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Polymorphism of omega-gliadins in durum wheat as revealed by the two-step APAGE/SDS-PAGE technique

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Abstract. Polymorphism of omega-gliadins was studied in 243 durum wheats from 27 countries using the two-step one-dimensional APAGE/SDS-PAGE technique. A total of 12 bands of different mobility were observed, and four of them were found to be different from those previously detected by Khelifi et al. (1992) in bread wheat. Fifteen alleles, six coded by the *Gli-A1* locus and nine coded by the *Gli-B1* locus, were identified, accounting for 19 different electrophoretic patterns. Seven new alleles were detected: two at the *Gli-A1* locus and five at the *Gli-B1* locus. The polymorphism found at the *Gli-A1* and *Gli-B1* loci was slightly greater than that found in bread wheat. Allelic differences between both species were higher at the *Gli-B1* locus. A comparison of the frequencies of alleles in both species was carried out. The null allele, *Gli-A1e*, was more common in durum wheat than in bread wheat. The *Gli-B1b* allele, present in 60% of the bread wheats, was found in only 2% of the durum wheats and *Gli-B1e*, very common in durum wheat (45%), was rare in bread wheat (4%). The *Gli-B11V* allele, common in durum wheat (28%), was not detected in bread wheat.

Key words: Durum wheat – Omega-gliadins – Allelic diversity

Introduction

Gliadins are a highly heterogenous group of proteins. They are monomeric proteins which when fractionated by gel electrophoresis at low pH separate into four groups, alpha-, beta-, gamma- and omega-gliadins (Woychik et al. 1961). Gliadin synthesis in both bread (Shepherd 1968) and durum wheat (du Cros et al. 1983) is controlled by chromosomes of homoeologous groups 1 and 6 (the *Gli-1* and *Gli-2* loci, respectively, Payne et al. (1984). There is a high multiple allelism for gliadin-coding loci of both bread (Sozinov and Poperelya 1980) and durum wheat (Metakovsky et al. 1989).

The development of the two-step one-dimensional APAGE/SDS-PAGE procedure (Khelifi and Branlard 1991) has provided an alternative method to reveal the omega-gliadins (Branlard et al. 1992). In the second gel, omega-gliadin mobility lies between that of the high- and the low-molecular-weight subunits of glutenin (Khelifi et al. 1992). Thus, Khelifi et al. (1992) analyzed a collection of 193 bread wheats and described 12 distinct bands, three controlled by the *Gli-A1* locus, six by *Gli-B1* and three by *Gli-D1*. The close relationship between bread and durum wheat led to the suggestion that the same arrangement of the omega-gliadin-encoding genes occurs in durum wheat. The present work describes the omega-gliadin variation in a wide array of durum wheats and its possible chromosome control.

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Materials and methods

Two hundred and forty three durum wheat varieties from 27 countries were analyzed (Table 1). Seed samples were from the durum wheat collection of the INRA Wheat Laboratory of Montpellier, France. At least six seeds per variety were analyzed in order to verify their protein homogeneity. The bread wheat cultivars Chinese Spring, Cappelle-Desprez, and Cheyenne, were used as standards to provide reference omega-gliadin bands for those in durum wheat. Their electrophoretic patterns are indicated in Fig. 1, according to the scheme of Khelifi et al. (1992).

Table 1. Omega-gliadin electrophoretic pattern classification of a collection of 243 durum wheats from 27 countries

