Environmental Factors Determining Carbon Isotope Discrimination and Yield in Durum Wheat under Mediterranean Conditions

J. L. Araus,* D. Villegas, N. Aparicio, L. F. García del Moral, S. El Hani, Y. Rharrabti,
J. P. Ferrio, and C. Royo

ABSTRACT

The effect of environment on the relationship between grain carbon isotope discrimination (Δ) and yield was studied for durum wheat (*Triticum turgidum* L. var. *durum*) under Mediterranean conditions. A group of 25 genotypes was grown under contrasting water regimes in two regions of Spain during three years. The first objective was to determine the environmental factors responsible for the strong positive relationship previously observed between Δ and yield across trials. Environmental factors tested were total water input (W_i), mean temperature, accumulated reference evapotranspiration (ET_0), and the ratio W_i/ET_0 during different periods of the crop cycle. Water input during grain filling was the variable most strongly correlated with grain Δ and yield across all the trials, as well as across the subset of trials in northeastern Spain. In southeastern Spain, the most drought prone of the two regions, W_i from sowing to heading explained the most variation in grain Δ and yield. The second objective was to study the effect of environment on the relationship between Δ and yield across genotypes. No significant correlation was found for trials with a mean yield up to about 2000 kg ha^{-1}, but the strength of the relationship increased sharply and attained significance in trials yielding 2500 kg ha^{-1}. When yield above 2500 kg ha^{-1} the correlation between Δ and yield remained relatively steady and positive, with an *r* value around 0.5. It is concluded that breeding to raise durum wheat yield in Mediterranean conditions could take advantage of selecting for higher Δ only in relatively wet years or under supplementary irrigation.

DROUGHT, defined as water deficit, and often combined with high temperature stress, is one of the greatest constraints to cereal grain yield in Mediterranean areas. For crops such as durum wheat, grown under rainfed conditions, agricultural practices are not sufficient to mitigate the effect of drought, and plant breeding has become the best tool for yield increases (Srivastava, 1991; Acevedo, 1991; Slafer et al., 1994; Ceccarelli and Grando, 1996; Royo et al., 1998).

Carbon isotope discrimination (Δ), when measured in plant dry matter, integrates transpiration efficiency, the ratio of net photosynthesis to water transpired, over the period during which the dry matter is assimilated, Δ and transpiration efficiency being negatively related (Farquhar and Richards, 1984; Condon et al., 1990). On dry matter basis, Δ has been proposed as a breeding criterion for increasing yield in temperate cereals and other crops, under either favorable or drought stress conditions. Although water status during growth dramatically affects yield and Δ, a specific environmental variable responsible for the positive relationship usually found between Δ and yield across growing conditions (i.e., trials) has not been unequivocally identified (Farquhar and Richards, 1984; Craufurd et al., 1991; Acevedo, 1993; Stewart et al., 1995; Araus et al., 1999a,b).

For breeding purposes, it is crucial to assess how the growing environment affects the relationship between Δ and yield across genotypes (Acevedo, 1993; Condon and Richards, 1993; Richards, 1996; Araus et al., 1999b). Patterns in this relationship may be masked by phenological differences among genotypes that may affect yield and also Δ, especially in drought-prone environments. For example, under Mediterranean conditions those genotypes with fewer days from sowing to heading or to anthesis show higher Δ values (Craufurd et al., 1991; Richards and Condon, 1993; Richards, 1996; Araus et al., 1998) probably because they attain grain filling with more water in the soil, whereas the evapotranspirative demand is lower. Nevertheless, in bread wheat (*Triticum aestivum* L., Sayre et al., 1995) and durum wheat (Araus et al., 1998) large genotypic variability in Δ, independent of phenology, has been reported.

This study was performed on a large collection of durum wheat genotypes cultivated in a wide range of Spain that differ in drought severity. The objective was to determine whether there is a common specific environmental variable which simultaneously affects the Δ of mature kernels and yield. That information may indicate the basis of the widely reported strong positive relationship between yield and Δ across trials. An additional objective was to assess how growing conditions affect the strength and sign (positive or negative) of the relationship between Δ and yield across genotypes.

MATERIALS AND METHODS

Plant Materials and Experimental Design

A total of 12 field trials were performed from 1997 to 1999 in two Mediterranean regions, northeastern (NE) and southeastern (SE) Spain, and under two contrasting water regimes (rainfed and irrigated) within each region. The trial locations and soil characteristics are summarized in Table 1, and environmental conditions are detailed in Table 2. Appropriate fertilization was provided to the seed bed, and trials were top-dressed at the onset of jointing depending on the

---

J.L. Araus and J.P. Ferrio, Unitat de Fisiologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain; D. Villegas, N. Aparicio and C. Royo, Area de Conreus Extensius, Centre UdL-IRTA, Alcalde Rovira Roure 191, 25198 Lleida, Spain; L.F. García del Moral, S. El Hani and Y. Rharrabti, Departamento de Biología Vegetal, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain. Received 5 Feb. 2002. *Corresponding author (josel@porthos.bio.ub.es).

Published in Crop Sci. 43:170–180 (2003).

Abbreviations: Δ, grain carbon isotope discrimination; CIMMYT, Centro Internacional de Mejoramiento de Maíz y Trigo; DA, days from sowing to anthesis; DH, days from sowing to heading; ET_0, accumulated reference evapotranspiration; ICARDA, International Center for Agricultural Research in the Dry Areas; NE-, northeastern; SE-, southeastern; W_i, total water input.

Table 1. Description and location of the sites used in the study. Cultivation was performed in two sites from NE Spain (Lleida province, Catalonia) and SE Spain (Granada province, East-Andalusia).

Site	Region	Water Regime	Coordinates	Altitude	Soil type (USDA)	Soil texture	Soil pH	Soil texture		
								Sand	Silt	Clay
				m above sea level					%	
Gimenells	NE Spain	Irrigated	41° 40′ N; 0° 20′ E	200	Calcixerolic-Xerochrept	fine-loamy	8.1	31.0	39.0	30.0
El Canós	NE Spain	Rainfed	41° 41′ N; 1° 13′ E	440	Fluventic-Xerochrept	loamy-fine	8.2	34.1	47.1	18.8
Granada	SE Spain	Irrigated	37° 21′ N; 3° 35′ W	650	Typic Xerofluvent	silty clay	8.0	40.0	49.6	10.4
Ventas de Huelma	SE Spain	Rainfed	37° 10′ N; 3° 50′ W	720	Loamy Carcixerolic Xerochrept	silty clay	8.2	17.8	54.9	27.3

water status of the crop. When applied, supplementary irrigation was given during late winter and spring (Table 2). Presowing subsoil moisture was not relevant for any of the trials assayed.

Trials consisted of 25 durum wheat genotypes grown in randomized complete blocks with four replicates in plots of 12 m^2 (six rows, 0.20 m apart). These genotypes included four commercial Spanish cultivars (Altar-aos, Jabato, Mexa, and Vitrón), and 21 genotypes from the eastern Mediterranean basin, including commercial cultivars and advanced lines of the CIMMYT/ICARDA durum wheat breeding program (Awalbit, Bicrecham-1, Chacan, Chahra-1, Haurani, Korifla, Krs/Haucan, Lagost-3, Lahn/Haucan, Massara-1, Moulchahba-1, Mousabil-2, Omlahn-3, Omrabi-3, Omruf-3, Quadalete//Erp/Mal, Sebah, Stojocri-3, Waha, Zeina-1, and Zeina-2). The genotypes were chosen to represent a wide range of genetic variability in terms of agronomical characteristics. Moreover, to minimize the potential interference of phenology on yield and Δ, these genotypes were selected with a relatively narrow range of variability in the number of days from planting to heading, days to anthesis, and days to physiological maturity (a mean range of 7 and 6 d for heading and anthesis dates, respectively). The times from sowing to heading (DH) and anthesis (DA) were recorded when more than half the plants in a plot reached the stages 55 and 65 of Zadoks' scale (Zadoks et al., 1974), respectively. Thermal time was calculated in growing degree-days (GDD) by summing the daily values of mean temperature, with a base temperature of 0°C (Gallagher, 1979). Physiological maturity was recorded when most of the plants had reached Zadoks' stage 87, representing kernels in hard dough stage. Plots were harvested mechanically when the grain was ripe, yield (kg ha^{-1}) expressed at a 100 g kg^{-1} moisture content (Table 2).

