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Amino-Acid Composition and Protein and Carbohydrate Accumulation in the Grain of Triticale Grown under Terminal Water Stress Simulated by a Senescing Agent

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ABSTRACT

Two field experiments involving two triticale genotypes and the application of a senescing agent (KI) to simulate the effect of a terminal drought stress were conducted at two sites during 1996. Although protein did not differ between genotypes, significant differences were found in methionine and lysine. The percentage of amino acids in the grain increased linearly with grain-protein content, this relationship being non-linear in the same degree for all amino acids. An inverse relationship ($r^2 = 0{\cdot}803$) was found between the rates of carbohydrate and protein accumulation in absence of the senescing agent, indicating competition in the transport of proteins and carbohydrates to the grain. Terminal drought stress induced by KI application increased the amino-acid concentration in the grain, mainly due to a higher protein content. Nevertheless, the amino-acid composition of the protein did not change after this application. The senescing agent significantly reduced dry weight as well as carbohydrate- and protein-accumulation rates in the grain, thus forcing the grain to be filled with carbohydrates assimilated before anthesis. Therefore, the grain-carbohydrate accumulation rate appeared highly dependent ($r^2 = 0{\cdot}884$) of the quantity of nonstructrural carbohydrates available at anthesis. The KI treatments could be of value not only for simulating terminal drought, but also for studying protein and carbohydrate accumulation in small-grain cereals.

Keywords: triticale, amino-acid, carbohydrate-accumulation, senescing agent.

INTRODUCTION

The first man-made cereal, triticale (× *Triticosecale* Wittmack), has considerable potential as a source of energy and protein. Thus, under Mediterranean conditions, triticale grain can provide from 300 kg/ha of crude protein under rainfed conditions to almost 900 kg/ha under irrigation[1]. Whereas the protein in the grain from wheat and barley is low in lysine and other amino acids, such as methionine and threonine, triticale grain contains high levels of lysine and easily digestible protein[2–4]. These nutritional advantages, together with the high grain and protein yields of modern varieties, make triticale a feasible alternative to

ABBREVIATIONS USED: GS = growth stage; HPLC = high performance liquid chromatography.

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traditional grains in the diets of monogastrics[5,6,7].

The protein content in grain of cereals depends upon the genotype and environmental factors, mainly temperature, moisture, soil fertility and N fertilization[8]. Recently, García del Moral *et al.*[1] found that, under Mediterranean conditions, protein content in the grain of triticale was inversely related to the rainfall measured during crop development, mainly due to reduced starch accumulation and decreased grain yield caused by limited moisture during grain filling.

Senescing agents can be used to simulate terminal drought stress on the plant by stopping current photosynthesis during grain filling, thereby forcing grain growth to depend increasingly on the contribution of vegetative reserves. Potassium iodide (KI), considered one of the best senescing agents for field use in cereals because of its efficacy and low toxicity[9], has been successfully used in previous studies for assessing the effects of water-stress on grain growth of triticale under Mediterranean conditions[10,11].

The objectives of the present study were: (1) to assess the differences in amino-acid composition between two genotypes of hexaploid triticale grown under two water regimes in a Mediterranean environment; (2) to determine the relationships between protein and amino-acid concentration; and (3) to analyse the terminal drought effects on protein and carbohydrate accumulation, as well as on amino-acid composition, in the grain.

EXPERIMENTAL

Material and experiments

During 1996, two field experiments were carried out at two sites in Granada, southern Spain (37°08′N, 3°37′W): Vega (600 m, irrigated silty clay; Typic Xerofluvent soil) as an irrigated environment; and Chimeneas (700 m, rainfed silty clay; loamy Calcixerollic Xerochrept soil) as a rainfed environment. Total water received by the crops during the season was 695 and 545 mm in the irrigated and rainfed experiments, respectively. In all cases, Trujillo, the most extensively cultivated triticale in Spain, and T-8-2, a chemically induced mutant from Trujillo, selected for its longer cycle duration, were sown at the end of November in plots of 12 m^2 with six rows 20 cm apart, separated by 0·5 m-wide uncultivated pathways. Each plot received 120, 70 and 60 kg/ha of N, P and K respectively at Vega and 80, 30 and 30 at Chimeneas, according to standard recommendations in the zone. The experimental design at each site was a randomised complete block with four replications.

