Breeding Effects on Grain Filling, Biomass Partitioning, and Remobilization in Mediterranean Durum Wheat

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ABSTRACT

This work assesses the changes resulting from durum wheat (*Triticum turgidum* L. var. *durum*) breeding in Italy and Spain during the 20th century on grain filling, biomass production, and allocation at anthesis, and the remobilization of preanthesis photosynthates to the grain of main stems. Field experiments, involving 12 Italian and 12 Spanish cultivars released at different periods, were conducted at two Spanish sites during three growing seasons. Breeding activities did not modify final grain weight (W_G), the maximum rate of grain filling (R), nor grain filling duration (D) of central grains of main spikes. Genetic variability existed for W_G and R within countries and periods. Cycle length (expressed as days from sowing to anthesis [DSA]) was reduced by 2 and 8 d in Italian and Spanish cultivars, respectively. Crop dry weight (CDW) decreased by 21% ($0.51\%\ yr^{-1}$) and green area index (GAI) by 28% ($-0.33\%\ yr^{-1}$) in Spanish cultivars. Green area index at anthesis did not differ between old and modern Italian cultivars, while CDW decreased by 9%. Leaf area index (LAI) remained unchanged in cultivars from both countries, while the spike:stem ratio increased by 8 and 10% in Italian and Spanish cultivars, respectively. Changes over time in DSA were positively associated with changes in CDW and GAI. Dry matter translocation (DMT) efficiency switched from 9% in old cultivars to 20% in modern ones. The contribution of preanthesis assimilates to grain yield (CPA) increased to values of 31 and 27% in Italian and Spanish modern cultivars, respectively. Opportunities for further increases in genetic yield potential in Mediterranean-type environments are discussed.

Italy and Spain are among the largest durum wheat producers in the Mediterranean basin. Despite the contrasting breeding strategies followed during the last century in both countries (Royo, 2005), it has been reported that genetic yield gains in Italian and Spanish durum wheat were attained through the modification of similar crop characteristics. Compared with old cultivars, modern Italian (De Vita et al., 2007) and Spanish cultivars have a shorter period from sowing to anthesis (Miralles et al., 2002), a greater harvest index, and more grains per unit area (Royo et al., 2007). Grain weight has been reported to remain unchanged (Waddington et al., 1987; De Vita et al., 2007; Royo et al., 2007) or to have slightly decreased (Pfeiffer et al., 2000).

Grain weight is the last yield component to be formed. Under Mediterranean conditions, grain filling is limited by several abiotic stresses, including rising temperatures and falling moisture supply, thus constraining yield potential (Simane et al., 1993). Final grain weight can be described as a function of the rate and duration of dry weight accumulation. High heritabilities for grain growth rate have been reported in wheat (May

and Van Sanford, 1992; Mov and Kronstrad, 1994), while it is generally accepted that D largely depends on environmental conditions (Royo et al., 2000; 2006), as its heritability is medium to low (Egli, 1998). However, genetic variability exists for both traits in durum wheat (Gebeyehou et al., 1982; Royo et al., 2006).

Grain filling rate has been associated with higher grain weight in durum wheat (Motzo et al., 1996) although contrasting results appear in the literature regarding its correlation with grain yield (Wardlaw and Moncur 1995; Frederick, 1997; Royo et al., 2006). Findings about the relationship between D and grain yield in small grain cereals are also contradictory (Gebeyehou et al., 1982; Bruckner and Frohberg, 1987). Although no consistent trends in grain weight over the last century have been reported, little is known about the effect of yield improvements on the rate and duration of grain filling, which have been reported to be mutually negatively correlated (Gebeyehou et al., 1982; Royo et al., 2006). Given this negative association, changes over time in phenology and sink size may have affected the rate and duration of grain filling without altering final grain weight.

Studies conducted under optimal conditions have failed to find significant associations between increases in yield during the 20th century and changes in bread wheat biomass at anthesis (Siddique et al., 1989). Contrasting effects of dwarfing genes on plant biomass have been reported for bread wheat

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Abbreviations: CDW, crop dry weight; CPA, contribution of preanthesis assimilates to grain yield; *D*, grain filling duration; DM, dry matter; DMT, dry matter translocation; DSA, days from sowing to anthesis; GA, gibberellic acid; GAI, green area index; GAP, green area per plant; GDD, growing degree-days; LAI, leaf area index; LAP, leaf area per plant; NPM2, number of plants per m²; PCA, principal component analysis; *R*, maximum rate of grain filling; W_G, final grain weight; W_{ST}, main stem weight at anthesis; WSC, water soluble carbohydrates.

experiments conducted in nonlimiting environments and those affected by drought stress. Under well-watered conditions, total plant biomass at anthesis does not seem to be reduced in semi-dwarf genotypes (Miralles and Slafer, 1997), while plants carrying the *Rht-B1* gene show lower plant biomass and green area than their taller isolines when grown in rainfed environments (Richards, 1992). However, shorter isolines seem to be more efficient at storing nonstructural assimilates (Richards, 1992).

Grain filling in wheat is supported by transient photosynthesis (primarily by the flag leaf and the inflorescence) and the translocation of stored reserves accumulated before anthesis (Blum, 1988; Gebbing et al., 1999). In Mediterranean environments, the hot and dry conditions occurring during grain filling reduce photosynthesis rate after anthesis, limiting the contribution of current assimilates to the grain (Johnson et al., 1981). In these circumstances, dry matter (DM) accumulation before anthesis in vegetative parts of the plant and its remobilization to the grain during grain filling becomes particularly important (Papakosta and Gagianas, 1991; Palta et al., 1994). Genetic variation exists in bread wheat for both the capacity to accumulate reserves in vegetative organs and the remobilization efficiency (Nicolas and Turner, 1993; López-Castañeda and Richards, 1994).

Inconsistent results have been published regarding the changes resulting from breeding on the remobilization of pre-anthesis assimilates to grain when comparing old and modern bread wheat cultivars. Siddique et al. (1989) did not find any significant trend over time in DMT, while Austin et al. (1980) and Pheloung and Siddique (1991) reported increases and decreases of DMT over time, respectively. In durum wheat, stem carbohydrate reserves have been estimated to contribute 25 to 34% of the final grain yield under optimal growing conditions (Arduini et al., 2006), while under drought or heat stress they are expected to be greater (Palta et al., 1994; Moragues et al., 2006). Durum wheat biomass at anthesis or during grain filling has been reported to be positively related to grain yield (Villegas et al., 2001), but not in old landraces (Moragues et al., 2006). Consequently, we hypothesized that breeding activities conducted on durum wheat during the last century may have modified the CPA.

The objectives of this work were to assess the changes resulting from durum wheat breeding during the 20th century in Italy and Spain on grain filling traits, biomass production at anthesis, and the remobilization of preanthesis assimilates to grain filling. Changes on yield, yield components, and associated traits have been recently published elsewhere (Royo et al., 2007) and in an upcoming publication (Royo et al. unpublished data).

