Universitat de Lleida

Contribución al conocimiento de la expresión fenotípica de variantes alélicas para los genes mayores Ppd-A1 y Ppd-B1 en trigo duro

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Departament de Producció Vegetal i Ciència Forestal

TESIS DOCTORAL

CONTRIBUCIÓN AL CONOCIMIENTO DE LA EXPRESIÓN FENOTÍPICA DE VARIANTES ALÉLICAS PARA LOS GENES MAYORES *Ppd-A1* y *Ppd-B1* EN TRIGO DURO

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Memoria de Tesis Doctoral presentada por:

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Para optar al grado de Doctor

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Discusión General

Resumen

La fenología del trigo es un carácter de gran importancia para su adaptación al ambiente donde se cultiva, y se basa en parte en las variantes alélicas para genes *Ppd-1*, asociadas a la respuesta al fotoperíodo. Con el objetivo de proveer de conocimiento para el diseño de ideotipos de trigo duro adaptados a diferentes condiciones ambientales, se trabajó con una colección de líneas con diferencias en sensibilidad al fotoperíodo basada en diversa dotación para los genes *Ppd-A1* y *Ppd-B1*. Este material vegetal fue evaluado en cuatro latitudes (entre 41°38'N y 19°31'N) en España y México durante los años 2007 y 2008, donde se estudió el desarrollo fenológico externo y el rendimiento, incluyendo algunos de sus componentes.

Los resultados obtenidos muestran que las principales variables ambientales que permitieron distinguir entre localidades fueron la temperatura hasta floración y la duración del día durante el período de llenado del grano. La expresión fenotípica de la insensibilidad en *Ppd-A1* aumentó en localidades en las que el fotoperíodo medio hasta floración fue inferior a 12 h. Los genes *Ppd-A1* y *Ppd-B1* explicaron una gran parte de la variabilidad genética de la duración entre siembra y floración, con un mayor efecto de *Ppd-A1b* que *Ppd-B1b.* De acuerdo a su efecto sobre la fecha de floración, los alelos que confieren insensibilidad al fotoperíodo se clasificaron como GS-100>GS-105>*Ppd-B1a*.

En base a los resultados referidos al rendimiento y su formación entre los genotipos portadores del alelo de insensibilidad en *Ppd-B1* (*Ppd-B1a*), los portadores del alelo *Ppd-A1b* dieron lugar a menores rendimientos en todos los ambientes debido a un menor número de espigas por unidad de superficie y un menor peso del grano a pesar de tener un mayor número de granos por espiga. El número de granos fue limitante para el rendimiento cuando su valor fue inferior a 14.000 granos m^{-2}, lo que ocurrió en ambientes con temperaturas medias de las mínimas diarias por encima de 6,9°C antes de floración e inferiores a 10,8°C durante el llenado del grano, acompañadas de fotoperíodos inferiores a 14,2 h.

Niveles de radiación durante el llenado del grano inferiores a 1,8 kJ $grano^{-1}$ $día^{-1}$ limitaron el peso del grano, proporcionando una explicación a la mayor influencia del

peso del grano en la determinación del rendimiento en latitudes altas en comparación con otras más bajas. Los efectos compensatorios entre componentes del rendimiento fueron menores en ambientes favorables que en los más limitantes.

El conocimiento de los genes de fotoperíodo y sus implicaciones sobre la adaptación del trigo duro suponen una gran oportunidad para contribuir a los desafíos actuales de la mejora de esta especie.

Abstract

Wheat phenology is a trait of great importance for environmental adaptation , and depends to some extent to allelic variants of *Ppd-1* genes associated to photoperiod response. In order to provide knowledge for ideotype design of durum wheat adapted to different environmental conditions, we worked with a collection of lines with contrasting photoperiod sensitivity based on different alleles for *Ppd-A1* and *Ppd-B1* genes. These lines were evaluated at four latitudes (between 41° 38'N and 19° 31'N), in Spain and Mexico during 2007 and 2008, where phenology and yield performance, including some of its components, were assessed.

The results showed that the main environmental variables that distinguish between locations were temperature to flowering and daylength during grain filling period. The phenotypic expression of insensitivity in *Ppd-A1* increased in locations where the average photoperiod until flowering was lower than 12 h. The *Ppd-A1b* and *Ppd-B1* genes explained a great part of the genetic variability of the duration between sowing and flowering, with a greater effect of *Ppd-A1* than *Ppd-B1*. According to their effect on flowering date, the effect of alleles conferring photoperiod insensitivity were classified as GS-100> GS-105> *Ppd-B1a*.

Based on the results related to yield and formation, among genotypes carrying the insensitivity allele at *Ppd-B1* (*Ppd-B1a)*, those carrying the *Ppd-A1b* led to lower yields in all environments due to fewer spikes per unit area and lighter grains, despite having more grains per spike. The number of grains limited performance when its value was less than 14,000 grains m^{-2}, which occurred in environments with average daily minimum temperatures above 6.9°C before flowering and below 10.8°C during the grain filling, accompanied by less than 14.2 h photoperiod.

Radiation levels during grain filling lower than 1.8 kJ $grain^{-1}$ day^{-1} limited grain weight, providing an explanation for the greater influence of grain weight in determining the performance at high latitudes compared with the lower one. Compensatory effects between yield components were lower in favorable environments than in more limiting ones.

Knowledge of photoperiod genes and implications for adaptation of durum wheat is an opportunity to address the current challenges of wheat breeding.

Resum

La fenologia del blat és un tret de gran importància per a la seva adaptació a l'ambient on es cultiva, i es basa en part en les variants al·lèliques per a gens *Ppd-1*, associades a la resposta al fotoperíode. Amb l'objectiu de proveir de coneixement per al disseny de ideotips de blat dur adaptats a diferents condicions ambientals, es va treballar amb una col·lecció de línies amb diferències en sensibilitat al fotoperíode basada en la diversa dotació per als gens *Ppd-A1* i *Ppd-B1*. Aquest material vegetal va ser avaluat en quatre latituds (entre 41°38'N i 19°31'N) a Espanya i Mèxic durant els anys 2007 i 2008, on es va estudiar el desenvolupament fenològic extern i el rendiment, incloent alguns dels seus components.

