Photoperiod response as a driver of flowering time in spring durum wheat and its influence on productivity, and environmental adaptabilityítol de la tesi

José María Arjona Rodríguez

Universitat de Lleida

IRTA RECERCA I TECNOLOGIA AGROALIMENTÀRIES

TESI DOCTORAL

Photoperiod response as a driver of flowering time in spring durum wheat and its influence on productivity, and environmental adaptability

Jose María Arjona Rodríguez

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Directora

Dolors Villegas Tort

Tutor

Carlos Cantero Martínez

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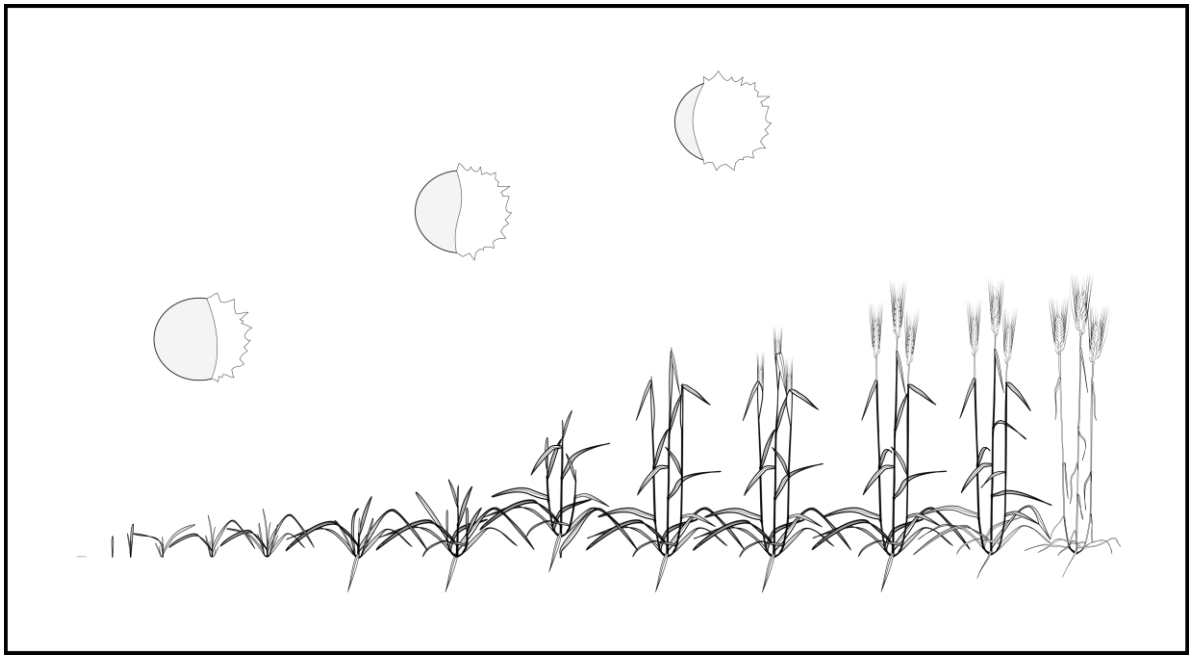
Doctoral Thesis

PHOTOPERIOD RESPONSE AS A DRIVER OF FLOWERING TIME IN SPRING DURUM WHEAT AND ITS INFLUENCE ON PRODUCTIVITY, AND ENVIRONMENTAL ADAPTABILITY



BY:

Jose M. Arjona Rodríguez

Director:
Dolors Villegas Tort

Tutor:
Carlos Cantero Martínez

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ABSTRACT

Wheat is a staple crop that provides 20% of proteins and calories to global human diets. Adapting flowering time to each particular environment is one of the stress avoidance mechanisms that could reduce the predicted impact of climate change. The general hypothesis underlying this research was that a change in flowering time would affect environmental conditions of spring durum wheat during critical developmental phases, which in turn would have an impact on yield formation.

A set of spring durum wheat lines with contrasting allele variants at *Ppd-1* loci, affecting photoperiod sensitivity, were tested at a range of Northern latitudes (41°N in Spain, 27°N in the northwest of Mexico, and 19°N in the south of Mexico, this last site with spring sowing time). Field experiments under irrigation were carried out between the years 2007 and 2012, to investigate the effect of allele variants at *Ppd-A1* and *Ppd-B1* loci on phenology and yield formation.

Genotypes carrying the allele *Ppd-A1a* GS100, causing photoperiod insensitivity, tended to have high grain weight (GW) and yield. Allele variants at *Ppd-B1* locus did not affect flowering time, but the *Ppd-B1b* allele causing photoperiod sensitivity increased grain number per unit area (GN) due to a higher number of spikelets spike^{-1}.

Early flowering (either due to *Ppd-1* or earliness *per se*, *Eps*) tended to be associated with high yield due to high GW. The allele combinations GS105/*Ppd-B1b* and *Ppd-A1b*/*Ppd-B1b* were associated with higher GN due to an increase in the number of grains spikelet^{-1}, but it did not translate as yield increase due to a trade-off between GN and GW. Early flowering caused by *Eps* genes resulted in a low number of spikelets spike^{-1}, but not a low GN. Yield stability was enhanced when alleles at *Ppd-1* loci conferred a similar photoperiod response (sensitive/sensitive or insensitive/insensitive).

The environmental conditions during the first half of the grain filling period were the most critical factors to define GW. Flowering time delays were associated with reductions in grain filling rate and GW. At autumn-sowing sites, an increase of 1°C in mean temperature reduced GW by 5.2 mg grain^{-1}. The analysis of phenotype-genotype associations showed that the regions at chromosomes 6A (114 cM) and 6B (126 cM) were associated with yield across sites, thus representing hotspots for QTL regulating yield performance. The detection of single markers-trait associations (MTAs) was highly affected by environment, and the interactions between pairs of markers showed a stronger effect than the corresponding single MTAs.

RESUMEN

El trigo es un cultivo que aporta el 20% de las proteínas y calorías para el consumo humano a nivel global. La adaptación de la fecha de floración a cada ambiente en particular forma parte de un mecanismo de escape al estrés, lo que podría reducir el impacto negativo esperado debido al cambio climático. La hipótesis general sobre la que se establece este estudio es que un cambio en la fecha de floración afectaría a las condiciones ambientales durante las fases críticas del desarrollo del trigo, lo que a su vez puede tener un impacto en la formación del rendimiento.

Un conjunto de líneas de trigo duro de primavera, con variaciones alélicas contrastantes para *loci* *Ppd-1*, que afectan la sensibilidad al fotoperiodo, fueron ensayadas en un amplio rango de latitudes del hemisferio Norte (41°N en España, 27°N en el noroeste de México, y 19°N en el sur de México, esta última con siembra de primavera). Se llevaron a cabo experimentos de campo en regadío, entre los años 2007 y 2012, para investigar el efecto que tuvieron las variantes alélicas en los loci *Ppd-A1* y *Ppd-B1* sobre la fenología y la formación del rendimiento.

Los genotipos con el alelo *Ppd-A1a* GS100, causante de insensibilidad al fotoperiodo, tendieron a presentar un peso de grano (PG) y un rendimiento superiores al resto. Las variantes alélicas para *Ppd-B1* no afectaron a la fecha de floración, pero el alelo causante de insensibilidad al fotoperiodo (*Ppd-B1b*) aumentó el número de granos por unidad de superficie (NG) debido a un aumento de número de espiguillas espiga^{-1}.

Una floración más temprana (ya fuera debida a *Ppd-1* o a precocidad intrínseca, *Eps*) tendió a estar asociada con rendimiento más alto debido a un PG mayor. Las combinaciones alélicas GS105/*Ppd-B1b* y *Ppd-A1b*/*Ppd-B1b* se asociaron con un incremento de NG debido a un aumento del número de granos espiguilla^{-1}, pero esto no se tradujo en un mayor rendimiento debido a la relación negativa entre NG y PG. Una floración más temprana producida por genes *Eps* tuvo como resultado un menor número de espiguillas espiga^{-1}, pero no un menor NG. Cuando ambos alelos en los *loci Ppd-1* poseían el mismo tipo de respuesta al fotoperiodo (sensible/sensible o insensible/insensible) se observó una mayor estabilidad en el rendimiento.

Las condiciones ambientales durante la primera mitad del periodo de llenado de grano fueron los factores más importantes para definir el PG. Un retraso en la fecha de floración se asoció con reducciones en la tasa de llenado de grano y el PG. En las latitudes donde la siembra se realizó en otoño, un incremento de 1°C en la temperatura media redujo el PG en 5,2 mg grano^{-1}. El análisis de asociación de fenotipo-genotipo mostró que las regiones en los cromosomas 6A (114

cM) y 6B (126 cM) se asociaron en general con el rendimiento, representando regiones críticas con QTLs importantes. La detección de marcadores únicos asociados a una característica estuvo muy ligada al ambiente, y la interacción entre pares de marcadores mostró mayor efecto que sus correspondientes marcadores únicos.

RESUM

El blat és un cultiu que aporta el 20% de les proteïnes i les calories per al consum humà a nivell global. L'adaptació de la data de floració a cada ambient en particular forma part d'un mecanisme d'escapament de l'estrès, cosa que podria reduir l'impacte negatiu esperat degut al canvi climàtic. La hipòtesi general sobre la que s'estableix aquest estudi és que un canvi en la data de floració afectaria les condiciones ambientals durant les fases crítiques de desenvolupament del blat, que al seu torn poden tenir un impacte en la formació del rendiment.

Un conjunt de línies de blat dur de primavera, amb variacions al·lèliques contrastants per als *loci Ppd-1*, que afecten la sensibilitat al fotoperíode, es van assajar en un ampli rang de latituds de l'hemisferi Nord (41°N a Espanya, 27°N al noroest de Mèxic, i 19°N al sud de Mèxic, aquesta darrera amb sembra de primavera). Es van portar a terme experiments de camp en regadiu, entre els anys 2007 i 2012, per a investigar l'efecte de les variants al·lèliques en els *loci Ppd-A1* i *Ppd-B1* sobre la fenologia i la formació del rendiment.

Els genotips portadors de l'al·lel *Ppd-A1a* GS100, causant d'insensibilitat al fotoperíode, van tendir a presentar un pes de gra (PG) i un rendiment superiors a la resta. Les variants al·lèliques per a *Ppd-B1* no van afectar la data de floració, però l'al·lel causant d'insensibilitat al fotoperíode (*Ppd-B1b*) es va associar a un major número de grans per unitat de superfície (NG), degut a un augment en el número d'espiguetes espiga^{-1}.

Una floració més precoç (tant si era degut a *Ppd-1* com a precocitat intrínseca, *Eps*), va tendir a una associació amb un rendiment més alt degut a un major PG. Les combinacions al·lèliques GS105/*Ppd-B1b* i *Ppd-A1b/Ppd-B1b* es van associar amb un increment del NG degut a un augment del número de grans espigueta^{-1}, però això no es va traduir en un rendiment major, degut a la relació negativa entre NG i PG. Una floració més precoç produïda per gens *Eps* va resultar en un menor número d'espiguetes espiga^{-1}, però no un menor NG. Quan ambdós al·lels en els *loci Ppd-1* presentaven el mateix tipus de resposta al fotoperíode (sensible/sensible o insensible/insensible) es va observar una major estabilitat en el rendiment.

Les condicions ambientals durant la primera meitat del període d'ompliment del gra van ser els factors més importants per definir el PG. Un retràs en la data de floració es va associar amb reduccions en la taxa d'ompliment de gra i amb PG. En les latituds amb sembra de tardor, un increment de 1°C en la temperatura mitjana va reduir el PG en 5,2 mg gra^{-1}. L'anàlisi d'associació genotip-fenotip va mostrar que les regions als cromosomes 6A (114 cM) i 6B (126 cM) tenien

associacions generals amb el rendiment, esdevenint regions crítiques amb QTLs importants. La detecció de marcadors únics associats a una característica va estar molt lligada a l'ambient, i la interacció entre parells de marcadors va mostrar major efecte que els corresponents marcadors únics.

GENERAL INTRODUCTION

0.1. DURUM WHEAT IN THE WORLD

A total of 2658 million tons of all cereals were produced around the world in 2017, of which wheat (*Triticum* spp.) represented close to 30%. Around 70% of wheat production was devoted to human consumption, while for coarse grains human consumption represented 15% of the total production (FAO, 2017). With the exception of Antarctica, wheat is grown on every continent on more than 220 million hectares, being the crop that most land occupies (Fig. 1). Wheat is the cereal that provides the most protein to the human diet around the world (CIMMYT, 2019; FAO, 2019).

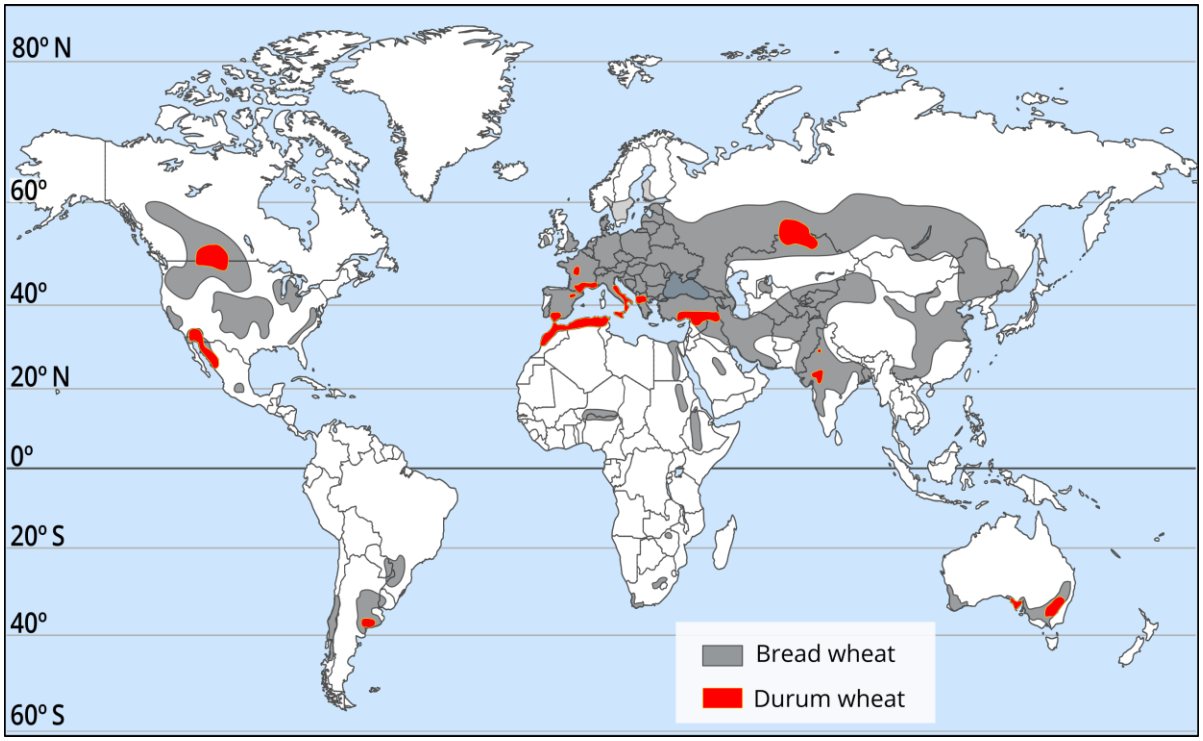

Figure 0.1. World map of wheat distribution. Based on: (Lantican et al., 2005; Ranieri, 2015).

The genus *Triticum* L. belongs to the family Poaceae Barnhart, which is comprised of the plants generally known as grasses. This genus is a complex one, with a rich number of wild and cultivated species. One of the centres of diversification and the place where the first archaeological remains appeared is the Fertile Crescent, where some wild species may still be found (Feldman and Kislev, 2007). Some of the members of *Triticum* L. evolved over time, mixing complex mechanisms of polyploidization and divergence that are not yet completely clear. In the case of *T. turgidum* L., tetraploid (2n=4x= 28; AABB), the most accepted theory consists of the hybridization of old relatives of *T. urartu* Thumanjan ex Gandilyan and *Aegilops speltoides* Tausch, contributing the A

and B genome respectively (Bozzini et al., 2012; Maestra and Naranjo, 1998). The second most important crop of *Triticum* species after bread wheat (*T. aestivum* L.) is durum wheat (*Triticum turgidum* L. var. *durum)*, which is considered to evolve from the wild emmer species of *T. turgidum* through a long process of domestication (Feldman and Kislev, 2007).

Durum wheat is an autogamous annual plant traditionally grown under rainfed conditions and adapted to dry conditions. About 60% of durum is grown in the Mediterranean basin, generally sown between November and December and harvested between May and July. In other regions, such as Canada, the second producer after the Mediterranean region, durum is grown in a summer cycle, being sown in spring and harvested between the end of the summer and the beginning of the autumn. Durum wheat represents between the 5% and 10% of the wheat cultivated, with around to 39 million tons per year. Despite having less proportion of the production than bread wheat, durum wheat is very important economically and socially because of its characteristics and end products. Pasta, couscous, bulgur, and flat breads are very relevant dishes in the Mediterranean basin. All of them are considered to be at the bottom of the food pyramid (Grant et al., 2012; Royo et al., 2017).

0.2. WHEAT PHENOLOGY

Phenology consists of the timing of the developmental phases that occur during the plant cycle, the interrelation among those phases when comparing genotypes or species, and the causes that affect that timing, such as intrinsic factors, extrinsic factors, and its interaction (Koch et al., 2009). As an intrinsic factor we consider the genotype. The external factors could be numerous, but the main ones affecting phenology are temperature, photoperiod, and water availability (Koch et al., 2009). The wheat developmental phases can be described in terms of internal or external morphological changes. Among these, there are three main consecutive phases: vegetative, reproductive, and grain filling. There are two ways of determining the actual phase, looking at external changes or growth, or those that check the stage of the apical meristem, for which dissection of the shoot apex is needed (Miralles and Slafer, 2000).

The vegetative phase (Fig. 2a) is considered to last from sowing to floral initiation, but since floral initiation is practically impossible to be visually determined, because it is not possible to differentiate the leaf primordia from the spikelet primordia, it has to be determined *a posteriori* from indirect calculations (Delécolle et al., 1989; Kirby et al., 1987). The moment of double ridge stage is considered as a reference. The reproductive phase takes place from floral initiation to flowering. In this phase, the floret development starts until the fertile florets are developed with

all their reproductive structures, and pollination occurs. The grain filling period ranges from the moment when the endosperm cells start to develop until physiological maturity (Miralles and Slafer, 2000). Other sub-phases have been used as indicators of development. Some of them, such as double ridge and terminal spikelet, need the dissection of the shoot apex. Double ridge is the first indubitable sign of the first reproductive structure, when the shoot apex shows a double ridge formed by spikelet and leaf primordia (Fig. 2b), and terminal spikelet when all spikelet primordia are visible and the embryo spike is complete (Fig. 2c) (Kirby and Appleyard, 1986). In other cases the scale of development is based on visual observations as in the determination of flowering time (Fig. 2d) and physiological maturity (Zadoks et al., 1974). Flowering and the time around it are considered to be one of the most critical stages of all the crop cycle. This period is when the pollination and fecundation of the florets occur, and just after, the final grain number is set (Draeger and Moore, 2017; Ferris et al., 1998).

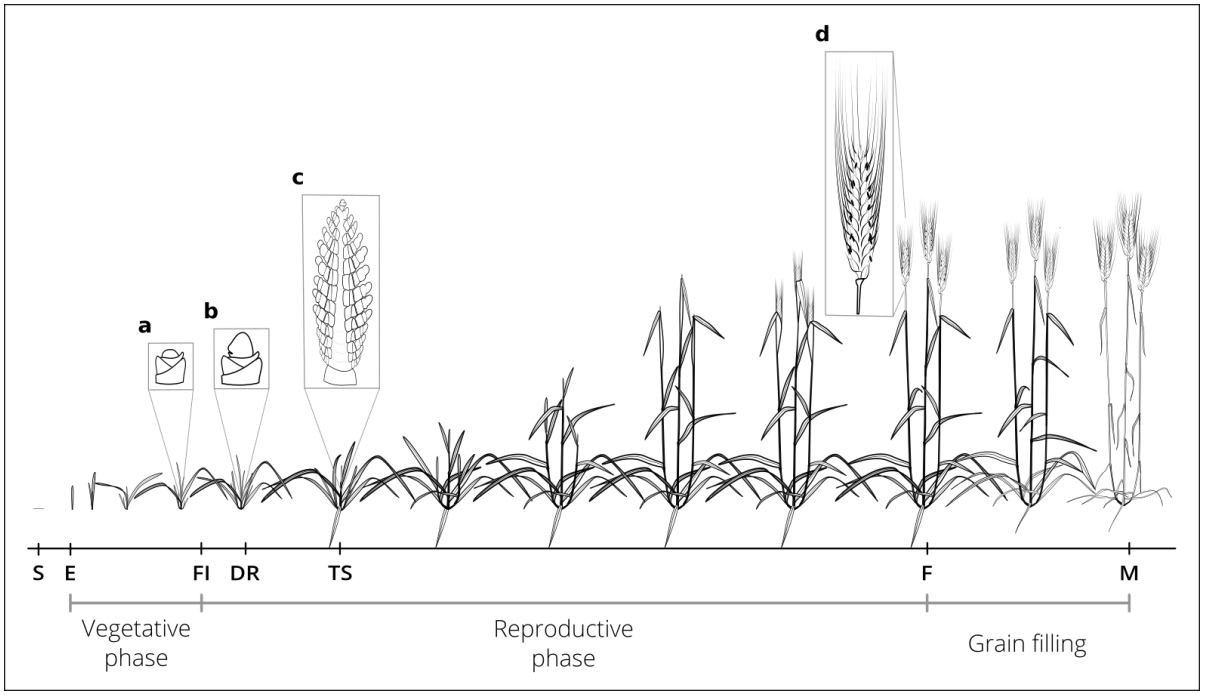

Figure 0.2. Representation of the durum wheat developmental phases. Based on (Slafer and Rawson, 1994). (S) Sowing, (E) emergence, (FI) floral initiation, (DR) double ridge, (TS) terminal spikelet, (F) flowering, (M) physiological maturity. **a**) Scheme of a vegetative apex, **b**) scheme of an apex at double ridge, **c**) scheme of an apex at terminal spikelet, and **d**) scheme of a spike with extruded anthers.

0.3. WHEAT COMPONENTS AND THEIR FORMATION

Grain yield may be considered to be the grain number per unit area (e.g. grain number m^{-2}), multiplied by the average weight of those grains. The grain number m^{-2} depends on two other variables, namely spikes m^{-2} and grains $spike^{-1}$. At the same time spikes m^{-2} is derived from plants

m^{-2} and shoots $plant^{-1}$, while grains $spike^{-1}$ is formed by spikelets $spike^{-1}$ and grains $spikelet^{-1}$ (Fig. 3).

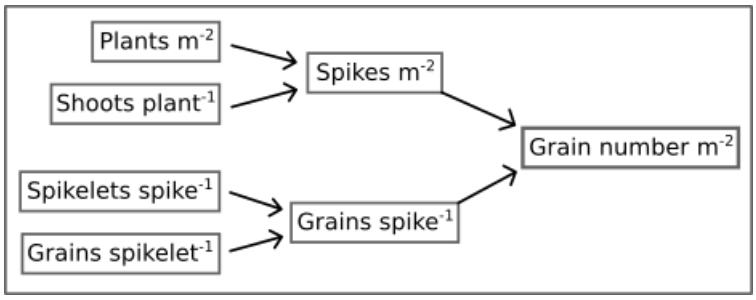

Figure 0.3. Grain number formation, and related components.

The number of plants m^{-2} is basically determined by the density of seeds sown, and the rate of germination. This component is the first to settle, between sowing and part of the vegetative phase. After the germination, the seedlings development starts, and at some point of the vegetative phase, the number of shoots is determined. Some of these shoots are going to carry a spike, but on some occasions the shoot could be aborted, or the spike never fully develops, which finally determines the number of spikes. The number of spikelets $spike^{-1}$ are mainly defined from floral initiation to terminal spikelet. The grains $spikelet^{-1}$ are determined in a sequence of phases. Firstly, it depends on the number of florets initiated in each spikelet, the phase that takes place from before the terminal spikelet to half way through the reproductive phase. After that, the florets develop until the moment when some of the florets are aborted. From all the fertile florets, only those that are pollinated will be able to generate a grain. Finally, during the grain setting process those pollinated florets will start to develop a full grain or will abort (Miralles and Slafer, 2000; Russell and Wilson, 1994).

Once the ovary has been pollinated the formation of the grain weight starts. This growth consists of three phases. In the first phase a rapid cell division period occurs, and the structures of the fruit and seed develop. There are no big changes in weight or in size at the beginning. After this period, the linear phase starts, where an exponential cell growth is led by the synthesis and deposition of starch and protein, without cell division. The protein deposition is slightly faster than that of starch, but a simultaneous process is carried out (Jenner et al., 1991). Finally the seed growth slows down and the maximum dry weight will be reached at physiological maturity (Farooq and Siddique, 2014). After that the seed will still lose moisture, but no changes in dry matter or yield would happen (Egli, 1998).

The majority of these phases are accelerated by increasing temperature and photoperiod (Slafer and Rawson, 1994). Although, in winter wheat, from sowing to double ridge the vernalization is

the major factor controlling development (Kirby et al., 1999). Every stage has its range of development temperatures with its optimum, at which the organs developing in that phase will be potentiated (Porter and Gawith, 1999). One of the most important phases is flowering, around which the final grain number is set. This stage is also one of the most sensitive to extreme temperatures, where cold temperatures under 9 ºC should be avoided, as well as temperatures higher than 31 ºC, and water stress (Porter and Gawith, 1999; Russell and Wilson, 1994). For a higher grain weight it is also important to have optimal conditions during the grain filling period, when it is also sensitive to drought and extreme temperatures (Dias and Lidon, 2009; Royo et al., 2006).

0.4. ENVIRONMENTAL CUES INTERACTION WITH PHENOLOGY

The most important factor that affects phenology is the environment. Inside the concept of environment the most important factors are variations in photo-thermal conditions, water availability, and soil composition. In this sense the crop land could be divided into agro-ecological regions. Each of these regions has a suitable period in which the environment allows the crop to develop (FAO, 1996). In a range from 19°N to 41°N latitudes, environment could account for more than 95% of the variation in length of days from sowing to flowering, and more than 48% in thermal time. For the period between flowering to physiological maturity the environmental factors could explain more than 60% of the variation in length for both days and thermal time (Villegas et al., 2016).

The temperature is one of the main influencers in plant development. In the model plant *Arabidopsis thaliana* (L.) Heynh. the temperature increases the expression of *FLOWERING LOCUS T* (*FT*) which stimulates flowering (Song et al., 2013). In Britain it has been shown that spring flowering plants have advanced their flowering date by 4.5 days during the last decade of the XX century due to global warming (Fitter and Fitter, 2002). In wheat, the first phenomenon in which the temperature plays an important role is in the vernalization (winter wheat). Once this period of low temperatures has passed in winter wheat, an increase in temperature will accelerate the developmental rate of the phenological phases, which also happens in spring wheat without the vernalization process (Asseng et al., 2015; Slafer and Rawson, 1994).

Drought stress can also accelerate the rate of development, among other negative physiological consequences. Water scarcity could reduce the length in days of heading to flowering and grain filling from 31 to 72%, being those the most sensitive phases (Ihsan et al., 2016). Apparently this

stress stimulates the production of ethylene, which enhances development and induction of senescence in plants (Farooq et al., 2009).

The photoperiod also has an important role on phenology determination, but in this case it seems to be more controlled by the interaction between photoperiod and the genotype, than by photoperiod itself. In Arabidopsis, longer daylength also stimulates *FT* expression and induction of flowering (Valverde et al., 2004).

0.5. PHENOLOGY FITTING AND PRODUCTIVITY

One of the main objectives in wheat production is the coincidence between the most sensitive phases and the best possible environmental conditions for them. As mentioned in the previous section, the environment is crucial to wheat development. The conditions during development determine grain yield, which is the final target of durum wheat breeding and production.

At each specific site, the cropping season has to be adapted to the local weather conditions. For example under Mediterranean conditions water scarcity and heat stress is more probable at the end of the spring and summer than in northern European latitudes (Kottek et al., 2006). On the other hand, in northern localities the possibility of spring frost is higher (FAO, 1996). In this context, two strategies could be considered: resistance and avoidance. The resistance mechanism consists of developing varieties that are able to cope with the stress, which could be frost, heat, or drought tolerant. The plant is not going to be in optimum conditions, but will be able to not decrease production dramatically (Kulkarni et al., 2017; Mickelbart et al., 2015). Stress avoidance or scape is a strategy that consists of developing the entire cycle in the most favourable conditions possible (Shavrukov et al., 2017). Another way of avoiding stresses is through agronomical practices, such irrigation, the most common practice to elude drought stress. Through genetic control, the most typical mechanism is the regulation of phenology by vernalization, which prevents the floral initiation during freezing temperatures (Kamran et al., 2014). The sowing date has been proved to have an effect on the phenological phases and on final grain yield. Depending on the winter type of wheat and the place of cropping, differences in full crop cycle could reach up to 90 days, around 20 days from sowing to heading, and 50 days from flowering to maturity. Yield components are also affected and yield differences can go above 3 t ha^{-1}. Modifications in the sowing date modified the environmental conditions of the developmental phases, and negative effects have been found in both too-early and too-late sowing times, where an optimal window has to be found for each place (Connor et al., 1992; Ortiz-Monasterio et al., 1994).

In the context of the climate change scenario, where temperatures are rising and drought events are more frequent, reductions of 5% to 6% by each degree °C have been predicted (Asseng et al., 2015). In this context, fitting phenology with the best environmental conditions is a key point to improve yield. Early flowering varieties tend to be more productive under the possibility of terminal drought, as in the typical Mediterranean climate or semi-arid croplands (Royo et al., 2016; Shavrukov et al., 2017). This is because high temperatures can affect yield components or yield itself. Temperatures higher than 31 °C around flowering affect fertility by reducing grain number (Draeger and Moore, 2017; Ferrise et al., 2010). The grain weight could be affected by high temperatures due to an impact on photosynthetic process and starch accumulation (Farooq et al., 2011). Drought will also severely affect grain weight, as it has an impact on many processes of the plant metabolism and development (Farooq et al., 2009; Russell and Wilson, 1994).

However, in years with better environmental conditions, where those final stresses are not produced, too-early genotypes could reduce their yield potential. This could be led by the reduction in biomass until flowering, and the lower grain number per unit area associated with early genotypes (Gonzalez et al., 2006; Royo et al., 2018). Early genotypes could also be exposed to late freezing temperatures, which can damage the reproductive structures (Fischer, 2016; Gott, 1961). Therefore, finding the optimal window for flowering is an important target in phenology adaptation. Nevertheless, with climate change and the randomization of extreme weather events, varieties with some flexibility could be favourable.

0.6. GENETIC CONTROL OF FLOWERING TIME IN WHEAT

In wheat, the control of flowering and pre-flowering phases consists of a complex interaction of environmental conditions and genotype. Three genetic systems control these phases: vernalization (*Vrn-1*), photoperiod response (*Ppd-1*), and intrinsic earliness or earliness *per se* (*Eps*). More effort should be made to fully understand the mechanism of control and interaction between *Vrn-1*, *Ppd-1,* and *Eps*. However, the accepted general scheme is shown in Figure 3. The circadian clock, a mechanism that regulates the response for rhythmic environment changes, such a day/night cycle and seasons of the year (Millar, 2004), is an important part of the mechanism of control. On short-days *Ppd-1* interacts with proteins of the circadian clock system, which downregulates the vernalization gene responsible of the flowering stimulation (VRN3). This path through *Ppd-1* has the opposite effect on long-days (Kitagawa et al., 2012). At the same time if the days are long, but no vernalization has taken place (typical condition at the beginning of autumn), the vernalization gene responsible for interacting with cold treatment and daylength

(VRN2) represses the expression of the VRN3 (Chen and Dubcovsky, 2012). After the vernalization has been fulfilled, the main gene controlling vernalization (VRN1) represses VRN2, which allows VRN3 to express. The expression of VRN3 stimulates VRN1, which creates a cascade effect. The mechanism of *Eps* genes is the mostly unknown, but is usually associated with the circadian clock regulation. Some interactions with photoperiod regulators are known, even though the exact path is not clear (Alvarez et al., 2016). Finally, the resultant signalling of VRN3 travels to the apex and in interaction with VRN1 it stimulates the floral transition (Chen et al., 2014) (Fig. 4).

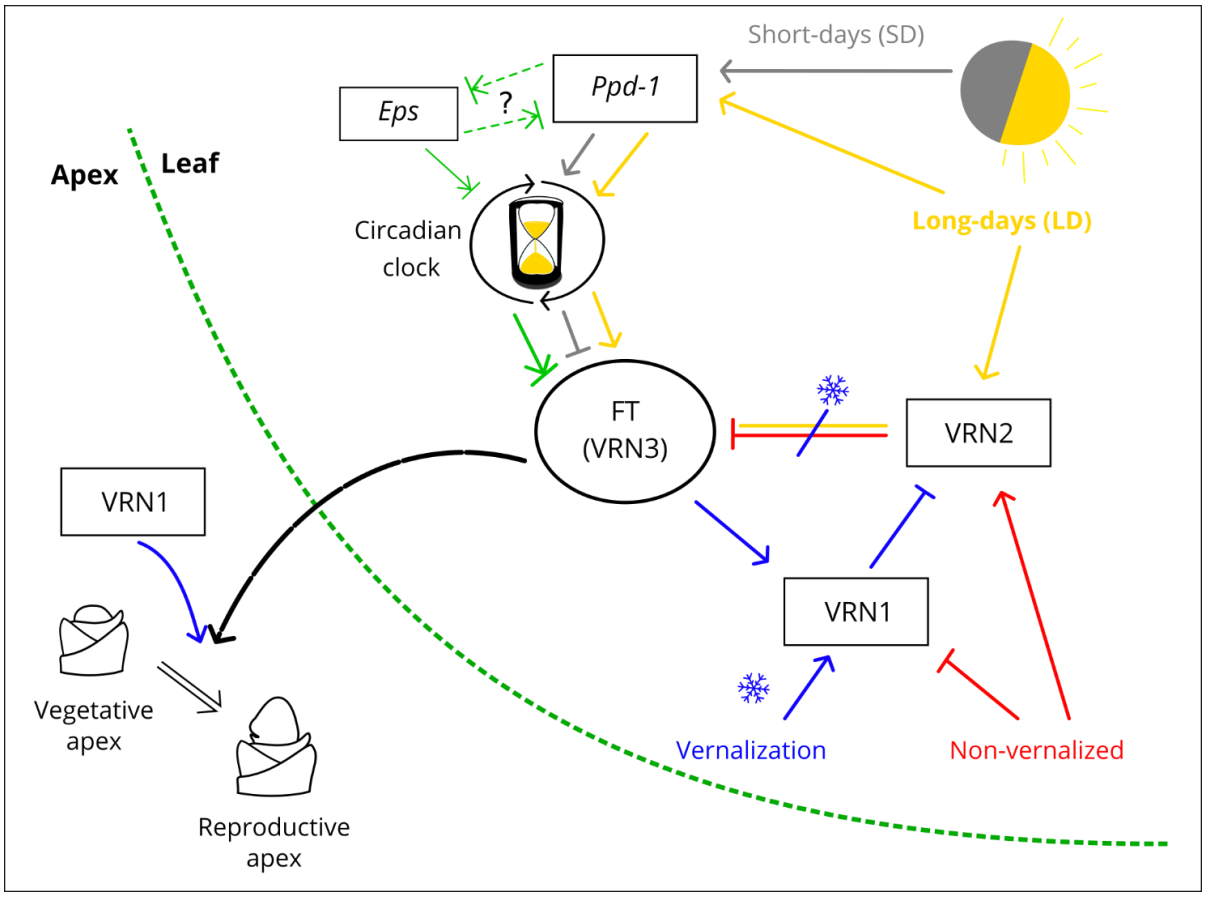

Figure 0.4. Scheme of the interaction between *VRN1*, *Ppd-1*, and *Eps* genes, into the transition from vegetative to reproductive phase. Based on: (Alvarez et al., 2016; Chen et al., 2014; Chen and Dubcovsky, 2012; Distelfeld et al., 2009a; Hill and Li, 2016; Kitagawa et al., 2012; Shimada et al., 2009). Segments ending in arrow head mean induction, segments with flat head indicates repression, and segments with both types of ending mean that the process is not clear. (FT) *FLOWERING LOCUS T*.

0.6.1. First research studies about vernalization and photoperiod requirements

In 1935, Lysenko described winter crops as those that if planted in spring were not able to form fruit. After different theories around 1925, finally the term vernalization was used, to describe the method of cold treatment to make them ready for spring sowing (Lysenko, 1935). This treatment

was first proposed by Klippart in 1857. He stated that "to convert winter into spring wheat, nothing more is necessary than that the winter wheat should be allowed to germinate slightly in the fall or winter, but kept from vegetation by a low temperature or freezing, until it can be sown in the spring" (Klippart, 1857). Therefore, winter wheat needs to go through a continuous chilling treatment during a determinate period of time, and spring wheat does not, even though in some spring wheat cases it could be beneficial. A third category is called facultative wheat, whose growth habit is between those of winter wheat and spring wheat. This process of vernalization has an optimum temperature of around 5 ºC and can be fulfilled between -1 to 15 ºC, and its duration varies between different genotypes (Chang et al., 2003; Porter and Gawith, 1999). Although the vernalization treatment could have an effect beyond floral initiation, the strongest effect would occur in the vegetative phase, which ends after the vernalization requirement is fulfilled and the development of the floral primordia starts (Slafer and Rawson, 1994). This vernalization control is considered an important adaptation to avoid frost damage to the floret primordia (Kamran et al., 2014; Worland and Snape, 2001).

Before the idea of vernalization was widely adopted, some authors mentioned the necessity of some plants to have an adequate daylength for their development, especially when concerning sexual reproduction (Garner and Allard, 1920). In 1922, Wanser first used the terms photoperiod and photoperiodism. He wrongly postulated that the different necessities of photoperiod was what differentiated winter wheat from spring wheat. But he also noticed that longer photoperiods were necessary for flowering (Wanser, 1922). Based on this theory, Mckinney and Sando observed that with chilled (vernalized) winter wheat seeds, and in spring wheat, if grown under higher temperatures and long days, they were able to cultivate two generations in a year (Mckinney and Sando, 1933). A more recent definition of the term photoperiodism states that daylength enables living organisms to adapt to the seasonal changes of their environment (Thomas and Vince-Prue, 1997).

In general, wheat is considered a facultative long days plant, which means that long days accelerate flowering but are not essential to complete the cycle (Thomas and Vince-Prue, 1997). However, the differential responses to daylength of different varieties has long been reported, and its mechanisms were tried to be understood (Pugsley, 1966). It is worth mentioning that we may use the expression long day plants and short day plants as convention, but it would be more appropriated to consider short night and long night plants. This is because what the plants detect is the duration of the night, since a short dark break during the day has no effect, but a short bright break during a long night will make it like a short night (Thomas and Vince-Prue, 1997).

From the first half of the XX century it has been noticed that there is an interaction between photoperiod and temperature (Mckinney and Sando, 1935). Because of that interaction, some authors proposed working with spring varieties for an easier understanding of the daylength effect (Pugsley, 1966). Studies carried out under these circumstances suggest the presence of two genes, which were involved in the sensitivity or insensitivity to photoperiod of those wheat plants that differed in the date of flowering under short days (Pugsley, 1966). In this context, two general groups of plants will be found sensitive and insensitive to photoperiod. The sensitive plants are those that have the mechanism of photoperiod control intact, and therefore they respond to changes in daylength. On the other hand, insensitive plants are those that do not change their behaviour when the daylength varies (Thomas and Vince-Prue, 1997).

