8144 *J. Agric. Food Chem.* **2007,** *55,* 8144–8151

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Environmentally Induced Changes in Amino Acid Composition in the Grain of Durum Wheat Grown under Different Water and Temperature Regimes in a Mediterranean Environment

LUIS F. GARCÍA DEL MORAL,*,† YAHIA RHARRABTI,† VANESSA MARTOS,† AND
CONXITA ROYO‡

Departamento de Fisiología Vegetal, Facultad de Ciencias, Instituto de Biotecnología, Universidad de Granada, 18071 Granada, Spain, and IRTA, Cereal Breeding, Rovira Roure, 191, 25198 Lleida, Spain

Amino acid composition is an important feature in determining the nutritional value of wheat grain for human and animal diets. Environmental conditions are known to influence protein quantity as well as grain production and, in turn, amino acid composition. In this study, grain yield, protein content, and amino acid composition were determined in 10 durum wheat genotypes under three water and temperature regimes in a Mediterranean environment. The highest value for grain-protein content (15.7%) was found in the warmer and driest environment and the lowest (12.8%) in the irrigated environment. Although amino acid composition showed significant variation for all genotypes, with the exception of arginine and cysteine, major changes in amino acid composition were caused by environmental conditions and in particular by water availability and temperature during the grain-filling period, which significantly altered the duration of grain development. The amino acids with the highest percentage of variation between environments were tyrosine (26.4%), lysine (23.7%), methionine (20.3%), threonine (19.3%), and valine (15.6%), whereas phenylalanine (5.1%), glycine (6.4%), and aspartic acid (6.8%) showed the least variation between environments. Whereas the content of glutamine, phenylalanine, and proline increased with the decrease in grain-filling duration, the remaining amino acids tended to diminish, presumably because high temperature and drought favored the deposition of gliadins (proteins particularly rich in glutamine and proline), to the detriment of albumins and globulins (proteins especially rich in threonine, lysine, methionine, valine, and histidine). Despite the negative correlations found between the percentage of protein and its content in essential amino acids, the results indicate that reductions in lysine per unit of food were not very pronounced (0.32 to 0.29 g/100 g of flour) with increases of up 22.7% in grain-protein content, whereas threonine did not change and valine even slightly increased.

KEYWORDS: Drum wheat; amino acid composition; lysine; protein content; grain-filling duration; water regime; Mediterranean environment

INTRODUCTION

Durum wheat (*Triticum turgidum* L. var. *durum*) is an important crop in human nutrition and animal feed. Grain-protein content and amino acid composition are the most important characteristics in determining the nutritional value of durum wheat for human and animal diets. Protein content and amino acid profiles are also relevant traits that largely determine the quality of the wheat grain, which depends both on its physicochemical characteristics (dough rheology) and nutritional attributes (protein content and amino acid composition) (*1*). Wheat protein is known to be low in some amino acids that are considered essential for the human diet, especially lysine (the most deficient amino acid) and threonine (the second most lacking amino acid), but they are rich in glutamine and proline (*2*), the functional amino acids in dough formation.

Grain-protein content and amino acid composition of wheat fluctuate largely with genotype and environmental characteristics, such as nitrogen-fertilization rate, nitrogen-application time, residual soil nitrogen, soil-moisture availability, and temperature during grain-filling (*2*–*8*).

Wheat-grain proteins can be divided into structural/metabolic and storage proteins. Structural/metabolic proteins consist of albumin, globulin, and amphiphilic proteins, whereas storage proteins are divided into gliadins (present as monomers) and

---

* To whom correspondence should be addressed: Tel +34 958 243253; Fax +34 958 259580; e-mail lfgm@ugr.es.

† Universidad de Granada.

‡ IRTA, Cereal Breeding.

10.1021/jf063094q CCC: $37.00

Table 1. Agronomic Details, Climatic Conditions, and Soil Characteristics of the Experiments

water regime	irrigated	rainfed (very dry)	rainfed (moderate dry)
soil characteristics			
classification	Typic Xerofluvent	Loamy Calcixerolic Xerochrept	Vertisol Uderts
texture	silty clay	silty clay	loamy
pH	8.0	8.2	7.7
P (ppm)	50	27	40
K (ppm)	88	210	155
organic matter (%)	2.01	1.86	2.50
residual N (kg ha^{-1})	33	31	38
agronomic practices			
fertilizers (kg ha^{-1})			
N (seed bed + top dressing)	60 + 40	45 + 20	45 + 40
P_2O_5	60	45	63
K_2O	60	45	40
climatic conditions during vegetative growth[a]			
seasonal rainfall + irrigation (mm)	311 + 150	188 + 0	330 + 0
mean temperature (°C)	10.8	12.0	13.1
maximum temperature (°C)[b]	16.9	18.7	19.1
minimum temperature (°C)[b]	4.8	5.3	7.1
climatic conditions during grain filling[c]			
rainfall (mm)	49.2	22.3	42.1
mean temperature (°C)	19.2	24.3	16.1
maximum temperatures (°C)[b]	26.0	31.2	22.6

[a] That is, from sowing to anthesis. [b] Mean values. [c] That is, from anthesis to physiological maturity.

glutenin subunits (which form polymers) (*2, 9*). The synthesis and accumulation of the different fractions of proteins in the wheat grain vary during kernel development and with the quantity of nitrogen applied. Thus, nitrogen fertilization (*10*) mainly influences the quantity of storage proteins (gliadins and glutenins) but has little effect on the amount of the other endosperm proteins (albumins and globulins). Structural/metabolic protein fractions accumulate mainly during the early phase of grain growth, when most endosperm cells are still dividing, whereas storage-protein fractions accumulate later, when cell division stops and grain growth is due only to cell expansion (*11, 12*). However, many of the albumins and globulins in the mature wheat grain are proteins with dual roles in abiotic stress or predator defence, such as the abundant amylases and amylase–trypsin inhibitors, proteins rich in essential amino acids, that tend to accumulate at the end of grain development following a similar pattern to that of the gluten storage proteins (*13*). Albumins and globulins have higher contents in basic amino acids (mainly lysine, arginine, and histidine), whereas glutenins and gliadins have higher contents in proline and glutamine (*2, 14*), reflecting their storage function. In effect, storage proteins must accommodate a maximum nitrogen reserve under low volume in the protein body, and hence, they have a low content of charged amino acids, limiting repulsion forces and thus favoring the formation of aggregates. Therefore, the amino acid composition of wheat grain varies over its development, especially under conditions that limit both the rate and duration of grain filling, as occurs in most Mediterranean environments.

