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Effect of gliadin and glutenin components on gluten strength in durum wheat

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SUMMARY - Electrophoretical analysis of seed storage proteins in a durum wheat collection has allowed identification of a landrace in which intralocus recombination at the Gli-B1 locus is present. Such a process has combined gamma gliadin component 45 with low molecular weight glutenin subunits LMW-1. Qualitative data have confirmed the positive functional relationship between low molecular weight glutenin subunits and gluten viscoelastic properties and that the gliadins gamma-42 and 45 are only genetic markers for technological properties in durum wheat.

Key words: *Triticum durum*, gliadins, glutenins, recombination, quality.

RESUME *- "Effet des composantes gliadine et gluténine sur la force du gluten chez le blé dur". L'analyse électrophorétique du contenu protéique qui se trouve dans le grain de blé dur a permis l'identification d'une population avec une recombinaison dans le Gli-B1. Cette procédure a été combinée avec la bande gliadine 45 et LMW-1. Le résultat de la qualité a confirmé qu'il y a une relation fonctionnelle positive entre le LMW et la propriété viscoélastique du gluten, et que les bandes 42 et 45 sont seulement des marqueurs génétiques pour la qualité chez le blé dur.*

Mots-clés : *Triticum durum, qualité, recombinaison, gliadines, gluténines.*

Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is an important food crop in the Mediterranean basin, not only because of its large acreage but also for its importance in the human diet (Williams *et al.*, 1984). In these regions besides for pasta a high proportion of durum wheat is used for making bread, burghul, couscous and frekeh. Several breeding programs at national and international level are emphasizing their activity not only towards aspects concerning the productivity, but also to the grain quality of durum wheat. It is now well recognised that characteristics of durum wheat that are related to end product quality are associated with medium to high protein content and protein components. In the last twenty years many scientists and researchers have focused their studies on the seed storage proteins, even if the first classification of these tissue-specific proteins was made by Osborne (1907), due to the increasing evidences of their impact on technological properties both in durum and bread wheat.

Gliadin and glutenin are the major seed storage protein fractions of durum endosperm, and relationship with viscoelastic properties of pasta have been found. Gliadins are monomeric protein molecules which have been subdivided into four groups termed: alpha, beta, gamma, omega according

to their decreasing mobility when separated on polyacrylamide gels at acidic pH (A-PAGE). The genes encoding gliadin components are located on the short arm of chromosomes of the homologous groups 1 and 6 of A and B genomes (Joppa *et al.*, 1983).

Genes coding for alpha and beta gliadins are clustered at homologous loci named *Gli-A2* and *Gli-B2* on the short arms of chromosomes of group 6; most of the genes coding for gamma and omega gliadins are clustered at homologous loci named *Gli-A1* and *Gli-B1* on the short arms of the chromosomes 1A and 1B. Glutenins contain different polypeptides connected by intermolecular disulphide bonds, the polypeptides are called subunits and are subdivided into low-molecular-weight (LMW) and high-molecular-weight (HMW) according to their molecular weight when separated on sodium dodecyl sulphate (SDS) PAGE. The genes coding for HMW glutenin subunits are located on the long arm of chromosomes 1A and 1B at the *Glu-1* loci, whereas the LMW glutenin subunits are controlled by genes tightly linked to *Gli-1* loci at the *Glu-3* loci (Jackson *et al.*, 1983).