Differences in flowering time were observed even when vernalization and photoperiod were completely fulfilled. It was deduced that other genes were modulating flowering time and generating earliness (Flood and Halloran, 1984; Ford et al., 1981; Yasuda and Shimoyama, 1965). This effect has been named in different ways ("Earliness *per se*", "Intrinsic earliness", "Narrow-sense earliness", etc. (Kato and Wada, 1999), but the most accepted lately is Earliness *per se* (*Eps*) (Alvarez et al., 2016; Lewis et al., 2008). The *Eps* is known to interact with *Ppd-1* in the same way that *Vrn-1* and *Ppd-1* genes do. For example, the *Eps* found in tetraploid wheat *Eps-A^{m}1* had a stronger effect if it was associated with a photoperiod-sensitive background, thus indicating an epistatic interaction with *Ppd-1* (Alvarez et al., 2016; Bullrich et al., 2002).

When all genes are at play, the *Vrn-1* genes account for 70% to 75 % of the total phenology variation, while to *Ppd-1* is attributed around 20%. The remaining variation (approximately 5%) would be due to *Eps* (Kamran et al., 2014). Because the existence of all these mechanisms, it is possible to fine tune flowering time in durum wheat, adapting it to the most suitable conditions (Snape et al., 2001).

0.6.2. Genetic control of vernalization

Vernalization requirement is controlled by three principal groups of genes: VRN1, VRN2, and VRN3. The VRN1 group of homologous genes are located in the middle of the long arms of chromosome 5A (*Vrn-A1*), and 5B (*Vrn-B1*) (Chu et al., 2011; Yan et al., 2004a). These genes are associated with *MADS-box* genes and more specifically with the gene of Arabidopsis promoter of meristem identity gene APETALA1 (*AP1*) (Yan et al., 2003). To fully understand the mechanism of vernalization genes more work has still to be done, but the general path has been studied. The *Vrn-A1* gene has more effect on controlling flowering time than the *Vrn-B1* does, and than the

remaining VRN2 and VRN3 systems (Muterko et al., 2016). The spring growth habit due to the VRN1 is associated with a modification in the promoter region of the *AP1* that will affect the recognition by the repressor, and being the spring habit dominant (Chu et al., 2011; Fu et al., 2005; Yan et al., 2003). Although in durum wheat the predominant modification was a deletion in the first intron (*Vrn-A1c*), which is also dominant, and the distribution of the different allele depends on the origin of the variety (Muterko et al., 2016). Less variation is observed for the *Vrn-B1* (Basualdo et al., 2015), but in studies with more genetic variability, it enables the differentiation of wheat from Russia and close countries from those grown in the rest of the world (Muterko et al., 2016).

The position of the VRN2 is less clear due to translocations between chromosomes (Tan and Yan, 2016), although in tetraploid wheat it has been located on the chromosome 5A (Distelfeld et al., 2009b). These genes have been associated with CO-like proteins from Arabidopsis, with two linked zinc finger-CCT domain genes, and finally linked with the gene *ZCCT1* (Yan et al., 2004b). In contrast with the mechanism of VRN1, VRN2 is down-regulated by vernalization and also by short days (Distelfeld et al., 2009b; Dubcovsky et al., 2006; Yan et al., 2004b). The function of VRN2 is the down-regulation of VRN3, preventing flowering during autumn (under long days, and without vernalization). The opposite profiles of transcription levels suggest that VRN1 down-regulates VRN2 when vernalization takes place (Distelfeld et al., 2009a; Yan et al., 2004b). The allelic variation for VRN2 is detected only when the winter VRN1 loci is present, which makes its study more difficult (Distelfeld et al., 2009b).

The VRN3 genes are located in the chromosome 7B (*VRN-B3*), and are linked to a gene similar to Arabidopsis *FLOWERING LOCUS T* (*FT*). The homozygous alleles for the dominant VRN3 promote early flowering, while recessive VRN3 alleles favour late flowering (Yan et al., 2006). The function of this gene is to accelerate flowering under long days producing a mobile protein that is transported from the leaves to the shoot apical meristem, where it stimulates the VRN1 system inducing flowering (Chen and Dubcovsky, 2012; Hill and Li, 2016). Dominant alleles of VRN3 are less frequent in durum wheat and the majority of the genotypes carrying it are grown mostly in Ukraine and Russia, and they are only revealed in combination with dominant alleles of VRN1 genes (Muterko et al., 2016).

In summary, the most plausible mechanism is that VRN2, without vernalization and long days, represses VRN3, and possibly the VRN1 genes. Then, with the ongoing of shorter days and cooler temperatures, VRN2 is repressed by VRN1. This way VRN3 stops being down-regulated by VRN2, but still needs long days to promote VRN1. When long days arrive VRN3 from the leaves starts to

promote VRN1 in the shoot apex towards flowering. It could be considered that VRN1 is the final target of the vernalization process (Distelfeld et al., 2009a; Yan et al., 2006).

0.6.3. Genetic control of photoperiod response

The *PHOTOPERIOD RESPONSE LOCI* (*Ppd-1*) are part of the pseudo-response regulators family. Those genes belong to the control mechanism of the circadian clock that interacts with *CONSTANS* (*CO*) genes (Beales et al., 2007; Valverde et al., 2004). It has recently been shown in Arabidopsis that under long days the *CO* gene stimulates flowering. The CO proteins activate the transcription of *FT*, which is responsible for inducing flowering. The evening light stabilises nuclear CO proteins, while morning light or dark conditions facilitate its degradation (Valverde et al., 2004).

Candidate clones of the barley *Ppd-H1* gene were found in chromosomes 2A, 2B and 2D in bread wheat (Beales et al., 2007). From these three genes, *Ppd-D1* has been the most widely used in bread wheat, due to it having a stronger effect than *Ppd-B1,* and the inappreciable effect of *Ppd-A1* (Beales et al., 2007; Worland et al., 1998). In durum wheat, there are two mapped genes in chromosomes 2A and 2B, *Ppd-A1* and *Ppd-B1* respectively. In the absence of the D genome the role of the *Ppd-D1* is replaced by *Ppd-A1*, which has a stronger effect than *Ppd-B1* (Royo et al., 2016; Wilhelm et al., 2009).

There are three known alleles of the *Ppd-A1* gene, the wild type that produces photoperiod sensitivity (*Ppd-A1b*), and two allele causing insensitivity: The GS100 allele has a deletion of 1027bp, and GS105, which has a deletion of 1117bp that partially overlaps with the fragment of GS100 (Wilhelm et al., 2009). The GS100 allele produces a stronger effect on shortening flowering time than GS105 (Royo et al., 2016). The sequence alteration that produces the effect on *Ppd-B1* is less clear. In bread wheat it was not associated with any modification of the sequence, but with the copy number of the gene (Diaz et al., 2012). In durum wheat no copy number has been detected, and two allele are recognised at the moment, the one causing insensitivity (*Ppd-B1a*), and the wild type producing photoperiod response (*Ppd-B1b*) (Royo et al., 2016).

0.6.4. Genetic control of Earliness *per se* (*Eps*) genes

The group of *Eps* genes includes all those genes that have not been clearly associated with vernalization or photoperiod responses (Snape et al., 2001). These genes are therefore not well defined by its control mechanism. It has been postulated that more than a dozen *Eps* could exist in different species of wheat (Griffiths et al., 2009; Snape et al., 2001).

There are some *Eps* genes already located mainly in diploid wheat (*T. monococcum*), such as: *Eps-A^{m}1* (Bullrich et al., 2002; Valárik et al., 2006), which has been associated with the wheat orthologue of Arabidopsis *EARLY FLOWERING 3* (*ELF3*) gene, part of the circadian clock control (Alvarez et al., 2016). This gene has been shown to have significant interactions with temperature for heading date (Bullrich et al., 2002; Lewis et al., 2008). The *Eps-3A^m* has as a candidate gene the wheat orthologue of *Arabidopsis LUX ARRHYTHMO/PHYTOCLOCK 1* (*LUX/PCL1*), which is also associated with the circadian clock regulators (Gawronski et al., 2014).

A large number of *Eps* candidates have been located in different zones of the genome of several wheat species: Nse-5A^m in the chromosome 5 of *T. monococcum* (Shindo et al., 2002); 1DL in the chromosome 1D of *T. aestivum*, and likely an orthologue of *Eps-A^{m}1* (Zikhali et al., 2014); *Eps-2B* located on the chromosome 2B of *T.aestivum* (Herndl et al., 2008; Scarth and Law, 1983), and several quantitative trait loci (QTL) associated with phenology, and distributed along the genome (Griffiths et al., 2009; Maccaferri et al., 2019; Snape et al., 2001, and citations therein). Even though the mechanism that controls these potential genes is not clear, the lack of epistatic interaction between some of the *Eps* genes suggests that more than one path of narrow-sense earliness controls are present in wheat (Shindo et al., 2002).

0.7. CHANGES ON WHEAT PHENOLOGY CAUSED BY BREEDING

A relevant figure in early wheat improvement was N. Strampelli at the end of the XIX century and beginning of the XX. As has been stated by other authors (Salvi et al., 2013; Tommaso and Mugnozza, 2005), this Italian plant breeder played an important role in the subsequent "Green Revolution". Under the necessity of increasing production, he identified the main problems of landraces being lodging, rust, and late flowering. He crossed some bread wheat varieties that had improvements in those characteristics with high yielding ones (Salvi et al., 2013; Tommaso and Mugnozza, 2005). His efforts still have relevance as the haplotype of the variety "Akakomugi" used by Strampelli is still widespread around the world (Guo et al., 2010). Later on, N.E. Borlaug led the period known as the "Green Revolution", in which genetic improvement jointly with agronomical upgrades significantly boosted yield (Borlaug, 2007). In his efforts to fight a stem rust epidemic, time to deliver a resistant variety was a very important factor, so the idea of shuttle breeding was applied, growing two crop seasons in a year. One crop season was grown in a winter cycle in the northwest of Mexico, and the other one in southern places in a summer crop cycle. As a consequence of coincidence the long days of the summer cycle derived into photoperiod

insensitive varieties that afterwards were suitable for their widespread use around the world (Borlaug, 2007).

In durum wheat, the most important programs of improvement were historically held in Italian institutions. The mutation breeding program led by F. D'Amato and G.T. Scarascia made a big step forward in reducing the yielding gap between bread wheat and durum wheat. One of the main objectives of this program was to reduce lodging to make the increase of nitrogen fertilization and disease resistance possible (Scarascia-mugnozza et al., 1993). In this context they obtained the variety Creso, considered the first in considerably reducing the yield gap between durum and bread wheat (Tommaso and Mugnozza, 2005). In those times no specific effort was made to fit phenology, as photoperiod sensitivity was not included as objective in CIMMYT´s program until 1989 (Braun et al., 1996). However, a tendency in earliness from old varieties to modern has been reported (Álvaro et al., 2008).

In Brazilian bread wheat, the changes from old to modern varieties represent a reduction of around 33% of the duration in two phases, from emergence to double ridge, and from double ridge to terminal spikelet. However, the period between terminal spikelet and flowering, and from flowering to maturity was increased by 22% and 7,6%, respectively (Beche et al., 2018). Similar results were found in bread wheat cultivated in Iran, although the grain filling extension was site dependant (Joudi et al., 2014).

In Spanish durum wheat, the period from sowing to flowering has been reduced in modern varieties irrespectively to old varieties, reducing 1.2 days in duration every 10 years of breeding (Álvaro et al., 2008). Similar to the Brazilian bread wheat case, the period from sowing to terminal spikelet was shortened, and the period between booting and flowering was enlarged from old to modern cultivars (Isidro et al., 2011). Although this aspect was not as clear in Italian varieties, a significant correlation was also found between the year of release and the time to flowering (Motzo and Giunta, 2007). Older Italian durum wheat cultivars responded less to vernalization under short daylength than modern cultivars, and a decrease in sensitivity to photoperiod was also observed in modern varieties (Motzo and Giunta, 2007). However, the earliness of flowering was not followed by a clear enlargement of the grain filling period, suggesting that old varieties were better adapted to high temperatures than modern ones (Motzo et al., 2010).

In general, some effect of earlier crop cycles could be attributed to climate change, although the 14 to 18 % change in thermal time was due to genotypic changes (Rezaei et al., 2018). In the context of higher probability of extreme weather events and drought (IPCC, 2014), fitting phenology has become an important aspect of the breeding effort to overcome expected

changes, such as drought (Forster et al., 2004). Early flowering genotypes are expected to avoid final drought, but some authors state that increasing the vegetative phase will increase the root mass and favour drought resistance (Wasson et al., 2012). Therefore, finding an optimal flowering date for each particular environment becomes very important for adaptation to overcome a climate change scenario (Semenov et al., 2014). Phenology control by *Vrn-1* genes is a basic step for adaptation to macro-environments differentiating the sites with frost risk to the apex from those without that risk. The photoperiod response, controlled for *Ppd-1* is a feature that differentiates between latitudes depending on the daylength of each site. In the case of *Eps* the adaptation could be more fine-tuned, which can be a good genetic control allowing the adaptation of the varieties to yearly changes. Consequently, *Vrn-1* and *Ppd-1* are more adequate mechanisms for coarse adjustment of phenology, while *Eps* should be considered for fine phenological adjustment (Zikhali and Griffiths, 2015).

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OBJECTIVES

The general objective of the current PhD dissertation is to analyse the effect of wheat phenology, particularly as affected by photoperiod response, on the yield of spring durum wheat at three sites located at latitudes ranging from 41° to 19° N.

The specific objectives are the following:

1. To study the effect of alleles at *Ppd-1* photoperiod sensitivity loci on wheat phenology, yield and the main yield components: grain number per unit area and grain weight. **Chapter I**
2. To analyse the effect of allele combinations at the *Ppd-A1* and *Ppd-B1* loci on durum wheat phenology, yield formation and yield stability. **Chapter II**
3. To analyse the effect of allele combinations at the *Ppd-A1* and *Ppd-B1* loci on the coefficients of the grain filling curve: grain filling rate, grain filling duration and final grain weight. **Chapter III**
4. To explore the relationships between flowering time due to earliness *per se* and yield formation. **Chapter II**
5. To explore the genetic variability, other than *Ppd-1*, associated with phenology, yield components, and yield itself, and how different molecular markers interact for those traits at contrasting latitudes. **Chapter IV**

The current research has been conducted under the INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, España) – CIMMYT (International Maize and Wheat Improvement Centre, México) agreement framework in the project 'Addressing the challenges for a sustainable wheat production in Spain and North Africa'.

This PhD Thesis is structured in four chapters written as scientific articles, so they can be read as individual entities. The Material and Methods sections may seem repetitive for this reason, as all the results were derived from the same experiments. At the moment of presenting this document, Chapter I was published as a paper in Frontiers in Plant Science 2018, Chapter II was submitted to the European Journal of Agronomy and Chapter III was an article published in the Journal of Agronomy and Crop Science 2019.

CHAPTER I

frontiers in Plant Science

ORIGINAL RESEARCH
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Check for updates

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

Jose M. Arjona[1], Conxita Royo[1], Susanne Dreisigacker[2], Karim Ammar[2] and Dolors Villegas[1]*

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

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Roberto Papa,
Università Politecnica delle Marche, Italy

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Ahmad M. Alqudah,
Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Germany
Ildikó Karsai,
Centre for Agricultural Research (MTA), Hungary

**Correspondence:*
Dolors Villegas
dolors.villegas@irta.cat

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The main yield components in durum wheat are grain number per unit area (GN) and thousand kernel weight (TKW), both of which are affected by environmental conditions. The most critical developmental stage for their determination is flowering time, which partly depends on photoperiod sensitivity genes at *Ppd-1* loci. Fifteen field experiments, involving 23 spring durum wheat genotypes containing all known allelic variants at the PHOTOPERIOD RESPONSE LOCUS (*Ppd-A1* and *Ppd-B1*) were carried out at three sites at latitudes ranging from 41° to 27° N (Spain, Mexico-north, and Mexico-south, the latter in spring planting). Allele GS100 at *Ppd-A1*, which causes photoperiod insensitivity and results in early-flowering genotypes, tended to increase TKW and yield, albeit not substantially. Allele *Ppd-B1a*, also causing photoperiod insensitivity, did not affect flowering time or grain yield. Genotypes carrying the *Ppd-B1b* allele conferring photoperiod sensitivity had consistently higher GN, which did not translate into higher yield due to under-compensation in TKW. This increased GN was due to a greater number of grains spike^{-1} as a result of a higher number of spikelets spike^{-1}. Daylength from double ridge to terminal spikelet stage was strongly and positively associated with the number of spikelets spike^{-1} in Spain. This association was not found in the Mexico sites, thereby indicating that *Ppd-B1b* had an intrinsic effect on spikelets spike^{-1} independently of environmental cues. Our results suggest that, in environments where yield is limited by the incapacity to produce a high GN, selecting for *Ppd-B1b* may be advisable.

Keywords: phenology, *Triticum turgidum* L. var. *durum*, photoperiod sensitivity, double ridge, terminal spikelet, yield components

Abbreviations: DR, double ridge; GN, grain number m^{-2}; GY, grain yield (g m^{-2}); QTL, quantitative trait locus; TKW, thousand kernel weight (g); TS, terminal spikelet.

1.1. INTRODUCTION

Wheat is one of the most widely cultivated crops in the world, with an average annual production, in the last decade, of more than 700 million tons (FAO, 2017). The production forecast for the 2017/18 season is close to 750 million tons, while the consumption is estimated at 720 million tons (FAO, 2017). The expected increase in world population, expected to reach 9.7 billion by 2050, suggests that the global agricultural production has to increase by 25% to 70% from the current levels (Hunter et al., 2017). This considerable challenge is even greater given the expected climate change scenarios. Therefore, further efforts should be devoted to increasing crop productivity, particularly that of wheat, in regions in which this crop is the most important source of calories and protein for humans. The probability of extreme climate episodes with a large effect on crop productivity, such as drought and heat waves, is increasing (IPCC, 2014). To improve wheat production, a mitigating strategy could be the tailoring of plant development cycles in order to avoid or escape from drought or heat events during the most sensitive phases of yield formation. To this end, among others measures, breeding programs could implement selection for a more efficient and precise phenology, maximizing yield in the prevalent environmental conditions (Hunter et al., 2017; IPCC, 2014).

The main yield components of wheat are grain number per unit area (GN) and thousand kernel weight (TKW), which are therefore important targets in breeding programs. However, the negative correlation between them (Sadras, 2007) limits the breeder's capacity to increase net yield via the improvement of these two components individually. When a reduction of this negative correlation has been achieved, grain yield (GY) has increased (Griffiths et al., 2015).

Different environmental conditions, during particular developmental phases, affect yield components differently. Low temperature and long pre-flowering periods favor GN (Prasad et al., 2008; Villegas et al., 2016). Temperatures above 31°C around flowering and the first stages of grain filling may affect grain setting, by reducing anther fertility (Draeger and Moore, 2017), thus reducing GN and consequently GY (Farooq et al., 2011; Ferris et al., 1998; Gibson and Paulsen, 1999). Heat stress during grain filling also negatively affects numerous physiological processes, such as membrane stability and metabolism, ultimately causing a reduction in TKW (Farooq et al., 2011). An increase in night temperature from 17 to 23°C has been reported to accelerate grain filling and decrease kernel weight (Prasad et al., 2008). As a result of its negative effect on photosynthesis and starch deposition (Farooq et al., 2011; Rezaei et al., 2015), heat stress reduces nitrogen mobilization efficiency, which is positively correlated with grain weight (Tahir and

Nakata, 2005). Therefore, for each particular environment, a balance must be found between a flowering time that is late enough to increase GN but not so late that flowering and grain filling take place under high temperature conditions or terminal drought.

After emergence, wheat development starts with leaf initiation. This vegetative phase ends at the double ridge (DR) stage, giving way to the beginning of the reproductive phase (Slafer and Rawson, 1994). Spikelets start to form from the DR to the terminal spikelet (TS) stages. Floret primordia develop during the stem elongation phase, some becoming actual fertile florets while others degenerate (González et al., 2005; Kirby and Appleyard, 1986). The duration of each phase as well as flowering time, is regulated by vernalization requirement, photoperiod sensitivity and earliness *per se* (Hanocq et al., 2004; Kamran et al., 2014). The PHOTOPERIOD RESPONSE LOCUS (*Ppd-1*) genes belong to the pseudo-response regulators family, which play an important role in controlling circadian cycles, increasing the expression of *CONSTANS* (*CO*) proteins under long days. The CO proteins interact with the *FLOWERING LOCUST T* (*FT*) enhancing their expression and promoting flowering (Valverde et al., 2004). This effect has been found in bread wheat (Beales et al., 2007), and in barley for the *Ppd-H1*, with differences in flowering time between different allelic variants ranging from 7 to 12 days' difference (Laurie et al., 1994; Turner et al., 2005). In winter barley, a second photoperiod sensitivity gene (*Ppd-H2*) has been characterized and mapped to chromosome 1 (*HvFT3*). The allele conferring insensitivity upregulates vernalization genes and triggers early flowering under short daylength, in some cases even when the vernalization requirements are not fulfilled (Casao et al., 2011).

In spring durum wheat (*Triticum turgidum* L. var. *durum*), two important genes, *Ppd-A1* and *Ppd-B1* (Laurie, 1997; Maccaferri et al., 2008; Wilhelm et al., 2009) on chromosomes 2A and 2B, respectively, have been found to control flowering time through differential response to photoperiod. The *Ppd-A1* gene has three alleles, two of them considered to confer insensitivity (GS100 and GS105), and the wild type allele, which confers sensitivity (*Ppd-A1b*) (Wilhelm et al., 2009). *Ppd-B1* in durum wheat was mapped to the same region as in bread wheat (Maccaferri et al., 2008), and it has only two known alleles, *Ppd-B1a* and *Ppd-B1b*, conferring sensitive and insensitive responses, respectively (Royo et al., 2016). Both genes affect flowering time but to a different extent. The *Ppd-A1* alleles conferring insensitivity cause a greater reduction in the pre-flowering phase duration than *Ppd-B1*, and among the *Ppd-A1* alleles, GS100 has a stronger effect than GS105 (Royo et al., 2016). Crop phenology can be adjusted to a certain extent, via the manipulation of photoperiod sensitivity genes, to better fit specific prevailing environmental conditions. Variation in these genes may become a tool for breeders to tailor crop phenology in

such a way that the most sensitive developmental phases occur under more favourable conditions.

This study is part of a project designed to analyse the effect of photoperiod sensitivity genes on durum wheat adaptation and productivity. Previous results have recently been published in Royo et al. (2016), Villegas et al. (2016), and Royo et al. (2018). The objective of the present study was to elucidate the effect of photoperiod sensitivity genes *Ppd-A1* and *Ppd-B1* on the formation of the main yield components in durum wheat, namely GN and TKW, and its possible effect on grain yield.

1.2. MATERIALS AND METHODS

1.2.1. Plant Material

Twenty-three spring durum wheat genotypes were used in this study (Supplementary Table 1). Twenty-one of these lines were derived from crosses between five late flowering genotypes from the breeding program of the University of Hohenheim, Germany [Durabon (*Ppd-A1b*, *Ppd-B1a*), 2716-25.94.01 (*Ppd-A1b*, *Ppd-B1a*), Megadur (*Ppd-A1b*, *Ppd-B1a*), 2805-49.94.02 (*Ppd-A1b*, *Ppd-B1b*), 2905-13.93.04 (*Ppd-A1b*, *Ppd-B1a*)] and five early-flowering advanced lines from the CIMMYT-Mexico program [Sooty_9/Rascon_37 (GS105 *Ppd-A1a*, *Ppd-B1a*), Cado/Boomer_33 (GS105 *Ppd-A1a*, *Ppd-B1b*), Dukem12/2*rascon_21 (GS100 *Ppd-A1a*, *Ppd-B1a*), Guanay GS105 *Ppd-A1a*, *Ppd-B1b*) and Snitan GS105 *Ppd-A1a*, *Ppd-B1b*)]. All crosses were advanced in CIMMYT as bulks without selection up to the F_3 Generation. Within these, spikes with highly contrasting heading time were selected and advanced as head rows up to the F_8 generation in Spain. Two well-known commercial cultivars with varying flowering dates were used as controls: Simeto (late-flowering in Mexico and medium to late-flowering in Spain) and Anton (late-flowering in both countries).

1.2.2. Molecular Characterization

Genotypes were analysed with a set of molecular markers detailed in Royo et al. (2016). In summary, genotypes were initially characterized for the *Vrn-1* and *Vrn-3* genetic loci (*Vrn-A1*, *Vrn-B1,* and *Vrn-B3*). Dominant spring alleles were identified in all genotypes on the basis of variation in the promoter and intron-1 region of the *Vrn-A1* locus, which was detected with gene-specific STS markers described by Yan et al. (2004) and Fu et al. (2005).

For *Ppd-A1,* two SNP KASP assays were applied to detect the 1027bp 'GS100' type and 1117 bp 'GS105' type deletion in durum wheat (Wilhelm et al., 2009). For *Ppd-B1*, linked SSR markers

gwm148 and gwm257 as described in Hanocq et al. (2004) were used. In addition, gene-specific KASP assays determining truncated copies, transposon-junction, and allele-specific SNPs observed in cv. 'Sonora64' (containing three copies of *Ppd-B1*), cv. 'Chinese Spring' (carrying four copies of *Ppd-B1*), and cv. 'Cheyenne' (carrying one copy of *Ppd-B1*) were tested to determine whether similar allele variation existed in durum wheat (Diaz et al., 2012). However, no copy number variation of *Ppd-B1* alleles was detected. Following Beales et al. (2007), the photoperiod-insensitive allele was designated as *Ppd-1a*. The alternative allele, which was assumed to confer some photoperiod sensitivity, was arbitrarily designed as *Ppd-1b*.

1.2.3. Experimental Field Setup

The current study involved 15 field experiments that were conducted in 2007, 2008, 2010, 2011, and 2012 at three sites with contrasting latitude: Spain (Gimenells in the north-east), Mexico-north (Ciudad Obregón), and Mexico-south (El Batán Experimental Station in Texcoco, in the Central Mexican Highlands) (Table 1 and Supplementary Table 2). The experiments were arranged in randomized complete block designs with three replications and plots of 12 m^2. Sowing density was adjusted at each site in order to obtain an approximate plant density of 450 spikes m^{-2}. Plots were managed according to the common cultural practices at each site, and were maintained free of weeds, diseases, and pests. Ten experiments were planted in autumn (from November 19 to December 23), while five experiments, corresponding to Mexico-south, were planted in late spring (from May 17 to 28) for a summer crop cycle. Temperatures (absolute maximum and minimum, and mean) and solar radiation (MJ m^{-2} day^{-1}) were recorded by meteorological stations placed within or near the experiments. Photoperiod (including twilight) for the emergence-flowering and flowering-maturity periods were calculated according to Forsythe et al. (1995) (Fig 1). Full irrigation was provided during the whole cycle in Mexico-north and when necessary to avoid water stress at the other two sites (Spain and Mexico-south).

1.2.4. Data Recording

In all experiments, the following developmental stages were determined on the central part of each plot according to the Zadoks' scale (Zadoks et al., 1974): emergence (GS10), flowering (GS65), and physiological maturity (GS87), as indicated by the loss of green colour in the spike peduncle. In the experiments conducted in 2010, 2011, and 2012, additional growth stages were determined on each plot: DR and TS (Kirby and Appleyard, 1986), booting (GS45), and heading (GS55). To assess the DR and TS stages, between 3 and 5 plants per plot were sampled 2 to 3 times a week and examined in the laboratory. Leaves were carefully removed, and the main apex of each plant

was observed under a binocular magnifier and compared with illustrations in Kirby and Appleyard (1986). A plot was considered to reach the DR or TS stages when 2 out of 3 or 3 out of 5 sampled plants were in the selected stage. A plot was considered to have reached a given developmental stage when at least 50% of the plants exhibited the stage-specific phenotypic characteristics. Thermal time (growing degree-days, GDD) was computed by summing averaged maximum and minimum daily temperatures with 0 and 37ºC as base and maximum temperatures, respectively, following Angus et al. (1981).

Table I-1. Relevant geographic and environmental descriptors for the three testing sites.

Site	Location	Experimental station (institution's acronym)	Coordinates		Altitude (m.a.s.l.)	Long-term rainfall (mm/year)	Environmental characteristics
			Lat.	Long.			
Spain	Gimenells, (Lleida)	Gimenells (IRTA)	41° 38'N	0º23'E	200	370	Moderate terminal stress. High to medium productivity
Mexico-north	Ciudad Obregón, (Sonora)	CENEB (CIMMYT)	27° 21'N	109º54'W	40	32	Very high terminal stress. Mandatory full irrigation. Very high productivity
Mexico-south	El Batán, (Texcoco)	El Batán (CIMMYT)	19° 31'N	98º50'W	2249	500	Initial stress eliminated with irrigation. Medium productivity

In all experiments (2007, 2008, 2010, 2011, and 2012), plots were divided into two sections of 6 m^2, one of which was used for destructive sampling, while the other one was left untouched and was mechanically harvested at commercial maturity. Grain yield (GY, g m^{-2}) was obtained, and subsequently adjusted to dry weight basis. Thousand kernel weight (TKW, g) was obtained by weighing a randomly drawn sample of 200 kernels from the harvested grain of each plot. The number of grains m^{-2} (GN) was calculated as the ratio of GY to TKW.

Additionally, in experiments performed in 2010, 2011, and 2012, a 1-m-long sample of representative central rows was taken, the spikes were counted and threshed, and their grains were counted. Spikelets $spike^{-1}$ were calculated as the average value of five main spikes randomly chosen on each sample. Grains $spike^{-1}$ was obtained by dividing the number of grains of the sample by the spike number. Grains $spikelet^{-1}$ was calculated as grains $spike^{-1}$ divided by spikelets $spike^{-1}$. Daylength from DR to TS (h) was calculated for each plot in the 2010, 2011, and 2012 experiments by averaging photoperiod between these two developmental stages. Maximum and minimum temperature at flowering ($T_{max}F$ and $T_{min}F$ ºC, respectively) were determined for each plot as the mean of the maximum or minimum temperatures recorded from 5 days before to 5 days after flowering date.

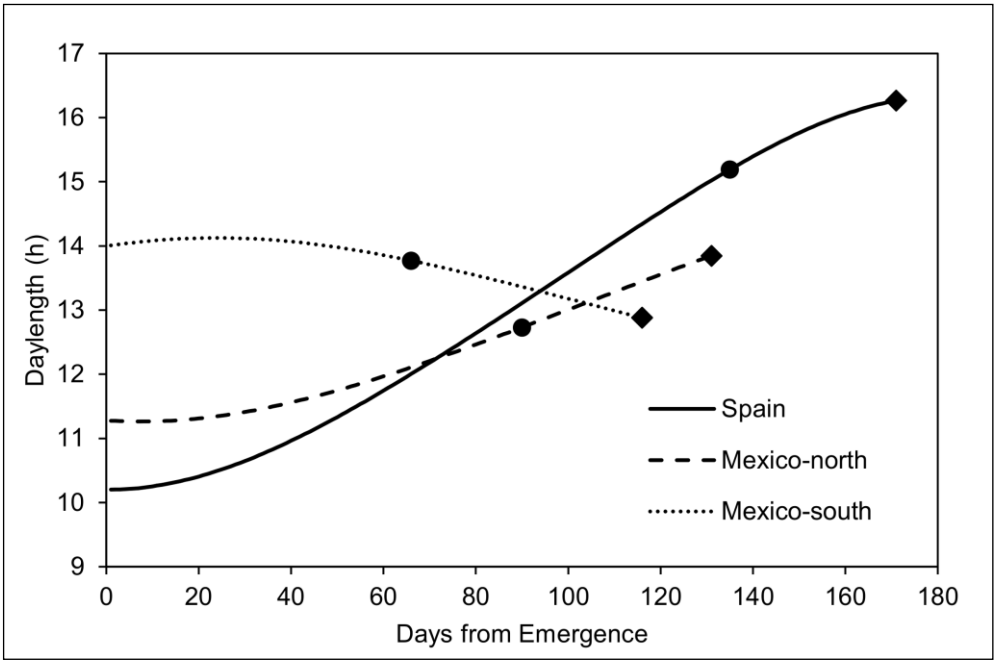

Figure I.1. Mean daylength (average of five years) during the crop cycles observed at the three testing sites, and mean duration of the emergence-flowering and flowering-maturity phases at each site. Dots: flowering time; Diamonds: maturity time.

1.2.5. Statistical Analysis

Combined ANOVAs were performed across experiments using the GLM procedure of the SAS statistical package (SAS, RRID:SCR_008567), considering year and genotype as random factors. The sum of squares of the genotype effect was partitioned into differences attributable to allelic variants at *Ppd-A1* and *Ppd-B1* (between allelic classes), and variability between genotypes within allelic classes. The error term used to test *Ppd-1* loci was the sum of squares of genotype within each locus. Means were compared using protected Fisher´s LSD (least significant differences) method at $P = 0.05$, using the sum of squares of genotype within each locus as the error term. A mixed model considering genotype, year and their interactions as random factors was also run using the Kenward-Roger correction, in order to check for the robustness of the significance of the effect of allele variants at *Ppd-A1* and *Ppd-B1* considering the different number of genotypes within each genetic group. Correlation analysis was performed with the pairwise correlation method used by default in JMP 12 Pro® (JMP, RRID:SCR_014242).

Table I-2. Allelic variants at *Ppd-A1* and *Ppd-B1* present in a collection of 23 spring durum wheat genotypes obtained through a divergent selection process for flowering time.

Genes	Alleles*	Photoperiod response	Number of genotypes
Ppd-B1			

	Ppd-B1b	Sensitive	9
	Ppd-B1a	Insensitive	14
Ppd-A1			
	Ppd-A1b	Sensitive	10
	GS105	Insensitive	10
	GS100	Insensitive	3

* Nomenclature described in Wilhelm et al. (2009)

1.3. RESULTS

1.3.1. Molecular Characterization

Table 2 shows the allelic composition at the *Ppd-A1* and *Ppd-B1* loci of the 23 genotypes used in this study. A previous study (Royo et al., 2016) showed that all genotypes were spring types, and a more detailed description of the molecular markers used can be found therein.

1.3.2. Environmental Conditions

Figure 1 shows the mean photoperiod from emergence to physiological maturity across the five years of experiments. In the autumn-sown experiments (Spain and Mexico-north), photoperiod increased during most of the crop cycle. In the late spring planting it increased slightly at the beginning, but decreased during most of the cycle. The mean length of the pre-flowering phase was 1218 GDD (135 days) in Spain, 1440 GDD (90 days in Mexico-north) and 1122 GDD (66 days) in Mexico-south. With regard to the duration from flowering to maturity, Spain had the shortest period with 692 GDD (36 days), followed by Mexico-north with 816 GDD (41 days), and Mexico-south, with 836 GDD (50 days, Fig.1).

1.3.3. Effect of *Ppd-1* Allelic Variants

The graphical ANOVA (Fig. 2) shows that site and genotype were the most important main factors affecting the studied traits, except for thermal time from flowering to maturity, which was affected mostly by the Site x Year interaction. TKW was the least influenced by the site (14.1%), while this source of variation was the most important for all remaining traits except thermal time from flowering to maturity. The site consistently explained a considerably larger proportion of the total variability than the year, the effect of the latter being significant only for yield. Genotypic variation explained a highly variable proportion of the total variability, very little for grain filling period, little in the case of GY, intermediate for GN and pre-flowering thermal time, and very high for TKW. Analyses of variance of the pre-flowering phases were also performed with data of experiments conducted in 2010, 2011, and 2012 (Supplementary Table 3).

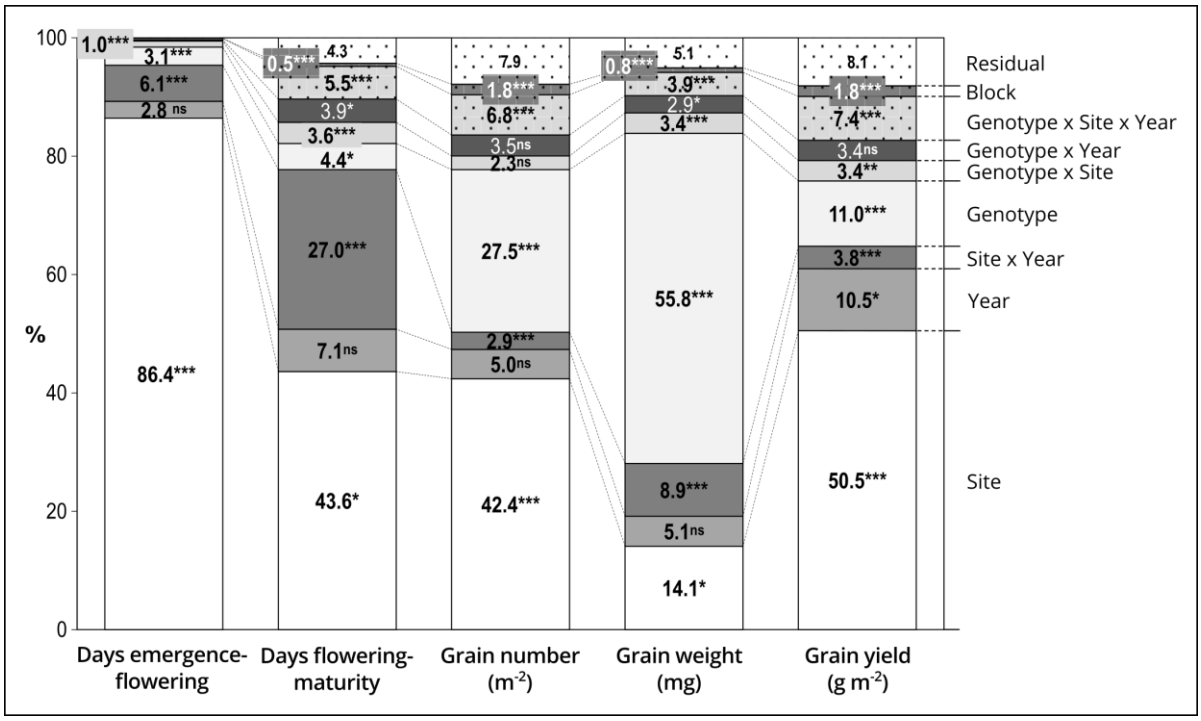

Figure I.2. Percentage of the total sum of squares corresponding to the different sources of variation in the ANOVA model obtained from the evaluation of 23 durum wheat genotypes grown in three sites of contrasting latitude during five years. *P<0.05; **P<0.01; ***P<0.001.

The genotype effect was further partitioned into differences that could be explained by variation between *Ppd-A1* allelic groups and differences within *Ppd-A1* allelic classes. The same partition was calculated for the *Ppd-B1* locus. The results shown in Table 3 indicate that differences between genotypes carrying the same allele (within allelic class variation) were generally greater than differences among allelic variants at *Ppd-A1* and *Ppd-B1* loci (between allelic classes variability) (Table 3).

The allelic composition at *Ppd-A1* did not explain any variation in yield, yield components, or grain filling duration. However, it significantly influenced pre-flowering duration. The *Ppd-A1* x Site interaction was significant only for phenology variables.

Allelic differences at *Ppd-B1* explained 7.2% and 10.8% of variations in GN and TKW, respectively, but did not significantly account for the differences observed in GY. On the other hand, the *Ppd-B1* x Site interaction was significant for GY.

Using the mean values of the 23 genotypes at each site across three replicates, and over five years, a strong negative correlation was found between GN and TKW at all latitudes, while the correlation between TKW and GY was positive only in Spain. No correlation was found between GN and GY at any of the study sites (Table 4).

Table I-3. Percentage of the genotype sums of squares from the ANOVA partitioned in differences between allelic variants at *Ppd-A1* and *Ppd-B1* genes and the differences within genotypes carrying a given allele. GDD: growing degree-days ns: non-significant; *P<0.05; **P<0.01; ***P<0.001.

