

Breeding effects on dry matter accumulation and partitioning in Spanish bread wheat during the 20th century

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Received: 12 June 2014 / Accepted: 26 September 2014 / Published online: 4 October 2014
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Abstract Increasing biomass accumulation is becoming a major objective of most bread wheat (*Triticum aestivum* L.) breeding programs worldwide. This study addresses the changes caused by breeding in the pattern of wheat growth and its relationship with the presence of *Rht* dwarfing alleles. A historical series of 16 varieties representative of the most widely grown during the twentieth century in Spain, including landraces (grown before 1940's), initial (1947–1955) and modern (1972–2001) cultivars, was characterized for *Rht8c*, *Rht-B1b* and *Rht-D1b* dwarfing alleles. Changes in biomass accumulation and partitioning, leaf area index (LAI) and leaf area duration (LAD) were studied in four field experiments conducted in NE Spain. Biomass and LAI at the beginning of jointing were reduced by ca. 20 and 40 % in varieties carrying one or two dwarfing

alleles, respectively. The rates of biomass accumulation from the beginning of jointing to anthesis were similar for wheats from the three historical periods (ca. $1.70 \text{ g m}^{-2} \text{ GDD}^{-1}$), and amongst varieties carrying a different number of dwarfing alleles. Almost 80 % of absolute yield gains during the last century were due to increases in the spikes weight per unit area from milk-grain to maturity. Initial varieties maintained and modern varieties increased above-ground biomass from milk-grain stage to maturity. The contribution of photosynthesis during this last phase was enhanced in modern varieties due to increases in LAI at milk-grain stage ($0.83 \% \text{ y}^{-1}$) and of LAD from it to maturity ($0.79 \% \text{ y}^{-1}$). Changes on the pattern of biomass accumulation and partitioning caused by the introduction of improved varieties were related to the number of dwarfing alleles present. The contribution of photosynthesis to grain filling was increased in modern varieties due to improved LAI values at milk-grain stage and, subsequently, a longer LAD.

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Keywords *Triticum aestivum* L. · Bread wheat ·
Crop biomass · Historical series · CDW · Dwarfing
alleles · *Rht* · LAI · LAD · Grain filling

Introduction

Wheat is the second most important staple crop in the world and is essential to global food security. Currently cultivated on 216.8 million ha worldwide,

it provides 18.3 % of the daily intake of calories and 20 % of protein for humans (FAOSTAT 2011). Yield improvement has been a primary objective of bread wheat (*Triticum aestivum* L.) breeding programs around the world. A conceptual framework frequently used to interpret historical yield gains consists in expressing yield as the product of biomass (crop dry weight, CDW) and harvest index (HI). It is generally accepted that wheat yield gains due to breeding during the twentieth century were accomplished through increases in HI, with few or no changes in CDW (Brancourt-Hulmel et al. 2003; Donmez et al. 2001; Royo et al. 2007). It has been suggested that increases in HI were a result of the selective pressure put on morphology and assimilate partitioning process when selecting for yield, which was not put on biomass (Blum 2011). Even though the theoretical maximum HI attainable in bread wheat has recently being revised and updated up to 0.64 (Foulkes et al. 2011), HI values above 0.50 have rarely been achieved (Fischer and Edmeades 2010). Therefore, it has been proposed that future international breeding efforts towards yield improvements should be based on increases in CDW production without decreasing HI (Parry et al. 2011) or, in other words, biomass and yield should be selected for, while retaining HI (Blum 2011).

Yield gains have been principally related to plant height reductions resulting from the introgression of major dwarfing genes (Worland and Snape 2001), intended as the mutant alleles of a series of loci grouped in the *Rht* loci complex, which showed a major effect promoting short height (Worland and Snape 2001). Basically, two types of *Rht* genes have been described, the ones which its mutant alleles promote short height by inducing the plant insensitivity to gibberellic acid (GA; e.g. *Rht-B1b* and *Rht-D1b*) and the ones that promote short height by affecting other physiological mechanisms (e.g. *Rht8c*, *Rht9* or *Rht12*) (Worland and Snape 2001). In spite of the yield increases accomplished, pleiotropic effects, such as decreased early vigour, have been reported with the introduction of the *Rht* dwarfing alleles, particularly with the ones providing GA insensitivity (Ellis et al. 2004).

Bread wheat yield improvements in Spain during the twentieth century took place in two steps. The first consisted in the introduction, from the late 1940's, of foreign varieties mostly issued from early Italian and French breeding programs, and the release of the first

improved varieties of local origin. The first *Rht* dwarfing alleles were introduced in the Spanish germplasm by these varieties, particularly in the GA sensitive loci *Rht8* and *Rht9* (Worland et al. 1998; Worland et al. 2001). Consequently, plant height was reduced and, as a pleiotropic effect, more resources were diverted from vegetative to reproductive organs, contributing to increases in HI (Sanchez-Garcia et al. 2013). The second step started with the introduction, from the 1970's, of modern semi-dwarf varieties mostly derived from CIMMYT (International Maize and Wheat Improvement Center) and French breeding programs. These varieties introduced in the Spanish wheat pool the gibberellic acid (GA) insensitive dwarfing alleles *Rht-B1b* and *Rht-D1b* that further reduced plant height and increased again HI (Sanchez-Garcia et al. 2013).

There is a large body of literature that describes the impact of selective breeding on HI and crop biomass, particularly the impact of dwarfing genes, (Brancourt-Hulmel et al. 2003; Royo et al. 2007; Clayshulte et al. 2007). However, less is known about the changes caused by historical breeding, and specially the introgression of dwarfing genes, on the patterns of growth and biomass allocation during specific phases of the growth cycle. For instance, the relative impact of dwarfing genes on biomass accumulation during the vegetative phase of the cycle, from jointing to anthesis when the plant accumulates the water soluble carbohydrates that will contribute to the filling grains, is unclear. Understanding these effects and their relationship with yield gains could be helpful for designing future breeding strategies based on crop biomass increases.