A strong relationship between the presence of certain seed storage protein components and gluten strength of durum wheat was first reported by Damidaux *et al.* (1978), who found that durum wheat cultivars possessing the gamma gliadin component designated 45, according to its relative mobility in A-PAGE (Bushuk and Zillman, 1978), exhibited a stronger gluten, compared to cultivars possessing the gamma gliadin component 42. The two gliadins were found to be encoded by two codominants alleles of a single gene on chromosome 1B (Damidaux *et al.*, 1984). It has also been shown that each of these two components belongs to a more complex group of proteins, whose genes are tightly linked, comprising omega gliadins and low molecular weight glutenin subunits, usually the gamma gliadin 45 is associated with the omega component 35 and a group of low molecular weight glutenin subunits termed LMW-2, while gamma 42 is associated with the omega components 33, 35 and 38 and LMW-1 glutenin subunits (Payne *et al.*, 1984). According to Payne *et al.* (1984) LMW glutenin subunits are the actual cause of qualitative differences in durum wheat with band 42 and 45 being only genetic markers. That LMW glutenin subunits are the actual cause of qualitative differences was established by Pogna *et al.* (1988) using the durum wheat cultivar Berillo in which an intralocus recombination at the *Gli-B1* locus was reported by Margiotta *et al.* (1987).

Another rare case of recombination has been discovered during the evaluation of the durum wheat collection maintained at ICARDA, giving the possibility to further confirm the role of LMW glutenin subunits in determining differences in gluten viscoelatic properties.

Materials and methods

The durum wheat landrace Australian Poulard, along with the durum wheat cultivars Belikh-2, Stork, Omrabi-5, Korifla, Waha, Akbash, Rozzi and Haurani Ayobieh were used in present study. Gliadin proteins were extracted from single seed with 1.5M dimethylformamide (DMF) and fractionated at pH 3.1 in aluminium lactate buffer by polyacrylamide-gel electrophoresis (A-PAGE) according to Khan *et al.* (1985) with minor modifications. Total seed storage proteins were extracted and fractionated by PAGE in sodium dodecyl sulphate (SDS-PAGE) according to the procedure described by Ciaffi *et al.* (1993).

Same genotypes were also analyzed for protein content, thousand kernel weight, vitreousness, flour pigment, SDS-sedimentation test, and SDS-sedimentation index (SDSi).

Results and discussion

Electrophoretic separation of gliadin components present in the durum wheat material used is reported in Fig. 1. Durum wheat cultivars Omrabi-5, Korifla, Belikh-2 and Stork possess the gamma gliadin component designated 45 and the linked omega gliadin 35, whereas cultivar Akbash, Haurani Ayobieh, Waha and Rozzi, possess the gamma gliadin 42 with the associated triplet of omega components 33-35-38. Landrace Australian Poulard, present on the same gel, shows a different electrophoretic patterns, in fact though band 45 is present, differently from the 45-type durum wheats, the triplet of omega components usually found associated with component 42 are present.