MATERIALS AND METHODS
Plant Material

Twenty-four durum wheat cultivars, 12 Italian and 12 Spanish, were selected to represent the germplasm grown in both Mediterranean countries during the last century. Based on the year of release, the cultivars were assigned to three periods: old (before 1945), intermediate (from 1950 to 1985), and modern (released from 1988 to 2000). Old varieties were landraces and, in the case of Italy, also selected genotypes from exotic populations such as Senatore Capelli. Within the intermediate varieties, some of the first semidwarf genotypes derived from CIMMYT materials such as Mexa and Creso, were included. The modern varieties were chosen in Spain from among the lately released by local breeding programs, avoiding the inclusion of varieties of foreign origin (Mexican and Italian), despite being the most cultivated in the country. About 20% of the durum wheat seed certified in Italy corresponds to Simeto, the variety most used by Italian farmers. The presence of the *Rht-B1* dwarfing gene in the cultivars had been previously determined (Royo et al., 2007) by analyzing the response of seedlings to gibberellic acid (GA) (Table 1).

Six field experiments were conducted during three growing seasons (2003, 2004, and 2005) at two Spanish sites representing contrasting weather conditions: Lleida in the Ebro Valley (northeast) and Granada in Andalusia (south). The experimental sites and environmental conditions are described in Table 2. Each experiment consisted of a randomized complete block design with three replications and plots of 12 m^2 (8 rows 0.15 m apart). Experiments were planted between 13 November and 23 December in all cases with a sowing rate of 400 and 350 seeds m^{-2} in the northern and the southern experiments, respectively. Plots were fertilized following the recommendations in each region, to prevent lodging and reduce diseases.

Table 1. Cultivars used in the study.

Italian	Year of release	*Rht-B1* presence	Plant height†	Spanish	Year of release	*Rht-B1* presence	Plant height†
			cm				cm
				Old			
Balilla Falso	<1930	–	117	Blanco Verdeal	<1930	–	143
Carlojucci	1945	–	117	Clarofino	<1930	–	140
Razza 208	1930	–	120	Pinet	<1930	–	128
Senatore Capelli	1930	–	136	Rubio de Belalcázar	<1930	–	141
				Intermediate			
Adamello	1985	–	74	Bidi 17	1950	–	104
Capeiti 8	1955	–	100	Camacho	1975	+	80
Creso	1974	+	72	Esquilache	1976	+	74
Trinakria	1970	–	106	Mexa	1980	+	81
				Modern			
Cirillo	1992	+	85	Ariesol	1992	+	74
Flavio	1992	+	65	Astigi	1999	+	83
Simeto	1988	+	76	Boabdil	2000	+	77
Zenit	1992	+	75	Senadur	1995	+	75

† Average of six experiments at two Spanish sites in 2003, 2004, and 2005.

Grain Filling Curve Coefficients

The mid-anthesis date was recorded on each plot and expressed as DSA. On that date, 50 main spikes per plot with simultaneous anthesis were tagged and 3 of them were sampled at random twice per week from each plot from 10 d after anthesis until ripening. To determine dry weight, the grains of three central spikelets were counted on each spike and oven dried at 70°C for 48 h. Grain dry weight data were fitted to the logistic curve proposed by Darroch and Baker (1990), using Proc NLIN of the

SAS-STAT package (SAS Institute, 2000). The suitability of fitting was tested by means of a pseudo-R^2 calculated as follows: $R^2 = 1 – $ (SS of the residual/SS of the corrected total).

The coefficients of the grain filling curves were determined for each plot. Final dry weight (W_G) was estimated in mg. Grain filling duration was considered to be the time in accumulated growing degree-days (GDD) required for grain weight to reach 0.95 W_G, using a base temperature of 9°C (Angus et al., 1981), and was derived from the curve parameters. The maximum rate of grain filling was mathematically determined from the curve parameters, as described in Royo and Blanco (1999), and was expressed as mg of grain GDD^{-1}.

Table 2. Localization and description of the sites and sowing dates for the experiments included in this study.

	North (Lleida)			South (Granada)		
Coordinates	41°40′ N, 0°20′ E			37°08′ N, 3°49′ W		
Altitude, masl	200			684		
Soil characteristics						
Classification	mesic Calcixerolic Xerochrept			loamy Calcixerolic Xerochrept		
Texture	fine-loamy			silty-clay		
Sowing rate (seeds m^{-2})	400			350		
	2003	2004	2005	2003	2004	2005
Seasonal rainfall + irrigation, mm	248 + 150	256 + 150	129 + 150	250 + 40	419 + 40	95.6 + 120
Water input during GFP, mm	77.6	84.2	66.3	1.8	81.4	7.6
Average air temperatures during GFP†						
Tmax, °C	29.0	28.9	29.1	32.3	28.9	34.0
Tmean, °C	21.3	21.1	21.0	23.3	20.6	24.5
Tmin, °C	13.8	13.8	13.6	14.6	13.3	15.3
Evapotranspiration during GFP, mm‡	266	278	282	349	326	537
Sowing date	26 Nov. 2002	16 Dec. 2003	26 Nov. 2004	23 Dec. 2002	13 Nov. 2003	10 Dec. 2004

† GFP, grain-filling period; T$_{max}$, T$_{mean}$, and T$_{min}$ are average maximum, mean, and minimum daily temperatures, respectively.

‡ Calculated by the Penman-FAO methodology (Allen et al., 1998).

Other Measurements

The plants contained in a randomly chosen 0.5-m-length section of a central row of each plot were uprooted at anthesis and physiological maturity. The number of plants and tillers were counted. The area of the leaves (one side laminae) and other green organs of the plant (stem projections including leaf-sheaths and the peduncles of the spike, and spike projections) was measured separately using a leaf-area meter (AT Delta-T-Dias II), excluding from the measurement yellow and senescent tissues. Leaf area index was calculated as LAP × NPM2; LAP is the leaf area per plant and NPM2 is the number of plants per m^2. Green area index was estimated as GAP × NPM2; GAP is the green area per plant, which included LAP and also the projected area of stems (multiplied by 3.14 according to Květ and Marshall [1971] and spikes (multiplied by 2). Samples were oven dried at 70°C for 48 h to determine CDW.

In the experiments of 2004 and 2005, a random subsample of five main stems was taken at the northern site. Green area and dry weight of the spikes, stems (including all leaf-sheaths), flag leaf blades, flag leaf sheaths, and remaining leaf blades were measured separately. Dry matter translocation (g) was computed for each main stem component as the difference between dry weights at anthesis and at physiological maturity, removing grain weight in the case of the spike. Dry matter translocation efficiency (DMT$_{efficiency}$, %), and contribution of preanthesis assimilates to the grain yield (CPA, %) were assessed following the methodology proposed by Papakosta and Gagianas (1991): DMT$_{efficiency}$ = (DMT/DM$_{anthesis}$) × 100 and CPA = (DMT/Main spike grain yield) × 100. Plots were mechanically harvested at ripening and grain yield was adjusted to 120 g/kg moisture content.

Statistical Analysis

Analyses of variance (ANOVA) were performed considering the environment as a random factor. Means were compared by a t test at the 5% significance level. Absolute and relative genetic gains were computed as the slope of the linear regression between the absolute or relative value of the trait and the year of release of the cultivar. Relative values were computed for each variety as a percentage to the average values of all the cultivars for a given country, as described in Royo et al. (2007). Genetic correlations were estimated from the genetic variances and covariances, following the method described by Hanson et al. (1956):

$$r_g = \sigma_{p1p2}/(\sigma^2_{p1} \times \sigma^2_{p2})^{1/2},$$

where σ_{p1p2} is the genetic covariance component for two traits, and σ^2_{p1} and σ^2_{p2} are the respective genetic variance components. Principal component analysis (PCA) was performed on the correlation matrix between means of each variety to determine the relationships between growth traits, final grain weight, and yield per hectare. Stepwise regression analyses were conducted independently for each group defined in the PCA, considering final grain weight and yield per hectare as the dependent variables and growth traits and the contribution of preanthesis assimilates to the grain by the different main stem components as independent variables. All analyses were performed with the SAS-STAT package (SAS Institute, 2000).