Environmental Parameters

For each trial, W_i was calculated as the sum of rainfall and irrigation (when applicable) for the following periods: sowing to heading, sowing to anthesis, sowing to maturity, heading to anthesis, heading to one week after anthesis, heading to maturity, anthesis to maturity (grain filling), and from one week after anthesis to maturity. Mean temperature and reference ET_0 for the same periods were also assessed. ET_0 was calculated from the mean maximal and minimal average temperatures of each period using the PC-program ETO (Subzero Evapotranspiration) (Snyder and Pruitt, 1991) version 1.04 (revised in February, 1994).

Carbon Isotope Discrimination

For each plot, a sample of about 2 g of mature kernels was oven dried and finely ground (mesh diameter of 0.5 mm). The $^{13}C/^{12}C$ ratio of samples was subsequently determined by mass spectrometry at the Serveis Científico-Tècnics de la Universitat de Barcelona, Spain. Samples of 0.7 to 0.9 mg were combusted in an elemental analyzer (EA1108, Series 1, Carlo Erba Instrumentazione, Milan, Italy) and the $^{13}C/^{12}C$ ratio was measured with an isotope ratio mass spectrometer (Delta C, Finnigan Mat, Bremen, Germany) operated in continuous flow mode. A system check for elemental analyses was achieved with an interspersed working standard of atropine. Stable carbon isotope composition was expressed as $\delta^{13}C$ values (Farquhar et al., 1989), where $\delta^{13}C(‰) = [(R\ sample/R\ standard)-1] \times 1000$,

Table 2. Growing conditions and main agronomical characteristics of the trials performed during this study. Days from sowing to either heading (DH) or anthesis (DA), total water input (Wi), reference evapotranspiration (ET_0), carbon isotope discrimination of kernels (Δ).

Environment	Year	Sowing date	DH	DA	Thermal time to heading	Thermal time to anthesis	W_i		Mean Temperature	Seasonal ET_0	Wi/ET_0	Δ	Yield	Thousand kernel weight
							Rainfall	Irrigation (times)						
			d		gdd		Mm		°C	mm		‰	kg ha^{-1}	g
NE Spain irrigated	1997	3 Dec. 96	135	143	1200	1300	258	150 (3)	11.5	497	0.82	18.36 b	4964 c	45.1 e
	1998	23 Nov. 97	150	157	1310	1411	285	100 (2)	10.3	414	0.93	16.74 e	5192 b	46.7 d
	1999	10 Nov. 98	162	172	1231	1369	255	150 (3)	9.6	482	0.84	18.72 a	7009 a	54.0 a
NE Spain rainfed	1997	3 Dec. 96	138	144	1260	1334	230	0	10.8	401	0.57	14.59 i	2062 g	42.5 f
	1998	17 Nov. 97	155	163	1294	1402	183	0	9.8	390	0.47	14.63 hi	2531 f	47.8 c
	1999	3 Nov. 98	175	184	1351	1496	256	0	10.0	473	0.54	17.10 d	3820 e	51.7 b
SE Spain irrigated	1997	5 Feb. 97	86	92	1149	1242	173	200 (2)	15.5	487	0.77	17.00 d	2624 f	37.7 h
	1998	11 Dec. 97	129	136	1296	1406	311	100 (1)	13.3	574	0.72	17.48 c	4312 d	46.4 d
	1999	15 Dec. 98	124	138	1157	1356	128	300 (3)	13.1	559	0.77	16.34 f	3628 e	31.3 i
SE Spain rainfed	1997	13 Dec. 96	84	93	1182	1326	134	0	16.0	490	0.27	15.51 g	1853 h	42.3 f
	1998	21 Jan. 98	116	122	1434	1538	180	0	14.6	508	0.35	15.62 g	2053 gh	40.1 g
	1999	25 Nov. 98	147	166	1394	1702	193	0	12.3	537	0.36	14.74 h	2547 f	42.4 f

and R is the $^{13}C/^{12}C$ ratio. Secondary standards of graphite, sucrose, and polyethylene foil (IAEA, Vienna, Austria) calibrated against Peedee belemnite (PDB) carbonate were used for comparison. The accuracy of the $\delta^{13}C$ measurements was ±0.1‰. Following Farquhar et al. (1989), Δ was further calculated from $\delta^{13}C$ as $\Delta = (\delta_a - \delta_p)/(1 + \delta_p)$, where δ_a and δ_p refer to air and plant, respectively. On the PDB scale, free atmospheric CO_2, δ_a, has a current composition of approximately −8‰ (Farquhar et al., 1989).

Statistical Analysis

Analyses were done with the SAS-STAT package (SAS Institute Inc., 1996). An analysis of variance was performed for grain yield and Δ, considering days from sowing to heading or from sowing to anthesis as the covariant. Means of trials for yield and Δ were compared by the LSD test at $P = 0.05$. Stepwise discriminant analysis was used to ascertain the relationship between either Δ or grain yield as the dependent variable and three different periods of the crop cycle (sowing to heading, heading to anthesis and anthesis to maturity) for each environmental parameter as the independent variables. The relationship between Pearson's correlation coefficients of individual trials and mean grain yield was fitted by Table Curve (Jandel Co., 1994).

RESULTS

The two regions differed in rainfall, temperature, and evaporative demand, SE Spain being markedly more arid, as inferred from the ratio W_i/ET_0 of the rainfed sites. Differences between irrigated and rainfed sites in this ratio were also evident (Table 2). The number of days from sowing to either heading (DH) or anthesis (DA) was higher in NE than in SE Spain (Table 2). Heading was at approximately 1235 growing-degree days and anthesis at approximately 1340 in all trials except two. The exceptions were both in one of the sites in SE Spain, and had a higher number of growing-degree days than the other sites (Table 2).

The effect of environment, understood as the combination of region and water regime, on grain yield and Δ of grain was much higher than that of genotypic variability (Table 3). Also, DH had a strong effect on yield. Although variation in phenology was limited within trials, DH probably also reflects an environmental effect as the trials differed dramatically in DH (Table 2). Therefore, environmental conditions were responsible by far for most of the variability observed in yield and Δ. The main environmental factor affecting yield and Δ was water regime, rainfed or irrigated, but the interaction between year and region also had a strong affect on Δ. Grain yield was doubled and Δ was 2.5‰ higher in irrigated than in rainfed trials in NE Spain and 64% and 1.6‰ higher, respectively, in SE-Spain trials (Table 2).

Table 3. Percentages of the sum of squares obtained in the analysis of variance for grain yield and carbon isotope discrimination of mature grains (Δ) of 25 durum wheat genotypes grown in two regions (NE and SE Spain) under two water regimes (rainfed and irrigated) during three years (1997 to 1999 seasons). For the analysis days from sowing to heading has been considered as a covariant.

Source of variation	df	Yield	Δ
Days to heading	1	23.4***	2.3***
Year	2	0.4ns	1.6***
Region	1	13.0***	9.0***
Year × Region	2	5.0***	22.7***
WR† (Region)	2	42.8***	51.2***
Year × WR (Region)	4	1.8**	5.4***
Block (Year × WR × Region)	36	3.3***	1.7***
Genotype	24	2.5***	1.9***
Genotype × Year	48	1.5***	1.0***
Genotype × Region	24	0.8**	0.4*
Genotype × Year × Region	48	1.6***	0.7**
Genotype × WR (Region)	48	1.3**	0.8***
Genotype × Year × WR (Region)	96	2.6***	1.3**
Residual	864	12.5	7.2

*, Significant at 0.05 probability level.
**, Significant at 0.01 probability level.
***, Significant at 0.001 probability level.
ns, Nonsignificant.
† WR, water regime.

