Effect of *Ppd-1* photoperiod sensitivity genes on dry matter production and allocation in durum wheat

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ABSTRACT

Understanding the effect of genetic factors controlling flowering time is essential to fine-tune phenological development and to maximize yield. Thirty-four spring durum wheat genotypes classified in five allelic combinations for *Ppd-A1/Ppd-B1* loci were grown for two years at three contrasting latitudes: Mexico-North, Spain-South and Spain-North. In all them, a delay in flowering date due to the presence of photoperiod sensitivity alleles *Ppd-A1b* and *Ppd-B1b* resulted in lower yields. The number of days to flowering, determined by an increasing number of photoperiod sensitivity alleles, accounted in all sites for more than 80% of the variation in the contribution of translocation of pre-flowering assimilates to grain yield. In Mexico and Spain-North late-flowering resulted in decreased harvest index as influenced by high temperatures during grain filling. In Mexico, where grain filling occurred under high temperatures and solar radiation, translocation of pre-flowering assimilates accounted from 55 to 63% of yield, independently of the flowering date of the genotype. In Spain-North, where water was available during grain filling, current photosynthesis was the main contributor to yield (57–73%), with independence of the allelic combination at *Ppd* loci. In Spain-South, the relative contribution of photosynthesis and translocation depended on the allelic composition at *Ppd* loci, with translocation increasing by 24% in the latest-flowering genotypes compared with the earliest ones. In all sites the limiting factor for attaining high yields was the capacity of the plant canopy to photosynthesize after anthesis. This study suggests that the expression of genes *Ppd-A1* and *Ppd-B1* regulating the response to photoperiod modulates the physiological strategy adopted by durum wheat to fill its grains, underlining the importance of phenology fitting in maximizing grain yield.

1. Introduction

Wheat is one of the major grain crops in the world and provides about 20% of the calories of the world's population (FAOSTAT, 2016). Durum wheat (*Triticum turgidum* L. var. *durum*) represents about 10% of total wheat production (Kantety et al., 2005), playing an important role in food security for urban populations in small geographical areas (Ammar et al., 2006). The Mediterranean Basin is the largest durum producing area worldwide, the most significant import market and the largest consumer of durum wheat products.

Durum wheat yield can only be maximized by growing varieties which flowering time allows the crop to avoid stresses during vegetative and grain-filling periods (Kamran et al., 2014). Flowering time is a critical stage that delimits the duration of spike formation and marks the transition into the grain-filling period during which kernels per spike and kernel weight are defined. Wheat grain growth is mainly supported by transient photosynthesis (primarily in the flag leaf and the inflorescence) and translocation of stored reserves accumulated in vegetative organs prior to flowering (Ehdaie et al., 2006; Blum, 1988). Dry matter accumulated prior to flowering is of particular importance when grain filling takes place under hot and dry conditions that limit photosynthesis (Papakosta and Gagianas, 1991; Villegas et al., 2001; Ehdaie et al., 2006; Álvaro et al., 2008). The relative proportion of stem reserves to wheat grain yield ranges from 6 to 100%, depending on the environment and genotypes under cultivation (Borrell et al., 1993; Blum et al., 1994). Under optimal conditions, stem carbohydrate reserves have been estimated to contribute from 10 to 12% of the final grain yield in wheat, but more than 40% under drought or heat stress

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during the grain filling period (Wardlaw and Porter, 1967; Bidinger et al., 1977; Ehdaie et al., 2008). Van Herwaarden et al. (1998) reported a 75–100% share of stem reserves in the grain yield of wheat under drought. It has been estimated that total dry matter remobilized from the main stem to the filling grains is greater in the modern durum wheat cultivars than in the landraces (Álvaro et al., 2008), possibly providing one basis for the increased harvest index of modern cultivars.

The genetic control of flowering time in wheat is complex. It is controlled primarily by three groups of loci: photoperiod sensitivity genes (*Ppd*), vernalization requirement genes (*Vrn*) and 'earliness *per se*' (*Eps*) or 'narrow-sense earliness'. The latter act on the developmental rate independently of vernalization and photoperiod (Snape et al., 2001; Distelfeld et al., 2009). Vernalization requirement is controlled by the *Vrn-1* genes, which in durum wheat consist of homoeologous copies designated as *Vrn-A1* and *Vrn-B1*, located on the long arms of chromosomes 5A and 5B, respectively (Yan et al., 2004; Fu et al., 2005).The major elite durum wheat gene pools are spring types showing no major vernalization requirements.

Photoperiod sensitivity in durum wheat is determined at the *Ppd-A1* and *Ppd-B1* loci, located on chromosomes 2AS and 2BS, respectively (Laurie, 1997). Wilhelm et al. (2009) found two large deletions within the *Ppd-A1* gene in durum wheat (1027 and 1117 bp, designated as allele 'GS-100′ and 'GS-105′, respectively), which remove a common region from the wild-type sequence. The presence of either deletion accelerated flowering, which led to the conclusion that these deletions are the likely causal basis of photoperiod insensitivity in tetraploid wheat (Wilhelm et al., 2009).

Ppd-1 genes play an important role in the regulation of wheat growth and development (Kirby, 1988; Miralles and Richards, 2000; Kamran et al., 2014). Allelic combinations at these loci modulate plant development, interacting with the environmental stimuli to advance or delay flowering time (Snape et al., 2001). This may affect indirectly the accumulation and distribution of dry matter within the wheat plant, modifying source-sink equilibrium (Foulkes et al., 2004). The intensive selection for photoperiod insensitivity conducted during the 20th century, particularly in the CIMMYT breeding programs, resulted in the selection of early types, most of them with little to no photoperiod sensitivity. The breeding-generated reduction in the number of days to flowering in durum wheat has been estimated to amount to 8 days in Spain and 2 days in Italy (Álvaro et al., 2008). Yield advantages resulting from photoperiod insensitivity in bread wheat have been estimated to represent up to 35% in Europe (Worland, 1996).

Current climate change scenario, predicting more drought events and increased temperature in Europe and northern latitudes (DePauw et al., 2011), require from wheat breeders to develop cultivars achieving high yields in spite of less than optimal growing conditions in order to ensure food security (Curtis and Halford, 2014). In this context, understanding the effect of allelic combinations at *Ppd-1* on flowering time and yield formation under different environmental conditions, through the analysis of the main physiological processes involved, becomes of prime importance to select allelic combinations, or fine tuning

phenological development, to maximize yield.

This study was conducted with a set of 34 spring durum wheat genotypes encompassing five of the six possible allelic combinations at *Ppd-A1* and *Ppd-B1* loci to estimate the contribution of current photosynthesis after flowering and translocation of stored reserves accumulated in vegetative organs prior to flowering on grain yield, as it is affected by flowering time.