RESULTS
Grain Filling Curve Coefficients

The logistic equation used in this study provided an appropriate fit to the grain weight data of the six field experiments (average $R^2 = 0.98 \pm 0.03$). The results of the ANOVA for the coefficients of the grain filling curves revealed that most of the variability was explained by the environment (Table 3). No significant differences between periods of release, countries, or the period × environment interaction were found for any of the three curve coefficients. The period × country interaction was significant for W_G, so mean data of the three curve coefficients are shown in Table 4 for each period within country. Final grain weight of the Italian cultivars differed between periods, the lowest values being those of the intermediate set. On the other hand, Spanish intermediate cultivars had the heaviest grains, although old and modern sets had grains of similar weight. Genetic variability existed for W_G and R within coun-

Table 3. Sum of squares of the ANOVA of the grain filling curve coefficients (W_G, final grain weight; D, grain filling duration; R, maximum rate of grain filling), DSA, days from sowing to anthesis, and growth traits at anthesis (LAI, leaf area index; GAI, green area index; CDW, crop dry weight).

Source of variation	df	W_G	D (×10³)†	R	DSA	Plants m⁻² (×10³)†	Tillers plant⁻¹	LAI	GAI	CDW (×10³)†
		mg	GDD	mg GDD⁻¹	d					g m⁻²
Environment (E)	5	17,223***	1953**	0.766**	50,362***	1272***	70.0***	312**	2145***	43,318***
Block (×E)	12	351***	27.4	0.008	23.7	54.5***	1.04	20.0***	96.2***	1051***
Period (P)	2	50.8	324	0.004	2628***	17.2	0.147	1.22	67.1*	3848***
Country (C)	1	109	0.009	0.001	409***	0.006	0.020	0.386	5.52	82.6
P × C	2	196*	51.9	0.021	786***	1.68	0.292*	11.4	26.6	749**
P × E	10	244	106	0.031	297*	28.3*	1.62**	21.4	98.5	1022
C × E	5	296*	26.7	0.011	25.7	18.1**	0.303	5.28	12.7	194
P × C × E	10	154	77.9**	0.027	82.6	6.1	0.261	22.4**	44.1	494
Variety (P × C)	18	4,084***	85.7	0.132***	2058***	46.8	3.49*	30.1**	43.7	860
Variety (P × C) × E	90	2,631***	266***	0.125***	858***	181***	10.0***	69.9***	247***	4321***
Residual	276	2,438	336	0.166	420	305	14.3	63.9	324	5311
Total	431									

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

† The actual numbers were multiplied by this to obtain the reported numbers.

Table 4. Means of the grain filling curve coefficients (W_G, final grain weight; D, grain filling duration; R, maximum rate of grain filling), DSA, and growth traits at anthesis (LAI, leaf area index; GAI, green area index; CDW, crop dry weight). Data are means of six experiments.

	Italian			Spanish		
	Old	Intermediate	Modern	Old	Intermediate	Modern
W_G, mg	50.4 a†	49.1 b	49.9 ab	48.6 b	50.0 a	47.6 b
D, GDD	400 a	390 a	412 a	427 a	393 a	382 a
R, mg GDD⁻¹	0.22 a	0.21 a	0.21 a	0.20 a	0.22 a	0.22 a
DSA	155 a	151 c	153 b	160 a	154 b	152 c
Plants m⁻²	225 a	231 a	239 a	221 a	236 a	239 a
Tillers plant⁻¹	1.24 a	1.25 a	1.26 a	1.33 a	1.26 ab	1.22 b
LAI	2.64 a	2.58 a	3.08 a	2.92 a	2.90 a	2.66 a
GAI	6.60 a	6.01 b	6.37 ab	7.52 a	6.26 b	5.95 b
CDW, g m⁻²	990 a	803 c	896 b	1112 a	858 b	806 c

† Means within a row and country followed by the same letter are not significantly different at the 0.05 probability level.

Table 5. Absolute and relative change of the grain filling curve coefficients (W_G, final grain weight; D, grain filling duration; R, maximum rate of grain filling), DSA, and growth traits at anthesis (LAI, leaf area index; GAI, green area index; CDW, crop dry weight). Data are means of six experiments.

	Italian			Spanish		
	R^2	Absolute change	Relative change	R^2	Absolute change	Relative change
		yr⁻¹	% yr⁻¹		yr⁻¹	% yr⁻¹
W_G, mg	1.0 × 10⁴†	0.001	0.002	0.04	−0.020	−0.041
D, GDD	0.06	0.199	0.049	0.72***	−0.779	−0.192
R, mg GDD⁻¹	0.10	−3.0 × 10⁴†	−0.132	0.16	2.0 × 10⁴†	0.106
DSA	0.15	−0.044	−0.029	0.78***	−0.125	−0.080
Plants m⁻²	0.12	0.208	0.091	0.10	0.116	0.051
Tillers plant⁻¹	2.4 × 10³†	1.4 × 10⁴†	0.011	0.03	−6.3 × 10⁴†	−0.050
LAI	0.22	0.008	0.283	0.03	−0.001	−0.052
GAI	0.05	−0.004	−0.059	0.74***	−0.021	−0.325
CDW, g m⁻²	0.19	−1.38	−0.155	0.88***	−4.64	−0.506

† The actual numbers were multiplied by this to obtain the reported numbers.

*** $P < 0.001$

tries and periods (Table 3). Maximum and minimum W_G values across environments were 58.5 and 42.7 mg grain⁻¹, both recorded in modern cultivars.

Despite that changes in D of Spanish cultivars were not significant over periods (Table 4), a decreasing tendency was observed since D of modern cultivars was 11 GDD shorter than that of the intermediate ones, which in turn had a D of 34 GDD shorter than that of the old cultivars. This decreasing tendency of D was reflected by the significant negative slope of the linear regression equation fitted to the relationship between D and the year of release of the cultivars (Table 5). Genetic correlations between grain filling curve coefficients were not significant, except for the relationship between R and W_G ($r_g = 0.80$, $P < 0.01$).

Cycle Length to Anthesis

Cycle length to anthesis was significantly affected by the environment, the period, and the country of release (Table 3). The period × country interaction for the length of the cycle from sowing to anthesis was also significant, due to the shortest cycle duration until anthesis being recorded on intermediate Italian cultivars and modern Spanish cultivars (Table 4). The rate of change of the number of days to anthesis was not significant for the Italian cultivars, but it was for the Spanish ones, with an absolute change of −0.125 d yr⁻¹ (Table 5).

Biomass at Anthesis

The environment explained most of the variation in the biomass traits (Table 3). The number of plants per unit area remained unchanged over periods in germplasm from both Italy and Spain (Tables 3 and 4). The period × country interaction was significant for the number of tillers per plant (Table 3), since it did not significantly change in Italian cultivars, whereas modern Spanish genotypes had less tillers per plant than the old ones (Table 4). The lack of significance of the linear regression line fitted to the relationship between the number of tillers per plant and the year of release of the Spanish cultivars (Table

5) indicates that the reduction of the tiller number from old to modern Spanish cultivars was not linear. Actually, the decrease was greater from old to intermediate than from intermediate to modern cultivars (Table 4). The period × environment interaction, significant for the number of plants per unit area and the number of tillers per plant, was quantitative in nature for both traits.