Effect of Environment on the Relationship between Δ and Yield across Trials

The Δ values of mature kernels and grain yield were strongly ($P < 0.001$) and positively correlated across all the trials (Fig. 1). Correlations between these traits in trials across either NE or SE Spain were similar in

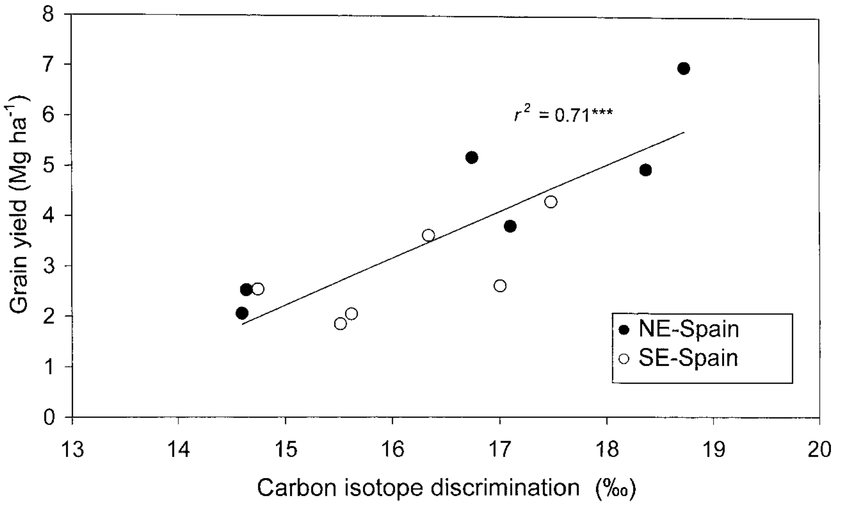

Fig. 1. Relationship across the whole set of trials between the carbon isotope discrimination of mature grains and grain yield. Each point represents a rainfed or irrigated trial (composed by 25 genotypes and four replicates per genotype) grown in NE Spain or SE Spain.

Table 4. Pearson's correlation coefficients of the relationship across trials between both grain yield and carbon isotope discrimination of mature grains (Δ), and some environmental variables recorded for different periods of the crop cycle. Correlations have been calculated with the whole set of trials (n = 12) used in this study and presented in Table 2.

	W_i†		Mean temperature		ET_0†		W_i/ET_0	
Growing period	**Yield**	**Δ**	**Yield**	**Δ**	**Yield**	**Δ**	**Yield**	**Δ**
Sowing to maturity	0.75**	0.78**	−0.54	−0.15	0.08	0.32	0.78**	0.72**
Sowing to heading	0.37	0.47	−0.65*	−0.28	−0.52	−0.49	0.44	0.54
Heading to anthesis	0.57	0.27	−0.11	0.21	−0.19	−0.28	0.59*	0.24
Heading to one week after anthesis	0.46	0.18	−0.36	−0.15	−0.17	−0.28	0.51	0.16
One week after anthesis to maturity	0.50	0.72**	−0.16	−0.02	0.33	0.60*	0.44	0.55
Heading to maturity	0.80**	0.75**	−0.18	0.05	0.24	0.46	0.81**	0.65*
Sowing to anthesis	0.60*	0.57	−0.66*	−0.30	−0.51	−0.54	0.64*	0.63*
Anthesis to maturity	0.69*	0.82**	−0.14	0.01	0.33	0.59*	0.69*	0.73**

*, Significant at 0.05 probability level.
**, Significant at 0.01 probability level.
† Wi, water input (rainfall plus irrigation); ET_0, accumulated reference evapotranspiration.

slope. To ascertain the basis of these relationships, Pearson's correlation coefficients were calculated across the 12 trials between these traits and several environmental parameters evaluated during the crop cycle (Table 4) plus DH and DA, these last being nonsignificant at the $P = 0.05$ level (DH, $r = 0.51$ and $r = 0.14$; DA, $r = 0.49$ and $r = 0.11$ for yield and Δ, respectively). The strongest relationships observed with either yield or Δ were those that involved $W_{i,}$ for the whole crop cycle or just from the last part of the cycle. The W_i/ET_0 ratio for the complete crop cycle, between heading and maturity, or only during grain filling, was also significantly correlated with yield and Δ. For the interval from sowing to heading, W_i and W_i/ET_0 showed much weaker relationships with yield and Δ. In general, mean temperature and accumulated evapotranspiration correlated less strongly with yield and Δ regardless of the stage of the crop cycle. Significant relationships of either W_i or W_i/ET_0 with yield and Δ were linear (Fig. 2 and Fig. 3).

Stepwise analysis showed W_i and W_i/ET_0 were again the parameters best correlated with yield and Δ. The combination of W_i or W_i/ET_0 for the three crop cycle periods explained about 70% of the total variability in yield and Δ across the 12 trials and about 90% across the six trials in NE and SE Spain separately (Table 5). Water input or W_i/ET_0 from anthesis to maturity were the independent variables selected first in the stepwise analysis for the whole set of trials and the subset of NE Spain. In the case of SE Spain, however, W_i or W_i/ET_0 from sowing to heading were the first choices in the analysis (Table 5), and were also linearly related with yield and Δ (Fig. 3). Moreover, W_i and W_i/ET_0, either from heading to maturity (Fig. 3), or anthesis to maturity, were correlated with yield and Δ only for NE Spain. The slope of all these relationships tended to be steeper in NE than in SE Spain.

Effect of Environment on the Relationship between Δ and Yield within Trials

To minimize the potential interference of phenology on the phenotypic relationship between grain Δ and yield, the set of genotypes chosen in this study had a relatively small variability in DH and DA within each trial. Analysis of variance for Δ and yield using DH or DA as covariables confirmed that the effect of genotype on these traits remained highly significant after subtracting the effect of phenology expressed as DH or DA (Table 3).

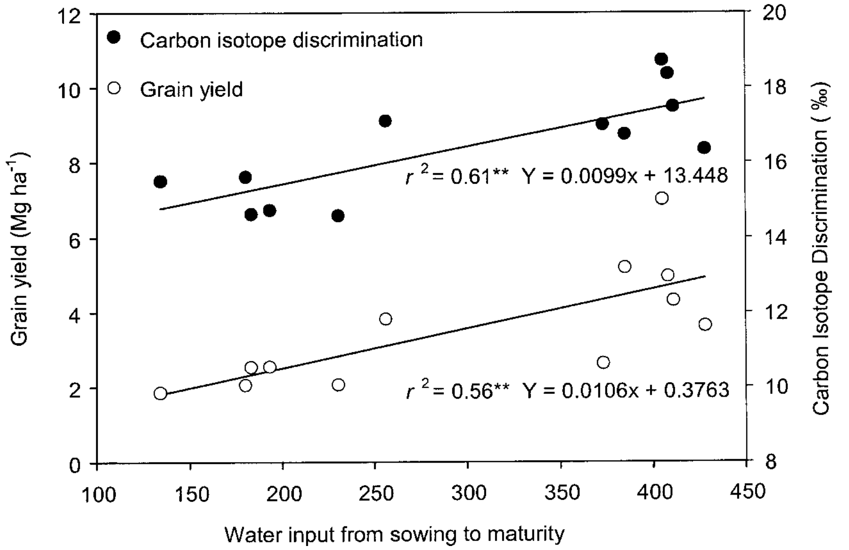

Fig. 2. Relationship across the whole set of trials between water input (including irrigation if applied) from sowing to maturity and either grain yield or carbon isotope discrimination. Each point represents either a rainfed or irrigated trial (composed by 25 genotypes and four replicates per genotype).

Table 5. Stepwise analysis taking yield and Δ as dependent variables, and three different periods of the crop cycle (sowing to heading, heading to anthesis and anthesis to maturity) for each environmental parameter as independent variables. Values are proportions (per one) of the total variability in grain yield and carbon isotope discrimination across the 12 trials attributable to either a given environmental variable or explained by the progressive combination of these variables.

