Indian J. Genet., **63**(4): 307-311 (2003)

Allelic variation of gliadin and glutenins in genetic stocks and advance lines of *Triticum turgidum* var. *durum*

S. V. Sai Prasad, V. S. Rao[1] and H. N. Pandey

Indian Agricultural Research Institute, Regional Station, Indore 452 001
[1]Agarkar Research Institute, Pune 411 004

(Received: June 2002; Revised: October 2003; Accepted: October 2003)

Abstract

In durum wheat genotypes, analysis of seed storage protein profiles of 30 genetic stocks and 30 advanced lines was done using Acid and SDS PAGE electrophoresis. More alleles were observed at *Glu-1* loci i.e., *Glu A1* (2*) and *Glu B1* (14+15, 7*+8 and a new type allele) in genetic stocks, while these alleles were not noticed in advanced lines. Most of the advance lines show null and 7+8 bands, while in genetic stocks, band 20 is common. Genetic stocks contain 12 different patterns at *Glu-3* loci, while only two were found in advance lines, indicating that the genetic stocks contain more diversity for this class of proteins in comparison to advance lines. The rust resistant lines do not posses LMW 2 / γ-45 type *Glu 3 / Gli-1* alleles, so these can be used as donors to introduce rust resistance in the good quality recently released varieties. Most of the advance lines showed LMW 2 / γ-45 type *Glu 3 / Gli-1* alleles, which is best for pasta making quality. So, LMW 2 / γ-45 type *Glu 3 / Gli-1* aliele can be used as a bio-chemical marker to select good quality lines in future breeding programmes. The presence of new LMW-B glutenin and γ-gliadin patterns is interesting and needs to be investigated further for their role in pasta making as well as overall technological quality of durum wheat.

Key words: *Triticum durum*, gliadin and glutenins

Introduction

Triticum durum is the second most important wheat species grown in India, which is generally considered the hardest of all wheats and have high protein content. It is well suited for production of pasta products, because of its high content of protein and virtreous kernals for satisfactorily cooking quality of pasta and high yellow pigment content required for attractive appearance of pasta in dry and cooked form. Presently, the improvement in processing quality of durum wheat, (mainly gliadin and gluten profiles) is gaining greater attention. The gluten complex is composed of two main groups of proteins, gliadins and glutenins. The gliadins are responsible for dough cohesiveness, whereas, glutenins for the property of resistance to extension. These proteins are recognised as the major wheat storage proteins, consisting about 80 to 85 % of the total grain proteins. Durum wheat with strong gluten strength generally gives (good pasta products. Variation in gluten strength and elasticity depends on the quality and quantity of gluten proteins i.e., glutenins and gliadins. In durum wheats, the chromosome 1B is primarily responsible for the qualitative traits, as genes coding for proteins correlated with strong or weak gluten are located on it. In the present study, analysis of seed storage protein profile was done using Acid PAGE and SDS PAGE of genetic stocks and advanced lines of Indian durum wheat genotypes, which will provide information on the direction for crossing as well as in selection programmes for improvement of durum wheat varieties.

Materials and methods

Sixty durum wheat genotypes from the collections at Indian Agricultural Research Institute, Regional Station, Indore, India were analysed. Among these, 30 were genetic stocks, while the remaining were advanced lines evolved through breeding efforts (Table 1 and 2). The lines *viz.*, Edmore, Langdon, Kalyansona, MACS 2496 and Marquis were used as standards for glutenin and gliadin sub-units.

Gliadin analysis: Single seed was crushed into fine powder to which 100 µl of 70% ethanol was added and incubated at 37°C for 30 minutes with brief vortexing. The tubes were centrifuged at 10,000 rpm for 10 minutes and the supernatant was collected. The residue left was used for glutenin extraction. 62.5 µl of the dilution buffer was added to 50 µl of the supernatant obtained. 20 µl of the sample was loaded into the well of the gel of Acid PAGE separation of gliadins. The electrophorograms of the gliadin proteins were conveniently evaluated according to Bushuk and Zillman (1978) based on relative mobility.