At each trial the natural field conditions were compared with the senescence effects caused by spraying the entire plant, including the spikes, with potassium iodide (KI) seven days after anthesis (Growth Stage 69[12]) of each genotype. The 0·3% KI solution, containing a surfactant to ensure full wetting of leaves and ears, was hand-sprayed at a rate of 280 mL/m^2. The mean concentrations of amino acids in the triticale grain were reported as milligrams of amino acid per gram dry matter and, on a protein-equivalent basis, as grams of amino acid per 100 g protein.

Analytical methods

For the characterisation of grain growth and dry-weight accumulation, 100 main stems were labelled in each plot at anthesis. Five tagged spikes were removed twice per week from anthesis to physiological maturity (GS 91[12]), and six grains were removed from the central spikelets of each spike. The grains were oven dried at 70 °C for 48 h to determine grain dry weight. The rate of dry-weight accumulation in the grain was obtained as the slope of the linear regression of the grain dry weight against time for the period from anthesis to physiological maturity. Image analysis of five mature grains per plot were carried out as described by García del Moral *et al.*[13] to quantify the proportion of the endosperm, embryo and aleurone layer. Grain-carbohydrate content was then calculated for each plot as the fraction of grain dry weight corresponding to percentage of endosperm, after discounting the appropriate protein content. Daily carbohydrate-accumulation rate was calculated by dividing this value by the duration of the grain-filling period. The protein-accumulation rate was considered to be the difference between the dry weight and carbohydrate-accumulation rates. Nonstructural-carbohydrates content was determined on the five stems sampled at anthesis following the method of McCaig and Clarke[14].

From each plot a grain sample was cleaned by passing it through a series of sieves and analysed individually. Chemical analyses from the Association of Official Analytical Chemists[15] included

dry matter (method 7.003), and Kjeldahl nitrogen (method 7.015). The crude protein of grain was estimated as nitrogen multiplied by 5.70. Amino acids were analysed quantitatively with high performance liquid chromatography (HPLC) using the Waters Pico-Tag Method, which involves pre-column derivatization with phenylisothiocyanate[16]. Protein was hydrolysed in 6 M hydrochloric acid + 1% phenol in sealed evacuated tubes at 110 °C for 24 h. Cysteine and methionine were determined as cysteic acid and methionine sulphone, respectively, obtained by oxidation with performic acid before 6 M HCl hydrolysis[17]. Tryptophan was not determined. α-aminoadipic acid was used as an internal standard.

Statistical analysis

The results were evaluated by means of integrative analysis of variance using the General Linear Model procedure, considering as main factors the site, genotype, treatment and replication (within each site). The significance of differences between means was determined using Duncan's multiple-range test. Error variance was derived from the table of analysis of variance. Correlation coefficients were calculated to detect significant relationships between the parameters under study.

RESULTS AND DISCUSSION

Genotype differences

The mean protein concentration did not differ between genotypes ($p<0{\cdot}05$; Table I). In contrast, the mean methionine concentration (expressed as mg amino acid/g d.m.) or methionine, histidine and lysine (expressed as g/100 g protein) significantly differed between genotypes. The protein in genotype T-8-2 was equal to or better than the protein in genotype Trujillo, in meeting the requirements for dietary indispensable amino acids of swine and poultry[18,19], especially for methionine and lysine. The availability of genotypes, such as those with a much higher concentration of essential amino acids[20], would allow for a differentiation between genotypes on the basis of the amino-acid content.

Environmental effects

The influence of environment on protein content and amino-acid composition was lower than that exerted by genotypes (Table II). The results, however, indicate that, in general, protein content and grain amino-acid composition tended to be slightly higher in the rainfed environment. Threonine was the only amino acid that showed statistical differences between environments both in grain and in protein (Table II), registering significantly higher values under rainfed conditions.