The partitioning of biomass between vegetative and reproductive organs, principally after anthesis, is a main process determining yield formation. Grain filling in wheat is supported by transient photosynthesis and the translocation of assimilates accumulated prior to anthesis and stored temporary in the vegetative organs (Maydup et al. 2010; Maydup et al. 2012). A previous study conducted in durum wheat under Mediterranean conditions demonstrated that the contribution of pre-anthesis assimilates to grain filling increased in modern varieties arriving to values close to 30 % (Álvarez et al. 2008). This result shows the importance of photosynthesis during grain filling—mostly by the spike and the flag leaf—on final grain weight, even under Mediterranean environments, where yield greatly depends on remobilization of

pre-anthesis assimilates to the grain. Increasing canopy light capture by promoting fast early-leaf area growth and/or delaying leaf senescence has been suggested as a potential key trait for future yield raises (Parry et al. 2011).

The aim of the present study was to investigate the changes on biomass accumulation and allocation caused by the introduction of improved bread wheat varieties in Spain during the twentieth century from a temporal perspective, through the involvement of varieties belonging to different breeding periods, and also from the genetic point of view, by considering the allelic structure of their dwarfing genes.

Materials and methods

Plant material

Plant material consisted in a collection of 16 bread wheat varieties representative of the most widely cultivated in Spain in different periods of the twentieth century. The collection included: (i) Spanish landraces grown before 1940; (ii) initial varieties (termed ‘old-bred’ in a previous study by Sanchez-Garcia et al. 2012), released and grown from the mid 1940s until the Green Revolution, and (iii) modern varieties, released and grown from 1970 until the end of the twentieth century. Most modern varieties had French origin or were derived from semi-dwarf CIMMYT germplasm (Table 1).

Molecular characterization

DNA was extracted from leaf samples obtained from young leaves pooled from three seedlings of each variety following the methodology proposed by Doyle and Doyle (1990).

PCR-based STS markers specific for the base pair change responsible for the semi-dwarf phenotype were used to identify specifically both wild-type (*Rht-B1a* and *Rht-D1a*) and mutant (*Rht-B1b* and *Rht-D1b*) allelic variants at *Rht-B1* and *Rht-D1* loci. This characterization was carried out in the Plant Industry laboratories (CSIRO, Canberra) following the methodology described by Ellis et al. (2002). For the *Rht8* locus, the WMS261 SSR marker products were obtained in the IRTA laboratories (Lleida, Spain) following the methodology proposed by Korzun et al.

(1998) and their size was determined with an ABI PRISM 3130 Genetic Analyzer (ABI; Foster City, California USA).

Experimental setup

Four field experiments were conducted during two crop seasons and under rainfed and irrigated conditions in Lleida (North-eastern Spain, 41°40'N, 0°20'E, 200 m a.s.l.) on a fine loam Calcic Cambisol soil. Rainfed experiments received 235 and 192 mm in 2007–2008 and 2008–2009, respectively, and supplementary irrigations provided 150 mm the first year and 96 mm the second (Table 2). Each experiment consisted of a randomized complete block design with three replications and plots of eight 5 m-length rows, 0.15 m apart. Sowing rate was 450 seeds m⁻². All the experiments were fertilized appropriately and pests, weeds and diseases were controlled according to standard cultural practices.

Field data recording

Zadoks's growth stage (Zadoks et al. 1974) was periodically determined on a plot basis. Biomass and leaf area were determined following the methodology described in Royo and Tribó (1997), and Villegas et al. (2001). Samples of plants within a 50-cm-long section on a central row per plot were pulled up at the first node-detectable stage (GS 31 of the Zadoks' scale), booting (GS 47), anthesis (GS 65) and milk-grain stage (GS 75), and a sample section of one m-length was taken at physiological maturity (GS 87). At each sampling occasion not less than 15 cm were left from a previous sampled area in the same row, and care was taken that adjacent rows had intact plant populations. In the laboratory the number of plants from each sample was counted and ten representative plants per plot were separated into vegetative (leaves + stems) and reproductive (spikes) organs. Except at GS47, leaf area projection (one side) was measured using a leaf-area meter (LI-3100E, LI-COR Inc., Lincoln, NE) and leaf area index (LAI) was computed as the ratio of green leaf area per sample area. Yellow and dry leaves were not considered. Samples were oven-dried at 70 °C for 48 h, then the dry weight of vegetative biomass and spikes per plant determined. Crop dry weight was expressed on a sample basis as g m⁻². Leaf area duration (LAD) was calculated by

Table 2 Description of the environments

Cropping season	2007–2008	2007–2008	2008–2009	2008–2009
Water regime	Rainfed	Irrigated	Rainfed	Irrigated
<i>Environmental conditions from sowing to anthesis</i>				
Accumulated GDD	1,311	1,357	1,265	1,258
Accumulated ET ₀ (mm)	273	259	243	239
Average minimum daily temperature (°C)	3.1	3.0	2.3	2.7
Average maximum daily temperature (°C)	14.1	14.2	13.4	12.9
Water input (rainfall + irrigation, mm)	102	252	183	234 ^a
<i>Environmental conditions from anthesis to physiological maturity</i>				
Accumulated ET ₀ (mm)	170	155	113	160
Average minimum daily temperature (°C)	11.5	11.3	11.8	12.7
Average maximum daily temperature (°C)	23.1	23.0	27.0	27.3
Water input (rainfall + irrigation, mm)	133	133	9.0	54 ^a
Sowing date	Nov. 20	Nov. 19	Nov. 24	Nov. 27
Mean yield (kg/ha)	4,065	5,416	5,434	7,201

ET₀ is the reference evapotranspiration computed by the Penman-FAO methodology (Allen et al. 1998)

^a Sprinkler irrigation was used in 2009

approximating the area from a trapezium to the curve of LAI against time (Aparicio et al. 2002), and was determined in growing degree-days (GDD). Plant height was measured at physiological maturity in ten main stems per plot, from the tillering node to the top of the spike excluding the awns. Plots were mechanically harvested at ripening and yield was expressed at 12 % of grain moisture.