Source of variation	d.f.	GDD emergence-flowering	GDD flowering-maturity	Grains m^{-2}	Thousand kernel weight (g)	Grain yield (g m-2)
Genotype	22	26.4 ***	5.5 *	27.5 ***	55.8 ***	11 ***
		Genotype sum of squares partition by *Ppd-A1*				
Between *Ppd-A1*	2	10.7 **	0.1 ns	0.5 ns	3.4 ns	1.9 ns
Within *Ppd-A1*	20	15.7 ***	5.4 ***	27.0 ***	52.4 ***	9.1 ***
		Genotype sum of squares partition by *Ppd-B1*				
Between *Ppd-B1*	1	1.5 ns	0.0 ns	7.2 *	10.8 *	0.0 ns
Within *Ppd-B1*	21	24.9 ***	5.5 ***	20.3 ***	45.0 ***	11.0 ***
Site x Genotype	44	9.3 ***	4.0 ***	2.3 ***	3.4 ***	3.4 **
		Site x Genotype sum of squares partition by *Ppd-A1*				
Between *Ppd-A1* x Site	4	2.1 *	1.5 ***	0.2 ns	0.7 ns	0.6 ns
Within *Ppd-A1* x Site	40	7.2 ***	2.5 *	2.1 ns	2.7 ***	2.8 *
		Site x Genotype sum of squares partition by *Ppd-B1*				
Between *Ppd-B1* x Site	2	0.2 ns	0.3 ns	0.0 ns	0.2 ns	0.6 *
Within *Ppd-B1* x Site	42	9.1 ***	3.7 ***	2.3 ns	3.2 ***	2.8 **

At *Ppd-A1,* allele GS100 was associated with the shortest emergence to flowering period, and *Ppd-A1b* the longest. None of the alleles affected the grain filling period (Table 5). The mean maximum temperature to which the crop was exposed five days before and after flowering also differed depending on the allele variant at *Ppd-A1*, with the lowest values corresponding to genotypes carrying GS100 allele. However, in no cases in this study did the temperature reach values that are considered to affect optimal seed set.

Table I-4. Pearson's correlation coefficients between yield (GY), grain number (GN) and thousand kernel weight (TKW), for experiments involving 23 durum wheat genotypes (n=23) and conducted at 3 sites over 5 years. ns: non-significant; ***P<0.001.

	Pearson's correlation coefficients		
Site	GN-TKW	GN-GY	TKW-GY
Spain	-0.88 ***	-0.27 ns	0.68 ***
Mexico-north	-0.79 ***	0.30 ns	0.32 ns
Mexico-south	-0.72 ***	0.40 ns	0.33 ns

Ppd-B1 alleles did not affect flowering date, but genotypes carrying the *Ppd-B1b* allele had higher GN with lower TKW than those with *Ppd-B1a*, which resulted in a similar average yield for the two allelic classes (Table 5). This *Ppd-B1*-related allelic effect on GN and TKW was consistent at all sites, with the exception of TKW in the summer crop of Mexico-south, for which the difference between the two allelic variants was not statistically significant (Table 6). In spite of these consistent differences in GN and TKW between the two allelic classes at *Ppd-B1*, the corresponding difference in yield was not statistically significant at any site.

Table I-5. Mean values (and coefficient of variance between brackets) across sites and years for thermal time emergence-flowering (GDD_{EF}) and flowering-maturity (GDD_{FM}), maximum ($T_{max}F$) and minimum temperature around flowering ($T_{min}F$), yield components and yield for each *Ppd-A1* and *Ppd-B1* allele.

Gene	Alleles	GDD_{EF} (°C)	GDD_{FM} (°C)	$T_{max}F$ (°C)	$T_{min}F$ (°C)	Grains m^{-2}	Thousand kernel weight (g)	Grain yield (g m^{-2})
Ppd-A1								
	Ppd-A1b	1324 (6.6) a	781 (3.4) a	25.5 (1.8) a	9.1 (1.0) b	13903 (2.7) a	41.0 (14.9) a	535 (8.9) a
	GS105	1225 (5.5) ab	782 (4.3) a	24.9 (2.2) ab	9.1 (3.6) ab	14493 (17.0) a	40.8 (17.6) a	545 (7.5) a
	GS100	1168 (5.3) b	788 (6.4) a	24.6 (1.4) b	9.6 (4.8) a	13991 (16.6) a	45.6 (15.5) a	600 (12.8) a
Ppd-B1								
	Ppd-B1b	1287 (7.2) a	782 (2.6) a	25.3 (2.3) a	9.3 (4.3) a	15529 (15.6) a	38.0 (16.6) b	549 (10.3) a
	Ppd-B1a	1243 (7.6) a	783 (4.8) a	25.0 (2.3) a	9.4 (4.8) a	13299 (11.5) b	43.8 (13.5) a	547 (8.9) a

$T_{max}F$: mean of the maximum temperatures of 5 days before and after flowering.
$T_{min}F$: mean of the minimum temperatures of 5 days before and after flowering.
Different letters between alleles at each gene indicate differences according to LSD test at P<0.05.
The number of genotypes carrying each allele is shown in Table 2.

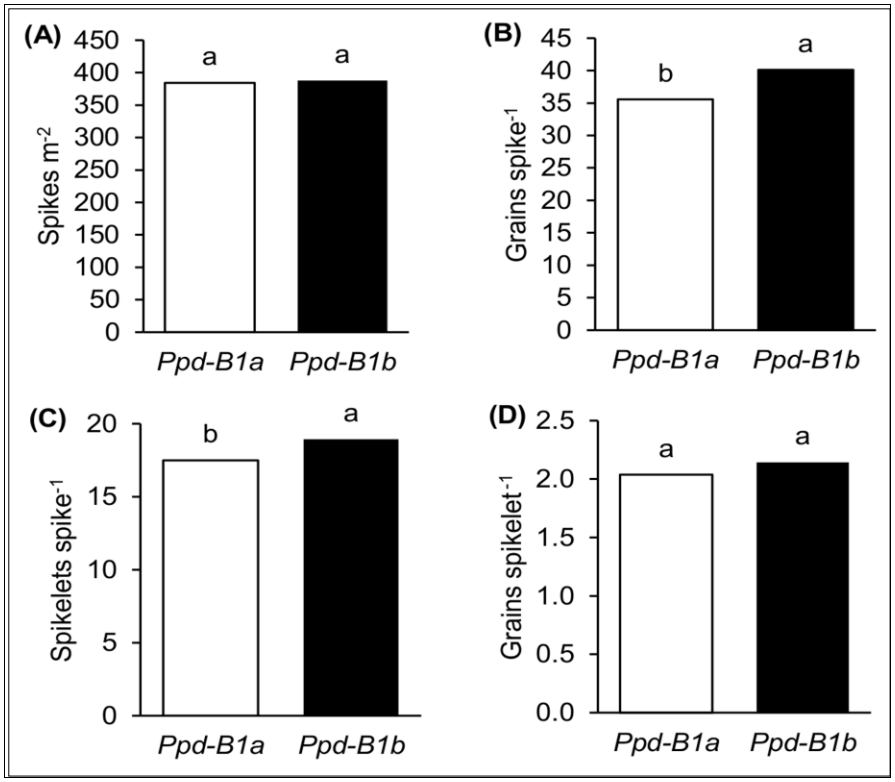

Figure I.3. Detailed yield components of 23 durum wheat genotypes grown in three sites of contrasting latitude during 2010, 2011 and 2012: (A) spikes m^{-2}, (B) spikelets $spike^{-1}$, (C) grains $spikelet^{-1}$, and (D) grains $spike^{-1}$. Each bar represents mean values of genotypes carrying *Ppd-B1a* or *Ppd-B1b*. Different letters indicate differences according to LSD test at P<0.05.

Detailed data on spike characteristics determined in the nine experiments conducted in 2010, 2011, and 2012 were used to elucidate the possible basis underlying the effects of *Ppd-B1* on GN. Results showed that genotypes carrying the *Ppd-B1b* allele had greater GN because they had more grains $spike^{-1}$, since the number of spikes per unit area was similar for the two allelic groups (Fig. 3 A, B). The dissection of grains $spike^{-1}$ into its individual components showed that the number of

spikelets spike^{-1} was related to its increase and not the number of grains spikelet^{-1} (Fig. 3 C, D). The effects of *Ppd-B1* on detailed yield components shown in Figure 3 were also observed at each site independently (Suppl. Fig. 1).

Table I-6. Mean values for yield components for each *Ppd-B1* allele. Values are means of experiments conducted over five years at each site. Different letters indicate differences according to LSD test at P<0.05.

Ppd-B1	**Grains m^{-2}**			**Thousand Kernel Weight (g)**			**Grain yield (g m^{-2})**		
	Spain	Mexico-north	Mexico-south	Spain	Mexico-north	Mexico-south	Spain	Mexico-north	Mexico-south
Ppd-B1b	19212 a	14135 a	13321 a	37 b	42 b	35 a	664 a	546 a	437 a
Ppd-B1a	17113 b	11820 b	11001 b	43 a	48 a	40 a	693 a	538 a	412 a

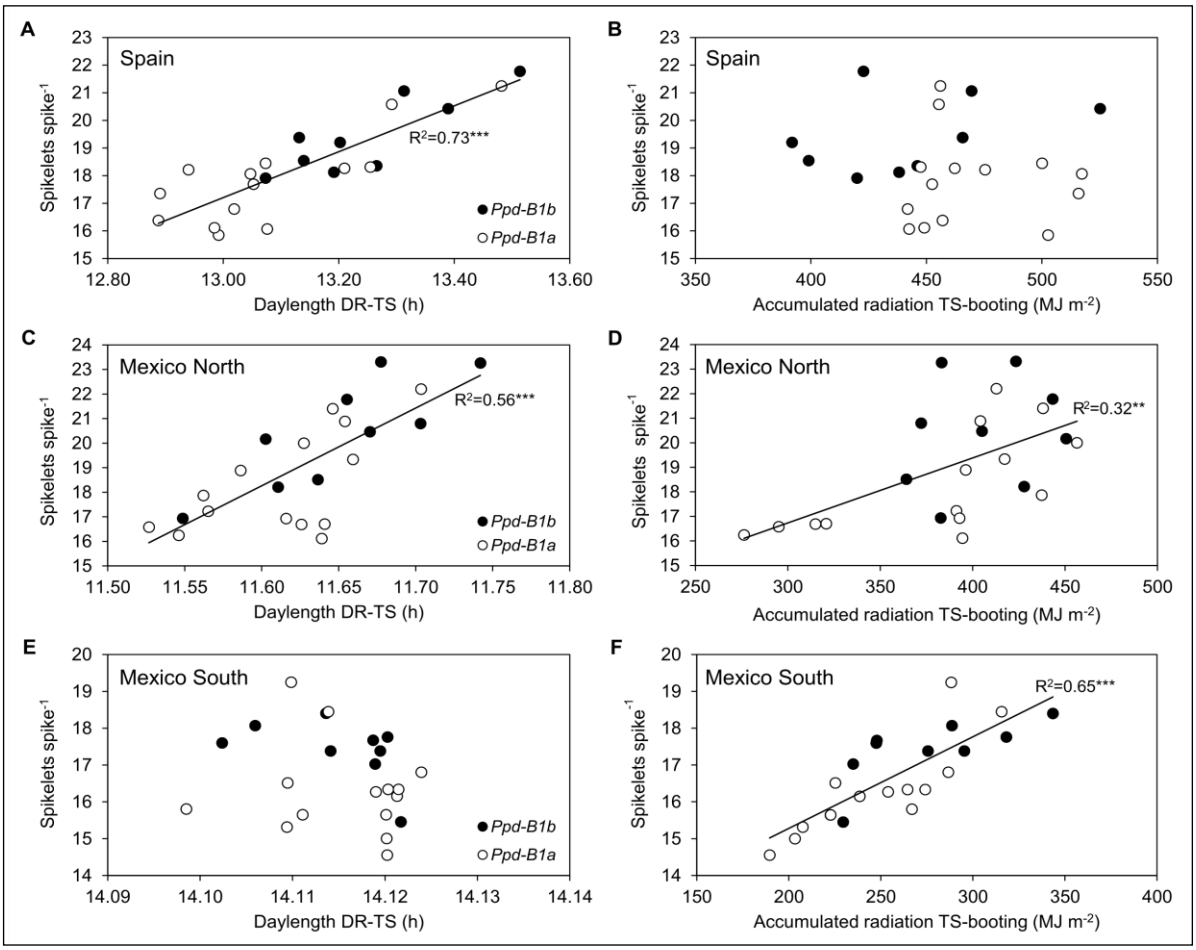

Figure I.4. Relationship between mean daylength (h) from double ridge to terminal spikelet (A, C, E) and from terminal spikelet to booting (B, D, F) and spikelets spike^{-1} for the 23 durum wheat genotypes carrying the *Ppd-B1a* (○) and *Ppd-B1b* (●) alleles, for each site across three years.

The length of the pre-flowering phases was analysed in the nine experiments conducted from 2010 to 2012. Differences between genotypes carrying *Ppd-B1a* and *Ppd-B1b* alleles were not statistically significant for the thermal time of each pre-flowering phase (Supplementary Tables 4 and 5). Genotypes carrying the *Ppd-B1b* allele tended to have longer phase duration than those

with *Ppd-B1a* in all developmental stages except for the duration from heading to flowering (Supplementary Table 5), which tended to be slightly longer in genotypes carrying the *Ppd-B1a* allele.

Given that the number of spikelets spike^{-1} is mainly determined in the phase from DR to TS, a linear regression was fitted for each site to the relationship between the average daylength during this phase and the number of spikelets spike^{-1}. The results showed that the average daylength during the DR to TS phase explained 73% of the variation observed in the number of spikelets spike^{-1} in Spain and 56% in Mexico-north (Fig. 4). However, *Ppd-B1* was significantly associated with differences in daylength during the DR to TS phase only in Spain ($P < 0.05$).

1.4. DISCUSSION

Most studies on the relationship between wheat adaptation and genes that confer photoperiod sensitivity have been conducted in bread wheat (Beales et al., 2007; Diaz et al., 2012; González-Navarro et al., 2015). Only a small number of papers (Maccaferri et al., 2008; Royo et al., 2016) have addressed the effect of *Ppd-1* genes on durum wheat yield and yield components. The current study was designed to unravel the main effects of *Ppd-A1* and *Ppd-B1* alleles on yield and its main components in durum wheat.

Experiments were conducted over five years at three contrasting latitudes, a factor that explained a high percentage of total variance in the ANOVA for thermal time from emergence to flowering, GN and GY, and a low, but significant, percentage of the variance for TKW. The influence of the main environmental variables on yield and yield components at the sites included in this study has been previously reported by Villegas et al. (2016).

The genotype effect accounted for a high percentage of the total variance of the model and was significant for all the traits studied. However, the fraction of effects attributable to *Ppd-1* was minor for yield components, but more important for thermal time from emergence to flowering. This result is in agreement with the genetic regulation of phenology and yield components previously stated by several authors (Kamran et al., 2014; Maphosa et al., 2014; Wang et al., 2011). As reported previously by Royo et al. (2016), the effect of *Ppd-A1* on durum wheat phenology was stronger than that of *Ppd-B1*. Alleles conferring photoperiod insensitivity were linked to earlier flowering time, with the GS100 allele conferring more earliness than GS105, in agreement with previous studies (Royo et al., 2016; Wilhelm et al., 2009). The percentage of total variance explained by allelic variants at *Ppd-A1* and *Ppd-B1* (between allelic classes) was small compared

with the variation existing between genotypes carrying the same allele (within allelic classes). For phenological traits, this within-classes variation can be attributed to *Eps* genes (Royo et al., 2016). In bread wheat, *Eps* genes have been estimated to be responsible for 5% of the genetic variability for heading time, whenever vernalization and photoperiod genes were also acting (Kamran et al., 2014). In other reports, when vernalization requirements were fulfilled and their effects accounted for, around 50% of genetic variation was attributed to intrinsic earliness (Cane et al., 2013; Eagles et al., 2010). In the case of the genotypes and the environments used in the present study, the percentage of variation attributable to genetic factors unrelated to *Vrn/Ppd* was over 50% for thermal time to flowering. This observation suggests that the influence of putative *Eps* genes on phenology in spring durum wheat would be of the same magnitude as in bread wheat.

The lack of a significant effect of allelic variation at *Ppd-A1* on GN and TKW may be due to the fact that these yield components are regulated by several QTLs (Wang et al., 2011), and the *Ppd-A1* gene act as modifier through the modification of growth cycle length. The substantial genotypic variance for GN and TKW observed in this study (Fig. 2) further supports this hypothesis. Several authors have reported a strong influence of phenology on GY and have detected additional variation explained by several genomic regions affecting yield and yield components (Edae et al., 2014; Kamran et al., 2014; Maphosa et al., 2014; Wang et al., 2011;Maccaferri et al., 2008).

Early flowering, due to the presence of alleles causing photoperiod insensitivity at *Ppd-A1*, would be expected to affect the yield components as noted by other authors (Kamran et al., 2014; Maphosa et al., 2014; Slafer et al., 2005), with insensitive types increasing yield and yield components at low to medium latitudes, such as those studied here (Kamran et al., 2014; Maphosa et al., 2014, and cites therein). However, in the current study, differences between alleles at *Ppd-A1* were not statistically significant for yield or yield components, even though, numerically, genotypes carrying allele GS100 yielded 12% more than those carrying the photoperiod sensitive allele *Ppd-A1b*.

However, differences in GN between allelic variants at *Ppd-A1* were small, possibly due to a compensation between the effects caused by alleles conferring photoperiod insensitivity on the potential number of grains and grain setting. It is known that a long pre-flowering period allows the crop to accumulate more biomass at flowering (Royo et al., 2018), produce a high number of grains, and gives it the chance to develop and allocate more resources to reproductive structures (Sinclair and Jamieson, 2006). Accordingly, genotypes carrying the GS100 allele would have a lower potential number of grains than the sensitive types. On the other hand, early flowering occurring under cooler temperatures is expected to be more favourable for grain filling at low latitudes,

where the high temperatures reached during the spring and summer may be limiting for grain setting. The optimal temperatures for flowering are considered to range between 18ºC and 21ºC (Porter and Gawith, 1999), and high temperatures can produce sterility, thereby reducing grain setting (Draeger and Moore, 2017). In the current study, genotypes carrying the GS100 allele experienced the lowest maximum temperatures at flowering, thus favouring superior grain setting. Therefore, the compensation between the reduction in the potential GN caused by a short pre-flowering period, and the theoretical increase in GN due to a superior grain setting favoured by cooler temperatures at flowering could explain the lack of significance of the effect of alleles causing photoperiod insensitivity at *Ppd-A1* on GN.

A shortening of the pre-flowering period is generally associated with a longer flowering-maturity period in some environments (Royo et al., 2016), and TKW could be expected to increase in earlier genotypes due to a longer grain filling period (Joudi et al., 2014). In the current study, differences in the duration of the grain filling period between genotypes with different alleles at *Ppd-A1* were minimal and not significant, with an average of 19 GDD (equivalent to one day), and therefore did not have a significant effect on TKW. Nevertheless, genotypes carrying the GS100 allele produced grains that were, numerically, 11% heavier than those carrying GS105 and *Ppd-A1b*. As 55.8% of the variation in TKW was explained by the genotype effect, with the sum of the site and year effect and their interactions accounting for 28.1% of the total variation for this trait, our results suggest that genetic factors other than photoperiod sensitivity caused the variations observed in TKW.

The results of the current study indicate that the *Ppd-A1* gene did not have a significant effect on the formation of yield components, but that early-flowering genotypes tended to produce more yield than late ones. The strong effect of *Ppd-A1* on flowering time was not translated into a greater GY, as reported by Maccaferri et al. (2008), who found a few environments across the Mediterranean Basin where early flowering was associated with higher yield. A previous study involving the germplasm used herein demonstrated that the limiting factor for attaining high yield was the capacity of the crop to photosynthesize during the grain filling period (Royo et al., 2018). It is well known that hot and dry conditions after flowering limit the capacity of the crop to support grain filling from transient photosynthesis (Bidinger et al., 1977; Ehdaie et al., 2008; Royo et al., 2018). The current study was conducted under irrigation, thus preventing the drought stress typical of many durum wheat growing environments. Under severe terminal drought stress, early-flowering genotypes would yield significantly more than the late ones, as reported by other authors (Kamran et al., 2014; Maccaferri et al.,2008; Maphosa et al., 2014; Royo et al., 2018, and references therein). In summary, the results of this study suggest that, in the absence of

knowledge of other known and well characterized factors, the presence of allele GS100 could be the most suitable for maximizing yield in environments considered to be close to optimal in terms of water availability.

Ppd-B1 had a non-significant effect on the flowering time, much smaller than that observed for *Ppd-A1*, in agreement with previous studies (Maccaferri et al., 2008; Royo et al., 2016). Since the effect of *Ppd-B1* on the duration of the emergence-flowering period was not significant, we studied in detail the different phases of this period. The results showed that, when measured in thermal time, none of the phases was significantly different for the two *Ppd-B1* allelic variants, but the *Ppd-B1a* tended to accelerate the initiation of DR stage and the remaining phases with it, as reported in bread wheat (Tanio and Kato, 2007).

However, differences between allelic variants at *Ppd-B1* were significant for both GN and TKW, with photoperiod sensitive genotypes consistently having a higher GN at all sites than the insensitive ones. Therefore, the higher GN achieved by genotypes carrying the *Ppd-B1b* allele would result in higher GY in environments that can satisfactorily sustain the adequate filling of a high number of grains. Genetic gains in GY have been historically been achieved by increasing GN under optimal conditions (Peltonen-Sainio et al., 2007). However, the TKW of genotypes carrying the *Ppd-B1b* allele was proportionally lower, counteracting the effect of higher GN, resulting in a GY equal to that of genotypes carrying the *Ppd-B1a* allele. These results, and the negative correlation between yield components found in the current study and previous ones (Bustos et al., 2013; Griffiths et al., 2015; Sadras, 2007; Sinclair and Jamieson, 2006), exemplify the well documented compensation effect between yield components and the difficulty of breeding for any of them individually and apart with the hope of dramatically increasing . The positive correlation between TKW and GY found in Spain has also been observed previously (García del Moral et al., 2003; Villegas et al., 2016).

A detailed analysis indicated that, when compared with the allele inducing photoperiod insensitivity, the higher GN of genotypes carrying the allele *Ppd-B1b* was not due to a different number of spikes per unit area, but to a greater number of grains spike^{-1} as a result of a greater number of spikelets spike^{-1}, as supported by the observation that grains spikelet^{-1} was not affected by *Ppd-B1* allele variation. Lewis et al. (2008) reported a similar effect attributed to *Eps-A^{m}1* in *Triticum monococcum* L., where different alleles were found responsible for early development differences linked to the number of spikelets spike^{-1}. In their study, however, *EpsA^{m}1* was also linked to significant differences in days to heading, as well as differences in days from sowing to DR and from DR to TS, which may constitute a difference between the action of *Ppd-B1*

in durum wheat and *Eps-A^{m}1* in *T. monococcum*. Edae et al. (2014) found at least two QTLs for number of spikelets spike^{-1} in chromosome 2B. Gao et al. (2015) identified a QTL for floret primordia in the same chromosome, suggesting that additional genes close to *Ppd-B1* may also have a role in determining the final number of spikelets spike^{-1}. The direct *Ppd-B1* effect on the number of spikelets spike^{-1} has not been reported in bread wheat. This could be attributed to the important role of the *Ppd-D1* gene (absent in durum wheat) in the determination of spikelets spike^{-1} through control of the expression of *FLOWERING LOCUS T* (*FT*) at early development stages, making any smaller effect of *Ppd-B1*. Since *Ppd-1* genes are part of the family of pseudo-response regulator (PRR) genes, which affect the circadian clock and control the flowering process (Boden et al., 2015), we hypothesize that, in durum wheat, in the absence of the influence of *Ppd-D1*, *Ppd-B1* plays a more important role, thus becoming a possible breeding target of choice to increase spikelets spike^{-1} in environments where this change could be advantageous. In bread wheat Maphosa et al. (2014) found that *Ppd-B1b* was associated with a higher GN, and lower TKW, than *Ppd-B1a*, which is consistent with the results obtained in durum wheat in the current study.

Our results suggest that the largest number of spikelets spike^{-1} due to the *Ppd-B1b* allele observed at the three sites had different causes. The initiation of the spikelet primordia occurs around the DR stage, and ends at the TS stage (Porter et al., 1987) and this phase duration was linked to photoperiod in Spain. Moreover, the development of spikelets continues after their initiation in a manner that is influenced by the environment, as observed in both Mexico sites. In Spain, genotypes carrying *Ppd-B1b* reached the DR stage later than those carrying *Ppd-B1a*, and were therefore exposed to a longer photoperiod during part of the DR-TS phase. Accordingly, the high accumulated radiation after this phase did not limit spikelet formation. In contrast, in the spring planting in Mexico-south, daylength during the DR-TS phase was similar for all genotypes regardless of their allelic constitution and radiation from TS to booting therefore must have determined the differences in spikelets spike^{-1}. Mexico-north showed an intermediate behaviour relative to the other two sites. A longer photoperiod during the DR-TS phase meant more hours of light during spikelet initiation. A possible explanation is that there is a more positive balance between photosynthesis and photorespiration when days are longer during this period, thereby improving the photosynthate source and allowing the plant to increase the sink capacity. However, at both Mexico sites, these environmental effects were unrelated to the significant effect of *Ppd-B1* on final spikelets spike^{-1}, since environmental variables shown in Figure 4 were not significantly different for *Ppd-B1* allelic classes.

Temperature, photoperiod, and their interaction affect the number of spikelets spike^{-1} by determining variations in the duration of the vegetative and early reproductive phases. Slafer and Rawson (1994) reported that the duration from DR to TS decreased with increasing temperature, up to a limit of 19°C. Above this threshold, the duration decreased. They observed the same trend for the number of spikelets spike^{-1}. On the other hand, shorter daylength has been associated with a higher number of days from sowing to DR and TS, and more spikelets spike^{-1} (Allison and Daynard, 1976; Miralles et al., 2000). Halse and Weir (1974) considered the interaction between temperature and photoperiod and found that short photoperiods (9 h) were associated with a high number of spikelets spike^{-1}, independently of the temperature. They also found that the most favourable temperature during this photoperiod was 15°C, in agreement with Slafer and Rawson (1994) and Allison and Daynard (1976). Our results show that Mexico-north, with a short photoperiod and mild temperatures during the emergence-DR period also had more spikelets spike^{-1} than Mexico-south and Spain. Mexico-south, on the other hand, experienced the longest daylength and showed the smallest number of spikelets spike^{-1}. In barley, Ejaz and von Korff (2017) reported an effect of temperature on the regulation of *Ppd-H1*, affecting the shoot apex development. They found that, at the same temperature, the *Ppd-H1* allele conferring sensitivity had an advanced apex development compared with the allele conferring insensitivity, and also reported a qualitative interaction with temperature. A higher number of spikelets spike^{-1} for the *Ppd-B1b* allele was consistently observed at all sites, but durations of pre-flowering phases and meteorological variables in these phases could not explain the effect of *Ppd-B1*. These observations would indicate that, in durum wheat, *Ppd-B1* acts like *Ppd-H1* in barley, as indicated by Ejaz and von Korff (2017).

The results of the present study suggest that *Ppd-B1* intrinsically affects the number of spikelets spike^{-1}, independently of environmental conditions. Boden et al. (2015) found relationships between *Ppd-1* genes and spikelet development in wheat, while other authors have located QTLs related to grain number in chromosome 2 close to the *Ppd-B1* position (Gao et al., 2015; Shi et al., 2017). Future studies will be useful to elucidate the exact mechanism underlying the interaction between *Ppd-B1* alleles and the determination of the number of spikelets spike^{-1}.

ABBREVIATIONS

GN, grain number m^{-2}

TKW, thousand kernel weight (g)

GY, grain yield (g m^{-2})

DR, double ridge

TS, terminal spikelet

QTL, quantitative trait locus

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AUTHOR CONTRIBUTIONS

CR, KA and DV designed the experiments. KA and DV purified and increased the germplasm. JA, CR, SD, KA and DV performed field evaluations, laboratory determinations and/or data analyses. CR, KA, and DV conceived the manuscript. JA, DV and CR wrote the manuscript. JA, CR, SD, KA and DV read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CHAPTER II

The influence of photoperiod genes and flowering time on yield and yield stability of durum wheat

ABSTRACT

The current study analysed the effect of flowering time, including the effect of photoperiod insensitivity or earliness *per se* (*Eps*), on yield and yield stability in durum wheat. Twenty-three spring durum wheat genotypes with different allele combinations at the photoperiod response loci (*Ppd-A1/Ppd-B1*) and with strong *Eps* were grown in 15 field experiments at three sites at latitudes ranging from 41° to 19° N (Spain, Mexico-North and Mexico-South). The low temperature and solar radiation before flowering and long daylength during grain filling characteristic of the Spanish site resulted in a high number of grains per unit area (GN) and yield (GY), while the short daylength before flowering combined with high temperature and solar radiation during grain filling characteristic of Mexico-North led to heavier grains. Allele combination GS100 *Ppd-A1a/Ppd-B1a* caused the earliest flowering time, reducing it by nine days on average in comparison with *Ppd-A1b/Ppd-B1a*. Owing to a compensation between GN and grain weight (GW), differences in flowering time caused by *Ppd-A1/Ppd-B1* allele combinations had no significant effect on yield. Allele combinations GS105*/Ppd-B1b* and *Ppd-A1b/Ppd-B1b* had the highest GN, because of a boost in the number of grains spike^{-1}, which was higher in the former because of a greater number of grains spikelet^{-1}. Flowering time due to *Eps* had a minor effect on GN and its components spikes m^{-2} and grains spike^{-1}, but late flowering resulted in reduced GW and GY. Allele combinations that carry alleles conferring a similar photoperiod sensitivity response at *Ppd-A1* and *Ppd-B1* (GS100 *Ppd-A1a/Ppd-B1a*, GS105*/Ppd-B1a* and *Ppd-A1b/Ppd-B1b*) resulted in greater yield stability than combinations that carry alleles conferring a different response (GS105*/Ppd-B1b* and *Ppd-A1b/Ppd-B1a*). Allele combination GS100 *Ppd-A1a/Ppd-B1a* was the most suitable for the study sites in terms of yield and yield stability.

2.1. INTRODUCTION

Wheat is a staple food providing about 20% of the calories of the world's population (FAO, 2016). Durum wheat (*Triticum turgidum* L. var *durum*) represents about 10% of global wheat production. In Mediterranean environments durum wheat is generally grown under rainfed conditions, in which yield is strongly affected by unpredictable fluctuations of temperature and erratic rainfall patterns across growing seasons. In a context of climate change, wheat yields are expected to fall as a consequence of rising temperatures, and more extreme weather events are predicted for the next few decades (Trnka et al. 2014). A decline in global wheat production of between 4.1% and 6.4% is expected for each °C of temperature increase (Liu et al. 2016). In this framework, understanding the genetic and environmental factors affecting yield formation is paramount for targeting breeding strategies to release new cultivars adapted to the upcoming environmental scenarios, thus ensuring food security (Curtis and Halford 2014).

Grain yield is a very complex trait governed by genotype, environment and the interaction between the two. Given the huge environmental effect on final yield, great variability between sites and crop seasons generally occur. The importance of yield stability in durum wheat has been highlighted in previous studies (De Vita et al. 2010, Royo et al. 2008), and there is a general consensus among breeders to pursue high-yielding and stable varieties. Whereas the concept of high yield is clear, there are several definitions and calculation methods for yield stability (Cubero and Flores 1994). Stability involves two different concepts: static (or biological) stability and dynamic (or agronomic) stability.

A genotype is considered statically stable when its performance remains unchanged regardless of the environmental variation, so the mean value of a phenotypic trait with static stability would not change between different conditions (Becker and Léon 1988). The static concept is the most valuable for quality or disease resistance traits, when the same response is desirable in all possible environments. On the other hand, for yield performance, dynamic stability is generally preferred. A genotype is considered agronomically stable when its performance can be predicted for different environments, with mean values for each environment being different. Several parametric and non-parametric statistical methods have been proposed for analysing yield stability. Parametric methods observe the genotypic responses of a sample to environmental conditions. Among the most common of these are the regression slope (b_i), which provides information on both stability and yield performance (Finlay and Wilkinson, 1963), the Lin and Binns (1988) superiority measure (*Pi*), which prioritizes high-yielding genotypes, Shukla's (1972) stability variance (σ^2) and Wricke's (1962) ecovalence (W_i^2). Non-parametric methods – including

Kang's (1993) yield stability (*YS*) – describe genotypic performance over ranks of data relative to environmental factors. In the current study we use the concept of dynamic stability, while adaptability is considered to be the ability to perform well in all testing environments.

Grain yield in wheat can be analysed in terms of two main yield components, grain number per unit area (GN) and grain weight (GW), which appear sequentially during crop development. Variations in yield components are closely related to environmental conditions, especially those before and around flowering time, the stage most sensitive to environmental variations. Flowering time is a critical stage that delimits the duration of spike formation and marks the transition into the grain filling period in which the number of grains spike^{-1} and GW are defined. Time to flowering is considered a primary trait determining wheat adaptation to a particular set of growing conditions (Snape et al., 2001; Worland et al., 1998) and a critical feature for increasing resilience to weather vagaries and achieving high yields (Casadebaig et al. 2016). Wheat phenology must be fine-tuned to a given environment to find the appropriate earliness to avoid excessively high temperatures during flowering time that would affect grain number by reducing fertility (Rezaei et al. 2015), but without risking frost damage during post-heading phases (Frederiks et al. 2015). It has been suggested that changes in allele frequencies in regions responsible for daylength and temperature responses will be critical for the adaptation of cropping systems to climate change (Atlin et al. 2017).

The complex genetic control of phenology is mainly based on vernalization requirement genes (*Vrn1*), photoperiod sensitivity genes (*Ppd-1*) and earliness *per se* (*Eps*) genes (Distelfeld et al., 2009). The effect of *Eps* genes on development rate is independent of vernalization and photoperiod (Distelfeld et al., 2009; Snape et al., 2001). Vernalization requirement is controlled by the *Vrn-1* genes, which in durum wheat consist of homologous copies designated as *Vrn-A1* and *Vrn-B1* and located on the long arms of chromosomes 5A and 5B, respectively (Fu et al. 2005, Yan et al. 2004). Photoperiod sensitivity in wheat is determined by *Ppd-1* genes, which in durum wheat are *Ppd-A1* and *Ppd-B1*. The *Ppd-A1* gene has three known alleles: the wild type conferring sensitivity (*Ppd-A1b*) and two alleles (GS100 and GS105) conferring photoperiod insensitivity (Wilhelm et al. 2009). The *Ppd-B1* gene has two alleles, the wild type conferring photoperiod sensitivity (*Ppd-B1b*) and *Ppd-B1*a causing photoperiod insensitivity (Maccaferri et al. 2008, Royo et al. 2016). Alleles at *Ppd-A1* causing photoperiod insensitivity have a stronger effect on shortening the pre-flowering period than the *Ppd-B1* alleles (GS100 > GS105 > *Ppd-B1a*) (Royo et al. 2016).

While vernalization genes are responsible for adaptability to mega-environments, photoperiod sensitivity may be considered a mechanism for fine-tuning the optimal flowering time in a given environment within mega-environments. In durum wheat, most varieties have the *Vrn-A1c* allele corresponding to the spring type called the 'Langdon type', with other possible alleles corresponding to genotypes from Russia, Ukraine, Azerbaijan and Hungary (Muterko et al. 2016). Therefore, once the appropriate *Vrn* alleles have been defined, a good strategy for setting an optimal flowering time associated with the best environmental conditions possible would be to use a suitable combination of photoperiod sensitivity alleles. Allele combinations at *Ppd-1* genes may modify flowering time by close to 40 days in bread wheat (Tanio and Kato 2007) and by close to 20 days in durum wheat in certain environments (Royo et al. 2016). Previous work shows interaction between *Ppd-1* genes as the flowering or heading date is modified depending on the allele combination. In bread wheat the interaction between *Ppd-D1* and *Ppd-B1* has been demonstrated by Tanio and Kato (2007) and Bentley et al. (2013), among others. Yield advantages resulting from photoperiod insensitivity have been estimated at over 35% in Southern Europe and 15% in Central Europe (Worland, 1996).

The present study is part of a comprehensive project aiming to understand the effect of flowering time as regulated by photoperiod sensitivity on the adaptability and productivity of durum wheat. For this purpose, a collection of durum wheat genotypes involving all known allele combinations for major genes at the *Ppd-A1* and *Ppd-B1* loci and with great variation in flowering time due to earliness *per se* was developed and tested under contrasting northern latitudes (Royo et al. 2016). Results have already been published regarding the effect of *Ppd-1* genes on patterns of phenological development (Royo et al. 2016), biomass production and allocation (Royo et al. 2018), and the effect of individual allele variants at the *Ppd-A1* and *Ppd-B1* loci on yield components and final yield (Arjona et al. 2018). The objectives of the current study were i) to ascertain the effect of changes in flowering time caused by allele combinations at the *Ppd-A1* and *Ppd-B1* loci on yield and yield stability across sites, ii) to identify the most suitable allele combination for yield and yield stability in the study environments, and iii) to explore the relationships between flowering time due to earliness *per se* and yield formation.

2.2. MATERIAL AND METHODS

2.2.1. Plant material

This study was conducted with 23 durum wheat (*Triticum turgidum* L. var. *durum*) genotypes including two commercial cultivars and 21 inbred lines derived from crosses between parents

with contrasting flowering dates. A detailed description of the process to obtain them may be found in Arjona et al. (2018). The molecular characterization for the *Vrn-1*, *Vrn-3* and *Ppd-1* genetic loci is explained in Royo et al. (2016). The 23 genotypes showed a spring growth habit (Royo et al. 2016). Their pedigrees and molecular characterization regarding the *Ppd-A1* and *Ppd-B1* loci are shown in Supplementary Table 1. A summary of the allele combinations for the two loci present in the collection used in the current study is presented in Table 1.

Table II-1. Allele combinations for *Ppd-A1* and *Ppd-B1* loci present in the durum wheat collection used in this study.

Ppd-A1		***Ppd-B1***		**Allele combination acronym**	**Number of lines**
Allele*	**Photoperiod response**	**Allele**	**Photoperiod response**		
Ppd-A1b	Sensitive	*Ppd-B1b*	Sensitive	SS	5
Ppd-A1b	Sensitive	*Ppd-B1a*	Insensitive	SI	5
GS105 *Ppd-A1a*	Insensitive	*Ppd-B1b*	Sensitive	I5S	4
GS105 *Ppd-A1a*	Insensitive	*Ppd-B1a*	Insensitive	I5I	6
GS100 *Ppd-A1a*	Insensitive	*Ppd-B1b*	Sensitive	I0S†	1
GS100 *Ppd-A1a*	Insensitive	*Ppd-B1a*	Insensitive	I0I	3

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

† Discarded from statistical analyses due to uniqueness in the collection

2.2.2. Experimental field setup

Field experiments were performed during five years (2007, 2008, 2010, 2011 and 2012) at three irrigated sites of contrasting latitude: Spain (Lleida, 41º 38'N), Mexico-North (Ciudad Obregón, 27º 21'N) and Mexico-South (El Batán Experimental Station, Texcoco, 19º 31'N). A detailed description of the sites and their environmental characteristics is found in Villegas et al. (2016) and is summarized in Supplementary Table 2. The experiments consisted of plots of 12 m^2 and three replications, arranged as randomized complete block designs. Sowing density and agronomic management were carried out according to the common cultural practices at each site, and the plots were kept free of weeds, diseases and pests. The ten experiments in Spain and Mexico-North were planted in autumn (from 19 November to 23 December), and the five experiments in Mexico-South were planted in spring (from 17 to 28 May) for a summer crop cycle. Temperature (daily maximum, minimum, and mean values) and solar radiation were recorded by meteorological stations within or near the experimental fields. Daily photoperiod including twilight was estimated according to Forsythe et al. (1995). In order to characterize the environments, the following environmental variables were calculated for each plot according to its phenology: average daily recorded mean temperature during the period from emergence to flowering ($Tmean_{EF}$,°C) and from flowering to physiological maturity ($Tmean_{FM}$,°C); average maximum daily temperatures from five days before to five days after the flowering date

($Tmax_F$,°C); mean daily solar radiation from emergence to flowering ($Rmean_{EF}$, MJ m^{-2} day^{-1}), from double ridge to flowering ($Rmean_{DRF}$, MJ m^{-2} day^{-1}) and from flowering to physiological maturity ($Rmean_{FM}$, MJ m^{-2} day^{-1}); accumulated solar radiation from emergence to flowering (Rac_{EF}, MJ m^{-2}); mean daylength from emergence to flowering ($DLmean_{EF}$, h) and from flowering to physiological maturity ($DLmean_{FM}$, h); and finally photo-thermal units from terminal spikelet to flowering (PTU_{TSF}, °C h), calculated according to Dalezios et al. (2002).