Linear relationships

In this study, crude protein in the grain ranged from 8·51 to 17·21%. Since the r^2 for the linear relationships between the percentage of a given amino acid and the percentage of protein ranged from 0.21 to 0.94 (left side in Table III), this relationship was not linear to the same degree for all amino acids in the grain. Thus, slopes in the regression equations indicated that glutamic acid was the amino acid most stimulated by an increase in grain-protein content, whereas methionine was the least affected.

A second set of linear-regression equations (Table III, right side) was also determined for the relationship between the concentration of amino acid expressed as g/100 g protein and the percentage of protein. Except for lysine, threonine, histidine, alanine and serine, the slopes for each of the amino acid-protein relationships were significantly different ($p<0{\cdot}05$) from zero. The quantity of aspartic and glutamic acid, as well as proline, showed an inverse relationship with percentage of protein. Nevertheless, the r^2 were 0.20 or lower, with numerous values less than 0.10. These regression equations with low r^2 indicate that for most of the amino acids the change in percentage of amino acid in a grain is relatively consistent with a change in percentage concentration of protein; that is, the amino-acid composition of the protein is independent of its amount in the grain.

Equations, which estimate the percentage of amino acid using the protein percentage as the independent variable, have been derived in order to estimate the amino-acid concentrations in a feedstuff[21]. The need for equations to predict the percentage of amino acid in cereals on the basis of a chemical analysis by the protein percentage must be questioned if the concentration of an amino acid in the protein does not change markedly with an increase in concentration of protein in a grain. The low r^2 for the linear regression of grams of amino acid per 100 g protein versus the

Table I Amino-acid composition of grain and protein of two triticale genotypes

	mg aa/g d.m.			g/100 g protein		
	T-8-2	TRUJILLO	Standard error	T-8-2	TRUJILLO	Standard error
Alanine	3·78[a]	3·90[a]	0·23	3·82[a]	3·98[a]	0·18
Arginine	5·35[a]	5·50[a]	0·16	5·43[a]	5·63[a]	0·12
Aspartic acid	11·27[a]	10·66[a]	0·40	11·44[a]	10·93[a]	0·38
Glutamic acid	34·35[a]	35·31[a]	1·10	34·79[a]	36·12[a]	0·52
Glycine	3·61[a]	3·48[a]	0·13	3·64[a]	3·55[a]	0·03
Histidine	2·09[a]	2·28[a]	0·11	2·12[b]	2·33[a]	0·06
Isoleucine	3·05[a]	2·76[a]	0·22	3·07[a]	2·79[a]	0·13
Leucine	4·94[a]	4·74[a]	0·38	4·98[a]	4·83[a]	0·32
Lysine	2·27[a]	1·97[a]	0·13	2·29[a]	1·98[b]	0·08
Methionine	2·02[a]	0·93[b]	0·09	2·04[a]	0·94[b]	0·08
Phenylalanine	3·42[a]	2·98[a]	0·24	3·44[a]	3·02[a]	0·18
Proline	8·14[a]	8·57[a]	0·25	8·24[a]	8·76[a]	0·26
Serine	5·29[a]	5·51[a]	0·19	5·34[a]	5·63[a]	0·07
Threonine	4·47[a]	4·14[a]	0·17	4·52[a]	4·25[a]	0·13
Tyrosine	2·12[a]	2·42[a]	0·12	2·14[a]	2·48[a]	0·13
Valine	2·73[a]	2·72[a]	0·17	2·78[a]	2·77[a]	0·11
SUM AA	97·82[a]	97·41[a]	3·10	87·83[a]	89·61[a]	3·08
CP %	11·21[a]	11·12[a]	0·37	—	—	—

[a,b] Means ($n=8$) within a row for mg aa/g d.m. or g/100 g protein bearing different superscripts are significantly different ($p<0·05$).