Glutenin analysis: 500 µl of 50 % (v/v) Propan-1-ol was added to the residue left after the extraction of gliadin and incubated for 30 minutes at 65 °C with

brief vortexing. The tubes were centrifuged at 600 rpm for 2 min and supernatant was discarded. The same process was repeated for 3 times. 100 µl of glutenin extraction buffer (pH = 8.0) containing 1.4 % of freshly mixed 4-Vinyl pyridine was added and incubated for 15 minutes for protein alkylation. The sample was then centrifuged at 10,000 rpm for 2 minutes. An aliquot of 100 µl of the supernatant was transferred to a new tube containing 100 µl of dilution sample buffer, vortexed briefly and incubated at 65 °C for 15 minutes before loading 10-20 µl of extract in the individual slots of the SDS - PAGE gel for glutenin fractionation.

Results and discussion

Allelic variation at Glu-1 loci: Eight different HMW banding patterns were observed (Fig. 1a and Table 1) in the genetic stocks. At *Glu-A1*, two alleles were observed i.e., *Glu-A1c* (null) and *Glu A1b* (2*), whereas, at *Glu-B1* loci, seven different alleles were observed *viz.*, 6+8 (d), 7+8 (b), 20 (e), 13+16 (f), 14+15 (h), 7*+8 and a new type (two bands were observed below 14+15). The advance lines showed four different HMW banding patterns. At *Glu-A1* loci, all advanced lines showed null allele, while, at *Glu-B1*, four alleles were observed i.e., 6+8 (d), 7+8 (b), 20 (e) and 13+16 (f).

In the analysis of frequency of band patterns (Table 3), it was observed that more alleles at *Glu-1* loci i.e., *Glu-A1* (2*) and *Glu-B1* (14+15, 7*+8 and a new type allele) were present in genetic stocks, while, these alleles were not noticed in advanced lines, indicating the extent of diversity in genetic stocks as compared to advance lines, which have been subjected to directed selection. Barnard *et al.*, 1989 classified HMW glutenin subunits into those encoded by loci *Glu-A1* and *Glu-B1* located respectively on chromosome *1A* and *1B*. Most of the advanced lines showed null and 7+8 bands, while, in genetic stocks, band 20 is common. It was observed in advance lines, that they are not possessing any glutenin sub-units encoded by *Glu-A1* and generally contain 6+8, 7+8, 13+16 and 20 at *Glu-B1* loci.

Allelic variation at Glu-3 loci: In genetic stocks, SDS-PAGE profiles of LMW glutenins showed ten different banding patterns considering only B sub units (Fig. 2a and Table 1). Each genotype had generally 3 to 6 LMW glutenin bands except B 662 (Fig. 2a, lane H arrow), which showed only one LMW glutenin band. LMW-1 and -2 were named as reported by Pogna *et al.* (1990) and the other eight variants of LMW B glutenin patterns were arbitrarily named as LMW-3 to -10 (Fig. 2a). Genotypes showing different LMW B glutenin patterns other than LMW-1 and -2 are to be studied for their effects on pasta making and overall quality. Four different LMW B glutenin patterns were observed in the advanced lines (Fig. 2b and Table 2). Most of the advanced lines showed LMW-2 (Payne, 1984), which is a protein marker for good pasta quality and each genotype showed 4 to 6 LMW B glutenin bands. Two variant patterns each were observed in the LMW-1 (LMW1-1 and -2) and LMW -2 (LMW2-1 and -2) glutenin types.

It was observed that genetic stocks contained 12 different patterns, while only two patterns were found in advanced lines, indicating that genetic stocks contain more diversity for this class of proteins in comparison to advance lines. Most of the advance durum lines are selected giving more priority to LMW-2 type of glutenin pattern, which is proved to be good for pasta making (Pogna *et al.*, 1990). It had been proved that LMW -2 / γ-45 is good for pasta making, while LMW -1 / γ-42 is poor. In the advance lines analysed, some of the lines indicated the presence of LMW -1 / γ-42, which are responsible for poor pasta quality. Hence, care should be taken to select for the presence of LMW -2 / γ-45 to ensure better quality durum wheat.