Pattern	Varieties
d2d4d9	Bezencukskaja 141, Bezostaja 116, Dritto, Ensat-398, Ensat-487
d2d5d6	Ensat-532
d3'd6'	Tripolitico
d3'd6'd8'	Kristall, Kubanca, Micurinka, Parus
d4'	Alaga, Alaga de Burgos, Beladia, Greece 2, Lez, Line 303, Maghrebi 72, Penatiel, Tun. Weizen
d4'd5	073/44, 11-4, Acme, Akmolinska 2, Apulicum 233, Bolu, BX IPI, Cando, Castel Delmonte, Celta, Coulter, Dalmatia 4, Derbentskaja, Enver Pascha, GK Bainieri, Hard Tangarog, Jairaj, Jabardo Blanco 9002, Kayseri, Kinee, Langdon, Latino, Line 68, Malav Raj, Monrisco Preto, Da Grao Escuro, P 424, Pellissier, Pombinho 0317, Professor Schiemann, Raineri, Rugby, Russo, Sadovo 01, Schwarzer Madonna, Sentry, Seorsonera, Sham 1, Syndiouk * Mahmoudi 52, Syndiouk * Mahmoudi 870, Tomclair, Tunesischer Weizen, Valnova, Well, Yozgat 2
d4'd5d8d9d10	Akrona, Bezencukskaja 139, Canoco 0277, Carleton, Carleton RL 1663, Kubanka, Mandon, Mindum, Minnesota II-19-203, Mondak, Novomicurinka, Stewart 63
d4'd5d9	Anatolien 6615, Arnautka Nemercanskaja, Candeal 2314, Cannizara, Kubanka, Madonna, Mondak, Paros, Preto Algarvio 10026, Sort NR 1048
d4'd8d9d10d12	Ensat-387
d4d8d9d10	Monrisco Preto, Ndac 7, Pentad, Purculu, Weihenstephan 5
d5	37.4, 44, 881, Agathe, Agrial, Akbasak 1, Akbasak 2, Albanien, Amarelo de Barba Preta, Ambral, Anatolien 6523, Arcour, Bohoth 1, Bohoth 3, Canne R, Capeiti, Capeiti 8, Cappelli, Castel Fusano, Castel Porciano, Castiglione Glabro, Chrysowitza, Cocorit 71, Creso, Cotrone, Belfuggito, Berillo, Bidi 17, Bitlis, Brindur, Bulgarischer 1, Dagestan, Damoiso, Diyarbakir, Dural, Duramba, Durandal, Edmor, Ensat-431, Espanhol 8914, Flodur, Grokaia-Keriger, Greece 20, Griechenland Grosskorning, Helvio, Hubice 47/3, Indian, Iran 2, Istanbul, Javardo 2530, Jazira 17, KU 0589/001, Kurzahriger, Line 304, Line 305, Lloyd, Mahmoudi 981, Medora, Melanopus 16, Mexicali 75, Mindum, Mohamed Ben Bachir, Mondur, Monrisco 063, Monrisco Fino 4364, Montferrier, Moskowskaja Tejskaja, Mugan, Neodur, Nuragus, Oued Zenati, Oued Zenati 368, Oued Zenati Cor. 26972, Pellissier 14, Plastovskaja 2, Primadur, Raj 1555, Sadovo 04, Schwarzer Hart Weiwen, Senator Cappelli, Shihani, Sinop 1, Topaz, Tuerdaja 931, Tunceli, URSS 1B, V. Z. 281, V. Z. 294, Valgerardo, Vic, Wascana
d5d12	Ensat-508
d5d8d9d10	Brumaire, Dalmatia 5
d5d9	Adapazari, Amidur, Beladi 118, Bezencukskaja 115, Briskri * Bouteille, Bufala Nera Corta, Espanhol, Gigantil, Kehla, Kyperounda, Kyperounda 1, Kyperounda 2, Kubanka 132, Sadobo 15, Tangarog Buck Balcarce
d6	Durazio Molar 9076, Grifoni 235, Hercules, Kislik-Koncine, Macoun, Melanopus 1528, Ramsey, Vatan, Wakooma
d8d9	Escuro 6141
d8d9d10	Arnautka, Weihenstephan 4
d9	28-4-9, Antalya, Cankiri, Tokat
Null	36.4, Amasya 2, Anatolien 6596, Anatolien 6591, Anatolien 18477, Ankara, Antalya 1517, Aric 581/1, Arnautka CI 1493, Azerbaidjan 18444, Candeal Grao Escuro, Corum, Durelle, Elazig 1495, Hedba, Iran 1, Kabul 2, Kastamonu, Kirmize, Kirsehir, Midge 1375, Professor Schiemann, Sivovka Besentchuk, Tunisweizen, Urfa 1366