Statistical analysis

Principal component analysis (PCA) was performed on the correlation matrix, calculated on the mean data across environments and replicates of CDW determined at different growth stages, LAD and yield. Standard analyses of variance were used to analyze the data obtained. The sum of squares of the variety effect was partitioned in the ANOVAs in differences between and within breeding periods, and also according to the groups formed by varieties carrying a common number of *Rht* dwarfing alleles (*Rht* DA), arisen from the PCA. All the terms in the model were considered fixed excepting the genotypic residual (genotypic variance retained within groups), used to test the differences between groups. Least-square means were computed and compared by Duncan multiple range tests. In order to ascertain whether the rate of biomass accumulation from jointing to anthesis changed across periods, three different linear regression models with increasing complexity, were

fitted to the relationships between CDW and thermal time. Firstly, the same regression line was used for the three breeding periods to fit the mean variety data across years. A second model forced a common slope for the three periods, and finally, three regression lines with different slopes were used. The models were compared through ANOVA. Slopes were only assumed to be different if the most complex model was significantly better ($P < 0.05$) than the simplest ones.

Stepwise linear regression analyses were conducted to explain the relationships between variables. Absolute and relative genetic gain estimations were computed as the slope of the linear regression between the absolute or relative value of the trait and the year of genotype release. Relative values were computed for each genotype as a percentage with regard to the average value of all genotypes. All analyses were carried out with the SAS-STAT (SAS Institute Inc. 2009) and R (R Development Core Team 2011) statistical packages.

Results

Molecular characterization for major dwarfing genes

Major dwarfing alleles present in the genotypes showed a trend across the different breeding periods

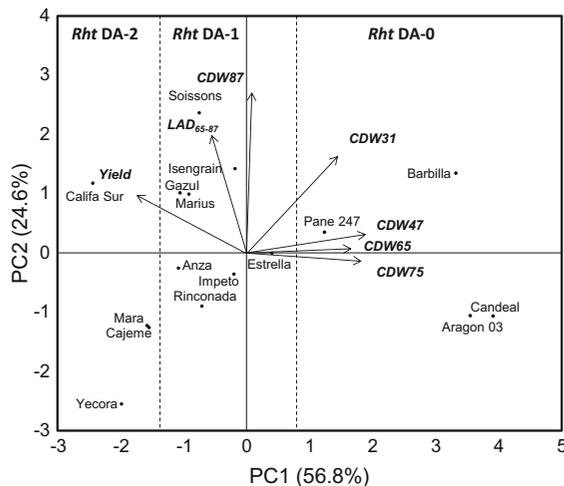


Fig. 1 Biplot of the first two axes of the PCA analysis summarizing the relationships between CDW at five developmental stages according to Zadoks' scale (jointing, 31; booting, 47; anthesis, 65; milk-grain, 75; and physiological maturity, 87), LAD from anthesis to physiological maturity (LAD_{65–87}) and grain yield. Discontinue lines separate the varieties according to the number of *Rht* dwarfing alleles (*Rht* DA) they carry

(Table 1). The three landraces and the initial variety 'Pané 247', with plant height ranging from 111 to 118 cm, carried wild-type alleles of the dwarfing genes, which are typically associated with tall stature (Table 1). Other initial varieties, with plant heights between 86 and 109 cm, carried mutant alleles of *Rht8* and *Rht9* that have previously been linked to semi-dwarf phenotypes. Modern Spanish wheat varieties carried mostly dwarfing alleles at *Rht-B1* and *Rht-D1* loci that typically result in strong reductions in plant height, consistent with the reduced stature of modern varieties (Table 1). Interestingly, the modern semi-dwarf variety 'Marius' did not appear to carry the mutant alleles *Rht-B1b*, *Rht-D1b* or *Rht8c* that have previously been linked to reduced plant height. This variety might carry novel alleles of the known dwarfing genes or could carry variation at alternative dwarfing genes.

Multivariate analysis

Multivariate analysis was used to examine the relationship between varieties for a set of growth traits and productivity, and the relationships between these variables. The first two axes of the PCA shown in Fig. 1 accounted for 81.4 % of the total PCA variance (axis 1, 56.8 %; axis 2, 24.6 %), suggesting that most

of the information contained in the data could be summarized by projecting the points in the plane determined by the first two axes. Principal component 1 was related with CDW at different stages, from jointing to milk-grain, on the positive direction, and with yield and LAD on the negative one. The location of the points corresponding to each variety on the plane determined by the first two axes showed that PC1 grouped the varieties according to the number of major *Rht* loci carrying the dwarfing allele (*Rht* DA). The four tall varieties (*Rht* DA-0) were located in the positive extreme of PC1, while the four ones carrying two *Rht* dwarfing alleles (*Rht* DA-2) were placed in the negative side. The intermediate position between these two groups was occupied by the varieties carrying one *Rht* dwarfing allele (*Rht* DA-1) (Fig. 1). These results suggest that tall varieties accumulated more biomass from the first node detectable stage (CDW₃₁) to milk-grain stage (CDW₇₅) than the others. Despite greater biomass accumulation during early growth stages, these tall varieties achieved the lowest yields. Maximum yields, but less biomass in the first growth stages, were recorded by the varieties carrying 2 *Rht* dwarfing alleles (*Rht* DA-2).