Els resultats obtinguts mostren que les principals variables ambientals que van permetre distingir entre localitats van ser la temperatura fins a floració i la duració del dia durant el període d'ompliment del gra. L'expressió fenotípica de la insensibilitat a *Ppd-A1* va augmentar en localitats en les que el fotoperíode mitjà fins a floració va ser inferior a 12 h. Els gens *Ppd-A1* i *Ppd-B1* van explicar gran part de la variabilitat genètica de la duració entre sembra i floració, amb un major efecte de *Ppd-A1* enfront *Ppd-B1*. D'acord amb el seu efecte sobre la data de floració, es al·lels que proporcionen insensibilitat al fotoperíode es va classificar com GS-100>GS-105>*Ppd-B1a*.

Considerant els resultats de rendiment i la seva formació, entre els genotips portadors de l'al·lel d'insensibilitat en *Ppd-B1* (*Ppd-B1a*), els portadors de l'al·lel *Ppd-A1b* van donar lloc a menors rendiments en tots els ambients, degut a un menor nombre d'espigues per unitat de superfície i un menor pes del gra, tot i tenir un major nombre de grans per espiga. El nombre de grans va ser limitant per al rendiment quan el seu valor va ser menor a 14.000 grans m^{-2}, el qual es va produir en ambients amb temperatures mínimes mitjanes diàries per sobre de 6,9°C abans de floració i inferiors a 10,8°C durant l'ompliment del grà, acompanyades de fotoperíodes inferiors a 14,2 h.

Nivells de radiació durant l'ompliment del gra inferiors a 1,8 kJ gra^{-1} dia^{-1} van limitar el pes del gra, proporcionant una explicació a la major influencia del pes del gra en la determinació del rendiment en latituds altes en comparació amb les més baixes.

Els efectes compensatoris entre components del rendiment van ser menors en ambients favorables en comparació amb els més limitants.

El coneixement dels gens de fotoperíode i les seves implicacions sobre l'adaptació del blat dur suposen una gran oportunitat per a contribuir als donar reposta al desafiaments actuals en la millora d'aquesta espècie.

INTRODUCCIÓN GENERAL

1. Importancia del trigo duro

El trigo es el cultivo que ocupa mayor superficie agrícola en el mundo, por lo que es fundamental para la seguridad alimentaria de la población mundial, a la que proporciona el 19% de la ingesta diaria de calorías y el 21% de la de proteína (FAOSTAT, 2011). La superficie mundial de trigo alcanza las 220 Mha, con un rendimiento medio de 3,2 t ha^{-1} (FAOSTAT, 2011).

El trigo duro (*Triticum turgidum* L. var. *durum*) representa alrededor del 10% de la superficie total de trigo (Kantety *et al.*, 2005), dedicada mayoritariamente a la producción de trigo harinero (*Triticum aestivum* L.). El trigo duro es una especie de gran relevancia en la Cuenca Mediterránea, particularmente en Italia, Grecia, España, Turquía, Argelia, Marruecos y Túnez. En estos países se elaboran y consumen productos con distintos grados de procesamiento. Por un lado, los productos tradicionales como el couscous, bulgur y otros tipos de pan (Troccoli *et al*., 2000; Shewry, 2009; Kezih *et al*., 2014) y por otro, la pasta, de mayor internacionalización fuera de la Cuenca Mediterránea (De Vita *et al.*, 2007). El trigo duro se produce en un rango de latitudes comprendidas entre 55°N y 40°S (Palamarchuck, 2005). Los principales exportadores a escala mundial son Canadá, EEUU y México. Por otra parte, los mayores productores y consumidores dentro de la Cuenca Mediterránea son Italia, Marruecos, Argelia y España (Royo *et al.*, 2009).

Las estimaciones de incremento de la población mundial sitúan en 9,3 mil millones el número de habitantes previstos en 2050. Ello hace que la demanda mundial de trigo se espere un 60% mayor respecto a la de 2010 (FAOSTAT, 2011), lo que representa que los rendimientos medios anuales deben aumentar desde el nivel del 1,1% alcanzado en el periodo 2001-2010, hasta el 1,6% entre 2011 y 2050.

La producción global de trigo es muy sensible a los estreses ambientales (Porter y Semenov, 2005), especialmente en condiciones mediterráneas, donde se encuentran la mayoría de productores y consumidores de trigo duro y donde el ambiente es responsable de hasta el 98% de la variación del rendimiento (Royo *et al.*, 2010). Los principales factores que limitan la producción de trigo duro son las altas temperaturas y la sequía (Lobell *et al.*, 2012; Semenov *et al.*, 2014), que aumentarán en el futuro

según predicen los modelos que estudian el cambio climático (IPCC, 2014). La previsión de una mayor incidencia de estos factores limitantes, junto con la necesidad de dar respuesta al aumento proyectado de la población en las próximas décadas (UN, 2012), hacen imprescindible un esfuerzo global para aumentar la productividad y sostenibilidad del cultivo, lo cual pasa forzosamente por identificar y comprender adecuadamente los factores genéticos y ambientales que limitan la producción de trigo (Reynolds *et al.*, 2011).

2. Desarrollo fenológico y adaptación

El ritmo con que ocurren los eventos biológicos de una planta se conoce como fenología (Lieth, 1974). El desarrollo vegetal se puede definir como la secuencia de acontecimientos fenológicos, controlados por factores genéticos y ambientales, que determinan los cambios morfológicos y funcionales de la planta (Landsberg, 1977) y que conducen a la acumulación de biomasa y a la formación de los componentes del rendimiento. Si bien el desarrollo del trigo es un proceso continuo, puede describirse en diversos periodos y fases, las cuales pueden estar a su vez definidas en términos de cambios morfológicos internos o externos. En el caso del trigo, es necesario un desarrollo adecuado para conseguir una adaptación al ambiente que permita la formación de un elevado número de granos con la mayor cantidad posible de reservas en el endospermo y, por tanto, el máximo rendimiento.

Para facilitar su estudio el ciclo del trigo puede dividirse en tres periodos: vegetativo, reproductivo y de maduración (García del Moral *et al*., 2003). El periodo vegetativo se inicia con la germinación de la semilla- Ésta promueve un incremento de la actividad fisiológica del grano, acelerando el crecimiento de los meristemos presentes en el embrión y la movilización de las reservas del grano. El período vegetativo termina en el estadío de doble arruga del ápice, momento en el cual la tasa de desarrollo es sensible a la temperatura y la luz.

El periodo reproductivo que externamente corresponde al inicio de elongación de tallos, se inicia con la formación de los primordios de espiguilla en el ápice meristemático y finaliza en la floración con la polinización de los ovarios en las espiguillas con flores fértiles. El periodo de maduración, conocido también como de

llenado del grano, abarca desde la antesis hasta la madurez fisiológica. Durante el proceso de llenado del grano se produce el depósito de almidón y proteínas en el endospermo. Todo este proceso se basa en la fotosíntesis, básicamente de la hoja bandera y la inflorescencia, además de la translocación de reservas de asimilados acumulados antes de la antesis (Royo *et al*., 1999).