The chromosomal location of the genes coding for the novel bands found amongst the durum wheats was inferred by determining which allelic subunits they replaced. The nomenclature used was that proposed by Khelifi et al. (1992).

Omega-gliadin identification was done using the two-step one-dimensional APAGE/SDS-PAGE procedure of Khelifi and Branlard (1991). Protein was extracted overnight from crushed half seeds using the extracting solution proposed by Khelifi et al. (1992): 50% (v/v) propanol-1, 1% (v/v) acetic acid, 0.5% (w/v) methyl green and 30% (v/v) glycerol.

Results and discussion

Relative electrophoretic mobilities of the omega-gliadins in durum wheat

A total of 12 bands with different mobilities could be identified (Figs. 1–3). Eight bands had the same mobility as those present in the bread wheat standards and were considered to be the same. The third band of Cheyenne (Fig. 3 l), initially identified as d8 by Khelifi et al. (1992), had a slightly higher mobility than the d8 of Cappelle-Desprez (Fig. 3 m), and was not considered in band identification. Band d12, associated with d11 in bread wheat and controlled by the 1D chromosome (Khelifi et al. 1992), was present in two Ethiopian cultivars (Fig. 1 k, l). No *Glu-D1*-encoded glutenins were present in the electrophoregrams of the two Ethiopian wheats and a cytological study was initiated to determine the possible presence of a 1Ds translocation. Among the four bands previously not detected in bread wheat, band d3' (Fig. 1 d, f, j; Fig. 2 j; Fig. 3 j), whose mobility lies between d3 and d4, was only detected in five cultivars, four from the Soviet Union and one from Cyprus. Band d4' (Fig. 1 c, k; Fig. 3 b, f), slower than d5, was very common in durum wheat. Band d6′ (Fig. 1 d, f, j; Fig. 2 j; Fig. 3 j), a little slower than d7, was found in the same five wheats in which band d4′ was detected. Band d8′ (Fig. 1 f, j; Fig. 3 j) migrates at a rate between bands d8 and d9 and was present only in four cultivars from the Soviet Union.

Allelic diversity

Each cultivar possessed from zero to five different omega-gliadin bands. A total of 15 different *Gli-A1*- and *Gli-B1*-encoded variants were identified in this durum wheat collection (Table 2). As in bread wheat, a null allele was detected at both loci. Seven of these variants, two controlled by *Gli-A1* and five by the *Gli-B1* locus, were different from those previously described in bread wheat by Khelifi et al. (1992). Novel alleles have been designated with progressive roman numerals prefixed by the gene symbol of the locus controlling their synthesis.