Principal component two was related with CDW at physiological maturity (CDW₈₇), LAD after anthesis (LAD_{65–87}), CDW at the first-node detectable stage (CDW₃₁) and, to a lesser extent, grain yield. The four French varieties (Soissons, Isengrain, Marius and Estrella), and two Spanish grown varieties derived from French breeding programs (Gazul and Califa Sur), were located from the edge to the positive side of PC2, whilst Italian and CIMMYT derived varieties were placed on its negative sense. These results suggest that CDW at physiological maturity (CDW₈₇), LAD, early biomass and yield tended to be greatest in French derived varieties.

Biomass production and partitioning

The variety effect explained ca. 40 % of the total variance for CDW at the first node detectable stage (GS31), but this value decreased in successive growth stages as the environmental effect became more important (Table 3). Except at GS31 in most cases the variety × environment interaction accounted less than the environment in explaining the variability of the experimental data. Varieties differed in the biomass allocated in vegetative organs at all growth

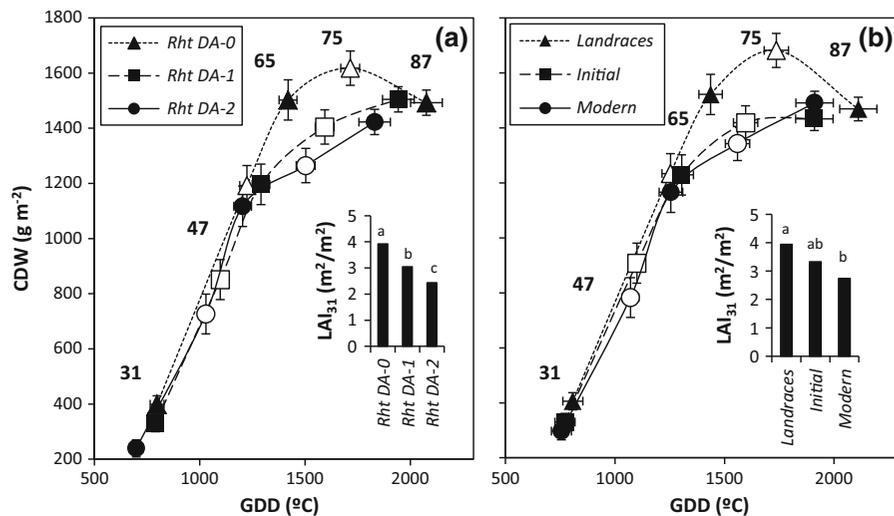


Fig. 2 Changes in the CDW of bread wheat varieties grouped according to: **a** the number of *Rht* dwarfing alleles (*Rht* DA) they carry, and **b** the breeding period (BP) in which they were released/grown. Points are means of four experiments. Numbers correspond to growth stages according to Zadok's scale: 31, first node detectable; 47, booting; 65, anthesis; 75, milk grain; 87, physiological maturity. Closed symbols correspond to stages 31,

65 and 87 and open symbols represent stages 47 and 75. *Inset* were depicted the differences in leaf area index (LAI) at the stage of first node detectable (31) between *Rht* DA and BP. Error bars indicate distances between groups within growth stages, and bars in inset figures followed by the same letter are not significantly different. Means were compared using Duncan multiple range tests at $P = 0.05$

stages, but at maturity differences in CDW between them were not statistically significant.

The partitioning of the variety effect allowed comparison of the variability induced by the general genotypic effect associated with different breeding periods versus the specific impact of the number of *Rht* dwarfing alleles present in the genotypes. Differences between the three groups of varieties with different number of *Rht* dwarfing alleles generally explained a greater percentage of the variety variance for CDW than more general differences across the breeding periods. For instance, 67.4 % of CDW variety variance at GS31 was explained by the presence of the *Rht* dwarfing alleles, while no significant differences were found between breeding periods (Table 3). The number of *Rht* dwarfing alleles present in the varieties also significantly affected LAI at GS31 (inset in Fig. 2a). Similarly, a decreasing tendency in LAI was apparent when breeding periods were compared (Fig. 2b).

From the first node detectable stage to milk-grain stage, the CDW of the varieties with the dwarfing allele at one or two *Rht* loci was lower than that of the tall varieties (*Rht* DA-0) (Table 4). When compared with the landraces, the reduction at GS31 was 17.5 % for the varieties with a mutant allele at one *Rht* locus and 40.4 % for the varieties carrying two *Rht* dwarfing

alleles (Table 4). Changes in CDW from GS31 to anthesis followed the same increasing trend for the three groups of *Rht* DA genotypes (wild type, single or double dwarf genotypes) (Fig. 2a), and the slopes of the three curves (ca $1.70 \text{ g m}^{-2} \text{ GDD}^{-1}$) were not significantly different (data not shown). In spite of showing the same crop growth rate from GS31 to GS65, the tall varieties needed more time and showed more CDW at anthesis than the other groups (Fig. 2a). Actually, tall varieties reached GS65 after 622 GDD from GS31, while the varieties carrying one mutant allele at the *Rht* loci needed 501 GDD, and those carrying two 505 GDD, the differences between *Rht* DA-0 and the two other groups being statistically significant ($P < 0.001$). Growth rate beyond anthesis decreased in the three groups. Tall varieties achieved the maximum CDW at milk-grain stage, while for varieties carrying dwarfing alleles the maximum CDW was recorded at physiological maturity (Table 4 and Fig. 2a). Except at physiological maturity, the number of *Rht* DA present in the studied varieties did not contribute to significantly explain in the ANOVA the variability in spikes weight per unit area due to the variety effect (Table 3).

Crop dry weight accumulation rates from the beginning of jointing to anthesis were not statistically different for the three breeding periods (data not

shown). However, differences in the CDW of varieties from distinct breeding periods were significant at booting, anthesis and milk grain stages (Table 4, Fig. 2b). In all these growth stages landraces accumulated more biomass than initial and modern varieties. Differences between the three groups were maximized at booting, when the CDW of initial and modern varieties were 26.4 and 36.5 %, respectively, lower than that of landraces (Table 4). Initial and modern varieties had similar CDW at all growth stages. The breeding period accounted for a large proportion of the variability in spikes weight per unit area due to the variety effect (Table 3). Spikes weight per unit area was greater in modern varieties than in landraces and initial varieties, being the maximum differences recorded at physiological maturity (Table 4).