2. Materials and methods

2.1. Plant material

Thirty-four spring durum wheat (*Triticum turgidum* L. var. *durum*) genotypes were used in this study. Thirty inbred lines resulted from a divergent selection process within the offspring of crosses between parents with contrasting flowering time. Five late-flowering German genotypes provided by the University of Hohenheim, Stuttgart, Germany ('Durabon', 'Megadur', 2716-25.94.01, 2805-49.94.02 and 2905-13.93-04), were crossed with five early-flowering advanced lines (Sooty_9/Rascon_37, Cado/Boomer_33, Dukem_12/2*Rascon_21, 'Guanay' and 'Snitan') from the CIMMYT (International Centre for Wheat and Maize Improvement, Mexico) durum wheat breeding program. The F₁, F₂ and F₃ populations were advanced in bulk at CIMMYT. From each F₄ population, an early-flowering and a late-flowering plants were selected in order to capture the maximum range for time to flowering. From generations F₅ to F₇, selected lines were selfed, purified and increased at the Institute for Food and Agricultural Research and Technology (IRTA) in Spain. At generations F₈ and F₉, the seed of fixed lines with contrasting flowering dates was used in field experiments. Two additional CIMMYT sister lines, derived from the cross CF4-JS 40/3/Stot//Altar84/Ald, and two commercial cultivars ('Simeto' and 'Anton') were also included in the collection.

The selected genotypes were analysed with a set of molecular markers associated with key *Vrn* and *Ppd* alleles as described in Royo et al. (2016). The molecular characterization revealed that all of the 34 genotypes were spring types, carrying the dominant allele *Vrn-A1c* with a deletion in intron-1 of *Vrn-A1* (Yan et al., 2004), and the recessive alleles *vrn-B1* and *vrn-B3* (Fu et al., 2005; Yan et al., 2006). The analysis of the allelic composition for *Ppd-1* identified three alleles at *Ppd-A1* (i.e. *Ppd-A1b* conferring photoperiod sensitivity in 16 genotypes, and alleles 'GS-105′ and 'GS-100′ causing photoperiod insensitivity in 12 and 6 genotypes, respectively), and two alleles at *Ppd-B1* (the wild-type allele *Ppd-B1b* conferring photoperiod sensitivity in 13 genotypes, and the mutation conferring photoperiod insensitivity *Ppd-B1a* in 21 genotypes (Table 1). Details on the allelic combinations present on each genotype at *Ppd-A1* and *Ppd-B1* loci is shown in Supplementary Table 1.

2.2. Experimental details

Field experiments were conducted in 2007 and 2008 at three sites, two in Spain: Lleida in the north (Spain-North), and Jerez de la Frontera

Table 1
Mean phenotypic values of 34 durum wheat genotypes classified according allelic combinations for *Ppd-A1*/*Ppd-B1* loci across three sites (Spain-North, Spain-South, and Mexico) and two years (2007 and 2008). (S) and (I) stand for sensitive and insensitive photoperiod response, respectively. DM$_F$ = dry matter at flowering, DMT = dry matter from translocation, DMP = dry matter in grain from current photosynthesis during grain filling, and CT = contribution of pre-anthesis assimilates to grain yield.

Ppd-A1 allele[1]	*Ppd-B1* allele	Acronym	Number of lines	Days emergence-flowering	Yield (g m^{-2})	DM$_F$ (g m^{-2})	Harvest index	DMT (g m^{-2})	DMP (g m^{-2})	CT (%)
Ppd-A1b (S)	*Ppd-B1a* (I)	SI	9	117[a]	597[b]	1019[b]	0.456[b]	316[a]	282[b]	54.5[a]
Ppd-A1b (S)	*Ppd-B1b* (S)	SS	7	117[a]	622[b]	1077[a]	0.456[b]	328[a]	294[b]	53.6[a]
GS-105 *Ppd-A1a* (I)	*Ppd-B1b* (S)	I5S	6	112[b]	611[b]	991[c]	0.470[a]	315[a]	296[b]	52.6[a]
GS-105 *Ppd-A1a* (I)	*Ppd-B1a* (I)	I5I	6	107[c]	623[b]	971[c]	0.467[ab]	251[b]	372[a]	42.9[b]
GS-100 *Ppd-A1a* (I)	*Ppd-B1a* (I)	I0I	6	105[d]	664[a]	975[c]	0.478[a]	260[b]	404[a]	40.8[b]

Means within columns with the same superscript latin letters are not significantly different ar *P* < 0.05
[1] Nomenclature described in Wilhelm et al. (2009).

Site
Location
Coordinates
Altitude, m.a.s.l.
Year
Seasonal rainfall + irrigation, mm
Mean temperature during grain filling, ˚C
Sowing date

in the south (Spain-South), and Ciudad Obregon in Northern Mexico (Mexico). Each experiment consisted of 12 m² plots (8 rows, 10 m long and 15 cm apart), arranged in a randomized complete block design with three replications. All the experiments were autumn-planted. Sowing densities were adjusted at each site in order to obtain approximately 450 spikes/m². Plots were managed to maximize yield at each site, to the extent allowed by local conditions. Plots were maintained free of weeds, diseases and pests and were irrigated when necessary (required full irrigation in Mexico) to prevent significant water deficit. Plots were kept disease and insect free with preventive pesticide applications. Experimental details are shown in Table 2.

2.3. Phenotypic data recording

Times to flowering and physiological maturity were recorded for each plot when approximately 50% of the main spikes reached Zadoks' stages 65 (anthesis half-way) and 87 (hard dough), respectively (Zadoks

et al., 1974). Plots were divided in two sections of 6 m², one used for destructive sampling and the other left untouched for bulk harvest and estimation of grain yield at commercial maturity (g m⁻²) subsequently expressed on a 10% moisture basis. Plants from a randomly chosen, representative section of 0.5 m were uprooted from a central row of each plot at flowering. At physiological maturity, a similar section of 1 m was taken. In both cases, the plants were oven dried at 70 ˚C for 48 h and weighed to estimate dry matter (DM) at flowering (DM_F) and dry matter at maturity (DM_M), respectively. Harvest index (HI) was calculated from the sample collected at maturity as the ratio between dry kernel weight and total aboveground DM.

