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Changes in duration of developmental phases of durum wheat caused by breeding in Spain and Italy during the 20th century and its impact on yield

Julio Isidro[1,*], Fanny Álvaro[2], Conxita Royo[2], Dolors Villegas[2], Daniel J. Miralles[3] and Luis F. García del Moral[1]

[1]*Departamento de Fisiología Vegetal, Facultad de Ciencias, Instituto de Biotecnología, Universidad de Granada, 18071 Granada, Spain,* [2]*IRTA, Cereal Breeding, Centre UdL-IRTA, Rovira Roure, 191, 25198 Lleida, Spain and* [3]*CONICET, IFEVA and Cátedra de Cerealicultura, Facultad de Agronomía, Universidad de Buenos Aires, Avda. San Martin 4453 (C 1417 DSE), Buenos Aires, Argentina*
* *For correspondence. E-mail jisidro@ugr.es*

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- *Background and Aims* Although the apical development of wheat has been widely described, studies analysing how genetic breeding over the 20th century influenced the developmental phases and its consequences on yield generation are lacking, especially for durum wheat under field conditions in Mediterranean environments. The aims of this study were to analyse the effects of breeding in Spain and Italy on crop development during the last century, to determine whether or not breeding significantly altered the developmental phases between sowing and maturity, and to evaluate the importance of each phase in determining the number of grains per spike of durum wheat (*Triticum durum*) cultivars representing the germplasm grown throughout the 20th century in Spain and Italy.
- *Methods* Eight field experiments were carried out during 4 years in two contrasting latitudes (Lleida and Granada, Spain). Plant material consisted of 24 durum wheat cultivars (12 Italian and 12 Spanish) grown throughout the 20th century in Spain and Italy.
- *Key Results* In Spanish materials, breeding reduced the duration of the period from sowing to anthesis, placing the grain-filling period in better conditions. In those cultivars, the sub-phase sowing–terminal spikelet formation was reduced while the duration of the period from booting to anthesis was increased. The number of grains per spike increased by 23 % from old to modern cultivars, by changes in the number of grains per spikelet in both Spanish and Italian cultivars. Floral abortion from booting to anthesis diminished by 24 % from old to modern cultivars, and grain setting increased by 13 %.
- *Conclusions* The results suggest that breeding reduced not only plant height, but also the time to anthesis. By extending the duration of the phase from booting to anthesis, which was associated with an increase in spike dry weight and grains per spike, it suggests that future increases in spike fertility could be achieved by enlarging that phase.

Key words: *Triticum durum*, apical development, terminal spikelet, durum wheat, grain and floret number, floral abortion, grain setting, breeding.

INTRODUCTION

It is well known that particular developmental phases are associated with different yield components (e.g. Rawson 1970, 1971; Rawson and Bagga, 1979); however, certain developmental phases are more critical than others in terms of yield generation (Kirby and Appleyard, 1984; Landes and Porter, 1989). In this sense, a critical phase for yield potential has been recognized during the period 20–30 d before anthesis, i.e. between terminal spikelet formation and anthesis (Fischer, 1984, 1985; Siddique *et al.*, 1989; Slafer *et al.*, 1994; Kirby, 1999). This indicates that the time coinciding with mortality of tillers and florets and with active growth of stems and spikes appears to be of critical importance for the determination of the final number of grains per unit of area (Thorne and Wood, 1987; Slafer and Andrade, 1989; Miralles *et al.*, 1998).

Thus, it becomes important to be able to manipulate the duration of these phases to customize cultivars for specific environments and to better understand their role in breeding applications (Reynolds *et al.*, 2009, 2011). Plant breeders have been doing this for centuries, in particular for the duration of the sowing to anthesis period. For example, it has been recently found that cycle length until anthesis was reduced in Spanish and Italian durum wheat as a consequence of breeding during the 20th century (Álvaro *et al.*, 2008*b*).

Reductions in plant height, the main feature altered by wheat breeding during the 20th century, by the introduction of *Rht* dwarfing genes, diminished the competition between the developing ear and stem, giving a more favourable partitioning of assimilates to growing reproductive organs during pre-anthesis, thus increasing floret fertility and thereby giving more, but smaller, grains per spike (Youssefian *et al.*, 1992*b*; Miralles *et al.*, 1998; Rebetzke and Richards, 2000). Floret degeneration or mortality usually coincides with the period when the stem and spike are growing at their maximum rate (Langer and Hanif, 1973; Kirby, 1988; Siddique *et al.*, 1989; Youssefian *et al.*, 1992*a*). The increased survival of florets observed with the introduction of the *Rht* genes was associated with heavier spikes at anthesis (Siddique *et al.*, 1989; Youssefian *et al.*, 1992*a*; Miralles *et al.*, 1998), suggesting that floral abortion (FLA) results mainly from a limitation in the translocation of resources to the growing spike due to competition with the elongating stem. As a result, spike growth is critical for the establishment of the number of fertile florets and grains in wheat (Fisher, 1985; Miralles *et al.*, 1998). The number of grains produced per ground area depends mainly on the number of fertile florets per square metre, which in turn is closely correlated with spike dry weight (SDW) per square metre at anthesis (Fischer, 1985; Abbate *et al.*, 1995; Miralles and Slafer, 1995). Manipulating the duration of the stem elongation phase may become an alternative avenue for increasing wheat yield, since a longer duration of stem elongation could increase SDW at anthesis and, thereby, the number of fertile florets and yield (Slafer *et al.*, 2005; Miralles and Slafer, 2007).

Although, as was indicated above, some evidence in durum wheat has shown that, in Spain and Italy, breeding has reduced the cycle up to anthesis, there is no evidence of the variability of the pre-anthesis phases in durum wheat cultivars released during different eras in the Mediterranean Basin and the eventual connection with the duration of those pre-anthesis phases and yield determination.

In this study we aimed (*a*) to analyse the effects of breeding in Spain and Italy on crop development during the last century to determine whether breeding significantly altered the developmental phases between sowing and maturity; and (*b*) to evaluate the importance of each phase in determining the number of grains per spike (NGS) in a wide range of durum wheat cultivars selected to represent the germplasm grown throughout the 20th century in Spain and Italy.

MATERIALS AND METHODS

Experimental set-up

Twenty-four durum wheat (*Triticum durum*) cultivars, 12 Spanish and 12 Italian, were used to investigate the changes caused by breeding over the last century in durum wheat from two Mediterranean countries (Italy and Spain). Cultivars were grouped into three classes: old (grown before 1945); intermediate (released between 1950 and 1985); and modern (released between 1988 and 2000) (Table 1). Old varieties were landraces or cultivars selected from exotic populations, such as Razza 208, and the intermediate group included varieties grown at around the time of the Green Revolution. Modern Spanish varieties were chosen from those recently released by local breeding programmes, avoiding the inclusion of varieties of foreign origin (Mexican and Italian), while the modern Italian set included some of the most cultivated varieties in the country (Table 1).

Eight field experiments were carried out during four seasons, i.e. 2002, 2003, 2004 and 2005, in two latitudes, representing contrasting environmental conditions within the Mediterranean area, i.e. Lleida in the Ebro Valley in the north-eastern part of the country (41°40′N, 0°20′E); and Granada in Andalusia in the south (37°08′N, 3°49′W). The northern area has a Mediterranean–continental climate with a mean yearly temperature of 17 °C and an average seasonal precipitation of 210 mm, while the southern area has a typical Mediterranean climate, with mild winters and hot, dry summers, with a mean yearly temperature of 21 °C and an average seasonal precipitation of 360 mm.