Table 1. Glutenin and gliadin bands in genetic stocks

Variety	*Gli-B1*	*Glu-3*	*Glu-A1*		*Glu-B1*	
			Band	Allele	Band	Allele
Sarangpur local	45	LMW 3	2*	b	14+15	h
Sawer local	45	LMW 3	N	c	20	e
Dahod local	45	LMW 3	2*	b	14+15	h
Mandsaur local	45	LMW 5	2*	b	20	e
Bansi local	45	LMW 3	2*	b	20	e
Malvi local	45	LMW 5	N	c	20	e
Motia	45	LMW 5	2*	b	20	e
Trinakaria	45	LMW 2	N	c	20	e
Kathia red	45	LMW 5	2*	b	20	e
Bijaga yellow	45	LMW 6	2*	b	20	e
IWP 5019	45	LMW 8	N	c	7+8	b
B 146	45	LMW 5	N	c	7+8	b
HG 110	45	LMW 9	N	c	7+8	b
IWP 5004-1	45	LMW 2	N	c	7+8	b
Guji	45	LMW 2	N	c	20	e
Kathia 25	44	LMW 6	N	c	13+16	f
NP 404	44	LMW 6	2*	b	20	e
N 59	44	LMW 6	N	c	13+16	f
Ju 12	44	LMW 6	N	c	13+16	f
Meghdoot	44	LMW 6	N	c	13+16	f
Bijaga red	44	LMW 6	2*	b	20	e
MACS 1967	44	LMW 6	N	c	13+16	f
Karnataka local	43.5	LMW 4	2*	b	14+15	h
A 206	43.5	LMW 4	2*	b	14+15	h
A 9-30-1	43.5	LMW 4	N	c	13+16	f
NIDW 15	42	LMW 1	N	c	6+8	d
Yuk	42	LMW 1	N	c	20	e
B 662	Null	LMW 7	c	N	20	e
ED 2398 A	47	LMW 9	N	c	7*+8	b*
Line 1172	47	LMW 10	N	c	New	h

Table 2. Glutenin and gliadin bands in advance lines

Variety	*Gli-B1*	*Glu-3*	*Glu-A1*		*Glu-B1*	
			Band	Allele	Band	Allele
HD 4672	45	LMW 2	N	c	7+8	b
HI 8498	45	LMW 2	N	c	7+8	b
HI 8381	45	LMW 2	N	c	7+8	b
PDW 233	45	LMW 2-1	N	c	6+8	d
WH 896	45	LMW 2	N	c	7+8	b
Raj 1555	45	LMW 2	N	c	7+8	b
MACS 2846	45	LMW 2	N	c	20	e
MPO 215	45	LMW 2	N	c	7+8	b
HI 8622	45	LMW 2	N	c	7+8	b
HI 8624	45	LMW 2	N	c	7+8	b
HD 4692	45	LMW 2	N	c	7+8	b
HI 8653	45	LMW 2	N	c	7+8	b
HD 4695	45	LMW 2	N	c	13+16	f
HD 4696	45	LMW 2	N	c	7+8	b
HD 4697	45	LMW 2	N	c	7+8	b
HD 4698	45	LMW 2	N	c	7+8	b
HI 8654	45	LMW 2	N	c	7+8	b
HI 8655	45	LMW 2	N	c	7+8	b
HI 8656	45	LMW 2	N	c	6+8	d
HI 8657	45	LMW 2	N	c	13+16	f
HD 4699	45	LMW 2	N	c	7+8	b
HI 8540	45	LMW 2	N	c	13+16	f
HI 7747	42	LMW 2	N	c	20	e
Jairaj	42	LMW 1	N	c	6+8	d
NIDW 9	42	LMW 1	N	c	6+8	d
HI 8591	42	LMW 1	N	c	7+8	e
HI 8620	42	LMW 1	N	c	7+8	b
HI 8625	42	LMW 1-1	N	c	20	e
HI 8623	42	LMW 1	N	c	7+8	b
HD 4694	42	LMW 1	N	c	7+8	b

Allelic variation at Gli-B1 loci: Six different *Glu-B1* alleles were found in genetic stocks (Fig. 3a and Table 1). Most of the local genotypes showed γ-45, γ-43.5 and γ-44 and only two genotypes showed γ-42 *viz.*, Yuk and NIDW 15, where Yuk is exotic and NIDW 15 is an old developed variety. None of the locals showed presence of γ-42 gliadin band. Two genotypes show γ-47 band (ED 2398-A and Line 1172) and one genotype, B 662 did not show any γ gliadin band (Null). MACS 1967, N 59, NP 404, JU 12, Meghdoot, Bijaga Red and Kathia 25 showed γ-44 gliadin band, which are all tall and grown in rainfed conditions. In this group, only Kathia 25 is a local type and rest of them are old released varieties, which have Gaza as one of the parent in their Pedigree (or parents derived from Gaza × local cross).