Period	Yield	Yield accumulated	Period	Δ	Δ accumulated
Water input (W_i) NE- and SE-Spain trials combined					
Anthesis–maturity	0.48*	0.48	Anthesis–maturity	0.67**	0.67
Heading–anthesis	0.13	0.61	Sowing–heading	0.10	0.77
Sowing–heading	0.06	0.67	Heading–anthesis	0.00	0.77
W_i/ET_0 ratio NE- and SE-Spain trials combined					
Anthesis–maturity	0.47*	0.47	Anthesis–maturity	0.53**	0.53
Heading–anthesis	0.13	0.60	Sowing–heading	0.20*	0.73
Sowing–heading	0.11	0.71	Heading–anthesis	0.01	0.74
Water input (W_i) NE-Spain trials					
Anthesis–maturity	0.85**	0.85	Anthesis–maturity	0.83*	0.83
Heading–anthesis	0.12*	0.97	Sowing–heading	0.01	0.84
Sowing–heading	0.01	0.98	Heading–anthesis	0.01	0.85
Water input (W_i) SE-Spain trials					
Sowing–heading	0.60	0.60	Sowing–heading	0.56	0.56
Heading–anthesis	0.10	0.70	Anthesis–maturity	0.33	0.89
Anthesis–maturity	0.05	0.75	Heading–anthesis	0.05	0.94
W_i/ET_0 ratio NE-Spain trials					
Anthesis–maturity	0.90**	0.90	Anthesis–maturity	0.69*	0.69
Heading–anthesis	0.02	0.92	Sowing–heading	0.05	0.74
Sowing–heading	0.00	0.92	Heading–anthesis	0.01	0.75
W_i/ET_0 ratio SE–Spain trials					
Sowing–heading	0.66*	0.66	Sowing–heading	0.60	0.60
Heading–anthesis	0.06	0.72	Anthesis–maturity	0.26	0.86
Anthesis–maturity	0.01	0.73	Heading–anthesis	0.03	0.89

*, Significant at 0.05 probability level.
**, Significant at 0.01 probability level.

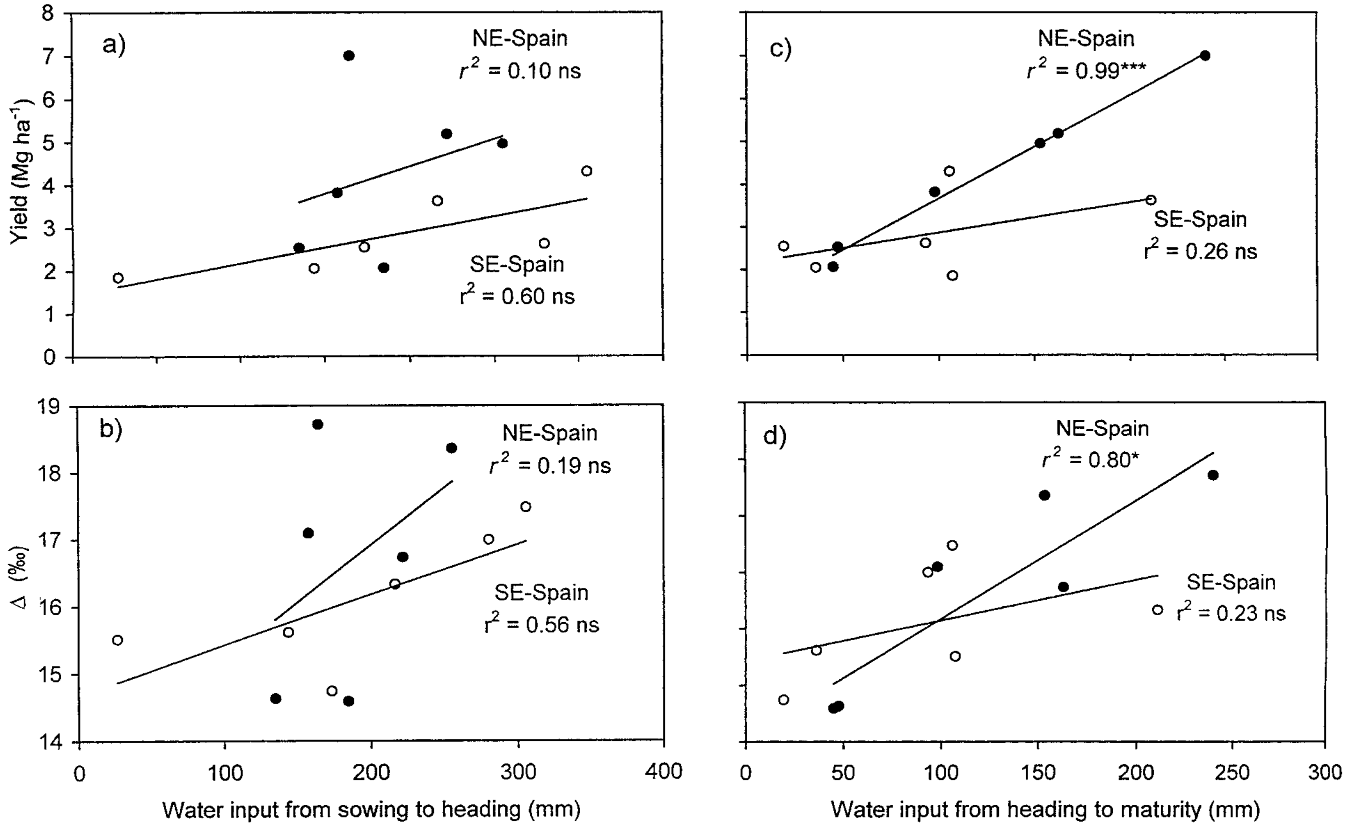

Fig. 3. Relationship across the subset of trials, from NE Spain or SE Spain, between water input from sowing to heading and (a) grain yield or (b) carbon isotope discrimination of mature grains (Δ), and between water input from heading to maturity and (c) grain yield or (d) carbon isotope discrimination. Each point represents either a rainfed or irrigated trial (composed by 25 genotypes and four replicates per genotype) grown in NE Spain or SE Spain.