In wheat, lysine (expressed as a percentage of protein) reportedly decreases as the total protein in the grain increases (*15, 16*). Under conditions that raise the grain-nitrogen content, most other essential amino acids also decline, except for phenylalanine, but there are large increases in the amounts of the nonessential glutamine and proline (*17–19*), amino acids particularly abundant in proteins with storage function, as discussed above.

Whereas the grain-protein content is usually measured in the grain of wheat, the amino acid composition is rarely assessed by the end user, despite its crucial impact upon nutritional quality and the welfare of human populations. In fact, as the world population increases, the requirements to produce good-quality protein available efficiently and economically becomes increasingly important. For this reason, the objectives of this work were (i) to study the variations in the amino acid composition of 10 durum wheat genotypes grown under different water and temperature regimes in a Mediterranean environment and (ii) to evaluate the influence that variations in the duration of the grain-filling period exert on the protein content and amino acid composition under Mediterranean conditions. Although the amino acid content of the grain of bread wheat has been described in classical works, there are very few modern studies that report the influence that water availability and temperature (two of the major environmental constraints to be expected from the climatic change) during grain growth exert on amino acid composition, especially for durum wheat grown under field conditions in Mediterranean environments.

MATERIALS AND METHODS

Plant Material and Experimental Details. During the 1998 growing season, three field experiments were conducted in southern Spain, a Mediterranean environment, including rainfed and irrigation conditions with three different water and temperature regimes during grain filling. **Table 1** summarizes agronomic details, climatic conditions, and soil characteristics of the experiments. Ten durum wheat (*Triticum turgidum* L. var. *durum*) genotypes were studied, including four Spanish commercial varieties (Altar-aos, Jabato, Mexa, and Vitron) and six advanced lines from the durum wheat selection program of CIMMYT/ICARDA (Awalbit, Korifla, Lagost-3, Omrabi-3, Sebah, and Waha). Genotypes were sown at an adjusted rate of 350 germinable seeds m^{-2} in plots 10 m long by 1.2 m wide, with six rows 20 cm apart. The experimental design at each site was a randomized complete block with three replications. At the time of sowing, soil samples were taken at a depth of 30 cm from 6 to 12 spots within each experimental area. The samples were air-dried quickly and were subsequently analyzed for their physicochemical properties (**Table 1**).

Analytical Methods. Plots were harvested mechanically at ripening, and grain yield (kg ha^{-1}) was determined and expressed at 12% moisture level. Average single kernel weight for mature grain was determined as mean weight of three sets of 100 grains per plot. Total nitrogen content for grains was determined using the standard Kjeldhal method (AACC method 46-12) (*20*). The grain-protein percentage was calculated after multiplying Kjeldhal nitrogen by a conversion factor of 5.7 and expressed on a dry basis. Amino acids were quantitatively

Table 2. Genotypic Means for Grain Yield, Protein Content, and Amino Acid Composition (g/100 g of Protein) of 10 Durum Wheat Genotypes Grown under Three Water Regimes in a Mediterranean Environment[a]

genotypes	yield[b]	SKW	GFD	PC	Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
Altar-aos	3514 b	38.7 d	33.0 a	14.3 c	3.13 ab	5.10 a	5.66 a	2.53 a	31.4 bc	4.51 ab	2.80 ab	3.57 ab	5.54 a	2.29 ab	1.90 ab	4.49 bc	9.18 bc	5.34 abc	3.24 bc	3.22 a	3.94 bc
Awalbit	2958 d	43.9 bc	31.1 d	14.9 b	3.00 d	5.12 a	5.58 ab	2.65 a	31.0 bc	4.40 b	2.65 bc	3.60 ab	5.32 ab	2.23 ab	1.80 cd	4.51 b	9.22 ab	5.07 c	3.17 cd	3.24 a	3.83 cd
Jabato	4066 a	43.8 bc	33.2 a	13.1 d	3.17 ab	5.09 a	5.55 b	2.67 a	31.0 bc	4.52 ab	2.92 a	3.70 a	5.57 a	2.36 a	1.95 a	4.50 b	8.98 c	5.27 abc	3.45 a	3.08 b	4.07 ab
Korifla	3342 bc	44.8 b	32.1 b	14.2 c	3.04 d	5.19 a	5.67 a	2.71 a	31.6 bc	4.47 b	2.72 bc	3.63 ab	5.26 b	2.04 c	1.81 cd	4.50 b	9.12 cd	5.37 ab	3.14 cd	2.97 b	3.89 bc
Lagost3	3137 cd	48.9 a	31.6 c	14.6 bc	3.08 bc	5.05 a	5.57 ab	2.51 a	32.0 ab	4.41 b	2.77 bc	3.47 bc	5.36 ab	2.18 b	1.75 de	4.49 bc	9.23 ab	5.24 abc	3.13 cd	3.03 b	3.91 bc
Mexa	2975 d	44.9 b	33.0 a	13.4 d	3.23 a	5.12 a	5.75 a	2.61 a	30.4 c	4.65 a	2.76 bc	3.68 a b	5.52 a	2.31 ab	1.87 bc	4.38 c	8.83 c	5.42 a	3.37 ab	3.26 a	4.17 a
Omrabi3	3260 cd	44.4 b	31.6 c	14.3 c	2.94 d	5.14 a	5.41 bc	2.59 a	32.2 ab	4.46 b	2.80 ab	3.49 bc	5.31 ab	2.02 c	1.84 bc	4.51 b	9.36 ab	5.25 abc	3.17 cd	2.83 cd	3.91 bc
Sebah	2945 d	42.7 c	31.1 d	15.3 a	3.12 ab	4.91 a	5.39 bc	2.58 a	31.9 b	4.36 b	2.60 c	3.57 ab	5.30 ab	2.01 c	1.68 e	4.52 b	9.38 a	5.09 bc	2.98 d	2.78 d	3.74 cd
Vitron	3469 b	43.6 b	32.7 ab	14.0 cd	3.10 b	5.12 a	5.49 b	2.63 a	31.8 bc	4.43 b	2.73 bc	3.62 ab	5.50 ab	2.26 ab	1.95 a	4.50 b	9.08 cd	5.12 bc	3.35 bc	3.34 a	3.97 ab
Waha	3590 b	43.2 bc	31.1 d	15.3 a	2.94 d	5.03 a	5.34 c	2.64 a	33.3 a	4.46 b	2.69 bc	3.32 c	5.39 ab	1.92 c	1.72 de	4.63 a	9.16 cd	5.07 c	3.19 cd	2.95 bc	3.62 d
S.E.	81.69	0.78	0.20	0.22	0.05	0.06	0.08	0.05	0.44	0.05	0.05	0.07	0.08	0.05	0.04	0.03	0.06	0.09	0.07	0.05	0.07