2.2.3. Data recording

Growth stages (GS) at emergence (GS10), flowering (GS65) and physiological maturity (GS87, indicated by the loss of green colour in the spike peduncle) were recorded on each plot according to the Zadoks scale (Zadoks et al. 1974) during the five years of the study. Additionally, the stages of double ridge, terminal spikelet (Kirby and Appleyard 1986) and heading (GS55) were also determined in the experiments conducted in 2011, 2012 and 2013, as described in Arjona et al. (2018). A developmental stage was recorded on a plot when at least 50% of the plants had reached it. Field plots were divided into two sections of 6 m^2, of which one was used for destructive sampling and the other was left intact for yield assessment through mechanical harvest at ripening. Grain yield (GY, g m^{-2}) is expressed on a dry weight basis; GW (mg $grain^{-1}$) was determined from a random sample of 200 dried grains; and GN, referred to here as grains m^{-2}, was computed as the ratio between GY and GW. In the experiments conducted in 2010, 2011 and 2012, a sample of a 1-m-long section of a representative central row of each plot was pulled out at maturity. In the laboratory, the spikes were counted and threshed, and their grains were counted. Spikelets $spike^{-1}$ were determined as the average of five main spikes randomly chosen on each sample. Grains $spike^{-1}$ were calculated by dividing the number of grains of the sample by the spike number. Grains $spikelet^{-1}$ were computed as the ratio between grains $spike^{-1}$ and spikelets $spike^{-1}$.

2.2.4. Statistical analysis

To characterize the environments, a principal component analysis (PCA) was carried out in JMP 13 Pro® (SAS institute Inc RRID:SCR_014242 2016), and a correlation matrix was calculated with the environmental variables that were not strongly correlated and with the above-mentioned yield-related traits averaged for each experiment across genotypes and replications (n=69). Combined ANOVAs across experiments were performed using the GLM procedure of the SAS® software (SAS RRID:SCR_008567, 2009), considering year and genotype as random factors. The sum of squares of the genotype effect was partitioned into differences attributable to *Ppd-A1*/*Ppd-*

B1 allele combinations and differences between genotypes within allele combinations. The error term used to test *Ppd-1* allele combinations was the sum of squares of genotype within each combination. Means were compared using Fisher's protected least significant differences (LSD) method at $P = 0.05$. To calculate the genetic variation within allele combinations (earliness *per se* in the case of phenology), the following model was considered for each experiment and run in JMP 13 Pro® (SAS institute Inc RRID:SCR_014242 2016):

$$Y_{ij} = AC_i + G(AC)_{ij} + R_j + E_{ij}$$

where:

Y_{ij} is the trait studied for genotype i in experiment j;

AC_i is the effect of allele combination for genotype i, considered as a fixed effect;

$G(AC)_{ij}$ is the genotype effect within its allele combination for genotype i on experiment j, considered as a random effect;

R_j is the effect of the replicate on experiment j, considered as a random effect; and

E_{ij} is the experimental error of genotype i in experiment j, considered as a random effect.

In order to analyse the earliness caused by the *Eps* genes existing in the collection, its effect on flowering time was detached from that of the *Ppd-1* genes. For this purpose, the best linear unbiased predictors (BLUPs) for the $G(AC)_{ij}$ effect were extracted from the standard least squares/restricted maximum likelihood (REML) method at each site and year. Pearson's correlation analysis was run on these values by site and year with PROC CORR of the SAS® software (SAS RRID:SCR_008567 2009). Five stability indices were calculated for each allele combination. The slope (*b*) of the join regression analysis (Finlay and Wilkinson, 1963) and the Lin and Binns (1988) superiority measure (*Pi*) were computed with SAS® software (SAS RRID:SCR_008567 2009). Shukla's (1972) stability variance (σ^2), Wricke's (1962) ecovalence (W_i^2) and Kang's (1993) non-parametric yield stability measure (YS_i) were calculated following Dia et al. (2017) using the R package "Agricolae" (Mendiburu 2019) executed in R (R Core Team RRID:SCR_001905 2018).

2.3. RESULTS

2.3.1. Environmental and genetic effects

Principal Component Analysis (PCA) was carried out with the environmental variables and yield-related traits calculated for each plot. The first two axes of the PCA shown in Figure 1 accounted for 85.5% of the total variance (axis 1, 61.0%; axis 2, 24.5%). The location of the points

corresponding to the experiments conducted in Spain close to the vectors related to daylength from flowering to maturity, GY and GN, but on the opposite side to the vectors related to temperature and solar radiation from emerging to flowering, indicates that this site was characterized by low temperature and radiation before flowering, long photoperiod during grain filling and a high number of grains m^{-2} and GY. The Mexico-South site showed a high photoperiod, temperature and radiation until flowering, and the lowest GY, while Mexico-North showed the highest temperature and radiation during grain filling, had the shortest daylength from emergence to flowering, and produced the heaviest grains. In all cases, yearly variations were much lower than differences between sites, as shown by the clustering in the PCA biplot of the points corresponding to the five years at each site (Fig. 1).

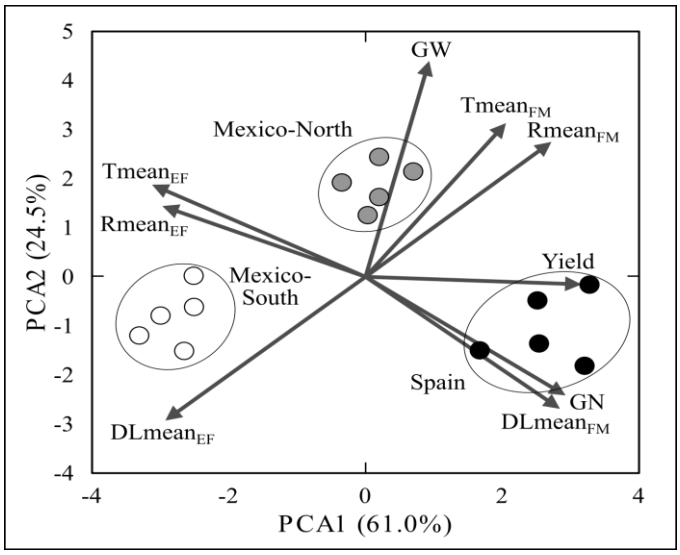

Figure II.1. Biplot of the first two axes of the principal component analysis summarizing the relationships between environmental variables and yield components for the three experimental sites. Eigenvalues of the correlation matrix are represented as vectors symbolizing environmental variables and yield associated traits. DLmean$_{EF}$: mean daylength from emergence to flowering, DLmean$_{FM}$: mean daylength from flowering to maturity, Tmean$_{EF}$: average of mean daily temperatures from emergence to flowering, Tmean$_{FM}$: average of mean daily temperatures from flowering to maturity, Rmean$_{EF}$, mean of daily solar radiation from emergence to flowering, Rmean$_{FM}$ mean of daily solar radiation from flowering to maturity, GN: grain number m^{-2}, GW: grain weight. Points correspond to average genotype values for each year at each site. Black dots: Spain; Grey dots: Mexico-North; White dots: Mexico-South.

The ANOVA showed that the site effect was the most important for explaining variations in phenology, GY and GN, accounting for 42.4% to 86.4% of the total sum of squares of these traits (Table 2). The year effect was only significant for GY, and explained 10.2% of its sum of squares. The site x year interaction was significant for all the analysed traits, but it accounted for much lower variability than the site effect for all of them. The genotype effect was also statistically significant for all traits and accounted for between 3.1% for days from emergence to flowering and 55.8% for GW of total variation. The partitioning of the genotype effect into its components,

i.e. differences between allele combinations at *Ppd-1* and differences between genotypes within each allele combination, revealed that the allele combination only affected flowering time and GN but had no effect on grain filling duration, GW or GY. Genotypes within allele combinations differed significantly for all traits (Table 2). The genotype x site interaction was significant for all traits except GN. Differences for the allele combination x site interaction were statistically significant for days from flowering to maturity (accounting for about 61% of the variability induced by the genotype effect) and for GW and GY (in both cases accounting for about 35% of the genotype variance) (Table 2). At all sites the longest cycle to flowering was recorded in the SI combination, but with no differences between SS and I5S (Table 3). There were no significant differences between allele combinations for GY at any site.

Table II-2. Percentage of the sum of squares of the ANOVA model for phenology and yield related traits of 23 durum wheat genotypes grown at three sites during five years. ns: non-significant; *P<0.05; **P<0.01; ***P<0.001.

Source of variation	d.f.	Days emergence-flowering	Days flowering-maturity	Grains m^{-2}	Grain weight (mg)	Grain yield (g m^{-2})
Site	2	86.4 ***	43.6 *	42.4 ***	14.1 *	50.5 ***
Year	4	2.8 ns	7.1 ns	5.0 ns	5.1 ns	10.5 *
Site × Year	8	6.1 ***	27.0 ***	2.9 ***	8.9 ***	3.8 ***
Genotype	22	3.1 ***	4.4 *	27.5 ***	55.8 ***	11.0 ***
Between allele combinations	4	1.5 *	0.3 ns	11.5 *	20.5 ns	2.7 ns
Within allele combinations	18	1.6 ***	4.1 ***	16.0 ***	35.3 ***	8.3 ***
Genotype × Site	44	1.0 ***	3.6 ***	2.3 ns	3.4 ***	3.4 **
Between allele combinations × Site	8	0.2 ns	2.2 ***		1.2 *	1.2 *
Within allele combinations × Site	36	0.8 ***	1.4 ns		2.2 ***	2.2 *
Genotype × Year	88	0.2 ns	3.9 *	3.5 ns	2.9 *	3.4 ns
Genotype × Site × Year	176	0.3 ***	5.5 ***	6.8 ***	3.9 ***	7.4 ***
Between allele combinations × Site × Year	32	0.1 ns	1.3 ns	1.6 ns	0.9 ns	1.9 *
Within allele combinations × Site × Year	144	0.2 ***	4.2 ***	5.2 ***	3.0 ***	5.5 ***
Block	30	0.0 ***	0.5 ***	1.8 ***	0.8 ***	1.8 ***
Residual	651	0.1	4.3	7.9	5.1	8.1

At all sites the largest GN and the smallest GW values corresponded to combination I5S, but it did not differ from SS for any of these traits. In order to elucidate the cause of the largest GN found for I5S, a detailed study of its components was carried out in 2010, 2011 and 2012. The results showed that the five allele combinations had a similar number of spikes m^{-2} at all sites (data not shown), but the greatest number of grains $spike^{-1}$ was always recorded in the I5S allele combination, although the differences were not statistically significant in Mexico-South (Table 4). The analysis of its components showed differences between allele combinations across sites for number of spikelets $spike^{-1}$ and grains $spikelet^{-1}$. The lowest number of spikelets $spike^{-1}$ was recorded in allele combinations I0I and I5I, while the highest values corresponded to a group

formed by combinations I5S, SS, and SI (Table 4). Allele combination I5S reached on average the highest absolute values for both spikelets spike^{-1} and grains spikelet^{-1}. A significant and positive relationship was found between mean solar radiation from double ridge to heading and spikelets spike^{-1} when they were calculated at each site with the mean values of each allele combination across years (Figure 2).

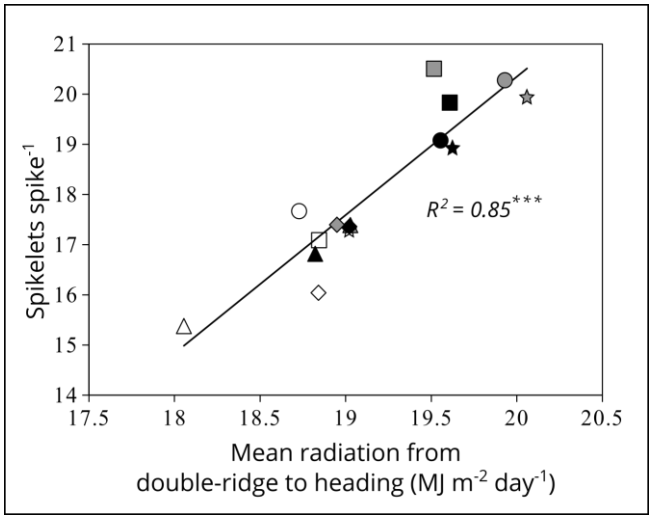

Figure II.2. Relationship between mean daily solar radiation from double ridge to heading and the number of spikelets spike^{-1}. Each point represents the mean of 3-9 durum wheat genotypes averaged over 3 years at each site. Allele combinations at *Ppd-A1* and *Ppd-B1* loci are represented with different symbols (see Table 1 for allele combination acronyms). ▵: I0I; ◇: I5I; □: I5S; ☆: SI; ○: SS. Black symbols: Spain; Grey symbols: Mexico-North; White symbols: Mexico-South. $^{***}P$<0.001.

2.3.2. Relationships between phenology variation due to *Eps* and yield formation

Although allele combinations at *Ppd-1* had no effect on GY within or across environments (Table 3), the ANOVA showed strong variability for flowering time and GY within allele combinations (Table 2). For this reason, the relationships between phenology and yield-related traits were explored, detaching the effect of the allele combination. Thus, Pearson correlation coefficients were calculated with the BLUP values that had removed the effect of the allele variants at *Ppd-1* by site and year. The results showed that, in agreement with the results of the ANOVA, in general the changes in flowering date within allele combinations did not affect GN, and the number of days from flowering to maturity did not affect yield-related traits (Table 5). However, long emergence to flowering periods were negatively associated with GW, and in 12 out of 15 experiments they were also negative associated with GY.

The analysis of the relationship between flowering time and the components of GN was conducted during three years. The results showed that the number of days from emergence to flowering did not affect spike number or number of grains spike^{-1} but was positively and significantly associated with number of spikelets spike^{-1} in eight out of nine experiments. Days

Table II-3. Mean comparison for phenology and yield related traits in a set of 23 durum wheat genotypes grouped according to five allele combinations at *Ppd-A1* and *Ppd-B1* loci and tested during five years at three sites of contrasting latitude. Means within columns with different letters are significantly different for a LSD test at $P<0.05$. See Table 1 for acronym description.

Allele combination	Days emergence-flowering			Days flowering-maturity			Grains m^{-2}			Grain weight (mg)			Grain yield (g m^{-2})		
	Spain	Mexico-North	Mexico-South	Mean	Spain	Mexico-North	Mexico-South	Mean	Spain	Mexico-North	Mexico-South	Mean	Spain	Mexico-North	Mexico-South	Mean
I0I	130^{b}	83^{b}	60^{b}	92^{c}	38^{a}	43^{ab}	49^{a}	43^{a}	18148^{ab}	12588^{b}	11270^{b}	14002^{b}	43.2^{a}	50.6^{a}	42.9^{a}	45.6^{a}	744^{a}	605^{a}	455^{a}	600^{a}
I5I	132^{ab}	84^{b}	63^{ab}	93^{bc}	36^{a}	43^{a}	50^{a}	43^{a}	16396^{b}	11254^{b}	10987^{b}	12879^{b}	43.6^{a}	50.2^{a}	41.8^{a}	45.2^{a}	677^{a}	534^{a}	430^{a}	547^{a}
I5S	135^{a}	90^{ab}	66^{ab}	97^{abc}	36^{a}	41^{abc}	49^{a}	42^{a}	20706^{a}	15540^{a}	14627^{a}	16958^{a}	32.8^{b}	37.4^{b}	32.1^{b}	34.1^{a}	642^{a}	544^{a}	441^{a}	542^{a}
SS	136^{a}	95^{a}	67^{ab}	99^{ab}	36^{a}	39^{bc}	52^{a}	43^{a}	18004^{ab}	13012^{ab}	12276^{ab}	14431^{ab}	40.4^{ab}	45.2^{ab}	37.6^{ab}	41.1^{a}	682^{a}	548^{a}	433^{a}	554^{a}
SI	136^{a}	97^{a}	70^{a}	101^{a}	37^{a}	39^{c}	51^{a}	42^{a}	17359^{b}	12040^{b}	10859^{b}	13419^{b}	42^{a}	45.2^{ab}	35.9^{ab}	41.0^{a}	682^{a}	504^{a}	363^{a}	516^{a}

to flowering was positively associated with number of grains spike^{-1} only in Mexico-South and was negatively correlated with number of grains spikelet^{-1} only in Mexico-North (Table 6). Grain number depended strongly on number of grains spike^{-1} and also on its components, grains spikelet^{-1} and spikelets spike^{-1}, as they were significantly correlated with GN in seven out of nine experiments (Table 6). At each site, using the within–allele combination values, number of spikelets spike^{-1} was positively and significantly correlated with photo-thermal units from terminal spikelet to flowering (Figure 3).

2.3.3. *Ppd-1* allele combinations and yield stability

The five statistical indices calculated with the data of the 15 experiments to assess the yield stability resulting for each allele combination gave fairly similar results (Table 7). The slopes of the joint regression analysis (*b*) ranged between 0.87 for I5S and 1.19 for SI, this latter value being the only one significantly different from 1. The superiority measure (*Pi*) was significantly different between combinations, with the lowest and highest value corresponding to I0I and SI, respectively, indicating that I0I was close to the best yielding standard and SI to the worst. Values for Shukla's stability, σ^2, that were not significantly different from 0 appeared for combinations I0I, I5I and SS, suggesting that they were the most stable. Wricke's ecovalence revealed similar patterns, recognizing I5I and SS as having the highest stability. Kang's YS_i was in accordance with the previous indices, indicating that the I5S and SI had a worse ratio between stability and yield than the pool formed by I0I, I5I and SS.

Table II-4. Means comparison for the spike components in a set of 23 durum wheat genotypes grouped according to five allele combinations at *Ppd-A1* and *Ppd-B1* loci and tested during five years at three sites of contrasting latitude. Means within columns with different letters are significantly different for a LSD test at *P*<0.05. See Table 1 for acronym description.

Allele combination	Grains spike^{-1}				Spikelets spike^{-1}				Grains spikelet^{-1}			
	Spain	Mexico-North	Mexico-South	Mean	Spain	Mexico-North	Mexico-South	Mean	Spain	Mexico-North	Mexico-South	Mean
I0I	36.8^{b}	36.3^{b}	32.9^{a}	35.4^{b}	16.8^{c}	17.3^{a}	15.4^{c}	16.5^{c}	2.2^{a}	2.1^{a}	2.1^{a}	2.1^{ab}
I5I	36.2^{b}	34.4^{b}	29.9^{a}	33.5^{b}	17.4^{bc}	17.4^{a}	16.0^{bc}	16.9^{bc}	2.1^{a}	2.0^{a}	1.9^{a}	2.0^{b}
I5S	47.4^{a}	47.7^{a}	38.9^{a}	44.6^{a}	19.8^{a}	20.5^{a}	17.1^{ab}	19.1^{a}	2.4^{a}	2.3^{a}	2.3^{a}	2.3^{a}
SS	38.9^{b}	40.1^{b}	33.4^{a}	37.4^{b}	19.1^{ab}	20.3^{a}	17.7^{a}	19.0^{a}	2.1^{a}	2.0^{a}	1.9^{a}	2.0^{b}
SI	36.6^{b}	40.4^{b}	32.8^{a}	36.6^{b}	18.9^{ab}	19.9^{a}	17.3^{ab}	18.7^{ab}	2.0^{a}	2.0^{a}	1.9^{a}	2.0^{b}

Table II-5. Pearson correlation coefficients between phenology and yield related traits of 23 durum wheat genotypes grown in 15 environments. This analysis was conducted with the BLUPs of each genotype after removing the *Ppd-1* allele combination effect. GN: grain number, GW: grain weight, GY: grain yield.

Year	Days emergence-flowering			Days flowering-maturity		
	GN	GW	GY	GN	GW	GY
			Spain			
2007	0.23^{ns}	-0.61**	-0.71***	-0.19^{ns}	0.45*	0.46*
2008	0.27^{ns}	-0.58**	-0.57**	-0.22^{ns}	0.45*	0.45*
2010	0.33^{ns}	-0.67***	-0.58**	-0.15^{ns}	0.31^{ns}	0.28^{ns}
2011	0.55**	-0.62**	0.01^{ns}	0.36^{ns}	-0.01^{ns}	0.38^{ns}
2012	-0.03^{ns}	-0.50*	-0.57**	0.28^{ns}	0.03^{ns}	0.37^{ns}
			Mexico-North			
2007	0.16^{ns}	-0.54**	-0.37^{ns}	0.16^{ns}	-0.14^{ns}	0.06^{ns}
2008	0.09^{ns}	-0.57**	-0.70***	-0.14^{ns}	0.13^{ns}	0.13^{ns}
2010	-0.09^{ns}	-0.41^{ns}	-0.55**	-0.03^{ns}	-0.06^{ns}	-0.14^{ns}
2011	-0.04^{ns}	-0.59**	-0.70***	-0.14^{ns}	0.68***	0.56**
2012	-0.34^{ns}	-0.74***	-0.95***	0.34^{ns}	0.57**	0.81***
			Mexico-South			
2007	-0.17^{ns}	-0.62**	-0.81***	0.24^{ns}	0.06^{ns}	0.36^{ns}
2008	-0.08^{ns}	-0.47*	-0.76***	0.15^{ns}	0.06^{ns}	0.32^{ns}
2010	0.74**	-0.51*	0.67***	-0.17^{ns}	0.03^{ns}	-0.18^{ns}
2011	0.39^{ns}	-0.78***	-0.49*	-0.04^{ns}	0.26^{ns}	0.40^{ns}
2012	0.29^{ns}	-0.62**	-0.23^{ns}	0.02^{ns}	-0.09^{ns}	0.02^{ns}

ns: non-significant; * P<0.05; **P<0.01; ***P<0.001

Table II-6. Pearson correlation coefficients between spike components and the number of days to flowering and grains m^{-2} of 23 durum wheat genotypes grown in nine environments. This analysis was conducted with the BLUPs of each genotype after removing the *Ppd-1* allele combination effect.

Year	Days emergence-flowering				Grains m^{-2}			
	Spikes m^{-2}	Grains spike^{-1}	Spikelets spike^{-1}	Grains spikelet^{-1}	Spikes m^{-2}	Grains spike^{-1}	Spikelets spike^{-1}	Grains spikelet^{-1}
				Spain				
2010	0.14^{ns}	-0.04^{ns}	0.83***	-0.29^{ns}	-0.12^{ns}	0.59***	0.22^{ns}	0.59**
2011	0.46*	0.10^{ns}	0.74***	-0.37^{ns}	0.38^{ns}	0.67***	0.66***	0.21^{ns}
2012	-0.41^{ns}	0.26^{ns}	0.66***	-0.12^{ns}	0.32^{ns}	0.72***	0.53**	0.57**
				Mexico-North				
2010	0.31^{ns}	-0.22^{ns}	0.70***	-0.74***	0.44*	0.58**	0.45*	0.28^{ns}
2011	-0.30^{ns}	0.21^{ns}	0.70***	-0.41*	0.36^{ns}	0.75***	0.44*	0.63**
2012	-0.50*	0.02^{ns}	0.75***	-0.54***	0.32^{ns}	0.65***	0.10^{ns}	0.72***
				Mexico-South				
2010	0.07^{ns}	0.63**	0.12^{ns}	0.68***	0.15^{ns}	0.69***	0.48*	0.55**
2011	-0.41^{ns}	0.56**	0.80***	0.34^{ns}	0.06^{ns}	0.83***	0.55**	0.74***
2012	-0.14^{ns}	0.45*	0.41*	0.32^{ns}	0.54***	0.78***	0.41*	0.74***

ns: non-significant; * *P*<0.05; ***P*<0.01; ****P*<0.001

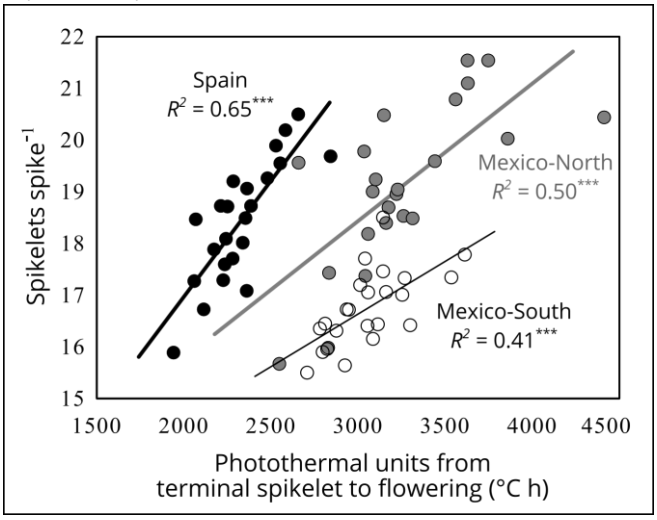

Figure II.3. Relationships between photo-thermal units following Dalezios et al. (2002) from terminal spikelet to flowering and the number of spikelets spike^{-1} at each experimental site. Each point is the average value of the BLUPs of each of 23 durum wheat genotypes across three years considering flowering time due to *Eps*. Black symbols: Spain; Grey symbols: Mexico-North; White symbols: Mexico-South. ***P<0.001.

Table II-7. Stability indices calculated for grain yield of 23 durum wheat genotypes grouped according five allele combinations for *Ppd-A1* and *Ppd-B1* and grown at three contrasting latitudes during five years. See Table 1 for acronym description.

Allele combination	Yield performance (g m^{-2})	Regression slope (*b*)	Superiority measure (*Pi*)	Shukla's stability (σ^2)	Wricke's Ecovalence (W_i^2)	Kang's Yield-Stability (YS_i)
I0I	600^{a}	1.12^{a}	2542^{e}	755^{ns}	1563	+8
SS	554^{a}	0.96^{b}	63966^{d}	-365^{ns}	219	+5
I5I	547^{a}	0.90^{b}	80228^{c}	-80^{ns}	561	+2
I5S	542^{a}	0.87^{b}	103535^{b}	4143**	5628	-7
SI	516^{a}	1.19^{a§}	139795^{a}	3754*	5162	-5

§: Significantly different from 1; *: Significantly different from 0 at P<0.05;
**: Significantly different form 0 at P<0.01; +: Stable, and -: Unstable allele combinations according to Kang's Yield-stability criterion.

2.4. Discussion

The wide range of latitudes and the different environmental conditions of the three experimental sites used in this study resulted in the site effect being the most important in the ANOVA model for explaining phenotypic variability for all the analysed traits except GW. Although other factors such as soil characteristics and agricultural practices could affect variability between sites, the results of the PCA showed that 85.5% of the information contained in the environmental data and yield attributes could be summarized by projecting the points in the plane determined by the first two axes of the multivariate analysis. The biplot of the PCA revealed that the environmental dissimilarities led to contrasting yield formation strategies. The low temperatures and solar radiation before flowering and the large daylength during grain filling were characteristic of the

Spanish site and led to the highest GN and GY. This result is in agreement with the reported interaction between the effect of temperature and solar radiation on GN during the pre-heading phase (Ortiz-Monasterio et al. 1994) and the positive effect of low temperatures in pre-anthesis on GN (Villegas et al. 2016). In contrast, the low daylength before flowering and the high temperature and solar radiation during grain filling recorded in Mexico-North produced the heaviest grains, while the high temperature, solar radiation and daylength before flowering characteristic of the spring sowing in Mexico-South led to the lowest yields. The importance of solar radiation during grain filling for achieving high GW has been reported previously (Villegas et al. 2016). In contrast, the variability induced by the year effect was lower than that caused by the site, as revealed by the ANOVA and the PCA.

Although the genotype had a much lower effect than the site for explaining differences in phenology, genotypes differed significantly for all the analysed traits. The longest period from emergence to flowering was consistently recorded for allele combinations *Ppd-A1b/Ppd-B1a* (SI) and *Ppd-A1b/Ppd-B1b* (SS), as shown in our previous study (Royo et al. 2016), but without significant differences from allele combination GS105*/Ppd-B1b* (I5S). Across sites and in Spain and Mexico-South individually, the allele combination had no effect on grain filling duration. The significant allele combination x site interaction observed in the ANOVA for this trait was due to the different duration of grain filling in Mexico-North, where differences between allele combinations carrying alleles causing photoperiod insensitivity at both loci and the ones having one locus with a sensitivity allele and the other with an insensitivity allele were maximized.

In contrast with the relatively low effect of the genotype on crop phenology, genotypic differences accounted for 55.8%, 27.5% and 11.0% of the total variation observed for GW, GN and GY, respectively. These results are in agreement with the high genetic control of GW (Arjona et al. 2018, Villegas et al. 2016) and GN (Roncallo et al. 2017, Soriano et al. 2017, Wang et al. 2011) shown in previous studies. The partitioning of the genotype effect into its components revealed that among the range of environments studied, major genes regulating flowering time had no effect on GY at any site or across them. However, differences between the *Ppd-A1/Ppd-B1* allele combinations were significant for GN, with combinations I5S (GS105*/Ppd-B1b*) and SS (*Ppd-A1b/Ppd-B1b*) consistently tending to have more grains per unit area than the remaining ones. The detailed analysis conducted to elucidate the reason for the largest GN recorded in genotypes carrying the allele causing photoperiod sensitivity at *Ppd-B1* (*Ppd-B1b*) revealed that it was due to a higher number of grains spike^{-1}, as differences were not found for number of spikes per unit area. However, the intensity of the effect of the *Ppd-B1b* allele depended on the allele present at

Ppd-A1, as the number of grains per spike was consistently higher for I5S (GS105*/Ppd-B1b*) than for SS (*Ppd-A1b/Ppd-B1b*). The dissection of the components of the number of grains per spike revealed that the number of spikelets spike^{-1} was similar in both combinations across sites (19.1 and 19.0 for I5S and SS, respectively) and at each site, in agreement with the similar mean solar radiation received from double ridge to heading by genotypes carrying one of the two allele combinations. However, although differences in the number of grains spikelet^{-1} were not statistically significant at any site, they were significant across them, with I5S having 15% more grains spikelet^{-1} than SS (from 14% to 21% depending on the site).The earlier flowering time of I5S (from 1 to 5 days depending on the site) may have provided better environmental conditions for grain setting (Draeger and Moore 2017, Terrile et al. 2017), thus resulting in a higher number of grains spikelet^{-1}. A previous study showed that when allele combinations carrying the *Ppd-B1a* allele (I0I, I5I and SI) were compared with those carrying allele *Ppd-B1b* (I5S and SS), the latter caused an increase in the number of grains spike^{-1} due to a consistently higher number of spikelets spike^{-1} (Arjona et al. 2018). The current study went a step further by analysing differences between alleles GS105 and *Ppd-A1b* in the genetic background of *Ppd-B1b*, and the results showed that grain setting was more relevant than spikelet number in the increase in number of grains spike^{-1}. The lack of significant differences between allele combinations for GY was due to a compensation effect on GW, in agreement with the common trade-off between these two components (Quintero et al. 2018).

Given the reduced effect of the *Ppd-A1/PpdB1* allele combinations on yield formation, a further analysis of the relationship between phenology and yield formation was conducted using the variability in flowering time existing within allele combinations, i.e. the effect of *Eps* genes. Despite the mathematical removal of *Ppd-1* effects, it has to be considered that some minor genes affecting photoperiod sensitivity and/or vernalization requirements could be present. Those minor effects, which would be caused by unknown genes, are not accounted for in the present study. The large variability for flowering time within allele combinations was not surprising, as a previous study revealed the presence of strong *Eps* in the set of genotypes used in the current study (Royo et al. 2016). The results of the correlation analyses showed that flowering time had a lower effect on GN, as correlation coefficients between the two traits were only significant in two of the fifteen experiments. In contrast, late flowering had a consistently negative effect on GW, in agreement with the findings of other authors (Fischer 2016, Ortiz-Monasterio et al. 1994), and on GY. Grain filling duration had in general no effect on yield formation. These results indicate that, in the range of environments studied, early flowering was a favourable trait for achieving high

yields. The negative effect of late flowering on GW and yield could be due to the higher temperatures suffered during grain filling by late flowering genotypes, as they may have limited the allocation of resources to grains, resulting in lower productivities (Bergkamp et al. 2018, García et al. 2016, Stone and Nicolas 1994). The dissection of GN into its components revealed that the number of grains per unit area mostly depended on the number of grains spike^{-1} and its components, spikelets spike^{-1} and grains spikelet^{-1}. The lack of effect of flowering time on the number of grains spike^{-1} was due to a compensation between the number of spikelets spike^{-1} and the number of grains spikelet^{-1}. This compensation was much lower at the spring-planting site (Mexico-South) because the number of spikelets spike^{-1} recorded at this site was lower than that of the other two sites. The large environmental effect on the number of spikelets spike^{-1} (Arjona et al. 2018) was also noticed here due to the strong and positive influence of the photo-thermal units from terminal spikelet to flowering on this value at all sites.

The study of the relationship between allele combinations and yield stability showed that allele combination *Ppd-A1b/Ppd-B1a* (SI) was the only one with a regression slope (Finlay and Wilkinson, 1963) significantly different from 1, thus revealing that it conferred the lowest yield stability but the greatest responsiveness to good growing conditions. Allele combination GS100 *Ppd-A1a*/*Ppd-B1a* (I0I) had a regression slope higher than 1, also suggesting good yield responsiveness to environmental improvements. The lowest value of the Lin and Binns (1988) superiority measure (P_i) recorded on this combination suggests good performance at all experimental sites. Shukla's (1972) stability variance (σ^2) and Wricke's (1962) ecovalence (W_i^2) emphasize yield stability against yield performance, with the closest values to zero denoting greatest stability. Accordingly, allele combinations *Ppd-A1b/Ppd-B1b* (SS) and GS105 *Ppd-A1a/Ppd-B1a* (I5I) showed the greatest yield stability. With regard to Kang's (1993) YS_i ratio, which gives the same weight to yield and stability, I0I stands out, because though a little less stable than SS and I5I, its better yield performance compensates for its instability.

As photoperiod-sensitive genotypes are generally grown at high latitudes, it was expected that genotypes carrying the *Ppd-A1b* allele would be well adapted to northern Spain. However, even at this site genotypes carrying I0I yielded 9.1% more than the ones carrying SS or SI. The high values of *Pi* and Wricke's ecovalence (W_i^2), the significance of the Shukla's stability, σ^2, and the negative values of Kang's YS_i ratio observed in SI and I5S revealed a low yield stability for them.

Allele combinations carrying alleles conferring the same response to photoperiod at both *Ppd-1* loci proved to be the most stable, with I0I being the most suitable for the study sites in terms of yield and yield stability. As it has been reported that allele GS100 is rare among modern durum

wheat varieties and has not been found in landraces (Bentley et al. 2011), it would be useful to integrate I0I in breeding programmes aiming to reduce flowering time while increasing genetic diversity, yield and yield stability.

2.5. CONCLUSIONS

The wide range of environmental conditions characteristic of the three sites where the experiments were carried out allowed strong relationships to be detected between environmental variables and yield formation traits. Large GN per unit area was associated with low temperatures and radiation before flowering. In addition, a large number of spikelets spike^{-1} resulted from high solar radiation from double ridge to heading and also from a high radiation:temperature ratio from terminal spikelet to flowering.

The range of flowering dates resulting from the different allele combinations at *Ppd-A1/Ppd-B1* loci were not sufficient to generate yield variations, owing to a compensation between GN and GW. A consistent effect of allele *Ppd-B1b* on GN per unit area was observed as a result of an increase in the number of grains spike^{-1}. Allele interaction caused the intensity of this effect to depend on the allele variant present at *Ppd-A1*. Allele combination GS100*/Ppd-B1a* was the most suitable for the range of environment considered here in terms of yield and yield stability. Within the variability of flowering dates used in this study, even when caused by *Ppd-1* genes or *Eps*, a delay in flowering date had no effect on the number of grains per unit area or for its components, i.e. spikes per unit area and grains spike^{1}. However, in all experiments, late flowering genotypes had lighter grains and in nearly all of them a delay in flowering time decreased yield.

The relationship between allele combination and yield stability classified the allele combinations into two groups. Combinations carrying alleles conferring the same response at both loci, i.e. GS100*/Ppd-B1a* (I0I), GS105*/Ppd-B1a* (I5I) and *Ppd-A1b/Ppd-B1b* (SS), resulted in greater yield stability than combinations carrying alleles conferring a different photoperiod sensitivity response at both loci, i.e. GS105*/Ppd-B1b* (I5S) and *Ppd-A1b/Ppd-B1a* (SI).

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Abbreviations: GN: Grains per unit area, GY: grain yield, GW: grain weight

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CHAPTER III

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HEAT STRESS

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

Jose M. Arjona[1] | **Conxita Royo**[1] | **Susanne Dreisigacker**[2] | **Karim Ammar**[2] | **Joan Subirà**[1] | **Dolors Villegas**[1]

[1]Sustainable Field Crops Programme, Institute for Food and Agricultural Research and Technology (IRTA), Lleida, Spain

[2]International 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

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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

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

Grain number per unit land area 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 (Rharrabti, Villegas, & Royo, 2002). 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, & Slafer, 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 i) adapting sowing dates to allow the crop to fill its grains under favourable environmental conditions (Ortiz-Monasterio, Dhillon, & Fischer, 1994), and ii) 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, & Spaner, 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 (Maccaferri 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-B1*a), 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.

3.2. MATERIALS AND METHODS

3.2.1. Plant material

Thirty-five spring durum wheat genotypes were used in this study (Supplementary 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).

Table III-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***		***Ppd-B1***	
		Allele†	Photoperiod response	Allele	Photoperiod response
I0I	5	GS100/*Ppd-A1a*	Insensitive	*Ppd-B1a*	Insensitive
I5I	7	GS105/*Ppd-A1a*	Insensitive	*Ppd-B1a*	Insensitive
I5S	10	GS105/*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)

3.2.2. 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 (November 17–December 23), while in southern Mexico the experiments were spring-sowing (May 17-28). 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.

Table III-2. Location and environmental descriptions of the three experimental sites.

Site	Location (state or province)	Experimental station (institution's acronym)	Coordinates		Altitude (m.a.s.l)	Environmental characteristics
			Lat	Long		
Spain	Gimenells, (Lleida)	Gimenells (IRTA)	41°38'N	0°23'E	200	Moderate terminal stress. High to medium productivity
Northern Mexico	Cd. Obregón, (Sonora)	CENEB (CIMMYT)	27°21'N	109°54'W	40	Very high terminal stress. Mandatory full irrigation. Very high productivity
Southern Mexico	El Batán, (Mexico)	El Batán (CIMMYT)	19°31'N	98°50'W	2249	Initial stress eliminated with irrigation. Medium productivity

Zadoks et al. (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 h 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 & Giltinan (1995). The model (Eq. 1) was fitted with the "NLIN" procedure and the Marquardt method of the SAS software (SAS RRID:SCR_008567, 2009):

$$\boldsymbol{GW_{ij}} = \frac{\boldsymbol{W_i}}{\mathbf{1+\exp\{-Rt_i(x_{ij}-midD_i)\}}} \qquad \text{[Equation 1]}$$

where

GW_{ij} is the weight of the grain for a sample i at time j;

W_i is the asymptote of the curve for sample i;

Rt_i 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

$midD_i$ is the value of growing degree days at the inflection point of the curve (midpoint 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* = *W*/*D.*

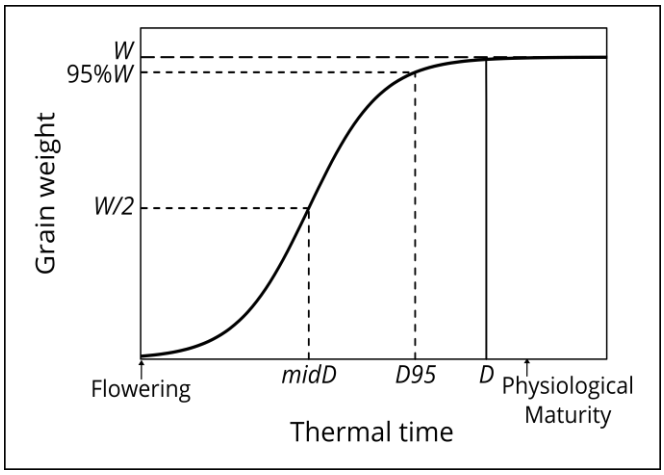

Figure III.1. Representation of the logistic curve: *W*, asymptote value; *W/2*, inflection point, where half the asymptote value is reached and corresponds to mid duration of grain filling (*midD*); *D95*, point where 95% of *W* is reached; and *D*, total grain filling duration.