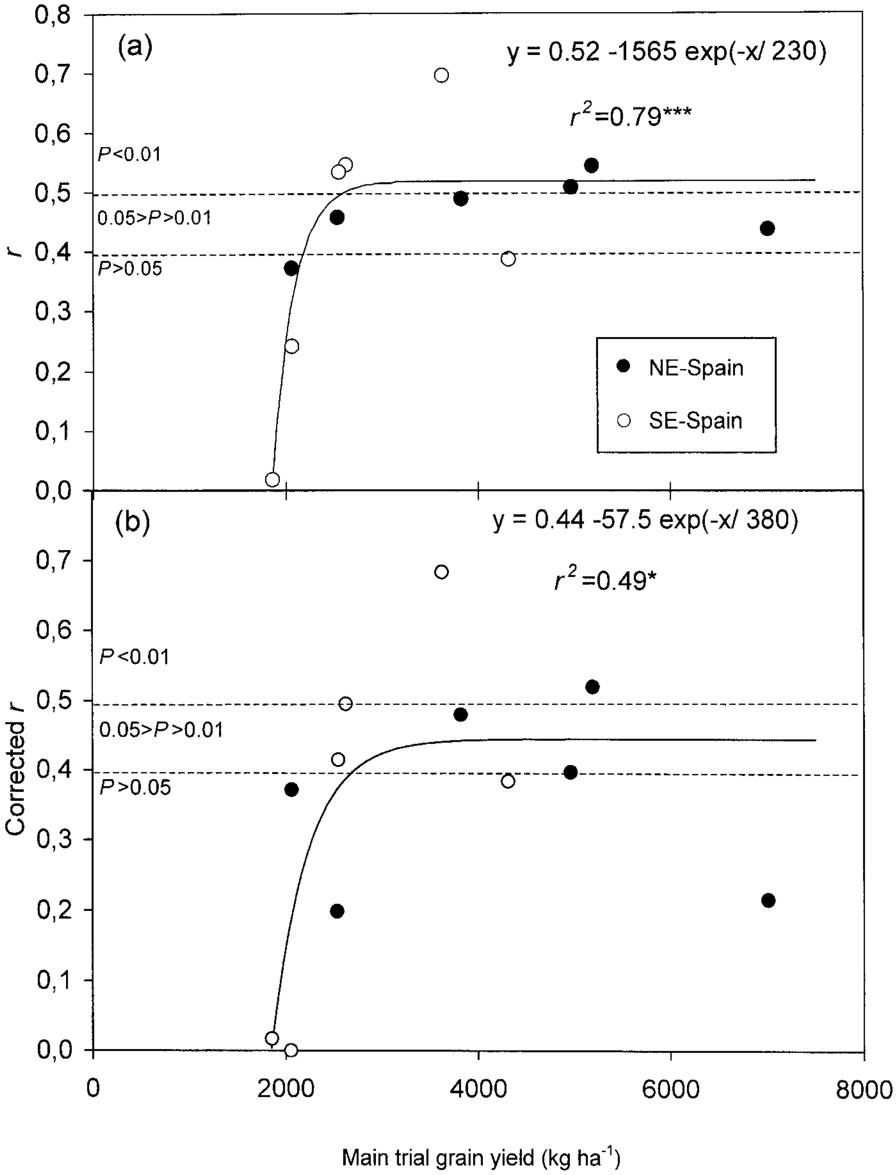

Fig. 4. Relationship between mean trial grain yield and the Pearson's correlation coefficient (*r*) of the relationship between grain yield and carbon isotope discrimination of mature kernels within the same trial (a). The correlation coefficients of the same relationships after subtracting the effect of DH are also shown (b). Each point represents a trial composed by 25 genotypes and four blocks per genotype. Trials were performed in NE Spain and SE Spain under two moisture regimes (irrigated and rainfed) and over 3 yr (1997–1999).

Genotype effects, although only accounted for about 2% of total variability in both yield and Δ, were highly significant. On the other hand, these phenological traits also had a highly significant effect, particularly on yield (Table 3). However, the effect of phenology in the analysis of variance may have incorporated an environmental bias. Moreover, no significant correlation across genotypes was observed between DH or DA and either Δ or grain yield in any of the trials.

For the 12 trials, mean trial yield was plotted against the Pearson's correlation coefficient of the phenotypic relationship between Δ and yield within the same trial (Fig. 4a). This coefficient was positive in all the trials. The relationship fitted an asymptotic function ($P <$ 0.01). Thus, trials yielding around 2000 kg ha^{-1} showed no significant ($P <$ 0.05) relationships, but coefficients of correlation increased sharply to attain significance for trials yielding about 2500 kg ha^{-1}. Above this yield, they remained steady at around 0.5, except for a trial under irrigation in SE Spain with r = 0.69. Many of these relationships remained almost unchanged after subtracting the effect of DH (Fig. 4b), although the correlation coefficient in the highest yielding trial was greatly decreased when the effect of DH was removed. The slope of the linear regression between yield and Δ showed the same pattern of changes as the growing conditions of the trials improved. It increased from values near zero in the lowest yielding trial (SE Spain rainfed 1997) (Table 2) to around 1000 kg ha^{-1} per 1‰ increase in Δ, for trials that yielded about 2500 kg ha^{-1}.

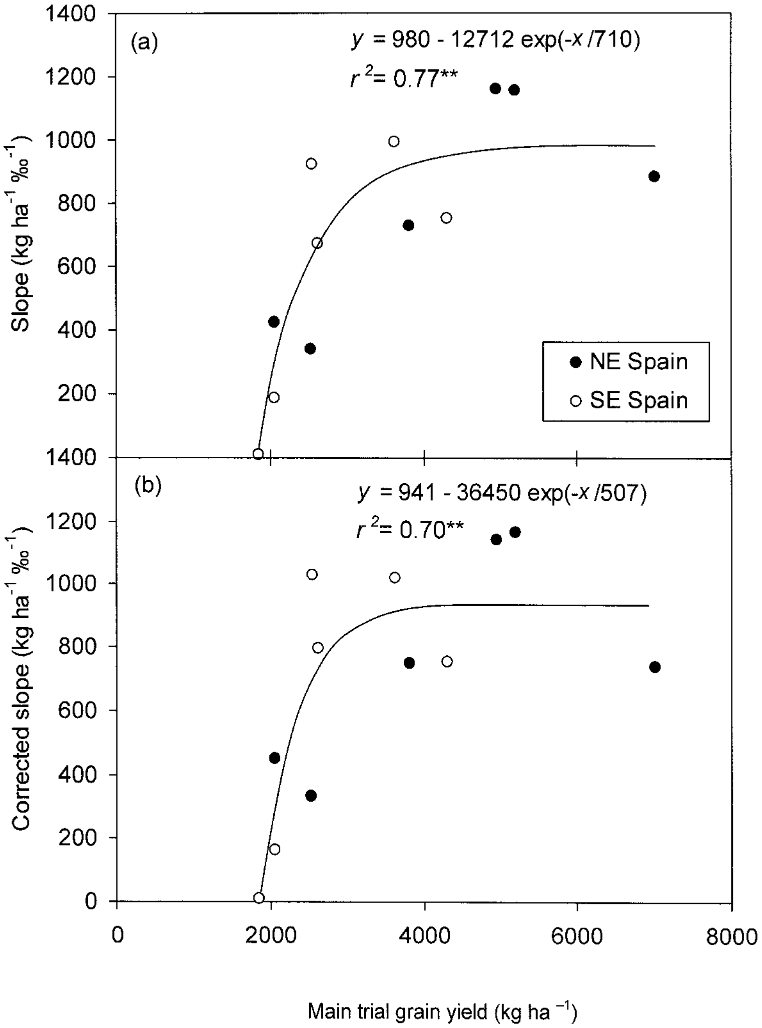

Fig. 5. Relationship between mean trial grain yield and the slope of the regression of the relationship between grain yield and carbon isotope discrimination of mature kernels within the same trial (a). Slopes of the same relationship after subtracting the effect of DH are also shown (b). Each point represents a trial composed by 25 genotypes and four blocks per genotype. Trials were performed in NE Spain and SE Spain under two moisture regimes (irrigated and rainfed) and over 3 yr (1997–1999).

Above these yields, slope values remained steady (Fig. 5a). Moreover, the slopes of these relationships were unaffected when the effect of DH was subtracted (Fig. 5b). From the set of 25 genotypes two groups of five genotypes each, having the extreme values of Δ at the two most productive trials were selected. Further, for each trial the percentage in yield as a result of picking based on these two groups was calculated as [mean yield high Δ group – mean yield low Δ group/mean yield low Δ group] $\times$ 100. The average yield differential showed a similar pattern to that on Fig. 4 (but $P < 0.10$). Thus, the high Δ group consistently showed a higher yield (about 10%) for trials yielding about 2500 kg ha^{-1} and above.

DISCUSSION

Role of Phenology in the Relationships between Δ and Yield across Trials

In spite of the large differences between trials in DH and DA, both parameters were only weakly correlated ($P < 0.1$) with yield and not correlated with Δ across trials. The lack of a stronger correlation between crop phenology and yield and its absence for Δ could be due to the dependence of DH and DA on temperature (Hay, 1999 and references herein). Indeed, mean temperature from sowing to heading and from sowing to anthesis was negatively correlated ($P < 0.05$) with yield but not with Δ. In our study, differences in DH and DA between trials were mainly caused by regional differences in temperature. Thus, the higher temperatures in the trials in SE Spain caused an accelerated accumulation of growing-degree days and therefore a shorter DH and DA than in those in NE Spain.

Role of Environmental Variables in the Relationships between Δ and Yield across Trials

Our results show that W_i for the whole crop cycle and specifically that comprising the part from heading onwards was the main environmental variable affecting grain yield and Δ. Sharing a common environmental variable would explain the strong positive relationship between grain yield and Δ across trials (Fig. 1). Similar results have been reported in durum wheat (Araus et al., 1999b) and other cereals (Condon et al., 1987; Romagosa and Araus, 1991; Acevedo, 1993).