[a] Yield: grain yield (kg ha^{-1}); SKW: single kernel weight (mg); PC: protein content (%); GFD: grain-filling duration (days); Ala: alanine; Arg: arginine; Asp: aspartic acid; Cys: cysteine; Glu: glutamine; Gly: glycine; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Tyr: tyrosine; Val: valine; S.E.: standard error of means. [b] Values followed by the same letter in a column are not significantly different according to Duncan's test ($p < 0.05$).

analyzed with high performance liquid chromatography (HPLC) using the Waters Pico-Tag method, which involves precolumn derivatization with phenylisothiocyanate (*21*). Protein was hydrolyzed in 6 N hydrochloric acid + 1% phenol in sealed evacuated tubes at 110 °C for 24 h. In order to prevent for losses of cysteine and methionine during hydrolysis, these sulfur-containing amino acids were converted into cysteic acid and methionine sulfone by preoxidation with performic acid prior to hydrolysis and derivatization. Tryptophane was not determined. α-Aminoadipic acid was used as an internal standard. The amino acid composition was expressed as percentage of protein content (i.e., in g/100 g of protein). Because most of the glutamine present in the flour protein is converted into glutamic acid during hydrolysis, the data for glutamine plus glutamic acid in all hydrolyzed samples have been reported as glutamine.

Statistical Analyses. Statistical Analysis System (*22*) procedures and programs were used for evaluating the data. Analyses of variance were calculated for amino acids, grain yield, grain-filling duration and protein content, and mean comparisons among genotypes and environments were performed. Correlations and regression analyses were performed to determine the relationships between duration of grain-filling, protein content, and amino acid composition.

RESULTS

Genotype Effects. Analyses of variance for amino acid composition showed the stronger influence of environment in comparison to those of genotype and genotype × environment interaction. **Table 2** presents the mean values of grain yield, single kernel weight, grain-filling duration, protein content, and amino acid composition over the three sites of the 10 genotypes studied. Grain yield varied significantly between genotypes from 2945 kg ha^{-1} in the genotype Sebah to 4066 kg ha^{-1} in Jabato (**Table 2**), the highest yielding genotype in the three experiments, which represents a variation for grain yield between genotypes of 27.6%. Single kernel weight significantly varied from 38.7 mg in the genotype Altar-aos to 48.9 mg in Lagost-3, the genotype with heaviest grains (**Table 2**). The duration of the grain-filling period was more stable between genotypes for the mean of the three experiments, varying significantly between 31.1 days in the genotypes Awalbit, Sebah, and Waha until 33.0 days in the genotypes Altar-aos and Mexa (**Table 2**), which constitutes an average variation between genotypes of only a 5.8% for this trait.

The grain-protein content showed a marked variation between the 10 genotypes, varying significantly from 13.1% in the genotype Jabato (the genotype with the highest grain yield) to 15.3% in the genotypes Sebah (the genotype with the lowest mean grain yield) and Waha. Moreover, in this study, the percentage of protein content in the grain tended to show the well-known negative relationship with grain yield ($r = -0.681$, $n = 30$, $P = 0.001$) found in wheat and in other small grains, such as barley and triticale, but whose physiological causes are not completely understood (*15*, *23*). It is argued that N accumulation and carbohydrate synthesis compete for energy and carbon skeletons during the reproductive growth phase of wheat (*23–25*) and that, under conditions that shorten the duration of the grain-filling period, starch deposition appears to be more sensitive than protein deposition, thus increasing the protein concentration in the grain as starch yield increases (*26*, *27*).

The values found for all amino acids were in the normal range reported for wheat in the literature (*2*, *17*, *18*, *28*). Amino acid composition showed significant variation for all genotypes, with the exception of arginine and cysteine, in which the differences did not reach statistical signification (**Table 2**). For the remaining amino acids the highest percentages of difference were found for the essential amino acids lysine (18.5% of variation between genotypes Waha and Jabato, **Table 2**), methionine (13.9% between genotypes Sebah and Jabato or Vitron, **Table 2**), threonine (13.6% between genotypes Sebah and Jabato, **Table 2**), and valine (13.0% between Waha and Mexa, **Table 2**) and for the nonessential amino acid tyrosine (16.6% between genotypes Sebah and Vitron, **Table 2**). The lowest range of averaged variation was found for the amino acid phenylalanine (5.4% between genotypes Mexa and Waha, **Table 2**), followed by proline (5.9% between Mexa and Sebah, **Table 2**). Variation in the remaining amino acid was between 6.4% for glycine and 10.8% for histidine (**Table 2**).