Remobilization of pre-flowering assimilates was assessed according to the following variables (Royo et al., 1999; Álvaro et al., 2008): DM from translocation (DMT), DM in grain from current photosynthesis during grain filling (DMP), and the contribution of pre-flowering assimilates to the grain (CT) as:

$$DMT\ (g\,m^{-2}) = DM_F - [DM_M - \text{grain yield}]$$

$$DMP\ (g\,m^{-2}) = \text{grain yield} - DMT$$

$$CT\ (\%) = [DMT/\text{grain yield}] \times 100$$

Data on daily mean temperature (T_{mean}), rainfall, and solar radiation were obtained from meteorological stations located less than 3 km away from the experimental plots. Environmental variables were calculated for each experimental plot for the period spanning from flowering to maturity.
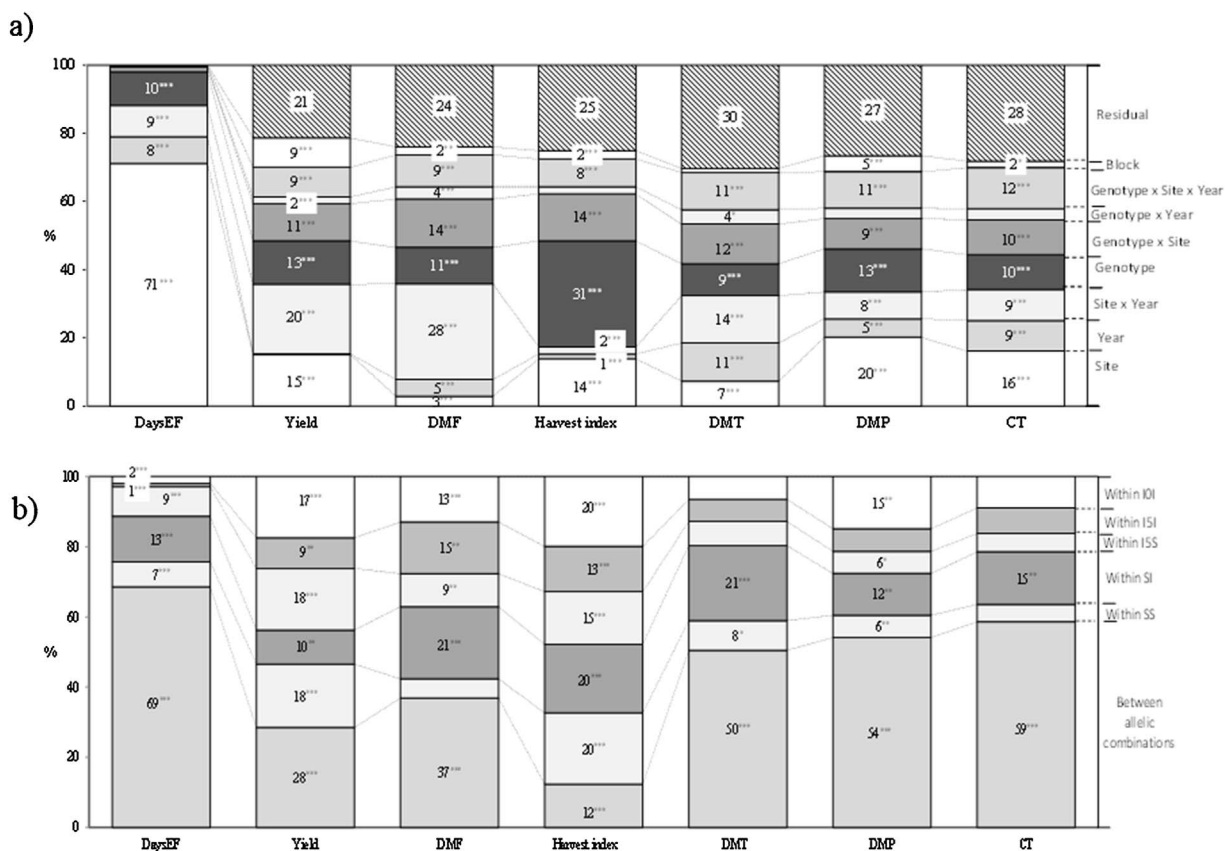
Fig. 1. Percentage of the sum of squares of the ANOVA model corresponding to the different components of variation in a set of 34 durum wheat genotypes grown in three sites during two years. Days$_{EF}$ = number of days from emergence to flowering, DM_F = dry matter at flowering, DMP = dry matter in grain from current photosynthesis during grain filling, DMT = dry matter from translocation, CT = contribution of pre-anthesis assimilates to grain yield. Fig. 1a refers to total variance and Fig. 1b is the percentage of the genotype effect partitioned in differences between allelic combinations at *Ppd-A1* and *Ppd-B1* loci and differences within each of them (see Table 1 for allelic combination acronyms). Values are only shown for the significant effects. * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$.

2.4. Statistical analyses

Combined ANOVA were performed across experiments using the GLM procedure in the SAS statistical package (SAS Institute Inc., 2009) with a fixed-factor model. The sum of squares of the genotype effect was partitioned into differences between allelic combinations at *Ppd-A1* and *Ppd-B1* loci, and differences within each of them. The sum of error terms of the differences between lines within each allelic combination was used to test the differences between allelic combinations. Differences between means were compared by the Student-Newman-Keuls test at $P < 0.05$. In order to ascertain the interaction between environmental variables after flowering and the balance between the two sources of assimilates for grain filling (current photosynthesis and translocation), principal component analysis (PCA) was performed on the correlation matrix, calculated on the mean data for each allelic combination at each site across replications and years. Linear regression models were fitted to the relationships between variables using the mean data across replications and years.

3. Results

The ANOVA revealed that the site effect was the most important in explaining the variance of the model for the number of days from emergence to flowering (Days$_{EF}$) as it accounted for 71% of this variable (Fig. 1a). For the remaining traits, the site effect explained from 3% (for DMF) to 20% (for DMP) of the total variance. The year effect was in general low. Except for Days$_{EF}$, the sum of environmental effects (site, year and site x year interaction) explained between 17% and 36% of total variance. Yield was similar in Spain-South and Mexico, and greater in Spain-North (Table 3). Dry matter at flowering (DM$_F$) reached the greatest and similar values in Spain-South and Mexico. The longest cycle length and the highest values for DMP and HI were reached in Spain-North although at this site DMT and CT were the lowest (Table 3).

The genotype effect explained between 9% (for DMT) and 31% (for HI) of the total variance (Fig. 1a). The partitioning of the genotype effect into its components, that is, differences between allelic combinations and differences within each of them, showed that the former accounted for more than 50% of the variance induced by the genotype effect for Days$_{EF}$, DMP, DMT and CT (Fig. 1b). This percentage was slightly lower, but still high for DM$_F$ and yield and much lower for HI. Except for HI variability for all traits within allelic combinations was lower than the variability due to differences between them (Fig. 1b), which differed for all studied traits (Table 1). For Days$_{EF}$ the largest differences were recorded between combinations carrying the sensitive allele at *Ppd-A1* (SI and SS) and combination *GS100/Ppd-B1a* (I0I), the latter resulting, on average, in 12 days less to flowering (Table 1). On average, genotypes carrying this allelic combination reached the greatest yields. Genotypes carrying the sensitive allele at *Ppd-A1* tended to produce more DM$_F$ and to have lower HI than the ones carrying one of the alleles conferring photoperiod insensitivity at this locus. Dry matter translocation (DMT) and CT were greatest, and DMP lowest in genotypes carrying allele *Ppd-A1b* (Table 1).

