HEAT STRESS

Effect of allele combinations at Ppd-1 loci on durum wheat grain filling at contrasting latitudes

Jose M. Arjona1 | Conxita Royo1 | Susanne Dreisigacker2 | Karim Ammar2 | Joan Subirà1 | Dolors Villegas1

1Sustainable Field Crops Programme, Institute for Food and Agricultural Research and Technology (IRTA), Lleida, Spain
2International Maize and Wheat Improvement Centre (CIMMYT), Mexico City, Mexico

Correspondence
Dolors Villegas, Sustainable Field Crops Programme, Institute for Food and Agricultural Research and Technology (IRTA), Lleida, Spain. Email: dolors.villegas@irta.cat

Funding information
INIA Spain, Grant/Award Number: RTA2015-00038, RTA2009-00085 and FPI2014-0022; Spanish Ministry of Economy and Competitiveness, Grant/Award Number: AGL2006-09226-C2-01; European Regional Development Fund (ERDF-FEDER); Generalitat de Catalunya (CERCA programme)

Abstract
Flowering time is the most critical developmental stage in wheat, as it determines environmental conditions during grain filling. Thirty-five spring durum genotypes carrying all known allele variants at Ppd-1 loci were evaluated in fully irrigated field experiments for three years at latitudes of 41°N (Spain), 27°N (northern Mexico) and 19°N (southern Mexico). Relationships between weight of central grains of main spikes (W) and thermal time from flowering to maturity were described by a logistic equation. Differences in flowering time between the allele combination causing the earliest (GS100/Ppd-B1a) and the latest (Ppd-A1b/Ppd-B1a) flowering were 7, 20 and 18 days in Spain, northern Mexico and southern Mexico, respectively. Flowering delay drastically reduced the mean grain filling rate (R) and W at all sites. At autumn-sowing sites, an increase of 1°C in mean temperature during the first half of the grain filling period decreased W by 5.2 mg per grain. At these sites, W was strongly dependent on R. At the spring-sowing site (southern Mexico), W depended on both R and grain filling duration. Our results suggest that incorporating the allele combinations GS100/Ppd-B1a and GS105/Ppd-B1a (alleles conferring photoperiod insensitivity) in newly released varieties can reduce the negative effects of climate change on grain filling at the studied latitudes.

KEYWORDS
flowering time, grain filling rate, grain weight, photoperiod sensitivity, solar radiation, temperature

1 | INTRODUCTION

Wheat is one of the staple foods of humankind, with global consumption during the last ten years reaching around 700 million tons per year. About 10% of total wheat production corresponds to durum wheat (Triticum turgidum L. var. durum) (Kantety, Diab, & Sorrells, 2005). Though a record wheat production was achieved in 2018, the forecast for 2019 suggests that use will exceed production (FAO, 2018). In most wheat-growing regions, around 36% of the annual variation in grain yield can be explained by climate changes (Ray, Gerber, MacDonald, & West, 2015). The mean temperature of the Earth’s surface has increased by between 0.8 and 1.2°C since the second half of the 18th century, and climate change models predict a mean increase of 0.2°C per decade in the next century (Allen et al., 2018). It has been estimated that an increase of 1°C could reduce wheat production by 6% (Asseng et al., 2015), so a decrease in wheat
TABLE 1  Allele combinations for Ppd-A1 and Ppd-B1 loci present in the collection of 35 durum wheat genotypes used in the current study, acronyms used and frequencies within the collection

| Allele combination acronym | Number of genotypes | Ppd-A1 | Photoperiod response | Ppd-B1 | Photoperiod response |
|----------------------------|---------------------|--------|----------------------|--------|----------------------|
| I0I                        | 5                   | GS-100/Ppd-A1a | Insensitive           | Ppd-B1a | Insensitive           |
| I5I                        | 7                   | GS-105/Ppd-A1a | Insensitive           | Ppd-B1a | Insensitive           |
| ISS                        | 10                  | GS-105/Ppd-A1a | Insensitive           | Ppd-B1b | Sensitive            |
| SI                         | 5                   | Ppd-A1b     | Sensitive             | Ppd-B1a | Insensitive           |
| SS                         | 8                   | Ppd-A1b     | Sensitive             | Ppd-B1b | Sensitive            |

*Nomenclature described in Wilhelm et al. (2009).*

stocks is expected in the future. Continuous efforts in crop and specifically yield improvement are therefore required (FAO, 2018).

Grain number per unit land and grain weight are the main components of wheat yield. Grain weight is not only an essential yield component but also an important quality trait that interacts with other quality standards, such as protein content and yellowness, which are usually negatively correlated with grain weight (Raharabiti, Villegas, Royo, Martins-Núñez, & García del Moral, 2003). Grain weight is also highly correlated with flour and semolina yield, bigger grains having higher milling yields per kg of grain than smaller grains (Baasandorj, Ohm, Manthey, & Simsek, 2015; Matsuo & Dexter, 1980).

In the context of climate change, yield reductions will be led by a significant decrease in one or both yield components. Reductions in grain number per unit land area due to an increase in temperature have been widely reported, as has a reduction in grain weight, which depends on the conditions before flowering and during grain filling (Bergkamp, Impa, Asebedo, Fritz, & Jagadish, 2018; Ferris, Ellis, Wheeler, & Hadley, 1998; Hlaváčová et al., 2018; Prasad, Pisipati, Momčilović, & Ristic, 2011; Terrile, Miralles, & González, 2017; Ugarte, Calderini, & Slifer, 2007). Although the environmental conditions in the pre-flowering period can have an effect on grain weight (Ugarte et al., 2007), the grain filling period is considered critical for the final grain weight (Royo et al., 2006). The two components of the grain filling period are the mean rate of grain filling (R) and the grain filling duration. Weather conditions such as drought and heat stress can modify the duration and the rate of grain filling. Crop senescence is usually accelerated and the starch accumulation phase is shortened, so R is reduced (Bergkamp et al., 2018; Dias & Lidon, 2009; García, Serrago, Dreccer, & Miralles, 2016; Royo et al., 2006). The effect of heat stress induced either during a short period of time or extended throughout the grain filling has been studied under controlled and semi-controlled conditions (Bergkamp et al., 2018; Dias & Lidon, 2009; Shirdelmoghanloo, Cozzolino, Lohraseb, & Collins, 2016). However, field studies analysing the effect of flowering date on grain filling are lacking in durum wheat.