Genotypes showing γ-47 and null type are found to be highly rust resistant and are derived from interspecific crosses. All these rust resistant genotypes do not show γ-gliadin 45, which is good for pasta making. It was observed that γ-gliadin 45 is not present in the rust resistant derived lines. So, it is necessary thereby to study γ-47 and null linked LMW B glutenins and their pasta making potential, or transfer of rust resistance to γ-45 containing genotypes. Three genotypes showed γ-43.5 *viz.*, A 206, Karnataka local and A 9-30-1, out of which, first two are local types and the latter is an old released variety, derived from a cross in which A 206 is one of the parent.

Table 3. Frequencies of alleles for the loci *Glu-A1*, *Glu-B1* and *Gli-B1* in genetic stocks and advance lines

Locus	Alleles	Genetic stocks (30) (%)	Advance lines (30) (%)
Glu-A1	2*	36.7	-
	Null	63.3	100
Glu-B1	6+8	3.3	10.0
	7+8	16.7	66.7
	13+16	20.0	10.0
	14+15	16.7	-
	20	43.3	13.3
Glu-3	1	6.7	23.3
	1-1	-	3.3
	2	10.0	7.0
	2-1	-	3.3
	3	13.3	-
	4	10.0	-
	5	16.7	-
	6	26.7	-
	7	3.3	-
	8	3.3	-
	9	6.7	-
	10	3.3	-
Gli-B1	47	6.7	-
	45	50.0	73.3
	44	23.3	-
	43.5	10.0	-
	42	6.7	26.7
	Null	3.3	-

In advanced lines, two different γ-gliadins were observed i.e., γ-45 and γ-42, of which γ-was most common. All the recently released durum varieties except HI 7747 and Jairaj were containing γ-45, which is a protein marker for good pasta making (Joppa *et al.*, 1983). Gamma gliadin types like 44, 43.5, 47 and null were noticed in genetic stocks and are totally absent in advanced lines. Most of the advance lines showed γ-45 gliadin band, which is tightly linked to LMW 2 and have good potential for pasta making and overall quality of durum wheat.

By observing the allelic frequencies of *Glu-1*, *Gli-1* and *Glu-3*, genetic stocks were found to have more diverse alleles at all three loci, while, the advance lines show less diversity because of directed selection for good quality. The rust resistant lines do not possess LMW-2 / γ-45 type *Glu-3* / *Gli-1* alleles, so these can

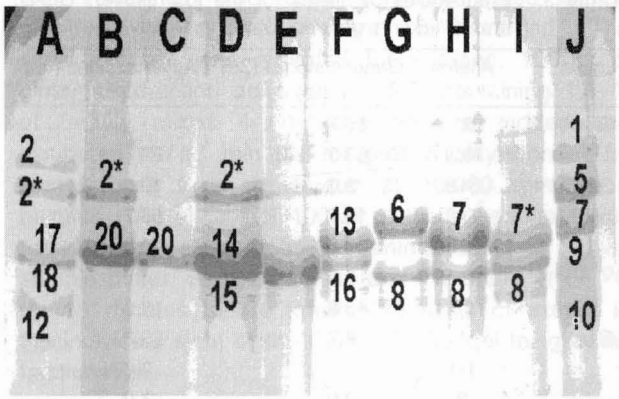

Fig. 1a. Variation at *Glu-1* loci in genetic stocks: (a) Kalyan Sona, (b) Mandsaur local, (c) Malvi local, (d) Sarangpur local, (e) Line 1172, (f) Kathia 25, (g) NIDW 15, (h) IWP 5019, (i) ED 2398-A, (j) MACS 2496

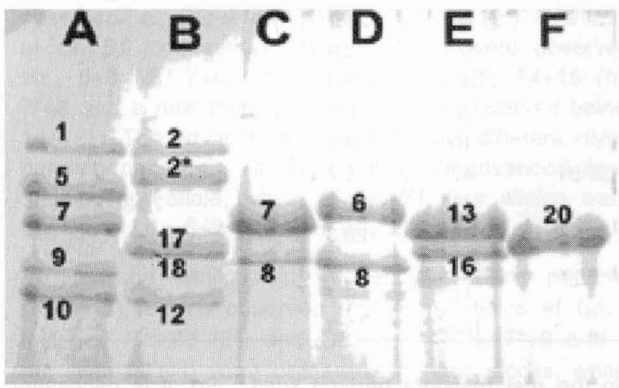

Fig. 1b. Variation at *Glu-1* loci in advance lines: (a) MACS 2496, (b) Kalyan Sona, (c) WH 896, (d) Jairaj, (e) HI 8540, (f) HI 8625