Table II Amino-acid composition of grain and protein of triticale grown under irrigated and rainfed conditions

	mg aa/g d.m.			g/100 g protein		
	Irrigated	Rainfed	Standard error	Irrigated	Rainfed	Standard error
Alanine	3·93[a]	3·77[a]	0·23	4·07[a]	3·77[a]	0·18
Arginine	5·26[a]	5·58[a]	0·16	5·47[a]	5·66[a]	0·12
Aspartic acid	10·99[a]	10·94[a]	0·40	11·31[a]	11·06[a]	0·38
Glutamic acid	34·74[a]	34·92[a]	1·10	35·97[a]	35·52[a]	0·52
Glycine	3·58[a]	3·52[a]	0·13	3·67[a]	3·56[a]	0·03
Histidine	2·08[a]	2·31[a]	0·11	2·18[a]	2·32[a]	0·06
Isoleucine	2·82[a]	2·99[a]	0·22	2·88[a]	2·98[a]	0·13
Leucine	4·83[a]	4·86[a]	0·38	4·97[a]	4·87[a]	0·32
Lysine	2·01[a]	2·21[a]	0·13	2·06[a]	2·21[a]	0·08
Methionine	1·51[a]	1·44[a]	0·09	1·56[a]	1·43[a]	0·08
Phenylalanine	3·17[a]	3·22[a]	0·24	3·26[a]	3·22[a]	0·18
Proline	8·37[a]	8·34[a]	0·25	8·70[a]	8·47[a]	0·26
Serine	5·26[a]	5·54[a]	0·19	5·47[a]	5·58[a]	0·07
Threonine	4·02[b]	4·59[a]	0·17	4·01[b]	4·68[a]	0·13
Tyrosine	2·18[a]	2·36[a]	0·12	2·27[a]	2·39[a]	0·13
Valine	2·72[a]	2·73[a]	0·17	2·78[a]	2·76[a]	0·11
SUM AA	96·72[a]	98·50[a]	3·10	97·52[a]	99·23[a]	3·08
CP %	11·11[a]	11·30[a]	0·37	—	—	—

[a,b] Means ($n=8$) within a row for mg aa/g d.m. or g/100 g protein bearing different superscripts are significantly different ($p<0·05$).

Table III Linear regression between amino-acid composition and protein content in the grain of two triticale genotypes

	Linear regression[a]									
	mg amino acid/g d.m. vs % protein					g amino acid/100 g protein vs % protein				
	y intercept		Slope			*y* intercept		Slope		
Amino acid	*a*	SE	*b*	SE	r^2	*a*	SE	*b*	SE	r^2
Alanine	0·167	0·389	0·303	0·030	0·78	3·474	0·282	0·022	0·022	0·03
Arginine	−0·828	0·517	0·535	0·040	0·85	5·157	0·449	0·040	0·036	0·04
Aspartic acid	1·491	0·866	0·778	0·069	0·83	12·753	0·795	−0·154	0·062	0·19
Glutamic acid	3·531	1·711	2·636	0·138	0·93	38·654	1·648	−0·249	0·133	0·12
Glycine	−0·731	0·256	0·362	0·020	0·92	3·080	0·217	0·047	0·017	0·20
Histidine	0·194	0·180	0·168	0·014	0·84	2·460	0·201	−0·015	0·016	0·03
Isoleucine	−0·080	0·420	0·239	0·033	0·81	2·245	0·418	0·049	0·033	0·07
Leucine	−0·733	0·585	0·454	0·045	0·77	4·137	0·553	0·052	0·043	0·05
Lysine	−0·692	0·482	0·221	0·028	0·56	2·275	0·357	−0·016	0·028	0·01
Methionine	−0·050	0·300	0·064	0·024	0·21	−0·998	0·677	0·145	0·053	0·20
Phenylalanine	0·318	0·587	0·232	0·047	0·46	3·00	0·508	0·008	0·040	0·13
Proline	0·677	0·753	0·626	0·059	0·83	9·390	0·897	−0·108	0·070	0·08
Serine	−0·248	0·293	0·477	0·023	0·94	5·497	0·217	0·0025	0·0169	0·00
Threonine	1·032	0·547	0·266	0·044	0·58	4·257	0·684	0·0110	0·053	0·00
Tyrosine	−0·600	0·504	0·250	0·040	0·57	1·921	0·482	0·041	0·038	0·04
Valine	−0·677	0·354	0·287	0·028	0·79	2·030	0·317	0·0636	0·0251	0·19