The coefficients of determination of the regression models fitted to

the relationships between variables using the mean genotype data across years (shown in Fig. 2, n = 34) revealed that Days$_{EF}$ was strongly and negatively associated with yield at all three sites, with Days$_{EF}$ explaining from 16% to 34% of yield variations depending on the site (Fig. 2a). In contrast, DM$_F$ increased as Days$_{EF}$ increased, with the coefficient of determination being significant in the two Spanish sites (Fig. 2b). A negative effect of flowering date was observed on HI in all three sites, the relationship between both traits being statistically significant in Mexico and Spain-North (Fig. 2c).

The relationships between Days$_{EF}$ and the sources of carbohydrates for filling the grains calculated with the mean data of allelic combinations across sites and years (n = 5) showed a consistent and significant negative relationship between Days$_{EF}$ and DMP ($R^2 = 0.88$, $P < 0.05$), but a positive relationship with DMT ($R^2 = 0.81$, $P < 0.05$). The trends were similar and statistically significant when these relationships were studied considering individual sites expressing in relative terms the contribution of current photosynthesis and dry matter from translocation to yield (Fig. 3). However, the relative position of the regression lines strongly depended on the site. In Mexico, where Days$_{EF}$ ranged from 86 to 98, the remobilization of pre-flowering assimilates was always the most important contributor to yield, independently of the allelic combination or of time to flowering. On the contrary, in Spain-North where the crop reached flowering in 127–134 days from emergence, current photosynthesis contributed the most to filling grains, in a manner that was also independent of the allelic combination or time to flowering. In Spain-South flowering date caused a qualitative change (cross-over) in the relative contribution of current photosynthesis or dry matter from translocation to yield, with allelic combinations that generated more than 110 days from emergence to flowering characterized by a drastically reduced photosynthesis (central part of Fig. 3).

The relationships between the sources of photosynthates and yield are shown in Fig. 4. The relationship between DMP and yield was positive and statistically significant in the three sites (Fig. 4a). Dry matter translocation was positively associated with yield in Mexico (Fig. 4b). Finally, the relationships between yield and CT had a negative trend in all sites, but it was only significant in Spain-North (Fig. 4c).

In order to identify the environmental variables affecting the relative importance of current photosynthesis *versus* dry matter from translocation as sources of assimilates for dry matter production and yield at each site, a multivariate analysis was conducted. On it temperature, solar radiation and water input data during grain filling were fused with variables related to biomass and the sources of photo-assimilates for grain filling (Fig. 5). The first two axes of the PCA accounted for ca. 88.8% of the total variance (axis 1, 65.6%; axis 2, 23.1%), indicating that most of the information contained in the data could be summarized by projecting the points on the plane determined by the first two axes. The length of each vector's projection on an axis is proportional to its contribution to the principal components of that axis, reflecting the extent to which each variable contributes to the two components. Principal component 1 was related to DM$_F$, DMT, CT, solar radiation and temperature during grain filling in the positive direction, and to DMP and water input in the negative direction (Fig. 5a). These results indicate a close relationship between DM$_F$ and DMT and a

Table 3

Mean values for grain yield and biomass production and allocation at each site across years and genotypes. DM$_F$ = dry matter at flowering, DMT = dry matter from translocation, DMP = dry matter in grain from current photosynthesis during grain filling, and CT = contribution of pre-anthesis assimilates to grain yield.

Site	Days emergence-flowering	Yield (g m^{-2})	DM$_F$ (g m^{-2})	Harvest index	DMT (g m^{-2})	DMP (g m^{-2})	CT (%)
Spain-North	132[a]	685[a]	965[b]	0.49[a]	243[c]	443[a]	36.2[c]
Spain-South	111[b]	591[b]	1022[a]	0.45[c]	303[b]	288[b]	52.9[b]
Mexico	92[c]	593[b]	1042[a]	0.46[b]	335[a]	258[c]	57.5[a]

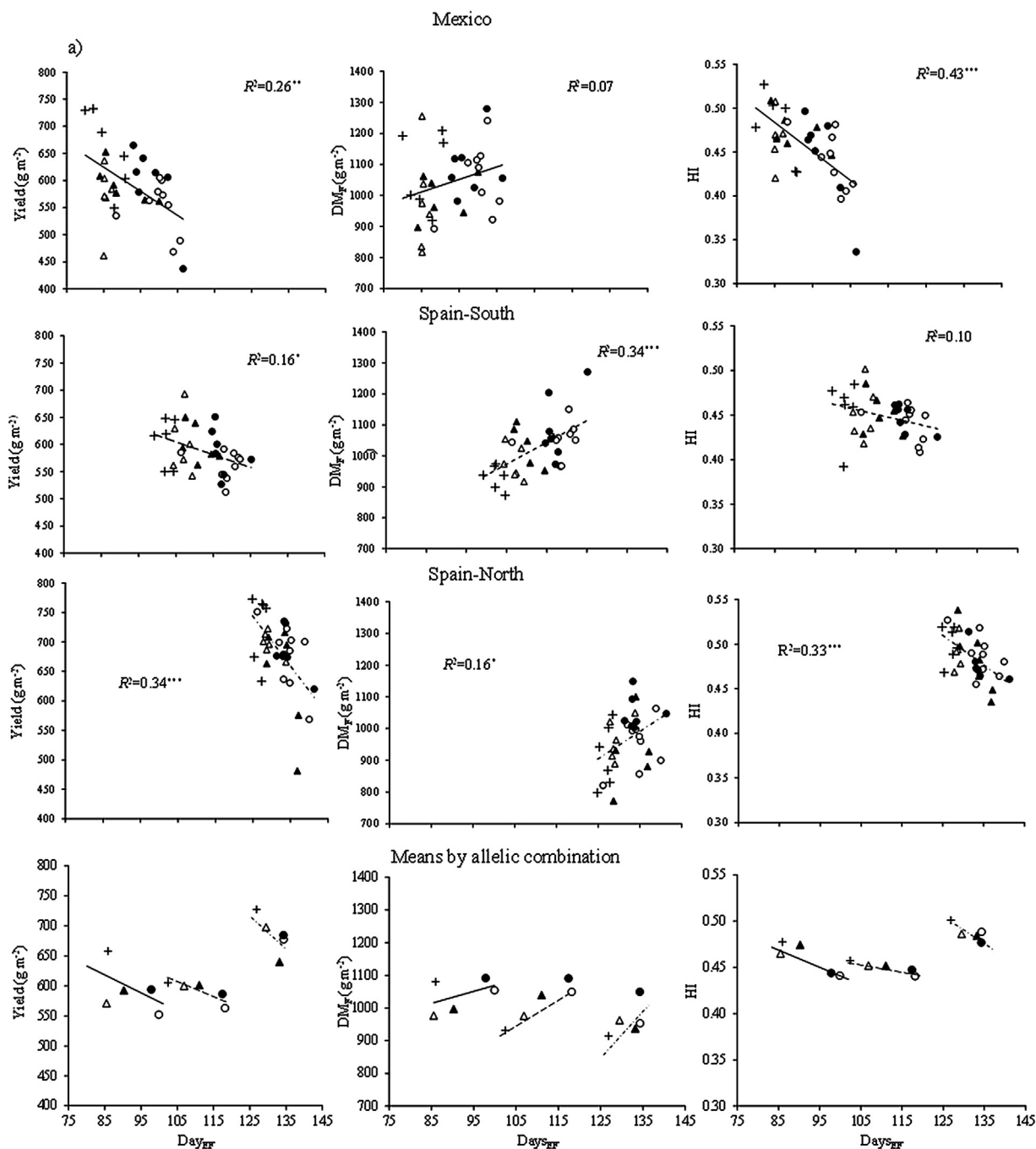Means within columns with the same superscript latin letters are not significantly different ar $P < 0.05$