The period of release did not exert any significant effect on LAI. Green area index and CDW decreased from old to intermediate cultivars in both Italian and Spanish sets. However, while GAI remained constant between intermediate and modern cultivars from both countries, CDW decreased from intermediate to modern Spanish cultivars, and increased in the modern period within the Italian set (Table 4). This change of the CDW tendency over time caused the linear regression between CDW and the year of release of Italian cultivars to be not significant (Table 5).

Absolute and relative changes in GAI were only significant for Spanish cultivars, which decreased at a rate of –0.32% yr^{-1} (Table 5). Changes over time in the length of the period until anthesis proved to be positively associated with changes in GAI ($R^2 = 0.33$, $P < 0.05$; $R^2 = 0.62$, $P < 0.01$) in Italian and Spanish cultivars, respectively, and in CDW ($R^2 = 0.59$, $P < 0.01$; $R^2 = 0.74$, $P < 0.001$) in Italian and Spanish cultivars, respectively.

Table 6. Mean values of traits measured in the whole main stem. Area and weight were calculated as the sum of leaves, stem, and spike values. †

	Italian			Spanish		
	Old	Intermediate	Modern	Old	Intermediate	Modern
Area, cm^2	217 a‡	187 b	191 b	256 a	184 b	178 b
Weight, g	3.27 a	2.77 b	2.84 b	3.90 a	2.77 b	2.57 b
MS, %	72.9 a	77.4 a	78.4 a	73.7 b	81.3 a	75.2 ab
DMT, mg	254 b	533 a	647 a	470 a	562 a	547 a
DMT efficiency, %	7.19 b	17.9 a	21.0 a	10.4 b	19.3 a	19.6 a
CPA, %	14.7 b	28.1 a	30.8 a	24.0 b	28.8 a	27.1 a

† MS, main stem weight with regard to total aboveground plant weight; DMT, dry matter translocation; CPA, contribution of preanthesis assimilates to grain yield. Data are means of two experiments conducted in northern Spain.

‡ Means within a row and country followed by the same letter are not significantly different at the 0.05 probability level.

Table 7. Means of the area and weight of the main stem components at anthesis and their dry matter translocation (DMT) efficiency. Leaf blades include the blade of the flag leaf. Stem + sheaths values include the sheath of the flag leaf. Data are means of two experiments conducted in northern Spain.

	Italian			Spanish		
	Old	Intermediate	Modern	Old	Intermediate	Modern
Area			cm^2			
Spike	16.8 b†	17.7 ab	19.5 a	22.9 a	22.1 a	21.1 a
Flag leaf blade	25.2 a	21.2 b	24.2 a	29.3 a	25.0 b	20.0 c
Flag leaf sheath	22.2 a	17.8 b	18.2 b	26.8 a	19.4 b	16.4 b
Leaf blades	91.4 a	78.3 b	87.0 a	101 a	78.9 b	75.8 b
Stem + sheaths	111 a	91.1 b	83.7 b	132 a	82.8 b	80.9 b
Weight			g			
Spike	0.467 b	0.463 b	0.520 a	0.594 a	0.581 a	0.526 b
Flag leaf blade	0.128 a	0.117 b	0.129 a	0.147 a	0.135 b	0.103 c
Flag leaf sheath	0.204 a	0.175 b	0.175 b	0.243 a	0.170 b	0.151 c
Leaf blades	0.431 a	0.375 b	0.444 a	0.470 a	0.399 b	0.364 b
Stem + sheaths	2.37 a	1.93 b	1.86 b	2.84 a	1.79 b	1.67 b
DMT$_{efficiency}$			%			
Spike	0.00 b	2.51 ab	2.86 a	1.74 a	2.99 a	3.62 a
Flag leaf blade	22.8 a	20.4 a	21.6 a	28.6 a	28.2 a	17.0 b
Flag leaf sheath	12.5 a	17.5 a	15.4 a	17.1 a	17.2 a	15.2 a
Leaf blades	20.5 a	26.3 a	26.4 a	16.8 b	25.4 ab	25.6 a
Stem + sheaths	5.84 b	19.7 a	24.9 a	10.8 b	22.6 a	22.6 a

† Means within a row and country followed by the same letter are not significantly different at the 0.05 probability level.

Biomass Allocation at Anthesis

In 2004 and 2005, five main stems per plot were dissected at anthesis in northern experiments to assess the changes caused by empirical breeding on main stem components and DMT. More than 72% of total plant biomass proved to be allocated on the main stem (Table 6).

The whole main stem area and weight, considering all its components, decreased from old to intermediate cultivars and remained unchanged during the last period (Table 6). The main stem structures responsible for these changes were the stem + sheaths in cultivars of both countries and the leaves of the Spanish subset (Table 6). The area and weight of the leaf blades of Italian cultivars also decreased between the first two periods, with a further increase in modern genotypes. Main spike biomass at anthesis increased from old to modern Italian cultivars, but it decreased in the Spanish ones. Differences in the pattern of changes in leaf-biomass allocation between Italian and Spanish cultivars were also noted in flag leaf blades (Table 7). On the other hand, the weight and green area of flag leaf sheaths decreased until the intermediate period in cultivars from both countries.

The relative contribution of the different main stem components to the total main stem green area and weight is shown in Fig. 1. The relative area and dry weight of the stem decreased across periods in cultivars from the two countries (Fig. 1), while the relative magnitude of the spike tended to increase. The pattern of changes of leaf blades was similar for the flag leaf and the remaining leaves within Italian cultivars. Their relative contribution to main stem biomass and green area did not change from old to intermediate periods, but increased in the last period. In Spanish cultivars, the relative contribution of flag leaf blade to main stem biomass increased initially but decreased in the last period (Fig. 1b and 1d). The flag leaf sheath remained unchanged over time in germplasm from both countries.