In each experiment, cultivars were sown in a randomized complete block design with three replications in plots of 12 m^2 (six rows 20 cm apart), on a mesic calcixerolic xerochrept soil with a fine-loamy texture in the southern area and on a loamy calcixerolic xerochrept soil with silty-clay texture in the northern area. All experiments were sown between 26 November and 16 December at a rate of 350 and 400 seeds m^{-2} in the southern and northern environments, respectively. Plots were fertilized (in kg ha^{-1}) before planting

TABLE 1. *Description of the Italian and Spanish durum wheat cultivars used in the study*

Italian	Year of release	Presence of *Rht-B1*	Plant height (cm)*	Spanish	Year of release	Presence of *Rht-B1*	Plant height (cm)*
Old							
Balilla Falso	<1930	No	115	Blanco Verdeal	<1930	No	141
Carlojucci	1945	No	115	Clarofino	<1930	No	139
Razza 208	1930	No	118	Pinet	<1930	No	127
Senatore Capelli	1930	No	134	Rubio de Belalcázar	<1930	No	139
Intermediate							
Adamello	1985	No	72	Bidi 17	1950	No	100
Capeiti 8	1955	No	98	Camacho	1975	Yes	79
Creso	1974	Yes	70	Esquilache	1976	Yes	75
Trinakria	1970	No	104	Mexa	1980	Yes	82
Modern							
Cirillo	1992	Yes	83	Ariesol	1992	Yes	75
Flavio	1992	Yes	62	Astigi	1999	Yes	80
Simeto	1988	Yes	73	Boabdil	2000	Yes	75
Zenit	1992	Yes	76	Senadur	1995	Yes	76

*Average of four experiments at each of two Spanish latitudes in 2002, 2003, 2004 and 2005.

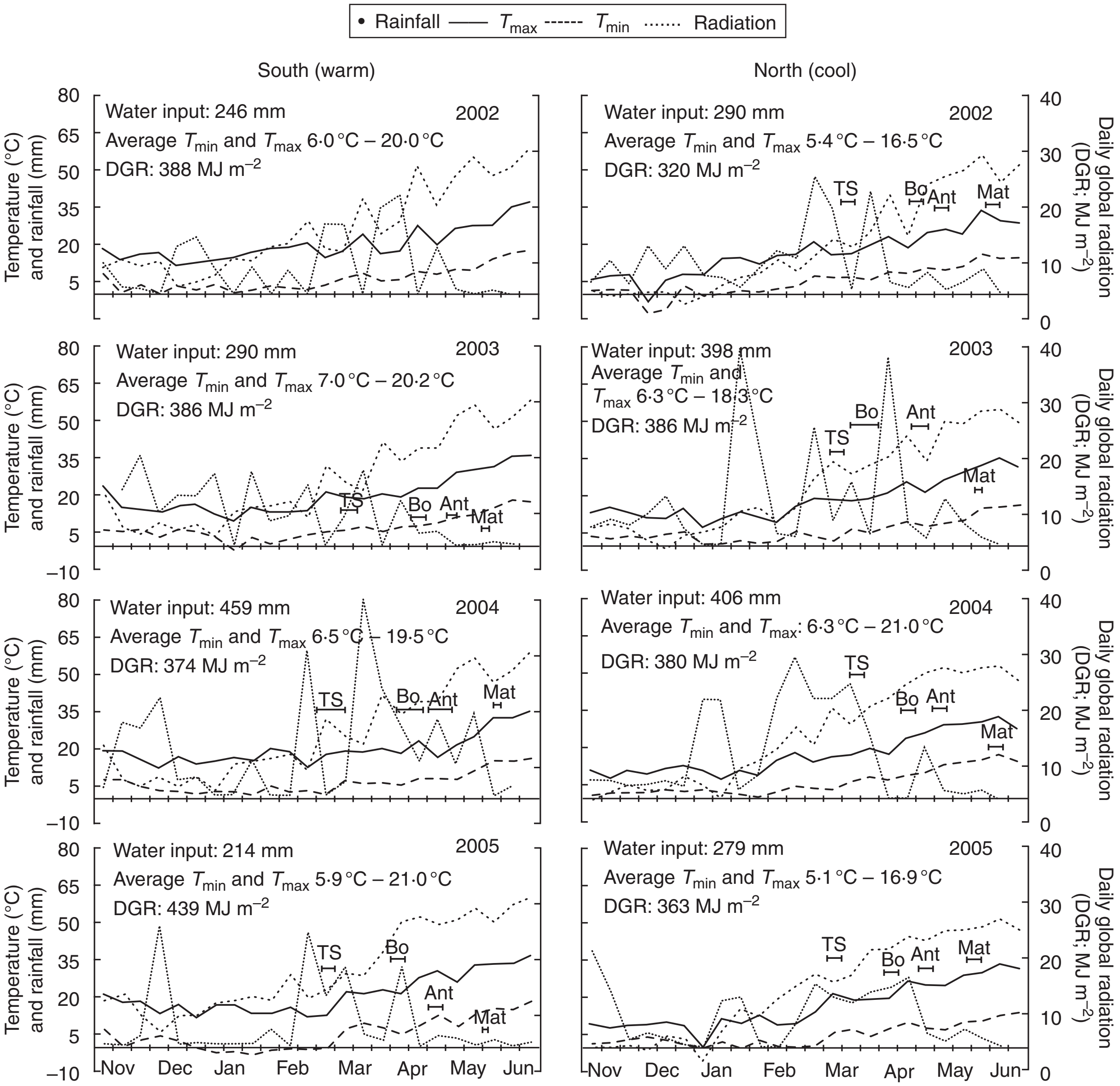


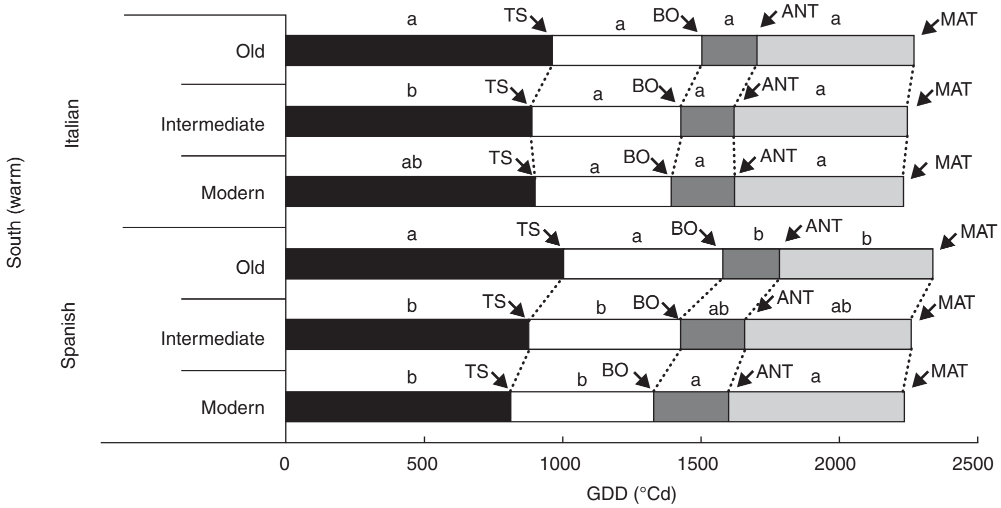
FIG. 1. Weather conditions during each crop cycle of both environments. Rainfall (mm), daily global radiation (MJ m^{-2}), maximum and minimum temperatures (°C) are presented. Average water input (includes rainfall plus irrigation), minimum and maximum temperature and global radiation during each year are also given. The range of variation among cultivars during the most important apical development states are indicated for each experiment: TS (terminal spikelet), BO (booting), ANT (anthesis) and MAT (maturity).