Fig. 2. Relationships between days from emergence to flowering (Days$_{EF}$) and: a) yield, b) dry matter at flowering (DM$_F$), and c) harvest index (HI) in field experiments conducted during two years in Mexico (— continuous line), Spain-South (- -), and Spain-North (-.-.-), involving 34 durum wheat genotypes grouped according to their allelic combinations at *Ppd-A1* and *Ppd-B1*. Symbols in upper figures (n = 34) represent mean data by genotype at each site and in the bottom figures (n = 5) they represent mean data by allelic combination for the three sites in the same figure. Allelic combinations are represented according to the acronyms shown in Table 1 as: ● = SS, ○ = SI, ▲ = I5S, △ = I5I, + = I0I. * P < 0.05 ** P < 0.01 *** P < 0.001.

negative relationship between DMP and the latter variables. Principal component 2 was mostly related to mean temperature, radiation and water input in the positive direction (Fig. 5a).

The points representing allelic combinations could be grouped in three clusters corresponding to the three experimental sites (Fig. 5b). The cluster corresponding to Mexico was located in the upper-right part of the graph, in the direction of the eigenvectors for mean temperature and radiation during grain filling, while allelic groups in Spain-North clustered in the opposite side of the figure, close to the eigenvectors for DMP and water input. Allelic combinations for Spain-South occupied an intermediate position, but its location towards the negative part of PC2

suggests a relationship with lower temperature, radiation and water input during grain filling at this site. The location of the points representing the five allelic combinations relative to each other within each cluster showed a quite consistent distribution across sites. The allelic combinations carrying the insensitive allele at both loci (I5I and I0I) were always located on the left side within each cluster in the direction of DMP and Wi, while the allelic combinations carrying at least one sensitive allele were constantly located on the right side within each cluster in the direction of DM$_F$, DMT and CT (Fig. 5b). Points representing the different allelic combinations were much closer to each other in the cluster corresponding to Mexico than in those representing
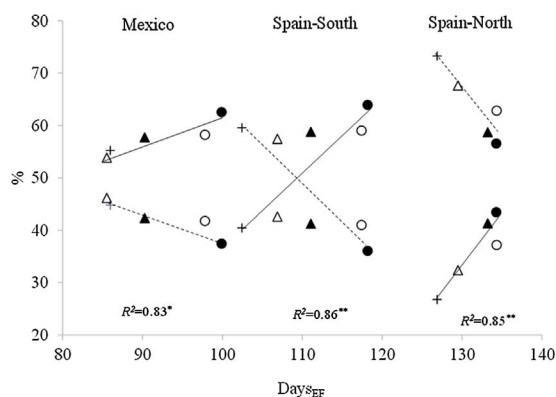
Fig. 3. Relationship between days from emergence to flowering (Days$_{EF}$) and the relative contribution of current photosynthesis during grain filling (- - -) and pre-anthesis assimilates (CT,–– continuous line) to grain yield in three contrasting latitudes. Allelic combinations are represented according to the acronyms shown in Table 1 as ● = SS, ○ = SI, ▲ = I5S, △ = I5I, + = I0I.

either of the Spanish sites.

The relationship between mean temperature from flowering to maturity and HI is shown in Fig. 6a. Genotypes flowering late were exposed to higher mean temperatures during grain filling, presumably causing a reduction in HI and an increase in CT (Fig. 6b).

4. Discussion

The 34 genotypes tested in this study had spring growth habit (non vernalization requiring allele at *Vrn-1*) and included all but one (*GS-100/Ppd-B1b*) of allelic combinations previously reported in durum wheat. A detailed discussion on the effect of *Ppd-1* genes on flowering time and grain filling duration in the collection used in this study can be found in Royo et al. (2016). We herein dissected the effects of five allelic combinations of *Ppd-1* genes on yield and its formation, as consequence of the modification of flowering date. The conceptual physiological framework used in this study considered grain yield as dependent on biomass production and the fraction of it allocated on grains (harvest index), by taking into consideration the relative importance of the two sources of photo-assimilates for grain filling, namely, current photosynthesis after flowering and the remobilization of carbohydrates accumulated prior to flowering.

A previous study showed that the three testing sites used in the current study had contrasting environmental conditions in terms of temperature, day length and solar radiation, with differences between sites being much larger than differences between crop seasons within a site (Villegas et al., 2016). In the current study the site effect was significant for all traits studied, and was particularly relevant for the number of days to flowering, with average differences of 40 days between the earliest (Mexico) and the latest (Spain-North) sites. Based on this observation, the relationships between traits were analysed within each site, individually.