Biomass accumulated in the spikes from anthesis to milk-grain stage was significantly greater in initial and modern varieties than in landraces (Fig. 3a). Increases in total CDW resulted from the difference between gains in spikes weight and losses in vegetative biomass. Although differences between breeding periods in the decrease of vegetative biomass from anthesis to GS75 were not statistically significant, a tendency towards greater losses was observed in improved varieties (Fig. 3a). Increase in spikes weight from milk-grain stage to physiological maturity was almost half of the amount accumulated in the preceding period for the landraces, but similar to previous gains for initial and modern varieties (Fig. 3b). While initial varieties maintained and modern varieties increased total CDW from milk-grain stage to maturity, landraces lost biomass during this period. Given that the weight of spikes per unit area increased, the loss of above ground biomass in landraces during the second part of the grain filling period could only be due to a decrease of biomass in vegetative organs, as shown in Table 4.

Growth traits contribution to spike weight gains

In order to study the relationship between yield gains during the twentieth century and changes in spikes weight through grain filling, a stepwise linear regression model was fitted, with yield as dependent variable and spike weight at anthesis (SW_{65}), and the gains in spikes weight during the two phases in which grain filling was divided, as independent variables (Table 5a). The results indicated that the increases in the weight of spikes per unit area from milk grain stage

to maturity (ΔSW_{75-87}) was the first variable entering in the model, explaining 55 % of yield variations, while the increases from anthesis to milk-grain stage (ΔSW_{65-75}) explained an additional 32 %.

With the aim of determining whether the observed differences in ΔSW_{75-87} were mostly attributable to the vegetative biomass at anthesis—source of assimilates to be remobilized to the growing grains during grain filling—or to the duration of leaf area after anthesis—associated to the current photosynthesis capacity during grain filling—, a second stepwise regression model was fitted. In this model ΔSW_{75-87} was the dependent variable and vegetative biomass at anthesis (VDW_{65}), and LAD, from anthesis to milk-grain stage (LAD_{65-87}), and from milk-grain stage to physiological maturity (LAD_{75-87}) were the independent variables. The results indicated that LAD_{75-87} was the only variable entered in the model explaining 58 % of the variability for ΔSW_{75-87} (Table 5b).

A third regression analysis was conducted to study the relationship between LAD_{75-87} and its associated variables LAI_{65} , LAI_{75} , and the thermal-time from sowing to milk-grain stage (GDD_{75}), from sowing to physiological maturity (GDD_{87}), and between these two growth stages (GDD_{75-87}). The results showed that the most important variable determining LAD_{75-87} was LAI_{75} , which accounted for 88 % of the observed variability, which increased until 96 % when GDD_{75-87} was added to the model (Table 5c).

Gains in spike weight from milk-grain stage to maturity and LAI_{75} , LAD_{75-87} and the year of release of the varieties were strongly related with the breeding period (Fig. 4a–c). Modern varieties had the greatest LAI at milk-grain stage, which increased from landraces during the twentieth century at a rate of 0.0055 y^{-1} (Fig. 4d) or, in relative terms, $0.83 \% \text{ y}^{-1}$ (data not shown). Similarly, LAD from milk-grain stage to maturity increased from landraces to modern varieties (Fig. 4b), with a relative gain of $0.79 \% \text{ y}^{-1}$ (data not shown). The increase in spikes weight per unit area was $3.14 \text{ g m}^{-2} \text{ y}^{-1}$ (Fig. 4c), and yield increases were estimated in $3.96 \text{ g m}^{-2} \text{ y}^{-1}$ (data not shown).

Discussion

The results of the present study indicate that, at maturity, total above ground biomass of bread wheat varieties grown in Spain was not significantly

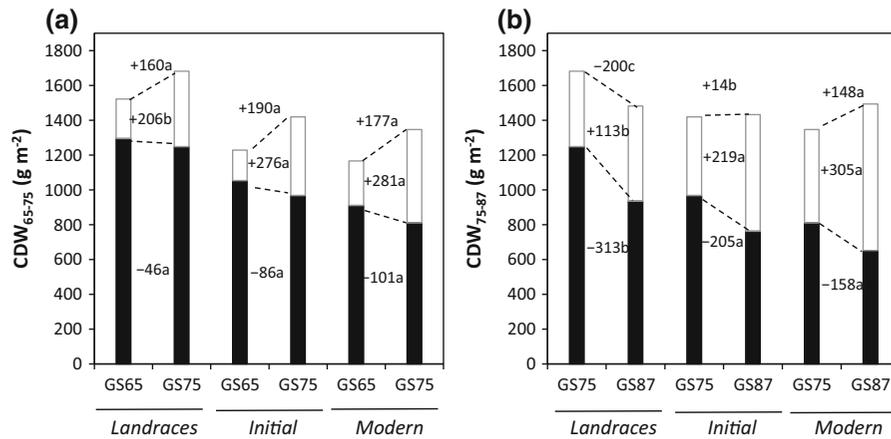


Fig. 3 Changes in CDW and its components, vegetative biomass in dark and spike weight in white, for bread wheat varieties belonging to different breeding periods. **a** Changes between anthesis (GS65) and milk-grain stage (GS75), and

b changes between milk-grain stage (GS75) and physiological maturity (GS87). Numbers within a component for different breeding periods followed by the same letter are not significantly different at $P = 0.05$