Previous reports on barley (*Hordeum vulgare* L.) and durum wheat under Mediterranean conditions have found a strong dependence of grain Δ on W_i during the later stages (from heading and anthesis to maturity) of the crop cycle (Araus et al., 1999a). In the present study, W_i explained the differences in Δ across growing environments better than ET_0 or even the ratio W_i/ET_0 (Table 4) as well as other related variables such as ET_0–W_i (data not shown). The strength of the relationships of W_i/ET_0 with yield and Δ was almost comparable to that obtained with W_i. However, because ET_0 alone related poorly to yield and Δ, the good performance of W_i/ET_0 seemed to be due only to the weight of W_i in this ratio. In fact, the range of variability in accumulated ET_0 across environments was smaller than that for W_i. Stewart et al. (1995), working with wild plant communities along a natural rainfall gradient, also concluded that W_i was the environmental variable best related to carbon isotope composition. Most of the studies on this topic, however, deal with tree species and not annual plants (see references below). In general, these studies are not conclusive in terms of agreement on a common, single environmental variable. They relate Δ to rainfall amounts, evapotranspiration, the W_i/ET_0 ratio, soil water status, or different drought stress indices either at the seasonal level or during a particular period of the growing season (Francey and Farquhar, 1982; Freyer and Belacy, 1983; Leavitt and Long, 1989; Dupouey et al., 1993; Guehl et al., 1995; Korol et al., 1999; Moore-Darrin et al., 1999). Water input during grain filling was not only the variable best correlated with yield and Δ for the whole set of trials (Table 4), but also for the subset of NE Spain (Fig. 3). However, whereas no significant correlations were attained, in SE Spain, W_i from the first part of the crop cycle (sowing to heading) tended to be the variable best related to yield and Δ (Fig. 3). On the other hand, no significant correlation was attained between W_i from sowing to heading and yield or Δ in NE-Spain trials (Fig. 3). These differences between regions may have an environmental basis. The Mediterranean environment of SE Spain is characterized by more severe drought stress during the last part of the crop cycle than is that of NE Spain (Table 6). The presence of a higher level of drought stress during grain filling in SE-Spain trials is further supported by the generally lower 1000-kernel weight in SE than in NE Spain (Table 2). Under the growing conditions of SE Spain, grain yield and Δ are defined earlier in the crop cycle because photoassimilates produced before anthesis may play a major role not only in defining the total number of grains per unit land, but also in the filling of these grains (Slafer and Araus, 1998; Royo et al., 1999). Interestingly, for two of the three years studied, the Δ for the rainfed site in SE Spain was higher than in that in the north in spite of the lower yield and greater drought in the former location (Table 2).

Table 6. Environmental conditions (means over three years) during the last part of the of crop culture (from heading to physiological maturity).

	W_i/ET_0†	Temperature	VPD‡
		°C	mbar
NE–Spain irrigated	**0.77**	**17.6**	**10.4**
NE–Spain rainfed	**0.31**	**16.9**	**12.4**
SE–Spain irrigated	**0.42**	**19.3**	**19.6**
SE–Spain rainfed	**0.21**	**19.9**	**21.2**

† W_i/ET_0, ratio of water input versus reference evapotranspiration.
‡ VPD, vapor-pressure deficit at anthesis.

The pattern of the relationships between W_i and yield and Δ suggest that for the same amount of water input, a higher grain yield and Δ were attained in NE Spain. These regional differences could initially be explained in terms of a higher ET_0 or vapor-pressure deficit in the trials done in the south (Table 6) or by differences in soil water-holding capacity and distinct seasonal patterns of rainfall. Canopy temperature depression at anthesis measured by infrared thermometry was about 5°C higher in SE than in NE Spain regardless of the water regime assayed (Royo et al., 2002). This observation suggests higher transpiration rates in the southern trials, irrespective of whether stomatal conductance was lower than in the northern trials as can be inferred from the lower Δ values of SE Spain (Table 2). Thus, Condon et al. (1992) reported a decrease in Δ in wheat which was attributed to stomatal closure in response to increasing VPD. However, complementary explanations may be sought because the relationships between the W_i/ET_0 ratio and grain yield or Δ in NE and SE Spain showed the same pattern as those with W_i illustrated in Fig. 3. A possible explanation could be a lower water holding capacity of the sandy soils in SE Spain (Table 1). Decreased soil water availability results in lower values of Δ in cereals (Farquhar and Richards, 1984; Hubick and Farquhar, 1989; Condon et al., 1992) primarily because of the decrease in stomatal conductance. Other soil characteristics, such as soil compaction, may also affect Δ in wheat (Masle and Farquhar, 1988).

Role of Environment on the Relationships between Δ and Yield within Trials

For all the trials, the slope of the regression between Δ and grain yield remained positive. In fact, for wheat and other cereals, Δ is frequently positively correlated with grain yield and/or total biomass not only under well-irrigated but also under rainfed conditions (Condon et al., 1987; Romagosa and Araus, 1991; Kirda et al., 1992; Araus et al., 1993, 1998; Merah et al., 2002; Morgan et al., 1993; Sayre et al., 1995). Most of these (and other studies reporting positive relationships) were conducted in Mediterranean or similar environments where there is strong reliance on "within-season" rainfall (Condon et al., 2002). However, unlike the results of Araus et al. (1998) in durum wheat, in the poor-yielding environments of our study (about 2000 kg ha^{-1}), the correlation between grain Δ and yield across genotypes was not significant, with correlation coefficients for two rainfed trials from SE Spain close to zero. Such a relationship attained significance in trials when yield was about 2500 kg ha^{-1} and greater. In addition, the slope of the regression, which represents the responsiveness of yield across genotypes to changes in Δ within a particular environment, increased to a maximum in trials yielding about 2500 kg ha^{-1}, above which it remained steady with values comparable to those reported before in durum wheat (Araus et al., 1997b). An increase in the strength of this positive relationship as the growing conditions improve has also been reported for barley (Voltas et al., 1999) under Mediterranean conditions.

Carbon isotope discrimination has largely been recommended as a selection criterion for transpiration efficiency (Farquhar and Richards, 1984), which holds true when the amount of captured water is the same for all genotypes. A negative relationship between Δ and either biomass or yield can then be expected (Slafer and Araus, 1998; Araus et al., 2002). This is frequently the case in pot studies where all the water provided is being used by genotypes (Richards, 1996; Slafer and Araus, 1998) or for Mediterranean field trials with very low water input, as is sometimes reported in barley (Romagosa and Araus, 1991; Voltas et al., 1999). It is also the case for wheat and other cereals in water limited environments where crop growth is most dependent on soil moisture stored from rain that falls outside the main crop growth phase, such as in the northern Australian wheat belt (Condon et al., 2002; Richards et al., 2002). Reports of a negative relationship in stressed Mediterranean environments between Δ and yield are less frequent in wheat than in barley. This is perhaps associated with the agronomical observation that the crossover for the genotype × environment interaction seems to be at higher yields (i.e., better environments) for barley (2000–2500 kg ha^{-1}) (Ceccarelli and Grando, 1991) than for wheat (1000–1500 kg ha^{-1}) (Laing and Fischer, 1977; Fischer, 1993). Barley is reported to have a more extensive root system than durum wheat and, consequently a higher capacity of water extraction from the soil (López-Castañeda and Richards, 1994). Therefore, in arid environments, a higher yield in barley would be associated with higher transpiration efficiency, and thus a lower Δ, because all the available water is extracted by the crop.

For trials with moderate stress, when genotypes may show their distinct ability to capture water, the positive effect of a higher transpiration efficiency is probably overridden by greater transpiration rate and water use. Genotypes that use more water keep their stomata more open and therefore discriminate more against ^{13}C than those that use less water (Condon et al., 1987; Richards and Condon, 1993; Turner, 1993; Richards, 1996) while showing higher photosynthesis and yield. In other words, as the growing conditions of trials improve, positive relationships between Δ and grain yield are attained because Δ indirectly reflects differences in water use in addition to those in transpiration efficiency. In fact, water use and transpiration efficiency, the two variables determining crop biomass in Passioura's equation (Passioura, 1977), would not behave as independent traits.