Strategies that could be followed to improve grain filling in wheat under climate change conditions include the development of heat-tolerant varieties and the use of avoidance mechanisms (Shavrukov et al., 2017). The most common among these are (a) adapting sowing dates to allow the crop to fill its grains under favourable environmental conditions (Ortiz-Monasterio, Dhillon, & Fischer, 1994) and (b) adjusting wheat phenology by modifying alleles of major genes responsible for crop development. Flowering time is controlled in wheat by three groups of loci affecting vernalization requirement (VRN), photoperiod sensitivity (Ppd-1) and earliness per se (Eps). Though vernalization genes exert the greatest influence on crop phenology (Kamran, Iqbal, & Spamer, 2014), most cultivated durum wheat has a spring growth habit, so flowering time is controlled by Ppd-1 and Eps genes.

In spring durum wheat, there are two known genes of photoperiod response (Ppd-1, Ppd-A1 and Ppd-B1), located in chromosome 2 of the A and B genomes, respectively (Maccarelli et al., 2008; Wilhelm, Turner, & Laurie, 2009). It has been reported that Ppd-A1 insensitive alleles shorten the pre-flowering phase to a greater extent than the insensitive allele of Ppd-B1 (Ppd-B1a), which in turn shortens pre-flowering time in comparison with the sensitive alleles of both genes at low to medium latitudes (Royo, Dreisigacker, Alfaro, Ammar, & Villegas, 2016). It is also known that Ppd-A1a ‘GS100’ allele has a stronger effect than Ppd-A1a ‘GS105’ (Arjona, Royo, Dreisigacker, Ammar, & Villegas, 2018; Royo et al., 2016; Wilhelm et al., 2009).

The objective of this study was to explore the effect of Ppd-1 genes on durum wheat development and yield formation at a range of northern latitudes. Results regarding the effect of Ppd-1 genes on flowering time (Royo et al., 2016), yield formation (Arjona et al., 2018; Royo et al., 2018) and yield constraints induced by environmental features (Villegas et al., 2016) have been published previously. As the shortening of the pre-flowering phase due to the presence of alleles causing photoperiod insensitivity may modify the environmental conditions after flowering, this study was carried out to examine the effect of allele combinations at Ppd-1 loci on grain filling in durum wheat.

2 MATERIALS AND METHODS

2.1 Plant material

Thirty-five spring durum wheat genotypes were used in this study (Table S1). The genotypes included 5 late-flowering German varieties and inbred lines from the University of Hohenheim, 5...
early-flowering inbred lines from the CIMMYT-Mexico breeding programme, and 25 lines obtained from crosses between a late genotype (used as a female parent) and an early genotype (used as a pollen donor). The set of markers and the methodologies used for the molecular characterization of the collection at \(Vrn-1\) and \(Ppd-1\) loci are described in Royo et al. (2016). The results revealed that the 35 genotypes used in this study were spring types, carrying the dominant allele \(Vrn-A1c\). For \(Ppd-1\) allele combinations, 8 genotypes carried the alleles conferring photoperiod sensitivity and 12 carried the mutations conferring photoperiod insensitivity at both \(Ppd-1\) loci (\(GS100/Ppd-A1a\) and \(Ppd-B1a\); \(GS105/Ppd-A1a\) and \(Ppd-B1a\)). Fifteen genotypes carried the photoperiod-insensitive allele only at one of the two loci (Table 1).

2.2 | Field experiments and phenotypic measures

Nine field experiments were conducted at three sites with contrasting latitude: 41°N (Spain), 27°N (northern Mexico) and 19°N (southern Mexico) (Table 2) during the growing seasons in the years 2010, 2011 and 2012. The experiments consisted of field plots of 12 m\(^2\) size with three replicates, arranged in a randomized complete block design. The plots were kept free of diseases, weeds and pests and were fully irrigated. Field management was conducted according to standard agronomic practices at each site. Sowing density was fitted to obtain an approximate density of 450 spikes/m\(^2\). The six experiments performed in Spain and northern Mexico were autumn-sowing (17 November–23 December), while in southern Mexico the experiments were spring-sowing (17–28 May). Daily maximum, minimum and mean temperatures (°C), as well as solar radiation (MJ/m\(^2\) day\(^{-1}\)), were recorded during the entire crop cycle with meteorological stations located on the field or nearby.

Zadoks, Chang, and Konzak (1974) growth stages 65 (flowering) and 87 (physiological maturity) were determined for each plot. At flowering, up to 60 main spikes in synchronous development and with similar size were tagged in the central part of each plot. On a weekly basis, five tagged spikes were removed at random, and six grains per spike were extracted from the central spikelets of each spike. The grains were oven-dried for 48 hr at 70°C and weighed with a precision scale (Mettler B-2002-S). For each plot, thermal time (growing degree days, GDD) was calculated from flowering to physiological maturity, assuming a base temperature of 9°C and a maximum temperature of 37°C (Weir, Bragg, Porter, & Rayner, 1984).