3.2.3. Statistical analyses

Combined ANOVA across sites (latitudes), experiments, years and genotypes was 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 (λ) 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 $p = 0.05$. 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_014242, 2007).

3.3. RESULTS

3.3.1. 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 III-3. Percentage of the sum of squares (SS) of the ANOVA for the number of days from emergence to flowering, and results of MANOVA for the curve coefficients final grain weight (*W*), mean rate of grain filling (*R*) and thermal time from flowering to 95% *W* (*D95*).

Source of variation	**ANOVA (days emergence to flowering)**		**MANOVA (*W*, *R*, *D95*)**				
	%SS	-Log *P*	*Wilks' λ*	*F*	n	d	-Log *P*
Site	84.7	>999	0.024	1096.95	6	1194	>999
Year	3.2	>999	0.144	325.56	6	1194	246
Site × Year	6.1	>999	0.050	277.73	12	1579.8	>999
Genotype	4.4	>999	0.016	52.24	102	1788.5	>999
Between Ppd-1	*58.5*	*7.6*	*0.148*	*139.33*	*12*	*1579.8*	*237*
Within Genotype (Ppd-1)	*41.5*	*>999*	*0.022*	*50.81*	*90*	*1787.5*	*>999*
Site × Genotype	1.2	>999	0.078	11.75	204	1791.1	214
Site × Ppd-1	*36.0*	*4.2*	*0.390*	*27.67*	*24*	*1732.1*	*103*
Site × Genotype (Ppd-1)	*64.0*	*>999*	*0.132*	*9.62*	*180*	*1790.8*	*162*
Year × Genotype	0.2	141.5	0.100	10.15	204	1791.1	186
Year × Ppd-1	*42.8*	*6.1*	*0.534*	*17.44*	*24*	*1732.1*	*64*
Year × Genotype (Ppd-1)	*57.2*	*96.5*	*0.139*	*9.25*	*180*	*1790.8*	*155*
Site × Year × Genotype	0.2	141.2	0.038	8.62	408	1791.8	232
Site × Year × Ppd-1	*28.9*	*4.2*	*0.400*	*13.35*	*48*	*1776.4*	*87*
Site × Year × Genotype (Ppd-1)	*71.1*	*114.4*	*0.056*	*8.04*	*360*	*1791.7*	*203*
Rep(Site × Year)	0.0	5.5	0.887	1.35	54	1779.6	1

n, degrees of freedom of the numerator; d, degrees of freedom of the denominator.

Table III-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^{-1})**	**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 ab	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

Different letters within columns and sites indicate significant differences according to protected Fisher's least significant difference at $p = 0.05$.

3.3.2. 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).

3.3.3. Relationships between traits

Exploring the relationships between flowering time and the coefficients in the grain filling curve revealed that flowering time accounted for 40% to 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% to 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).

Table III-5. Summary of the regression models fitted to the relationships between variables.

Dependent variable	Independent variable	Spain			Northern Mexico			Southern Mexico		
		b	b S.E.	R^2	b	b S.E.	R^2	b	b S.E.	R^2
W	$Days_{EF}$	-0.95	0.201	0.40^{***}	-0.59	0.091	0.56^{***}	-0.57	0.090	0.55^{***}
R	$Days_{EF}$	-0.00028	0.0005	0.51^{***}	-0.0012	0.0002	0.47^{***}	-0.0009	0.0003	0.25^{**}
D95	$Days_{EF}$	0.14	0.66	0.01^{ns}	0.23	0.32	0.02^{ns}	-2.19	0.38	0.50^{***}
W	*R*	355	28.0	0.82^{***}	409	31.2	0.84^{***}	382	38.3	0.75^{***}
W	*D95*	0.108	0.066	0.07^{ns}	0.001	0.074	0.01^{ns}	0.127	0.038	0.04^{**}

W, final grain weight (mg $grain^{-1}$); *R,* mean rate of grain filling (mg GDD^{-1}); *D95*, grain filling duration (GDD); $Days_{EF}$, number of days from emergence to flowering. Genotype mean data across years was used at each site (n=35). The slope of the linear regression equation (*b*), its standard error (S.E.), the coefficient of determination (R^2), and the statistical significance ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{ns}p > 0.05$) are shown.

3.3.4. 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 I0I, I5I and I5S. 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 during the first half of the grain filling period of 0.18°C in Spain and 0.09ºC 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 year-dependent, 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 R^2 value (Figure 2 B, C).

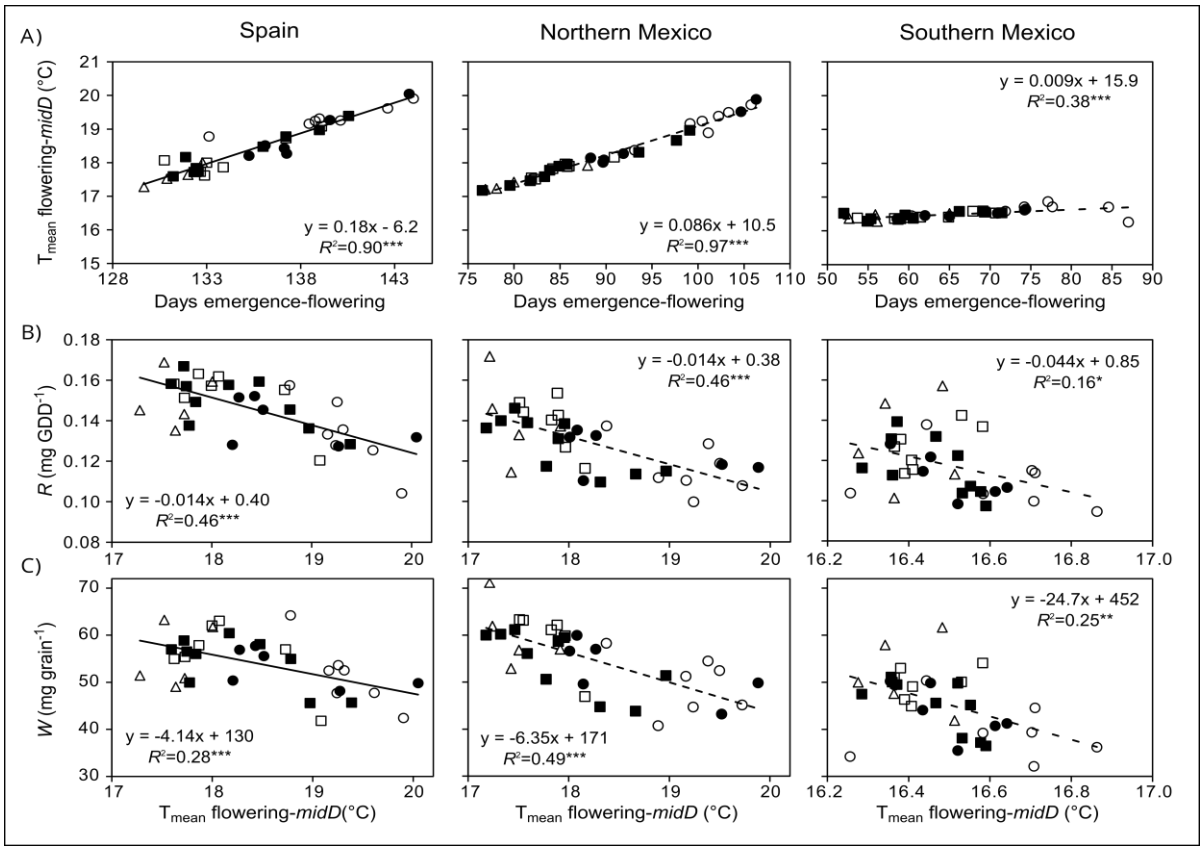

Figure III.2. Relationships between A) days from emergence to flowering and mean temperature (Tmean) from flowering to mid–grain filling (*midD*), B) Tmean from flowering to *midD* and mean grain filling rate (*R*), and C) Tmean from flowering to *midD* and final grain weight (*W*) in field experiments 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. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

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 3 A-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 3 B, C).

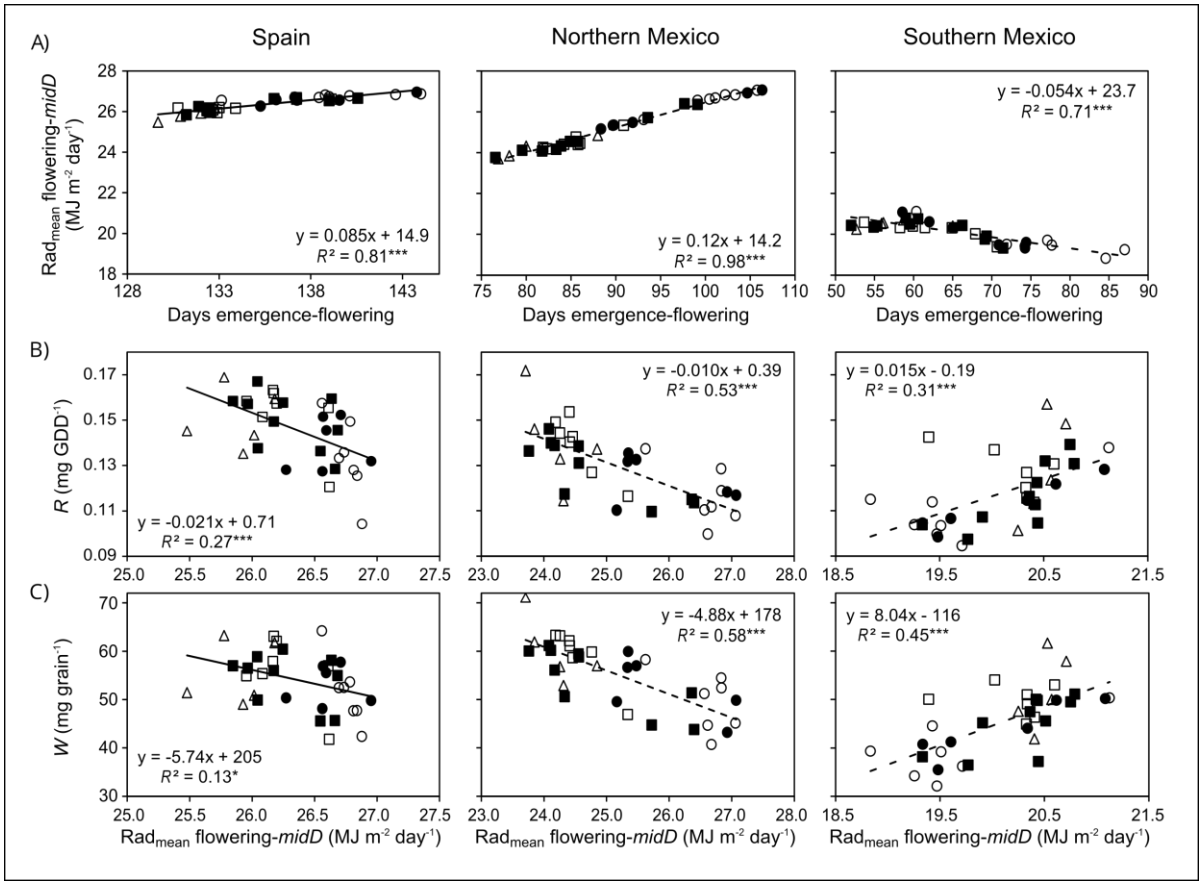

Figure III.3. Relationships between A) days from emergence to flowering and mean radiation (Rad_{mean}) from flowering to mid–grain filling (*midD*), B) Rad_{mean} from flowering to *midD* and mean grain filling rate (*R*), and C) Rad_{mean} from flowering to *midD* and final grain weight (*W*) in field 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. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

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).

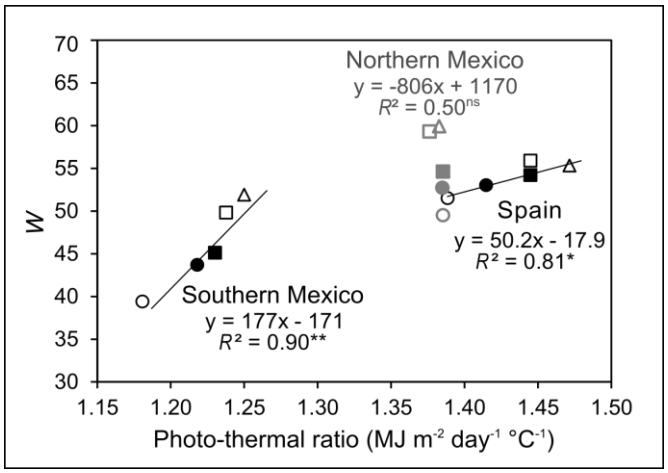

Figure III.4. Relationship between the photo-thermal ratio from flowering to mid–grain filling (*midD*) and final grain weight (*W*) in field experiments conducted in Spain, northern Mexico and southern Mexico, involving durum wheat genotypes grouped in five allele combinations at *Ppd-A1* and *Ppd-B1* loci. Allele combinations are represented according to the acronyms shown in Table 1 as ∆ = I0I, □ = I5I, ■ = I5S, ● = SS, ○ = SI. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3.4. 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 were a consequence of the contrasting latitudes and weather conditions at the experimental sites, such

as daylength 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 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 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, reduce 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.

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CHAPTER IV

Identification of marker-trait associations responsible for phenology, yield, and yield components in spring durum wheat cultivars grown at contrasting latitudes

ABSTRACT

Understanding the genetic variation responsible for the differences in phenology, yield, and yield components, is fundamental to guide selection in breeding programs. The interaction of this genetic variation with the environment (G×E) is a major subject to deal with. Nine experiments were carried out at three contrasting latitudes, in Spain, northern Mexico, and southern Mexico (41°N, 27°N, and 19°N, respectively), across three years. A set of 40 spring durum wheat lines was grown in the field experiments and genotyped with DArTseq® markers, in addition to specific markers for *Ppd-A1* and *Ppd-B1* loci. A phenotype-genotype association analysis by multiple ANOVAs, for individual markers and for marker pairs interactions, was performed to explore the genetic variability. Differences between sites in the genetic architecture of the analysed traits was also studied. Significant MTAs and MTA interactions for phenology, and yield components were found throughout the genome. Chromosomes 6A (114cM) and 6B (126cM) harboured an interesting region significant for yield across sites. Special attention should be paid to the interaction between MTAs, since some MTAs with lower effect increase when combined with other MTAs. In both single MTAs and combinations between them, site was an important factor for their detection.

4.1. INTRODUCTION

The global harvested area of wheat between the years 2016 and 2018 was around 225 million hectares, producing above 725 million tons, which accounts for 28% of the total cereal production (FAO, 2019). Durum wheat (*Triticum turgidum* L. var. *durum*) represents between 5 to 10% of total world wheat production. This cereal represents an important proportion of the diet, especially in Mediterranean regions, where pasta, couscous, and other derived products are important (Royo et al., 2017). This region is expected to be more affected by climate change than other places in the world (Hoegh-Guldberg et al., 2018). A reduction of around 6% in wheat yield has been predicted globally. Therefore, if the Mediterranean region suffers from climate change more severely, a worse scenario for durum wheat could be expected (Hoegh-Guldberg et al., 2018).

The increasingly extreme weather conditions makes the understanding of the interaction between the genotype and the environment (G×E) more important than ever. In the context of the climate change scenario it is important to elucidate how the interactions G×E work.

The decreasing prices of genotyping technologies in recent years have facilitated the access to more accurate and a bigger amount of genotypic data. Next-generation genotyping gives the possibility of obtaining thousands of molecular markers without prior sequence information. The genotyping by sequencing (GBS) approach of DArTseq technology developed by the company Diversity Arrays Pty Ltd (Canberra, Australia) (Sansaloni et al., 2011) can be used to analyse population diversity (Li et al., 2015), fine mapping of quantitative trait loci (QTLs), and trait association mapping (Poland and Rife, 2012). Finally, the tools for a better understanding of candidate genes have also improved with the publication of annotated genomes. A fully annotated reference genome of bread wheat was published in 2018 by (IWGSC, 2018), and more recently the assembly of the genome of durum wheat Svevo has been released (Maccaferri et al., 2019).

The main target of the genetic improvement in durum wheat is grain yield. Yield is a complex trait highly influenced by the environment, genetic factors, and their interaction. To facilitate the task of understanding the final yield, it could be dissected into two main yield components, grain number per unit area (GNm^{-2}) and grain weight (GW). In turn, GNm^{-2} can be explained as spike number per unit area (Spm^{-2}) and grains per spike (GSp), and the latter by spikelets per spike (SpklSp) and grains per spikelet (GSpkl). Numerous studies of trait association have been conducted to find QTLs related to yield and yield components. However, in most of them, the experiments were carried out in Mediterranean conditions (Golan et al., 2015; Maccaferri et al.,

2008; Mangini et al., 2018; Soriano et al., 2017), or in latitudes between 30°N and 45°N (Thanh et al., 2013; Zhang et al., 2012). Some of the studies were conducted in latitudes from around 11 to 18°N in Ethiopia and India (Mengistu et al., 2016; Patil et al., 2013) or in the southern hemisphere (Roncallo et al., 2017), but in general the individual studies have been carried out in a narrow latitudinal range.

One of the important aspects affecting yield and its components is crop phenology. Phenology is controlled by different types of genes such as vernalization genes (*Vrn*), photoperiod sensitivity (*Ppd-1*) and earliness *per se* (*Eps*), which is defined as the genetic variation due to other factors aside from photoperiod and vernalization. In previous studies developed in our group, the effect of known mayor genes of *Ppd-1* on phenology and yield components was studied (Arjona et al., 2018; Royo et al., 2016). However, in these reports it was found that the genetic variation not controlled by these genes was noticeably high, thus other genes must be involved in controlling the phenology fitting.

The objective of this work was to explore the genetic variation for phenology, yield, and yield components in a set of durum wheat cultivars and its interaction with the environment at three contrasting latitudes, exploring possible bilateral interactions between chromosome regions affecting developmental stages and increasing agronomic performance.

4.2. Material and Methods

4.2.1. Plant material and growing conditions

A collection of forty spring durum wheat genotypes was used. Five lines were provided from the University of Hohenheim, Germany (Durabon, 2716- 25.94.01, Megadur, 2805-49.94.02, and 2905-13.93.04), and were crossed with five advanced lines from the CIMMYT durum wheat program (Sooty_9/Rascon_37, Cado/Boomer_33, Dukem12/2∗rascon_21, Guanay, and Snitan). Thirty lines were derived from these crosses (Supplementary table 1). These populations were cultivated in CIMMYT (El Batán, Mexico) without selection in bulks up to the third generation (F_3). Spikes were randomly chosen in F_3 and were sown as head-row to F_4. At this stage, spikes with contrasting heading time were selected. The lines were advanced as head rows and increased up to the F_8 generation in Spain.

Field experiments were performed for three seasons (2010, 2011, and 2012), at three sites with contrasting latitudes: Spain (41°N), Mexico-north (27°N), and Mexico-south (19°N). The experimental field setup consisted of a randomized complete block design with three replicates.

Plots of 12 m^2 were sown, with a plant density adjusted to obtain around 450 spikes m^{-2}. Sowing time was November or December in Spain and Mexico-north, while in Mexico-south experiments were sown in May. Full irrigation was provided and standard agronomic practices were applied in order to avoid pests, diseases, and weeds.

4.2.2. Phenotyping

The following phenology stages were recorded according to Zadoks' scale (Zadoks et al., 1974): emergence (GS10), flowering (GS65), and physiological maturity (GS87), when at least 50 % of the plot reached the corresponding stage. The stages of double ridge (DR), and terminal spikelet (TS), according to the illustrations in (Kirby and Appleyard, 1986), were also recorded. For this purpose, 3 to 5 plants were sampled and dissected 2 to 3 times per week on each plot.

Temperature (absolute maximum, minimum, and mean) was recorded daily in each experiment by a meteorological station located in the experimental field. Thermal time (growing degree-days, GDD) was calculated using the daily temperature and the exact date when the corresponding phenological state was reached. Daily values of GDD were computed using 37°C as a threshold for maximum temperature and 0°C for minimum temperature. Daily GDD was computed using the maximum and minimum temperatures as reported in Angus et al. (1981). By using this methodology, GDD from emergence to double ridge (GDD_EDR), emergence to terminal spikelet (GDD_ETS), emergence to flowering (GDD_EF), and flowering to maturity (GDD_FM) were calculated. Daylength was calculated for each day including twilight (Forsythe et al., 1995). For each site, and counting from emergence, daily data points of each meteorological variable were fitted with the LOESS method.

Plots were divided into two identical sets; one used for destructive sampling, and the other one was harvested at commercial maturity. Plots were mechanically harvested and grain yield (GY, g m^{-2}) was expressed as dry weight. Grain weight (GW, mg) was estimated from a random sample of 200 dried grains. Grain number per unit area (GNm^{-2}, grains m^{-2}) was calculated as GY/GW. A 1m sample was taken from a central row, and the spikes (Spm^{-2}, m^{-2}) were counted and threshed, and grains were counted. For each sample, five random main spikes were selected, and their spikelets were counted. The number of grains per spike (GSp) were obtained dividing the total number of grains of the sample by the spikes. Grains per spikelet (GSpkl) were calculated as the grains per spike (GSp) divided by the spikelets per spike (SpklSp).

4.2.3. Genotyping

DNA isolation was performed from young leaf samples following the method of Doyle and Doyle, (1987). High-throughput genotyping was performed at Diversity Arrays Technology Pty Ltd (Canberra, Australia) (http://www.diversityarrays.com) with the DArTseq genotyping by sequencing platform (Sansaloni et al., 2011). A total of 46,161 markers were used to genotype the collection. Markers with missing values were discarded. A total of 1060 markers mapped in the Svevo physical map (Maccaferri et al., 2019) were considered and used for mapping purposes. The physical distance (Mb) was transformed into genetic distance (cM) using the physical *vs* genetic ratio distance (Mb/cM) (Maccaferri et al., 2019). The photoperiod sensitivity alleles located in the chromosome 2A (*Ppd-A1*) were genotyped following (Wilhelm et al., 2009), and the locus located in the chromosome 2B (*Ppd-B1*) following (Hanocq et al., 2004). The position of the *Ppd-A1* and *Ppd-B1* was assigned based on the position in the Svevo consensus map (Maccaferri et al., 2019).

4.2.4. Data analysis

4.2.4.1. Association analysis

Combined ANOVAs were performed across experiments using *aov* function from base R (R Core Team RRID:SCR_001905 et al., 2018), and the percentage of the sum of squares for each factor was computed. To explore the association between markers and phenotypic traits, ANOVA test was performed for each marker using a complete model with all 3 sites, years, and replicates, and then repeated individually for each site including the 3 years and replicates. Genetic variability was dissected into the variability between the groups formed by the marker alleles, and the variability within allele variants. The significance of a marker was tested using the within allele variation as error:

F test = SS between alleles / SS within alleles

Where SS means sum of squares of the ANOVA analysis.

Linkage disequilibrium (LD) was calculated for the whole set of markers using TASSEL 5.0 software (Bradbury et al., 2007), and graphically represented in R (R Core Team RRID:SCR_001905 et al., 2018). A LOESS curve was calculated and the decay of LD was considered at the distance where the r^2 mean value intercepts the curve.

The significance threshold for association analysis was established using the Bonferroni multiple comparison correction at p <0.05. The effect of the markers was calculated subtracting the mean value of the allele with lower value from the mean value of the allele with higher score for the trait.

4.2.4.2. QTL interaction

To study the interaction between marker-trait associations (MTAs), those markers with associations with a -log $p \geq 3$ for each trait were combined in pairs. A more permissive threshold was selected to be able to detect markers that even with a lower probability of being considered associated, could have a higher effect considering the interaction. In order to avoid spurious interactions, a threshold was established using a Bonferroni multiple comparison correction at $p < 0.005$. All calculations were carried out using R software (R Core Team RRID:SCR_001905 et al., 2018). Graphical representations were made with the software *Inkscape* (Inkscape Team, 2019).

4.3. RESULTS

4.3.1. Phenotypic data

Spain showed the lowest temperatures and radiation in the first part of the cycle, as well as the longest period from emergence to double ridge (DR) and the shortest period from flowering to maturity. This site showed the highest variation in daylength, from almost 10h at emergence to more than 16h at maturity, and the highest temperatures from flowering to maturity. Mexico-north showed the highest temperatures in the majority of the cycle. The shortest period from emergence to DR took place in Mexico-south, with the highest temperatures in this period. This site presented the longest flowering to maturity phase, and the lowest radiation during this period. In Mexico-south, given that sowing was carried out during the spring, the daylength had a trend of shortening during the majority of the cycle (Fig 1).

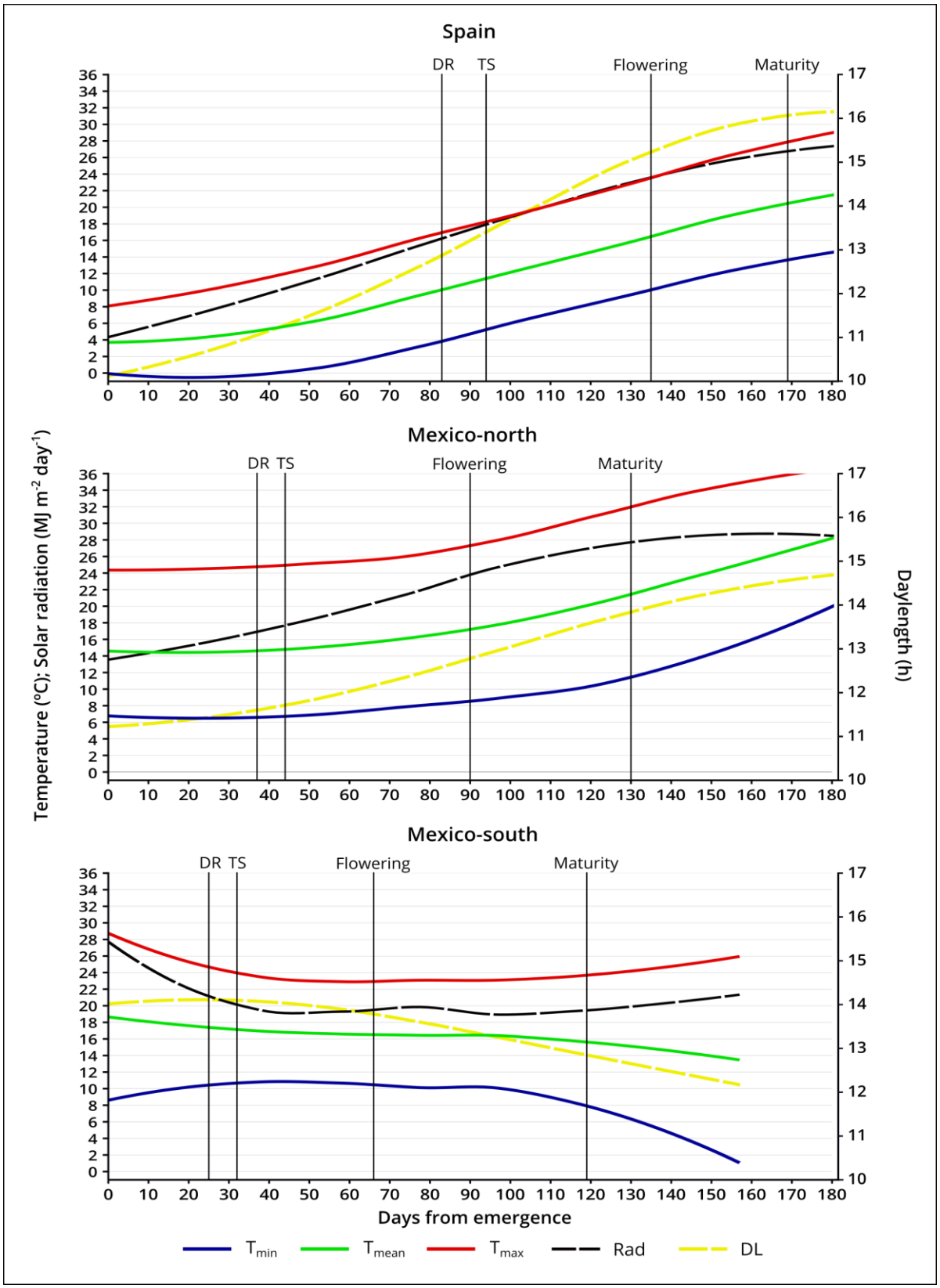


Figure IV.1. Average environmental conditions for each site during the years 2010, 2011 and 2012. Smooth line drawn with the Loess method fitted to the 3 year points from emergence. Tmin: absolute minimum temperature of the day (ºC), Tmean: mean temperature of the day (ºC), Tmax: absolute maximum temperature of the day (ºC). Rad: accumulated radiation in the day (MJ m^{-2} day^{-1}), DL: daylength (h) including twilight. Vertical bars indicate the mean of days needed to reach each developmental phase: double ridge (DR), terminal spikelet (TS), flowering, and maturity.

The ANOVA showed wide variation between traits. Variability was mainly explained by the site for six out of eleven traits, ranging from 2 to 62% of the sum of squares (SS) for GSpkl and GDD_FM respectively (Fig. 2). The year effect explained less than 10% of SS except for GDD_ETS (19%), becoming more important than site x year interaction for this trait. The environment (combination of site and year) represented between 2 % and 18 % of the SS for all traits, and it was more important than the genotype effect only for GDD_FM and Spm^{-2}. The Genotype effect explained the largest variation for GDD_ETS, GSp, SpklSp, GSpkl and GW, whereas for GDD_FM, Spm^{-2}, and yield explained the lowest variation. The interaction genotype x site was more stable between traits, with values from 4 to 12% of the phenotypic variation. Genotype x year explained between 1 and 7 % of total variation, and the interaction genotype x site x year represented between 2 to 12 %, and it was more important than genotype and genotype x year for Spm^{-2} (Fig. 2).

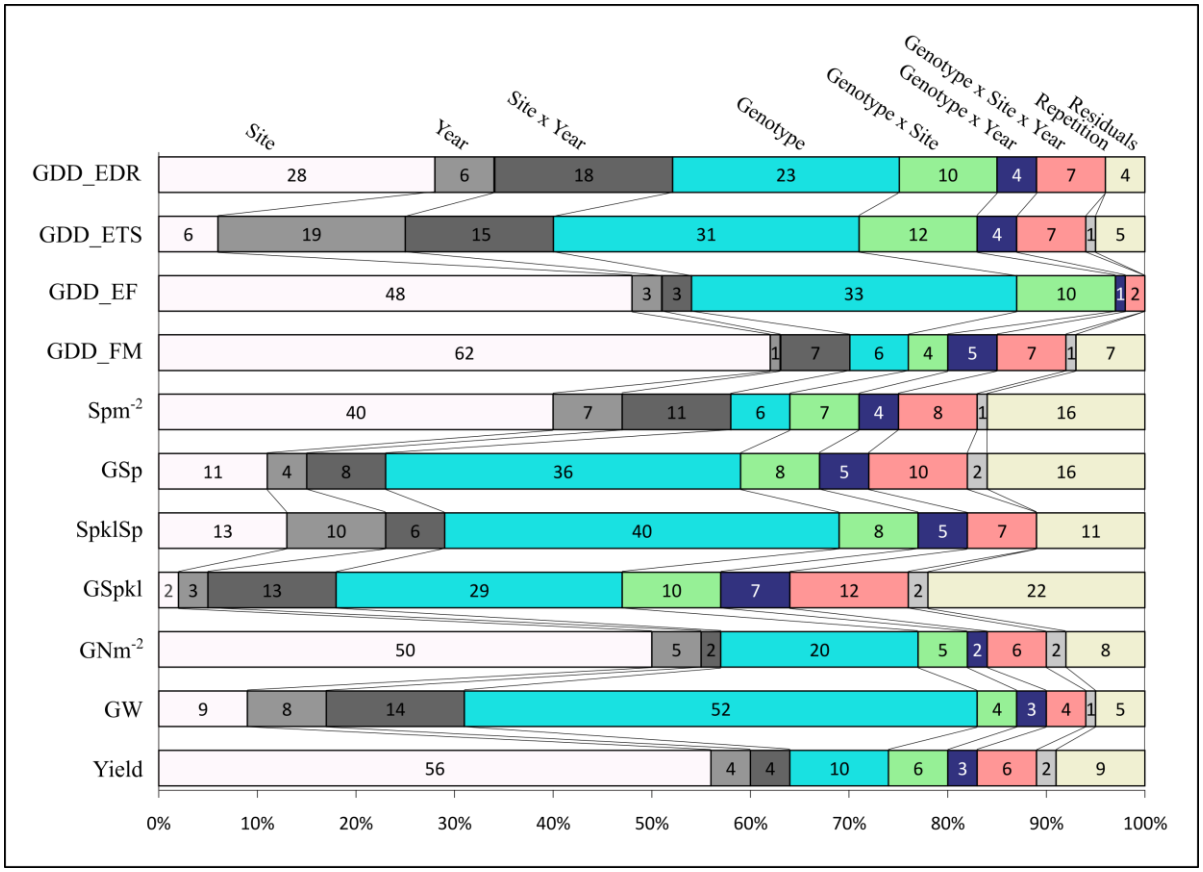


Figure IV.2. Percentage of the sum of squares of the ANOVAs for each trait. All percentages were significant at $p < 0.001$. The percentage values that explain less than 0.5 % of the SS are not represented. GDD_EDR: GDD from emergence to double ridge. GDD_ETS: GDD from emergence to terminal spikelet. GDD_EF: GDD from emergence to flowering. GDD_FM: GDD from flowering to maturity. Spm^{-2}: spikes m^{-2}. Gsp: grains $spike^{-1}$. SpklSp: spikelets $spike^{-1}$. GSpkl: Grains $spikelet^{-1}$. GNm^{-2}: grains number m^{-2}. GW: grain weight (g).

4.3.2. Linkage disequilibrium

Linkage disequilibrium was estimated for locus pairs in the whole genome for the 1060 markers. A total of 404,750 possible pair-wise loci were observed. Of these locus pairs, 5.7 % showed significant linkage disequilibrium at $p < 0.01$. A critical value of $r^2 = 0.1$, corresponding to the mean of LD for the whole genome, was determined as the threshold for LD due to physical linkage. Markers were in LD up to a distance of 18 cM (Fig. 3).

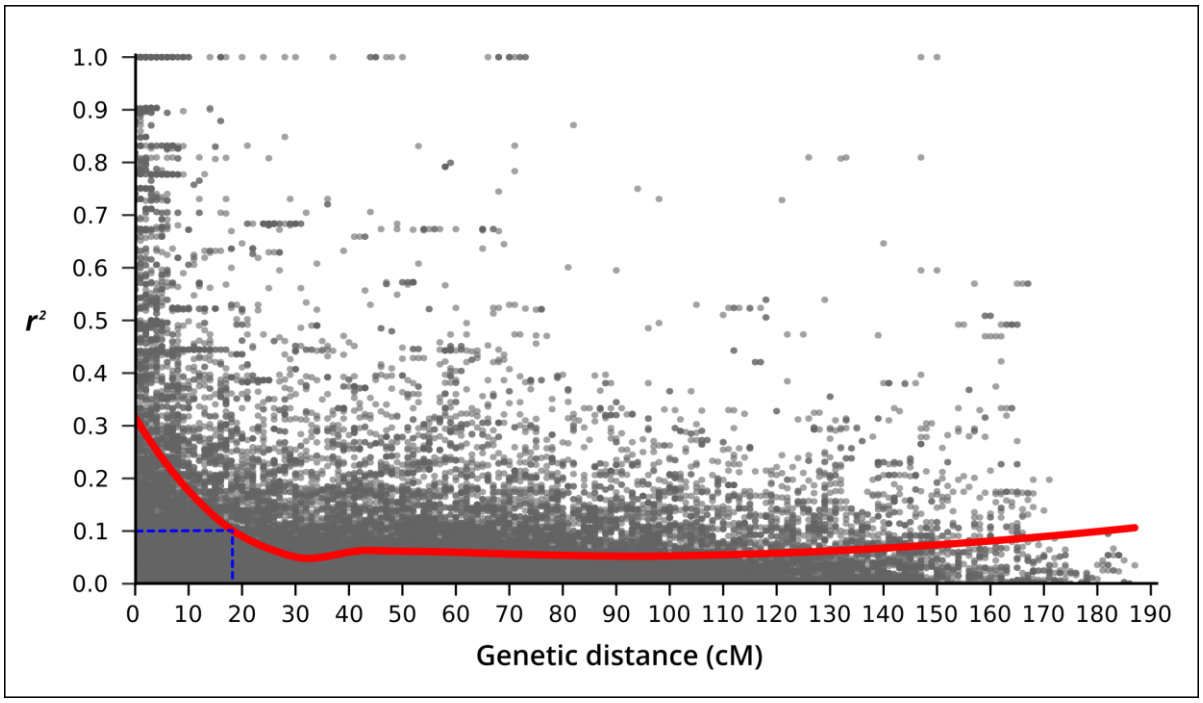


Figure IV.3. Linkage disequilibrium plot. The LOESS curve is represented in red. The horizontal blue dashed line corresponds to the r^2 mean, and the vertical one the distance at which LD decays.

4.3.3. Association analysis

A total of 46,161 DArTseq markers and the *Ppd-A1* and *Ppd-B1* loci were used to genotype the set of 40 durum wheat genotypes. Markers with minor allele frequency lower than 5%, and missing values were removed from the analysis, with a total of 1062 remaining markers. An FDR (false discovery rate) threshold using the Bonferroni correction at $P < 0.05$ was defined at 4.3, taking into account the 1062 markers according to the Svevo reference genome (Maccaferri et al., 2019). According to the LD decay, those MTAs within a genetic distance of 18 cM were considered as belonging to the same QTL (Table 1). Following this criteria, a total of 82 MTAs were found, 19 MTAs across sites, 43 for Spain, 12 for Mexico-north, and 8 for Mexico-south. Across sites, only *Ppd-A1* was significant for phenology, while all yield components except Spm^{-2} had at least 2 MTAs. Yield only had significant MTAs across sites. Spain was the only site that had significant MTAs for all the phenology traits. The MTAs for phenology in Mexico-north comprise the phases between

emergence and terminal spikelet, and emergence to flowering measured in days, and also from flowering to maturity if measured in GDD. For yield components Mexico-north only reported significant MTAs for GW, GNm^{-2}, Spm^{-2} and GSp. Mexico-south did not show significant MTAs for phenology, and for the yield components GNm^{-2}, and Spm^{-2} (Table 1).

No significant markers were found in chromosomes 5A, 5B, and 7A. On the other hand, chromosome 2A harboured most of the significant associations (37), followed by 7B with 11 significant MTAs. The chromosome 3A and 6B harboured 7 MTAs each, and the remaining chromosomes less than 5 MTAs. For phenology, only the chromosomes 2A, 2B, 3B, 6B, and 7B had significant MTAs. In the case of yield and yield components, the chromosome 2B did not show significant MTAs (Table 1).

The distal part of chromosome 6B reported associations for phenology in a region where no other MTAs were previously found. The remaining significant MTAs were consistent with information found in the literature, if not of the same traits, at least related ones (Fig. 4a and 4b).