Whereas the genotype effect accounted for only 10% of the total variance of the model for the number of days to flowering, 69% of the genotype-dependent variance was explained by differences between allelic combinations at *Ppd-1* loci. The significant and negative relationship between DAYS$_{EF}$ and yield, and harvest index underline the importance of fitting phenology, via selection of adequate allelic combinations, for durum wheat to maximize both traits in any given environment. However, as substantial variability for HI was observed within allelic combinations there may be various suitable allelic options to choose from in a given environment and, most likely, additional characteristics to consider in order to maximize this yield component. Allelic combination SI (*Ppd-A1b/Ppd-B1a*) showed the largest internal variability for HI and other traits, possibly due to the higher number of genotypes included in this group. The largest percentages of total

variance of the model explained by differences between allelic combinations corresponded to DM in grain produced from current photosynthesis during grain filling (7.4%) and the contribution of pre-flowering assimilates to grain yield (5.9%). These results emphasize the importance of flowering date, as determined in a substantial extent by allelic composition at *Ppd* loci, on the balance between, or the relative importance of, the sources of photosynthates used by the plant to fill its grains.

In all sites yield was reduced with delayed flowering date, with allelic combinations increasing levels of photoperiod sensitivity being characterized by lower yields. As expected, a delay in flowering time increased DM$_F$ by allowing more time for vegetative biomass production. This effect was particularly significant in Spain-South where the number of days to flowering explained 34% of variations in DM$_F$ indicating that late-flowering genotypes drastically increased their biomass at flowering compared with early-flowering ones. However, the negative relationship found between Days$_{EF}$ and HI in Mexico and Spain-North indicates that late flowering genotypes limited biomass allocation to the grains in sites with high temperature and solar radiation during grain filling. In these sites, late flowering genotypes had probably to invest a large part of their energy for transpiration reducing the energy available for accumulating photosynthates in the grain, hence reducing HI. Late flowering resulting in large biomass accumulation with low harvest index, and ultimately reduced yield, has been observed in grain sorghum (Wallace and Yan 1998; Hammer and Broad 2003).

A delay in flowering time had also important effects on the relative contributions of current photosynthesis during grain filling *versus* that of translocation of previously synthetized assimilates to grains. At the three sites, allelic combinations involving photoperiod sensitivity delaying flowering date resulted in an increased relative contribution from 8% to 24% depending on the environment (Fig. 3), of translocation to grain-fill and consequently, a proportional reduction of the role of current photosynthesis. As shown by Fig. 6b this was a direct effect of the increasing temperatures under which late flowering genotypes had to fill their grains (Wahid et al., 2007). However, large site-related differences were observed. In Mexico, a site characterized by the shortest-cycle with an average of 92 days from emergence to flowering, the remobilization of pre-flowering assimilates contributed from 55% to 63% to fill the grains depending on the allelic combination (Fig. 3), thus showing that DMT was the main source for grain filling, independently of the flowering date of the genotype and therefore of the allelic combination at *Ppd* loci. Principal component analysis indicated that, in Mexico, temperature and radiation were very high during grain filling, which stimulated the relocation of assimilate reserves to the developing grains (Palta et al., 1994; Yang et al., 2000), thereby making pre-flowering assimilates to be the main source of carbohydrates for grain filling. This result is in line with those reported by Reynolds et al. (2007) for bread wheat grown in heat stressed environments, attributing yield superiority to an optimization of the remobilization of stem carbohydrates to the grain. It has been reported that translocation mitigates the effects of hastened leaf senescence (Plaut et al., 2004; Ehdaie et al., 2006). However, the fact that in Mexico, DMP explained 45% of yield variations while DMT only explained 16%, suggest that the limiting factor for attaining high yields at this site was the capacity of the plant canopy to photosynthesize after anthesis. It is well known that the principal reasons for yield losses caused by terminal drought are reduced rates of net photosynthesis owing to metabolic limitations and oxidative damage to chloroplasts, stomata closure and poor grain set and development (Farooq et al., 2014). For this reason, water soluble carbohydrates (WSC) accumulated primarily in leaves and stems (culm and leaf sheaths) prior to flowering, have been considered a fundamental physiological trait indicative of drought tolerance. Water soluble carbohydrates act not only as the principal (or even the only) dominant carbon source for filling the grains when active photosynthesis is inhibited, as discussed above, but also support canopy and grain
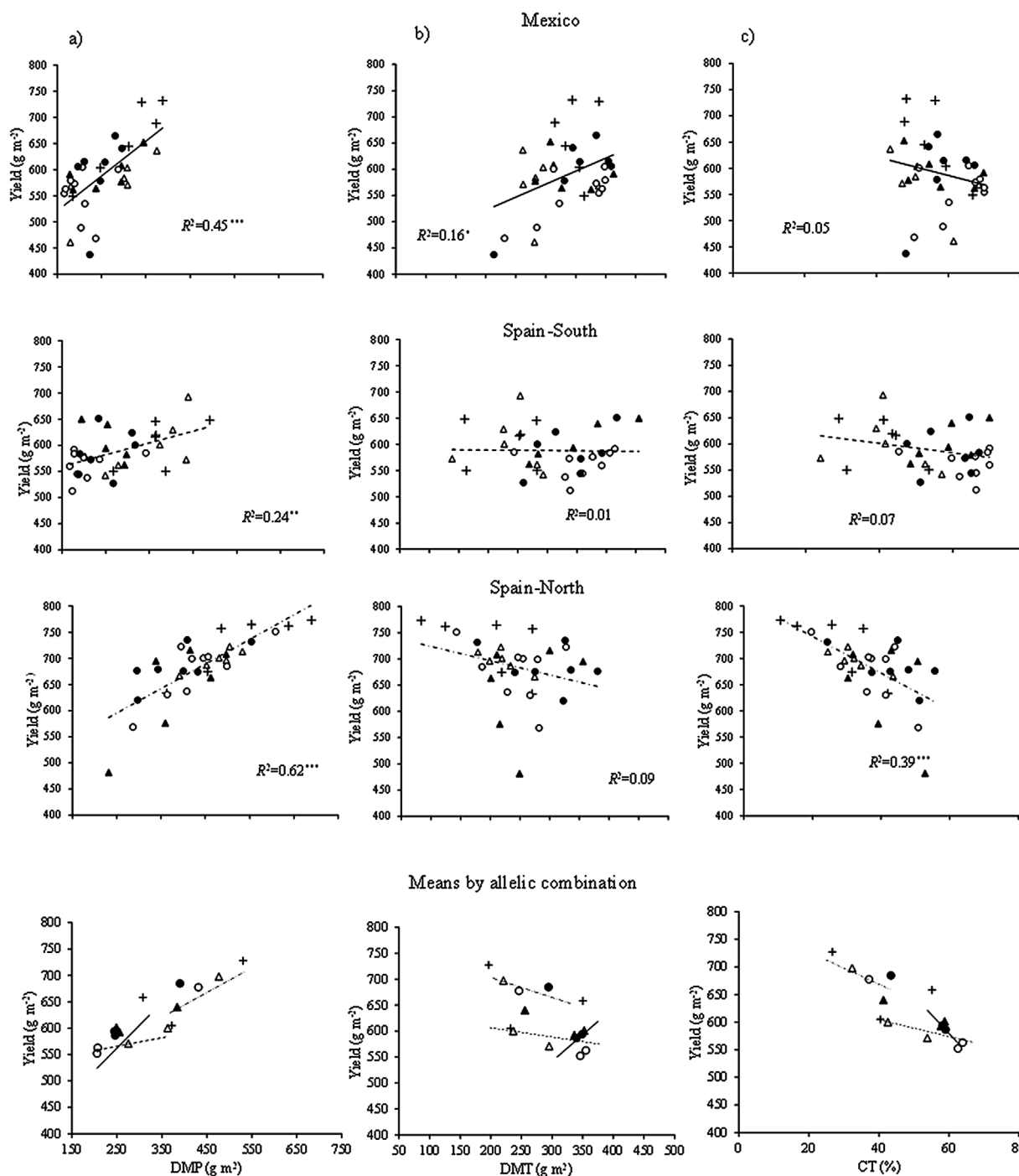
Fig. 4. Relationship between yield and: a) dry matter in grain from current photosynthesis during grain filling (DMP), b) dry matter from translocation (DMT), and c) contribution of pre-flowering assimilates to grain yield (CT) in field experiments conducted during two years in Mexico (— continuous line), Spain-South (- -), and Spain-North (-.-.-) involving 34 durum wheat genotypes grouped according to their allelic combinations at *Ppd-A1* and *Ppd-B1*. Symbols in upper figures (n = 34) represent mean data by genotype at each site and in the bottom figures (n = 5) they represent mean data by allelic combination for the three sites in the same figure. Allelic combinations are represented according to the acronyms shown in Table 1 as: ● = SS, ○ = SI, ▲ = I5S, △ = I5I, + = I0I. * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$.