Environmental Effects. The mean comparison among environments (**Table 3**) showed that all traits studied were significantly influenced by water availability and temperatures during crop growth. Thus, grain yield ranged from 2162 kg ha^{-1} under the more unfavorable conditions of the severe rainfed environment to 4523 kg ha^{-1} under irrigated conditions (**Table 3**), which represents a variation of 52.2% between environments. Single kernel weight varied from 40.8 mg in the driest environment to 46.2 mg under irrigated conditions, which represents an average variation of 11.7% between environments (**Table 3**). The grain-filling duration varied significantly according to the water availability and environmental temperature, ranging from 28.0 days under severe rainfed conditions to 37.7 days under the irrigated environment (**Table 3**), this representing 25.7% variation between environments. As indicated above, the highest value for grain-protein content (15.7%) was found in

Table 3. Environmental Means for Grain Yield, Protein Content, and Amino Acid Composition (g/100 g of Protein) of 10 Durum Wheat Genotypes Grown under Three Water Regimes in a Mediterranean Environment[a]

environment	yield[b]	SKW	GFD	PC	Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
irrigated	4523 a	46.2 a	37.7 a	12.8 c	3.24 a	5.33 a	5.71 a	2.83 a	30.1 c	4.66 a	2.96 a	3.89 a	5.82 a	2.45 a	2.02 a	4.38 c	8.68 c	5.52 a	3.57 a	3.56 a	4.29 a
rainfed (moderate dry)	3292 b	44.7 b	30.4 b	14.3 b	3.05 b	5.05 b	5.59 a	2.51 b	31.8 b	4.38 b	2.74 b	3.55 b	5.27 b	2.11 b	1.86 b	4.53 b	9.16 b	5.16 b	3.20 b	3.02 b	3.80 b
rainfed (very dry)	2162 c	40.8 c	28.0 c	15.7 a	2.94 c	4.88 c	5.32 b	2.50 b	33.1 a	4.36 b	2.54 c	3.25 c	5.12 c	1.87 c	1.61 c	4.61 a	9.61 a	4.99 c	2.88 c	2.62 c	3.62 c
S.E.	44.75	0.43	0.11	0.12	0.03	0.03	0.05	0.03	0.24	0.03	0.03	0.04	0.04	0.03	0.02	0.02	0.03	0.05	0.04	0.03	0.04

[a] Yield: grain yield (kg ha^{-1}); SKW: single kernel weight (mg); PC: protein content (%); Ala: alanine; Arg: arginine; Asp: aspartic acid; Cys: cysteine; Glu: glutamine; Gly: glycine; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Tyr: tyrosine; Val: valine; SumAA: total amino acid content; S.E.: standard error of means. [b] Values followed by the same letter are not significantly different according to Duncan's test ($p < 0.05$).

the warmer and driest environment and the lowest (12.8%) in the irrigated environment (**Table 3**).

Amino acid composition varied significantly between environments, the highest values being in general found under irrigated conditions for all amino acids and the lowest values under very dry rainfed conditions, although with the exceptions of glutamine, proline, and phenylalanine, which showed the reverse trend (**Table 3**). As in the case of the genotypes, tyrosine (26.4%), lysine (23.7%), methionine (20.3%), threonine (19.3%), and valine (15.6%) were the amino acids with the highest percentage of average variation between environments, whereas phenylalanine (5.1%), glycine (6.4%), and aspartic acid (6.8%) showed least variation between environments (**Table 3**).

Protein Content and Amino Acid Composition in Relation to Grain-Filling Duration. The duration of grain-filling exerted a strong influence on both the grain-protein content and amino acid composition for the 10 genotypes studied (**Figure 1**). Thus, the protein content significantly decreased as environmental conditions favoured a longer grain-filling duration (**Figure 1a**), whereas the concentration of all amino acids significantly increased, but with the exceptions of glutamine (**Figure 1f**), proline (**Figure 1n**), and phenylalanine (**Figure 1m**), the concentration of these amino acids decreasing significantly in those environments in which water availability diminished the duration of grain-filling. The slopes of the regression equations (**Figure 1**) indicate that the level of increase in amino acid concentration per day of grain-filling was very similar for most amino acids, oscillating from 0.034% day^{-1} in the amino acids alanine and glycine to 0.092% day^{-1} in the amino acid tyrosine. For the amino acids in which concentration diminished with the increase in grain-filling duration, the slopes varied from −0.024% day^{-1} for phenylalanine (**Figure 1m**) to −0.303% day^{-1} for glutamine (**Figure 1f**).

Relationship between Amino Acid Composition and Protein Content. In our study, the amino acid composition (in g/100 g of protein) was negatively associated to grain-nitrogen content (**Table 4**), with the exception of glutamine, proline, and phenylalanine, which showed a positive relationship. The regression slopes, which reflect the quantitative change in amino acid composition per unit of change in nitrogen content, indicated that glutamine, proline, and tyrosine were the amino acids most affected by variations in the nitrogen content of the grain (**Table 4**). Among the rest of the amino acids, the slopes of the regression equations were close to −1 for lysine, threonine, and valine (**Table 4**), indicating a stronger trend to reduction than in the other amino acids when the grain-nitrogen content increased.