Table IV-1. List of significant MTAs

Trait	Site	Marker	Chromosome	Distance (cM)	-log *p*	Effect
D_EDR	Spain	SNP1077397	7B	136.6	5.3	4.4
D_EDR	Spain	*Ppd-A1*	2A	38.8	5.0	6.8
D_EDR	Spain	SNP1125985	6B	109.7	4.5	4.5
D_ETS	Across	*Ppd-A1*	2A	38.8	4.4	5.7
D_ETS	Spain	*Ppd-A1*	2A	38.8	5.5	7.8
D_ETS	Spain	SNP1077397	7B	136.6	5.5	5.0
D_ETS	Spain	SNP1045660	7B	5.6	4.9	6.1
D_ETS	Spain	SNP1088346	7B	31.6	4.4	5.4
D_ETS	Spain	PAV1724214	2A	102.9	4.3	4.8
D_ETS	Mexico-north	PAV1667148	2A	158.8	4.9	5.6
D_EF	Across	*Ppd-A1*	2A	38.8	6.0	11.6
D_EF	Spain	PAV1308762	7B	138.5	5.2	4.7
D_EF	Spain	*Ppd-A1*	2A	38.8	5.0	6.8
D_EF	Spain	SNP1125985	6B	109.7	4.3	4.7
D_EF	Mexico-north	*Ppd-A1*	2A	38.8	7.0	16.3
D_FM	Spain	SNP1021742	2B	72.5	6.4	2.3
D_FM	Spain	PAV1216270	3B	41.5	5.6	2.4
D_FM	Spain	SNP991212	2A	69.0	4.4	2.0
GDD_EDR	Spain	PAV1308762	7B	138.5	5.2	49.8
GDD_EDR	Spain	*Ppd-A1*	2A	38.8	4.8	76.8
GDD_EDR	Spain	SNP1125985	6B	109.7	4.3	49.5
GDD_ETS	Spain	PAV1308762	7B	138.5	5.4	61.0
GDD_ETS	Spain	*Ppd-A1*	2A	38.8	5.3	95.2
GDD_ETS	Spain	SNP1045660	7B	5.6	4.8	72.4
GDD_ETS	Spain	PAV1073035	7B	31.6	4.3	64.3
GDD_ETS	Mexico-north	PAV1667148	2A	158.8	4.9	90.3
GDD_EF	Across	*Ppd-A1*	2A	38.8	6.1	203
GDD_EF	Spain	PAV1308762	7B	138.5	5.1	77.2
GDD_EF	Spain	*Ppd-A1*	2A	38.8	4.8	114
GDD_EF	Mexico-north	*Ppd-A1*	2A	38.8	7.0	291
GDD_FM	Spain	SNP1021742	2B	72.5	5.5	42.9

Trait	Site	Marker	Chromosome	Distance (cM)	-log *p*	Effect
GDD_FM	Spain	PAV1216270	3B	41.5	4.8	44.3
GDD_FM	Spain	PAV1113052	2A	90.1	4.3	40.7
GDD_FM	Mexico-north	SNP1021742	2B	72.5	4.3	46.8
GW	Across	SNP1060708	2A	81.2	7.7	10.4
GW	Across	PAV1724214	2A	102.9	6.0	7.8
GW	Across	SNP1109210	3A	143.9	4.5	7.7
GW	Spain	PAV2293689	2A	79.7	9.5	8.9
GW	Spain	PAV1724214	2A	102.9	5.9	7.4
GW	Spain	SNP1109210	3A	143.9	4.4	7.3
GW	Mexico-north	SNP984567	2A	81.3	7.3	12.1
GW	Mexico-north	PAV1724214	2A	102.9	5.7	9.1
GW	Mexico-north	SNP1109210	3A	143.9	4.3	9.0
GW	Mexico-south	SNP1060708	2A	81.2	6.0	9.0
GW	Mexico-south	SNP1089380	4B	55.3	5.2	8.3
GW	Mexico-south	PAV1724214	2A	102.9	5.0	6.9
GNm^{-2}	Across	SNP1091747	2A	79.9	8.2	2954
GNm^{-2}	Across	PAV1126966	3A	57.3	4.7	3178
GNm^{-2}	Across	SNP1109210	3A	143.9	4.6	2609
GNm^{-2}	Spain	SNP984567	2A	81.3	7.6	4447
GNm^{-2}	Spain	SNP1109210	3A	143.9	4.8	3400
GNm^{-2}	Spain	PAV1229305	4A	36.6	4.7	2924
GNm^{-2}	Spain	PAV1126966	3A	57.3	4.6	4175
GNm^{-2}	Spain	PAV1165987	1A	58.3	4.4	2885
GNm^{-2}	Mexico-north	PAV2293689	2A	79.7	6.8	2917
Yield	Across	SNP2276353	6A	114.5	4.6	63.9
Yield	Across	PAV1106411	6B	126.0	4.6	63.9
Spm^{-2}	Spain	PAV977865	4B	0.1	4.3	73.3
Spm^{-2}	Mexico-north	PAV1138184	1B	70.4	4.5	46.1
GSp	Across	SNP1091747	2A	79.9	9.6	8.8
GSp	Across	SNP1022127	3B	116.3	4.5	6.5
GSp	Across	PAV992973	6A	3.1	4.3	6.2
GSp	Spain	PAV2293689	2A	79.7	7.0	9.4
GSp	Mexico-north	PAV2293689	2A	79.7	7.5	9.7
GSp	Mexico-north	SNP1022127	3B	116.3	5.7	8.5
GSp	Mexico-south	PAV2293689	2A	79.7	7.4	7.6
GSp	Mexico-south	PAV992973	6A	3.1	4.5	6.0
SpklSp	Across	*Ppd-A1*	2A	38.8	4.7	3.1
SpklSp	Across	PAV1040965	1A	1.3	4.6	2.1
SpklSp	Across	SNP1125985	6B	109.7	4.3	2.2
SpklSp	Spain	PAV1308762	7B	138.5	4.9	2.0
SpklSp	Spain	SNP1054888	4B	89.1	4.8	2.2
SpklSp	Spain	PAV1119715	6B	112.4	4.8	2.0
SpklSp	Spain	PAV1040965	1A	1.3	4.4	2.0
SpklSp	Spain	PAV1032504	6B	0.3	4.4	2.1
SpklSp	Spain	SNP999959	4A	131.8	4.4	2.2
SpklSp	Mexico-south	SNP1127702	2A	46.0	4.3	1.9
SpklSp	Mexico-south	*Ppd-A1*	2A	38.8	6.2	2.7
GSpkl	Across	PAV2293689	2A	79.7	7.4	0.4
GSpkl	Across	PAV1140854	1A	73.8	4.5	0.4
GSpkl	Spain	PAV2293689	2A	79.7	7.3	0.4
GSpkl	Mexico-south	PAV2293689	2A	79.7	5.9	0.4

4.3.4. MTA interaction

A total of 439 MTAs with a -log $p \geq 3$ with no missing values were chosen to study the interaction among chromosome regions (Supplementary table 2).

After considering the interaction of all the previously selected markers two by two for each site and trait, and eliminating redundant combinations, a total of 77 interactions between chromosome regions were selected (Table 2). All of these interactions have a value of -log *p* higher than 5.3. No MTAs interacted for Spm^{-2}. In the case of GSp, SpklSp, and GW all had 10 or more MTA interactions, followed by GNm^{-2} with 9 MTA interactions, while all the remaining traits had 5 or less interactions (Table 2). Based on the site, the number of MTA interactions across sites was 24, of which 11 were for phenology traits and the remaining 13 for yield and yield components. Spain was the site with more MTAs interactions with 31, of which 17 were related with yield components. Mexico-north had the lowest interactions with 6. Mexico-south was the only site with more significant MTA interactions (14) than single MTAs (Table 1 and 2).

The highest number of MTA interactions was found in the chromosome 2A with 41 interactions. The chromosomes 6B and 3A both reported 13 interactions. The chromosomes 1A and 4B showed 12 interactions, chromosome 5A showed 10 interactions, and finally, the remaining chromosomes 9 or fewer MTA interactions. The chromosome 4A had 7 interactions, all for yield related traits. The chromosome 5B had only 1 interaction, and it was at the Spain site (SpklSp). The chromosome 7A did not report any interaction in Spain. Chromosomes 1A, 4A, and 7B were not present in any interaction in north and Mexico-south. Chromosomes 1B and 4A were not present in any of the MTA interactions regarding phenological traits (Table 2).

The strongest improvements in MTA significance when considering interactions occurred in yield and yield components. The interactions between chromosomes 2A (37-44 cM) and 4B (13-26 cM) improved the *-log p* of the 4B region by 3 points (Supplementary table 2). In Mexico-south, the interaction of regions 2A (103-126 cM) with 4B (55 cM) had 3 units of the -log *p* with respect to the individual MTAs for GW. In Spain, for GNm^{-2} the interaction of the region 2A (103-126 cM) with the region 4A (37cM), and 1A (43-58 cM) improved the -log *p* in 4 and 3.7 units, respectively. For yield, an improvement of 3.2 units was achieved by the interactions between regions 1B (27-29 cM) and 6B (64 cM) (Table 2).

Table IV-2. Significant MTA Interactions. The improvement shows the difference between the significance of the interaction and the highest of the single MTAs. It also shows the absolute effect between the lowest and higher values of the different allele combinations between MTAs.

Trait	Site	Chr. region 1	Chr. Region 2	-log *p*	Improvement	Effect
D_EDR	Across	2A/37-44	6B/1-8	6.0	2.0	8.0
D_EDR	Spain	6B/110	7B/5-6	7.1	2.6	6.4
D_EDR	Spain	1A/101	1A/1	5.8	2.0	8.3
D_ETS	Across	2A/103-126	6B/64	6.1	2.8	6.4
D_ETS	Across	6B/1-8	7B/5-6	5.6	2.2	7.4
D_ETS	Spain	2A/37-44	2A/71-81	7.5	2.0	9.1
D_ETS	Spain	6B/110	7B/5-6	7.0	2.1	7.2
D_ETS	Spain	1A/1	1A/101	6.5	2.5	8.5
D_EF	Across	5A/111-116	6A/44-53	5.5	2.3	13.4
D_EF	Spain	3B/73-76	5A/111-116	5.8	2.0	7.9
D_EF	Spain	2A/103-126	5A/194	5.7	2.3	6.8
D_EF	Mexico-north	5A/111-116	6B/64	6.4	2.3	17.9
D_FM	Mexico-north	2A/37-44	2B/71-72	5.8	2.5	7.5
GDD_EDR	Across	2A/37-44	6B/1-8	5.6	2.0	120
GDD_EDR	Spain	6B/110	7B/5-6	7.0	2.7	70
GDD_EDR	Spain	2A/37-44	2A/71-81	6.8	2.0	94.1
GDD_ETS	Spain	2A/37-44	2A/71-81	7.3	2.0	111
GDD_ETS	Spain	6B/110	7B/5-6	6.8	2.0	86
GDD_ETS	Spain	1A/1	1A/101	6.1	2.2	102
GDD_EF	Across	5A/111-116	6A/44-53	5.6	2.4	237
GDD_EF	Spain	3B/73-76	5A/111-116	5.8	2.0	133
GDD_EF	Spain	2A/103-126	5A/194	5.7	2.3	115
GDD_EF	Mexico-north	5A/111-116	6B/64	6.3	2.3	319
GDD_FM	Across	2A/71-81	2B/71-72	6.6	2.6	61
GDD_FM	Across	2A/71-81	7A/118	6.4	2.5	65
GDD_FM	Across	2A/71-81	3A/2	6.4	2.5	66
GDD_FM	Across	2A/71-81	2B/165	6.0	2.1	62
GDD_FM	Across	4B/13-26	5A/26	5.9	2.1	72
GW	Across	2A/37-44	4B/13-26	6.4	3.0	14.3
GW	Across	2A/37-44	3A/57	6.0	2.3	14.1
GW	Across	2B/120	6B/1-8	5.5	2.0	14.7
GW	Spain	2A/71-81	2A/103-126	12.0	2.5	11.7
GW	Spain	2A/103-126	3B/116	6.3	2.2	11.5
GW	Mexico-south	2A/103-126	4B/55	8.2	3.0	11.9
GW	Mexico-south	2B/120	4B/55	7.8	2.6	11.1
GW	Mexico-south	2A/37-44	4B/13-26	5.9	2.5	12.9
GW	Mexico-south	2B/120	6B/1-8	5.8	2.1	13.4
GW	Mexico-south	2A/37-44	3A/57	5.5	2.0	12.6
GNm^{-2}	Across	2A/103-126	4A/37	5.9	2.2	3477
GNm^{-2}	Across	4A/37	4B/13-26	5.8	2.1	4029
GNm^{-2}	Across	1A/43-58	2A/103-126	5.6	2.3	3333
GNm^{-2}	Spain	2A/103-126	4A/37	8.6	4.0	5335
GNm^{-2}	Spain	1A/43-58	2A/103-126	8.1	3.7	5192
GNm^{-2}	Spain	3A/144-147	4A/37	6.9	2.1	4911
GNm^{-2}	Spain	3A/144-147	4A/140	6.7	2.0	5413
GNm^{-2}	Spain	4A/37	4B/13-26	6.7	2.1	5354
GNm^{-2}	Spain	2A/103-126	4A/140	6.3	2.2	4923
Yield	Mexico-north	1B/27-29	6B/64	6.3	3.2	162
Yield	Mexico-south	2A/71-81	2A/103-126	6.0	2.1	138
Yield	Mexico-south	2A/103-126	5A/111-116	5.5	2.2	139
GSp	Across	3B/116	6B/1-8	6.9	2.4	11.6
GSp	Across	2A/103-126	3B/116	6.6	2.1	11.3

Trait	Site	Chr. region 1	Chr. Region 2	-log *p*	Improvement	Effect
GSp	Spain	2A/103-126	2B/45	6.4	2.4	13.0
GSp	Spain	1A/43-58	2A/103-126	6.4	2.4	13.0
GSp	Spain	2B/45	3A/57	6.4	2.4	14.3
GSp	Spain	1A/43-58	3A/57	6.4	2.4	14.3
GSp	Spain	2B/45	3A/144-147	6.0	2.0	13.5
GSp	Spain	1A/43-58	3A/144-147	6.0	2.0	13.5
GSp	Mexico-north	2A/144	3A/144-147	6.3	2.8	18.1
GSp	Mexico-north	3A/144-147	7A/138	6.0	2.5	14.3
GSp	Mexico-south	2A/103-126	3B/73-76	5.7	2.3	10.4
GSp	Mexico-south	1B/75-76	2A/103-126	5.7	2.3	10.4
SpklSp	Across	2A/37-44	2A/71-81	7.2	2.5	4.1
SpklSp	Across	4B/55	7B/137	6.7	2.7	4.6
SpklSp	Across	4B/55	7A/169	6.6	2.6	4.8
SpklSp	Across	4B/55	7A/7	6.0	2.0	4.2
SpklSp	Across	1B/27-29	2A/71-81	5.8	2.0	3.9
SpklSp	Spain	1A/1	6A/44-53	6.5	2.1	4.4
SpklSp	Spain	2A/71-81	5B/75	6.1	2.1	3.7
SpklSp	Spain	1B/75-76	2A/71-81	6.0	2.2	3.8
SpklSp	Mexico-north	2A/37-44	6B/110	6.6	2.6	3.9
SpklSp	Mexico-north	2A/37-44	2A/71-81	6.1	2.1	5.1
SpklSp	Mexico-south	4B/55	7A/169	6.6	2.9	4.0
SpklSp	Mexico-south	2B/71-72	6A/19	5.5	2.0	2.9
SpklSp	Mexico-south	4B/55	7A/7	5.3	2.0	2.9
GSpkl	Mexico-south	2A/71-81	3A/124	8.7	2.9	0.6
GSpkl	Mexico-south	2A/71-81	3A/82	8.4	2.5	0.6

4.3.5. Phenology

In Spain, when the region 6B (110 cM) interacted with 7B (5-6 cM) the effect on D_EDR increased by 1.9 days in comparison with the 6B (110 cM) alone. The absolute strongest effect changing D_EDR was found in the interaction between the regions at 1 cM and 101 cM in the chromosome 1A, with 1.9 days with respect to the next higher value. The same interaction between 6B (110 cM) and 7B (5-6 cM) regions improved the effect in D_ETS, but in this case, regarding the 7B (5-6 cM) region. In addition, the same interactions at 1 cM and 101 cM in the chromosome 1A had the strongest effect. The same MTAs that were significant for D_EDR were also significant for D_EF, but the regions in the interactions were all different. Significant interactions for D_FM were found only in Mexico-north. In Spain, the MTAs and the interactions observed in phenology were almost identical when measured in days or GDD (Tables 1 and 2).

In Mexico-north, no phenology traits had either significant MTAs or interactions, except for *Ppd-A1*. Similarly to what happened in Spain, the results of phenology measured in days were the same as in GDD, with the exception of GDD_FM. For this trait, the MTA at the chromosome 2B was not significant either in GDD or in days, but it took part in a significant interaction.

Across sites, only *Ppd-A1* was significant for phenology, while 11 MTA interactions were found to be significant. Phenology traits measured in days instead of GDD had more MTAs in Spain than in Mexico-north sites. Mexico-south did not have significant MTAs or interactions between them (Tables 1 and 2).

4.3.6. Yield components

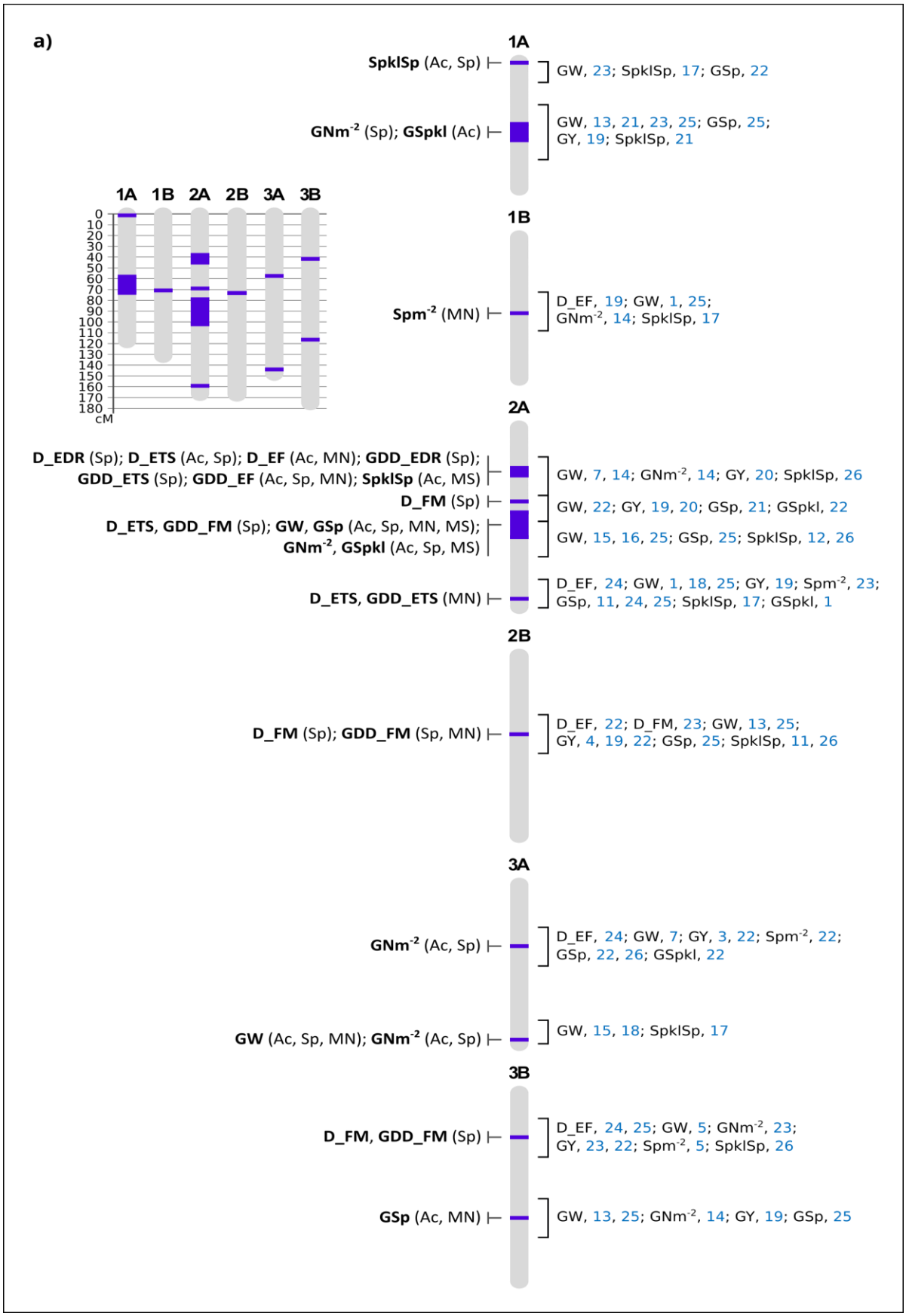
The MTAs in the chromosome 2A (80 cm and 103cM) in Spain interacted with GW and became the most significant interaction of all traits and sites (12.0). Its effect when interacting increased in 2.8 mg/grain regarding the best of them alone. For GNm^{-2} the two MTAs 3A (144 cM) and 4A (37 cM) improved the effect by 1511 grains/m^2 when interacting, and reached an effect improvement of between 2000 and 2430 GNm^{-2} when they interacted with other regions. At this site, for GSp the MTA in the chromosome 2A did not participate in any MTA interaction. For SpklSp the MTA in 1A (1 cM) increased the effect in 2.4 spikelets, which was more than double of the effect on its own (Table 1 and 2).

Mexico-north only had MTAs and interactions at the same time for GSp, and no mutual region was found between them. The best interaction occurred between regions 2A (144 cM) and 3A (144-147 cM), and increased the effect in 8 grains regarding the best single MTA. The strongest effect on SpklSp of all sites was due to the interaction of the regions 2A (37-44 cM) and 2A (71-81 cM), which had an effect of 5.1 SpklSp (Table 1 and 2).

In Mexico-south, the interaction between regions 2A (103-126 cM) and 4B (55 cM) improved the effect on GW in 3.6 mg/grain regarding the regions alone. For GSp both MTAs and interactions were found, but no common regions were present. The Highest -log *p* value (7.4) corresponded with the MTA in the chromosome 2A. The same situation of non-mutual regions between MTAs and interactions occurred for SpklSp. In the case of GSpkl the only significant MTA in south Mexico was involved in the two interactions for this trait, which meant a mean increase of 0.2 GSpkl.

Across sites, GW did not share any mutual region between significant MTAs and their interactions. The MTA found in the chromosome 2A at 81 cM had the highest value of -log *p* (7.7), while the interactions between the region 2B (120 cM) and the 6B (1-8 cM) showed the highest effect with 14.7 mg/grain of difference. For GNm^{-2} the MTAs and interactions did not share any mutual region either. The region in chromosome 2A (80 cM) had the greatest value of -log *p* (8.2) of any interaction between other regions. Similar effects in yield were found in the chromosomes 6A and 6B. The MTA in chromosome 2A (80 cM) had the highest value of GSp, and the effect of the MTA in chromosome 3B (116 cM) was increased in 5 grains when interacted with 6B (1-8 cM). For

SpklSp no MTAs were considered significant for the interaction with the exception of the region where the *Ppd-A1* is located. In all cases the five interactions doubled the effect of the single MTAs for SpklSp (Tables 1 and 2).


a)
1A
SpklSp (Ac, Sp)
GW, 23; SpklSp, 17; GSp, 22
GNm-2 (Sp); GSpkl (Ac)
GW, 13, 21, 23, 25; GSp, 25;
GY, 19; SpklSp, 21
1A 1B 2A 2B 3A 3B
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180
cM
1B
Spm-2 (MN)
D_EF, 19; GW, 1, 25;
GNm-2, 14; SpklSp, 17
2A
D_EDR (Sp); D_ETS (Ac, Sp); D_EF (Ac, MN); GDD_EDR (Sp);
GDD_ETS (Sp); GDD_EF (Ac, Sp, MN); SpklSp (Ac, MS)
GW, 7, 14; GNm-2, 14; GY, 20; SpklSp, 26
D_FM (Sp)
GW, 22; GY, 19, 20; GSp, 21; GSpkl, 22
D_ETS, GDD_FM (Sp); GW, GSp (Ac, Sp, MN, MS);
GNm-2, GSpkl (Ac, Sp, MS)
GW, 15, 16, 25; GSp, 25; SpklSp, 12, 26
D_ETS, GDD_ETS (MN)
D_EF, 24; GW, 1, 18, 25; GY, 19; Spm-2, 23;
GSp, 11, 24, 25; SpklSp, 17; GSpkl, 1
2B
D_FM (Sp); GDD_FM (Sp, MN)
D_EF, 22; D_FM, 23; GW, 13, 25;
GY, 4, 19, 22; GSp, 25; SpklSp, 11, 26
3A
GNm-2 (Ac, Sp)
D_EF, 24; GW, 7; GY, 3, 22; Spm-2, 22;
GSp, 22, 26; GSpkl, 22
GW (Ac, Sp, MN); GNm-2 (Ac, Sp)
GW, 15, 18; SpklSp, 17
3B
D_FM, GDD_FM (Sp)
D_EF, 24, 25; GW, 5; GNm-2, 23;
GY, 23, 22; Spm-2, 5; SpklSp, 26
GSp (Ac, MN)
GW, 13, 25; GNm-2, 14; GY, 19; GSp, 25

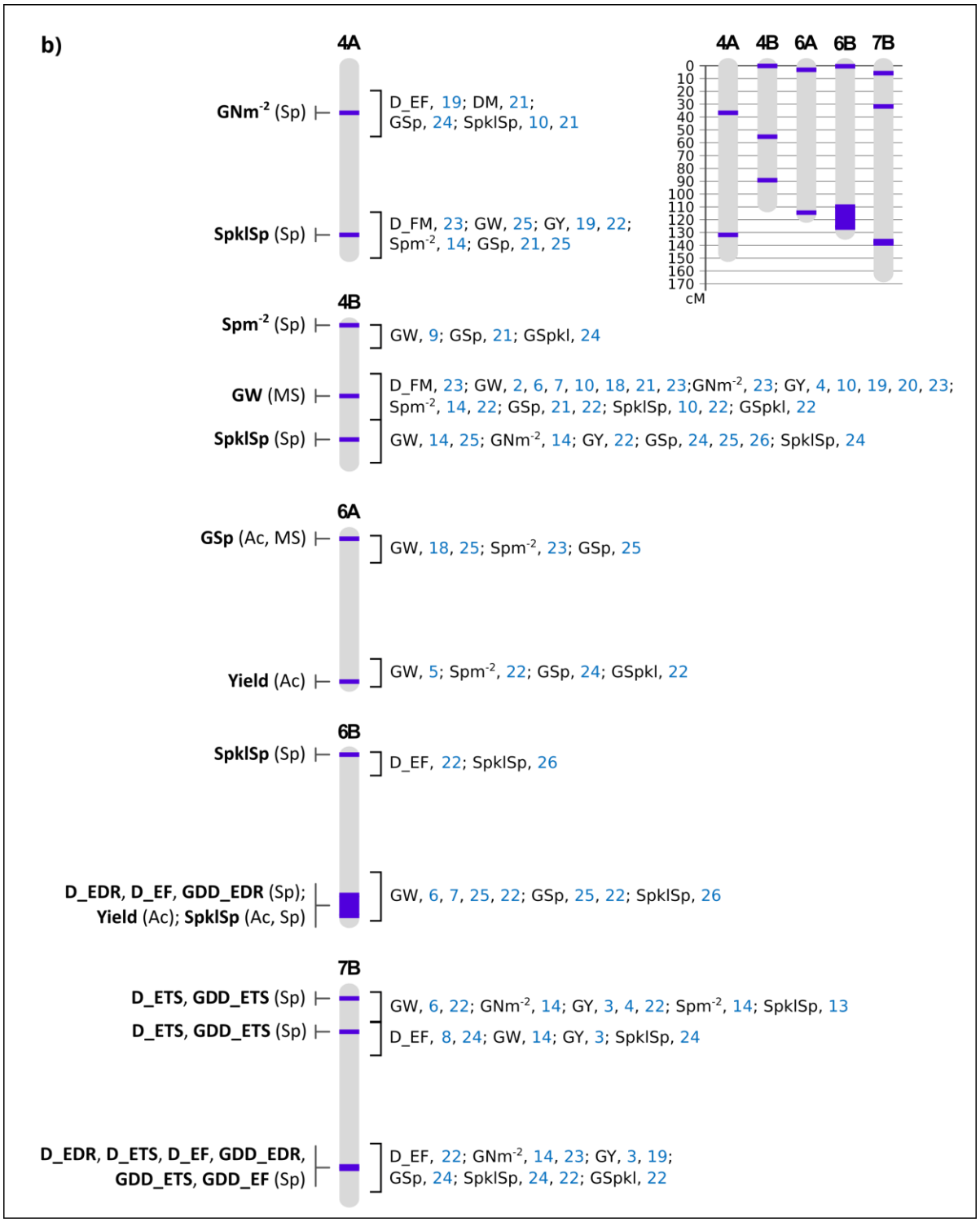


Figure IV.4. **a**) and **b**). Schematic representation of the chromosomes and the regions where MTAs were found. Left annotations in bold are traits for which MTAs were found, left annotations between brackets reference the site where MTAs were significant: Spain (Sp), Mexico-north (MN), and Mexico-south (MS). Right annotations reference the trait found in bibliography in this region, the numbers are the article references: 1. (Peng et al., 2003), 2. (Elouafi and Nachit, 2004), 3. (Maccaferri et al., 2008), 4. (Peleg et al., 2009), 5. (Golabadi et al., 2011), 6. (Peleg et al., 2011), 7. (Blanco et al., 2012), 8. (Buerstmayr et al., 2012), 9. (Zhang et al., 2012), 10. (Patil et al., 2013), 11. (Thanh et al., 2013), 12. (Faris et al., 2014b), 13. (Faris et al., 2014a), 14. (Graziani et al., 2014), 15. (Tzarfati et al., 2014), 16. (Golan et al., 2015), 17. (Giraldo et al., 2016), 18. (Maccaferri et al., 2016), 19. (Mengistu et al., 2016), 20. (Milner et al., 2016), 21. (Kidane et al., 2017), 22. (Roncallo et al., 2017), 23. (Soriano et al., 2017), 24. (Giunta et al., 2018), 25. (Mangini et al., 2018), 26. (Maccaferri et al., 2019).

4.4. DISCUSSION

A large number of studies demonstrated the effect of phenology on yield related traits. Selecting for important agronomical traits resulted in a change in phenology genes, which indirectly affected yield (Kamran et al., 2014). The effect of spring durum wheat phenology in yield traits was dissected in a previous study (Arjona et al., 2018). In this work, authors observed that *Ppd-1* allele combination only explained a fraction of phenology, yield, and yield component variation. Genetic variability other than *Ppd-1* genes was therefore worth being considered and studied.

GWAS studies in durum wheat have been performed worldwide, covering a wide range of latitudes: Argentina (Roncallo et al., 2017), Ethiopia (Mengistu et al., 2016), Mediterranean basin (Maccaferri et al., 2008; Soriano et al., 2017), or Canada (Maccaferri et al., 2019). Most of the studies covered at most 5° of latitudinal range. The advantage of this study is that a wide latitudinal range was considered, from 41°N to 19°N, with contrasting photoperiod and temperature, giving an idea of how the different MTAs depend on the environment, although the low number of samples may reduce the power of predicting significant MTAs. It is also not common to find studies with data of pre-flowering phases in the literature, while in this study we report data about the time needed to reach double ridge and terminal spikelet. The point of having the time measured in GDD and days is also a feature not usual in the literature. The aim of this study was to explore the genetic variability present in our population in relation to phenology, yield, and yield traits under contrasting environmental conditions.

The use of a low number of genotypes comprising offspring from different families could lead to spurious associations due to their genetic structure and relatedness. We performed association analysis through combined ANOVAs, thus we did not take into account the genetic structure and kinship matrix. Based on these premises, in order to avoid spurious associations the significance threshold for association analysis was established using the Bonferroni multiple comparison correction at p <0.05.

Site was one of the more determinant factors to explain variation for most of the traits, and especially for yield, the ultimate breeding target. The genotype x environment interaction became an important factor to consider, because yield stability is important for food security, and this interaction opens new opportunities to exploit the positive interaction between both factors (Annicchiarico, 2002). Together, the effects of site, year, and their interaction explained more than 60% of yield variations. When yield was dissected into its main components, the genotype explained most of the variation for GW. Additionally, the low percentage of variation attributable

to genotype interactions indicated that GW genotypic values were stable between sites. In the case of GNm^{-2}, a lower proportion of variability was explained by genotype than in GW. The effect of the latitude on GNm^{-2} was previously reported, with differences of up to 5,000 GNm^{-2} between sites, and a negative correlation was found between minimum temperatures from sowing to flowering and GNm^{-2} (Villegas et al., 2016). It could be expected that more effort applied to genetic GW improvement could raise yield, but since GW and GNm^{-2} are closely linked (Quintero et al., 2018) none of them should be left aside. Generally it is accepted that GNm^{-2} has more influence than GW in the final yield, because the photosynthetic resources are less constraining than the sink capacity (Fischer, 2011) and the plasticity of GNm^{-2} is higher than that of GW (Sadras, 2007). However, a strong debate about this interplay between sink-source balance exist (Fischer, 2008; Sinclair and Jamieson, 2006). For GNm^{-2} sub-components (GSp, SpklSp, and GSpkl) genotype was an important source of variation, and site became less important. This observation had the exception of Spm^{-2}, whose numbers are determined in the first phases of development (Slafer and Rawson, 1994).

For phenology, the marker corresponding to *Ppd-B1* was not significant at any site. This result is in agreement with previous reports where the effect of this locus was considered weak (Arjona et al., 2018; Royo et al., 2016). In the case of the *Ppd-A1*, it was significant for D_EF at all sites with the exception of Mexico-south. This site was the one with the shortest period from emergence to flowering and the longest photoperiod until terminal spikelet. Thus, the combination of both factors did not cause strong differences in phenology for this site. However, in previous studies significant differences were found at this site although with a low effect as in the case of *Ppd-B1* (Arjona et al., 2018; Royo et al., 2016). Single MTAs for phenology were found to be significant in Mexico-north, Spain, and across sites. In Mexico-north the marker at 2A (158 cM) was significantly associated to time to terminal spikelet in both days and GDD. Close to the same position (Giunta et al., 2018) a significant marker was found for days to flowering. However, this was true only in treatments under short days, no longer than 13h by flowering, a condition that, in our environments, was only met in Mexico-north. For this site, an MTA in the chromosome 2B (72.5 cM) for GDD_FM was also found, which was shared with Spain. In the same region (Roncallo et al., 2017) reported the presence of a QTL modifying days to flowering, and (Soriano et al., 2017) a QTL linked to decreasing days from flowering to maturity. Previous to these studies, (Hanocq et al., 2007) identified a meta-QTL in this position for vernalization requirements. The presence of this association at more sites suggests the possibility that this region could be effective in a wide range of latitudes. However, in Mexico-south this association was not significant, indicating that

the effect may be evident in latitudes higher than 19°. In Mexico-north, the region of chromosome 5A and 6B had the strongest effect on phenology of all MTAs, interactions and localities (18 days). The 5A (111-116 cM) region is close to where the Vrn-A1 (121 cM) is located in the reference map of Svevo (Maccaferri et al., 2019). Although the varieties were considered to be spring habit, some interactions could have happened downstream related with temperature control. This region was involved in interactions for phenology across sites and in Spain, the strongest effect in these sites were shown. The main difference between sites was the region interacting with 5A (111-116 cM), being in Mexico-north the 6B (64 cM). Close to the last region of 6B Giunta et al. (2018) found a significant marker for phenology in non-vernalized treatment. This region in the chromosome 6B was also involved in interactions with other chromosomal regions across sites for pre-flowering phases. This could mean that this region is involved in phenology control in different environments, but the effect and the phase in which it is involved depends on the site. In Spain, there were numerous MTAs and interactions related to phenology in the chromosome 7B. The region in this chromosome at 6 cM is close to the *Vrn-B3*, which was mapped in Svevo at 7cM of the beginning of chromosome 7B (Maccaferri et al., 2019). This region was also involved in interactions for phenology across sites. The other regions of the chromosome 7B could be related to the *Ppd-B2* located in this chromosome (Khlestkina et al., 2009), and having effect under long days as happened in Spain close to flowering date (Villegas et al., 2016). The regions in the chromosome 1A that interact in Spain could be related with the *Eps-A^{m}1* found in *T. monococcum* L. (Lewis et al., 2008; Valarik et al., 2006) but no position for this gene has been reported for durum wheat. The interactions reported in chromosome 2A (37-44cM, 71-81 cM, and 103-126 cM) suggested the importance of this chromosome in controlling phenology, above all in Spain, the three of them interacting and modifying the grain filling duration and affecting GW. But it was in Mexico-south where the interaction between these regions had an effect on yield performance.

The number of interactions and MTAs in Spain was substantially larger than in the other sites. This could be due to the conditions at this site, with larger differences of daylength from emergence to maturity (from 10.5 to 15 h/day), a drastic change that was similar in temperature as reported by (Villegas et al., 2016). On the other hand Mexico-south did not have markers or interactions for phenology, which could be explained by a shorter crop cycle, which may not allow the different genotypes to express their differences. The conditions in Mexico-south were similar during the whole crop cycle, with around 14h of daylength and warmer temperatures (Villegas et al., 2016).

Different authors reported the importance of chromosome 2A for increasing grain weight (Blanco et al., 2012; Golan et al., 2015; Graziani et al., 2014; Maccaferri et al., 2016; Mangini et al., 2018; Peng et al., 2003; Roncallo et al., 2017; Tzarfati et al., 2014). In our study, we identified significant associations for GW at chromosome 2A (80 and 103 cM), being the region at 80 cM, the MTA with strongest effect in GW (10.4 mg grain^{-1} across sites, 8.9 mg grain^{-1} at Spain, 12.1 mg grain^{-1} at Mexico-north, and 9 mg grain^{-1} at Mexico-south) detected in the present work. However, with the exception of Mexico-south this region was also significant for GNm^{-2}, which means that the effect on GW could be driven by the negative trade-off between GNm^{-2} and GW (Quintero et al., 2018). The effect of this MTA on GNm^{-2} was associated at all sites with an effect on GSp, and with the exception of Mexico-north, with an effect on GSpkl. In the literature this region has also been associated with GW (Roncallo et al., 2017), GSp (Kidane et al., 2017), and GSpkl (Roncallo et al., 2017). On some occasions it was even associated with Yield (Mengistu et al., 2016; Milner et al., 2016), which means that depending on the site and its GNm^{-2} limitation, the change on these alleles could make the difference. The region at 2A (103 cM) had no MTAs for GNm^{-2}, but it was present in the interaction with other regions affecting GNm^{-2} in Spain and Mexico-south. Something similar happened with the region at chromosome 3A, where the trade-off was also noticeable across sites and Spain, which makes it difficult to find an MTA or interaction at all sites for increasing GNm^{-2} without negatively affecting GW. In this sense, it would be easier to report interactions than single MTAs due to the possibility of fine tuning some effects, as well as finding the proper combination for each environment.