with 96 N, 124 P_2O_5, 120 K_2O in the northern experiment and 80 N, 45 P_2O_5, 45 K_2O in the southern experiment. At the end of tillering, the plots were top dressed with ammonium nitrosulfate at a rate of 35 kg N ha^{-1}. Pests, diseases and weeds were chemically controlled, following the recommendations at each site, and plant growth promoters were not applied. Details of the meteorological conditions in both environments are presented in Fig. 1. As temperature varied among years, and with the objective of including the temperature effects on the length of the crop phases, duration of phases was calculated in thermal time units as the sum of the daily average temperature considering a base temperature of 0 °C, below which development does not proceed (Gallagher, 1979; Baker *et al.*, 1980; Kernich and Halloran, 1996) between sowing and anthesis, and 9 °C from anthesis to maturity (Angus *et al.*, 1981).

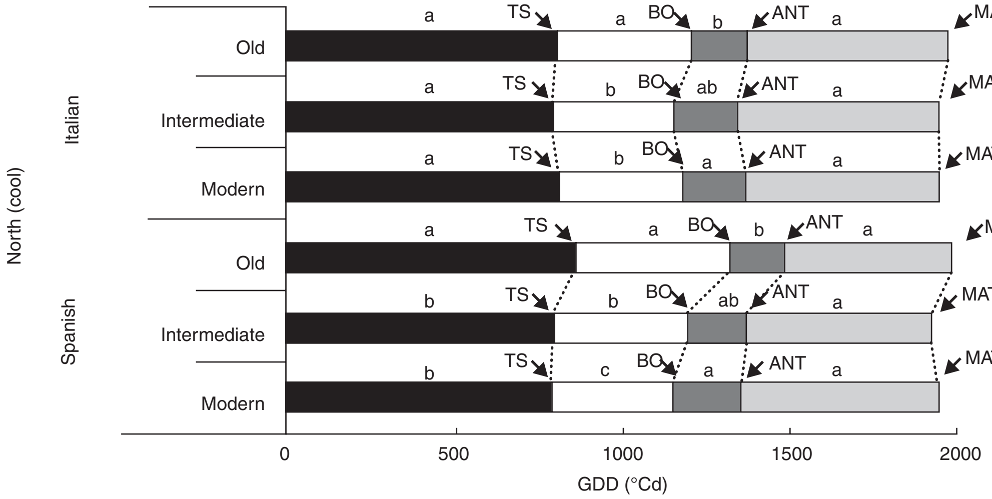
Measurements

Main shoots were monitored regularly, to determine the duration of different phenological phases from emergence to flowering, and to evaluate the dynamics of spike initiation and its development. For this, three plants per plot were randomly sampled and dissected under a binocular stereoscopic microscope once or twice weekly, depending on the rate of development. Main shoot apices were examined to detect the most

	S–TS ■	TS–BO □	BO–ANT ▨	S–ANT ANT	ANT–MAT ░	S–MAT MAT
Old	982·2^{a} (100%)	563·0^{a} (100%)	200·0^{b} (100%)	1745^{a} (100%)	580·0^{a} (100%)	2304^{a} (100%)
Intermediate	878·0^{b} (–10·6%)	551·3^{a} (–2·1%)	208·0^{ab} (+3·9%)	1637^{b} (–6·1%)	617·0^{a} (+10·1%)	2253^{b} (–2·2%)
Modern	854·0^{c} (–13·1%)	505·5^{b} (–10·2%)	252·2^{a} (+26·2%)	1608^{b} (–7·8%)	621·0^{a} (+10·9%)	2229^{b} (–3·2%)
l.s.d. (0·05)	14·5	40·6	44·2	38·3	63·7	35·6
Spain	897·5^{b} (100%)	550·1^{a} (100%)	235·0^{a} (100%)	1680^{a} (100%)	594·1^{a} (100%)	2274^{a} (100%)
Italy	912·0^{a} (+1·6%)	530·0^{b} (–3·6%)	205·0^{a} (–12·8%)	1645^{b} (–2·0%)	604·2^{a} (+1·9%)	2250^{a} (–1·1%)
l.s.d. (0·05)	11·8	12·3	92·9	14·8	12·7	29·8



	S–TS ■	TS–BO □	BO–ANT ▨	S–ANT ANT	ANT–MAT ░	S–MAT MAT
Old	749·0^{a} (100%)	442·4^{a} (100%)	175·4^{b} (100%)	1366^{a} (100%)	613·5^{a} (100%)	1980^{a} (100%)
Intermediate	704·4^{b} (–5·9%)	393·0^{b} (–11·2%)	194·5^{ab} (+10·9%)	1291^{b} (–5·4%)	661·0^{a} (+7·7%)	1955^{a} (–1·2%)
Modern	714·0^{b} (–4·7%)	378·1^{c} (–14·5%)	203·5^{a} (+16%)	1295^{b} (–5·2%)	663·2^{a} (+8·1%)	1956^{a} (–1·2%)
l.s.d. (0·05)	20·4	17·4	17·6	21·2	52·1	30·5
Spain	730·0^{a} (100%)	420·2^{a} (100%)	189·5^{a} (100%)	1339^{a} (100%)	638·5^{a} (100%)	1978^{a} (100%)
Italy	715·3^{b} (–2·0%)	389·0^{b} (–7·4%)	192·2^{a} (+1·4%)	1296^{b} (–3·2%)	653·2^{a} (+0·8%)	1949^{a} (–1·5%)
l.s.d. (0·05)	14·1	20·7	14·9	19·3	44·7	30·6



important stages during the ontogeny of the spike (vegetative, double ridge, terminal spikelet, green anther, yellow anther and anthesis) according to Kirby and Appleyard (1984). From these observations, the duration of the following phases was determined: sowing to terminal spikelet (S–TS), terminal spikelet to booting (TS–BO), booting to anthesis (BO–ANT), sowing to anthesis (S–ANT) and anthesis to maturity (ANT–MAT). Booting was recorded when 50 % of main shoot spikes were at Zadoks stage 45, while anthesis was recorded when 50 % of main shoot spikes had visible anthers (Zadoks stage 65). Physiological maturity was recorded when the peduncle of the main spike had turned yellow in 50 % of the plants.

At anthesis (Zadoks stage 65) radiation absorption was obtained by measuring the PAR above and at ground level below the canopy by using a 1 m long linear ceptometer (Accupar DECAGON Inc., USA), and the fraction of radiation absorbed (R_a) was calculated as:

$$R_a = [(I_o - I - I_r)/Io] \times 100$$

where I_o is the incident radiation, I is the transmitted radiation through the canopy to the soil surface, and I_r is the light reflected by the crop.