Translocation of Preanthesis Assimilates to the Growing Grains

The amount of DMT from the main stem structures to growing grains increased significantly in Italian cultivars. In Spanish cultivars, changes over time in DMT were not significant, although modern genotypes translocated 16% more DMT than old ones (Table 6). Dry matter translocation efficiency and the CPA increased significantly over time in cultivars from both countries (Table 6). The main stem component
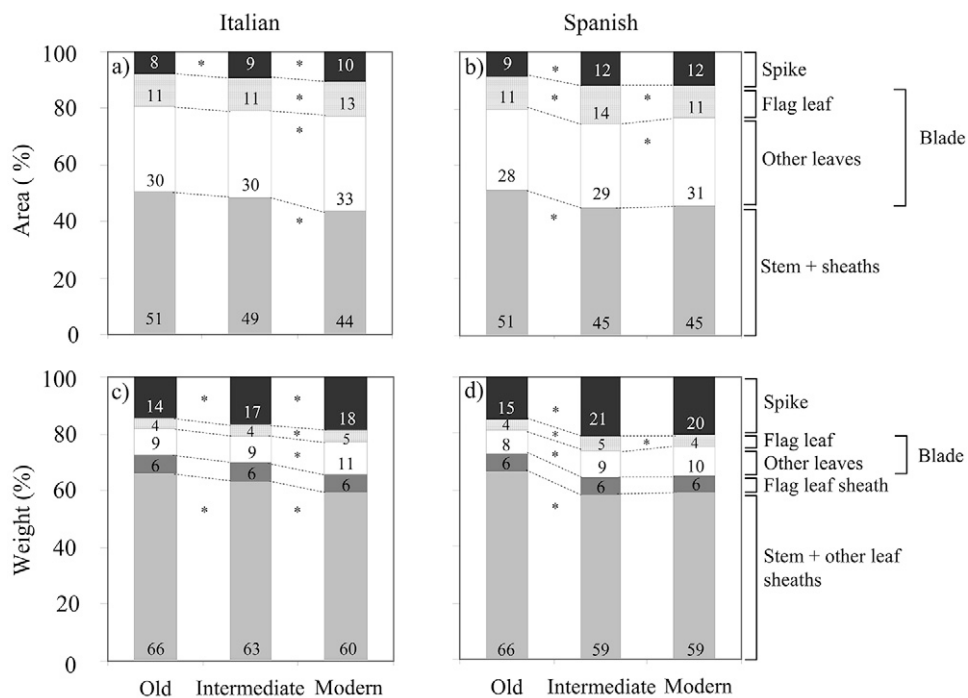
Fig. 1. Partitioning of main stem area and weight at anthesis on its components for Italian and Spanish cultivars released in different periods.
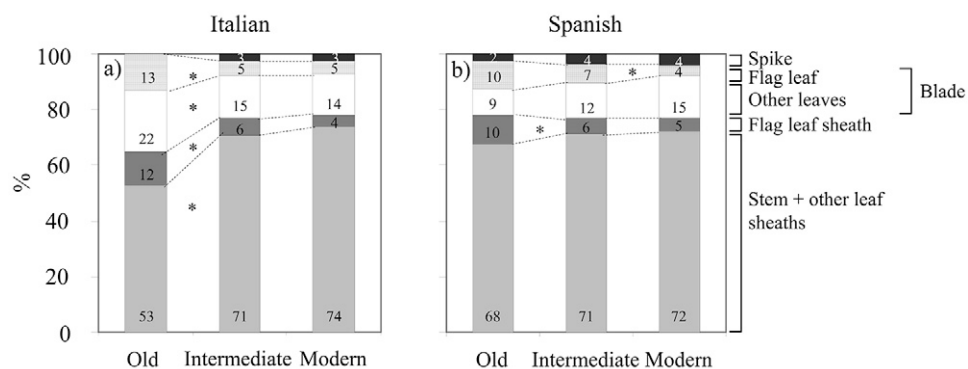

Fig. 2. Relative contribution of preanthesis assimilates accumulated on main stem components to the grain yield of the main spike for Italian and Spanish cultivars released in different periods.

axis measures the weight (loading or eigenvalue) of its influence on that axis, whereas the cosine of the angle between any two vectors is inversely proportional to their correlation. The first axis demonstrates a positive influence of flag leaf and main stem biomass, as well as the contribution of preanthesis assimilates stored in the flag leaf to grain filling, while a negative influence of grain yield. The traits with greatest weight on PC2 were the relative weight of the main stem in relation to total plant biomass, grain yield, and the contribution to grain filling of assimilates accumulated before anthesis on the main stem and spike. Figure 3b shows the projection on PC1 and PC2 of the points of the mean genotype values across environments. Two big clusters were formed: one including old Italian and Spanish cultivars, and the other the intermediate and modern ones. Old cultivars tended to have negative values for PC2, while intermediate and modern cultivars were mostly located in the positive direction of this axis, suggesting that yield, the relative weight of the main stem as a percentage of total plant biomass, as well as the contribution to grain yield of preanthesis assimilates accumulated in the main stem, leaves, and spike were the most important traits contributing to the separation of the two groups. The modern Italian variety Simeto was

that showed the largest increase over time in $DMT_{efficiency}$ was the stem (Table 7). It also accounted for most of the DMT translocated to grains in cultivars from all periods and countries (Fig. 2). The contribution of leaves was much less, and that of the spike practically negligible. The greatest changes in the percentage of DMT by the different main stem components occurred between old and intermediate Italian cultivars: the relative contribution of main stem increased significantly by 21% from old to modern cultivars, while the contribution of the flag leaf blade, other leaf blades, and flag leaf sheath each decreased by about 8% (Fig. 2).

Multivariate Analyses

Principal component analysis was performed to determine the relationship between traits measured at the main stem level, the number of days from sowing to anthesis, and yield per unit area. The first two axes of the PCA explained 60.4% (41.6 and 18.8%, respectively) of the variability found. Figure 3a shows the eigenvectors of the traits influencing each axis. The length of the projection of each vector on each principal component

an outlier due to its exceptional grain weight. On the other hand, the group formed by old cultivars showed more variability in flag leaf and main stem biomass than intermediate and modern cultivars.

To determine which traits contributed most to final grain weight, stepwise regression analyses were performed independently for each group formed in the PCA. The only variable that significantly entered the regression analysis for old cultivars was the relative weight of the main stem (%MS), which explained 71% ($P < 0.01$) of final grain weight variations. In the regression conducted with data of intermediate and modern cultivars, the only trait that entered was main stem weight at anthesis (W_{ST}), which explained 43% ($P < 0.01$) of grain weight variations. However, when the variety Simeto was removed from the analysis no variable entered the regression equation significantly.

Stepwise regression analyses were also conducted considering yield per hectare as a dependent variable. When cultivars of the three periods were included in the analysis, the only variable that entered in the regression equation was the number of days

from sowing to anthesis. It had a negative relationship with yield and explained 58.6% ($P < 0.0001$) of yield variations. When the same analysis was conducted for the two groups of Fig. 3b separately, the number of days to anthesis entered negatively in the equation for old cultivars, explaining 74.4% of yield variations ($P < 0.01$). However, no variable entered the regression equation for modern and intermediate varieties.

DISCUSSION

The results of this study indicate that W_G, R, and D of central grains of main spikes were not modified by plant breeding during the 20th century. However, small variations were found in W_G of intermediate Italian and Spanish cultivars compared with the old and modern genotypes. The slight increase in W_G of intermediate Spanish cultivars may be attributed to the introduction in the 1970s of improved semidwarf germplasm from CIMMYT (Royo, 2005), where empirical breeding for grain yield per se was complemented with selection for larger sink sizes through an increased number of grains per spike and grain weight (Waddington et al., 1987). The slight decrease in W_G of Italian cultivars may be explained by the earlier breeding activities conducted in Italy during the first few decades of the 20th century (Borghi, 2001). This assumption is supported by the high grain weight of old Italian cultivars. The genetic variability existing for W_G may allow further grain weight improvements. Moreover, the strong genetic correlation that appears between W_G and R ($r_g = 0.80$, $P < 0.01$) reveals that grain weight may also be improved by selecting for greater rates, without a penalty on D, as is suggested by the lack of genetic correlation between rate and duration.

Grain filling duration was the trait most affected by the environment, as previously stated in durum wheat (Royo et al., 2006) and other cereals under Mediterranean conditions (Royo et al., 2000). In Spanish genotypes, D tended to decrease following a linear pattern, but the change was not large enough to result in significant differences between old and modern cultivars. Total cycle length of modern Spanish cultivars was about 11 d shorter than that of old landraces. This was due to decreases of 8 d in the duration of the cycle to anthesis, which decreased at a rate of -0.12 d yr^{-1}, and 45 GDD, or about 3 d in D, both coinciding with the introduction of improved germplasm. The later flowering of old cultivars may have been a disadvantage in our conditions, since they were more exposed to terminal drought than the modern earlier-flowering cultivars.