Under favorable growing conditions, positive associations between yield and Δ have also been reported for several cereals (Condon et al., 1987; Romagosa and Araus, 1991; Richards, 1996; Araus et al., 1998; Voltas et al., 1999). This arises when the highest yielding genotypes have a lower assimilation capacity per unit area than the lowest yielding lines (Araus et al., 1997a, 1997b) or when lines with a higher stomatal conductance have the highest yields, which seems to be the most frequent situation (Richards, 2000). For an historic set of bread wheats, released through several decades and bred at CIMMYT, progress in potential yield was associated with changes in gas exchange characteristics of photosynthesis and transpiration (Fischer et al., 1998). Among these characteristics, correlation with leaf conductance was strongest and Δ was also positively associated with yield progress. Similar results were reported by Richards (2000) and Barbour et al. (2000). Several explanations have been proposed for the positive relationship between stomatal conductance and yield in this CIMMYT set: decreased stomatal sensitivity to vapor-pressure deficit or to subtle water stress, extra cooling particularly at warmer temperatures, or increased sink strength (Fischer et al., 1998, Richards, 2000). Whatever the reason, higher stomatal conductance leads to more photosynthesis and assimilation.

There are, however, reports in barley of a strong positive relationship between grain Δ and yield in the very dry rainfed conditions of northern Syria, whereas negative, although weaker, relationships were recorded in the very productive environments of Cambridge, England (Austin et al., 1990; Acevedo, 1993). Differences in phenology may also explain the positive relationships between grain Δ and yield within Mediterranean rainfed environments (Richards, 1996; Araus et al., 1998; Condon et al., 2002). However, this factor was limited in our study. Thus, the relationship between Δ and yield were positive and still significant for most of the trials after subtracting the effect of DH (Fig. 4).

Implications for Breeding in Mediterranean Conditions

It has been suggested that positive relationships between Δ and yield are attained when the differential ability of the genotypes for taking up available water is the main determinant of differential yields (Richards, 1996; Slafer et al., 1999) even when another factors discussed above and elsewhere (Condon et al., 2002) could also account. The relationship is expected to be negative when the main factor determining genotypic differences in yield is transpiration efficiency and thus its translation into agronomic terms, water use efficiency, the ratio of biomass accumulated to water transpired. Therefore, in the Mediterranean environments of this study, the rainfed conditions may have resulted in the expression of both abilities simultaneously. Thus, some cultivars give improved yields because of their higher water use efficiency while others perform better because of their differential ability to use more water (beyond differences in crop phenology), and therefore the relationship between Δ and yield across genotypes was weak, regardless of whether it was positive or negative. Additionally, a large contribution of preanthesis reserves to the filling of grains (Blum, 1988; Loss and Siddique, 1994), thereby affecting the isotope signature of mature kernels, may also explain the weakness of this relationship in more stressed environments.

Breeding to raise yield could take advantage of selecting for higher Δ only in relatively wet years (or under supplementary irrigation), but selection for this trait should not take place in the drier years under rainfed conditions, particularly in SE Spain.

ACKNOWLEDGMENTS

This study was supported by research projects CICYT AGF-96-1137-C02, CICYT AGF99-0611-C03 and INIA SC97-039-C2-01 (Spain). D. Villegas and N. Aparicio were recipients of PhD grants from the *Generalitat de Catalunya*, and from the *Ministerio de Educación y Cultura* (Spain) respectively.

REFERENCES

Acevedo, E. 1991. Improvement of winter cereal crops in Mediterranean environments. Use of yield, morphological and physiological traits. p. 273–305 *In* E. Acevedo et al. (ed.) Physiology-Breeding of winter cereals for stressed mediterranean environments. INRA, Paris.

Acevedo, E. 1993. Potential of carbon isotope discrimination as a selection criterion in barley breeding. p. 399–417 *In* J.R. Ehleringer et al. (ed.) Stable isotopes and plant carbon/water relations. Academic Press, New York.

Araus, J.L., M.P. Reynolds, and E. Acevedo. 1993. Leaf posture, grain yield, growth, leaf structure and carbon isotope discrimination in wheat. Crop Sci. 33:1273–1279.

Araus, J.L., J. Bort, S. Ceccarelli, and S. Grando. 1997a. Relationship between leaf structure and carbon isotope discrimination in field grown barley. Plant Physiol. Biochem. 35:533–541.

Araus, J.L., T. Amaro, Y. Zuhair, and M.M. Nachit. 1997b. Effect of leaf structure and water status on carbon isotope discrimination in field-grown durum wheat. Plant Cell Environ. 20:1484–1494.

Araus, J.L., T. Amaro, J. Casadesús, A. Asbati, and M.M. Nachit. 1998. Relationships between ash content, carbon isotope discrimination and yield in durum wheat. Aust. J. Plant Physiol. 25:835–842.

Araus, J.L., A. Febrero, M. Catala, M. Molist, J. Voltas, and I. Romagosa. 1999a. Crop water availability in early agriculture: Evidence from carbon isotope discrimination of seeds from a tenth millennium BP site on the Euphrates. Global Change Biol. 5:233–244.

Araus, J.L., G.A. Slafer, and I. Romagosa. 1999b. Durum wheat and barley yields in antiquity estimated from ^{13}C discrimination of archaeological grains: a case study from the Western Mediterranean Basin. Aust. J. Plant Physiol. 26:345–352.

Araus, J.L., G.A. Slafer, M.P. Reynolds, and C. Royo. 2002. Plant breeding and water relations in C_3 cereals: What to breed for? Ann. Bot. (London) 89:925–940.

Austin, R.B., P.Q. Craufurd, B. da Silveira Pinheiro, M.A. Hall, E. Acevedo, and E.C.K. Ngugi. 1990. Carbon isotope discrimination as a means of evaluating drought resistance in barley, rice and cowpeas. Bulletin Société Botanique Français 137. Actual. Bot. 1:21–30.

Barbour, M.M., R.A. Fischer, K.D. Sayre, and G.D. Farquhar. 2000. Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and yield in irrigated, field-grown wheat. Aust. J. Plant Physiol. 27:625–637.

Blum, A. 1988. Plant breeding for stress environments. CRC Press, Boca Raton, FL.

Ceccarelli, S., and S. Grando. 1991. Selection environment and environmental sensitivity in barley. Euphytica 57:157–167.

Ceccarelli, S., and S. Grando. 1996. Drought as a challenge for the plant breeder. Plant Growth Regul. 20:149–155.

Condon, A.G., R.A. Richards, and G.D. Farquhar. 1987. Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field grown wheat. Crop Sci. 27:996–1001.

Condon, A.G., G.D. Farquhar, and R.A. Richards. 1990. Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat. Leaf gas exchange and whole plant studies. Aust. J. Plant Physiol. 17:9–22.

Condon, A.G., R.A. Richards, and G.D. Farquhar. 1992. The effect of variation in soil water availability, vapour-pressure deficit and nitrogen nutrition on carbon isotope discrimination in wheat. Aust. J. Agric. Res. 43:935–947.

Condon, A.G., and R.A. Richards. 1993. Exploiting genetic variation in transpiration efficiency in wheat: an agronomic view. p. 435–450. *In* J.R. Ehleringer et al. (ed.) Stable isotopes and plant carbon/water relations. Academic Press, New York.

Condon, A.G., R.A. Richards, G.J. Rebetzke, and G.D. Farquhar. 2002. Improving intrinsic water-use efficiency and crop yield. Crop Sci. 42:122–131.

Craufurd, P.Q., R.B. Austin, E. Acevedo, and M.A. Hall. 1991. Carbon isotope discrimination and grain-yield in barley. Field Crops Res. 27:301–313.

Dupouey, J.L., S. Leavitt, E. Choisnel, and S. Jourdain. 1993. Modeling of carbon isotope fractionation in tree rings based on effective evapotranspiration and soil water status. Plant Cell Environ. 16: 939–947.

Farquhar, G.D., and R.A. Richards. 1984. Isotopic composition of plant carbon correlates with water-use-efficiency of wheat genotypes. Aust. J. Plant Physiol. 11:539–552.

Farquhar, G.D., J.R. Ehleringer, and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40:503–537.

Fischer, R.A. 1993. Cereal breeding in developing countries: Progress and prospects. p. 201–209. *In* D.R. Buxton et al. (ed.) International crop science I, CSSA, Madison, WI.

Fischer, R.A., D. Rees, K.D. Sayre, Z.-M. Lu, A.G. Condon, and A. Larqué-Saavedra. 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Sci. 38:1467–1475.