Floral development, FLA and grain setting were determined in 2003, 2004 and 2005. The number of spikelets per spike (NsS) and fertile florets per spikelet were counted in all the spikelets of the spike at both booting (sSBO) and anthesis (sSANT). In the booting stage, all florets that presented green anthers (stage 7·5–8·5 on the scale of Waddington *et al.*, 1983) were considered potentially fertile, whereas at anthesis only florets that had developed green or yellow anthers and *bifidum* stigma were actually considered fertile (stage 10 on the scale of Waddington *et al.*, 1983). The percentage of FLA between booting and maturity (FLA BO–MAT %) was calculated as the quotient between the number of fertile florets per spike at booting and the NGS at maturity. The percentage of FLA between booting and anthesis (FLA BOT–ANT %) was calculated as the quotient between the number of fertile florets at booting and the number of fertile florets at anthesis. Finally, the percentage of FLA between anthesis and maturity (FLA ANT–MAT %) was calculated as the difference between the total FLA (FLO BOT–MAT %) and FLA between booting and anthesis (FLO BO–ANT %). The percentage of grain setting (GS) was calculated as the NGS at maturity over the number of fertile florets per spike at anthesis. The NGS, the number of grains per spikelet (NGs) and the NsS were measured from a sub-sample of ten plants randomly collected from a 1 m long segment of a central row at ripening during the years 2003, 2004 and 2005. Spike dry weight was determined in main shoots at harvest without grains, and the spike fertility index (SFI) was calculated as the ratio between the number of grains established per unit of SDW without grains at harvest.

Statistical analysis

Analyses of variances (ANOVAs) were performed across years and latitudes. All factors were regarded as fixed effects, except the blocks, which were nested to year and latitude. Adjusted means were compared by least significant difference (l.s.d.) test at $P = 0{\cdot}05$. All the analyses were performed with the Statgraphics plus 5·1 software.

RESULTS

Environmental characterization

The maximum, minimum and mean temperatures, global radiation and rainfall during crop growth are shown in Fig. 1. An overview of the prevailing weather conditions during the growing season indicates that the northern environment was cooler, more humid and with lower daily global radiation than the southern environment. The most important differences between years were the amount and the distribution of rainfall, always higher in the northern environment, except in 2004, and the radiation as well as the maximum temperature, always higher in the southern experiment, except in 2004. For instance, during the 2005 growing season, the crop in the southern environment was exposed to high levels of irradiance during April and May, with low rainfall, compared with other years. Maximum temperatures were higher during the entire experiment in the southern environment than in the northern environment, with differences up to 4 °C in 2005.

Duration of development

The duration of the phases during the ontogeny of apical development is illustrated in Fig. 2. The ANOVAs for the duration of the developmental phases revealed that, with the exception of the ANT–MAT phase, the period of release significantly influenced the duration of all apical phases in both the North and South (Fig. 2). Cycle length from sowing to anthesis showed similar trends in both latitudes, being longer in the old than in the modern cultivars. However, this situation was evident only in the Spanish cultivars, as the length of the crop cycle was slightly shortened in the Italian cultivars, and in those materials the S–ANT phase remained practically the same independent of the year of release (Fig. 2).

Consistently, in both latitudes, the old Spanish cultivars showed a longer S–ANT phase (+6·5 %) than in the intermediate and modern cultivars (Fig. 2). When the duration of the S–ANT phase was related to the year of release, in Spanish cultivars it was shortened $-2{\cdot}4\ ^{\circ}\mathrm{Cd\ year}^{-1}$ while in the Italian cultivars the reduction was $-0{\cdot}83\ ^{\circ}\mathrm{Cd\ year}^{-1}$, determining reductions of approx. 160 and 45 °Cd for that phase in Spanish and Italian cultivars, respectively. The earliness of anthesis observed in the modern Spanish germplasm, and of a much lower magnitude in the modern Italian cultivars, in both latitudes, was due to changes in two phases prior to

FIG. 2. Thermal time (growing degree days; GDD) from sowing to terminal spikelet (S–TS), terminal spikelet to booting (TS–BO), booting to anthesis (BO–ANT), sowing to anthesis (S–ANT) and anthesis to maturity (ANT–MAT) of 24 durum wheat cultivars released in different periods in Italy and Spain. Data are the means of four experiments at each latitude. Arrows indicate the occurrence of anthesis (A), terminal spikelet (TS), booting (BO) and maturity (M). Means followed by the same letter in each column of the table and figure do not differ significantly according to l.s.d. test ($P > 5$ %). Percentages in parentheses represent the difference (+ or −) between old cultivars and the other cultivars.

TABLE 2. *Number of fertile florets (FF) at booting and at anthesis for 24 durum wheat cultivars released in different periods in Italy and Spain, determined in four experiments at each of two contrasting latitudes*

	At booting		At anthesis	
Environment	FFB (per spike)	FFsB (per spikelet)	FFA (per spike)	FFsA (per spikelet)
(a) South (warm)				
Cultivar age				
Old	100·5^{a} (100 %)	4·96^{a} (100 %)	54·1^{b} (100 %)	3·10^{b} (100 %)
Intermediate	99·0^{a} (−1·5 %)	5·25^{a} (+5·8 %)	57·7^{ab} (+6·6 %)	3·30^{ab} (+6·4 %)
Modern	98·8^{a} (−1·7 %)	5·25^{a} (+5·8 %)	61·4^{a} (+13·5 %)	3·53^{a} (+13·9 %)
l.s.d. (0·05)	4·5	0·48	4·9	0·28
Country				
Spain	100·5^{a} (100 %)	5·12^{a} (100 %)	58·7^{a} (100 %)	3·35^{a} (100 %)
Italy	98·8^{a} (−1·7 %)	5·19^{a} (+1·4 %)	56·7^{a} (−3·4 %)	3·26^{a} (−2·7 %)
l.s.d. (0·05)	3·65	0·34	7·45	0·26
(b) North (cool)				
Cultivar age				
Old	107·6^{a} (100 %)	5·46^{a} (100 %)	55·5^{b} (100 %)	2·75^{a} (100 %)
Intermediate	100·2^{a} (−6·9 %)	5·52^{a} (+1·1 %)	56·6^{b} (+2·0 %)	3·05^{ab} (+10·9 %)
Modern	96·2^{a} (−10·6 %)	5·33^{a} (−2·4 %)	60·4^{a} (+8·8 %)	3·24^{b} (+17·8 %)
l.s.d. (0·05)	14·6	0·83	2·56	0·42
Country				
Spain	104·1^{a} (100 %)	5·51^{a} (100 %)	60·1^{a} (100 %)	3·11^{a} (100 %)
Italy	98·6^{a} (−5·3 %)	5·36^{a} (−2·7 %)	54·9^{a} (−8·6 %)	2·91^{a} (−6·4 %)
l.s.d. (0·05)	8·51	0·56	5·60	0·36

Means within a column and group followed by the same letter are not significantly different according to least significant difference test (P >5 %). The percentage change in relation to old cultivars appears in parentheses.

anthesis, i.e. S–TS and TS–BO. Variations in the S–TS phase explained most of the differences between old and modern cultivars, accounting for 56 and 54 % of the duration of S–ANT in the South and North sites, respectively. Similarly to that observed in the S–ANT phase, the Spanish cultivars showed a higher rate of shortening of the S–TS phase (−2·0 °Cd year^{-1} of release) than the Italian cultivars (−0·6 °Cd year^{-1}). The TS–ANT phase was also reduced in the modern cultivars with respect to the older cultivars; thus the rate of reduction was −0·39 and −0·22 °Cd year^{-1} of release for Spanish and Italian cultivars, respectively. The variation in the TS–BO phase explained 32 and 30 % of the changes in the S–ANT, when considering South and North, respectively. While old cultivars had longer durations of the phases S–TS and TS–BO than modern cultivars, the duration of the BO–ANT phase showed an opposite trend. Thus, this phase lengthened from old to modern cultivars. However, there were some differences between North and South. In the southern experiments the duration of all phases was longer, with increases in the BO–ANT phase of 3·9 and 26·2 % from old to intermediate and modern cultivars, respectively.