Breeding activities performed during the time elapsed between old and intermediate cultivars (about the first half of the 20th century) reduced crop biomass and GAI at anthesis, while LAI was not significantly affected. Reductions in crop biomass and GAI were a consequence of the reduction in plant biomass and GAP, since no changes were observed in the number of plants per unit area. The reduction in plant biomass and GAP was a direct consequence of the incorporation of dwarfing genes, which occurred in the interval from old to intermediate cultivars. Actually, the Norin10-derived gene *Rht-B1* is carried by one and three out of four Italian and Spanish intermediate cultivars, respectively. Moreover, the shorter plant height of the variety Adamello suggests that other GA-sensitive dwarfing genes, from alternative sources, were also used by Italian breeders to reduce plant height (Royo et al.,
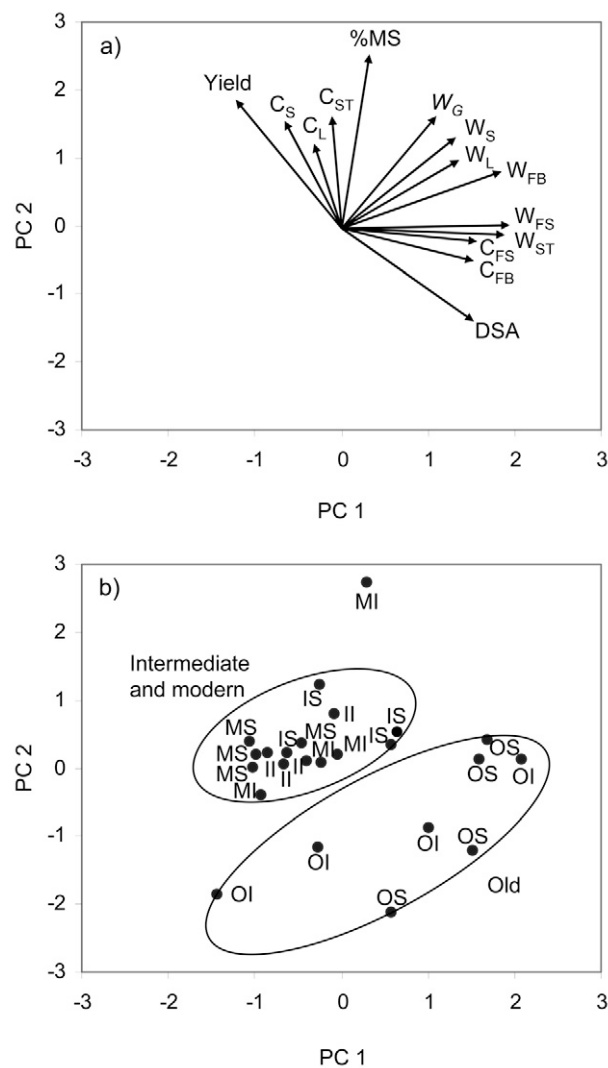

Fig. 3. Principal component (PC) analysis projections on axes 1 and 2. (a) Eigenvalues are presented as vectors for the following traits: contribution of preanthesis assimilates to the grain by the spike (C_S), flag leaf blade (C_{FB}), flag leaf sheath (C_{FS}), other leaf blades (C_L), and stem (C_{ST}), final grain weight (W_G), dry weight of the spike (W_S), flag leaf blade (W_{FB}), flag leaf sheath (W_{FS}), other leaf blades (W_L), and stem (W_{ST}), days from sowing to anthesis (DSA), relative main stem weight with regard to total aboveground plant biomass (%MS), and grain yield per hectare. (b) Values of the cultivars are symbolized by their period and country: old Italian (OI), intermediate Italian (II), modern Italian (MI), old Spanish (OS), intermediate Spanish (IS), and modern Spanish (MS).

2007). The significant relationship found in this study between time until anthesis and biomass accumulation is in agreement with previous findings of Richards (1992), who reported that the dwarfing gene *Rht-B1* reduced biomass at anthesis due to its pleiotropic effect on shortening cycle length, given that semidwarf isolines had similar growth rates to the tall ones. The slight changes in biomass and green area between intermediate and modern cultivars observed in this study may also be explained by changes in cycle length until anthesis.

On average, main stem accounted for 76% of total plant biomass. The relative weight of the main stem (%MS) and the number of tillers per plant did not change over time in Italian cultivars. Consequently, the biomass per tiller must have decreased by 13%, the same percentage as main stem biomass.

The scenario was different for the Spanish cultivars, in which a reduction of 8% in the number of tillers per plant took place from old to modern genotypes. Since the relative weight of the main stem was not significantly lower in modern genotypes, biomass per tiller must have been reduced in Spanish cultivars, and this reduction must have been smaller than the 34% decrease observed in main stem biomass. The cause may have been an improvement in the percentage of fertile tillers since a previous study, involving the same set of cultivars, found that the number of spikes per plant increased over time in Spanish durum wheats (Royo et al., 2007).

Main stem (stem + sheaths) weight and area decreased from old to modern Italian genotypes by 21 and 25%, respectively. These decreases were compensated by increases of 11 and 16% in spike weight and area, respectively, while leaf blades remained unchanged. In Spanish cultivars, however, the weight and area of all main stem components decreased, but to a different extent. The largest reductions corresponded to the weight and area of stems (around 40%), followed by those of leaf blades (around 25%) and spikes (around 10%). Nevertheless, independently of the modifications that occurred in each country, the distribution of biomass within the main stem was similar in Italian and Spanish modern cultivars (Fig. 1), which suggests that an analogous plant ideotype was sought by breeders from both countries. Differences in the changes of biomass allocation between Italian and Spanish durum wheats are the consequence of the contrasting main stem configuration of the old sets of cultivars used in this study, since the main stem of Spanish landraces outweighed that of the Italian old ones by 20%. The changes, mainly due to the reduction in plant height, resulted in increases of 8 and 10% in the spike-to-stem ratio in Italian and Spanish cultivars, respectively. A lesser demand of photosynthates by the stem during jointing may have increased the availability of assimilates for the growing spikes.

Changes in the photosynthesis/translocation ratio occurred during the 20th century in cultivars from both countries. The CPA increased significantly over time, suggesting that the net assimilation of CO_2 during the grain filling period did not increase sufficiently to cover the rising demand of the larger sinks of modern cultivars (Royo et al., 2007; Álvaro et al., 2008). Modern durum wheats tended to translocate more preanthesis assimilates to filling grains and, due to the reduction in main stem biomass, they were much more efficient in translocation than the old genotypes. The increase in DMT of Italian cultivars was mostly due to the enlarged contribution of assimilates from the stem, since the role of the blade and sheath of the flag leaf in translocation decreased significantly over time. In accordance with our results, studies based on growth analyses have reported larger losses of DM from vegetative organs during grain filling in modern than in old cultivars (Austin et al., 1980; Mehrhoff and Kühbauch, 1990). These findings are also supported by research based on the content of water soluble carbohydrates (WSC) in the stem, which showed higher WSC concentrations (Ehdaie et al., 2006) and more rapid and complete WSC mobilization (Mehrhoff and Kühbauch, 1990) in modern than in old bread wheat cultivars.