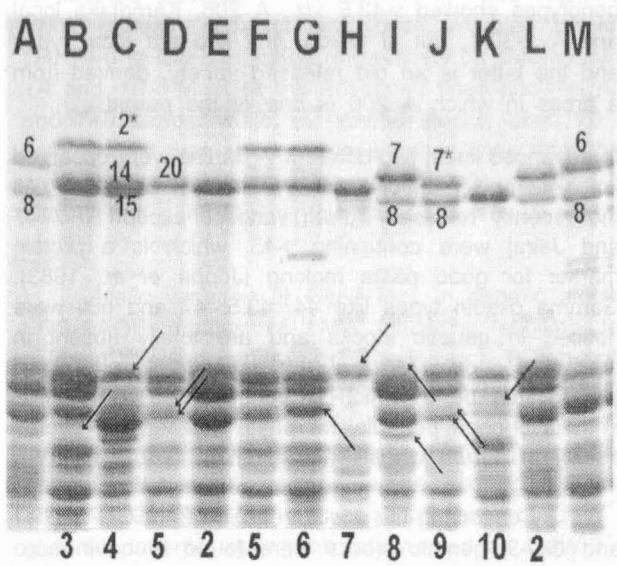

Fig. 2a. Variation at *Glu-3* loci in genetic stocks: (a) Edmore, (b) Sarangpur local, (c) Karnataka local, (d) Malvi local, (e) Trinakaria, (f) Kathia red, (g) NP 404, (h) B 662, (i) IWP 5019, (j) ED 2398-A, (k) Line 1172, (l) IWP 5004-1, (m) Langdon

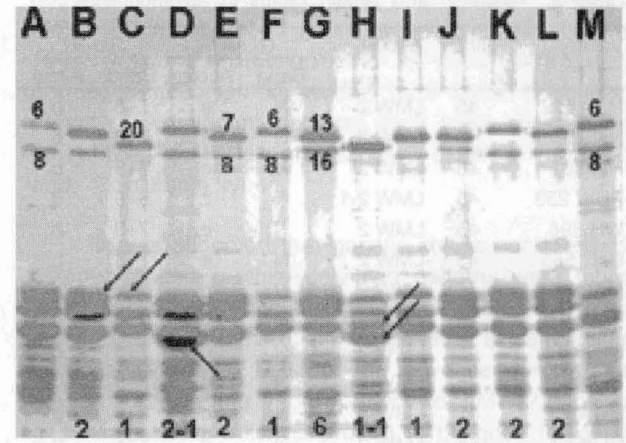

Fig. 2b. Variation at *Glu-3* loci in advance lines: (a) Edmore, (b) HI 8498, (c) HI 7747, (d) PDW 233, (h) WH 896, (f) Jairaj, (g) MACS 1967 (GS), (h) HI 8625, (i) HI 8623, (j) HI 4695, (k) HI 8656, (l) HD 4699, (m) Langdon

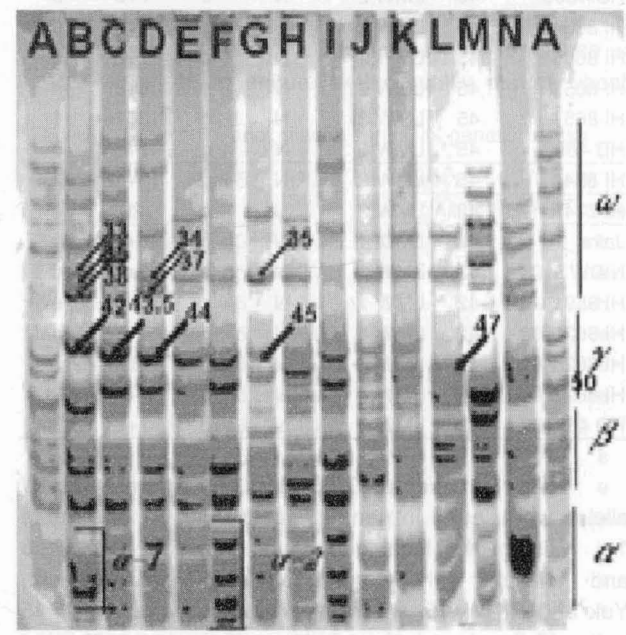

Fig. 3a. Variation at *Gli-B1* loci in genetic stocks: (a) Marquis, (b) Yuk, (c) Karnataka local, (d) NP 404, (e) MACS 1967, (f) Sarangpur local, (g) Sawer local, (h) Dahod local, (i) Kathia red, (j) IWP 5019, (k) B 146, (l) ED 2398-A, (m) B 662, (n) Line 1172

