of Carthage, National Agricultural Research Institute Of Tunisia, Rue Hédi Karray 2049 Ariana, Tunisia*
[3]*Genetic And Plant Breeding Laboratory, Department Of Agronomy And Biotechnology. National Agronomic Institute Of Tunisia*

**R**eceived; 06 October 2016 Accepted; 21 November 2016; 

***Abstract:*** *The variability of high and low molecular weight glutenin (HMW and LMW) of 12 tunisians varieties (3 modern varieties and 9 landraces) was investigated using SDS-PAGE electrophoresis. Results showed significant differences between genotypes in protein compostion. Three alleles were found at Glu-B1 locus, being 6 + 8 and 7 + 8 the most frequent (66.66 and 16.66%, respectively). For LMW subunits LMW1 and LMW 2 were the most frequent (33.33 and 66.66 %, respectively). The slowest and widest band of patterns LMW type 2 was the most important determinant influencing positively the strength of gluten. Genotypes Mahmoudi and Azizi, present 6+8 HMW subunit and the LMW-2 type of SDS-PAGE pattern, showed the best quality. These varieties were good sources of favorable glutenin subunits that would be desirable in breeding programs.*
***Keywords****: Bands, quality, protein composition*

## I. INTRODUCTION

Durum wheat breeding programs in the major North Africa countries are focusing on quality requirements. The selection from landraces and varieties with traditionally superior quality is important for increase genetic variability and improving new genotypes. Gluten proteins namely gliadins and glutenins (80% of the total protein) are the major storage protein fraction in wheat grain. It presents an important role in dough properties and in bread making quality in various wheat varieties. It has been suggested that the gliadins generally contribute to dough viscosity and glutenins contribute to dough elasticity (Shewry et al., 1995). Glutenin may be composed of either high molecular weight (HMW) or low molecular weight (LMW) subunits (Ahmad, 2000). The distinction of LMW and HMW has been based on their mobility on Sodium Dodecyl Sulphate PolyAcrylamide Gel Electrophoresis (SDS-PAGE) (Zhen and Mares 1992). HMW subunits are encoded at the Glu-1 loci located on the long arms of chromosomes 1A, 1B, 1D and LMW subunits are mostly encoded by genes located at the *Glu-3* loci (Shewry et al., 1992; D'Ovidio et al., 1999). Pasta-making quality of the semolina of durum wheat cultivars is strongly correlated with the presence of LMW-2 type relates with superior quality characteristics and the LMW-1 type have a poor pasta-making quality of the semolina of durum wheat cultivars (Pogna et al.,1990). The LMW-2 and LMW-1 differ mainly by the presence in the LMW-2 group SDS-PAGE pattern of a band with molecular weight 42.000 (42 K subunit). It is the largest protein in the group and is absent from the LMW-1 type of pattern. This 42 K subunit is coded on chromosome 1B at the *Glu-B3* locus (Ruiz and Carrillo, 1995). In the present study, electrophoretic patterns of HMW and LMW glutenin subunits were examined for 12 tunisian genotypes.

## II. Material And Methods

### 2.1 Plant materials

Twelve Tunisian varieties**:** 3 modern varieties (Nasr, Selim, Maali) and 9 landraces (Bidi, Agili, Chili, Azizi, Mahmoudi, Hmira, Ward Bled, Souri and Derbassi) were analysed by SDS-PAGE according to Lammeli (1970)

*Corresponding Author: Ayed Sourour[1],
[1]*University Of Carthage, Regional Research Development Office Of Agriculture In Semi Arid North West Of Kef, Boulifa 7100-Kef, Tunisia*

method. All the durum wheat accessions analyzed in this work was obtained from National Agronomic Institute of Tunisia.

### 2.2 SDS PAGE electrophoresis

The 12 durum wheat accessions were examined for their prolamins composition (low-molecular-weight and high molecular- weight glutenin subunits). The seeds were crushed finely and the flour was mixed in an extraction buffer of 1% Tris Base (hydroxymethyl aminomethane) (pH 6.8), 1% Sodium Dodecyl Sulfate (SDS), 0.03% bromophenol blue and 10% mercaptoethanol and 15% Glycérol. Samples were boiled for 2 min at 100°C and then centrifuged for 15 min at 10000 rpm before being fractionated by SDS-PAGE. According to Lammeli (1970) method, a separating gel containing 30% acrylamide, 1% Bis acrylamide, 10% SDS and 1.5 M Tris (pH = 8.8), and stacking gel containing 30% acrylamide, 1% Bis acrylamide, 10% SDS and 0.5 M Tris-Hcl (pH 6.8) were used. The two gels were polymerised in the presence of Tetramethylethylenediamine (TEMED) and 10% ammonium peroxodisulfate. After denaturation, glutenin subunits were fractionated by SDS-PAGE slabs in a discontinuous system. Gels were stained overnight with 25% Methanol, 10% Acetic acid glacial, 65% water and 0.1% comassie Brilliant Blue R. De-staining was carried out overnight in tap water.