Para el correcto estudio y seguimiento del ciclo del trigo se han propuesto escalas que describen las distintas fases de desarrollo. Entre las más usadas se encuentra el código decimal de Zadoks *et al.* (1974), quienes modificaron la escala de Feekes-Large (Large, 1954) generando códigos decimales, donde el primer dígito identifica la fase y el segundo el estadio dentro de cada fase: 00 germinación, 10 emergencia, 20 ahijamiento, 30 elongación del tallo, 40 embuchado o zurrón, 50 emergencia de la espiga, 60 antesis o floración, 70 grano lechoso, 80 grano pastoso y 90 madurez. La escala de Zadoks-Chang-Konzak es de gran ayuda para apreciar externamente la expresión morfológica en condiciones de campo (Mellado, 2006). Sin embargo, para el estudio del desarrollo interno o apical se utiliza la escala propuesta por Kirby y Appleyard (1984), que incluye diferentes fases de desarrollo de la espiga, incluyendo desarrollo apical, desarrollo de espiguillas y desarrollo del grano.

La duración de cada fase y el total del ciclo, así como el número de primordios de hoja y de espiguilla que pueden iniciarse, vienen definidos por la interacción de factores genéticos y ambientales. Esta interacción se produce cuando las diferencias entre genotipos dependen del ambiente en que éstos se ensayan (Romagosa *et al.*, 2008), principalmente como respuesta frente a los estreses biótico y abiótico (Kang, 2002). Además juega un papel fundamental en los programas de mejora, puesto que determina el tipo de adaptación, comportamiento y número de años que debe evaluarse el material antes de ser utilizado como variedad (Fox *et al*., 1997; Baenziger y DePauw, 2009).

2.1 Efecto de la temperatura y el fotoperíodo sobre la fenología del cultivo

Los principales factores ambientales que modifican el desarrollo del trigo son la temperatura y el fotoperíodo (Klepper *et al*., 1982; Baker y Gallagher, 1983; McMaster, 2009). La temperatura afecta a la tasa de desarrollo de la planta, y en consecuencia,

influye sobre la duración de todas las fases (Klepper *et al*., 1982). Las temperaturas demasiado altas alteran a la planta a nivel morfológico, fisiológico y bioquímico, afectando el crecimiento y desarrollo, y disminuyendo el rendimiento (Wahid *et al*., 2007). La sensibilidad a la temperatura varía no solo entre los órganos de la planta sino que también cambia durante su desarrollo (Musich *et al.*, 1981; Porter y Gawith, 1999). En trigo la temperatura óptima para un adecuado desarrollo y rendimiento oscila entre 18 y 24°C (Almeselmani *et al.*, 2011). Por otra parte, se han reportado rangos óptimos de temperaturas para las diferentes fases: inicio de la fase reproductiva, entre 9,3 y 11,9°C (Porter y Gawith, 1999); floración 18-24°C (Russell y Wilson, 1994) y llenado de grano >25°C (Slafer y Rawson, 1995), en tanto que temperaturas superiores a 32°C durante el llenado de grano producen disminuciones del tamaño y el peso de los granos (Labuschagne *et al*., 2009).

En cereales de invierno las bajas temperaturas pueden inducir el fenómeno conocido como vernalización, consistente en la adquisición de la capacidad de cambiar el ápice vegetativo a reproductivo, o la aceleración del proceso, que tiene lugar cuando la planta se expone a un periodo de temperaturas adecuadamente bajas (Chouard, 1960; Amasino, 2005; Li *et al.*, 2011). Específicamente en trigo las temperaturas vernalizantes se dan en un rango de -1,3 a 15,7°C (Porter y Gawith, 1999). La vernalización es un mecanismo natural que mejora la supervivencia en ambientes con inviernos muy fríos, pues evita que el espigado y la antesis tengan lugar en momentos con riesgo de heladas (Flood y Halloran, 1986; Fowler *et al*., 1996 y 2001).

El trigo es una especie de día largo, que manifiesta una respuesta cuantitativa al incremento de las horas de luz (Thomas y Vince-Prue, 1997), es decir, adelanta su floración en la medida que se expone a días largos (Yan, 2009). El trigo originalmente es sensible al fotoperíodo, y esta característica se ha utilizado en la mejora de esta especie como mecanismo de adaptación a diferentes condiciones (Slafer y Rawson, 1994; Bentley *et al*., 2011).

2.2 Ajuste fenológico y adaptabilidad

El ajuste fenológico es uno de los mecanismos más conocidos de adaptación a las condiciones ambientales inestables, particularmente a temperaturas desfavorables y sequía terminal (Loss y Siddique, 1994). La posibilidad de ajustar la fecha de siembra, floración y el llenado de grano provee a los productores y mejoradores de una herramienta para diseñar una estrategia de escape a los diferentes tipos de estrés (Richards, 1996; Banzinger y Cooper, 2001; Sylvester-Bradley *et al.*, 2012). Esta estrategia consiste en hacer coincidir las fases críticas que determinan el rendimiento, tales como la formación de espiguillas (Sreenivasulu y Schnurbusch, 2012), antesis (Fischer, 1985; Reynolds *et al*., 2009) y llenado de grano (Fischer, 2011), con los momentos en que el ambiente es menos desfavorable (Reynolds *et al*., 2012).

La adaptabilidad se define como la capacidad de una especie para afrontar el estrés ambiental (Schlegel, 2003), y se sabe que existe variabilidad genética para la adaptabilidad a distintos ambientes a nivel de especie y de variedad dentro de la especie (Dencic *et al.*, 2000; Banzinger y Cooper, 2001). Según Finlay y Wilkinson (1963), existen dos tipos de adaptabilidad: adaptabilidad amplia y adaptabilidad específica. Mientras que la adaptabilidad amplia se manifiesta como un mejor desempeño de ciertas variedades en la mayoría de ambientes, la adaptación específica consiste en la expresión de mejores resultados en ciertos ambientes en particular (Cooper y Byth, 1996; Annichiarico, 2002).