In each experiment, changes in dry weight per grain were fitted for each individual plot to a logistic model with three parameters (Figure 1), chosen on the basis of previous studies (Robert, Huet, Hennequet, & Bouvier, 1999) and with the modification suggested by Davidian and Giltinan (1995). The model (Equation 1) was fitted with the ‘NLIN’ procedure and the Marquardt method of the SAS software (SAS RRID:SCR_008567, 2009):

\[
GW_i = \frac{W_i}{1 + \exp\{-R_t(x_{ij} - midD)i\}}
\]

where

\(GW_i\) is the weight of the grain for a sample \(i\) at time \(j\);
\(W_i\) is the asymptote of the curve for sample \(i\);
\(R_t\) is the factor that relates in constant proportion the growing rate and the current size of sample \(i\);
\(x_{ij}\) are the growing degree days of sample \(i\) at time \(j\); and
midD, is the value of growing degree days at the inflection point of the curve (mid-point of duration of the grain filling).

Final grain weight (W) was estimated in mg. Grain filling duration (D95) was considered to be the thermal time (GDD) required for grain weight to reach 0.95 W. The mean rate of grain filling (R, mg GDD⁻¹) was calculated as $R = \frac{W}{D}$.

2.3 | Statistical analyses

Combined ANOVA across sites (latitudes), experiments, years and genotypes were performed using a fixed model to analyse the number of days from emergence to flowering and from flowering to physiological maturity, as well as the mean temperature and solar radiation from flowering to midD. The genotype effect was partitioned into differences between allele combinations at the Ppd-1 loci and differences between genotypes within each allele combination. This last factor was considered as the error term used to test differences between allele combinations (SAS Institute Inc., 2010). Multivariate analysis of variance (MANOVA) was used to analyse W, R and D95 to deal with the association between variables. The GLM procedure of the SAS software (SAS RRID:SCR_008567, 2009) was used for these analyses, and the Wilks lambda ($\lambda$) values and the log $P$ for the $F$-values were obtained. Means of allele combinations were compared using the protected Fisher’s least significant differences method at $P = .05$. A photo-thermal ratio was calculated at each site for the first part of the grain filling period (flowering to midD) as the ratio between solar radiation and temperature (MJ m⁻² day⁻¹°C⁻¹). Linear regression equations were used to study the relationships between variables at each site (JMP RRID:SCR_008567 14242, 2007).

3 | RESULTS

3.1 | Phenology

The ANOVA for the number of days from emergence to flowering revealed that all factors in the analysis were statistically significant, but the site effect explained most of the variation of the model (84.7%), followed by the site × year interaction (6.1%) and the genotype (4.4%) effect (Table 3). Differences between allele combinations accounted for 58.5% of the variation induced by the genotype and 2.6% of the total variation of the model, while the site × allele combination interaction explained 36% of the site × genotype interaction (Table 3).

On average across sites and years, the number of days from emergence to flowering ranged from 90 for the allele combination I0I to 105 for the combination SI (Table 4). The same pattern of flowering delay derived from photoperiod-sensitive alleles was observed at each site. Differences in the number of days to flowering between the allele combinations showing the earliest and the latest flowering dates were 7 days in Spain, 20 days in northern Mexico and 18 days in southern Mexico. At all sites, allele combinations I0I, I5I and I5S led to similar earlier flowering dates in comparison with allele combinations SS and SI. Only in southern Mexico were flowering dates of allele combinations SI and SS significantly different (Table 4).
**TABLE 4** Mean values for each allele combination at Ppd-1 across sites and at each site for days from emergence to flowering, final grain weight (W), mean rate of grain filling (R), thermal time from flowering to 95% W (D95), days from flowering to 95% W, mean temperature and mean solar radiation from flowering to mid-grain filling duration (midD). See Table 1 for acronym list.

| Ppd-1 allele combination | Days emergence to flowering | W (mg) | R (mg GDD⁻¹) | D95 (GDD) | Days to D95 | Mean temperature from flowering to midD (°C) | Mean solar radiation from flowering to midD (MJ m⁻² day⁻¹) |
|--------------------------|----------------------------|--------|--------------|-----------|------------|-------------------------------------------|--------------------------------------------------|
| I0I                      | 90 b                       | 55.7 a | 0.140 a      | 366 a     | 41.5 a     | 17.2 b                                    | 23.5 b                                           |
| I5I                      | 94 b                       | 55.0 a | 0.139 a      | 362 a     | 40.6 a     | 17.5 b                                    | 23.7 b                                           |
| I5S                      | 94 b                       | 51.3 a | 0.132 a      | 362 a     | 40.2 a     | 17.5 b                                    | 23.8 b                                           |
| SS                       | 100 a                      | 49.8 a | 0.125 a      | 367 a     | 39.5 ab    | 18.0 a                                    | 24.2 a                                           |
| SI                       | 105 a                      | 46.7 a | 0.120 a      | 358 a     | 37.9 b     | 18.4 a                                    | 24.3 a                                           |
| Ppd-1 × site interaction |                           |        |              |           |            |                                           |                                                  |
| Spain                    |                            |        |              |           |            |                                           |                                                  |
| I0I                      | 132 b                      | 55.3 a | 0.150 a      | 350 a     | 35.0 a     | 17.6 d                                    | 25.9 c                                           |
| I5I                      | 134 b                      | 55.9 a | 0.152 a      | 345 a     | 33.7 a     | 18.2 cd                                   | 26.3 b                                           |
| I5S                      | 135 b                      | 54.2 a | 0.150 a      | 345 a     | 33.6 a     | 18.2 bc                                   | 26.3 b                                           |
| SS                       | 138 a                      | 53.0 a | 0.139 a      | 360 a     | 34.3 a     | 18.8 ab                                   | 26.6 a                                           |
| SI                       | 139 a                      | 51.5 a | 0.134 a      | 360 a     | 33.9 a     | 19.3 a                                    | 26.8 a                                           |
| Northern Mexico          |                            |        |              |           |            |                                           |                                                  |
| I0I                      | 81 b                       | 59.9 a | 0.140 a      | 388 a     | 41.7 a     | 17.5 b                                    | 24.2 b                                           |
| I5I                      | 85 b                       | 59.3 a | 0.139 ab     | 386 a     | 40.1 ab    | 17.8 b                                    | 24.5 b                                           |
| I5S                      | 87 b                       | 54.6 abc| 0.129 abc    | 388 a     | 39.7 ab    | 17.9 b                                    | 24.8 b                                           |
| SS                       | 95 a                       | 52.7 bc| 0.124 bc     | 395 a     | 37.4 bc    | 18.7 a                                    | 25.9 a                                           |
| SI                       | 101 a                      | 49.5 c | 0.116 c      | 395 a     | 35.1 c     | 19.2 a                                    | 26.6 a                                           |
| Southern Mexico          |                            |        |              |           |            |                                           |                                                  |
| I0I                      | 58 c                       | 51.9 a | 0.129 a      | 361 a     | 48.0 a     | 16.4 a                                    | 20.5 a                                           |
| I5I                      | 62 bc                      | 49.8 ab| 0.127 a      | 356 a     | 48.0 a     | 16.4 a                                    | 20.3 a                                           |
| I5S                      | 62 bc                      | 45.1 bc| 0.117 a      | 353 a     | 47.3 a     | 16.5 a                                    | 20.3 a                                           |
| SS                       | 67 b                       | 43.7 bc| 0.113 a      | 347 a     | 46.6 a     | 16.5 a                                    | 20.1 a                                           |
| SI                       | 76 a                       | 39.4 c | 0.110 a      | 321 a     | 44.6 a     | 16.6 a                                    | 19.6 a                                           |