## III. Results

SDS-PAGE of grain proteins was performed to determine composition of the gluten protein fractions in order to investigate genetic diversity among durum wheat genotypes. The electrophoretic separation of glutenins components of the durum wheat cultivars (‘Karim’, ‘Cocorit’ and ‘Kyperounda’) used as controls is presented in Figure 1. The cultivar ‘Kyperounda’ has HMW-GS of 20x + 20y, and LMW-GS of type 2 (LMW2); and the cultivar ‘Karim’ has HMW-GS of 7 + 8, and LMW-GS of type1 (LMW1). The cultivar Cocorit has HMW-GS of 6 + 8 and LMW-GS of type2 (LMW2). The allelic composition of glutenins subunits for each accession is given in the Table 2. Electrophoregram showing protein banding pattern of different durum wheat varieties are presented in figure 1. The presence or absence of bands is mentioned in Table 1.

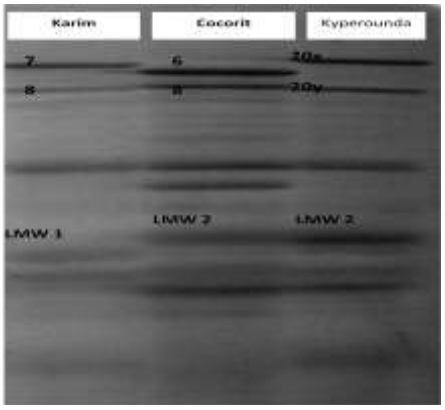


**Figure 1:** SDS-PAGE pattern of durum wheat cultivars: Karim, Cocorit and ‘Kyperounda’

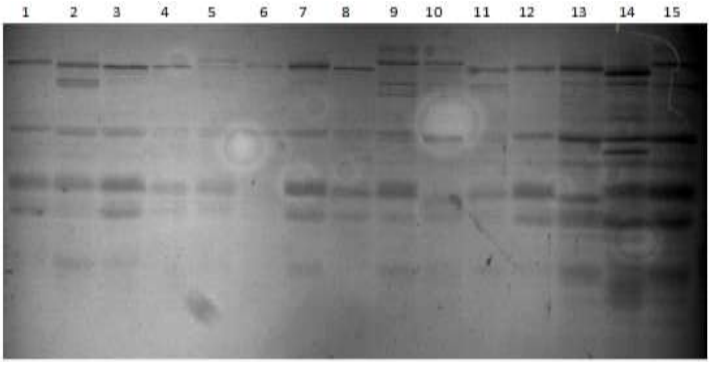


**Figure 2:** SDS-PAGE fractionation of total endosperm protein from different durum wheat genotypes, (1) Nasr, (2) Bidi, (3) Agili, (4) Chili, (5) Azizi, (6) Salim, (7) Maali, (8) Mahmoudi, (9) Hmira, (10) Ward bled, (11) Souri, (12) Derbassi, (13) Karim, (14) Cocorit et (15) Kyperounda

**Table 1:** Matrix of presence or absence HMW-GS and LMW-GS bands of different durum wheat genotypes

| Varieties | HMW-GS and LMW-GS bands | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **20x** | **20y** | **6** | **7** | **8** | **12** | **LM1** | **LM2** |
| 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 |
| 2 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 |
| 3 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 |
| 4 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 |
| 5 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 |
| 6 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 |
| 7 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 |
| 8 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 |
| 9 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| 10 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 |
| 11 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 |
| 12 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 |

The allelic composition of glutenins subunits for each accession is given in the Table 2. Three different alleles were detected at Glu-A1 and 2 at Glu-B1, which are responsible for the high molecular weight glutenin subunits (Table 2). The allele was found at Glu-B1 locus, being 7 + 8 the most frequent with 66.66 %. In this study, HMW and LMW glutenin loci showed high variability for allelic composition. For LMW subunits LMW1 and LMW2 were the most frequent (33.33 and 66.66 %, respectively) (Table 1).

**Table 2:** Allelic diversity of HMW-GS of durum wheat genotypes studied

| Locus | Allele | Sous-unité | Number of Materiel | Frequence (%) |
|---|---|---|---|---|
| Glu- A1 | A | 1 | 0 | 0.00 |
| | B | 2 | 0 | 0.00 |
| | C | Null | 12 | 100.00 |
| Glu- B1 | B | 7+8 | 3 | 66.66 |
| | D | 6+8 | 2 | 16.66 |
| | E | 20 | 0 | 16.66 |

## IV. DISCUSSION

The 12 durum wheat genotypes used showed various banding pattern. Results showed that a large variability in patterns of glutenins was found among the 12 durum wheat genotypes analyzed by SDS-PAGE. Because of the relatively important size of the HMW subunits of glutenin, they appear well separated from all other low molecular weight polypeptides in the HMW region of the profiles. According to results there were significant differences between genotypes in protein compostion. Our results are in agreement with the genetic variability reported for durum wheat (Carrillo, 1991). However, other studies showed low degree of diversity between wheat genotypes tested (Mohd et al., 2007; Siddiqui and Naz, 2009). This low level of genetic diversity may be attributed to the low number of varieties used.