[a] Linear equations are in the format $y = a + bx$, where y is the dependent variable, mg amino acid per gram or g amino acid per 100 g protein; a is the intercept, b is the slope of the line; and x is the independent variable, percent protein; r^2 is the proportion of the variance in amino-acid concentration that can be attributed to its linear regression on the concentration of protein.

protein percentage for all amino acids calculated in this study (Table III) reveals that the concentration of the amino acids in the protein is quite constant. The alternative to these regression equations is to estimate the concentration of amino acid as grams of amino acid/100 g protein, in order to quantify the amino-acid composition from a given grain-protein content. The use of such equations may provide an estimate that is no better than expressing the concentration of an amino acid as g/100 g protein with its associated variation[22]. Regression equations, when there is a low r^2, are not useful because the concentrations of the amino acids in the protein do not change markedly as the concentration of protein in the grain increases (Table III).

Protein in a grain is composed of numerous fractions that constitute the storage and metabolically active proteins located in the cells of the grain[20,23]. Each of these fractions has a unique amino-acid composition that may differ from that in the grain. A change in balance between protein fractions differing in amino-acid content results in a change in concentration of amino acids in the grain. In our case, imbalances did not appear between protein fractions in the grain. No other studies to our knowledge have developed equations to predict the amino-acid concentrations in triticale grown under Mediterranean conditions.

Senescing agent

The effect of KI, detected one week after the treatment, took the form of yellowing of the leaves and spikes. Table IV shows the average values of the amino-acid composition of grain and protein content.

The chemical treatment significantly raised the protein and amino-acid concentration (expressed as mg aa/g d.m) for all amino acids in the grain, with the exception of methionine ($p<0·05$). This result was apparently due to a lack of carbohydrates caused by the inhibition of photosynthesis, and also to the fact that the main source of the grain protein in temperate cereals is protein in leaves, particularly the enzyme RuBisCo[24], the hydrolysis of which is enhanced by KI application.

When the data for amino acids were expressed on a protein-equivalent basis (g/100 g protein),

Table IV Amino-acid composition of grain and protein of triticale untreated (control) or treated with a senescing agent (KI)

	mg aa/g d.m.			g aa/100 g protein		
	KI	CONTROL	Standard error	KI	CONTROL	Standard error
Alanine	5·17[a]	3·85[b]	0·20	3·97[a]	3·90[a]	0·19
Arginine	7·61[a]	5·42[b]	0·20	5·78[a]	5·53[a]	0·12
Aspartic acid	14·25[a]	10·97[b]	0·32	10·9[a]	11·20[a]	0·28
Glutamic acid	46·45[a]	34·84[b]	0·89	35·53[a]	35·46[a]	0·56
Glycine	4·88[a]	3·54[b]	0·15	3·70[a]	3·60[a]	0·06
Histidine	3·00[a]	2·19[b]	0·01	2·30[a]	2·23[a]	0·05
Isoleucine	3·74[a]	2·91[b]	0·21	2·82[a]	2·93[a]	0·12
Leucine	6·31[a]	4·84[b]	0·27	4·80[a]	4·91[a]	0·17
Lysine	2·79[a]	2·11[b]	0·17	2·08[a]	2·14[a]	0·10
Methionine	2·69[a]	1·47[a]	0·59	1·94[a]	1·49[a]	0·38
Phenylalanine	4·22[a]	3·192[b]	0·29	3·18[a]	3·23[a]	0·17
Proline	10·34[a]	8·36[b]	0·38	7·86[b]	8·50[a]	0·25
Serine	7·28[a]	5·40[b]	0·20	5·54[a]	5·49[a]	0·08
Threonine	5·83[a]	4·31[b]	0·20	4·44[a]	4·40[a]	0·15
Tyrosine	3·09[a]	2·27[b]	0·20	2·48[a]	2·31[a]	0·11
Valine	3·69[a]	2·72[b]	0·14	2·81[a]	2·76[a]	0·09
SUMAA	131·32[a]	98·41[b]	2·93	91·4[a]	89·8[a]	1·63
CP %	14·90[a]	11·21[b]	0·31	—	—	—

[a,b] Means ($n=16$) within a row for mg aa/g d.m. or g/100 g protein bearing different superscripts are significantly different ($p<0·05$).

the mean concentrations did not differ between control and treated plants (right part of Table IV). There was no apparent change in the profile of storage proteins located in the cells of the grain, except for proline, considering that each fraction has a unique amino-acid composition.