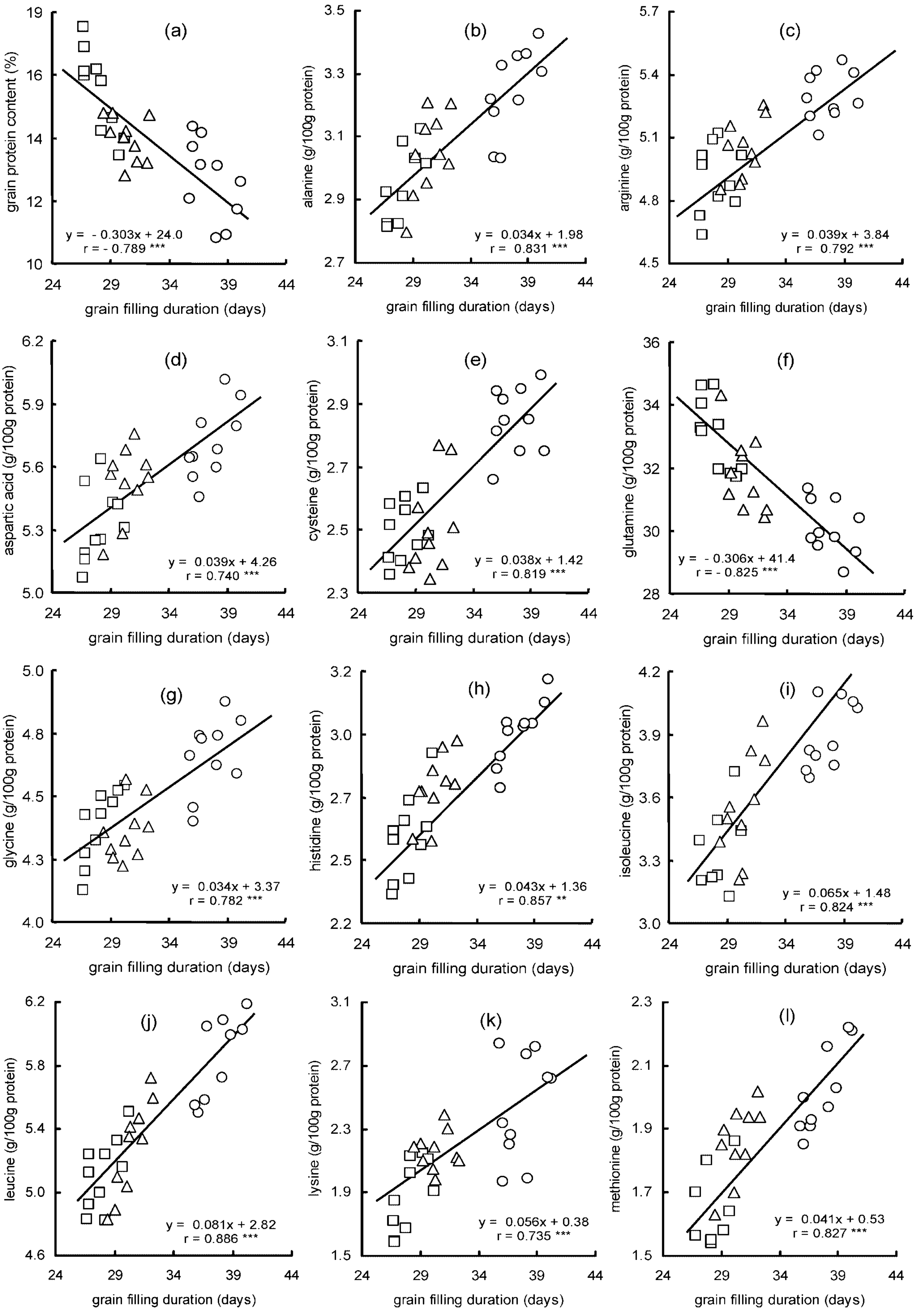
DISCUSSION

It is well known that environmental factors, especially temperature, nitrogen supply, and water availability, influence yield, protein content, and the composition of wheat grain. In this study, grain-protein content showed an inverse relationship with the grain-filling duration, diminishing an average value of 0.3% per day of lengthening in grain-filling period from the more severe rainfed to irrigated conditions. This result confirms that the deposition of carbohydrates is more sensitive to high temperatures and drought in the postanthesis period than is protein deposition (*15, 25, 27*), thereby triggering the dilution of grain protein by non-nitrogen compounds as grain yield increases from rainfed to irrigated conditions.

Durum wheat genotypes in our study were rich in glutamine and proline (which accounted for 32.5% and 9.4% of total grain protein averaged between environments, respectively), but they showed small amounts of methionine (1.9%), lysine (2.2%), cysteine (2.7%), histidine (2.8%) threonine, (3.3%), and alanine (3.2%). Moreover, the content of glutamine, phenylalanine, and proline in the protein increased with the decline in the grain-filling duration and the faster leaf senescence caused by higher temperatures and reduction in water availability during grain growth in the rainfed environments. On the contrary, under irrigated conditions, which tend to lengthen the grain-filling period, the remaining amino acids tended to diminish, this relative reduction being especially significant for tyrosine, lysine, methionine, threonine, and valine.

These differences in amino acid composition probably reflect the highly asynchronous pattern of accumulation of the main protein fractions in the wheat grain, causing changes in the protein composition during grain development (*12*). Actually, the albumin–globulin fraction accumulates from anthesis to ∼20 days after anthesis (DAA), remaining at an almost constant level afterward, whereas storage proteins accumulate from ∼6 DAA to the end of grain-filling (*2, 12, 29*). Thus, conditions that shorten grain-filling, such as high temperatures or drought, affect the balance of protein fractions, the gliadin:glutenin ratio increasing with the quantity of nitrogen in the grain (*30, 31*). There is experimental evidence (*28, 30*) of conditions that hasten wheat-leaf senescence and shorten the grain-filling duration (high temperatures and water scarcity), which seems to promote an earlier synthesis of gliadins, proteins especially abundant in glutamine and proline, as found in our study under rainfed conditions. Moreover, the composition of the individual gliadins and glutenins fractions changes with the environment, particularly with regard to the sulfur amino acids cysteine and methionine (*32*), as found in our study.

Recent comparisons of experimental and simulation results under wide ranges of nitrogen fertilization, temperatures, and water supply have suggested that during the first 10–15 DAA grain-nitrogen accumulation was sink limited or colimited by both source and sink (*11*). Several studies have shown that the amino acid composition (g aa/100 g protein) varies with the quantity of protein deposited in the grain (*18, 19, 28*), owing to the preferential increase in storage protein (mainly gliadins) as grain development and protein accumulation progress (*11, 33*), although without strict dependency on kernel developmental


(a)
grain protein content (%)
grain filling duration (days)
y = - 0.303x + 24.0
r = - 0.789 ***
(b)
alanine (g/100g protein)
grain filling duration (days)
y = 0.034x + 1.98
r = 0.831 ***
(c)
arginine (g/100g protein)
grain filling duration (days)
y = 0.039x + 3.84
r = 0.792 ***
(d)
aspartic acid (g/100g protein)
grain filling duration (days)
y = 0.039x + 4.26
r = 0.740 ***
(e)
cysteine (g/100g protein)
grain filling duration (days)
y = 0.038x + 1.42
r = 0.819 ***
(f)
glutamine (g/100g protein)
grain filling duration (days)
y = - 0.306x + 41.4
r = - 0.825 ***
(g)
glycine (g/100g protein)
grain filling duration (days)
y = 0.034x + 3.37
r = 0.782 ***
(h)
histidine (g/100g protein)
grain filling duration (days)
y = 0.043x + 1.36
r = 0.857 **
(i)
isoleucine (g/100g protein)
grain filling duration (days)
y = 0.065x + 1.48
r = 0.824 ***
(j)
leucine (g/100g protein)
grain filling duration (days)
y = 0.081x + 2.82
r = 0.886 ***
(k)
lysine (g/100g protein)
grain filling duration (days)
y = 0.056x + 0.38
r = 0.735 ***
(l)
methionine (g/100g protein)
grain filling duration (days)
y = 0.041x + 0.53
r = 0.827 ***
24
29
34
39
44