Across sites, two MTAs located in the chromosomes 6A and 6B were significant for Yield. In the case of the 6A MTA, they were not related to any yield component in our study. However, closely linked effects have been reported on GW (Golabadi et al., 2011), Spm^{-2} , GSpkl (Roncallo et al., 2017), and GSp (Giunta et al., 2018). Regarding the region of 6B, close associations with phenology in Spain, and SpklSp across sites and Spain were found. Other authors also associated this region to yield components (Maccaferri et al., 2019) and cites therein), and yield itself (Maccaferri et al., 2008). This association across sites could indicate a wide ranging effect, although it was not noticeable in a single site. As with a complex trait such as yield, it is expected that the interactions of different MTAs perform better than single associations, which is what happened in Mexico-north and Mexico-south. In Mexico-north the interaction between 1B and 6B was significant for yield. The region in the chromosome 1B was present in an interaction across sites with significant associations for SpklSp, and was also associated with GY by other authors (Mengistu et al., 2016; Roncallo et al., 2017) in Ethiopia and Argentina, respectively. The 6B region, at 64 cM, was far from

the one that was significant such as single MTA across sites (126 cM). This region interacted with the 5A chromosome with an effect on phenology in Mexico-north and other sites, and it was associated with yield by Mengistu et al. (2016). In Mexico-south, two interactions were significant for yield (2A 103-126 cM with 5A 111-116 cM, and with 2A 71-81 cM). The regions of the chromosome 2A were involved in different sites in phenology and yield components. An increase of GNm^{-2} in Mexico-south was found favorable in other studies, due to the limiting GNm^{-2} associated at this site (Villegas et al., 2016). The other region of the chromosome 5A was also found associated with phenology in other sites in our study, and with yield components, such as SpklSp, by other authors (Peng et al., 2003).

4.5. CONCLUSIONS

Results of this work revealed that other chromosomal regions beyond the *Ppd-1* genes showed important effects on phenology, as well as complex interactions between developmental stages and yield components. The results of this work pointed out the importance of the site when association analysis is performed, indicating that different chromosome regions reported significant associations with phenology and yield components according to the environmental conditions, revealing the importance of the interaction between genotype and environment in the expression of these traits.

Special attention should be brought to the chromosomes 6A and 6B, which were significantly associated with yield across sites, even though was not significant for any specific site. In these regions, around 114 and 126 cM could be an interesting target for a more specific and close up study. The final environment targeted should be highlighted in breeding programs, due to the G×E specificity found in the MTAs. This also indicates the importance of the environment when trying to map targeted genes, or in GWAS analysis.

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GENERAL DISCUSSION

The objective of this PhD dissertation is to broaden the knowledge about the genetics responsible for spring durum wheat phenology and its influence on yield, yield components and yield stability. The research is centred on the effect of photoperiod sensitivity genes, although genetic variation of *Eps* minor genes and their effect is also explored.

The general hypothesis we tested in this study was that the differential developing time, particularly flowering time, due to the presence of diverse allele combinations of *Ppd-1,* and/or the effect of *Eps* genes are expected to modify the environmental conditions during wheat development. In turn, these conditions are supposed to have an effect on the physiological processes of durum wheat growth and yield formation.

This report is encompassed in the project 'Addressing the challenges for a sustainable wheat production in Spain and North Africa', within the agreement framework between INIA (*Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria*, Spain) and CIMMYT (International Maize and Wheat Improvement Centre, Mexico). This work was implemented by the research institutions IRTA (Institute for Food and Agricultural Research and Technology, Spain) and CIMMYT.

The experiments were carried out at three sites with contrasting latitudes. The northern site was in Spain (Lleida, 41° 38´N), and the other two sites were located in Mexico, in Cd. Obregón (state of Sonora, northern Mexico, 27° 21´N), and at El Batán experimental station (Texcoco, 19° 31´). At these sites, field experiments were performed in 5 cropping seasons (year of harvest 2007, 2008, 2010, 2011, and 2012) with an experimental design of randomized complete block with three replicates. In all experiments, irrigation was applied to avoid water stress. Most studies found in the literature about photoperiod variation were conducted under controlled conditions, and field studies are usually limited to a narrow range of latitudes (He et al., 2012; Penrose et al., 1996). One of the most valuable assets of this work relies on results arisen from carefully curated data obtained in such a wide range of different latitudes, with homogeneous protocols and the same plant materials at all sites.

It is critical to understand the characteristics differentiating the studied sites in order to interpret the genetic by environment (G×E) interaction. When fitting daily data to a LOESS curve, no apparent differences were found either fitting 3 years of data (2010, 2011, and 2012) or 5 years

of data (also including 2007 and 2008, data not shown). As previously reported by (Villegas et al., 2016), Spain had the longest crop cycle (Chapter IV). This length could be explained mostly by the difference in days from emergence to double ridge, where Spain had a noticeable long phase. This phenomenon could be explained by the lower temperatures and shorter photoperiod observed from emergence to double ridge at this site. The length of grain filling also differed between sites. While minimum temperatures during grain filling in Spain ranged from 10 to 14ºC, in Mexico-north they ranged from 8 to 12°C, and in Mexico-south from 10 to 8°C in a decreasing tendency (Chapter I). This would explain the long period of grain filling in Mexico-south, and the shortest period in Spain, as grain filling length is known to be affected by the minimum temperature (García et al., 2016).

Under such contrasting latitudes, the effect of the environment is expected to be very high. This is the case of the period from emergence to flowering, which in thermal time explained 55% of the variation. This percentage was higher in previous studies which included one additional site and with durations measured in days ranging from 85% up to 96% (Royo et al., 2016; Villegas et al., 2016). For the period from flowering to maturity the site effect explained less proportion of the variation, but the effect of the interaction site × year was highly increased, which is in agreement with previous results (Royo et al., 2016; Villegas et al., 2016). The variation explained by site was reduced to 42% for grain number per unit area (GN), and to 14% for grain weight (GW), which was also observed in the literature (Villegas et al., 2016).

The plant material used in this work was specifically designed and developed to address the objectives of this Thesis. Spring durum wheat lines were obtained by crossing 5 late-flowering varieties from the University of Hohenheim (Germany), with 5 early-flowering varieties from CIMMYT-Mexico. The crosses were advanced as bulks, without selection, up to the F_3 generation in CIMMYT-Mexico. In the F_4 generation, spikes with contrasting flowering date were selected, and advanced until F_8 in the IRTA experimental fields.

Once the experimental trials had started, the molecular characterization was carried out in the germplasm, in order to detect the alleles of the two known *Ppd-1* genes: *Ppd-A1* (Wilhelm et al., 2009) and *Ppd-B1* (Hanocq et al., 2004). The fact that the markers for *Ppd-A1* were available once the experiments were ongoing did not allow the creation of balanced groups of varieties with the different allele combinations. The parental lines from CIMMYT included a sub-set present in most of the CIMMYT genetic materials and they may be considered representative of a vast majority of durum wheat modern varieties as insensitive, or with low sensitivity to photoperiod. Fortunately, one of the parental lines carried the *Ppd-A1a* GS100 allele, which is rare in the durum germplasm,

which allowed the study of the effect of this allele on phenology, yield, and yield components. The German lines were used as donors of photoperiod sensitivity and were selected due to their genetic distance to CIMMYT breeding materials. All the German lines carried the *Ppd-A1b* allele, but they were polymorphic for the *Ppd-B1* gene. The set of lines derived from the crosses, however, became a group with a trend of having a later flowering time than the general set of durum wheat varieties commonly grown in the sites of study. This phenomenon may indicate the strength of *Eps* genes in the control of phenology.

In the first chapter of this dissertation (Chapter I), the effect of the differences in photoperiod sensitivity of each allele of *Ppd-A1* and *Ppd-B1* is studied. Previous information about how these alleles affect phenology (Royo et al., 2016) is complemented, allowing the understanding of how the differences in phenology produce changes in yield or yield components. Additionally, the possible intrinsic effect of these alleles in yield formation is suggested.

The second chapter (Chapter II) includes three main parts: 1) the understanding of how the allele combination of *Ppd-A1* and *Ppd-B1* loci affect yield components and yield itself; 2) how the modification of phenology due to *Eps* affects yield and yield components formation; and 3) how the allele combination in *Ppd-A1* and *Ppd-B1* affect yield stability in the agronomical sense.

The third chapter (Chapter III) investigates the effect of *Ppd-A1* and *Ppd-B1* allele combinations on the grain filling process, due to their effect on phenology.

The aim of the fourth chapter (Chapter IV) is to understand and explore the large genetic variability in phenology and yield components observed in the previous chapters but not explained by *Ppd-A1*, *Ppd-B1* or their allele combination.

5.1. EFFECT OF THE *PPD-1* IN PHENOLOGY

In spring durum wheat, phenology largely depends on the effect of the photoperiod response genes (*Ppd-1*) and the earliness *per se* (*Eps*). This dissertation is more focused on the study of the *Ppd-1* genes, although the variation by *Eps* was also explored. The effect of the *Ppd-A1* represented 40% of the genetic variation in growing-degree days (GDD) from emergence to flowering, while the effect of the *Ppd-B1* represented 6% (Chapter I). When considering the combination of both loci (Chapter II), the proportion explained by allele combinations in days from emergence to flowering was 48%, a lower value than the 66% and above reported in previous studies on durum wheat (Royo et al., 2016). The difference could be due to the Spain-south location included in the study by Royo et al. (2016), in which the genotypes were highly responsive to changes in

photoperiod. These results showed that an important part of the variation in phenology depended on other genes such as *Eps* (Chapter II). This observation was in accordance with previous studies in bread wheat, reporting that when vernalization requirement was fulfilled, the variation due to *Eps* was around 50% (Cane et al., 2013; Eagles et al., 2010). This can also be appreciated in the analysis of the general variation of the MTAs, as many MTAs were related with phenology in the different sites (Chapter IV). The strongest effect on shortening the pre-flowering phase was associated with the presence of the GS100 allele, followed by GS105 and finally by *Ppd-A1b* in agreement with previous results (Royo et al., 2016). *Ppd-B1* had no effect or it was very soft (Chapter I), which is also in accordance with previous studies (Maccaferri et al., 2008; Royo et al., 2016; Wilhelm et al., 2009).

When the two loci were considered together, the tendency indicated that genotypes insensitive for *Ppd-A1* flowered earlier (GS100/*Ppd-B1a* < GS105/*Ppd-B1a* < GS105/*Ppd-B1b* < *Ppd-A1b*/*Ppd-B1b* < *Ppd-A1b*/*Ppd-B1a*), with only a small modulation due to the *Ppd-B1,* and with almost all the variation being attributable to *Ppd-A1* (Chapter II). However, a study in Germany attributed more variation explained by *Ppd-B1* than by *Ppd-A1* (Würschum et al., 2019). In the germplasm used in their study, they found polymorphism for *Ppd-B1* copy number, while in our lines no variation was detected. However, in the three latitudes considered in this dissertation, the effect of the *Ppd-B1* on flowering time varied between two and four days, a similar amount to the four day variation found in Würschum et al. (2019). In our results the *Ppd-A1* variation explained between 8 and 12 days in flowering time (deduced from Chapter I). Additionally, Würschum et al. (2019) didn't take into account the allele variation existing in *Ppd-A1*. The differences in effect between the two insensitive alleles (GS100 and GS105; Wilhelm et al., 2009), were not negligible according to our results, but in the study by Würschum et al. (2019), the softer effect of the GS105 could have masked the stronger effect of the GS100 allele. As the frequency of the GS100 allele is expected to be lower than the GS105 allele (Bentley et al., 2011), its effect could be diluted in that of GS105.

In the current study, we did not detect significant differences, but the insensitive alleles of *Ppd-A1* tended to shorten all pre-flowering phases, with the exception of the heading-flowering phase. A similar phenomenon occurred in the case of *Ppd-B1*, but it was only noticeable for the period from emergence to double ridge (Chapter I).

The differences in phenology were translated into differences in weather conditions, confirming the validity of the general hypothesis. These differences were more noticeable at sites such as Mexico-north, where the environmental conditions change more progressively and were more stable across consecutive days. The *Ppd-A1* effect was associated with a change of about 1°C of

the maximum temperatures, as well as 0.5°C in minimum temperature around flowering (Chapter I). In Mexico-south the variation in temperature was low, but in Spain and Mexico-north sites the mean temperature in the first half of grain filling changed by 1.7°C between the earliest allele combination (GS100/*Ppd-B1a*) and the latest one (*Ppd-A1b*/*Ppd-B1b*, Chapter III). However, in Mexico-south genotypes carrying different allele combinations experienced similar temperatures. Instead, the genotypes with the earliest allele combinations were exposed to close to a 1 MJ m^{-2} day^{-1} more of radiation, which was considered the limiting factor for grain growth in the first half of the grain filling period (Chapter III).

The genetic variability explained by the interaction *Ppd-1* × site for the period between flowering and maturity represented 61% of the variation of genotype × site. This effect was mainly driven by the results of Mexico-north, where the genotypes carrying the earlier *Ppd-1* allele combinations delayed their grain filling by four days rather than the allele combinations causing later flowering time (Chapter II). This could be due to the mentioned stability of the weather in Mexico-north, which ensured that earlier genotypes developed their grains under cooler temperatures at the beginning of grain filling (Chapter III). In Spain and Mexico-south the same tendency was observed, but the temperature fluctuations were more random along the crop cycle, thus making it more difficult to predict lower temperatures when flowering time was earlier.

5.2. EFFECT OF ALLELES AND ALLELE COMBINATIONS AT *PPD-1* LOCI AND PHENOLOGY ON YIELD, YIELD FORMATION, AND YIELD STABILITY

In order to study the effect of *Ppd-1* genes on yield formation, the individual yield components were analysed. As such, grain weight (GW) and grain number (GN) were studied. The GN was also broken down into its components: Spikes per unit area, grains $spike^{-1}$, spikelets $spike^{-1}$, and grains $spikelet^{-1}$.

As mentioned in the previous section, the effect on modifying the phenology of the allele combinations at *Ppd-1*, jointly with *Eps* effects was the emplacement of certain phenology phases in different environmental conditions. It was expected that those changes in the environmental conditions would affect the formation of some yield components.

5.2.1. Grain number

The percentage of the ANOVA variation explained by genotype was greater for GN than for phenology. However, the site effect was still very important, accounting for 42.4% of the sum of squares in the ANOVA in our case and no less than 32% in previous studies (Villegas et al., 2016).

For GN, the variability explained by *Ppd-A1* was 2% of the variation attributed to genotype, and non-significant. On the other hand, the effect of *Ppd-B1* on GN was around 26%. This result appeared to be contradictory to the strongest effect on phenology of the *Ppd-A1*, which would be expected to affect GN due to a modification of the stem elongation period and the number of fertile florets (Gonzalez et al., 2003; Miralles et al., 2000). However, in this study the effect of *Ppd-A1* and *Ppd-B1* genes on GN was principally associated with *Ppd-B1*. This effect was due to an increase in spikelets spike^{-1} when the allele conferring sensitivity was present (Chapter I).

The *Ppd-B1b* allele tended to increase the phase from emergence to double ridge. Considering the error on the prediction of floral initiation from the observation of double ridge that goes after the floral initiation (Delécolle et al., 1989), the possibility exists that the *Ppd-B1* had more effect on the phase from floral initiation to terminal spikelet than the observed herein. Since the spikelets' primordia are formed from floral initiation to terminal spikelet (Kirby, 1990), this tendency to lengthen the phase and increase the number of light hours around this period could favour the spikelet primordia production. In Mexico-north and Mexico-south, the *Ppd-B1b* allele tended to result in more radiation accumulated from terminal spikelet to booting (Chapter I). It is well known that during this phase, abortion of spikelets occurs in barley (Alqudah and Schnurbusch, 2014). Therefore, more radiation could provide more energy, thus reducing the aborted spikelets and increasing spikelet fertility (Gonzalez et al., 2003; Miralles et al., 2000). More irradiance during this phase was also associated with more grains spike^{-1} in other studies, although not with spikelets spike^{-1} (Evans, 1978). However, the effect of the *Ppd-B1* on phenology, and the phenology on the environmental conditions during the individual developmental phases, did not explain all the variation due to the *Ppd-B1*. An intrinsic effect of the *Ppd-B1* was expected, due to its interaction with the *FLOWERING LOCUS T* (*FT*), as was reported in other cases (Boden et al., 2015). It is also possible that the *Ppd-B1* locus was linked to other loci with an intrinsic effect on spikelets spike^{-1} (Chapter I). In Mexico-south at the position 71-72cM of the chromosome 2B, an interaction with the chromosome 6A increased by 2.9 spikelets spike^{-1} (Chapter IV). Other authors have also found quantitative trait loci (QTLs) related with GN components close to the genomic position of the *Ppd-B1* (Maccaferri et al., 2019), and more specifically for spikelets spike^{-1} (Giunta et al., 2018).

The allele combination of the two *Ppd-1* loci explained 41% of the genetic variation for GN. This is much more than the sum of the effects of the two loci separately (28%), indicating that there was a synergic effect between the two loci when explaining GN (Chapter II). As mentioned before,

Ppd-B1b had an effect on increasing the spikelets spike^{-1}. The late flowering genotypes also had more spikelets spike^{-1}, but in this case, the difference relied on the grains spikelet^{-1}.

The *Ppd-A1α* allele shortening the pre-flowering phase was expected to reduce GN, but this was not the case (Chapter I). Therefore, the negative effect of shortening the pre-flowering phase could be compensated with a better conditions during grain setting (Draeger and Moore, 2017; Terrile et al., 2017). This phenomenon was observed on the higher grains spikelet^{-1} found in the I5S allele combination with regard to the SS (Chapter II).

The variation in phenology due to the effect of *Eps* was only correlated with GN in 2 out of 15 experiments. This result suggests that the *Eps* effect on phenology did not explain the remaining 59% of the genetic variation in GN (Chapter II). This variation could be attributed to the numerous MTAs found along the genome (Chapter IV), as other authors have shown (Maccaferri et al., 2019, and cites therein).

5.2.2. Grain weight

A very important aspect to take into consideration about the main yield components GN and GW is the trade-off existing between them. An increase in GN is, in most cases, negatively correlated with GW (Bustos et al., 2013; Griffiths et al., 2015; Quintero et al., 2018), as observed in the current study, where *Ppd-B1* exerted on and affected GW. The *Ppd-B1α* allele had a slight effect increasing the GW, but it was due to the lower GN associated. This was also reinforced by the lack of effect of *Ppd-B1* in yield, due to compensation.

On the other hand, the *Ppd-A1* GS100 allele tended to increase GW. The differences in GN with the other alleles was small, and this tendency was translated as a trend on increasing yield (Chapter I). These tendencies were not significant, probably because of the small size of population in some allele combinations. However, the tendencies were considered solid. When the allele combinations of *Ppd-1* were considered, similar results were found. The earlier genotypes tended to have greater GW. Even when it was hard to distinguish if it was due to a real increase in GW or rather to a decrease in GN, yield followed the same tendency (Chapter II). The increase in GW due to the effect of *Ppd-1* was associated with a modification in the flowering time that placed the grain filling phase to be developed under better environmental conditions (Chapter III). This was highlighted when the effect of *Eps* in flowering time was considered. It was observed that in 14 out of 15 experiments the increase in days from emergence to flowering negatively correlated with GW, and in 11 cases with yield (Chapter II).

Grain filling was studied as a function of phenology, independently of whether the effect was due to *Ppd-1* or *Eps*. It was observed that late-flowering time was negatively correlated with GW, as it was associated with higher temperatures during the first half of grain filling (Chapter III). The increase in temperature could affect different metabolic pathways and organ functionality, photosynthesis, thylakoid membrane stability, nitrogen mobilization, etc. (Farooq et al., 2011; Prasad et al., 2011; Rezaei et al., 2015). Grain filling rate decreased when flowering time was delayed, and so did GW (Chapter III). Other authors have attributed the reduction in GW to a shortening in grain filling duration (Bergkamp et al., 2018; García et al., 2016). The main difference with those studies is that they measured the time in days, while thermal time was used in the current study. An increase in temperature usually increases GDD, or maintains GDD if the grain filling period is reduced proportionally in days. In bread wheat, the reduction measured in GDD was also associated with grain filling rate and grain filling duration reductions (Liu et al., 2016). Usually, when grain filling rate is measured in days, day number is reduced by the increasing temperatures, and when dividing the final grain weight by days, the rate (mg day^{-1}) increases or remains stable (Bergkamp et al., 2018; García et al., 2016; Shirdelmoghanloo et al., 2016). In the autumn sowing sites considered in this study, the increase in radiation in the first half of grain filling was negatively associated with the grain filling rate, and consequently GW (Chapter III). It could be speculated that photo-inhibition or photo-damage due to an excess of radiation is at play (Takahashi and Badger, 2011). Some efforts have been devoted to understanding the photo-inhibitory process of light in wheat (Li et al., 2017), but more information about the thresholds and the possibility of photo-inhibition occurrence or lack of irradiation at each site are needed. When only the effect of the *Ppd-1* allele composition was considered, its effect on phenology modified the relationship between temperature and radiation (Photo-thermal ratio, PTR). The increment of the PTR between flowering and the time of half of grain filling increased GW. In Spain and Mexico-south the earliness produced by the effect of the *Ppd-1* increased the PTR, but for different reasons. In Spain, after flowering, the temperature increased more rapidly than the radiation, which reduced the PTR. On the other hand, in Mexico-south, after flowering, the temperature increased and the radiation decreased, which made the PTR reduction stronger (Chapter III).

Most of the variability for GW due to the genotype was attributed to genes different to *Ppd-1*, as noticed in the amount of marker-trait associations (MTAs) found. The chromosome 2A stands out in a number of MTAs, interactions, and in their importance. However, most of the MTAs for GW were associate also with GN (Chapter IV), which shows the complexity of breeding to increase

both yield components at the same time (Quintero et al., 2018). The amount of MTAs was also site dependent, as well as the dimension of the trade-off between GN and GW. Therefore, in breeding efforts it is very important to consider the G×E interaction and the environmental conditions of the target site, besides understanding how this environmental variables are going to affect the yield components balance

5.2.3. Yield

The effect of *Ppd-1* on yield could be deduced from the effect in yield components shown previously. In general, the compensation between yield components resulted in no effect on final yield according to previous results (Bustos et al., 2013). However, there was a clear tendency of earlier genotypes to have greater GW and consequently higher yield, independently of the cause of earliness (*Ppd-1* or *Eps*, Chapter II). Consistently, the *Ppd-B1* did not have any effect, the opposite being true for *Ppd-A1* (Chapter I), as well as the *Eps* tendencies found (Chapter II).

The genome variation explored showed that the MTAs significantly related to yield were not strongly related to any yield component. This fact shows the importance of fine tuning the balance between yield components, where small changes finally add up to yield increases. However, the MTAs related with yield tended to be associated with GN components more often than to GW (Chapter IV). As has been previously mentioned, usually the increases in yield came through an increase in GN while maintaining an acceptable value of GW. In durum wheat, Milner et al. (2016) found in one out of 6 environments that in regions close to *Ppd-A1* and *Ppd-B1,* there were QTLs significant for yield. This reinforces the assumption that *Ppd-1* can have an effect on yield, but it strongly depends on the environment. This coincides with the negative correlation found in our study between flowering time and yield (Chapter II).

5.2.4. Yield stability

Yield stability is an important feature for adaptation, particularly in its agronomic definition (Cubero and Flores, 1994). The most stable genotype will be the one with higher yield in low productive environments, but also responding well to environmental improvements. As mentioned in Chapter II, yield stability was better achieved by the genotypes carrying both alleles of *Ppd-1* loci with the same effect (either sensitive or insensitive). We hypothesise that this could be due to a better synchronization of the flowering signalling when both genes work in the same direction. Previous studies conducted in barley have reported that yield stability was associated with phenology, specifically with *Ppd-H1*, and some *Eps* genes have also been reported to be

significant QTLs for yield stability. However, it is not clear if the stability was associated with early or late flowering genotypes (Emebiri and Moody, 2006). The asynchrony may be observed in the allele combination SI (*Ppd-A1b*/*Ppd-B1a*), which was expected to flower earlier than the SS (*Ppd-A1b*/*Ppd-B1b*), but tended to have a later flowering time. The effect of sensitivity is not genome specific (Shaw et al., 2012), which means that the molecular regulators produced in one genome can regulate pathways in other genomes. If alleles of different sensitivities are present in both genomes, a certain disruption in pathways responsible for development may be expected. A genome predominance was found in bread wheat, which means that one genome has more influence in the control of a pathway than the other ones (Shaw et al., 2012, 2013). This interplay could be part of the complex interaction that seems to exist between the *Ppd-1* loci in durum wheat related to yield stability. In our case, for phenology, the amount of single MTAs and those that interacted was very similar between the two genomes (Chapter IV). On one hand the strongest effect of the *Ppd-A1* suggests a stronger control of the A genome in phenology (Chapter I). However, the unexpected lateness of the SI allele combination regarding SS, when the GS105/*Ppd-B1a* was earlier than the GS105/*Ppd-B1b,* may indicate the opposite - a dominance of the B genome in the control of phenology (Chapter II), which needs further investigation.

5.3. RECOMMENDATIONS FOR BREEDING PROGRAMMES AND FUTURE PERSPECTIVES

In some cases and for some characteristics, breeding durum wheat for wide adaptation is possible, but in general, looking for specific adaptation to a target environment would be more practical and better results are expected. In the range of sites tested in this study, earliness in flowering time could be recommended, and *Ppd-A1* GS100 insensitive allele is advisable. Because this allele is nowadays rare in the genetic material, an enrichment in its frequency is suggested. Since the *Ppd-B1* was not clearly affecting flowering time, the *Ppd-B1a* insensitive allele could be recommended, because the same kind of photoperiod sensitivity in both *Ppd-1* loci will contribute with yield stability. However, if more grains m^{-2} were desirable in the target environment, then the *Ppd-B1b* allele would be a better option.

The *Eps* effect should be studied more deeply in order to identify the genes responsible for phenology modification, as well as the pathway of its control and the environmental cues involved. In order to do so, the elimination of every effect due to vernalization control and photoperiod should allow an understanding of the strength of the effect of *Eps*. However, the earliness produced by *Eps* correlated positively with GW and yield. Therefore, earliness due to *Eps* is also advisable. In the context of climate change, where it is expected to be harder to predict

weather conditions, genotypic responses due to *Eps* could be a good option that could facilitate heat and drought stress avoidance.

As mentioned before, a better understanding and more information about the interaction of durum wheat with solar radiation would be desirable. How this lack or excess of radiation can interact with other external conditions as temperature and water availability needs more attention. Broadening the basic knowledge of the paths of control mechanism, as well as the interaction between genes and their regulation will also be an important task that should be shared among physiologists, geneticists, breeders, agronomists and modellers.

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CONCLUSIONS

From the results of this PhD dissertation, the following conclusions could be yielded:

1. Allele GS100 at *Ppd-A1*, causing photoperiod insensitivity and resulting in early-flowering genotypes, tended to increase grain weight and yield, albeit not substantially.
2. Genotypes carrying the *Ppd-B1b* allele conferring photoperiod sensitivity had a consistently higher grain number per unit area, which was not translated into higher yield due to an under-compensation in grain weight. This under-compensation of grain weight was reduced in sites where yield was limited by grain number per unit area.
3. The increase of grain number per unit area due to the *Ppd-B1b* allele was produced by an increase in grains spike^{-1} as a result of a higher number of spikelets spike^{-1}, associated with an intrinsic effect.
4. Genotypes carrying the allele combination GS100 *Ppd-A1a*/*Ppd-B1a* showed the earliest flowering time, and tended to be the most productive and stable although differences in flowering time caused by *Ppd-A1*/*Ppd-B1* allele combinations had no significant effect on yield due to compensation between grain number per unit area and grain weight.
5. Allele combinations GS105/*Ppd-B1b* and *Ppd-A1b*/*Ppd-B1b* had the highest grain number per unit area due to an increase in spikelets spike^{-1}.
6. Yield stability was enhanced when alleles at *Ppd-1* loci conferred a similar photoperiod response (sensitive/sensitive or insensitive/insensitive).
7. Flowering delay reduced the mean grain filling rate and grain weight. Duration of grain filling was independent of flowering time when measured in thermal time, and reduced in later flowering genotypes, if measured in days.
8. Environmental conditions in the first half of grain filling were the most determinant for final grain weight. In the autumn-sowing sites, an increase of 1°C in mean temperature at this phase decreased grain weight by 5.2 mg grain^{-1}.
9. Earliness *per se* (*Eps*) accounted for 52% of the genetic variability for phenology from emergence to flowering, and 93% for the grain filling period. Eps had a minor effect on grain number per unit area, but the associated earliness increased grain weight and yield.

10. The regions at 114cM and 126cM, on chromosomes 6A and 6B respectively, represent hotspots for QTL regulating yield performance.
11. Interactions between pairs of marker-trait associations showed stronger effect than the corresponding single marker-trait associations.
12. The detection of marker-trait associations was highly affected by the environment.

SUPPORTING INFORMATION

SUPPORTING INFORMATION CHAPTER I

Supplementary table 1. Pedigrees and corresponding allelic combinations for *Ppd-A1* and *Ppd-B1* loci present in the genotypes used in the study.

Genotype	*Allele at Ppd-A1*	*Allele at Ppd-B1*	Pedigree
Line 1	GS100 *Ppd-A1a*	*Ppd-B1a*	2905-13.93.04//DUKEM_12/2*RASCON_21
Line 2	GS100 *Ppd-A1a*	*Ppd-B1a*	MEGADUR//DUKEM_12/2*RASCON_21
Line 3	GS100 *Ppd-A1a*	*Ppd-B1a*	MEGADUR//DUKEM_12/2*RASCON_21
Line 4	GS105 *Ppd-A1a*	*Ppd-B1a*	2716-25.94.01/3/SNITAN
Line 5	GS105 *Ppd-A1a*	*Ppd-B1a*	2716-25.94.01/3/SNITAN
Line 6	GS105 *Ppd-A1a*	*Ppd-B1a*	2805-49.94.02/GUANAY
Line 7	GS105 *Ppd-A1a*	*Ppd-B1a*	2905-13.93.04//CADO/BOOMER_33
Line 8	GS105 *Ppd-A1a*	*Ppd-B1a*	DURABON//SOOTY_9/RASCON_37
Line 9	GS105 *Ppd-A1a*	*Ppd-B1a*	DURABON//SOOTY_9/RASCON_37
Line 10	GS105 *Ppd-A1a*	*Ppd-B1b*	2805-49.94.02//CADO/BOOMER_33
Line 11	GS105 *Ppd-A1a*	*Ppd-B1b*	2905-13.93.04//CADO/BOOMER_33
Line 12	GS105 *Ppd-A1a*	*Ppd-B1b*	2905-13.93.04//CADO/BOOMER_33
Line 13	GS105 *Ppd-A1a*	*Ppd-B1b*	2905-13.93.04/SNITAN
Line 14	*Ppd-A1b*	*Ppd-B1b*	2716-25.94.01/GUANAY
Line 15	*Ppd-A1b*	*Ppd-B1b*	2716-25.94.01/GUANAY
Line 16	*Ppd-A1b*	*Ppd-B1b*	2905-13.93.04//CADO/BOOMER_33
Line 17	*Ppd-A1b*	*Ppd-B1b*	2905-13.93.04//CADO/BOOMER_33
Line 18	*Ppd-A1b*	*Ppd-B1b*	2905-13.93.04/SNITAN
Line 19	*Ppd-A1b*	*Ppd-B1a*	2805-49.94.02//CADO/BOOMER_33
Line 20	*Ppd-A1b*	*Ppd-B1a*	2905-13.93.04//CADO/BOOMER_33
Line 21	*Ppd-A1b*	*Ppd-B1a*	2905-13.93.04//CADO/BOOMER_33
Anton	*Ppd-A1b*	*Ppd-B1a*	Anton
Simeto	*Ppd-A1b*	*Ppd-B1a*	Simeto

Supplementary Table 2. Environmental variables during the field experiments

	Emergence-flowering			Flowering-maturity			
Experiment	Minimum temperature (ºC)	Maximum temperature (ºC)	Mean radiation (MJ m^{-2} day^{-1})	Minimum temperature (ºC)	Maximum temperature (ºC)	Mean radiation (MJ m^{-2} day^{-1})	Total water input during growing season (mm)
Spain 2007	3.2	13.6	11.1	11.7	26.2	25.7	463
Spain 2008	4.4	16.4	14.2	11.8	23.5	21.7	640
Spain 2010	3.3	13.2	12.0	12.0	25.5	26.0	675
Spain 2011	3.4	15.7	13.8	11.2	26.6	25.9	357
Spain 2012	1.9	15.2	14.0	13.2	28.4	26.6	299
Mexico North 2007	7.0	24.1	16.2	9.4	29.2	24.2	384
Mexico North 2008	7.1	25.4	19.7	11.2	32.1	27.6	507
Mexico North 2010	7.7	24.5	17.0	9.5	28.5	25.8	444
Mexico North 2011	6.4	26.0	20.0	11.7	31.9	26.5	420
Mexico North 2012	7.0	25.2	17.4	8.6	28.0	25.7	404
Mexico South 2007	10.3	24.9	21.4	10.4	23.4	18.2	670
Mexico South 2008	10.3	23.2	19.3	10.9	23.6	18.2	482
Mexico South 2010	11.0	24.5	21.0	10.4	22.6	18.3	637
Mexico South 2011	9.0	24.7	20.7	8.0	24.7	21.9	525
Mexico South 2012	10.7	23.9	21.2	10.6	23.0	19.6	489

GDD: growing degree-days. ns: non-significant; *P<0.05; **P<0.01;***P<0.001.

Supplementary Table 3. Percentage of the total sum of squares for pre-flowering phases, corresponding to the different sources of variation in the ANOVA model obtained from the evaluation of 23 durum wheat genotypes grown in three sites of contrasting latitude during three years (2010, 2011 and 2012).

Source	d.f.	GDD emergence-double ridge (ºC)	GDD double ridge - terminal spikelet (ºC)	GDD terminal spikelet - booting (ºC)	GDD booting - heading (ºC)	GDD heading - flowering (ºC)
Genotype	22	17.9 ***	9.6 ns	8.9 *	19.7 ***	11.1 ns
Site	2	27.5 ns	4.2 ns	26.3 ns	42.0 *	13.1 *
Site x Genotype	44	9.2 ***	12.6 ns	6.9 *	11.1 ***	12.1 ns
Year	2	3.7 ns	10.3 ns	8.3 ns	5.9 ns	10.2 *
Year x Genotype	44	3.6 ns	8.5 ns	5.8 ns	3.7 ns	8.3 ns
Site x Year	4	24.4 ***	16.8 ***	24.0 ***	9.1 ***	2.2 *
Site x Year x Genotype	88	7.1 ***	18.8 ***	9.2 ***	4.8 ***	18.8 ***

Supplementary Table 4. Percentage of the genotype sums of squares from the ANOVA for pre-flowering phase duration, partitioned in differences between allelic variants at *Ppd-A1 and Ppd-B1* genes and the differences within genotypes carrying a given allele. Data from 3 sites and 3 years.

Source of variation	d.f.	GDD emergence-double ridge	GDD double ridge-terminal spikelet	GDD terminal spikelet-booting	GDD booting-heading	GDD heading-flowering
Genotype	22	17.9 ***	9.6 ns	8.9 *	19.7 ***	11.1 *
Genotype sum of squares partition by *Ppd-A1*						
Between Ppd-A1	*2*	*3.4 ns*	*2.0 ns*	*2.6 ns*	*4.4 ns*	*0.3 ns*
Within Ppd-A1	*20*	*14.5 *****	*7.6 ns*	*6.3 **	*15.3 ***	*10.8 **
Genotype sum of squares partition by *Ppd-B1*						
Between Ppd-B1	*1*	*1.9 ns*	*0.0 ns*	*0.2 ns*	*0.2 ns*	*0.2 ns*
Within Ppd-B1	*21*	*16.0 *****	*9.6 ns*	*8.7 **	*19.5 *****	*10.9 ns*
Site x Genotype	44	9.1 ***	12.6 ns	6.9 ns	11.0 ***	12.2 ns
Site x Genotype sum of squares partition by *Ppd-A1*						
Between Ppd-A1 x Site	*4*	*0.7 ns*	*0.2 ns*	*1.6 ns*	*2.1 ns*	*2.8 ns*
Within Ppd-A1 x Site	*40*	*8.4 *****	*12.4 ns*	*5.4 ns*	*8.9 *****	*9.4 ns*
Site x Genotype sum of squares partition by *Ppd-B1*						
Between Ppd-B1 x Site	*2*	*0.9 ns*	*0.3 ns*	*0.8 ns*	*0.3 ns*	*0.2 ns*
Within Ppd-B1 x Site	*42*	*8.2 *****	*12.3 ns*	*6.1 ns*	*10.7 *****	*12.0 ns*

GDD: growing degree-days. ns: non-significant; **P*<0.05; ***P*<0.01;****P*<0.001.

Supplementary Table 5. Mean values across 3 sites and 3 years for pre-flowering phenological phases for each *Ppd-A1* and *Ppd-B1* allele, expressed as growing degree-days (GDD). The number of genotypes carrying each allele is shown in Table 2. Different letters between alleles at each gene indicate differences according to LSD test at $P<0.05$.

Gene	Alleles	GDD emergence-double ridge (ºC)	GDD double ridge - terminal spikelet (ºC)	GDD terminal spikelet - booting (ºC)	GDD booting - heading(ºC)	GDD heading - flowering(ºC)
Ppd-*A1*						
	Ppd-A1b	519 a	126 a	314 a	243 a	113 a
	GS105	496 a	120 a	296 a	205 a	110 a
	GS100	471 a	107 a	274 a	200 a	112 a
Ppd-B1						
	Ppd-B1b	519 a	122 a	305 a	226 a	110 a
	Ppd-B1a	493 a	120 a	298 a	218 a	112 a

SUPPORTING INFORMATION CHAPTER II

Supplementary Table 1. Pedigrees and allelic combinations for *Ppd-A1* and *Ppd-B1* loci present in the genotypes used in the study.

Line number/ Cultivar name	Pedigree	Allele variant at ***Ppd-A1***	Allele variant at ***Ppd-B1***	Allele combination acronym
1	2905-13.93.04//DUKEM_12/2*RASCON_21	GS100 *Ppd-A1a*	*Ppd-B1a*	I0I
2	MEGADUR//DUKEM_12/2*RASCON_21	GS100 *Ppd-A1a*	*Ppd-B1a*	I0I
3	MEGADUR//DUKEM_12/2*RASCON_21	GS100 *Ppd-A1a*	*Ppd-B1a*	I0I
4	2716-25.94.01/3/SNITAN	GS105 *Ppd-A1a*	*Ppd-B1a*	I5I
5	2716-25.94.01/3/SNITAN	GS105 *Ppd-A1a*	*Ppd-B1a*	I5I
6	2805-49.94.02/GUANAY	GS105 *Ppd-A1a*	*Ppd-B1a*	I5I
7	2905-13.93.04//CADO/BOOMER_33	GS105 *Ppd-A1a*	*Ppd-B1a*	I5I
8	DURABON//SOOTY_9/RASCON_37	GS105 *Ppd-A1a*	*Ppd-B1a*	I5I
9	DURABON//SOOTY_9/RASCON_37	GS105 *Ppd-A1a*	*Ppd-B1a*	I5I
10	2805-49.94.02//CADO/BOOMER_33	GS105 *Ppd-A1a*	*Ppd-B1b*	I5S
11	2905-13.93.04//CADO/BOOMER_33	GS105 *Ppd-A1a*	*Ppd-B1b*	I5S
12	2905-13.93.04//CADO/BOOMER_33	GS105 *Ppd-A1a*	*Ppd-B1b*	I5S
13	2905-13.93.04/SNITAN	GS105 *Ppd-A1a*	*Ppd-B1b*	I5S
14	2716-25.94.01/GUANAY	*Ppd-A1b*	*Ppd-B1b*	SS
15	2716-25.94.01/GUANAY	*Ppd-A1b*	*Ppd-B1b*	SS
16	2905-13.93.04//CADO/BOOMER_33	*Ppd-A1b*	*Ppd-B1b*	SS
17	2905-13.93.04//CADO/BOOMER_33	*Ppd-A1b*	*Ppd-B1b*	SS
18	2905-13.93.04/SNITAN	*Ppd-A1b*	*Ppd-B1b*	SS
19	2805-49.94.02//CADO/BOOMER_33	*Ppd-A1b*	*Ppd-B1a*	SI
20	2905-13.93.04//CADO/BOOMER_33	*Ppd-A1b*	*Ppd-B1a*	SI
21	2905-13.93.04//CADO/BOOMER_33	*Ppd-A1b*	*Ppd-B1a*	SI
Anton	Anton	*Ppd-A1b*	*Ppd-B1a*	SI
Simeto	Simeto	*Ppd-A1b*	*Ppd-B1a*	SI

Supplementary Table 2. Geographic and environmental descriptors for the three testing sites

Site	Spain	Mexico-North	Mexico-South
Location	Gimenells	Ciudad Obregón	El Batán
Experimental station (institution)	Lleida (IRTA)	CENEB (CIMMYT)	El Batán (CIMMYT)
Coordinates			
Latitude	41° 38'N	27° 21'N	19° 31'N
Longitude	0° 23'E	109° 54'W	98° 50'W
Altitude (m asl.)	200	40	2249
Soil characteristics			
Texture	Fine-loamy	Clay	Clay
pH	8.1	8.5	5.9
P (ppm)	16	2.4	65
K (ppm)	134	273	312
Organic matter (%)	2.4	1.2	5

SUPPORTING INFORMATION CHAPTER III

Supplementary table 1. Pedigree and corresponding allele variants for *Ppd-A1* and *Ppd-B1* loci present in the genotypes used in the study.