*Note: Different letters within columns and sites indicate significant differences according to protected Fisher’s least significant difference at p = .05.*
3.2 | Grain filling curve coefficients

The results of MANOVA showed that although all effects and interactions were statistically significant, the site, year and genotype effects and the site × year interaction resulted in a p-value close to zero (Table 3). Mean values of the allele combinations across sites and years showed no significant statistical differences for W, R or D95 (Table 4). However, differences between allele combinations were significant in northern Mexico for W and R and in southern Mexico for W, with the allele combinations leading to an earlier flowering date showing higher values for both coefficients. All allele combinations led to a similar D95 at all sites (Table 4).

3.3 | Relationships between traits

Exploring the relationships between flowering time and the coefficients in the grain filling curve revealed that flowering time accounted for 40%–56% of W variations depending on the site (Table 5). The values of the slopes of the regression equations fitted to these relationships indicated that each day of delay in flowering resulted in a decrease of 0.57 mg per grain in southern Mexico and 0.95 mg per grain in Spain. In northern Mexico and Spain, this was due to a significant reduction in R, as D95 was not significantly affected by flowering time. However, in southern Mexico both W and R were significantly reduced when flowering date was delayed (Table 5).

Variations in R explained 75%–84% of W, depending on the site. Grain filling duration had no effect on W at the two autumn-sowed sites, but a longer grain filling period significantly increased W in southern Mexico (Table 5).

3.4 | Allele combinations and associated environmental conditions during grain filling

The ANOVA revealed that the allele combination affected flowering time and thus the mean temperature and solar radiation during the first half of the grain filling period of the crop (Table 4). On average across sites and years, genotypes carrying allele combinations SS and SI received higher temperatures and solar radiation levels during the first part of the grain filling period than genotypes carrying allele combinations 10I, 15I and 15S. Though this tendency was observed at the two autumn-sowing sites, it was not observed in southern Mexico, where allele combinations did not significantly affect temperature or solar radiation to midD (Table 4).

To further explore the influence of flowering time on the shift of temperature and solar radiation during the first half of the grain filling period, regression models were fitted for each site to the relationships between them, and the same methodology was used subsequently to analyse the effect of the two environmental variables on R and W. The results showed that, in Spain and northern Mexico, flowering delay increased significantly the temperature and solar radiation to midD and reduced R and W drastically (Figures 2 and 3). The slopes of the regression equations showed that each day of delay in flowering time caused an increase in the mean temperature.
ARJONA et al.

During the first half of the grain filling period of 0.18°C in Spain and 0.09°C in northern Mexico (Figure 2a). Moreover, an increase of 1°C in this period caused a decrease in R of 0.014 mg GDD-1 at both sites (Figure 2b) and a decrease in W of 4.14 mg per grain in Spain and 6.35 mg per grain in northern Mexico (Figure 2c). In southern Mexico, genotypes consistently experienced the lowest temperatures during midD. At this site, a delay in flowering time did not always cause a clear pattern of temperature increase. However, mean data across years revealed a temperature increase of about 0.01°C per day (Figure 2a). The effect of this temperature increase was also yeardependent, but on average it was associated with higher decreases of R and W than in the other two sites. However, the model was not as explanatory as in Spain and northern Mexico, with a worse R² value (Figure 2b,c).

A longer pre-flowering period significantly increased the solar radiation during midD, which had a similar effect as temperature on reducing R and W in both Spain and northern Mexico (Figure 3a–c). In southern Mexico, the effect of flowering delay on solar radiation depended on the year. However, data across years showed significant increases in R and W as solar radiation increased (Figure 3b,c).