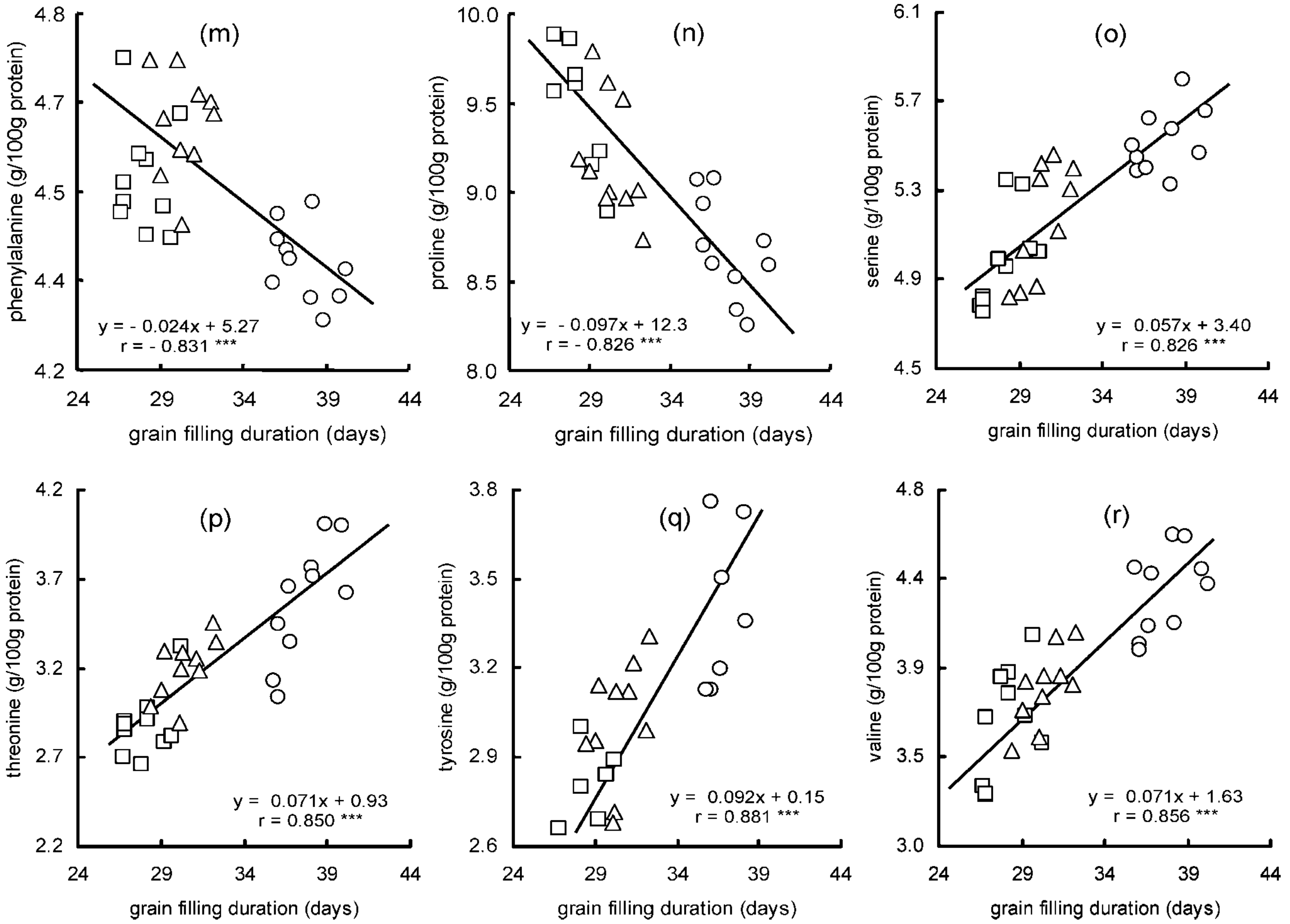


Figure 1. Linear relationships between protein content or amino acid composition and grain-filling duration for 10 durum wheat genotypes grown under three water and temperature regimes in a Mediterranean environment: □, severe rainfed; △, moderate rainfed; ○, irrigated. Data are means of three replications.

Table 4. Regression Equations and Correlation Coefficients (r) between Amino Acid Content (g/100 g of protein) and Grain-Nitrogen Content (% Dry Matter) in 10 Durum Wheat Genotypes Grown under Three Water Regimes in a Mediterranean Environment[a]

	r^b	S.E.	regression equation
alanine	-0.771^{***}	0.12	$AA = 4.25 - 0.47N$
arginine	-0.639^{***}	0.17	$AA = 6.24 - 0.46N$
aspartic acid	-0.665^{***}	0.18	$AA = 6.84 - 0.52N$
cysteine	-0.553^{**}	0.17	$AA = 3.56 - 0.37N$
glutamine	0.733^{***}	1.13	$AA = 21.5 + 4.03N$
glycine	-0.671^{***}	0.14	$AA = 5.56 - 0.44N$
histidine	-0.773^{***}	0.14	$AA = 4.20 - 0.58N$
isoleucine	-0.710^{***}	0.25	$AA = 5.65 - 0.83N$
leucine	-0.663^{***}	0.31	$AA = 7.66 - 0.90N$
lysine	-0.864^{***}	0.17	$AA = 4.6 - 0.97\ \mathrm{N}$
methionine	-0.766^{***}	0.14	$AA = 3.23 - 0.56\ \mathrm{N}$
phenylalanine	0.788^{***}	0.08	$AA = 3.66 + 0.34\ \mathrm{N}$
proline	0.779^{***}	0.33	$AA = 5.74 + 1.36N$
serine	-0.715^{***}	0.22	$AA = 7.06 - 0.73N$
threonine	-0.782^{***}	0.19	$AA = 5.67- 0.98N$
tyrosine	-0.756^{***}	0.30	$AA = 6.02- 1.17N$
valine	-0.805^{***}	0.22	$AA = 6.40 - 0.99N$