Grain yield and rate of carbohydrate and protein accumulation

Grain yield per hectare was 17·6% higher in the genotype Trujillo (Table V), due to a higher mean grain weight and longer grain-filling period. The irrigated environment produced an 81·2% higher grain yield than the rainfed one. This increase was due to a significantly higher number of spikes per m^2 (63·2%, data not shown) and number of grains per spike (35·3%, data not shown), given that no statistical differences appeared in the mean grain weight nor grain-filling period between environments. The KI application reduced grain yield by 56·7% in relation to control, mainly due to a limited supply of photosynthates that diminished grain weight by 42·0%. These results agree with those of Royo *et al.*[10,11], who also found reductions in grain yield and mean grain weight of triticale grown under Mediterranean conditions, in response to natural drought and KI application.

There were not significant differences in the rate of dry-weight accumulation between genotypes or environments (Table V). The carbohydrate-accumulation rate, however, was significantly higher for genotype Trujillo, in comparison with genotype T-8-2 (Table V), but the protein-accumulation rate in this last genotype was higher than in Trujillo. This fact explains the higher grain-protein content found in genotype T-8-2 in comparison to genotype Trujillo (Table I). In the rainfed environment, the carbohydrate-accumulation rate was significantly higher than in the irrigated one, the opposite being true for protein-accumulation rate (Table V). The effect of a KI spraying was to reduce, to a similar extent, both the carbohydrates and protein-accumulation rates; as a result, the rate of dry-weight accumulation and the final grain weight significantly diminished in relation to controls (Table V). Protein content, however, increased in the grain of treated plots due mainly to the enhanced degradation in RuBisCo, as discussed above.

A strongly significant inverse relationship (Fig. 1) was found between the rates of carbohydrate

Table V Mean values* of grain yield, grain weight, grain filling period, and rates of accumulation in dry weight, carbohydrates and proteins in the grain of triticale.

	Genotype (†)			Environment (‡)			Treatment (‡)		
	T-8-2	Trujillo	Standard error	Irrigated	Rainfed	Standard error	KI	Control	Standard error
Grain Yield (dm, kg/ha)	5447[b]	6468[a]	110	7645[a]	4270[b]	110	2687[a]	5958[b]	79·6
Grain Weight (dm, mg)	39·0[b]	43·8[a]	1·01	40·8[a]	42·0[a]	1·01	24·0[a]	41·4[b]	0·62
Grain filling period (d)	28·1[b]	31·9[a]	0·13	30·6[a]	29·4[a]	0·13	—	—	—
Dry weight accumulation rate (mg/d)	1·612[a]	1·655[a]	0·02	1·633[a]	1·634[a]	0·02	0·777[a]	1·635[b]	0·02
Carbohydrate accumulation rate (mg/d)	1·102[b]	1·305[a]	0·02	1·152[b]	1·255[a]	0·02	0·566[a]	1·204[b]	0·01
Protein accumulation rate (mg/d)	0·513[b]	0·350[a]	0·01	0·480[a]	0·384[b]	0·01	0·211[a]	0·432[b]	0·01
Carbohydrates at anthesis (mg/g)	291·2[a]	282·9[a]	10·5	259·5[b]	314·5[a]	10·5	282·0[a]	287·0[a]	7·78

* Least squares means were calculated for 8 (†) or 16 (‡) samples of each cultivar.
[a,b] Means within a row in each section bearing different superscripts are significantly different ($p<0·05$).