The landraces Azizi and Mahmoudi showed the presence of (6+8) subunits. This HMW-GS associated to good quality better than 7+8. In contrast, Tahir (2008) found that 6+8 was inferior to 7+8. According to Sissons et al. (2005), 6+8 and 7+8 are not different, but both have a significantly higher gluten index than subunit 20 (6+8= 7+8>20). These results are in concordance with the results obtained by Kaan et al. (1993).

In this study, the majority of material tested presents the LMW2 type of SDS-PAGE pattern. In fact, the 42 K LMW-GS typical of LMW2 patterns was found in the patterns of 66.66 % of durum wheat varieties analysed by SDS-PAGE. The slowest and widest band of patterns type 2 (LMW 2) was the most important determinant influencing positively the strength of gluten. According Masci et al. (2000), good quality durum wheats usually related to the presence of the LMW2 type in SDS-PAGE pattern, whereas the LMW1 type of pattern is usually correlated with poor quality of durum wheats.

The wide variation in gluten strength suggests that the presence of a particular allelic pattern is an indicator of quality performance. Variations in gluten HMW-GS provide markers of gluten strength with the exception of HMW-GS 20.

## V. CONCLUSION

SDS-PAGE is widely used due to its simplicity and effectiveness for describing the genetic structure of durum wheat germplasm. Based on the storage protein composition, the variety Mahmoudi and Azizi appears to have good quality, containing 6+8 HMW and LMW type 2. These genotypes could be source of quality for breeding programs for improving pasta quality.

## REFERENCES


[1]. Shewry PR, Tatham AS, Barro F, Barcelo P, Lazzeri P (1995) Biotechnology of breadmaking: unraveling and manipulating the multi-protein gluten complex. Biotechnology 13 : 1185-1190

[2]. Ahmad, M., 2000. Molecular marker-assisted selection of HMW glutenin alleles related to wheat bread quality by PCR-generated DNA markers. Theoretical and Applied Genetics 101, 892–896.

[3]. Zhen Z, Mares D (1992) A simple extraction and one-step SDSPAGE system for separating HMW and LMW glutenin subunits of wheat and high molecular weight proteins of rye. J Cereal Sci 15:63–78.

[4]. Shewry PR, Halford NG, Tatham AS (1992) J Cereal Sci 15:105–120

[5]. D'Ovidio R, Marchitelli C, Ercoli Cardelli L, Porceddu E (1999) Sequence similarity between allelic *Glu-B3* genes related to quality properties of durum wheat. Theor Appl Genet 98: 455–461

[6]. Pogna N.E., Autran J.C., Mellini F., La Fiandra D. and Feillet P. (1990). J. Ceral Sci. 11: 15-34

[7]. Ruiz M., Carrillo JM (1995) Separate effects on gluten strength of Gli-1 and Gli-3 prolamin genes on chromosomes 1A and 1B in durum wheat. J cereal Sci 21 : 137-144

[8]. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 277: 680-685.

[9]. Carrillo JM, Vazquez JF, Ruiz M and Albuquerque MM (1991) Relationships between gluten strength and gluten components in Spanish durum wheat landraces. In: Bushuk, W., Tkachuk, R. (Eds.), Gluten Proteins. AACC, St Paul, MN, 268–277.

[10]. Mohd S, Alam Z, Zahir A, Waqar A, Taufiq A, Ikhtiar K (2007). Characterization of wheat varieties by seed storage protein electrophoresis. Afr. J. Biotechnol. 6: 497-500.

[11]. Siddiqui MF, Naz N (2009). Protein landmarks for diversity assessment in wheat genotypes. Afr. J. Biotechnol. 8: 1855-1859.

[12]. Tahir NAR. (2008) Characterization and glutenin diversity in tetraploid wheat varieties in Sulaimanyah by wheat storage proteins. African J. Biotech. 7: 4031-4036

[13]. Sissons MJ, Ames NP, Hare RA, Clarke JM. (2005) Relationship between glutenin subunit composition and gluten strength measurements in durum wheat. J. Sci. Food Agr. 85: 2445-2452

[14]. Kaan F., Branlard G., Chihab B., BorrieS C., Monneveux P., 1993. Relations between genes coding for grain storage protein and two pasta cooking quality criteria among durum wheat (T. durum Desf.) genetic resources. J Genet Breed 47, 151-156.

[15]. Carrillo J.M. 1995. Variability for glutenin proteins in Spanish durum wheat landraces. Options Mediterraneennes Serie A, Seminaires Mediterraneens, pp. 143–147.

[16]. Masci S., D'Ovidio R., Lafiandra D., Kasarda D.D. (2000) A 1B-coded low-molecular-weight glutenin subunit associated with quality in durum wheats shows strong similarity to a subunit present in some bread wheat cultivars Theor Appl Genet 100:396–400