[a] *AA*: amino acid; *N*: grain nitrogen content (% DM); SE: standard error of the regression slope. [b] **,*** indicate significant at 0.01 and 0.001 probability level, respectively.

timing (*34*). Therefore, the amino acid composition of the grain varies with the total quantity of nitrogen per grain (*11, 19, 33*). In our study, the relationships between grain-protein content and amino acid composition were positive for glutamine, proline, and phenylalanine but negative for the other amino acids. High nitrogen content of the grain may be due either to better remobilization of nitrogen from the vegetative parts (amino acid resulting from protein hydrolysis) and from direct nitrogen assimilation during grain development. However, factors that influence nitrogen source/sink relationships, leaf senescence, and grain development interact in ways that are not yet well understood.

Under conditions that significantly shorten the duration of grain growth, simultaneously limiting nitrogen uptake during grain-filling, as observed under very dry rainfed conditions in our study, the change in the gliadin:glutenin ratio seems to be a consequence mainly of shifts in the total amount of nitrogen remobilized early to grains, whereas the processes leading to the synthesis of storage proteins seem to be less affected by the total quantity of protein remobilized to grains, as pointed out recently by Martre et al. (*11*). According to these authors, an important practical consequence of these results is that protein fractions and amino acids composition of the bread-wheat grain could be deduced directly from the total nitrogen content per grain. Our results confirm these findings for the grain of durum wheat grown under field conditions and under different temperature and water regimes during grain growth.

In the present experiment, changes in amino acid composition were caused mainly by environmental conditions and particularly water availability and temperature during the grain-filling period, which significantly altered the duration of grain development. Whereas the content of glutamine, phenylalanine, and proline increased with the decline in grain-filling duration, the remaining amino acids tended to diminish, probably because high temperature and drought favored the deposition of gliadins (proteins particularly rich in glutamine and proline), at the expense of albumins and globulins (proteins especially rich in threonine, lysine, methionine, valine, and histidine).

As found in other studies, the negative correlations between the percentage of protein and the content of the essential amino acid appear to indicate that increases in protein content of durum wheat are achieved to detriment of high protein quality in terms of good amino acid balance and especially for lysine, threonine, and valine content. In our study, however, the reduction in lysine due to the increase of up 22.7% in grain-protein content under severe rainfed conditions was not very pronounced (0.32 to 0.29 g per 100 g of flour), whereas the threonine content did not changed (0.45 g/100 g of flour) or even increased in the case of valine (from 0.55 to 0.57 g/100 g of flour). These results agree with opinions of several authors who have pointed out that increasing the protein content could be a useful strategy for improving the nutritional value of cereals grains, despite that the percentage of lysine and other essential amino acids could decreases.

Finally, the results found in the present study concerning the influence of environmental conditions on grain protein and amino acid composition confirm for durum wheat under field conditions some of the previous findings reported for bread wheat but in studies carried out mainly under controlled conditions.

ACKNOWLEDGMENT

The authors thank Dr. Marinetto, J. Aracil, C. Conradi, and A. Cabello for management of field trials and Mr. Alvarez Yaque for its assistance in preparing samples for amino acid determination as well as to Mr. D. Nesbitt for revising the English version of the text. The authors also thank anonymous reviewers 1 and 3 for their valuable comments and suggestions.

LITERATURE CITED

(1) Troccoli, A.; Borrelli, G. M.; De Vita, P.; Fares, C.; Di Fonzo, N. Durum wheat quality: A multidisciplinary concept. *J. Cereal Sci.* **2000**, *32*, 99–113.

(2) Bénétrix, F.; Autran, J. C. Protein synthesis in grains and seeds. In *Nitrogen Assimilation by Plants. Physiological, Biochemical and Molecular Aspects*; Morot-Gaudry, J. F., Ed.;Science Publishers: Enfield, NH, 2001; pp 343–360.

(3) Boila, R. J.; Stothers, S. C.; Campbell, L. D. The relationships between the concentrations of individual amino acids and protein in wheat and barley grain grown at selected locations throughout Manitoba. *Can. J. Anim. Sci.* **1996**, *76*, 163–169.

(4) Lopez-Bellido, L.; Fuentes, M.; Castillo, J. E.; López-Garrido, F. J. Effects of tillage, crop rotation and nitrogen fertilization on wheat-grain quality grown under rainfed Mediterranean conditions. *Field Crop Res.* **1998**, *57*, 265–276.

(5) Rharrabti, Y.; Villegas, D.; García del Moral, L. F.; Aparicio, N.; Elhani, S.; Royo, C. Environmental and genetic determination of protein content and grain yield in durum wheat under Mediterranean conditions. *Plant Breed* **2001**, *120*, 381–388.

(6) Rharrabti, Y.; Royo, C.; Villegas, D.; Aparicio, N.; García del Moral, L. F. Durum wheat quality in Mediterranean environments. I. Quality expression under different zones, latitudes and water regimes across Spain. *Field Crops Res.* **2003**, *80*, 123–131.

(7) Dupont, F. M.; Hurkman, W. J.; Vensel, W. H.; Tanaka, Ch.; Kothari, K. M.; Chung, O. K.; Altenbach, S. B. Protein accumulation and composition in wheat grains: Effects of mineral nutrients and high temperature. *Eur. J. Agron.* **2006**, *25*, 96–107.

(8) Spiertz, J. H. J.; Hamer, R. J.; Xu, H.; Primo-Martin, C.; Don, C.; van der Putten, P. E. L. Heat stress in wheat (*Triticum aestivum* L.): Effects on grain growth and quality traits. *Eur. J. Agron.* **2006**, *25*, 89–95.