In the northern environment the increases in the BO–ANT phase from old to intermediate and modern cultivars were 11 and 16 %, respectively; moreover, no significant differences among cultivars were found in the duration of the TS–ANT phase (Fig. 2). The shortest cycle duration until anthesis was recorded in intermediate Italian cultivars in both environments.

The grain-filling duration, the ANT–MAT phase, accounted for from 23 to 28 % of the duration from sowing to maturity for old and modern cultivars in both latitudes, respectively. No significant differences were found between cultivars and environments in the duration of the grain-filling phase, except for the Spanish cultivars in the southern experiment.

Floral development and abortion, grain setting and yield components

The number of fertile florets per spike (FFB) and per spikelet (FFsB), at booting, did not show any change between environments or period of release of cultivars (Table 2). Conversely, the total number of fertile florets per spike (FFA) and per spikelet (FFsA), at anthesis, significantly increased from old to modern cultivars (Table 2). Thus, modern cultivars showed increases of 11·5 and 15·8 % in FFA and FFsA, respectively, compared with the old cultivars. Regarding country of origin, Spanish cultivars consistently showed higher values for both traits in both latitudes, except in FFsB where the Italian cultivars showed a slight increase in the southern experiment.

Floral abortion was reduced by 24 % from old to modern cultivars, from booting to anthesis (Table 3), but did not change from anthesis to maturity. Thus, the reduction in FLA from booting to maturity in the modern cultivars was explained by the changes that occurred before anthesis. In fact, FLA during the BO–ANT phase was negatively and linearly related to the duration of that phase (Fig. 3A), suggesting that the longer the duration of the BO–ANT phase the lower the FLA and the greater the number of florets capable of fertility at anthesis. The duration of the BO–ANT phase was shorter in old cultivars than in the modern cultivars, and it appears to be related to the greater FLA of old cultivars compared with modern cultivars. As the year of release strongly modified plant height, the duration of the BO–ANT phase

TABLE 3. *Main spike components, floret abortion and grain setting for 24 durum wheat cultivars released in different periods in Italy and Spain, determined on six experiments at each of two contrasting latitudes*

				Floral abortion			
	NsS	NGS	NGs	BO–ANT (%)	ANT–MAT (%)	BO–MAT (%)	GS (%)
(a) South (warm)							
Cultivar age							
Old	18·2^{a} (100 %)	27·6^{c} (100 %)	1·46^{b} (100 %)	45·1^{a} (100 %)	27·4^{a} (100 %)	72·6^{a} (100 %)	51·6^{b} (100 %)
Intermediate	17·7^{a} (−2·7 %)	30·8^{b} (+11·6 %)	1·70^{ab} (+16·4 %)	40·2^{b} (−10·8 %)	28·3^{a} (+3·3 %)	68·6^{ab} (−5·5 %)	54·3^{ab} (+5·2 %)
Modern	17·2^{a} (−5·5 %)	35·5^{a} (+28·6 %)	2·03^{a} (+39·0 %)	36·5^{c} (−19·1 %)	26·6^{a} (−2·9 %)	63·2^{b} (−12·9 %)	59·6^{a} (+15·5 %)
l.s.d. (0·05)	2·70	1·86	0·35	2·76	4·86	5·09	5·65
Country							
Spain	18·0^{a} (100 %)	32·0^{a} (100 %)	1·76^{a} (100 %)	40·0^{a} (100 %)	27·3^{a} (100 %)	67·4^{a} (100 %)	55·6^{a} (100 %)
Italy	17·6^{a} (−2·2 %)	30·6^{b} (−4·4 %)	1·70^{a} (−3·4 %)	41·1^{a} (+2·7 %)	27·6^{a} (+1·1 %)	68·8^{a} (+2·1 %)	54·9^{a} (−1·2 %)
l.s.d. (0·05)	0·92	1·14	0·12	5·79	4·91	5·67	5·89
(b) North (cool)							
Cultivar age							
Old	17·2^{a} (100 %)	29·9^{b} (100 %)	1·52^{b} (100 %)	48·2^{a} (100 %)	23·5^{a} (100 %)	71·6^{a} (100 %)	54·9^{b} (100 %)
Intermediate	17·0^{a} (−1·2 %)	32·8^{ab} (+9·7 %)	1·80^{ab} (+18·4 %)	41·4^{b} (−14·1 %)	22·1^{a} (−5·9 %)	66·5^{ab} (−7·1 %)	59·5^{ab} (+8·4 %)
Modern	16·9^{a} (−1·7 %)	35·3^{a} (+18·1 %)	2·01^{a} (+32·2 %)	34·2^{c} (−29·0 %)	22·8^{a} (−3·0 %)	62·2^{b} (−13·1 %)	60·9^{a} (+11·0 %)
l.s.d. (0·05)	2·10	3·24	0·23	5·01	9·36	6·22	9·33
Country							
Spain	17·2^{a} (100 %)	33·4^{a} (100 %)	1·74^{a} (100 %)	38·7^{a} (100 %)	22·6^{a} (100 %)	66·6^{a} (100 %)	57·7^{a} (100 %)
Italy	16·2^{a} (−5·8 %)	32·0^{a} (−4·2 %)	1·78^{a} (+2·3 %)	44·0^{a} (+13·7 %)	23·1^{a} (+2·2 %)	67·0^{a} (+0·6 %)	59·1^{a} (+2·4 %)
l.s.d (0·05)	1·32	1·50	0·1	11·8	14·3	4·03	9·54

Means within a column and group followed by the same letter are not significantly different according to l.s.d. test ($P > 5$ %).The percentage change in relation to old cultivars appears in parentheses.

NsS, number of spikelets per spike; NGS, number of grains per spike; NGs, number of grains per spikelet; BO–ANT, from booting to anthesis; ANT–MAT, anthesis to maturity; BO–MAT, booting to maturity; GS, grain setting.