2.3 Relación entre la fenología y el rendimiento

El rendimiento del trigo duro puede expresarse como el producto del número de granos por unidad de superficie y el peso medio de estos granos (Perry y D`Antuono, 1989). La formación de cada uno de estos componentes ocurre en diferentes momentos del ciclo de vida de la planta de trigo. En el período reproductivo, se determina el número de espigas por unidad de superficie además de el número potencial de granos por espiga (Sreennivasulu y Schnurbusch, 2012), mientras que durante el período de llenado del grano se determina el peso de los granos (Fischer, 2011). La formación de los componentes del rendimiento tiene lugar de forma secuencial, proporcionando una gran plasticidad al permitir compensar efectos

adversos sobre los primeros componentes del rendimiento que se forman mediante la elevación de los siguientes (García del Moral *et al*., 1991; 2003). De esta manera se consiguen cosechas similares bajo una gran diversidad de ambientes y circunstancias, lo cual constituye una de las razones por la que los cereales han sido cultivos preferentes desde el inicio de la agricultura.

En trigo y cebada se ha demostrado que la fecha de espigado es crítica para obtener altos rendimientos en un ambiente determinado, especialmente en aquellos donde el rendimiento es limitado por sequías terminales o bajas temperaturas (Ford *et al.*, 1981; Acevedo *et al.*, 1991; Van Oosterom y Acevedo, 1992). El conocimiento actual de los efectos de la fenología, en particular la fecha de floración, sobre la formación del rendimiento en trigo duro y su interacción con las señales ambientales es insuficiente.

Sadras *et al.* (2009) estudiaron la relación entre la plasticidad del rendimiento y la fenología en ambientes con distintos niveles de estrés hídrico y térmico. Como resultado identificaron tres caracteres asociados a la fenología como principales responsables del alto rendimiento en condiciones de estrés: floración temprana, larga duración del llenado de grano y baja plasticidad de la misma.

Por otra parte se han identificado QTLs que controlan la fecha de floración y que tienen efectos en la duración del llenado de grano. Bogard *et al*. (2011) reportaron un total de 40 QTLs asociados a la regulación de la fecha de antesis en una población de dobles haploides de trigo harinero, de hábito invernal. Además sugieren la necesidad de plantear una hipótesis que tenga en cuenta la variabilidad genética para la fecha de antesis, senescencia de las hojas durante el llenado de grano y el rendimiento o concentración de proteína en grano. Se ha sugerido que la floración tiene un rol importante en la determinación del rendimiento de grano y su contenido de proteína, y por tanto incide en la seguridad alimentaria mundial (Kamran *et al*., 2014).

3. Bases moleculares de la fecha de floración

La fecha de floración es un carácter complejo que manifiesta variación continua (Worland *et al.*, 1998; Snape *et al.*, 2001; Kamran *et al.*, 2014). El control genético de este carácter está determinado por genes que afectan la sensibilidad al fotoperíodo (*Ppd*), vernalización (*Vrn*) y precocidad intrínseca (*Eps)* (Fischer, 2011). Estos genes

interactúan entre sí y con el ambiente, causando efectos pleiotrópicos sobre otros aspectos del crecimiento y desarrollo de la planta (Snape *et al.*, 2001).

3.1 Requerimiento de vernalización

El requerimiento de vernalización en trigo está controlado por 3 sistemas denominados *Vrn1, Vrn2* y *Vrn3,* ubicados en los cromosomas 5A, 5B (Worland, 1996) y 7B respectivamente (Distelfeld *et al*., 2009; Snape *et al.,* 2001, Trevaskis *et al*., 2007). Existe interacción entre los tres sistemas de genes (Li *et al.*, 2011). Las proteínas involucradas en la expresión de *Vrn1* desencadenan la transición del ápice vegetativo al reproductivo, proceso que termina en el estadio de espiguilla terminal (Trevaskis, 2010). Este proceso está apoyado por la acción de *Vrn3*, gen que promueve el aumento de la concentración de proteínas de *Vrn1* (Brown *et al.*, 2013). Por otra parte se ha demostrado en cebada que *Vrn2* actúa reprimiendo la floración, retrasándola cuando las plantas no han sido vernalizadas (Karsai *et al.*, 2005; Casao, 2011). La presencia del alelo dominante da lugar a un genotipo de hábito primaveral, en caso contrario el genotipo será de hábito invernal (Yan *et al.*, 2004).

3.2 Sensibilidad al fotoperíodo

En trigo duro se han mapeado dos genes de sensibilidad al fotoperíodo: *Ppd-A1* y *Ppd-B1*, ubicados en los cromosomas 2A y 2B, respectivamente (Snape *et al.*, 1996). Sus alelos dominantes, denotados con el sufijo "*a*" confieren baja sensibilidad al fotoperíodo (Pugsley, 1966), mientras que los alelos recesivos, denotados con el sufijo "*b*" confieren sensibilidad (Mc Intosch *et al.*, 2007). Se ha sugerido que el alelo de insensibilidad sería una mutación del alelo original sensible del gen *Ppd-1* (Bentley *et al.,* 2011). Por otra parte la detección de alelos para los genes de fotoperíodo está todavía en desarrollo, y hasta la fecha se dispone de marcadores moleculares para identificar las mutaciones en *Ppd-A1* (Wilhelm *et al.*, 2009), mientras que para *Ppd-B1* se pueden utilizar los microsatélites *Xgwm 148 y Xgwm 257* cercanos al gen descrito en Hanocq *et al.*, (2004).

La sensibilidad al fotoperíodo permite utilizar la variación genética existente para los genes *Ppd-1* con el objetivo de generar variedades con diferentes grados de adaptación (Worland *et al.*, 1998). La respuesta al fotoperíodo es, después de la

vernalización, el carácter genético más importante para la determinación de la fecha de floración y la adaptación del trigo a diferentes condiciones agroclimáticas (Kamran *et al.*, 2014). Stelmakh (1998) calculó que el sistema de genes *Ppd* explica alrededor de 20-25% de la variabilidad genética en la fecha de espigado en trigo harinero. Se ha sugerido que los mejoradores de trigo lograron identificar las mejores combinaciones de genes *Ppd* para una zona determinada basándose en una selección indirecta de caracteres de importancia agronómica (Kosner y Zurkova, 1996).

Considerando los efectos pleiotrópicos que tiene este grupo de genes es posible realizar un ajuste cada vez más preciso y directo de la fenología para alcanzar rendimiento óptimo. Esto es importante sobre todo en un ambiente impredecible en términos de regímenes de luz y temperatura (Reynolds *et al.*, 2012). Entre los efectos indirectos de los genes *Ppd-1*, destacan la reducción del número de hijuelos (Miralles *et al*., 2000), desarrollo de la espiguilla (Rawson y Richards, 1993), número de espiguillas por espiga (Snape *et al.*, 2001), número potencial de granos (Slafer y Whitechurch, 2001), número final de hojas (Brooking *et al*., 1995; Dyck *et al.*, 2004), reducción área foliar (Foulkes *et al.*, 2004) y altura de la planta (Worland, 1996).