(9) Shewry, P. R.; Halford, N. G. Cereal seed storage proteins: Structures, properties and role in grain utilization. *J. Exp. Bot.* **2002**, *53*, 947–958.

(10) Wieser, H.; Seilmeier, W. The influence of nitrogen fertilisation on quantities and proportions of different protein types in wheat flour. *J. Sci. Food Agric.* **1998**, *76*, 49–55.

(11) Martre, P.; Porter, J. R.; Jamieson, P. D.; Triboi, E. Modeling grain nitrogen accumulation and protein composition to understand the sink/source regulations of nitrogen remobilization for wheat. *Plant Physiol.* **2003**, *133*, 1959–1967.

(12) Triboi, E.; Martre, P.; Triboi-Blondel, A. M. Environmentally-induced changes in protein composition in developing grains of wheat are related to changes in total protein content. *J. Exp. Bot.* **2003**, *54*, 1731–1742.

(13) Vensel, W. H.; Tanaka, C. K.; Cai, N.; Wong, J. H.; Buchanan, B. B.; Hurkman, W. J. Developmental changes in the metabolic protein profiles of wheat endosperm. *Proteomics* **2005**, *5*, 1594–1611.

(14) Shewry, P. R.; Napier, J. A.; Tatham, A. S. Seed storage proteins: Structures and biosynthesis. *Plant Cell* **1995**, *7*, 945–956.

(15) Feil, B. The inverse yield-protein relationship in cereals: possibilities and limitations for genetically improving the grain protein yield. *Trends Agron.* **1997**, *1*, 103–119.

(16) Rharrabti, Y.; Elhani, S.; Martos-Nuñez, V.; Garcia del Moral, L. F. Protein and lysine content, grain yield, and other technological traits in durum wheat under Mediterranean conditions. *J. Agric. Food Chem.* **2001**, *49*, 3802–3807.

(17) Sosulski, F. W.; Paul, E. A.; Hutcheon, W. L. The influence of soil moisture, nitrogen fertilization, and temperature on quality and amino acid composition of Thatcher wheat. *Can. J. Soil Sci.* **1963**, *43*, 219–228.

(18) Eppendorfer, W. H. Effects of nitrogen, phosphorus and potassium on amino acid composition and on relationships between nitrogen and amino acids in wheat and oat grain. *J. Sci. Food Agric.* **1978**, *29*, 995–1001.

(19) Mossé, J.; Huet, J. C.; Baudet, J. The amino acid composition of wheat grain as a function of nitrogen content. *J. Cereal Sci.* **1985**, *3*, 115–130.

(20) Approved Methods of AACC, 8th ed.; American Association of Cereal Chemist: St Paul, MN, 1989.

(21) Cohen, S. A.; Meys, M.; Tarvin, T. L. The Pico-Tag Method. A Method of Advanced Techniques for Amino-Acids Analysis; Millipore Corp.: Bedford, MA, 1989.

(22) SAS Institute Inc. The SAS system for Windows. Release. Version 8.0. SAS Institute Inc., Cary, NC, 2000.

(23) Simmonds, N. The relation between yield and protein in cereal grain. *J. Sci. Food Agric.* **1995**, *67*, 309–315.

(24) Bänziger, M.; Fiel, B.; Stamp, P. Competition between nitrogen accumulation and grain-growth for carbohydrates during grain filling of wheat. *Crop Sci.* **1994**, *34*, 440–446.

(25) Fernandez-Figares, I.; Marinetto, J.; Royo, C.; Ramos, J. M.; Garcia del Moral, L. F. Amino-acid composition and protein and carbohydrate accumulation in the grain of triticale grown under terminal water stress simulated by a senescing agent. *J. Cereal Sci.* **2000**, *32*, 249–258.

(26) Campbell, C. A.; Davidson, H. R.; Winkelman, G. E. Effect of nitrogen, temperature, growth stage and duration of moisture stress on yield components and protein content of Manitou spring wheat. *Can. J. Plant Sci* **1981**, *61*, 549–563.

(27) Jenner, C. F.; Ugalde, T. D.; Aspinall, D. The physiology of starch and protein deposition in the endosperm of wheat. *Aust. J. Plant Physiol.* **1991**, *18*, 211–226.

(28) Martin del Molino, I. M.; Rojo, B.; Martinez-Carrasco, R.; Perez, P. Varietal differences in amino acid composition of wheat (*Triticum aestivum* L) grain. *J. Sci. Food Agric.* **1989**, *48*, 177–188.

(29) Panozzo, J. F.; Eagles, H. A.; Wooton, M. Changes in protein composition during grain development in wheat. *Aust. J. Agric. Res.* **2001**, *52*, 485–493.

(30) Daniel, C.; Triboi, E. Effects of temperature and nitrogen nutrition on the grain composition of winter wheat: Effects on gliadin content and composition. *J. Cereal Sci.* **2000**, *32*, 45–56.

(31) Jamieson, P. D.; Stone, P. J.; Semenov, M. A. Towards modelling quality in wheat- from grain nitrogen concentration to protein composition. *Aspects Appl. Biol.* **2001**, *64*, 111–126.

(32) DuPont, F. M.; Hurkman, W. J.; Vensel, W. H.; Chan, R.; Lopez, R.; Tanaka, C. K.; Altenbach, S. B. Differential accumulation of sulphur-rich and sulphur-poor wheat flour proteins is affected by temperature and mineral nutrition during grain development. *J. Cereal Sci.* **2006**, *44*, 101–112.

(33) Martin del Molino, I. M.; Rojo, B.; Martinez-Carrasco, R.; Perez, P. Amino acid composition of wheat grain. 1: Changes during development. *J. Sci. Food Agric.* **1988**, *42*, 29–37.

(34) Altenbach, S. B.; DuPont, F. M.; Kothari, K. M.; Chan, R.; Johnson, E. L.; Lieu, D. Temperature, water and fertilizer influence the timing of key events during grain development in a US spring wheat. *J. Cereal Sci.* **2003**, *37*, 9–20.

Received for review October 26, 2006. Revised manuscript received July 21, 2007. Accepted July 26, 2007. This work was funded by Spanish CICYT AGL2002-04285-C03 and partially by AGL2006-09226-C02-02.

JF063094Q