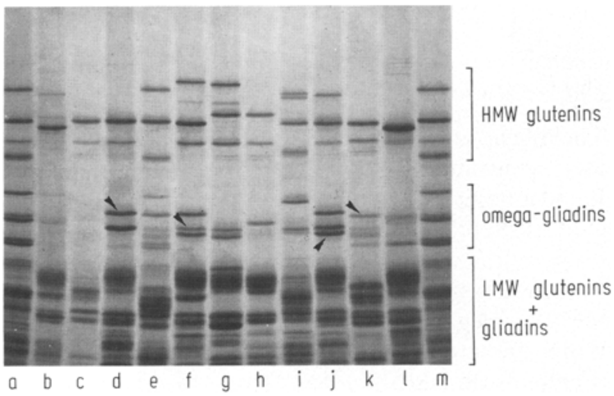

Fig. 1. Two-step one-dimensional APAGE/SDS-PAGE electrophoretic patterns of the omega-gliadins of the bread wheat standards Chinese Spring (bands d1, d5, d6, d11, d12, *Tracks a and m*), Cappelle-Desprez (bands d2, d4, d8, d9, d10, d11, d12, *track e*), and Cheyenne (bands d3, d7, *track i*), and of the durum wheats Ensat-532 (*b*), Latino (*c*), Tripolitico (*d*), Micurinka (*f*), Escuro 6141 (*g*), Macoun (*h*), Kristall (*j*), Ensat-387 (*k*) and Ensat-508 (*l*). Novel bands, d3′ (*d*), d4′ (*k*), d6′ (*f*) and d8′ (*j*) are *arrowed*

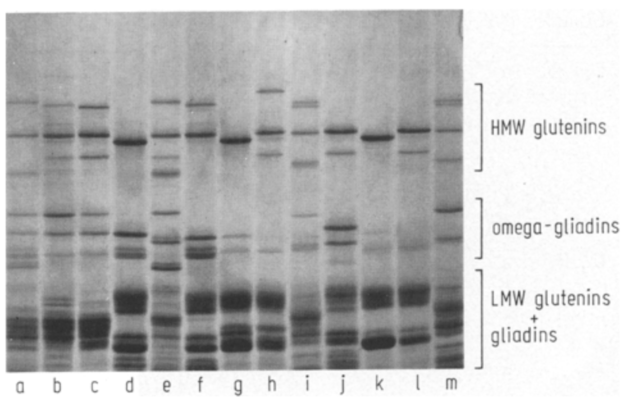

Fig. 2. Two-step one-dimensional APAGE/SDS-PAGE electrophoretic patterns of the omega-gliadins of the bread wheat standards Chinese Spring (*e*), Cappelle-Desprez (*a*) and Cheyenne (*i*, *m*), and the durum wheats Ensat-398 (*b*), Bezostaja 116 (*c*), Weihenstephan 5 (*d*), Dalmatia 5 (*f*), Tangarog (*g*), Tokat (*h*), Tripolitico (*j*), Monrisco Preto (*k*) and Arnautka (*l*)

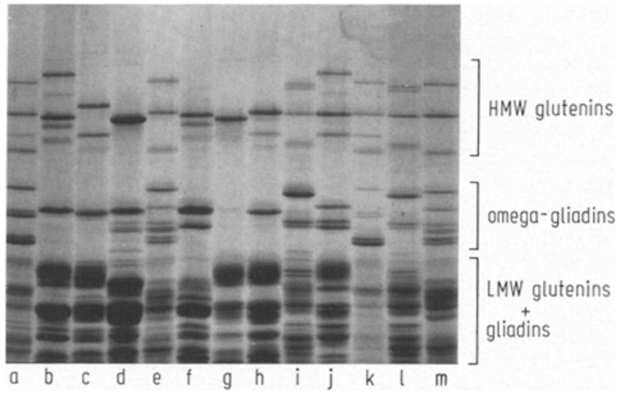

Fig. 3. Two-step one-dimensional electrophoretic patterns of the omega-gliadins of the bread wheat standards Chinese Spring (*a*, *k*), Cappelle-Desprez (*e*, *m*) and Cheyenne (*i*, *l*), and of the durum wheats Alaga (*b*), Cotrone (*c*), Mandon (*d*), Cannizara (*f*), Durelle (*g*), Mexicali 75 (*h*) and Micurinke (*j*)

At the *Gli-A1* locus six alleles were identified. *Gli-A1b*, coding for band d9, was present in 14% of the durum wheats sampled. *Gli-A1c*, coding for two bands, d8 and d9, was only found in one cultivar from Portugal. *Gli-A1d*, coding for the group of bands d8, d9 and d10, was detected in 9% of the wheats analyzed. The null allele *Gli-A1e* was the most common and was present in 74% of the total sample. Among the new alleles found, *Gli-A1I*, coding for the single band named d6′ (Fig. 1 d), was only detected in one variety from Cyprus. *Gli-A1II*, coding for two bands, d6′ and d8′ (Fig. 1 f, j; Fig. 3 j), was present in four wheats from the Soviet Union. The bread wheat allele *Gli-A1a*, coding for band d8 (Khelifi et al. 1992), was not found in this durum wheat collection.