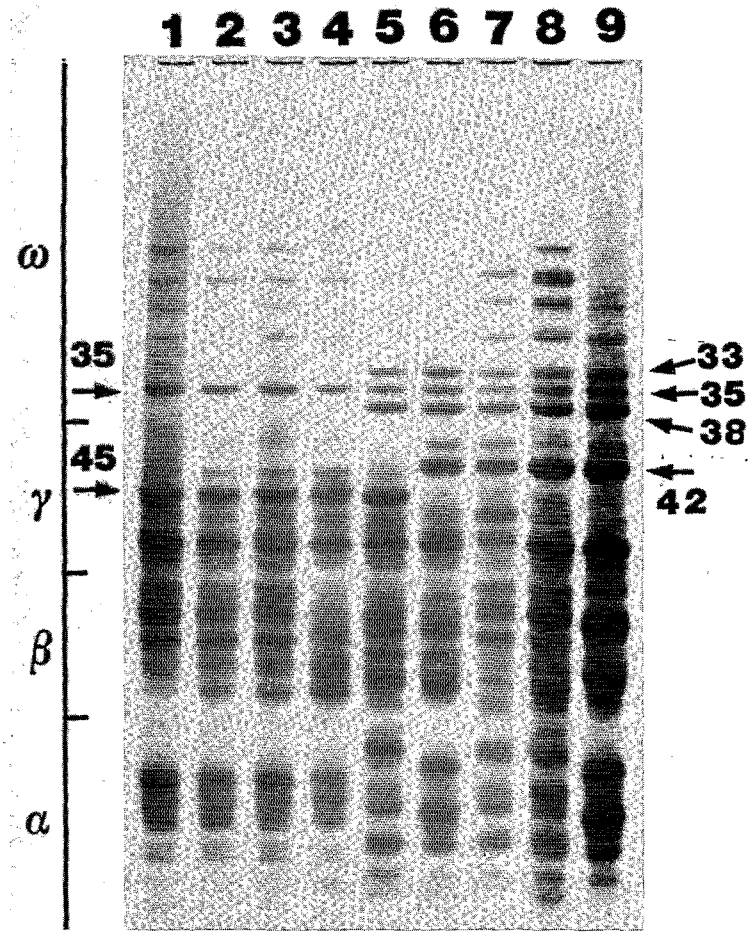

Fig. 1. One dimensional electrophoretic separation (A-PAGE) of gliadins. 1) Omrabi-5, 2) Korifla, 3) Belikh-2, 4) Stork, 5) Australian Poulard, 6) Akbash, 7) Haurani Ayobieh, 8) Waha and 9) Rozzi.

SDS-PAGE analyses of total proteins on the same genotypes (Fig. 2) indicated that all cultivars with gamma gliadin component 45 possess low-molecular weight glutenin subunits indicated as LMW-2, whereas low molecular weight glutenin subunits LMW-1 are present in those cultivars with gamma gliadin 42 (Fig. 2). Australian Poulard shows also the LMW-1 though contains the gamma gliadin component 45. This results indicates that an intralocus recombination has occurred at the *Gli-B1* locus in Australian Poulard resulting in the new combination omega-33-35-38/gamma-45/ LMW-1.

Quality traits determined on the same material are reported in Table 1. Australian Poulard produced the highest kernel weight, while Waha produced the lowest one. In contrast Waha showed the highest kernel protein content followed by Haurani Ayobieh and Australian Poulard. Most of the genotypes used in this study had high levels of vitreousness. As far as flour pigment concern the highest scores were showed by Akbash, Omrabi-5 and Rozzi and the lowest ones by Belikh-2 and Australian Poulard. The highest levels of SDS-sedimentation values and SDS-sedimentation index (SDSi) were found in Belikh-2 and Korifla, and the lowest ones in Australian Poulard and Akbash.

Cultivars have been grouped according to the type of LMW possessed, with Australian Poulard included in the group of genotypes possessing LMW-1, and mean values for all the traits were calculated (Table 2), differences were found to be significant only for SDS-sedimentation values and SDS-sedimentation index.

Results of the SDS-sedimentation test are in agreement with earlier findings indicating that quality differences in durum wheat is associated to the LMW glutenin subunits with genotypes possessing

LMW-1 being inferior to genotypes with LMW-2 (Pogna *et al.*, 1988). In fact Australian Poulard, which possesses gamma gliadin component 45 and LMW-1 has weak gluten strength further confirming the positive functional relationship between LMW-2 and gluten strength.