3.3 Precocidad intrínseca

Una vez cubiertas las necesidades de vernalización y fotoperíodo entran en acción un grupo de genes que se han denominado genes de precocidad intrínseca o *earliness per se (Eps)* (Hoogendoorn, 1985; Snape *et al.*, 2001). Estos genes pueden ser responsables de la variación en la fecha de espigado incluso en presencia de genes que confieren sensibilidad al fotoperíodo (Van Beem *et al*., 2005), aunque diversos autores los definen como genes de efectos relativamente pequeños (Miura y Worland, 1994; Miura *et al*., 1999). En trigo se han identificado en los cromosomas 1B, 3A, 3B, 4A, 4B, 6A y 6B (Griffiths *et al.*, 2009), entre otros.

Kamran *et al.* (2013) detectaron un QTL asociado a la precocidad intrínseca que denominaron *QFlt.dms-5B.1* y que induce una floración temprana, además contribuye a alargar el periodo de llenado de grano. Estos resultados indican que los genes de precocidad intrínseca tienen potencial para ser manipulados para aumentar el rendimiento del trigo.

3.4 Ideotipo y adaptación

Donald (1968), propuso el concepto de ideotipo o planta modelo integrando todos los conocimientos disponibles para el desarrollo de cultivares adaptados. Se basó principalmente en la selección de caracteres morfológicos que darían como resultado una planta de baja altura, tallo único y grueso, de espiga larga y hojas erectas. Algunos de estos caracteres se han integrado en las variedades modernas, tales como la reducción de talla mediante la introducción de genes de enanismo, que han reducido la altura de la planta, mejorando a su vez la fertilidad floral y el índice de cosecha (Austin *et al*., 1980; Royo *et al.*, 2007; Sánchez *et al.*, 2012).

Por otra parte, un ejemplo de cómo el ambiente donde se desarrolla germoplasma influye en su adaptación y utilización alrededor del mundo, se puede encontrar en el método de mejora conocido como "shuttle breeding" (Ortiz *et al.*, 2007). Esta estrategia utilizada por CIMMYT desde los años 60, tuvo como propósito inicial acelerar el avance generacional del germoplasma realizando para ello dos cosechas al año en latitudes contrastantes (Borlaug, 2007). Bajo estas condiciones se seleccionaron sistemáticamente genotipos insensibles al fotoperíodo, que se convirtieron en la base de la Revolución Verde (Baenzinger y DePauw, 2009) y del aumento sostenido del rendimiento en el Siglo XX (Rajaram, 2001).

En la actualidad es posible incorporar los avances de la biología molecular y la fisiología, con el propósito de incrementar la comprensión de los mecanismos de expresión de los genes involucrados en la floración del trigo (Brown *et al*., 2013). De esta manera, es posible potenciar el concepto de ideotipo para el desarrollo de variedades adaptadas, garantizando una producción sostenible en el uso de los recursos y que provea de alimentos suficientes en cantidad y calidad (Curtis y Halford, 2014).

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OBJETIVOS

Objetivos

El objetivo general de esta tesis Doctoral fue proveer de conocimiento para el diseño de ideotipos de trigo duro adaptados a diferentes condiciones ambientales en base a variantes alélicas para genes *Ppd-1* asociadas a la respuesta al fotoperíodo.

Este objetivo general se concretó en los siguientes objetivos específicos:

1) Evaluar el efecto de las principales variables ambientales asociadas a la latitud sobre la fenología del trigo duro y profundizar en el conocimiento de aquellas que limitan el rendimiento y su formación.
2) Estudiar el efecto de diversas combinaciones alélicas para los genes mayores *Ppd-A1* y *Ppd-B1* sobre la duración de los períodos emergencia-floración y llenado de grano, evaluando el efecto relativo de supuestos genes de precocidad intrínseca (*Eps*).
3) Investigar la influencia ejercida por variantes alélicas en *Ppd-A1*, en presencia del alelo *Ppd-B1a*, sobre la estrategia de formación del rendimiento en ambientes con diferentes regímenes de temperatura y fotoperíodo.

La presente Memoria de Tesis Doctoral se compone de tres capítulos elaborados como entidades independientes de información, al objeto de que sean publicados como artículos científicos en revistas de impacto.

El trabajo aquí reflejado es fruto de la colaboración del IRTA, el INIA-España y el CIMMYT, siendo parte del Proyecto titulado "*Addressing the challenges for a sustainable wheat production in Spain and North Africa*".

CHAPTER I

ENVIRONMENTAL EFFECTS ASSOCIATED WITH LATITUDE AND THEIR IMPACT ON WHEAT PHENOLOGY AND YIELD FORMATION

ABSTRACT

The effect of environmental variables on the agronomic performance of durum wheat was studied on eight field experiments carried out in four sites at contrasting latitudes (from 41º38'N in the north of Spain to 19º31'N in the south of Mexico) during two years. Each experiment involved 42 durum wheat genotypes of divergent phenology. The main environmental variables discriminating between sites were the minimum and maximum temperatures from sowing to anthesis, which increased from northern to southern latitudes, and the daylength and minimum temperatures from anthesis to maturity, which followed the opposite trend. The site effect explained 96% of the variation in the number of days from sowing to anthesis, which was much shorter in southern sites. Average minimum daily temperatures below 6.9 ºC before anthesis and above 10.8 ºC during grain filling accompanied by photoperiods of more than 14.2 h, frequent in autumn planting in northern environments, resulted in large number of kernels per unit area. In these environments radiation during grain filling lower than 1.8 kJ kernel^{-1}day^{-1} limited kernel weight, thus giving a reasonable explanation for the greatest influence of kernel weight in yield determination in northern sites when compared with the southern ones, found in this and previous studies. The high minimum temperatures in pre-anthesis registered in southern latitudes resulted in a low number of kernels, which below 14,000 kernels m^{-2} became a limiting factor for yield, while in these environments the high solar radiation per grain during grain filling favored grain weight. The results suggest that successful durum wheat yield improvements could be reached in breeding programs by emphasizing the selection for kernel size on target environments located in northern sites, but focusing on kernels per unit area at southern sites.

Keywords: Temperature; radiation; daylength; yield components; harvest index.