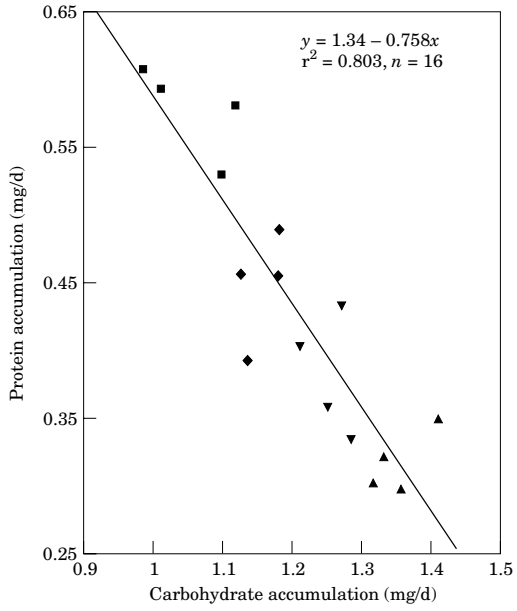

Figure 1 Regression of protein-accumulation rate on carbohydrate-accumulation rate in the grain of two genotypes of triticale not treated with KI, under irrigated (I) or rainfed (R) conditions. ■: T-8-2 (I); ▼: Trujillo (I); ◆: T-8-2 (R); ▲: Trujillo (R).

and protein accumulation in the absence of the senescing agent (control plants), indicating the existence of a competition in the transport of proteins and sugars to the grain of triticale under Mediterranean conditions. On the average, an increase of 1 mg/day in carbohydrate-accumulation rate was associated with a decrease of 0·76 mg/day in the respective protein-accumulation rate. This competition could be responsible for the negative relationship between grain yield and grain-protein content commonly found in triticale[1,25] and in other cereals, such as barley and wheat[24,26], but whose physiological causes remained still unascertained (see for this aspect the excellent summary in Simmonds, 1995[27]). It is argued that N accumulation and carbohydrate synthesis compete for energy and carbon skeletons during the reproductive growth phase of wheat[27,28]. Penning de Vries *et al.*[29] calculated that, in theory, the assimilation of 1 g of N would require 11·8 g of carbohydrates, although in the study of Bänzinger *et al.*[28], using four genotypes of wheat and three N treatments, a ratio of 8·5 : 1 was calculated. In the present study, the negative relationship in remobilisation of proteins and carbohydrates could be caused, moreover, by

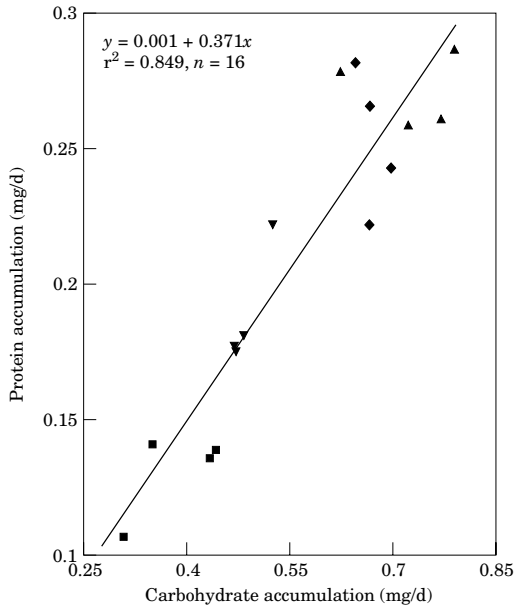

Figure 2 Regression of protein-accumulation rate on carbohydrate-accumulation rate in the grain of two genotypes of triticale treated with KI seven days after anthesis, under irrigated or rainfed conditions. Symbols as in Figure 1.

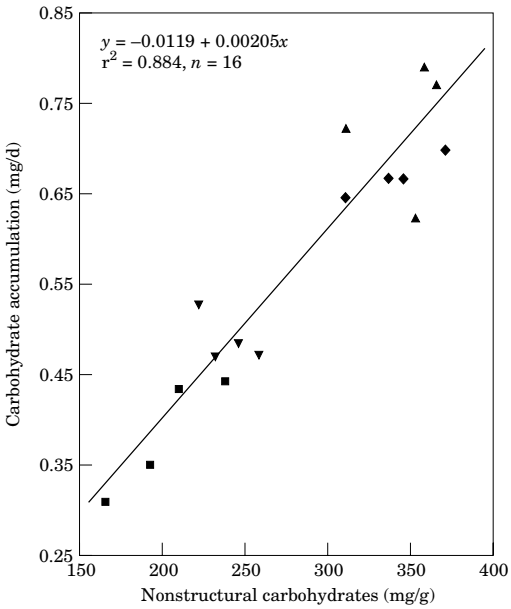

Figure 3 Relationship between carbohydrate-accumulation rate in the grain of triticale and nonstructural-carbohydrate content at anthesis in plants treated with KI seven days after anthesis, under irrigated or rainfed conditions. Symbols as in Figure 1.