be used as donors to introduce rust resistance in the good quality recently released varieties, which are containing LMW-2 / γ-45 type *Glu-3* / *Gli-1* alleles. Most of advance lines showed LMW-2 / γ-45 type *Glu-3* / *Gli-1* alleles, which is the best type for pasta making quality (Ruiz and Carrillo, 1993). As some of the advance lines are showing LMW-1 / γ-42 type *Glu-3* / *Gli-1* allele, which are not good for pasta making, it is advisable to select for LMW-2 / γ-45 as a biochemical marker in the future breeding programmes, The presence

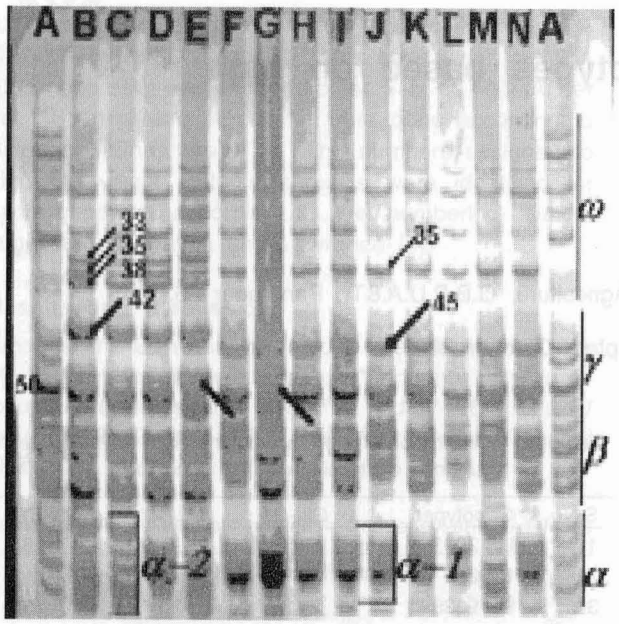

Fig. 3b. Variation at *Gli-B1* loci in advance lines: (a) Marquis, (b) HI 7747, (c) Jairaj, (d) NIDW 9, (e) HI 8625, (f) HI 8498, (g) PDW 233, (h) WH 896, (i) HI 8540, (j) HD 4696, (k) HD 4698, (l) HI 8656, (m) HI 8657, (n) HD 4699

of new LMW B glutenin and γ-gliadin patterns is interesting and needs to be investigated further for their role in pasta making as well as overall technological quality of durum wheat.

Acknowledgements

The first author is thankful to the Indian National Science Academy, New Delhi, for financial help provided in the form of Visiting Scientist Fellowship and to the Director, Indian Agricultural Research Institute, New Delhi for allowing to undergo the training. The help Tendered by Scientist, Shubhada Tamhankar and Research Associate, Manoj Oak of Agharkar Research Institute, Pune during the work was highly acknowledged.

References

1. **Branlard G., Autran J. C. and Monneveux P.** 1989. High molecular weight glutenin subunit in durum wheat (*T. durum*). Theor. Appl. Genet., **78**: 353-358.
2. **Bushuk W. and Zillman R. R.** 1978. Wheat cultivar identification by gliadin electrophoregrams. I Apparatus, method and nomenclature. Can. J. Plant Sci., **58**: 505-515.
3. **Joppa L. R., Khan K. and Williams N. D.** 1983. Chromosomal location of genes for gliadin polypeptides in durum wheat (*Triticum turgidum* L.) Theor. Appl. Genet., **64**: 289-293.
4. **Payne P. I.** 1984. The association between γ-gliadin 45 and gluten strength in durum wheat varieties : a direct causal effect or the result of genetic linkage? J. Cereal Sci., **11**: 15-34.
5. **Pogna N. E., Autran J. C., Mellini F., Lafiandra D. and Feillet P.** 1990. Chromosome 1B-encoded gliadins and glutenin subunits in durum wheat : genetics and relationship to gluten strength. J. Cereal Sci., **11**: 15-34.
6. **Ruiz M. and Carrillo R. M.** 1993. Linkage relationships between prolamin genes on chromosome 1A and 1B of durum wheat. Theor. Appl. Genet., **87**: 353-360.