INTRODUCTION

Wheat is one of the most important staple crops in the world (FAOSTAT, 2010), and is essential to global food security. Increases in wheat production will be needed to match the expected population growth in the next decades, and both agronomic improvements and genetic progress through breeding will be needed to meet the resulting increase in global demand (Spiertz, 2012). Planning of breeding strategies likely to lead to important genetic yield gains will depend on the precise knowledge of both genetic and environmental yield-limiting factors (Reynolds et al., 2011).

Most climate change scenarios are predicting increases in temperatures and in the frequency of extreme events, such as heat-waves and longer lasting droughts, as well as more erratic water availability in several of the major wheat producing areas worldwide (IPCC, 2014). Realized grain yield in wheat is primarily determined by environmental factors, particularly under Mediterranean conditions where they have been shown to be responsible for 98% of variations in durum wheat yields (Royo et al., 2010). The uncertainty associated with weather patterns is one of the most important causes of the existing gap between potential and actual productivities (Zhang et al., 2013), estimated at 20% according to Lobell et al. (2009). Luo et al. (2005) reported that climate change is likely to cause a reduction between 13.5 and 32% of wheat yield in Mediterranean-type environments, mostly due to changes in rainfall and temperature (Asseng et al., 2011). Solar radiation is the main driver in dry matter accumulation (Lobell et al., 2009), and a global dimming has been reported during the last 50 years (Stanhill and Cohen, 2001), but its effects are dependent on particular environmental constraints (Yang et al., 2013).

Latitude is an important and integrative environmental driver, since it is associated with variations in temperature regimes, photoperiod and radiation intensity, all determining growth and development (Craufurd and Wheeler, 2009) and ultimately productivity. Within the range of latitudes of spring cereals' growing areas, in addition to causing changes in development, latitude may affect biomass production and tiller density (Peltonen-Sainio et al., 2009), grain number (Fischer, 1985; 2011) and kernel weight (Menéndez and Satorre, 2007). It is also known that latitude-determined photoperiod affects biomass production (Hay, 1990). Most studies relating photoperiod or temperature variation to production have considered fixed photoperiod or temperature treatments which do not occur under natural conditions (Farooq et al., 2011; Giunta et al., 2001), and field studies are often limited to a small range of latitudes (He et al., 2012; Penrose et al., 1996).

Environment and crop relationships are very complex and the environmental limiting factors differ from site to site (Wu et al., 2006). The study of the genotype x environment interaction has critical implications for breeding programs (Rebetzke et al., 2013; Sanchez-Garcia et al., 2012), and new genetic improvements should take into account environmental limitations of the target environment (Spiertz, 2012). Phenological adjustment, or the optimization of the duration of the different developmental phases, has been one of the most useful strategy for adaptation to harsh or/and highly erratic environmental conditions (Gouache et al., 2012). It is considered that time to anthesis is a primary trait determining wheat adaptation to a particular set of growing conditions (Snape et al., 2001; Worland et al., 1998). Variability in time to anthesis can be used to fine-tune growth and development patterns to the most prevailing environmental conditions in any particular environment (Blum, 2011).

The objective of this study was to ascertain the effect of environmental variables associated with latitude on phenology and to learn about the constraints imposed by latitude-related environmental factors on yield formation in durum wheat.

MATERIAL AND METHODS

Experimental setup

Eight field experiments involving 42 durum wheat genotypes were carried out at four sites with contrasting latitude in Spain and Mexico (Table 1) in 2007 and 2008. Each experiment consisted of 12 m^2 plots arranged in a randomized complete block design with 3 replications. Experiments were planted in autumn except in the south of Mexico, where they were planted in May (summer crop cycle). Sowing densities were adjusted to 400 and 275 viable seeds m^{-2} in Spain and Mexico respectively, which was chosen to obtain approximately 450 spikes/m^2. Plot management was implemented to maximize yield at each location, to the extent allowed by local conditions. Soil analyses were performed and fertilization was provided to cover crop extractions. Plots were irrigated when necessary (mandatory full irrigation in the North of Mexico) to avoid any significant water deficit. Plots were kept disease and insect free with preventive pesticide applications. Lodging was prevented, when needed, using networks of strings to support lodging prone or tall genotypes.

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

Site	Site, state or autonomous community	Experimental station (institution's	Coordinates		Altitude (m asl)	Long-term rainfall (mm/year)	Environmental characteristics
			Lat	Long			
North Spain	Gimenells, Cataluña	Commercial farmer's fields	41° 38'N	0° 23'E	200	370	Moderate terminal stress High to medium productivity
South Spain	Jerez de la Frontera, Andalucía	Rancho de la Merced (IFAPA)	37° 0'N	3° 40'W	30	600	Very high terminal stress Medium productivity
North Mexico	Ciudad Obregón, Sonora	CENEB (CIMMYT)	27° 21'N	109° 54'W	40	32	Very high terminal stress Mandatory full irrigation Very high productivity
South Mexico	El Batán - Texcoco, State of Mexico	El Batán (CIMMYT)	19° 31'N	98° 50'W	2249	500	Initial stress eliminated with irrigation Medium productivity

2.2. Plant material

Thirty-nine inbred lines and three checks (Mexa-early, Simeto-medium late and Anton-late) of contrasting time to anthesis were used in this study. Thirty-seven inbred lines resulted from divergent selection for time to anthesis initiated in F_4 and continued until F_8 in crosses between five German genotypes (Durabon, Megadur, 2716-25.94.01, 2805-49.94.02, 2905-13.93-04), and five CIMMYT (International Maize and Wheat Improvement Center) lines (Sooty_9/Rascon_37, Cado/Boomer_33, Dukem_12/2*Rascon_21, Guanay and Snitan). Two CIMMYT unrelated sister lines, derived from the cross CF4-JS 40/3/Stot//Altar84/Ald, were also included in the collection.

Data recording

Anthesis and physiological maturity dates were recorded in each experimental plot when approximately 50% of the main spikes reached Zadoks stages 65 and 87, respectively (Zadoks et al., 1974). Plots were divided in two sections of 6 m^2, one used for destructive sampling and the other left untouched for bulk harvest and estimation of grain yield at full maturity (g m^{-2}), subsequently adjusted to a 10% moisture basis.

At maturity, 1 m row-length of a central representative section was uprooted and a sub-sample of 10 randomly-selected stems was weighed after being oven-dried at 70°C for 48 h. Harvest index (HI) was calculated from the sub-sample as the ratio between kernel weight and total stem weight. Biomass (crop dry weight, CDW g m^{-2}) was computed for each plot as the product of average dry stem weight and number of stems m^{-2}. Thousand-kernel weight (TKW, g) was obtained by weighing a randomly drawn sample of 200 kernels from harvested grain of each plot. The number of kernels m^{-2} was computed as the ratio between grain yield and TKW.