Nine alleles were found at the *Gli-B1* locus. *Gli-B1b*, coding for bands d2 and d4, was only present in five wheats, one from Italy, two from Ethiopia and two from the Soviet Union. *Gli-B1c*, coding for band d4, was present in five varieties of different origin. *Gli-B1e*, coding for band d5, was very common being present in 45% of the total sample. The null allele *Gli-B3g* was present in 13% of the durum wheats analyzed. The novel allele *Gli-B1I*, coding for band d4′ (Fig. 1 k; Fig. 3 b), was present in ten wheats of different origin. *Gli-B1II*, coding for three bands, d2, d5 and d6 (Fig. 1 b), was present only in one wheat from Ethiopia. *Gli-B1III*, coding for band d3′ (Fig. 1 d, f, j; Fig. 2 j;

Table 2. Omega-gliadin electrophoretic patterns observed among a collection of 243 durum wheats and their allelic classification[a]

Phenotype	No.	*Gli-A1*		*Gli-B1*	
		Band	Allele	Band	Allele
d2d4d9	5	d9	b	d2d4	b
d2d5d6	1	–	e	d2d5d6	II
d3′d6′d8′	4	d6′d8′	II	d3′	III
d3′d6′	1	d6′	I	d3′	III
d4′	9	–	e	d4′	I
d4′d5	45	–	e	d4′d5	IV
d4′d5d8d9d10	12	d8d9d10	d	d4′d5	IV
d4′d5d9	10	d9	b	d4′d5	IV
d4′d8d9d10d12[b]	1	d8d9d10	d	d4′	I
d4d8d9d10	5	d8d9d10	d	d4	c
d5	91	–	e	d5	e
d5d12[b]	1	–	e	d5	e
d5d8d9d10	2	d8d9d10	d	d5	e
d5d9	15	d9	b	d5	e
d6	9	–	e	d6	V
d8d9	1	d8d9	c	–	g
d8d9d10	2	d8d9d10	d	–	g
d9	4	d9	b	–	g
Null	25	–	e	–	g

[a] Nomenclature of bands and alleles was that of Khelifi et al. (1992)

[b] These two varieties, from Ethiopia, possessed band d12, coded for by the 1D chromosome in bread wheat (Khelifi et al. 1992)

Fig. 3j), was detected only in five wheats from the Soviet Union. *Gli-B1IV*, coding for bands d4′ and d5 (Fig. 1c; Fig. 3d, f), was found in 28% of the varieties. Finally, *Gli-B1V*, coding for band d6 (Fig. 1h), was detected in nine wheats of different origin. The bread wheat alleles *Gli-B1a*, coding for bands d1, d5 and d6, *Gli-B1d*, coding for band d3, and *Gli-B1f*, coding for bands d5 and d6, were not detected among the 243 durum wheats analyzed.

Electrophoretic patterns

A total of 19 different electrophoretic patterns were identified. Table 1 shows the omega-gliadin classification of the durum wheats analyzed. Table 2 shows the phenotypes observed and their allelic classification. Sixty six per cent of the population can be characterized by only three omega-gliadin electrophoretic patterns: d5 (37%), d4′ d5 (18%) and the absence of bands (10%). Five of the patterns were only detected in one variety each; d2d5d6 in Ensat-532; d4′d8d9d10d12 in Ensat-387, and d5d12 in Ensat-508, all from Ethiopia; d3′d6′ in Tripolitico, from Cyprus, and d8d9 in Escuro 6141, from Portugal.