The greatest changes in DMT and related traits occurred between old and intermediate genotypes, thus coinciding with the introduction of dwarfing genes in the germplasm of

both countries. This could lead to the conclusion that it was a pleiotropic effect of dwarfing genes. However, *Rht* alleles have not been found to confer the ability to increase DMT, while $DMT_{efficiency}$ of the stem has proved to be higher in *Rht-B1* lines (Borrell et al., 1993). Therefore, greater DMT capacity may have been unconsciously increased when empirical breeding for yield led to the selection of genotypes able to fill the large number of grains per unit area typical of modern cultivars (Royo et al., 2007). Studies conducted on bread wheat have demonstrated that reductions in sink size by de-graining reduced the amount of assimilates remobilized from the stem, thus suggesting that increased sink demand determined stem reserve mobilization (Kühbauch and Thome, 1989). The improved DMT capacity and efficiency of modern released cultivars may also account for the higher yields of high-responsive genotypes, even when they are grown in mildly to moderately drought-stressed Mediterranean environments (Richards, 2000; Richards et al., 2002).

The results of the PCA showed the divergence between old cultivars and improved cultivars, most of them carrying dwarfing genes. Several traits helped to differentiate the two groups, but the most important were DSA and grain yield. Days from sowing to anthesis, the contribution of flag leaf assimilates to grain yield and stem weight, were greater in old cultivars, while yield per unit area, the contribution to grain yield of assimilates accumulated in the main spike and stem, reached higher values in cultivars carrying dwarfing genes. Main stem biomass was better than leaf blade biomass in discriminating between tall and dwarf genotypes because dwarfing genes have been reported to affect internode growth more than leaf blade expansion (Calderini et al., 1996; Miralles et al., 1998). The traits most positively associated with yield per unit area were the contribution of preanthesis assimilates from the spike, other leaves apart from the flag leaf and the stem. Principal component analysis confirmed that the role of the flag leaf on the remobilization of assimilates to grain has largely decreased as a result of empirical yield improvement.

Stepwise regression confirmed the negative relationship between yield and DSA in the whole set of cultivars and within the old group. Our results indicate that during the last century breeding efforts devoted to durum wheat improvement in Mediterranean countries were largely concentrated in matching phenology to avoid terminal stresses.

The relative weight of main stem explained 71% of the variation in the weight of central grains from main spikes of old cultivars. This result suggests that those cultivars with fewer tillers achieved greater grain weight, probably due to the competition for assimilates between main stem and tillers, because wheat plants with inhibited tillering produce heavier grains (Duggan et al., 2005). In intermediate and modern cultivars, main stem weight accounted for 43% of the variability observed in the final grain weight of main spikes, thus suggesting that selection for tall plants within the semidwarf genotypes would improve grain weight. However, the fact that none of the traits entered in the model when the variety Simeto, with exceptional grain weight, was removed from the analysis suggests that the relationship found was caused by the increased range of varia-

tion introduced by Simeto. Moreover, it also indicates that phenotype variability for grain weight among the remaining cultivars was not enough for any of the studied traits to be entered in the regression equation.

Breeding Implications

Yield progress in durum wheat during the last century has been related to an increase in the number of grains per unit area (Royo et al., 2007; De Vita et al., 2007; Álvaro et al., unpublished data), although, as demonstrated by our results, it has not involved significant changes in grain growth. The results of our experiments indicate that the greater demand of assimilates from the sink of modern cultivars was compensated by an increase in the amount and efficiency of DMT to the spike, especially from the stem. However, terminal stresses in our experiments may have limited photosynthesis during grain filling, enhancing the role of translocation in grain growth.

One of the ways to achieve future durum wheat yield increases under Mediterranean conditions is to keep improving DMT to grains, which may result from an enlargement of the source size (biomass) at anthesis or from increases in DMT efficiencies. Even though a positive relationship between grain yield and biomass at anthesis has been reported for improved durum wheat cultivars (Villegas et al., 2001), variability for the rate and duration of biomass production has still to be demonstrated (Villegas et al., 2001). On the other hand, this study showed that DMT efficiency increased as much as 21% in modern cultivars, but higher values (around 30%) have been observed for this trait in bread wheat grown under nonlimiting conditions (Austin et al., 1980). Therefore, it seems that there is still room for further improvements in the efficiency of DMT, to ensure grain filling without increasing plant biomass in those environments where transient photosynthesis after anthesis is inhibited.

REFERENCES

Allen, R.G., L.S. Pereira, D. Raes, and M. Smith. 1998. Crop evapotranspiration. Guidelines for computing crop water requirements. FAO Irrigation and Drainage Paper 56. FAO, Rome.

Álvaro, F., J. Isidro, D. Villegas, L.F. García del Moral, and C. Royo. 2008. Old and modern durum wheat varieties from Italy and Spain differ in spike components. Field Crops Res. 106:86–93.

Angus, J.F., D.H. Mackenzie, R. Morton, and C.A. Schafer. 1981. Phasic development in field crops II. Thermal and photoperiodic responses of spring wheat. Field Crops Res. 4:269–283.

Arduini, I., A. Masoni, L. Ercoli, and M. Mariotti. 2006. Grain yield, and dry matter and nitrogen accumulation and remobilization in durum wheat as affected by variety and seeding rate. Eur. J. Agron. 25:309–318.

Austin, R.B., J. Bingham, R.D. Blackwell, L.T. Evans, M.A. Ford, C.L. Morgan, and M. Taylor. 1980. Genetic improvements in winter wheat yields since 1900 and associated physiological changes. J. Agric. Sci. 94:675–689.

Blum, A. 1988. Physiological selection criteria for drought resistance. In G. Wittmer (ed.) The future of cereals for human feeding and devel-

opment of biotechnological research. p. 191–199. Int. Fair of Agric., 39th, Foggia, Italy.

Borghi, B. 2001. Italian wheat pool. In A.P. Bonjean et al. (ed.) The world wheat book, a history of wheat breeding. Lavoisier Publ., Paris. p. 289–309.

Borrell, A.K., L.D. Incoll, and M.J. Dalling. 1993. The influence of *Rht1* and *Rht2* alleles on the deposition and use of stem reserves in wheat. Ann. Bot. 71:317–326.

Bruckner, P.L., and R.C. Frohberg. 1987. Rate and duration of grain fill in spring wheat. Crop Sci. 27:451–455.

Calderini, D.F., D.J. Miralles, and V.O. Sadras. 1996. Appearance and growth of individual leaves as affected by semidwarfism in isogenic lines of wheat. Ann. Bot. 77:583–589.

Darroch, B.A., and R.J. Baker. 1990. Grain filling in three spring wheat genotypes: Statistical analysis. Crop Sci. 30:525–529.

De Vita, P., O.L.D. Nicosia, F. Nigro, C. Platani, C. Riefolo, N. Di Fonzo, and L. Cattivelli. 2007. Breeding progress in morpho-physiological, agronomical and qualitative traits of durum wheat cultivars released in Italy during the 20th century. Eur. J. Agron. 26:39–53.

Duggan, B.L., R.A. Richards, A.F. van Herwaarden, and N.A. Fettell. 2005. Agronomic evaluation of a tiller inhibition gene (*tin*) in wheat. I. Effect on yield, yield components, and grain protein. Aust. J. Agric. Res. 56:169–178.