Table 5 Regression equations for the relationships between: (a) Yield as a dependent variable and spike weight per unit area at anthesis (SW_{65}), spike weight gain between anthesis and milk-grain stage (ΔSW_{65-75}), and spike weight gain between milk grain and physiological maturity (ΔSW_{75-87}) as independent variables, (b) Spike weight gain between milk-grain stage and physiological maturity (ΔSW_{75-87}) as a dependent variable, and vegetative biomass at anthesis (VDW_{65}), LAD from anthesis to physiological maturity (LAD_{65-87}) and LAD

between milk-grain stage and physiological maturity (LAD_{75-87}) as independent variables and (c) Leaf area duration between milk-grain stage and physiological maturity (LAD_{75-87}) as a dependent variable and leaf area index at anthesis (LAI_{65}), milk-grain (LAI_{75}) and physiological maturity (LAI_{87}), thermal time from sowing to milk-grain (GDD_{75}), thermal time from sowing to physiological maturity (GDD_{87}), and from milk grain to physiological maturity (GDD_{75-87}) as independent variables

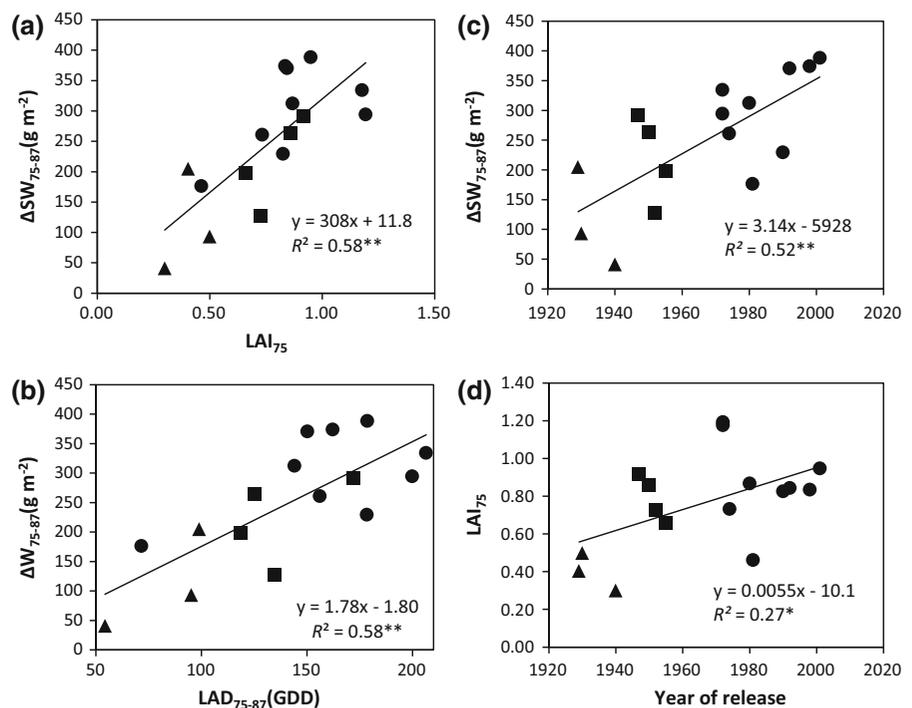
Dependent variable	Independent variables	Regression equation	Variable	Partial R^2	Model R^2	P
a)	Yield	$Y = 0.675 + 74.7 \cdot 10^{-3} \Delta SW_{75-87} + 11.6 \cdot 10^{-3} \Delta SW_{65-75}$	ΔSW_{75-87}	0.55	0.55	<0.001
			ΔSW_{65-75}	0.32	0.87	<0.001
			ΔSW_{75-87}			
b)	ΔSW_{75-87}	$Y = -1.80 + 1.78 LAD_{75-87}$	LAD_{75-87}	0.58	0.58	<0.001
			VDW_{65}			
			LAD_{65-87} LAD_{75-87}			
c)	LAD_{75-87}	$Y = -78.1 + 172 LAI_{75} + 0.249 GDD_{75-87}$	LAI_{65}	0.88	0.88	<0.001
			LAI_{75}	0.08	0.96	<0.001
			GDD_{75}			
			GDD_{87}			
			GDD_{75-87}			

Only variables that met the 5 % probability level entered into the model

modified by the introduction of improved varieties during the twentieth century. However, significant changes occurred in dry matter accumulation and partitioning through the crop cycle.

The set of varieties used in this study reflected the history of introduction of *Rht* dwarfing alleles in the wheats grown in Spain in the last century. Great differences in plant height existed between the

Fig. 4 Relationship between increases in spike weight per unit area from milk-grain stage to physiological maturity (ΔSW_{75-87}) and **a** leaf area index at milk-grain stage (LAI_{75}), **b** leaf area duration from milk-grain stage to physiological maturity (LAD_{75-87}), and **c** year of release; **d** relationship between LAI_{75} and the year of release. Each point corresponds to the mean data of a variety across four experiments. Breeding periods are identified with triangles for local landraces, squares for initial varieties and circles for modern varieties. * $P < 0.05$; ** $P < 0.01$



unimproved landraces—characterized by the absence of reduced height alleles of the major dwarfing genes, compared to the initial varieties from the early Italian and French breeding programs that carried the first reduce height alleles at *Rht* loci. These early improved varieties showed a great plant height variability that, in this study, was noticed by the differences existing between the Italian GA sensitive variety ‘Mara’ (86 cm height), carrying *Rht8c* and *Rht9* dwarfing alleles (Worland et al. 2001), and the Spanish variety ‘Pané 247’ (115 cm height), the only improved variety in which *Rht* dwarfing alleles were missing. This points out the importance that the pioneer breeders, especially Nazareno Strampelli, gave to reductions in plant height to improve the agronomic performance of wheat varieties (Salvi et al. 2013). Further height decreases occurred after the introduction of modern varieties from the Green Revolution, most of them derived from CIMMYT germplasm, thus carrying the Norin-10/Brevor major dwarfing GA insensitive alleles *Rht-B1b* and/or *Rht-D1b* (Worland and Snape 2001).