Pattern d3′d6′d8′ was found only in four varieties: Kristal, Kubanca, Micurinca and Parus, all from the Soviet Union. Pattern d5d8d9d10 was detected in only two wheats, Brumaire, from France, and Dalmatia 5, from Yugoslavia. Pattern d9 was present in four wheats, 28-4-9 from France, and Antalya, Cankiri and Tokat from Turkey. Only two varieties had the d8d9 d10 pattern; Arnautka, from the Soviet Union, and Weihenstephan 4, from Germany.

The diversity found among the durum wheat collection analyzed was low. Eighty one per cent of the population can be defined by only six patterns and seven others were extremely rare (one or two wheats). Thus, the utilization of the omega-gliadin patterns for varietal identification does not look promising. Further work should be done to correlate the omega-gliadins to pasta-making quality in durum wheat.

Comparison of allele frequencies between durum and bread wheat

The allelic variability found at the *Gli-A1* and *Gli-B1* loci in durum wheat was slightly greater than that found among the 168 bread wheats analyzed by Khelifi et al. (1992). This can be explained by the narrower origin of the wheats they analyzed, which were mostly French. Their survey probably understimates the extent of omega-gliadin variation worldwide. Furthermore, modern breeding can lead to genetic erosion (Feldman and Sears 1981). Thus, Branlard and Le Blanc (1985) demonstrated a strong decrease in the polymorphism of high-molecular-weight glutenins in French bread wheat cultivars during the last 30 years.

A comparison of the allelic frequencies for the *Gli-A1* and *Gli-B1* loci between bread and durum wheat are given in Table 3. At the *Gli-A1* locus, the

Table 3. Frequencies (%) of alleles at the *Gli-A1* and *Gli-B1* loci between a collection of 168 bread wheat cultivars (Khelifi et al. 1992) and 243 durum wheats

Locus	Allele	Bands	Bread wheat	Durum wheat
Gli-A1	a	d8	4.8	0.0
	b	d9	16.7	14.0
	c	d8d9	4.2	0.4
	d	d8d9d10	32.7	9.0
	e	–	41.7	74.5
	I	d6′	0.0	0.4
	II	d6′d8′	0.0	1.6
Gli-B1	a	d1d5d6	0.6	0.0
	b	d2d4	60.1	2.1
	c	d4	2.4	2.1
	d	d3	19.0	0.0
	e	d5	4.2	44.9
	f	d5d6	1.2	0.0
	g	–	12.5	13.2
	I	d4′	0.0	4.1
	II	d2d5d6	0.0	0.4
	III	d3′	0.0	2.1
	IV	d4′d5	0.0	27.6
	V	d6	0.0	3.7

Gli-A1a allele, absent in durum wheat, was present at a low frequency (<5%) in bread wheat, while *Gli-A1I*, absent in bread wheat, was rare (<2%) in durum wheat. The null allele *Gle-A1e* was present in a higher proportion in durum (74%) than in bread wheat (42%). The *Gli-A1d* allele, very common in bread wheat (33%), was only present in 9% of the durum wheats analyzed. At the *Gli-B1* locus differences between the two species were higher. *Gli-B1b*, present in 60% of the bread wheat sample, was found in only 2% of the durum wheats. *Gli-B1d* was present in 19% of the bread wheats and was not found in durum wheat. Otherwise, *Gli-B1e*, present in 45% of the durum wheats, was only detected in 4% of the bread wheats; and *Gli-B1IV*, not detected in bread wheat, was present in 28% of the durum wheats. Allelic differences between bread and durum wheat were higher at the *Gli-B1* than at the *Gli-A1* locus, in agreement with the results of Metakovsky et al. (1989). The differences found between the two species in the allelic frequencies of equivalent alleles may be due to the narrow genetical background currently utilized in breeding programs.

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