Francey, R.J., and G.D. Farquhar. 1982. An explanation of ^{13}C/^{12}C variations in three rings. Nature (London) 290:232–234.

Freyer, H.D., and N. Belacy. 1983. ^{13}C/^{12}C records in northern hemispheric trees during the past 500 years-anthropogenic impact and climatic superpositions. J. Geophys. Res. 88:6844–6852.

Gallagher, J.N. 1979. Field studies of cereal leaf growth: I. Initiation and expansion in relation to temperature and ontogeny. J. Exp. Bot. 117:625–636.

Guehl, J.M., C. Fort, and A. Ferhi. 1995. Differential response of leaf conductance, carbon isotope discrimination and water use efficiency to nitrogen defficiency in marine pine and pedunculate oak plants. New Phytol. 131:149–157.

Hay, R.K.M. 1999. Physiological control of growth and yield in wheat: analysis and synthesis. p. 1–38 *In* D.L. Smith and C. Hamel (ed.) Crop yield. Physiology and processes. Springer, Berlin.

Hubick, K.T., and G.D. Farquhar. 1989. Genetic variation in carbon isotope discrimination and the ratio of carbon gained to water loss in barley. Plant Cell Environ. 12:795–804.

Jandel Co. 1994. Table curve 2D. User's manual. Jandel Corporation, San Rafael, CA.

Kirda, C., A.R.A.G. Mohamed, K.S. Kumarasinghe, A. Montenegro, and F. Zapata. 1992. Carbon isotope discrimination at vegetative stage as an indicator of yield and water use efficiency of spring wheat (*Triticum turgidum* L. var. *durum*). Plant Soil 7:217–223.

Korol, R.L., M.U.F. Kirschbaum, G.D. Farquhar, and M. Jeffreys. 1999. Effects of water status and soil fertility on the C-isotope signature in *Pinus radiata.* Tree Physiol. 19:551–562.

Laing, D.R., and R.A. Fischer. 1977. Adaptation of semidwarf wheat cultivars to rainfed conditions. Euphytica 26:129–139.

Leavitt, S.W., and A. Long. 1989. The atmospheric Δ^{13}C record as derived from 56 pinyon trees at 14 sites in the southwestern United States. Radiocarbon 31:469–474.

López-Castañeda, C., and R.A. Richards. 1994. Variation in temperate cereals in rainfed environments. I. Grain yield, biomass and agronomic characteristics. Field Crops Res. 37:51–62.

Loss, S.P., and K.H.M. Siddique. 1994. Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. Adv. Agron. 52:229–276.

Masle, J., and G.D. Farquhar. 1988. Effects of soil strength on the relations of water use efficiency and growth to carbon isotope discrimination in wheat seedlings. Plant Physiol. 86:32–38.

Merah, O., E. Deléens, I. Souyris, M. Nachit, and P. Monneveux. 2002. Stability of Carbon isotope discrimination and grain yield in durum wheat. Crop Sci. 41:677–681.

Moore-Darrin, J., S. Nowak-Robert, and J. Tausch-Robin. 1999. Gas exchange and carbon isotope discrimination of *Juniperus osteosperma* and *Juniperus occidentalis* across environmental gradients in the Great Basin of western North America. Tree Physiol. 19:421–433.

Morgan, J.A., D.R. LeCain, T.N. McCaig, and J.S. Quick. 1993. Gas exchange, carbon isotope discrimination and productivity in winter wheat. Crop Sci. 33:178–186.

Passioura, J.B. 1977. Grain yield, harvest index and water use of wheat. J. Aust. Inst. Agric. Sci. 43:117–120.

Richards, R.A. 1996. Defining selection criteria to improve yield under drought. Plant Growth Regul. 20:157–166.

Richards, R.A. 2000. Selectable traits to increase crop photosynthesis and yield of grain crops. J. Exp. Bot. 51:447–458.

Richards, R.A., and A.G. Condon. 1993. Challenges ahead in using carbon isotope discrimination in plant breeding programs. p. 451–462 *In* J.R. Ehleringer et al. (ed.) Stable isotopes and plant carbon/water relations. Academic Press, New York.

Richards, R.A., G.J. Rebetzke, A.G. Condon, and A.F. van Herwaarden. 2002. Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. Crop Sci. 42:111–121.

Romagosa, I., and J.L. Araus. 1991. Genotype-environment interaction for grain yield and ^{13}C discrimination in barley. p. 563–567. *In* L. Munck et al. (ed.) Proceedings of the Sixth International Barley Genetics Symposium, Helsingborg, Sweden. 22–27 July 1991. Munksgaard International Publishers Ltd., Copenhagen.

Royo, C., A. Michelena, J.M. Carrillo, P. García, J. Juan-Aracil, and C. Soler. 1998. Spanish durum breeding program. p. 80–87. *In* M.M. Nachit et al. (ed.) Proceedings of the SEWANA Durum Network Workshop. 20–23 March 1995. ICARDA, Aleppo.

Royo, C., J. Voltas, and I. Romagosa. 1999. Remobilization of preanthesis assimilates to the grain for grain only and dual-purpose (forage and grain) triticale. Agron. J. 91:312–316.

Royo, C., D. Villegas, L.F. García del Moral, S. El Hani, N. Aparicio, Y. Rharrabti, and J.L. Araus. 2002. Comparative performance of carbon isotope discrimination and canopy temperature depression as predictors of genotype differences in durum wheat yield in Spain. Aust. J. Agric. Res. 53:561–569.

SAS Institute Inc. 1996. SAS/STAT Guide for personal computers, Version 6th. Cary, NC.

Sayre, K.D., E. Acevedo, and R.B. Austin. 1995. Carbon isotope discrimination and grain yield for three bread wheat germplasm groups grown at different levels of water stress. Field Crops Res. 41:45–54.

Slafer, G.A., E.H. Satorre, and F.H. Andrade. 1994. Increases in grain yield in bread wheat from breeding and associated physiological changes. p. 1–68. *In* G.A. Slafer (ed.) Genetic improvement of field crops: Current status and development. Marcel Dekker Inc., New York.

Slafer, G.A., and J.L. Araus. 1998. Keynote address: Improving wheat responses to abiotic stresses. p. 201–213. *In* A.E. Slinkard (ed.) Proceedings of the 9th International Wheat Genetics Symposium Vol. 1, (Section 7 Abiotic Stresses– Keynote address), Saskatoon. 2–7 Aug. 1998. University of Saskatchewan, Saskatoon Univ. Extension Press, Saskatoon, SK.

Slafer, G., J.L. Araus, and R.A. Richards. 1999. Promising traits for future breeding to increase wheat yield. p. 379–415. *In* E.H. Satorre and G.A. Slafer (ed.) Wheat ecology and physiology of yield determination. Food Products Press, New York.

Stewart, G.R., M.H. Turnbull, S. Schmidt, and P.D. Erskine. 1995. ^{13}C natural abundance in plant communities along a rainfall gradient: a biological integrator of water availability. Aust. J. Plant Physiol. 22:51–55.

Snyder, R.L., and W.O. Pruitt. 1991. ETO, Version 1.04. Department of land, air and water resources. Univ. of California, Davis.

Srivastava, J.P. 1991. Improving winter cereals in unfavorable environments. Need for coordinated multidisciplinary research approach. p. 17–32. *In* E. Acevedo et al. (ed.) Improvement and management of winter cereals under temperature, drought and salinity stresses. Monogr. 76. INIA, Madrid.

Turner, N.C. 1993. Water use efficiency of crop plants: potential for improvement. p. 75–82. *In* D.R. Buxton et al. (ed.) International crop science I. CSSA, Madison, WI.

Voltas, J., I. Romagosa, A. Lafarga, A.P. Armesto, A. Sombrero, and J.L. Araus. 1999. Genotype by environment interaction for grain yield and carbon isotope discrimination of barley in Mediterranean Spain. Aust. J. Agric. Res. 50:1263–1271.

Zadoks, J.C., T.T. Chang, and C.F. Konzak. 1974. A decimal code for the growth stage of cereals. Euphytica Bull. 7:42–52.