the fact that the photosynthetic rate of leaves can show an acute feedback response to internal changes in the demand for assimilates[26]. In this way, conditions that promote high rates of carbohydrate accumulation in the grain tend to delay leaf senescence and the onset of RuBisCo hydrolysis, thus limiting the N available to be remobilised to the grain. In the plants treated with KI, on the contrary, carbohydrates and protein-accumulation rates appeared directly related (Fig. 2), due to the fact that after KI treatment the enzyme RuBisCo was rapidly degraded, thus favoring higher N redistribution to growing grains.

Nonstructural carbohydrates at anthesis

Since soluble carbohydrates in the stem represent the bulk of carbohydrate reserves of cereals during grain filling[24,30], the quantity of nonstructural carbohydrates at anthesis was used to assess the carbohydrate status of the plants at the beginning of grain filling. Whereas no statistical differences were found between genotypes, nonstructural carbohydrates were significantly higher in the rainfed environment (Table V), probably due to an osmotic effect, because soluble carbohydrates usually accumulate in the stem of cereals in response to periods of limited moisture in the soil[31]. To investigate the influence of nonstructural carbohydrates on grain growth, we computed a regression both for controls and treated plants. The results indicated (Fig. 3) that, after inhibition of photosynthesis caused by KI, grain-carbohydrate accumulation was highly dependent on the quantity of nonstructural carbohydrates available at anthesis ($r^2 = 0{\cdot}884$, $n = 16$). On the average, for each 100 mg/g of increase in nonstructural-carbohydrate content at anthesis, the carbohydrate-accumulation rate in the grain could increase by 0·193 mg/g. This relationship, however, was not significant in the absence of KI ($r^2 = 0{\cdot}013$, $n = 16$), suggesting that in the absence of stress conditions, most grain growth in triticale in Mediterranean environments is based on current photosynthesis, as observed in other small cereals[24,26].

CONCLUSIONS

The concentration of methionine and lysine in the protein differed between the grains from selected genotypes of triticale grown under Mediterranean conditions, being higher for genotype T-8-2. The percentage of amino acids in triticale increased linearly as the concentration of protein increased,

with r^2 ranging from 0·21 to 0·94. These ranges for r^2 indicated that the relationship was not linear to the same degree for all amino acids. Equations to estimate the amino-acid content of triticale could be of restricted use, because there is not a marked change in amino-acid concentration in the protein as the concentration of protein increases. Indeed, the use of these equations when expressing mean concentrations of amino acids in the protein as g/100 g protein must be tempered, because the variation in the estimate provided by the equations may be no different than the associated error. The use of KI, as a simulator of terminal drought, increases the amino-acid concentration of the grain, explained by a higher protein content, lower grain weights and lesser proportion of endosperm in the treated grains. Nevertheless, the amino-acid composition of the protein expressed on a protein-equivalent basis did not differ between treatments.

Under non-stress conditions, carbohydrates and protein-accumulation rates showed an inverse relationship, probably due both to competition between N and carbohydrates for energy and carbon skeletons, as well as to a delay in leaf-protein hydrolysis to maintain photosynthetic rate in response to a high demand of assimilates by growing grains. The KI application significantly reduced dry weight, carbohydrates and protein-accumulation rates, forcing the grain to be filled with carbohydrates assimilated before anthesis. In this way, in the plants treated with KI, the accumulation rate of grain carbohydrate proved highly dependent on the quantity of nonstructrural carbohydrates available at anthesis. From data presented in this study, we also conclude that KI treatments could be of value not only to simulate terminal drought, but also to study protein and carbohydrate accumulation in the grain of small cereals.

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