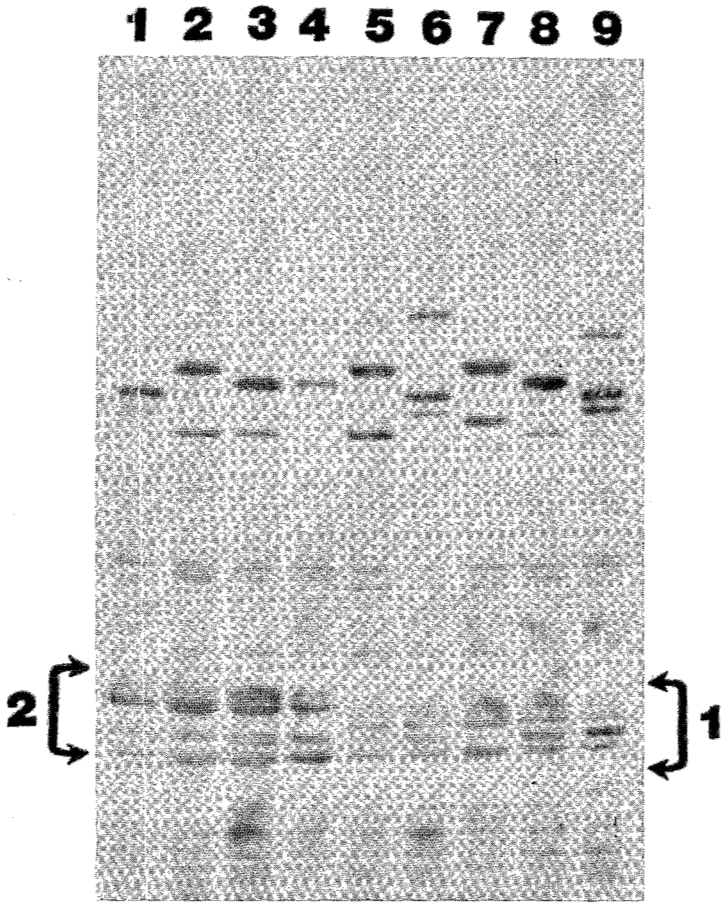

Fig. 2. SDS-PAGE separation of total proteins extracted from the same genotypes described in Fig. 1.

Table 1. Quality traits of cultivars used with different protein type at the Gli-B1/Glu-B3 loci

Variety	TKW	Prot.	Vitr.	Fl. Pig.	SDS	SDSi
Omrabi-5	42.68	12.33	99.67	6.67	28.83	2.33
Korifla	38.65	13.07	100.00	4.33	38.17	2.93
Belikh-2	40.58	13.17	100.00	3.67	39.50	2.98
Stork	44.60	12.60	99.00	5.17	30.17	2.34
Australian Poulard	51.42	14.00	99.00	3.50	19.58	1.39
Akbash	43.50	10.81	96.00	8.00	18.20	1.60
Haurani Ayobieh	46.90	14.30	99.80	5.50	27.00	1.90
Waha	34.48	15.20	100.00	6.33	23.67	1.54
Rozzi	39.80	12.87	99.00	6.50	23.00	1.80
LSD	5.72	1.13	1.35	1.19	6.97	0.36
CV%	7.77	4.98	0.79	12.48	14.61	9.99

TKW: Thousand kernel weight (g); Prot.: Protein content (%); Vitr.: Vitreousness (%); Fl. Pig.: Flour pigment (ppm); SDS: Sedimentation test (ml); SDSi: SDS (ml)/protein content (%)

Table 2. Comparison between cultivars with LMW-1 and LMW-2 for grain quality traits

Quality trait	LMW-2	LMW-1	Diff.
TKW	41.63	43.22	1.59 ns
Prot. cont.	12.79	13.44	0.65 ns
Vitreousness	99.67	98.76	0.91 ns
Flour pigment	4.96	5.97	1.01 ns
SDS	34.17	22.29	11.88*
SDSi	2.65	1.65	1.00**

*Differences significant at 1% and
**Differences significant at 0.1%
ns: non significant

Conclusions

Margiotta *et al.* (1987) first reported the presence of recombination at the *Gli-B1* locus in the durum wheat cultivar Berillo. In fact electrophoretic analyses indicated the presence of the gamma gliadin component 42, omega component 35 and LMW-2. Pogna *et al.* (1988) showed that gluten extracted from Berillo had high elastic recovery and gluten firmness similar to durum wheat cultivars with gamma gliadin 45 and LMW-2 glutenin subunits.

The detection of a different recombinant at the *Gli-B1* locus in the Egyptian landrace Australian Poulard has given us the opportunity to further confirm these results formulating the conclusion that differences in quality properties in durum wheat is a property conferred by low molecular weight (LMW) glutenin subunits, due to their greater ability to aggregate with gliadins being only genetic markers.

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