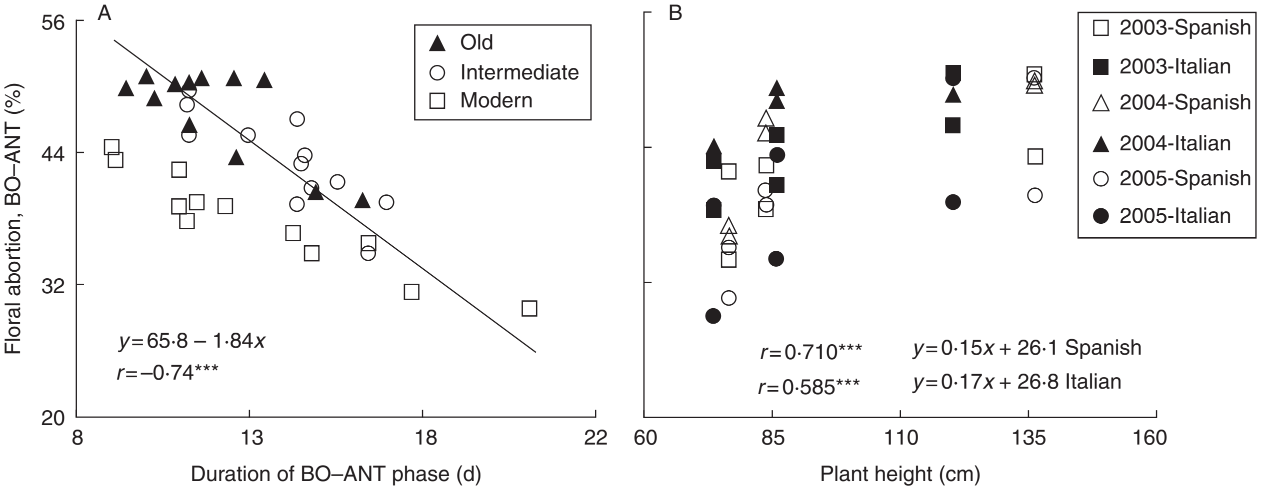


FIG. 3. Relationship between the percentage of floral abortion from booting to anthesis and (A) the duration of the BO–ANT phase (d), and (B) plant height (cm). In (A) each point represents the mean value from six experiments conducted in northern and southern Spain for old, intermediate and modern sets of durum wheat cultivars. In (B) each point represents the mean value from two experiments conducted in northern and southern Spain in 2003, 2004 and 2005 (right box) for Spanish and Italian sets of durum wheat cultivars.

represents only one part of the origin of the differences observed in floral abortion. In fact, a positive relationship ($P < 0·01$) was found when FLA during BO–ANT was related to plant height (Fig. 3B), determining that the NGS was negatively related to plant height in both Spanish ($r = 0·64$, $P < 0·05$) and Italian ($r = 0·59$, $P < 0·05$) cultivars, suggesting that carbon partitioning between spike and stem was promoted as plant height was reduced. Additionally, a strong and significant relationship was observed between NGS and SDW at harvest when all cultivars released in different periods were included ($r = 0·90$, $P < 0·01$; data not shown) as well as when cultivars from Spain ($r = 0·95$, $P < 0·01$) and Italy ($r = 0·93$, $P < 0·01$) were considered (Fig. 4A, B), supporting that the higher the amount of carbohydrates acquired by the spike the higher the NGS. On the other hand, the relationship between NGS and the SFI was

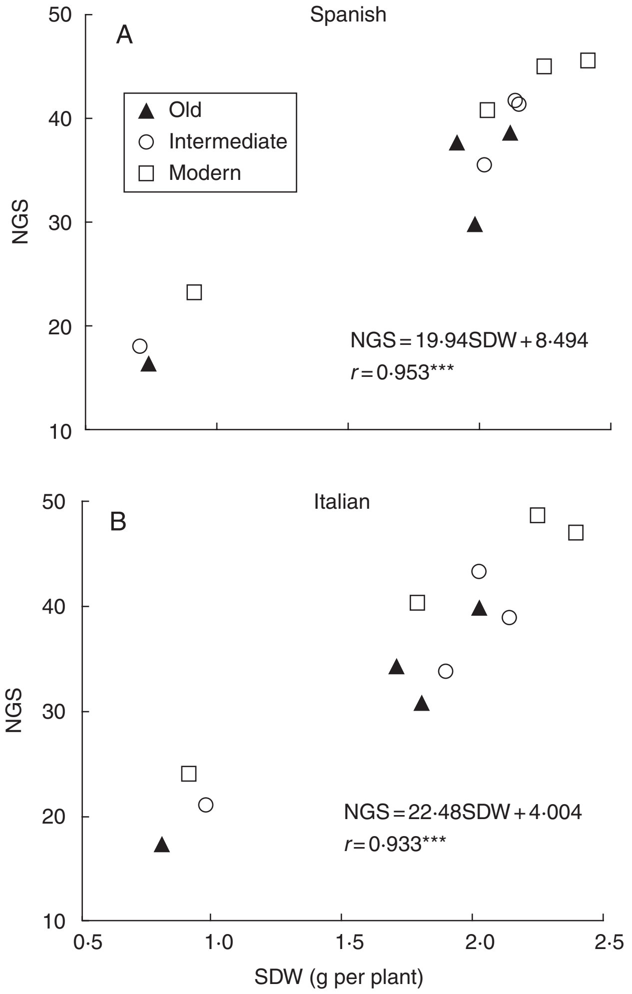


FIG. 4. Relationship between number of grains per spike (NGS) and spike dry weight (SDW) for (A) Spanish and (B) Italian cultivars. Each point represents the mean value from four experiments conducted in southern Spain (Granada) for old, intermediate and modern sets of durum wheat cultivars, as indicated.

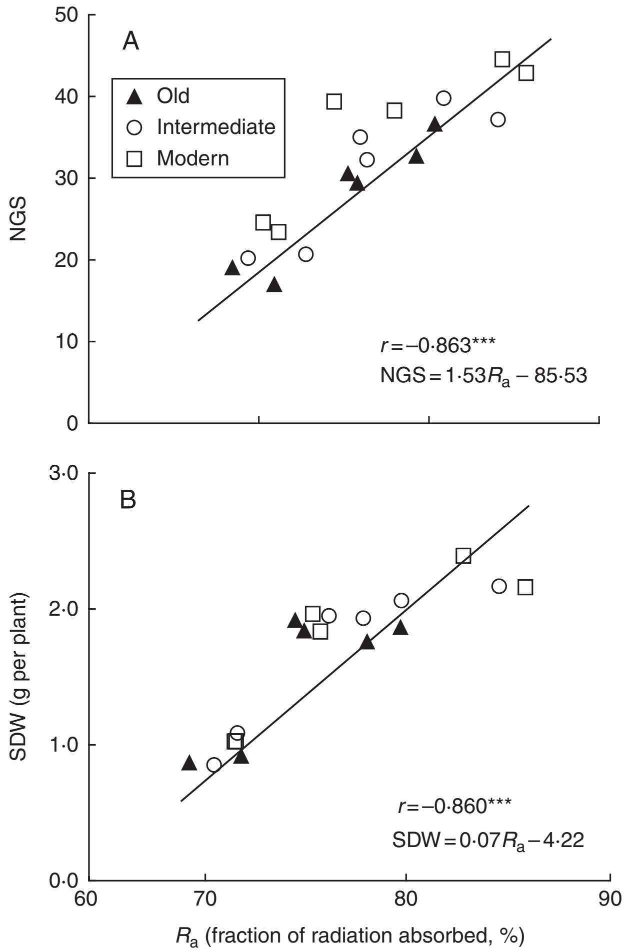


FIG. 5. (A) Number of grains per spike (NGS) and (B) spike dry weight (SDW) in relation to the fraction of absorbed radiation by the crop (R_a) at anthesis. Each point represents the mean value from four experiments conducted in southern Spain (Granada) for old, intermediate and modern sets of durum wheat cultivars, as indicated.