It is well known that the introduction of dwarfing genes helped boosting wheat HI worldwide during the twentieth century (Austin et al. 1989; Worland and Snape 2001) and particularly in Spain (Royo et al. 2007; Sanchez-Garcia et al. 2013). The present study revealed that within the set of modern varieties, a consistent trend of HI increases did not exist through the period elapsed from the introduction of ‘Yecora’, in 1972, with a HI of 0.47 until the release of ‘Califa Sur’, in 2001, with a HI of 0.48. Seeking further improvements in HI through new reductions in plant height may be difficult to achieve, or even undesirable, since the height of the newest variety of the historical series, ‘Califa Sur’ (71 cm), was close to the lower limit of the optimum plant height range (Flintham et al. 1997; Addisu et al. 2010; Foulkes et al. 2011). Since shorter heights may be detrimental for optimum canopy architecture and, therefore, for an efficient photosynthetic performance (Foulkes et al. 2011), future yield gains seem to be better attained by increasing CDW without modifying the

HI, as proposed by Parry et al. (2011) and Blum (2011).

The results of the multivariate analysis indicated that the relationship between dwarfing genes, biomass and yield depended more on the number of dwarfing alleles at the *Rht* loci than on the presence of any specific allele or genotypic combination. In addition, the distribution of varieties along PC1 was more related to the number of dwarfing alleles they carry than to their growth habit. Thus, varieties carrying a mutant allele at one *Rht* gene, such as the French winter cultivars ‘Soissons’, ‘Isengrain’ and ‘Estrella’, and the spring types ‘Anza’, ‘Rinconada’, ‘Impeto’ and ‘Gazul’ (Sanchez-Garcia et al. 2012), were clustered together according to PC1. These results justified the study of the pattern of biomass accumulation and partitioning on the basis of the number of *Rht* dwarfing alleles (*Rht* DA) present in the historical series.

Multivariate analysis and the differences observed in CDW and LAI between *Rht* DA groups revealed a detrimental effect of dwarfing alleles on biomass and leaf area at the beginning of jointing. This detrimental effect was larger in varieties carrying two of them. Previous studies have reported a negative effect of the GA insensitive dwarfing alleles *Rht-B1b* and *Rht-D1b*—the most common in our set of genotypes—on early vigour. These effects involve shortening of the coleoptile length and leaf elongation rate (Ellis et al. 2004). Despite differences between breeding periods in CDW at the beginning of jointing were not significant, a decrease trend was observed on this trait, which was further confirmed by the progressive reduction of LAI in initial and modern varieties when compared with the landraces. The fact that varieties with a different number of *Rht* DA were included in the initial, and particularly in the modern group of varieties, was probably the reason that the changes in CDW at the beginning of jointing across periods were not significant. It has been demonstrated that early crop development may be determinant for adaptation to some Mediterranean-like environments, since a faster rate of seedling development can accelerate soil coverage and prevent soil moisture losses (Condon et al. 2004). We offer here the speculation that the reduction of biomass at the beginning of jointing caused by the introduction of *Rht* dwarfing alleles could be behind the worse adaptation of initial and, specially, Spanish modern varieties to poor and dry environments when compared with the landraces,

reported in a previous study including most of the varieties used here (Sanchez-Garcia et al. 2012).

Similar rates of dry matter accumulation from the beginning of jointing to anthesis were observed in the three *Rht* DA groups and the three breeding periods. Therefore, differences in CDW between *Rht* DA or breeding periods at anthesis and booting relied upon the initial differences in CDW at the beginning of jointing, probably due to the detrimental effect of the GA insensitive dwarfing alleles on early vigor (Ellis et al. 2004), and the thermal time needed to attain anthesis. A similar conclusion was obtained in previous studies carried out in Mediterranean-like environments with bread (Richards 1992; Acreche et al. 2009) and durum wheat (Álvarez et al. 2008). Phenology, one of the main adaptation mechanisms, has been related to wheat yield gains in Spain during the twentieth century (Sanchez-Garcia et al. 2013). The earliness of initial and modern varieties when compared with the landraces was the main cause of their lower biomass accumulated prior to anthesis, but it also most likely allowed grains to fill under better environmental conditions avoiding, at least in part, the typical terminal stresses of Mediterranean environments.

Biomass at anthesis was significantly greater in landraces (*Rht* DA-0) than in improved varieties, independently of the breeding period they belong or the number of *Rht* DA they carry. However, these differences disappeared at physiological maturity, when all groups had similar CDW. These results suggest contrasting strategies in landraces and improved varieties for biomass formation and allocation during grain filling. Despite biomass accumulation was better explained by the number of *Rht* DA than by breeding periods, they were not useful to understand the partitioning of biomass to grains during grain filling.

The study of biomass allocation from anthesis to maturity revealed that initial and modern varieties accumulated 176 g m^{-2} (55 %) and 267 g m^{-2} (84 %), respectively, more biomass in the spikes during this period than the landraces. On the other hand, relative losses of vegetative biomass during this period were -68 g m^{-2} (-19 %) and -100 g m^{-2} (-27 %) in initial and modern varieties, respectively, when compared with the landraces. Since grain growth is supported by transient photosynthesis and translocation of stored reserves accumulated prior to anthesis in vegetative organs (Álvarez et al. 2008; Maydup et al. 2010; Sanchez-Bragado et al. 2014), our results

suggest that the introduction of the first improved varieties increased the contribution of photosynthesis to grain filling.

Changes in biomass partitioning were studied by fractioning grain filling in two phases, one from anthesis to milk-grain stage, and the other from it to physiological maturity. Increases in spikes weight from anthesis to milk-grain stage were greater in initial and modern varieties than in the landraces. However, in spite of the observed tendency towards a higher remobilization of vegetative biomass and, also an increase in current photosynthesis resulting in higher CDW accumulation, differences between breeding periods were not statistically significant. Thus, we could not attribute the gains in spikes weight per unit area during this phase to a specific increase in photosynthesis or translocation, because they were likely due to both of them.