Given that both temperature and solar radiation significantly affected R and W at all sites, we tried to ascertain which of them had the greatest effect at each site. For this purpose, the relationship between the photo-thermal ratio during midD and W was calculated at each site with the mean values of each allele combination across genotypes and years. The results showed that the relationships were significant and positive in Spain and southern Mexico, with the allele combinations causing a regular decrease in the photo-thermal ratio associated with a delay in flowering time (Figure 4). At these two sites, the photo-thermal coefficient increased steadily for genotypes with allele combinations SI to I0I, but in northern Mexico, it was similar for all five combinations (Figure 4).
DISCUSSION

It has been demonstrated that Ppd-1 genes have a significant influence on flowering time (Royo et al., 2016). Early and late genotypes could be expected to experience different weather conditions close to flowering and during the grain filling period, particularly in environments where springs have an increasing pattern of temperature. Under this assumption, nine experiments were carried out at three contrasting latitudes for three years with the aim of quantifying the effect of allelic combination for Ppd-1 (Ppd-A1 and Ppd-B1) on grain filling traits and final grain weight.

Two important aspects must be considered when interpreting the results of the current study. First, drought stress was avoided in our experiments, so the impact of temperature and solar radiation on grain filling traits was not associated with water scarcity, as generally occurs in many environments such as the Mediterranean (Royo, Nazco, & Villegas, 2014). Second, it has been reported that grains from the lower and upper parts of main spikes and from spikes at tillers are more affected by temperature than grains from the centre of the main spikes (Tashiro & Wardlaw, 1990). Therefore, the effect of the allele combinations on W described here could underestimate the average grain weight corresponding to all grains and spikes of crop canopies.

The environmental effect on the coefficients of the grain filling curve observed in this study was a consequence of the contrasting latitudes and weather conditions at the experimental sites, such as day-length and temperature during the grain filling period (Villegas et al., 2016). As reported previously, allele variants that cause photoperiod insensitivity exert a significant effect on flowering time (Royo et al., 2016). When we compared the mean values of five allele combinations across sites and years, we observed no significant effect on W, R or D95. The lack of statistical significance was assumed to be due to the great annual variability. Consistent and negative correlations were found between the days from emergence to flowering and W and R at the three experimental sites, thus indicating that a delay in flowering time significantly reduced R and W. Although the

![Figure 3](image-url)

**Figure 3** Relationships between (a) days from emergence to flowering and mean radiation (Rad mean) from flowering to mid-grain filling (midD), (b) Rad mean from flowering to midD and mean grain filling rate (R) and (c) Rad mean from flowering to midD and final grain weight (W) in field experiments conducted in Spain (— continuous line), northern Mexico (---) and southern Mexico (--), involving 35 durum wheat genotypes grouped according to their allele combination at Ppd-A1 and Ppd-B1 loci. Allele combinations are represented according to the acronyms shown in Table 1 as △ = I0I, □ = I5I, ■ = I5S, ● = SS, ○ = SI. * p < .05, ** p < .01, *** p < .001

4 | DISCUSSION
FIGURE 4  Relationship between the photo-thermal ratio from flowering to mid-grain filling (midD) and final grain weight (W) in field experiments conducted in Spain, northern Mexico and southern Mexico, involving durum wheat genotypes grouped in five allele combinations at Ppd-A1 and Ppd-B1 loci. Allele combinations are represented according to the acronyms shown in Table 1 as △ = I0I, □ = I5I, ■ = I5S, ● = SS, ○ = SI. * p < .05, ** p < .01, *** p < .001.

differences between allele combinations were not significant for W and R in Spain, or for R in southern Mexico, the tendency was the same at all three sites and across sites. The differences in days to flowering between the allele combinations causing the earliest (I0I) and the latest flowering date (SI) were 7, 20 and 18 days in Spain, northern Mexico and southern Mexico, respectively. The flowering time delay resulted in decreases in R of 10.7%, 17.1% and 14.7% in Spain, northern Mexico and southern Mexico, respectively, and decreases in W of 6.8%, 17.4% and 24.1% at the same sites. Our results indicated that the effect of the allele combination on flowering time differed between sites, but at all sites the flowering delay reduced R and W, although with different intensity, so the site × allele combination interaction was quantitative in nature for the two traits.

On the other hand, D95 measured in thermal time was not affected by flowering time in Spain and northern Mexico, as only small increases in D95 (≤2.8%) were caused by a flowering delay at these two sites. However, in southern Mexico each day of flowering delay reduced D95 by 2.19 GDD. The positive and significant relationship between D95 and W found at this site reveals that the short grain filling period of spring planting in southern Mexico constrained the achievement of high grain weight. The analyses of the relationships between W and its components, R and D95, showed that W strongly depended on R in Spain and northern Mexico, but in southern Mexico the two components were important for final grain weight, though R was more important.