Data of daily maximum and minimum temperatures, rainfall, daylength and radiation were obtained from meteorological stations located at less than 3 km away from the experimental plots. Thermal time (growing degree-days, GDD) was calculated by summing the daily values of mean temperatures (T_m, °C) minus the base temperature. The limits for the maximum and minimum temperatures used to calculate T_m were 37°C and 0°C, respectively (Gallagher, 1979). Daylength including civil twilight (h) was computed daily following Forsythe et al. (1995). Environmental variables were averaged from sowing to anthesis (SA) and from anthesis to maturity (AM) considering the mean phenological data of each experiment. The daily post-anthesis radiation per kernel was computed for each experiment by dividing the mean radiation received during the anthesis-maturity period (MJ m^{-2} day^{-1}) by the mean number of kernels m^{-2}.

Statistical analyses

Environmental variables were coupled with crop phenology by averaging their values on each plot to the length of the periods sowing-anthesis and anthesis-maturity. Procedure GLM of the SAS statistical package (SAS Institute Inc. 2009) was used to perform a combined ANOVA for environmental and agronomic variables. A mixed model was fitted, in which year and site were fixed factors, while genotype and block (nested to site and year), were considered random factors. Means were compared by Duncan's multi-range test at P=0.05.

Pearson correlation coefficients between yield and yield components were calculated at each experiment with the genotype means. In order to identify the environmental variables discriminating among sites, principal component analysis (PCA) was performed on the correlation matrix, calculated on the mean data of each experiment. Linear regression models were fitted to the relationships between variables by using the mean data of each experiment and the forward selection option of the REG procedure of the SAS-STAT statistical package (SAS Institute Inc. 2009). From the

regression equations, boundary values for environmental variables were those at which the coefficients of correlation between yield and the corresponding yield component became significant at *P*=0.05.

RESULTS

Environmental

Mean seasonal temperatures ranged from 10.0°C, recorded in the north of Spain in 2007 to 17.7°C, registered in the north of Mexico in 2008 (Table 2), with the latter site showing the greatest difference between years for this variable. Daily minimum averages increased as site latitude decreased, with South Spain and North Mexico being relatively similar while there was close to a two-fold difference for this variable between North Spain and South Mexico. A similar trend was observed for daily maximum averages for the 3 first sites, but this variable decreased while moving from North Mexico (40 m asl) to South Mexico, where altitude was 2249 m asl (Table 1). Thermal amplitude (average daily maximum-minimum temperatures) also increased with decreasing site latitude, with a substantial jump going down from South Spain to North Mexico and then decreased moving down to South Mexico.

Table I.2. Summary of environmental conditions prevailing at each testing site and main agronomic data

Latitude	Year	Sowing date	Data from sowing to maturity							Range of days sowing-anthesis	Yield ($g\ m^{-2}$)
			Water input (mm)	Temperature (°C)			Net radiation ($MJ\ m^{-2}$)	Daylength[1](h)			
				Max	Min	Mean		Min	Max		
North Spain	2007	24/11/2006	463	16	4.8	10	2576	10.2	16.2	152-171	661
	2008	19/11/2007	640	16	5.5	10.3	2990	10.2	16.3	159-177	705
South Spain	2007	12/12/2006	299	20	6.9	12.9	2667	10.6	15.4	116-139	648
	2008	30/11/2007	343	20	7.7	13.7	2706	10.6	15.3	107-139	525
North Mexico	2007	30/11/2006	384	25	7.7	15.9	2390	11.3	13.3	81-114	526
	2008	22/12/2007	507	27	8.1	17.7	3212	11.3	14.4	83-115	646
South Mexico	2007	18/05/2007	670	24	10	17.3	2481	12.9	13.9	59-87	402
	2008	28/05/2008	482	24	10	17	2319	12.8	14.1	65-90	485

Daylength increased during growth cycle except in the spring planting site of south Mexico (Fig. 1). Average daylength amplitude from sowing to maturity narrowed sharply with decreasing latitude, from 6.05 hours in North Spain to 1.15 hours in South Mexico (two-year average, deduced from Table 2). Sharp differences were observed between sites and close similarity between years at each site. A similar trend was observed when considering average daylength from anthesis to maturity (data not shown).

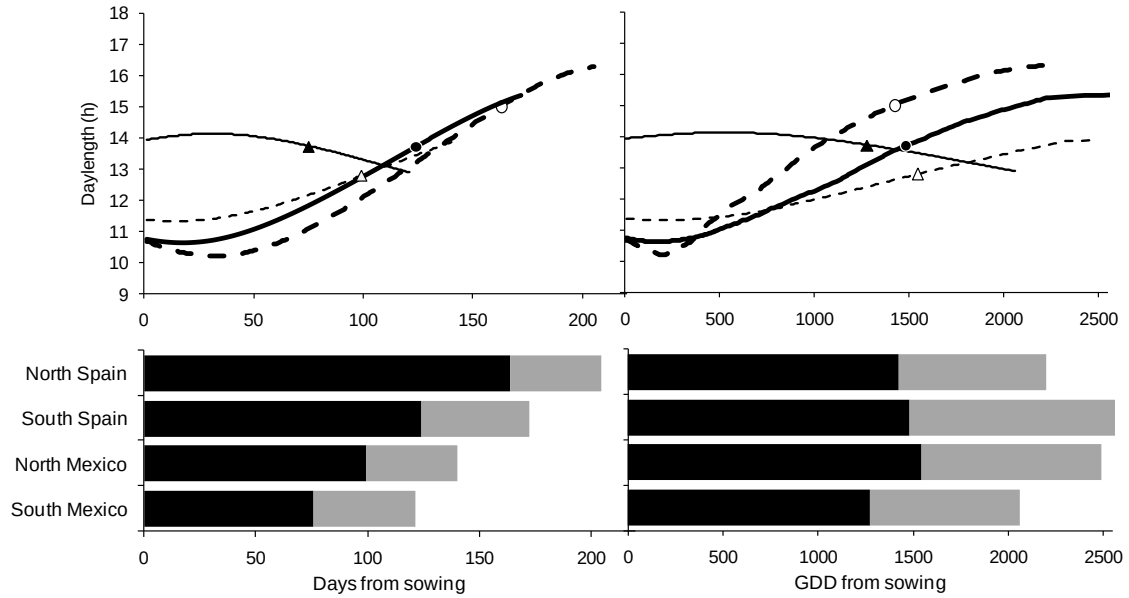

Figure I.1. Mean daylength and cycle duration at each testing site. Data are means of experiments conducted in 2007 and 2008, and involving 42 durum wheat inbred lines. For daylength, North Spain: ● and thick solid line; South Spain: ○ and thick discontinuous line; North Mexico: △ and light discontinuous line; and South Mexico: ▲ and light continuous line. Black bars indicate cycle duration from sowing to anthesis; grey bars indicate duration from anthesis to maturity.