Genotype	Allele at ***Ppd-A1***	Allele at ***Ppd-B1***	Allele combination acronym	Pedigree
Line 1	GS100 *Ppd-A1a*	*Ppd-B1a*	*I0I*	2905-13.93.04//DUKEM_12/2*RASCON_21
Line 2	GS100 *Ppd-A1a*	*Ppd-B1a*	*I0I*	2905-13.93.04//DUKEM_12/2*RASCON_21
Line 3	GS100 *Ppd-A1a*	*Ppd-B1a*	*I0I*	MEGADUR//DUKEM_12/2*RASCON_21
Line 4	GS100 *Ppd-A1a*	*Ppd-B1a*	*I0I*	MEGADUR//DUKEM_12/2*RASCON_21
Parental 8	GS100 *Ppd-A1a*	*Ppd-B1a*	*I0I*	DUKEM_12/2*RASCON_21
Line 5	GS105 *Ppd-A1a*	*Ppd-B1a*	*I5I*	2716-25.94.01/3/SNITAN
Line 6	GS105 *Ppd-A1a*	*Ppd-B1a*	*I5I*	2716-25.94.01/3/SNITAN
Line 7	GS105 *Ppd-A1a*	*Ppd-B1a*	*I5I*	2905-13.93.04//CADO/BOOMER_33
Line 8	GS105 *Ppd-A1a*	*Ppd-B1a*	*I5I*	DURABON//SOOTY_9/RASCON_37
Line 9	GS105 *Ppd-A1a*	*Ppd-B1a*	*I5I*	DURABON//SOOTY_9/RASCON_37
Line 10	GS105 *Ppd-A1a*	*Ppd-B1a*	*I5I*	DURABON//GUANAY
Parental 6	GS105 *Ppd-A1a*	*Ppd-B1a*	*I5I*	SOOTY_9/RASCON_37
Line 11	GS105 *Ppd-A1a*	*Ppd-B1b*	*I5S*	2805-49.94.02//CADO/BOOMER_33
Line 12	GS105 *Ppd-A1a*	*Ppd-B1b*	*I5S*	2905-13.93.04//CADO/BOOMER_33
Line 13	GS105 *Ppd-A1a*	*Ppd-B1b*	*I5S*	2905-13.93.04//CADO/BOOMER_33
Line 14	GS105 *Ppd-A1a*	*Ppd-B1b*	*I5S*	2905-13.93.04//CADO/BOOMER_33
Line 15	GS105 *Ppd-A1a*	*Ppd-B1b*	*I5S*	2905-13.93.04/SNITAN
Line 16	GS105 *Ppd-A1a*	*Ppd-B1b*	*I5S*	2716-25.94.01/3/SNITAN
Line 17	GS105 *Ppd-A1a*	*Ppd-B1b*	*I5S*	2716-25.94.01/3/SNITAN
Parental 7	GS105 *Ppd-A1a*	*Ppd-B1b*	*I5S*	CADO/BOOMER_33
Parental 9	GS105 *Ppd-A1a*	*Ppd-B1b*	*I5S*	GUANAY
Parental 10	GS105 *Ppd-A1a*	*Ppd-B1b*	*I5S*	SNITAN
Line 18	*Ppd-A1b*	*Ppd-B1b*	*SS*	2716-25.94.01/GUANAY
Line 19	*Ppd-A1b*	*Ppd-B1b*	*SS*	2716-25.94.01/GUANAY
Line 20	*Ppd-A1b*	*Ppd-B1b*	*SS*	2905-13.93.04//CADO/BOOMER_33
Line 21	*Ppd-A1b*	*Ppd-B1b*	*SS*	2905-13.93.04//CADO/BOOMER_33
Line 22	*Ppd-A1b*	*Ppd-B1b*	*SS*	2905-13.93.04/SNITAN
Parental 4	*Ppd-A1b*	*Ppd-B1b*	*SS*	2805-49.94.02
Line 23	*Ppd-A1b*	*Ppd-B1a*	*SI*	2716-25.94.01// GUANAY
Line 24	*Ppd-A1b*	*Ppd-B1a*	*SI*	2905-13.93.04//CADO/BOOMER_33
Line 25	*Ppd-A1b*	*Ppd-B1a*	*SI*	2905-13.93.04//CADO/BOOMER_33
Parental 1	*Ppd-A1b*	*Ppd-B1a*	*SI*	DURABON
Parental 2	*Ppd-A1b*	*Ppd-B1a*	*SI*	MEGADUR
Parental 3	*Ppd-A1b*	*Ppd-B1a*	*SI*	2716-25.94.01
Parental 5	*Ppd-A1b*	*Ppd-B1a*	*SI*	2905-13.93.04

SUPPORTING INFORMATION CHAPTER IV

Supplementary table 1. Pedigree of the genotypes used in the study.

Genotype	Pedigree
Line 1	2716-25.94.01/GUANAY
Line 2	2716-25.94.01/GUANAY
Line 3	2716-25.94.01/SNITAN
Line 4	2716-25.94.01/SNITAN
Line 5	2716-25.94.01/SNITAN
Line 6	2716-25.94.01/SNITAN
Line 7	2805-49.94.02//CADO/BOOMER_33
Line 8	2805-49.94.02//CADO/BOOMER_33
Line 9	2805-49.94.02/GUANAY
Line 10	2805-49.94.02/GUANAY
Line 11	2905-13.93.04//CADO/BOOMER_33
Line 12	2905-13.93.04//CADO/BOOMER_33
Line 13	2905-13.93.04//CADO/BOOMER_33
Line 14	2905-13.93.04//CADO/BOOMER_33
Line 15	2905-13.93.04//CADO/BOOMER_33
Line 16	2905-13.93.04//CADO/BOOMER_33
Line 17	2905-13.93.04//CADO/BOOMER_33
Line 18	2905-13.93.04//CADO/BOOMER_33
Line 19	2905-13.93.04//DUKEM_12/2*RASCON_21
Line 20	2905-13.93.04//DUKEM_12/2*RASCON_21
Line 21	2905-13.93.04/SNITAN
Line 22	2905-13.93.04/SNITAN
Line 23	DURABON//SOOTY_9/RASCON_37
Line 24	DURABON//SOOTY_9/RASCON_37
Line 25	DURABON/GUANAY
Line 26	DURABON/GUANAY
Line 27	MEGADUR//DUKEM_12/2*RASCON_21
Line 28	MEGADUR//DUKEM_12/2*RASCON_21
Line 29	MEGADUR//DUKEM_12/2*RASCON_21
Line 30	MEGADUR//DUKEM_12/2*RASCON_21
Parental 1	DURABON
Parental 2	MEGADUR
Parental 3	2716-25.94.01
Parental 4	2805-49.94.02
Parental 5	2905-13.93.04
Parental 6	SOOTY_9/RASCON_37
Parental 7	CADO/BOOMER_33
Parental 8	DUKEM_12/2*RASCON_21
Parental 9	GUANAY
Parental 10	SNITAN

Supplementary table 2: MTAs with a -log *p* > 3.

Site	Trait	Mrk	Chr Svevo	cM Svevo	-Log *p*
Across	D_EDR	*Ppd-A1*	2A	38.8	4.0
Across	D_EDR	PAV1724214	2A	102.9	3.3
Across	D_EDR	PAV1667148	2A	158.8	3.3
Across	D_EDR	PAV1117036	6B	7.6	3.5
Across	D_EDR	SNP982140	6B	63.6	3.0
Across	D_ETS	*Ppd-A1*	2A	38.8	4.4
Across	D_ETS	PAV1724214	2A	102.9	3.3
Across	D_ETS	PAV1667148	2A	158.8	3.6
Across	D_ETS	SNP1089380	4B	55.3	3.5
Across	D_ETS	PAV1117036	6B	7.6	3.1
Across	D_ETS	SNP982140	6B	63.6	3.3
Across	D_ETS	PAV1766561	7B	4.8	3.4
Across	D_EF	*Ppd-A1*	2A	38.8	6.0
Across	D_EF	SNP1062525	2A	152.6	3.1
Across	D_EF	PAV1223252	5A	115.9	3.2
Across	D_EF	PAV1211379	6A	52.8	3.1
Across	D_EF	SNP982140	6B	63.6	4.1
Across	D_FM	SNP1058666	2A	43.1	3.9
Across	D_FM	SNP1124767	2A	90.7	3.1
Across	D_FM	PAV1122444	2A	153.1	3.1
Across	D_FM	SNP1021742	2B	72.5	3.7
Across	D_FM	PAV1250890	2B	145.1	3.1
Across	D_FM	SNP1036721	4B	16.5	3.2
Across	D_FM	PAV1074583	7A	118.5	3.7
Across	D_FM	PAV1262904	7A	175.1	3.2
Across	D_FM	PAV1080449	7B	142.9	3.3
Across	GDD_EDR	*Ppd-A1*	2A	38.8	3.6
Across	GDD_EDR	PAV1667148	2A	158.8	3.5
Across	GDD_EDR	PAV2255749	3B	25.1	3.1
Across	GDD_EDR	SNP1089380	4B	55.3	3.1
Across	GDD_EDR	PAV1117036	6B	7.6	3.5
Across	GDD_EDR	SNP982140	6B	63.6	3.2
Across	GDD_ETS	*Ppd-A1*	2A	38.8	4.2
Across	GDD_ETS	PAV1667148	2A	158.8	3.8
Across	GDD_ETS	SNP1089380	4B	55.3	3.4
Across	GDD_ETS	PAV1117036	6B	7.6	3.0
Across	GDD_ETS	SNP982140	6B	63.6	3.6
Across	GDD_ETS	PAV1766561	7B	4.8	3.1
Across	GDD_EF	*Ppd-A1*	2A	38.8	6.1
Across	GDD_EF	PAV1259751	2A	152.6	3.1
Across	GDD_EF	PAV1223252	5A	115.9	3.2
Across	GDD_EF	PAV1211379	6A	52.8	3.2
Across	GDD_EF	SNP982140	6B	63.6	4.2
Across	GDD_FM	PAV1105852	2A	78.5	3.9
Across	GDD_FM	PAV1122444	2A	153.1	3.1
Across	GDD_FM	PAV994059	2B	45.0	3.4
Across	GDD_FM	SNP1021742	2B	72.5	4.0
Across	GDD_FM	SNP1055075	2B	165.3	3.2
Across	GDD_FM	PAV1161677	3A	1.6	3.6
Across	GDD_FM	PAV982680	3B	10.1	3.1
Across	GDD_FM	PAV3064759	3B	39.2	3.6
Across	GDD_FM	SNP1092216	4B	12.9	3.8
Across	GDD_FM	SNP1233081	5A	25.6	3.7
Across	GDD_FM	SNP1019499	5A	189.0	3.3
Across	GDD_FM	SNP1086577	5B	135.8	3.1
Across	GDD_FM	SNP1075915	6B	84.9	3.2

Site	Trait	Mrk	Chr Svevo	cM Svevo	-Log *p*
Across	GDD_FM	PAV1074583	7A	118.5	3.6
Across	GDD_FM	PAV1254480	7A	186.7	3.1
Across	GDD_FM	PAV1080449	7B	142.9	3.1
Across	GW	PAV985860	2A	37.3	3.3
Across	GW	SNP1060708	2A	81.2	7.7
Across	GW	PAV1724214	2A	102.9	6.0
Across	GW	SNP2260254	2A	126.3	4.2
Across	GW	PAV1106792	2B	120.0	3.4
Across	GW	PAV1126966	3A	57.3	3.7
Across	GW	SNP1109210	3A	143.9	4.5
Across	GW	PAV2255749	3B	25.1	3.6
Across	GW	PAV1053370	4A	141.2	3.1
Across	GW	PAV1279588	4B	25.7	3.3
Across	GW	SNP1089380	4B	55.3	3.8
Across	GW	PAV1117036	6B	7.6	3.5
Across	GW	PAV1314665	7A	5.9	3.1
Across	GNm2	PAV1165987	1A	58.3	3.1
Across	GNm2	SNP1091747	2A	79.9	8.2
Across	GNm2	PAV1724214	2A	102.9	3.3
Across	GNm2	SNP2260254	2A	126.3	3.0
Across	GNm2	PAV1126966	3A	57.3	4.7
Across	GNm2	SNP1109210	3A	143.9	4.6
Across	GNm2	PAV1229305	4A	36.6	3.6
Across	GNm2	PAV1064199	4A	140.1	3.0
Across	GNm2	PAV1279588	4B	25.7	3.8
Across	GNm2	PAV2278451	6B	0.6	3.4
Across	Yield	PAV1101698	4A	122.4	3.3
Across	Yield	SNP1070974	4B	40.4	3.5
Across	Yield	SNP2276353	6A	114.5	4.6
Across	Yield	PAV1106411	6B	126.0	4.6
Across	Yield	SNP1013644	7A	32.0	3.3
Across	GSp	SNP1091747	2A	79.9	9.6
Across	GSp	PAV1724214	2A	102.9	4.0
Across	GSp	SNP2260254	2A	126.3	3.5
Across	GSp	PAV1126966	3A	57.3	3.1
Across	GSp	SNP1021077	3A	145.8	3.7
Across	GSp	SNP1022127	3B	116.3	4.5
Across	GSp	PAV992973	6A	3.1	4.3
Across	GSp	PAV2278451	6B	0.6	3.6
Across	SpklSp	PAV1040965	1A	1.3	4.6
Across	SpklSp	PAV1071220	1B	29.0	3.6
Across	SpklSp	SNP1209708	1B	87.5	3.2
Across	SpklSp	*Ppd-A1*	2A	38.8	4.7
Across	SpklSp	SNP984567	2A	81.3	3.8
Across	SpklSp	PAV1724214	2A	102.9	3.7
Across	SpklSp	PAV1249417	2A	143.7	3.4
Across	SpklSp	PAV1108695	2B	71.7	3.2
Across	SpklSp	SNP1059438	4A	141.6	3.6
Across	SpklSp	SNP1089380	4B	55.3	4.0
Across	SpklSp	SNP1054888	4B	89.1	3.6
Across	SpklSp	SNP1101715	5A	113.5	3.0
Across	SpklSp	PAV2276852	5B	75.3	3.2
Across	SpklSp	PAV1032504	6B	0.3	3.9
Across	SpklSp	SNP1125985	6B	109.7	4.3
Across	SpklSp	SNP1695340	7A	6.8	3.0
Across	SpklSp	SNP1008088	7A	168.8	3.3
Across	SpklSp	SNP1077397	7B	136.6	4.0
Across	GSpkl	SNP1088268	1A	25.3	3.0

Site	Trait	Mrk	Chr Svevo	cM Svevo	-Log *p*
Across	GSpkl	PAV1140854	1A	73.8	4.5
Across	GSpkl	PAV2258791	1B	24.0	3.0
Across	GSpkl	PAV2293689	2A	79.7	7.4
Across	GSpkl	SNP1100914	3B	102.9	3.1
Across	GSpkl	PAV1064199	4A	140.1	3.2
Across	GSpkl	PAV1162937	6A	3.9	3.7
Spain	D_EDR	PAV1040965	1A	1.3	3.6
Spain	D_EDR	SNP2253156	1A	101.0	3.8
Spain	D_EDR	PAV2277115	1B	75.1	3.5
Spain	D_EDR	SNP1119258	1B	128.7	3.0
Spain	D_EDR	*Ppd-A1*	2A	38.8	5.0
Spain	D_EDR	SNP1060708	2A	81.2	3.0
Spain	D_EDR	PAV1724214	2A	102.9	4.1
Spain	D_EDR	SNP1068560	3A	50.3	3.4
Spain	D_EDR	SNP999959	4A	131.8	3.6
Spain	D_EDR	SNP1101715	5A	113.5	4.1
Spain	D_EDR	PAV1032504	6B	0.3	3.0
Spain	D_EDR	SNP1387282	6B	67.0	3.2
Spain	D_EDR	SNP1125985	6B	109.7	4.5
Spain	D_EDR	SNP1045660	7B	5.6	4.2
Spain	D_EDR	SNP1088346	7B	31.6	3.8
Spain	D_EDR	SNP979836	7B	70.1	3.2
Spain	D_EDR	SNP1077397	7B	136.6	5.3
Spain	D_ETS	PAV1040965	1A	1.3	4.0
Spain	D_ETS	SNP2253156	1A	101.0	4.0
Spain	D_ETS	PAV2277115	1B	75.1	3.5
Spain	D_ETS	SNP1119258	1B	128.7	3.2
Spain	D_ETS	*Ppd-A1*	2A	38.8	5.5
Spain	D_ETS	SNP1060708	2A	81.2	3.1
Spain	D_ETS	PAV1724214	2A	102.9	4.3
Spain	D_ETS	PAV1385093	3B	143.9	3.1
Spain	D_ETS	SNP999959	4A	131.8	3.3
Spain	D_ETS	SNP1101715	5A	113.5	4.0
Spain	D_ETS	PAV1032504	6B	0.3	3.2
Spain	D_ETS	SNP1125985	6B	109.7	3.9
Spain	D_ETS	SNP1045660	7B	5.6	4.9
Spain	D_ETS	SNP1088346	7B	31.6	4.4
Spain	D_ETS	SNP1077397	7B	136.6	5.5
Spain	D_EF	PAV1040965	1A	1.3	4.1
Spain	D_EF	PAV2277115	1B	75.1	3.2
Spain	D_EF	*Ppd-A1*	2A	38.8	5.0
Spain	D_EF	PAV1724214	2A	102.9	3.4
Spain	D_EF	PAV2258114	3B	72.8	3.5
Spain	D_EF	PAV1385093	3B	143.9	3.6
Spain	D_EF	SNP1266998	5A	52.3	3.5
Spain	D_EF	SNP1101715	5A	113.5	3.8
Spain	D_EF	PAV1281528	5A	193.7	3.4
Spain	D_EF	PAV2276852	5B	75.3	3.2
Spain	D_EF	PAV1094527	5B	144.2	3.3
Spain	D_EF	SNP1125985	6B	109.7	4.3
Spain	D_EF	SNP1008088	7A	168.8	3.7
Spain	D_EF	SNP1045660	7B	5.6	3.1
Spain	D_EF	PAV1308762	7B	138.5	5.2
Spain	D_FM	SNP1058666	2A	43.1	3.2
Spain	D_FM	SNP991212	2A	69.0	4.4
Spain	D_FM	PAV2278113	2A	82.0	3.7
Spain	D_FM	PAV1122444	2A	153.1	4.1
Spain	D_FM	SNP1021742	2B	72.5	6.4

Site	Trait	Mrk	Chr Svevo	cM Svevo	-Log *p*
Spain	D_FM	SNP1125555	2B	145.0	4.1
Spain	D_FM	PAV1216270	3B	41.5	5.6
Spain	D_FM	PAV2279877	5A	184.4	3.3
Spain	D_FM	SNP1025472	6A	7.6	3.1
Spain	D_FM	SNP993262	6B	93.8	3.0
Spain	D_FM	PAV1254480	7A	186.7	4.1
Spain	GDD_EDR	PAV1040965	1A	1.3	3.6
Spain	GDD_EDR	SNP2253156	1A	101.0	3.5
Spain	GDD_EDR	PAV2277115	1B	75.1	3.5
Spain	GDD_EDR	*Ppd-A1*	2A	38.8	4.8
Spain	GDD_EDR	SNP1060708	2A	81.2	3.1
Spain	GDD_EDR	PAV1724214	2A	102.9	4.2
Spain	GDD_EDR	SNP1068560	3A	50.3	3.4
Spain	GDD_EDR	PAV1385093	3B	143.9	3.0
Spain	GDD_EDR	SNP999959	4A	131.8	3.4
Spain	GDD_EDR	SNP1101715	5A	113.5	4.1
Spain	GDD_EDR	PAV1032504	6B	0.3	3.1
Spain	GDD_EDR	SNP1387282	6B	67.0	3.3
Spain	GDD_EDR	SNP1125985	6B	109.7	4.3
Spain	GDD_EDR	SNP1045660	7B	5.6	4.2
Spain	GDD_EDR	SNP1088346	7B	31.6	3.7
Spain	GDD_EDR	SNP979836	7B	70.1	3.3
Spain	GDD_EDR	PAV1308762	7B	138.5	5.2
Spain	GDD_ETS	PAV1040965	1A	1.3	3.9
Spain	GDD_ETS	SNP2253156	1A	101.0	3.9
Spain	GDD_ETS	PAV2277115	1B	75.1	3.5
Spain	GDD_ETS	SNP1119258	1B	128.7	3.1
Spain	GDD_ETS	*Ppd-A1*	2A	38.8	5.3
Spain	GDD_ETS	SNP1060708	2A	81.2	3.1
Spain	GDD_ETS	PAV1724214	2A	102.9	4.2
Spain	GDD_ETS	PAV1385093	3B	143.9	3.2
Spain	GDD_ETS	SNP999959	4A	131.8	3.2
Spain	GDD_ETS	SNP1101715	5A	113.5	4.0
Spain	GDD_ETS	PAV1032504	6B	0.3	3.1
Spain	GDD_ETS	SNP1125985	6B	109.7	3.8
Spain	GDD_ETS	SNP1045660	7B	5.6	4.8
Spain	GDD_ETS	PAV1073035	7B	31.6	4.3
Spain	GDD_ETS	PAV1308762	7B	138.5	5.4
Spain	GDD_EF	PAV1040965	1A	1.3	4.0
Spain	GDD_EF	PAV2277115	1B	75.1	3.2
Spain	GDD_EF	*Ppd-A1*	2A	38.8	4.8
Spain	GDD_EF	PAV1724214	2A	102.9	3.4
Spain	GDD_EF	PAV2258114	3B	72.8	3.5
Spain	GDD_EF	PAV1385093	3B	143.9	3.7
Spain	GDD_EF	SNP1266998	5A	52.3	3.5
Spain	GDD_EF	SNP1101715	5A	113.5	3.8
Spain	GDD_EF	PAV1281528	5A	193.7	3.4
Spain	GDD_EF	PAV2276852	5B	75.3	3.1
Spain	GDD_EF	PAV1094527	5B	144.2	3.3
Spain	GDD_EF	SNP1125985	6B	109.7	4.2
Spain	GDD_EF	SNP1008088	7A	168.8	3.8
Spain	GDD_EF	SNP1045660	7B	5.6	3.0
Spain	GDD_EF	PAV1308762	7B	138.5	5.1
Spain	GDD_FM	SNP1173915	1B	28.2	3.2
Spain	GDD_FM	PAV1674297	2A	44.1	3.0
Spain	GDD_FM	SNP991212	2A	69.0	3.3
Spain	GDD_FM	PAV1113052	2A	90.1	4.3
Spain	GDD_FM	PAV1122444	2A	153.1	3.3

Site	Trait	Mrk	Chr Svevo	cM Svevo	-Log *p*
Spain	GDD_FM	SNP1021742	2B	72.5	5.5
Spain	GDD_FM	SNP1125555	2B	145.0	3.3
Spain	GDD_FM	SNP1025974	3A	52.7	3.0
Spain	GDD_FM	PAV982680	3B	10.1	3.6
Spain	GDD_FM	PAV1216270	3B	41.5	4.8
Spain	GDD_FM	PAV2279877	5A	184.4	3.8
Spain	GDD_FM	SNP1025472	6A	7.6	3.7
Spain	GDD_FM	PAV1127936	6B	104.4	3.2
Spain	GDD_FM	PAV1254480	7A	186.7	3.3
Spain	GDD_FM	SNP1216332	7B	2.3	3.6
Spain	GW	PAV2293689	2A	79.7	9.5
Spain	GW	PAV1724214	2A	102.9	5.9
Spain	GW	SNP2260254	2A	126.3	4.1
Spain	GW	PAV1106792	2B	120.0	3.5
Spain	GW	PAV1126966	3A	57.3	4.1
Spain	GW	SNP1109210	3A	143.9	4.4
Spain	GW	PAV2255749	3B	25.1	3.6
Spain	GW	SNP1022127	3B	116.3	3.0
Spain	GW	PAV1279588	4B	25.7	3.7
Spain	GW	PAV2278451	6B	0.6	3.8
Spain	GNm2	PAV1165987	1A	58.3	4.4
Spain	GNm2	SNP984567	2A	81.3	7.6
Spain	GNm2	SNP2260254	2A	126.3	4.0
Spain	GNm2	PAV1126966	3A	57.3	4.6
Spain	GNm2	SNP1109210	3A	143.9	4.8
Spain	GNm2	PAV2255749	3B	25.1	3.1
Spain	GNm2	PAV1229305	4A	36.6	4.7
Spain	GNm2	PAV1064199	4A	140.1	3.9
Spain	GNm2	PAV1279588	4B	25.7	3.3
Spain	GNm2	SNP1089380	4B	55.3	3.1
Spain	GNm2	PAV2278451	6B	0.6	3.2
Spain	Yield	SNP1070974	4B	40.4	3.2
Spain	Yield	SNP1686062	5B	70.9	4.1
Spain	Yield	SNP1220436	6B	126.6	3.1
Spain	Spm2	PAV977865	4B	0.1	4.3
Spain	GSp	PAV1111225	1A	43.4	4.0
Spain	GSp	SNP1025439	1B	34.4	3.0
Spain	GSp	PAV2293689	2A	79.7	7.0
Spain	GSp	PAV1724214	2A	102.9	3.9
Spain	GSp	SNP2260254	2A	126.3	3.3
Spain	GSp	PAV2323735	2B	45.1	4.0
Spain	GSp	PAV1126966	3A	57.3	3.3
Spain	GSp	SNP998662	3A	147.2	3.7
Spain	GSp	PAV2277637	3B	4.2	3.2
Spain	GSp	SNP1087823	3B	127.9	3.5
Spain	GSp	PAV992973	6A	3.1	3.5
Spain	GSp	PAV2278451	6B	0.6	3.1
Spain	GSp	SNP1075915	6B	84.9	3.0
Spain	SpklSp	PAV1040965	1A	1.3	4.4
Spain	SpklSp	PAV1299157	1B	75.4	3.2
Spain	SpklSp	SNP1060708	2A	81.2	3.7
Spain	SpklSp	PAV1724214	2A	102.9	3.5
Spain	SpklSp	SNP2256686	2A	140.8	3.0
Spain	SpklSp	PAV994059	2B	45.0	3.5
Spain	SpklSp	PAV1086078	2B	70.9	3.1
Spain	SpklSp	SNP999959	4A	131.8	4.4
Spain	SpklSp	SNP1054888	4B	89.1	4.8
Spain	SpklSp	PAV2276852	5B	75.3	4.1

Site	Trait	Mrk	Chr Svevo	cM Svevo	-Log *p*
Spain	SpklSp	SNP1007285	6A	44.0	3.6
Spain	SpklSp	PAV1032504	6B	0.3	4.4
Spain	SpklSp	SNP1222885	6B	84.5	3.3
Spain	SpklSp	PAV1119715	6B	112.4	4.8
Spain	SpklSp	SNP1008088	7A	168.8	3.2
Spain	SpklSp	PAV1308762	7B	138.5	4.9
Spain	GSpkl	PAV1140854	1A	73.8	3.1
Spain	GSpkl	PAV2293689	2A	79.7	7.3
Spain	GSpkl	PAV1064199	4A	140.1	3.0
Spain	GSpkl	PAV992973	6A	3.1	3.8
Mexico-north	D_EDR	PAV1667148	2A	158.8	4.0
Mexico-north	D_EDR	PAV2255749	3B	25.1	3.6
Mexico-north	D_EDR	PAV1117036	6B	7.6	3.6
Mexico-north	D_ETS	*Ppd-A1*	2A	38.8	3.9
Mexico-north	D_ETS	PAV1667148	2A	158.8	4.9
Mexico-north	D_ETS	SNP1089380	4B	55.3	3.3
Mexico-north	D_ETS	PAV1117036	6B	7.6	3.1
Mexico-north	D_ETS	SNP982140	6B	63.6	3.6
Mexico-north	D_EF	PAV1040965	1A	1.3	3.0
Mexico-north	D_EF	*Ppd-A1*	2A	38.8	7.0
Mexico-north	D_EF	PAV1223252	5A	115.9	4.1
Mexico-north	D_EF	SNP1141161	6A	100.0	3.3
Mexico-north	D_EF	SNP982140	6B	63.6	3.7
Mexico-north	D_EF	SNP1125985	6B	109.7	3.1
Mexico-north	D_FM	*Ppd-A1*	2A	38.8	3.3
Mexico-north	D_FM	SNP1021742	2B	72.5	3.2
Mexico-north	D_FM	PAV1211379	6A	52.8	3.2
Mexico-north	D_FM	SNP986941	6B	26.9	3.2
Mexico-north	D_FM	SNP982140	6B	63.6	3.8
Mexico-north	GDD_EDR	PAV1667148	2A	158.8	3.9
Mexico-north	GDD_EDR	PAV2255749	3B	25.1	3.7
Mexico-north	GDD_EDR	PAV1117036	6B	7.6	3.7
Mexico-north	GDD_ETS	*Ppd-A1*	2A	38.8	3.9
Mexico-north	GDD_ETS	PAV1667148	2A	158.8	4.9
Mexico-north	GDD_ETS	PAV2255749	3B	25.1	3.1
Mexico-north	GDD_ETS	SNP1089380	4B	55.3	3.3
Mexico-north	GDD_ETS	PAV1117036	6B	7.6	3.2
Mexico-north	GDD_ETS	SNP982140	6B	63.6	3.6
Mexico-north	GDD_EF	PAV1040965	1A	1.3	3.0
Mexico-north	GDD_EF	*Ppd-A1*	2A	38.8	7.0
Mexico-north	GDD_EF	PAV1223252	5A	115.9	4.0
Mexico-north	GDD_EF	SNP1141161	6A	100.0	3.3
Mexico-north	GDD_EF	SNP982140	6B	63.6	3.7
Mexico-north	GDD_EF	SNP1125985	6B	109.7	3.1
Mexico-north	GDD_FM	SNP1124767	2A	90.7	3.4
Mexico-north	GDD_FM	SNP1021742	2B	72.5	4.3
Mexico-north	GDD_FM	PAV1114413	3A	2.7	3.5
Mexico-north	GDD_FM	SNP1019499	5A	189.0	3.7
Mexico-north	GDD_FM	PAV1095260	5B	143.0	3.2
Mexico-north	GW	PAV985860	2A	37.3	3.4
Mexico-north	GW	SNP984567	2A	81.3	7.3
Mexico-north	GW	PAV1724214	2A	102.9	5.7
Mexico-north	GW	SNP2260254	2A	126.3	3.7
Mexico-north	GW	SNP1109210	3A	143.9	4.3
Mexico-north	GW	PAV2255749	3B	25.1	3.0
Mexico-north	GW	SNP1089380	4B	55.3	3.2
Mexico-north	GW	PAV1032504	6B	0.3	3.6

Site	Trait	Mrk	Chr Svevo	cM Svevo	-Log *p*
Mexico-north	GNm2	PAV2293689	2A	79.7	6.8
Mexico-north	GNm2	PAV1126966	3A	57.3	3.8
Mexico-north	GNm2	SNP1109210	3A	143.9	3.3
Mexico-north	GNm2	PAV1229305	4A	36.6	3.0
Mexico-north	GNm2	PAV1064199	4A	140.1	3.6
Mexico-north	Yield	SNP1115716	1B	26.6	3.0
Mexico-north	Yield	SNP2276353	6A	114.5	4.1
Mexico-north	Yield	SNP982140	6B	63.6	3.1
Mexico-north	Yield	PAV1106411	6B	126.0	4.1
Mexico-north	Spm2	PAV1161137	1A	69.2	3.2
Mexico-north	Spm2	SNP999471	1B	12.5	3.5
Mexico-north	Spm2	PAV1138184	1B	70.4	4.5
Mexico-north	Spm2	SNP1141161	6A	100.0	3.5
Mexico-north	Spm2	SNP1077397	7B	136.6	3.0
Mexico-north	GSp	SNP1056211	1A	54.0	3.0
Mexico-north	GSp	PAV2293689	2A	79.7	7.5
Mexico-north	GSp	SNP1049262	2A	143.9	3.0
Mexico-north	GSp	SNP1021077	3A	145.8	3.5
Mexico-north	GSp	SNP1022127	3B	116.3	5.7
Mexico-north	GSp	SNP997641	7A	138.0	3.3
Mexico-north	SpklSp	PAV1040965	1A	1.3	4.1
Mexico-north	SpklSp	PAV1071220	1B	29.0	3.9
Mexico-north	SpklSp	*Ppd-A1*	2A	38.8	4.0
Mexico-north	SpklSp	PAV1674297	2A	44.1	3.5
Mexico-north	SpklSp	SNP984567	2A	81.3	3.4
Mexico-north	SpklSp	PAV1724214	2A	102.9	3.2
Mexico-north	SpklSp	SNP999293	2A	143.0	3.6
Mexico-north	SpklSp	SNP1089380	4B	55.3	4.1
Mexico-north	SpklSp	SNP1101715	5A	113.5	3.4
Mexico-north	SpklSp	PAV1032504	6B	0.3	3.2
Mexico-north	SpklSp	SNP1125985	6B	109.7	4.0
Mexico-north	SpklSp	PAV1308762	7B	138.5	3.2
Mexico-north	GSpkl	SNP1088268	1A	25.3	3.9
Mexico-north	GSpkl	PAV1140854	1A	73.8	3.6
Mexico-north	GSpkl	PAV1269704	1B	18.6	3.9
Mexico-north	GSpkl	SNP1108327	2A	71.9	3.6
Mexico-north	GSpkl	PAV1106247	6B	56.5	3.0
Mexico-south	D_EDR	PAV1135710	4A	51.9	3.7
Mexico-south	D_EDR	PAV1150328	4B	19.2	3.2
Mexico-south	D_EDR	SNP1089380	4B	55.3	3.9
Mexico-south	D_ETS	PAV1150328	4B	19.2	3.4
Mexico-south	D_ETS	SNP1089380	4B	55.3	3.6
Mexico-south	D_EF	*Ppd-A1*	2A	38.8	3.7
Mexico-south	D_EF	PAV1259751	2A	152.6	3.3
Mexico-south	D_EF	PAV1211379	6A	52.8	3.3
Mexico-south	D_EF	SNP982140	6B	63.6	4.1
Mexico-south	D_FM	PAV1105852	2A	78.5	3.5
Mexico-south	GDD_EDR	PAV1135710	4A	51.9	3.5
Mexico-south	GDD_EDR	PAV1150328	4B	19.2	3.1
Mexico-south	GDD_EDR	SNP1089380	4B	55.3	3.8
Mexico-south	GDD_ETS	PAV1150328	4B	19.2	3.3
Mexico-south	GDD_ETS	SNP1089380	4B	55.3	3.4
Mexico-south	GDD_EF	*Ppd-A1*	2A	38.8	3.7
Mexico-south	GDD_EF	PAV1259751	2A	152.6	3.3
Mexico-south	GDD_EF	PAV1211379	6A	52.8	3.3
Mexico-south	GDD_EF	SNP982140	6B	63.6	4.1
Mexico-south	GDD_FM	PAV1105852	2A	78.5	4.0

Site	Trait	Mrk	Chr Svevo	cM Svevo	-Log *p*
Mexico-south	GDD_FM	SNP2257519	2B	13.8	3.1
Mexico-south	GDD_FM	PAV3064759	3B	39.2	3.4
Mexico-south	GDD_FM	SNP985312	4B	37.8	3.1
Mexico-south	GW	PAV985860	2A	37.3	3.1
Mexico-south	GW	SNP1060708	2A	81.2	6.0
Mexico-south	GW	PAV1724214	2A	102.9	5.0
Mexico-south	GW	SNP2260254	2A	126.3	4.0
Mexico-south	GW	PAV1106792	2B	120.0	3.7
Mexico-south	GW	PAV1126966	3A	57.3	3.5
Mexico-south	GW	SNP1109210	3A	143.9	3.8
Mexico-south	GW	PAV2255749	3B	25.1	3.7
Mexico-south	GW	PAV1219738	4A	139.8	3.8
Mexico-south	GW	PAV1279588	4B	25.7	3.4
Mexico-south	GW	SNP1089380	4B	55.3	5.2
Mexico-south	GW	PAV1060931	5B	107.9	3.2
Mexico-south	GW	PAV1117036	6B	7.6	3.7
Mexico-south	GW	PAV1314665	7A	5.9	3.3
Mexico-south	GNm2	PAV2293689	2A	79.7	4.2
Mexico-south	GNm2	PAV2276537	3A	147.6	3.4
Mexico-south	GNm2	PAV1279588	4B	25.7	3.6
Mexico-south	GNm2	PAV2278451	6B	0.6	3.7
Mexico-south	Yield	SNP1137911	2A	71.0	3.8
Mexico-south	Yield	PAV2299734	2A	113.8	3.3
Mexico-south	Yield	SNP1019805.1	2A	143.6	3.1
Mexico-south	Yield	PAV3027493	2B	122.0	3.5
Mexico-south	Yield	PAV1165946	5A	111.4	3.1
Mexico-south	Yield	PAV1269450	7A	114.0	3.1
Mexico-south	Spm2	SNP981396	2B	13.5	3.1
Mexico-south	Spm2	PAV1110164	3B	149.8	3.0
Mexico-south	GSp	PAV2256230	1B	76.4	3.4
Mexico-south	GSp	PAV2293689	2A	79.7	7.4
Mexico-south	GSp	PAV1724214	2A	102.9	3.5
Mexico-south	GSp	SNP2260254	2A	126.3	3.1
Mexico-south	GSp	PAV1228181	3B	76.1	3.4
Mexico-south	GSp	PAV992973	6A	3.1	4.5
Mexico-south	GSp	PAV2278451	6B	0.6	4.0
Mexico-south	SpklSp	PAV1040965	1A	1.3	3.7
Mexico-south	SpklSp	*Ppd-A1*	2A	38.8	6.2
Mexico-south	SpklSp	SNP1127702	2A	46.0	4.3
Mexico-south	SpklSp	SNP1060708	2A	81.2	3.1
Mexico-south	SpklSp	PAV1724214	2A	102.9	3.3
Mexico-south	SpklSp	SNP1275924	2A	135.4	3.1
Mexico-south	SpklSp	PAV1086078	2B	70.9	3.0
Mexico-south	SpklSp	PAV3027493	2B	122.0	3.1
Mexico-south	SpklSp	PAV2288900	3B	27.5	3.0
Mexico-south	SpklSp	SNP1059438	4A	141.6	3.6
Mexico-south	SpklSp	SNP1089380	4B	55.3	3.4
Mexico-south	SpklSp	SNP1054888	4B	89.1	3.0
Mexico-south	SpklSp	PAV2326083	6A	19.2	3.5
Mexico-south	SpklSp	PAV1119715	6B	112.4	3.1
Mexico-south	SpklSp	SNP1695340	7A	6.8	3.2
Mexico-south	SpklSp	SNP1008088	7A	168.8	3.7
Mexico-south	GSpkl	PAV1140854	1A	73.8	3.0
Mexico-south	GSpkl	PAV2293689	2A	79.7	5.9
Mexico-south	GSpkl	PAV1665854	3A	81.6	3.7
Mexico-south	GSpkl	SNP1092164	3A	123.6	3.8
Mexico-south	GSpkl	PAV992973	6A	3.1	3.8