The relationship between flowering time, temperature and solar radiation that occurred during the first half of the grain filling period showed clear differences between the two sowing times. In Spain and northern Mexico, where sowing was carried out in autumn, both temperature and solar radiation increased significantly after flowering, and these increases significantly reduced R and W in the late-flowering genotypes. At the spring-sowing site in southern Mexico, the effect of flowering delay on temperature and solar radiation depended strongly on the year, as it coincided with the rainy season. However, on average, a slight increase in temperature after flowering also caused reductions in R and W in the late-flowering genotypes. The negative effect of high temperatures on grain weight has been previously reported in wheat, either durum (Ferrise, Triossi, Stratonovitch, Bindi, & Martre, 2010) or bread wheat (Gibson & Paulsen, 1999; Ortiz-Monasterio et al., 1994; Shirdelmoghanloo et al., 2016; Tashiro & Wardlaw, 1990; Thomason et al., 2018). The effect of temperature on grain development has been deeply studied in bread wheat. Lower grain weight has been attributed to a shorter grain filling period (Bergkamp et al., 2018; García et al., 2016) and to both shorter grain filling periods and lower grain filling rates (Liu et al., 2016). Previous studies conducted in bread and durum wheat also reported lower grain filling rates as a consequence of temperature rises after flowering when grain filling rate was measured in mg GDD⁻¹ (Dias & Lidon, 2009; Liu et al., 2016). However, increased grain filling rates were described when measured in mg day⁻¹ (García et al., 2016; Shirdelmoghanloo et al., 2016). The lower R could be due to temperature effects on starch enzymes, stability of membranes and photosynthetic activity (Jener, 1994; Keeling, Banisadr, Barone, Wasserman, & Singletary, 1994; Thomason et al., 2018). The fact that our results agree with the reported by studies conducted on bread wheat indicates that the effect of temperature on grain filling is a general trend for both species. However, the effect of allele combinations presented in this study cannot be translated directly to bread wheat. The absence of the D genome in durum wheat is the main difference, as it has been reported to have the strongest effect on bread wheat development (Beales, Turner, Griffiths, Snape, & Laurie, 2007). While any allele combination leading to earlier flowering time would be desirable both in durum and in bread wheat under the environmental conditions considered in the current study, the specific allele combination would therefore be species-dependent.

The relationship between the photo-thermal ratio and W was useful to understand the relative effect of changes in temperature and solar radiation on final grain weight at each site. In Spain, where both temperature and solar radiation increased after flowering, the photo-thermal ratio decreased significantly when flowering was delayed, suggesting that the increase in temperature was more important for reducing W than the increase in solar radiation. In northern Mexico, this ratio remained stable independently of the flowering date, which indicates that temperature and radiation had a similar impact on reducing W. In southern Mexico, as in Spain, the photo-thermal ratio decreased as flowering was delayed. At this site, both reductions in solar radiation and increases in temperature contributed to the reduction of the photo-thermal ratio, but the greater effect of flowering date on decreasing radiation than on increasing temperature shown by the slopes of the regression models fitted to these relationships suggests that limiting radiation contributed the most to reducing final grain weight at this site. This result is supported by previous studies demonstrating that solar radiation was a limiting factor at the spring-sowing site in southern Mexico (Arjona et al., 2018; Villegas et al., 2016).
The allele combinations I0I and I5I tended to cause the earliest flowering time, hence associated with the most favourable environmental conditions for grain filling and increased W values. However, it has been demonstrated that Ppd-B1a allele, causing photoperiod insensitivity, reduces the number of grains per unit area (Arjona et al., 2018). Therefore, this should be taken into account in sites where increasing grain number would be desirable. This is the case of the southern Mexico site where the high minimum temperatures cause a very low grain number that constrains yield (Villegas et al., 2016).

This study was carried out at three sites with contrasting conditions of photoperiod, temperature and solar radiation. In order to extrapolate the results to other locations worldwide, it is worth mentioning that the northern Mexico site (CENEB in Ciudad Obregón) has been considered representative of high-yielding irrigated sites. On the other hand, the Spain site (Gimenells) has a typical Mediterranean climate and is representative of the Mediterranean regions, where durum wheat is a widely grown crop (Ammar et al., 2008). Broadly, when facing the unfavourable conditions during and after flowering time predicted by climate change models, two different strategies could be considered to avoid crop stress: tolerance and escape. In this study, we focused on the escape strategy: the early-flowering genotypes performed better in terms of grain filling because of more favourable environmental conditions. An earlier flowering time could also be achieved by an earlier sowing time, but too early sowing may also cause yield reductions caused by frost or unfavourable conditions during the growth cycle (Fischer, 2016; Ortiz-Monasterio et al., 1994). Furthermore, changing the sowing date is not always an option for farmers. It may depend on precipitation after a dry summer, an unsuitable temperature regime or a previous crop still to be harvested. The selection of the optimum sowing date for each particular site will be an important crop operation, jointly with the variety selection for each site.

Fine-tuning flowering time for each site by using developmental important genes such as Ppd-1 will become one of the important choices in future farming (Wasson et al., 2012). The results obtained in the current study are in line with predicted declines of grain yield in wheat caused by temperature increases as a consequence of climate change (Asseng et al., 2015; Bergkamp et al., 2018; García et al., 2016; Gibson & Paulsen, 1999; Liu et al., 2016; Vignjevic, Wang, Olesen, & Wollenweber, 2015).

On average, across the two autumn-sowing sites, a temperature increase of 1°C during the first half of the grain filling period resulted in a decrease in the mean rate of grain filling of 0.014 mg GDD⁻¹ and in a reduction of about 5.2 mg per grain, which is about 10% of the average weight of the grains from the central main spikes. In this context, the late-flowering genotypes would be the most damaged by temperature rises during the grain filling period. Our results therefore suggest that incorporating the allele combinations GS100/Ppd-B1a (I0I) and GS105/Ppd-B1a (I5I), which confer photoperiod insensitivity, at the two Ppd-1 loci in newly released varieties could help reduce the negative effects of climate change.

ACKNOWLEDGEMENTS

This work was conducted within the framework of the CIMMYT-INIA agreement under the project ‘Addressing the Challenges for a Sustainable Wheat Production in Spain and North Africa’. Support was in particular received from INIA Spain (projects RTA2015-00038 and RTA2009-00085) and the Spanish Ministry of Economy and Competitiveness (AGL2006-09226-C2-01). J.M. Arjona was a recipient of an FPI grant from INIA and the European Regional Development Fund (ERDF-FEDER). The field experiments in Mexico were supported by PIEAES (Patronato por la Investigación y Experimentación Agrícola del Estado de Sonora) and CIMMYT. Thanks are due to Dr Christian Alfaro for his assistance in the field sampling. The authors also acknowledge the contribution of the CERCA Programme (Generalitat de Catalunya) and special thanks are given to Dr Kling from the University of Hohenheim and Dr Wolfgang Pfeiffer for providing the parental germplasm and the resulting segregating populations used.