significantly influenced by the year and only showed a significant association when year 2005 was considered separately ($r = 0{\cdot}88$, $P < 0{\cdot}01$) from the other years ($r = 0{\cdot}74$, $P < 0{\cdot}1$). When all the data were considered together, NGS was not significantly associated with SFI ($P > 0{\cdot}1$). Most of the variation in SDW, that explained the changes in NGS, was a consequence of variations in the capacity of produce carbohydrates, as a significant positive relationship was found between NGS and SDW at harvest ($r = 0{\cdot}86$ $P < 0{\cdot}001$) (data not shown) and with the FR_a by the crop at anthesis ($r = 0{\cdot}86$, $P < 0{\cdot}001$) (Fig. 5A). In addition, a significant correlation was observed when SDW was plotted against R_a ($r = 0{\cdot}86$ $P < 0{\cdot}001$; Fig. 5B).

In accordance with the changes observed in FLA during the BO–ANT phase, the final NGS was significantly ($P < 0{\cdot}001$) related to the percentage of GS (Fig. 6). Grain setting explained >77 % of the observed variation in the NGS, being greater in modern cultivars than in the old cultivars (Table 3 and Fig. 6). Between environments, GS was 5·2 and 15·5 % higher for the intermediate and modern

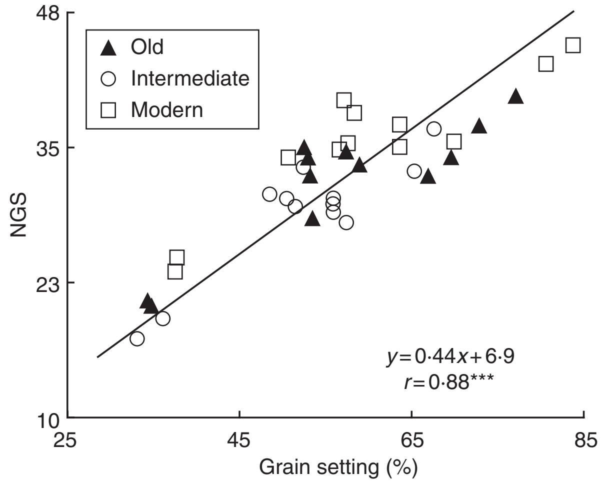


FIG. 6. Relationship between the number of grains per spike and grain setting. Each point represents the mean value across six experiments conducted in northern and southern Spain for old, intermediate and modern sets of durum wheat cultivars, as indicated.

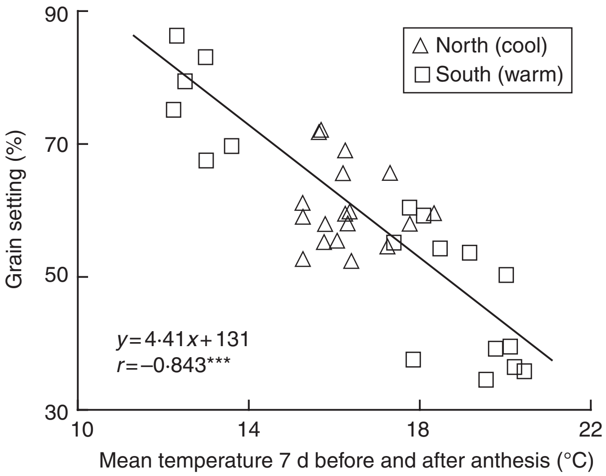


FIG. 7. Relationship between grain setting and the mean temperature 7 d before and after anthesis. Each point represents the mean value across six experiments conducted in the northern and in the southern regions of Spain, as indicated.

cultivars, respectively, than for the old cultivars in the southern environment, and 8·4 and 11 % in the northern experiment (Table 3). Most of the differences observed in GS were explained by changes in mean temperature during grain filling ($r = -0{\cdot}84$, Fig. 7), and therefore the lower the temperature the higher the GS. Thus, the northern experiments showed higher percentages of GS than in the southern experiments.

In line with what was observed in the number of fertile florets at anthesis, the NGS was higher in the modern than in the old cultivars in both latitudes. Thus, Spanish cultivars in each latitude had significantly more grains per spike (5 %) in both environments than did Italian cultivars. In addition, the northern environments registered higher NGS than did the southern ones (Table 3). No significant differences were found between Spanish and Italian cultivars in the NsS at anthesis, as well as among cultivars of the same origin released at different times (Table 3). The NGs increased significantly from old to modern cultivars, with mean values of 39·0 and 32·2 % under the southern and the northern environments, respectively (Table 3).

DISCUSSION

Duration of the developmental phases

The results reported here indicate that breeding during the last century reduced the duration of the entire biological cycle of durum wheat, especially in the Spanish cultivars. The phenological adaptation, manifested by the shortening of the developmental phases, affected the period between sowing and anthesis. Possibly, the reduction in the period up to anthesis caused by breeding places the modern cultivars under better environmental conditions (less water restriction), with respect to the old cultivars, thereby avoiding (though only partially) the negative effect of water deficiency during the grain-filling period, as usually occurs under Mediterranean conditions (Royo *et al.*, 2006). Our results showed that the BO–ANT phase increased in modern cultivars, compared with the old ones, which is particularly relevant given that most of the floret mortality occurs during this phase (Kirby, 1988; Miralles *et al.*, 1998, 2002). Although, it is not possible to attribute all of the effects to the phenological changes, since there are other factors, such as the reduction in plant height, determining more carbon partitioning to the spikes (i.e. heavier SDW) and the harvest index, the results of the present work support the assumption that the longer the duration between booting and anthesis, the larger the floret survives to form the spikes (Halloran and Pennell, 1982; Slafer and Rawson, 1994; Miralles *et al.*, 2000; Miralles and Slafer, 2007) and, therefore, an increase in the NGS will be expected.

Our results indicate that durum wheat breeding during the last century shortened the time to flowering, at the expense of the vegetative and early reproductive phases. In fact, the S–TS and TS–BO phases were significantly reduced throughout the breeding process, especially in the Spanish materials. However, the period when the number of fertile florets is generated was lengthened, allowing more floret primordia to become fertile at anthesis, contributing to a greater GS. The result of this strategy, which was more evident in Spain than in Italy, together with the plant height reduction determined that modern cultivars developed more grains per unit area and had superior yields (Royo *et al.*, 2008) compared with the durum wheat cultivars grown at the beginning of the century. As was demonstrated in a recent study with the same germplasm that was used in the present study (see Royo *et al.*, 2007), the yield increased during the last century at a rate of 0·36 and 0·44 % year^{-1} and the number of grains per square metre improved by 39 and 55 % in Italian and Spanish varieties, respectively. Although it is well established that not all developmental phases are equally important for yield formation, our results clearly show that the period between terminal spikelet initiation and anthesis, and more specifically the BO–ANT phase, has been the most relevant for grain per spike gains. Despite that, it has been pointed out that floret survival could be enhanced by increasing the duration from terminal spikelet initiation to anthesis (Slafer *et al.*, 2001). Our results go further in specifying that the lengthening of the phase between booting and anthesis was the one most closely related to the reduction in FLA.