Changes in biomass accumulation and partitioning from milk-grain stage to physiological maturity revealed contrasting strategies in landraces and improved varieties of different periods. Landraces lost above ground biomass during the second phase of the grain filling, due to the senescence of leaves and the loss of stems weight (313 g m^{-2}) that was much greater than the increases in spikes weight. Thus, part of the decreases of stems weight was not attributable to translocation, but most probably to the dead of secondary tillers. It has been reported that the number of unfertile tillers is much greater in landraces than in improved varieties (Sanchez-Garcia et al. 2013), and probably the warm and dry environmental conditions at the end of the grain filling period accelerated their senescence. Initial varieties maintained total above ground biomass after milk-grain stage, and the gains in spikes weight during this phase corresponded to the loss of vegetative biomass. This result suggests that translocation was in this phase the main contributor to fill the grains of the first improved varieties grown in Spain. In contrast with the patterns followed by previous cultivars, modern varieties increased total above ground biomass from milk-grain stage to maturity. In addition, their decrease of vegetative biomass was much lower than the increases in the weight of the spikes, thus indicating that the contribution of photosynthesis to grain filling in this last phase was enhanced in modern varieties when compared with the precedent ones. This was probably the

cause why the gain in spikes weight from milk-grain stage to physiological maturity was the most relevant to explain yield genetic gains along the century. Actually, our results showed that 79.3 % of absolute yield gains of bread wheat in Spain during the last century were due to the increases in spikes weight from milk-grain stage to physiological maturity, while the remainder 20.7 % corresponded to the gains in the anthesis to milk-grain phase.

The study of the relationship between growth traits and the increases in spikes weight after milk-grain stage revealed that they were strongly linked to the greater LAD of modern varieties. LAD has been considered an important mechanism for increasing total crop photosynthesis and, thereby, crop biomass (Richards 2000) and yield performance in non water-limited Mediterranean-like environments (Christopher et al. 2008). When compared with the landraces, the higher LAD of improved varieties could be attributed to their early heading, as it has been previously described (Naruoka et al. 2012). However, the higher LAD of modern varieties in relation to the initial cultivars could not be explained in terms of phenological differences, since both sets of genotypes had similar developmental patterns. The reason of the superior LAD of the most recent cultivars was their greater leaf area available at milk-grain stage. These results, and the positive and significant relationship found from milk-grain stage to physiological maturity between the year of release and both, LAI at milk-grain stage and increases in spikes weight, support the previous assumption that enhanced current photosynthesis helped boosting the gains on spikes weight during the last phase of grain filling in modern varieties. However, improvements in the contribution of photosynthetic organs other than leaves known to play a role in yield formation like the spike (Maydup et al. 2010, Maydup et al. 2012; Sanchez-Bragado et al. 2014), or in the photosynthetic rates during grain filling may have also played a role in the observed yield gain in Spain during the twentieth century.

Finally, in the present scenario of climate change the increased importance of photosynthesis during the last part of the grain filling on yield formation in modern varieties and its sensitivity to heat and drought stress events (Dias and Lidon 2009; Sanchez-Bragado et al. 2014) will need further studies and special attention from breeders, especially the ones working in dry areas.

Concluding remarks

Genetic gains in bread wheat yield in Spain during the twentieth century were closely related to changes in biomass accumulation and partitioning over the crop cycle. These changes were directly associated to the introduction of mutant alleles at the *Rht* major dwarfing alleles, which caused a strong decrease in crop biomass and LAI at the beginning of jointing. Plant height reductions and early anthesis of improved varieties increased biomass partitioning to the spikes and HI.

Although the number of *Rht* DA explained changes in CDW during all the growth cycle better than the breeding periods, the last were more useful to interpret biomass allocation from anthesis to maturity. The reason may be that the introduction of dwarfing genes was only one of the strategies used by breeders to improve yields, but increases in LAI during grain filling and LAD also significantly contributed to productivity increases during the last decades of the century. The maintenance of more green leaf area from milk-grain stage to maturity—the main period determining yield gains during the past century—appeared to be the main contributor to gains on spikes weight. Since no evidence was found that increases on delayed senescence reached a plateau, enhancing LAI at milk-grain stage could be a strategy for further boosting yield gains.

Acknowledgments This work was partially supported by the Spanish National Institute for Agriculture and Food Research and Technology (INIA) [RTA2004-058-C04 and RTA2009-061-C03] and Spain's Inter-ministerial Commission for Science and Technology (CICYT) [AGL-2009-11187]. The Centre UdL-IRTA is part of the Centre CONSOLIDER INGENIO 2010 on Agrigenomics funded by the Spanish Ministry of Education and Science. M. Sanchez-Garcia was a recipient of a PhD grant from INIA.

Appendix

ΔSW_{65-75} : spike dry weight gain per m^2 between growth stages 65 and 75 (anthesis and milk-grain stage); ΔSW_{75-87} : spike dry weight gain per m^2 between growth stages 75 and 87 (milk-grain and physiological maturity); BP: breeding period; CDW: crop dry weight; GDD: growing degree days; GDD_{75} : thermal time from sowing to milk-grain; GDD_{87} : thermal time from sowing to physiological maturity; GDD_{75-87} : thermal time from milk-grain to physiological maturity; GS:

growth stage; LAI_{65} : leaf area index at anthesis; LAI_{75} : leaf area index at milk-grain; LAD_{65-87} : leaf area duration from anthesis to physiological maturity; LAD_{75-87} : leaf area duration between milk-grain stage and physiological maturity; *Rht* DA: *Rht* dwarfing alleles; SW_{65} : spike dry weight per unit area at growth stage 65 (anthesis); VDW_{65} : vegetative biomass dry weight per unit area at anthesis.