ORCID

Jose M. Arjona https://orcid.org/0000-0002-7386-1560
Conxita Royo https://orcid.org/0000-0001-7939-1451
Susanne Dreisigacker https://orcid.org/0000-0002-3546-5989
Dolores Villegas https://orcid.org/0000-0003-2961-6346

REFERENCES

Allen, M. R., Dube, O. P., Solecki, W., Aragón-Durand, F., Cramer, W., Humphreys, S.,...Zickfeld, K. (2018). Farming and Context. In Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, (V. Masson, pp. 47–92). Retrieved from https://www.ipcc.ch/site/assets/uploads/sites/2/2018/11/SR15_Chapter1_Low_Res.pdf
Ammar, K., Lage, J., Villegas, D., Crossa, J., Hernandez, H., & Alvarado, G. (2008). Association among durum wheat international testing sites and trends in yield progress over the last twenty-two years. In M. P. Reynolds, & P. J., & H.-J. Braun, (Eds.), International symposium on wheat yield potential: Challenges to international wheat breeding. Mexico, DF: CIMMYT.
Arjona, J. M., Royo, C., Dreisigacker, S., Ammar, K., & Villegas, D. (2018). Effect of Ppd-A1 and Ppd-B1 allelic variants on grain number and thousand kernel weight of durum wheat and their impact on final grain yield. Frontiers in Plant Science, 9, 888. https://doi.org/10.3389/fpls.2018.00888
Asseng, S., Ewert, F., Martre, P., Rötter, R. P., Lobell, D. B., Cammarano, D.,...Zhu, Y. (2015). Rising temperatures reduce global wheat production. Nature Climate Change, 5(2), 143–147. https://doi.org/10.1038/nclimate2470
Baasandorj, T., Ohm, J.-B., Manthey, F., & Simsek, S. (2015). Effect of kernel size and mill type on protein, milling yield, and baking quality of hard red spring wheat. Cereal Chemistry Journal, 92(1), 81–87. https://doi.org/10.1094/CCHEM-12-13-0259-R
Beales, J., Turner, A., Griffiths, S., Snape, J. W., & Laurie, D. A. (2007). A Pseudo-Response Regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (Triticum aestivum L.). Theoretical
and Applied Genetics, 115(5), 721–733. https://doi.org/10.1007/s00122-007-0603-4

Bergkamp, B., Impa, S. M., Asebedo, A. R., Fritz, A. K., & Jagadish, S. V. K. (2018). Prominent wheat variety response to post-flowering heat stress under controlled chambers and field based heat tents. Field Crops Research, 222, 143–152. https://doi.org/10.1016/j.fcr.2018.02.008

Davidian, M., & Giltinan, D. M. (1995). Nonlinear models for repeated measurement data (1st ed.). London, UK: Chapman & Hall.

Dias, A. S., & Lidon, F. C. (2009). Evaluation of grain filling rate and nonlinear models for repeated measurement data. Journal of Agronomy and Crop Science, 195(2), 137–147. https://doi.org/10.1111/j.1439-037X.2008.00347.x

FAO (2018). Food outlook-biannual report on global food markets – November 2018. (104 pp). Licence: CC BY-NC-SA 3.0 IGO. Rome, Italy: FAO. Retrieved from http://www.wipo.int/amc/en/media tion/}

Ferris, R., Ellis, R. H., Wheeler, T. R., & Hadley, P. (1998). Effect of high temperature stress on anthesis on grain yield and biomass of field-grown crops of wheat. Annals of Botany, 8(5), 631–639. https://doi.org/10.1006/anbo.1998.0740

Ferrise, R., Triossi, A., Stratonovitch, P., Bind, M., & Martre, P. (2010). Sowing date and nitrogen fertilisation effects on dry matter and nitrogen dynamics for durum wheat: An experimental and simulation study. Field Crops Research, 117, 245–257. https://doi.org/10.1016/j.fcr.2010.03.010

Fischer, R. A. (2016). The effect of duration of the vegetative phase in irrigated semi-dwarf spring wheat on phenology, growth and potential yield across sowing dates at low latitude. Field Crops Res., 198, 188–199. https://doi.org/10.1016/j.fcr.2016.06.019

García, G. A., Serrago, R. A., Dreccer, M. F., & Miralles, D. J. (2016). Post-anthesis warm nights reduce grain weight in field-grown wheat and barley. Field Crops Research, 195, 50–59. https://doi.org/10.1016/j.fcr.2016.06.002

Gibson, L. R., & Paulsen, G. M. (1999). Yield Components of wheat grown under high temperature stress during reproductive growth. Crop Science, 39(6), 1841. https://doi.org/10.2135/cropsci1999.3961841x

Hlaváčová, M., Klem, K., Rapantová, B., Novotná, K., Urban, O., Hlavinka, P., … Trnka, M. (2018). Interactive effects of high temperature and drought stress during grain filling on plant yield and chloroplast EF-Tu expression in spring wheat. Journal of Agronomy and Crop Science, 197(6), 430–441. https://doi.org/10.1111/j.1439-037X.2011.00477.x

Ray, D. K., Gerber, J. S., MacDonald, G. K., & West, P. C. (2015). Climate variation explains a third of global crop yield variability. Nature Communications, 6(1), 5989. https://doi.org/10.1038/ncomms6989

Prasad, P. V. V., Pisipati, S. R., Momčilović, I., & Ristic, Z. (2011). Independent and combined effects of high temperature and drought stress during grain filling on plant yield and chloroplastic EF-Tu expression in spring wheat. Journal of Agronomy and Crop Science, 197(6), 430–441. https://doi.org/10.1111/j.1439-037X.2011.00477.x