respiration under water deficit. Moreover, WSC play a significant contribution to osmotic regulation under terminal drought, acting as compatible osmolytes, thus helping to maintain turgor and delaying senescence of photosynthetically active organs under terminal drought (Blum 1998; Ehdaie et al., 2006). Actually, a high remobilization efficiency of stem WSC (mainly fructans of low molecular weight) could contribute to high water use efficiency (Passioura, 2012; Zhang et al., 2015) and may also help to resistance and recovery mechanisms under abiotic stresses (Valluru and Vanden Ende, 2008; Livingston and

Hincha, 2009).

On the other hand, in Spain-North, the site characterized by the largest water input after flowering and with an average of 132 days from emergence to flowering, current photosynthesis was the most important contributor to yield formation, across phenology and allelic combinations at *Ppd-1* loci. Dry matter accumulated in the grain via current photosynthesis during grain-fill was positively and significantly associated with yield, explaining 62% of yield variations at this site. These results indicated that the high yields obtained in Spain-North
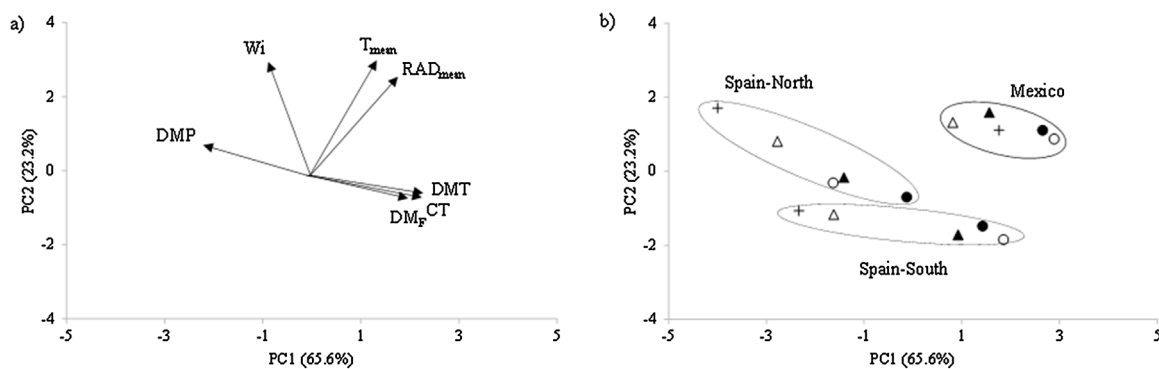
Fig. 5. Principal component analysis biplot. a) Eigenvalues of the correlation matrix symbolized as vectors representing daily mean temperature (T_{mean}), daily mean solar radiation (RAD_{mean}) and water input (Wi) from flowering to maturity, dry matter at flowering (DM_F), dry matter in grain from current photosynthesis during grain filling (DMP), dry matter from translocation (DMT) and the contribution of pre-flowering assimilates to grain yield (CT). b) Points plotted on the plane determined by the first two axes for durum wheat genotypes grouped according to their allelic combinations at *Ppd-A1* and *Ppd-B1* in the three sites: ● = SS, ○ = SI, ▲ = I5S, △ = I5I, + = I0I. See Table 1 for acronyms interpretation.

were a consequence of the large amount of photosynthates coming from photosynthesis, which were largest in genotypes carrying the earliest flowering allelic combination I0I, which were those filling their grains under the most favourable environmental conditions (before onset of water limitations or temperature stress). In this site CT was greater in late flowering genotypes, but this increase resulted in yield decreases as it did not compensate for the reduction on current photosynthesis caused by increasing temperatures and reduction in water supply as shown by Fig. 5 and 6. This is in agreement with the accepted assumption that an increased photosynthesis would result in increased yields, provided that other constraints are not limiting (Parry et al., 2011), and with the relationship reported between the production of assimilates from photosynthesis and wheat harvest index (Araus et al., 2002; Parry et al., 2011).

At the intermediate site (Spain-South) with an average of 111 days from emergence to flower (ranging from 102 to 117 days), a cross-over of the linear regression lines corresponding to DM translocation and current photosynthesis was observed. At this site, genotypes carrying alleles conferring photoperiod insensitivity at both loci, the earliest flowering ones, filled their grains primarily from current photosynthesis. In contrast, for genotypes with late flowering, due to the presence of allele *Ppd-A1b,* DM translocation was much more important than current photosynthesis as the source of photosynthates for grain filling. These results indicated that under the environmental conditions of the south of Spain, where *Ppd-1* genes generated differences in flowering date were maximized, flowering date and therefore allelic composition at *Ppd-1* loci played an essential role in the relative contribution of current photosynthesis *versus* that of DM translocation for grain filling. Late flowering genotypes had more biomass at flowering than the earliest ones, but filled their grains under warmer temperatures, thus increasing the relative contribution of translocation to grains, which although it did not reduce HI, resulted in yields decreases. The more favourable environmental conditions during grain filling in Spain-south compared with those recorded in Mexico and Spain-North give a reasonable explanation to the smooth effect of delaying flowering time on HI and yield at this site. However, as in the other two sites the contribution to photosynthesis to grain filling was the limiting factor for attaining high yields.