Egli, D.B. 1998. Seed biology and the yield of grain crops. CAB International, Oxford.

Ehdaie, B., G.A. Alloush, M.A. Madore, and J.G. Waines. 2006. Genotypic variation for stem reserves and mobilization in wheat: II. Postanthesis changes in internode water-soluble carbohydrates. Crop Sci. 46:2093–2103.

Frederick, J.R. 1997. Winter wheat leaf photosynthesis, stomatal conductance, and leaf N concentration during reproductive development. Crop Sci. 37:1819–1826.

Gebbing, T., H. Schnyder, and W. Kühbauch. 1999. The utilization of preanthesis reserves in grain filling of wheat. Assessment by steady-state $^{13}CO_2/^{12}CO_2$ labelling. Plant Cell Environ. 22:851–858.

Gebeyehou, G., D.R. Knott, and R.J. Baker. 1982. Rate and duration of grain filling in durum wheat cultivars. Crop Sci. 22:337–340.

Hanson, C.H., H.F. Robinson, and R.E. Comstock. 1956. Biometrical studies of yield in segregating populations of Korean Lespedeza. Agron. J. 48:268–272.

Johnson, R.C., R.E. Witters, and A.J. Ciha. 1981. Daily patterns of apparent photosynthesis and evapotranspiration in a developing winter wheat crop. Agron. J. 73:414–418.

Kühbauch, W., and U. Thome. 1989. Nonstructural carbohydrates of wheat stems as influenced by sink-source manipulations. J. Plant Physiol. 134:243–250.

Kvĕt, J., and J.K. Marshall. 1971. Assessment of leaf area and other assimilating plant surfaces. In Z. Šesták et al. (ed.) Plant photosynthetic production. Manual of methods. Dr. W. Junk N.V., Publ., The Hague, Chapter 14, p. 517–555.

López-Castañeda, C., and R.A. Richards. 1994. Variation in temperate cereals in rainfed environments. II. Phasic development and growth. Field Crops Res. 37:63–75.

May, L., and D.A. Van Sanford. 1992. Selection for early heading and correlated response in maturity of soft red winter wheat. Crop Sci. 32:47–51.

Mehrhoff, R., and W. Kühbauch. 1990. Yield components in old and new German winter-wheat varieties with respect to the storage and remobilization of fructan in the wheat-stem. J. Agron. Crop Sci. 165:47–53.

Miralles, D.J., D.F. Calderini, K.P. Pomar, and A. D'Ambrogio. 1998. Dwarfing genes and cell dimensions in different organs of wheat. J. Exp. Bot. 49:1119–1127.

Miralles, D.J., Y. Rharrabti, C. Royo, D. Villegas, and L.F. Garcia del Moral. 2002. Grain setting strategies of Mediterranean durum wheat cultivars released in different periods (1900–2000). In Genotype-phenotype: Narrowing the gaps. The Royal Agric. College, Cirencester, UK. 16–18 Dec. Assoc. of Applied Biologists, ellesbourne, Warwick, UK.

Miralles, D.J., and G.A. Slafer. 1997. Radiation interception and radiation use efficiency of near-isogenic wheat lines with different height. Euphytica 97:201–208.

Moragues, M., L.F. García del Moral, M. Moralejo, and C. Royo. 2006. Yield formation strategies of durum wheat landraces with distinct pat-

tern of dispersal within the Mediterranean basin. II. Biomass production and allocation. Field Crops Res. 95:182–193.

Motzo, R., M. Giunta, and F. Deidda. 1996. Relationships between grain-filling parameters, fertility, earliness and grain protein of durum wheat in a Mediterranean environment. Field Crops Res. 47:129–142.

Mov, B., and W.E. Kronstrad. 1994. Duration and rate of grain filling in selected winter wheat populations. I. Inheritance. Crop Sci. 34:833–837.

Nicolas, M.E., and N.C. Turner. 1993. Use of chemicals desiccants and senescing agents to select wheat lines maintaining stable grain size during post-anthesis drought. Field Crops Res. 31:155–171.

Palta, J.A., T. Kobata, I.R. Fillery, and N.C. Turner. 1994. Remobilization of carbon and nitrogen in wheat as influenced by postanthesis water deficits. Crop Sci. 34:118–124.

Papakosta, D.K., and A.A. Gagianas. 1991. Nitrogen and dry matter accumulation, remobilization, and losses for Mediterranean wheat during grain filling. Agron. J. 83:864–870.

Pfeiffer, W.H., K.D. Sayre, and M.P. Reynolds. 2000. Enhancing genetic grain yield potential and yield stability in durum wheat. In C. Royo et al. (ed.). Durum wheat improvement in the Mediterranean region: New challenges. Options Méditerranéennes 40:83–93.

Pheloung, P.C., and K.H.M. Siddique. 1991. Contribution of stem dry matter to grain yield in wheat cultivars. Aust. J. Plant Physiol. 18:53–64.

Richards, R.A. 1992. The effect of dwarfing genes in spring wheat in dry environments. II. Growth, water use and water-use efficiency. Aust. J. Agric. Res. 43:517–527.

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

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.

Royo, C. 2005. Durum wheat improvement in Spain. In C. Royo et al.(ed.). Durum wheat breeding: Current approaches and future strategies.

Food Products Press, New York. p. 883–906.

Royo, C., M. Abaza, R. Blanco, and L.F. García del Moral. 2000. Triticale grain growth and morphometry as affected by drought stress, late sowing and simulated drought stress. Aust. J. Plant Physiol. 27:1051–1059.

Royo, C., F. Álvaro, V. Martos, A. Ramdani, J. Isidro, D. Villegas, and L.F. García del Moral. 2007. Genetic changes in durum wheat yield components and associated traits in Italian and Spanish varieties during the 20th century. Euphytica 155:259–270.

Royo, C., and R. Blanco. 1999. Growth analysis of five spring and five winter triticale genotypes. Agron. J. 91:305–311.

Royo, C., D. Villegas, Y. Rharrabti, R. Blanco, V. Martos, and L.F. García del Moral. 2006. Grain growth and yield formation of durum wheat grown at contrasting latitudes and water regimes in a Mediterranean environment. Cereal Res. Commun. 34:1021–1028.

SAS Institute. 2000. SAS OnlineDoc. v. 8. SAS Inst., Cary, NC.

Siddique, K.H.M., R.K. Belford, M.W. Perry, and D. Tennant. 1989. Growth, development and light interception of old and modern wheat cultivars in a Mediterranean-type environment. Aust. J. Agric. Res. 40:473–487.

Simane, B., J.M. Peacock, and P.C. Struik. 1993. Differences in developmental plasticity and growth rate among drought-resistant and susceptible cultivars of durum wheat (*Triticum turgidum* L. var. *durum*). Plant Soil 157:155–166.

Villegas, D., N. Aparicio, R. Blanco, and C. Royo. 2001. Biomass accumulation and main stem elongation of durum wheat grown under Mediterranean conditions. Ann. Bot. 88:617–627.

Waddington, S.R., M. Osmanzai, M. Yoshida, and J.K. Ransom. 1987. The yield of durum wheats released in Mexico between 1960 and 1984. J. Agric. Sci. 108:469–477.

Wardlaw, I.F., and L. Moncur. 1995. The response of wheat to high temperature following anthesis. I. The rate and duration of kernel filling. Aust. J. Plant Physiol. 22:391–397.