As was stated above, differences between the cycle length of old and modern cultivars were far greater within Spanish germplasm than within the Italian material. This may be attributed to the different breeding strategies followed in both countries over the 20th century (Royo *et al.*, 2007; Alvaro *et al.*, 2008*a*). Italian breeders probably did not face the need to reduce the cycle length of their varieties, as even the old Italian cultivars had an optimum crop phenology, as a consequence of past breeding efforts. Conversely, the length of the cycle of the old Spanish cultivars was too long compared with the modern cultivars, forcing the BO–ANT phase and grain filling to occur under unfavourable climatic conditions (i.e. higher air temperature and drought conditions). Thus, breeding in Spain tended to reduce the length of the cycle so that the critical phases for grain number determination would be exposed to better environmental conditions, and thus lead to improved grain yield.

Breeding effects on floral development, floral abortion, grain setting and yield components

The introduction of dwarfing genes and the selection of shorter cultivars in durum wheat, with a longer BO–ANT phase, reduced plant height and decreased the competition between spike and stem elongation before anthesis, thus improving the allocation of assimilates to the spike (Fischer, 1984; Álvaro *et al.*, 2008*c*), and allowing a higher proportion of flowers to survive and set grain. The fact that the rapid spike growth period is concentrated in the BO–ANT phase and that more carbohydrates can be allocated in the spikes during that particular phase in the modern cultivars is due to a (*a*) more favourable partitioning to the spike (given by a reduction in plant height) that determined heavier spikes, and (*b*) an extended period during which the spike can acquire carbohydrates during the BO–ANT phase promoted a higher spike fertility (i.e. higher NGS) in modern than in old cultivars in both countries. The results of the present study support the large body of evidence published in the literature (Fischer, 1984, 1985, 2007; Fischer and Edmeades, 2010) that the reduction of FLA and the establishment of the NGS are closely linked to pre-anthesis spike growth. In fact, independently of the environments (years, localities) and genotypes (released in different periods) the changes in NGS were significantly explained by variations in the SDW.

The results of the present study suggest that the longer duration of the BO–ANT phase reduced FLA; as a consequence, the longer duration of spike growth allowed more assimilates to be available to the spike. Thus, more fertile florets per spikelet at anthesis, due to the breeding process, resulted from a higher number of relatively distal primordia that reached the stage of fertile floret at anthesis (Gónzalez *et al.*, 2005; Álvaro *et al.*, 2008*a*). The number of potential florets per spike at booting and the NsS did not change over the periods, thus reinforcing the assumption that increased partitioning to the spikes given by a plant height reduction and by the longer duration of the BO–ANT phase observed in the modern cultivars was responsible for their reduced FLA.

The slightly higher values achieved by NGS and NGs in the northern experiments, compared with in the southern environments, could be due, as pointed out previously in an ontogenic study about grain yield formation (García del Moral *et al.*, 2003), to the fact that the northern and wetter environment enabled the genotypes better expression of their yield potential.

Our study also shows that the NsS remained unchanged during the last century, and that the rise in the NGS was due to a higher number of fertile florets and GS, in agreement with other studies (Miralles and Slafer, 1998; Royo *et al.*, 2007; Álvaro *et al.*, 2008*a*). In addition, the percentage of GS was significantly correlated with the NGS. Our results indicate that GS was higher in modern than in old cultivars, in both latitudes, because the number of fertile florets that reached anthesis was also higher in modern cultivars. As FLA between anthesis and maturity did not show significant differences between periods of breeding, the attainment of a high NGS depended more on the number of fertile florets that reached anthesis than on the grain-setting percentage from anthesis to maturity. However, the temperature also played an important role in determining the florets that effectively set grains, as shown by the negative and significant relationship existing between the mean temperature during grain filling and the percentage of GS. The temperature was abnormally low during grain filling in the southern experiments in 2004 (Fig. 1), resulting in the highest GS values.

These results support the evidence reported by Calderini *et al.* (1999) who demonstrated that GS and grain weight potential was reduced due to short periods of high temperature immediately pre-anthesis or during post-anthesis.

Our results are in line with previous reported evidence (Youssefian *et al.*, 1992*b*; Miralles and Slafer, 1995; Alvaro *et al.*, 2008*a*), indicating that an increase in the NGS was associated with a greater NGs, rather than with more spikelets per spike (Fisher, 1973; Holmes, 1973). Future yield improvement, therefore, should be reached by increasing the capacity for setting grains after anthesis (Álvaro *et al.*, 2008*a*), as the percentages of grain setting of modern durum cultivars are still far from those of modern bread wheat. This could be achieved by selecting cultivars with fast nascence and emergence in order to reduce the duration until anthesis, by shortening the duration of the S–TS phase and by increasing the BO–ANT duration. This strategy would probably increase the number of fertile florets per spike, resulting in a greater number of them setting grains.

Conclusions

Breeding during the 20th century reduced mainly the time from sowing to anthesis, in both Italian and Spanish cultivars, although in particular in the latter. This reduction was caused principally by the shortening of the S–TS and TS–BO phases, although the BO–ANT phase was significantly lengthened. Plant height reduction and extending the period during which the spikes are grown at their maximum rate were the two main breeding strategies in both Spain and Italy that determined increases in SDW and, thereby, in the NGS. Considering that modern cultivars have already reached harvest indexes that are close to the theoretical upper limit of 0·62, estimated by Austin (1980), future genetic improvement will depend upon the success of other strategies to achieve greater biomass (Foulkes *et al.*, 2007) and/or to lengthen the duration of the developmental phases (Miralles *et al.*, 2000; Slafer *et al.*, 2001), as well as increasing yield potential by new breeding strategies (Reynolds *et al.*, 2011). Taking into account that reducing plant height more than the actual values reached by modern cultivars (i.e. 70–100 cm) could determine penalizations in yield (see Miralles and Slafer, 2007), extending the duration of the BO–ANT phase, at the expenses of the previous phases to avoid changes in flowering time, could be a strategy to be continued in modern cultivars for improving the number of grains per unit area and yield. This strategy would cause less degeneration of distal flowers within the spike as a consequence of a higher partitioning to the spike during the pre-flowering critical period, thereby increasing the fertility of the spikes. Since it has been demonstrated that lengthening the emergence–ANT phase could cause yield reductions due to higher water restrictions and/or higher temperatures during the grain-filling period, the extension of the BO–ANT phase should be made at the expense of shortening the other phases to maintain the duration of the cycle up to anthesis. Photoperiod sensitivity manipulation of the phases could be an avenue by which to change differentially the relative duration of the phases (see Miralles and Richards, 2000; Slafer *et al*, 2001; Gonzalez *et al.*, 2005). The differences found in the phenology between Spanish and Italian cultivars may be a consequence of the contrasting breeding strategies used by the two countries during the last century. The total FLA was better explained by FLA from booting to anthesis, than from anthesis to maturity, and so less FLA would result in more fertile florets at anthesis and a superior GS. As was expected, the final NGS depended on the number of fertile florets reached at anthesis more than the grain abortion after anthesis as was reported earlier by Bingham (1969) and Fischer (1975). In addition, this study complements previous works conducted under similar conditions in which the different strategies of breeding during the last century were studied.

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