5. Concluding remarks

The results of this study revealed that *Ppd-A1* and *Ppd-B1* genes regulating the photoperiod response in durum wheat had, through their dominating effect on time to flowering, important consequences on the plant strategy to fill its grains, produce high harvest index and ultimately achieve high yield. Early flowering genotypes, with the lowest levels of photoperiod sensitivity, were the most consistently high

yielding in all three environments tested. Whereas late flowering, induced by photoperiod sensitivity due to the presence of alleles *Ppd-A1b* and *Ppd-B1b*, resulted in more dry matter accumulated at flowering in the Spanish environments, it did not confer the crop any advantage in terms of grain yield. The contribution to grain filling of current photosynthesis after flowering was enhanced in genotypes carrying photoperiod insensitive alleles *Ppd-A1a* and *Ppd-B1a*, particularly when they were present at both loci. This resulted in the earliest genotypes showing a tendency to have superior HI and yield.
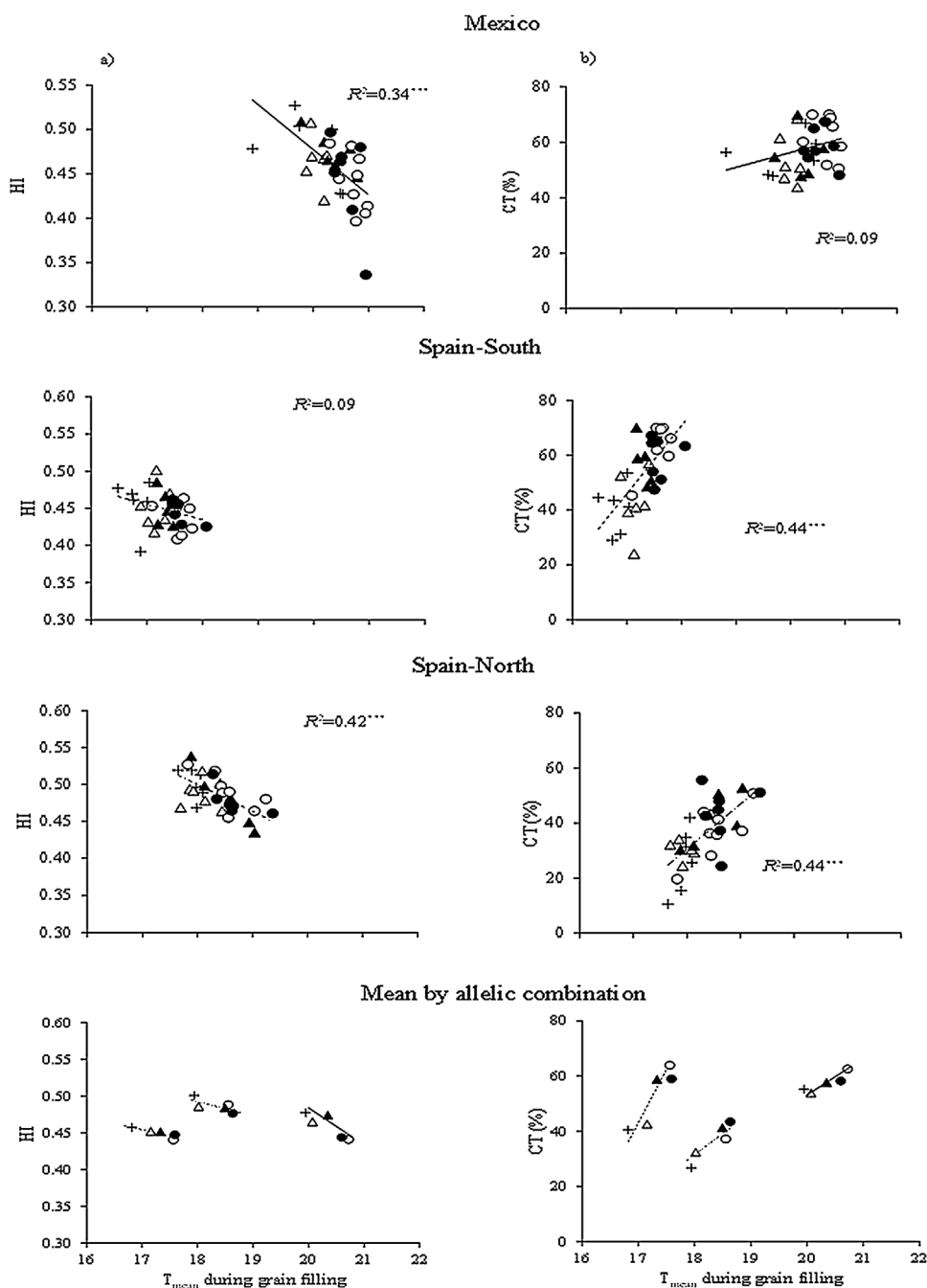
The contribution of pre-flowering assimilates to grain filling increased steadily as flowerings date was delayed as determined by an increasing number of photoperiod sensitivity alleles. This increase in the relative contribution of dry matter translocation to yield strongly was site- dependent and related with the average temperature during grain filling, which, in turn, caused a decrease in HI and a reduction in yield.

This study suggests that the expression of genes *Ppd-A1* and *Ppd-B1* regulating the response to photoperiod modulates the physiological strategy adopted by durum wheat to fill its grains. The results presented underline the importance of selecting the right allelic combination at *Ppd-1* loci to target the crop phenology and the resulting relative contribution to grain-fill of current photosynthesis during grain filling *versus* dry matter translocation in a manner that maximizes grain yield. The results of this study provide genetic and physiological bases underlying the widespread success of photoperiod insensitive germplasm developed by CIMMYT for many parts of the world (Borlaug, 1995).

Fig. 6. Relationship between: a) mean temperature during grain filling and harvest index (HI), and b) mean temperature during grain filling and the contribution of pre-flowering assimilates to grain yield (CT) in field experiments conducted during two years in Mexico (— continuous line), Spain-South (- -), and Spain-North (-.-.-) involving 34 durum wheat genotypes grouped according to their allelic combinations at *Ppd-A1* and *Ppd-B1*. Symbols in upper figures (n = 34) represent mean data by genotype at each site and in the bottom figures (n = 5) they represent mean data by allelic combination for the three sites in the same figure. Allelic combinations are represented according to the acronyms shown in Table 1 as: ● = SS, ○ = SI, ▲ = I5S, △ = I5I, + = I0I. * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fcr.2017.06.005.

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