Robert, N., Huet, S., Hennequet, C., & Bouvier, A. (1999). Methodology for choosing a model for wheat kernel growth. Agronomie, 19(5), 405–417. https://doi.org/10.1051/agro:19990507

Royo, C., Ammar, K., Alfaro, C., Dreisigacker, S., García Del Moral, L. F., Villegas, D., … Villegas, D. (2018). Effect of Ppd-1 photoperiod sensitivity genes on dry matter production and allocation in durum wheat. Field Crops Research, 221, 358–367. https://doi.org/10.1016/j.fcr.2017.06.00

Royo, C., Dreisigacker, S., Alfaro, C., Ammar, K., & Villegas, D. (2016). Effect of Ppd-1 genes on durum wheat flowering time and grain filling duration in a wide range of latitudes. The Journal of Agricultural Science, 154(4), 612–631. https://doi.org/10.1017/S002188910000507

Royo, C., Naczo, R., & Villegas, D. (2014). The climate of the zone of origin of Mediterranean durum wheat (Triticum durum Desf.) landraces affects their agronomic performance. Genetic Resources and Crop Evolution, 61(7), 1345–1358. https://doi.org/10.1007/s10722-014-0116-3

Royo, C., Villegas, D., Rharrabit, Y., Blanco, R., Martos, V., García del Moral, L. F., … del Moral, L. F. G. (2006). Grain growth and yield formation of durum wheat grown at contrasting latitudes and water regimes in a Mediterranean environment. Cereal Research, 34(2-3), 1021–1028. https://doi.org/10.1556/CRC.34.2006.2-3.233

SAS RRD: SCR 008567. (2009). Statistical analysis system software. SAS Institute Inc. (2010). SAS/STAT® 9.22 user’s guide. Chapter 60: “The NLIN Procedure.” Cary, NC: SAS Institute Inc.

Shavrukov, Y., Kurishbayev, A., Jatayev, S., Shvidchenko, V., Zotova, L., Koekemoer, F., … Langridge, P. (2017). Early flowering as a drought escape mechanism in plants: How can it aid wheat production? Frontiers in Plant Science, 8(November), 1–8. https://doi.org/10.3389/fpls.2017.01950

Shirdelmoghanloo, H., Cazzolino, D., Lohraseb, I., & Collins, N. C. (2016). Truncation of grain filling in wheat (Triticum aestivum) triggered by brief heat stress during early grain filling: Association with senescence responses and reductions in stem reserves. Functional Plant Biology, 43, 919–930. https://doi.org/10.1071/FP15384

Tashiro, T., & Wardlaw, I. F. (1990). The effect of high temperature at different stages of ripening on grain set, grain weight and grain dimensions in the semi-dwarf wheat “banks”. Annals of Botany, 65, 51–61. https://doi.org/10.2307/42771373
Terrile, I. I., Miralles, D. J., & González, F. G. (2017). Fruiting efficiency in wheat (Triticum aestivum L): Trait response to different growing conditions and its relation to spike dry weight at anthesis and grain weight at harvest. *Field Crops Research*, 201, 86–96. https://doi.org/10.1016/j.fcr.2016.09.026

Thomason, K., Babar, M. A., Erickson, J. E., Mulvaney, M., Beecher, C., & MacDonald, G. (2018). Comparative physiological and metabolomics analysis of wheat (Triticum aestivum L.) following post-anthesis heat stress. *PLoS One*, 13(6), e0197919. https://doi.org/10.1371/journal.pone.0197919

Ugarte, C., Calderini, D. F., & Slafer, G. A. (2007). Grain weight and grain number responsiveness to pre-anthesis temperature in wheat, barley and triticale. *Field Crops Research*, 100, 240–248. https://doi.org/10.1016/j.fcr.2006.07.010

Vignjevic, M., Wang, X., Olesen, J. E., & Wollenweber, B. (2015). Traits in spring wheat cultivars associated with yield loss caused by a heat stress episode after anthesis. *Journal of Agronomy and Crop Science*, 201(1), 32–48. https://doi.org/10.1111/jac.12085

Villegas, D., Alfaro, C., Ammar, K., Cátedra, M. M., Crossa, J., García del Moral, L. F., & Royo, C. (2016). Daylength, temperature and solar radiation effects on the phenology and yield formation of spring durum wheat. *Journal of Agronomy and Crop Science*, 202(3), 203–216. https://doi.org/10.1111/jac.12146

Wasson, A. P., Richards, R. A., Chatrath, R., Misra, S. C., Prasad, S. V. S., Rebetke, G. J., ... Watt, M. (2012). Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *Journal of Experimental Botany*, 63(9), 3485–3498. https://doi.org/10.1093/jxb/ers111

Weir, A. H., Bragg, P. L., Porter, J. R., & Rayner, J. H. (1984). A winter wheat crop simulation model without water or nutrient limitations. *The Journal of Agricultural Science*, 102(02), 371–382. https://doi.org/10.1017/S0021859600042702

Wilhelm, E. P., Turner, A. S., & Laurie, D. A. (2009). Photoperiod insensitive Ppd-A1a mutations in tetraploid wheat (Triticum durum Desf.). *Theoretical and Applied Genetics*, 118(2), 285–294. https://doi.org/10.1007/s00122-008-0898-9

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research*, 14(6), 415–421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Arjona JM, Royo C, Dreisigacker S, Ammar K, Subirà J, Villegas D. Effect of allele combinations at Ppd-1 loci on durum wheat grain filling at contrasting latitudes. *J Agro Crop Sci*. 2020;206:64–75. https://doi.org/10.1111/jac.12363