In terms of solar radiation, the lowest and highest values accumulated during the entire growth cycle were recorded in 2008 in South Mexico and North Mexico, respectively (Table 2). A substantial year-to-year variability was observed for this trait, especially in the North Mexico site. Water input ranged from 299 mm in South Spain (2007) to 670 mm in North Mexico (2007). All experiments were irrigated either fully (North Mexico) or in a complementary manner, except in South Spain, where the long-term yearly rainfall averages 600 mm (Table 1). These values of total water input and the distribution of watering events should not have resulted in significant water stress on the experiments, even in the case of Spain-south where the 2-years average yield level was equal to that of the typically high yielding site of North Mexico (Table 2).

In order to identify the combination of variables which better explained the existing environmental variation, principal component analysis (PCA) was conducted on the mean values of environmental variables for each experiment. The first two axes of the PCA shown in Fig. 2 accounted for ca. 77.1% of the total variance (axis 1, 52.8%; axis 2, 24.3%), indicating that most of the information contained in the data could be summarized by projecting the points on the plane determined by these two axes. The eigenvectors of the various components are represented in Fig. 2a. Principal component 1 was related to all the variables included in the analysis, but with a negligible effect of the average daily maximum temperature from anthesis to maturity. Increases in principal component 1 were related to increases in average daily minimum and maximum temperatures and daylength from sowing to anthesis. The negative

direction of PC1 was related to mean daylength and average daily minimum temperature from anthesis to maturity, and accumulated radiation both before and after anthesis (Fig. 2a). Increases in principal component 2 were mostly related to average daily maximum temperatures and accumulated radiation. However, it should be noted that the variance information content of axis 2 is less than half than that of axis 1.

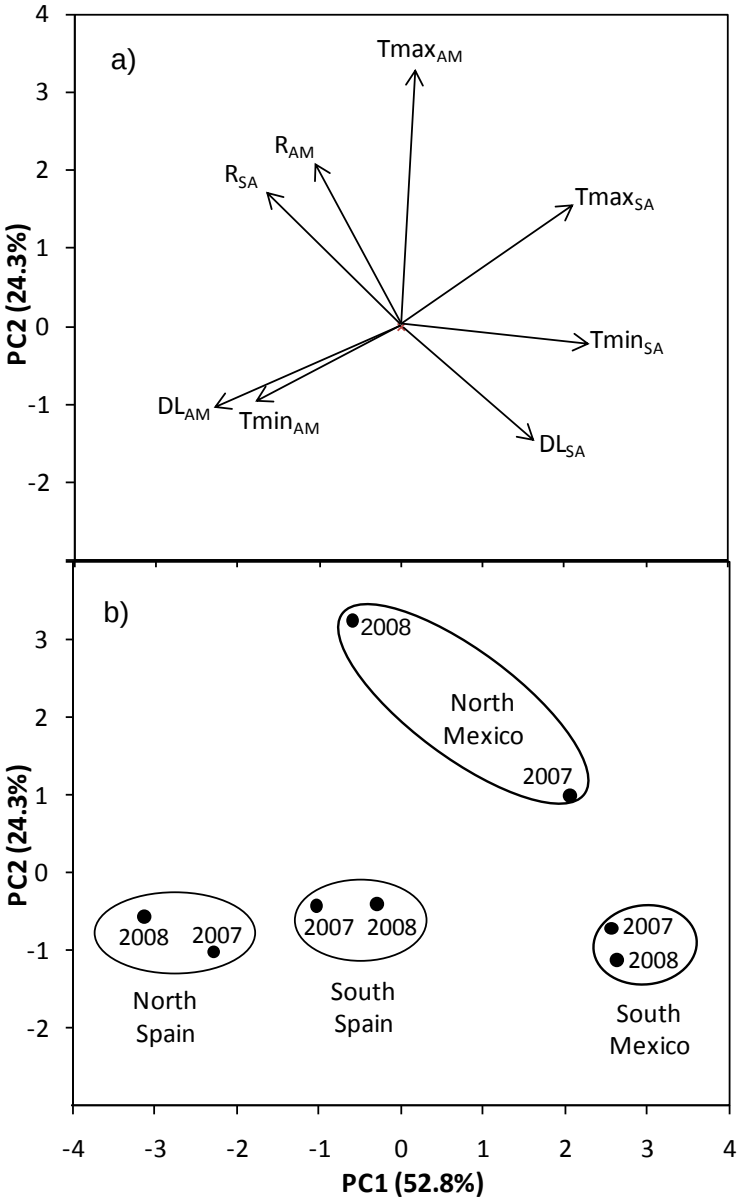

Figure I.2. Plot of the first two axes of the Principal Component Analysis for the following environmental variables from sowing to anthesis (subscript SA) and from anthesis to maturity (subscript AM): Tmin, mean of daily minimum temperatures; Tmax, mean of daily maximum temperatures; DL, mean daylength, including twilight; R, accumulated solar radiation; DL, mean daylength, including twilight; R, accumulated solar radiation. a) Eigenvectors of the variables considered. b) Eigenvalues for the experiments conducted at each site and year.

The points corresponding to each experiment are plotted in Fig. 2b. The relatively lower distance between points representing years within sites compared to the distances between sites indicates a generally weaker effect of years compared to that of sites. The first axis was related with site, going from the highest to the lowest latitude moving from negative to positive values along the axis. The site in the bottom left part of Fig. 2b of the points corresponding to North Spain indicates that this site is characterized by large daylength and high minimum temperatures from anthesis to maturity, in agreement with the daylength data shown in Fig. 1. The points corresponding to the South Spain site were located in between the ones of North Spain and South Mexico. These last were located in the right part of Fig. 2b, indicating long days and high temperatures from sowing to anthesis in this location, in agreement with the spring planting at this site. The points corresponding to the North of Mexico were located in the upper part of Fig. 2b mostly due to the high maximum temperatures recorded at this site.

The combined ANOVA across experiments showed that site was the most important variation factor influencing temperature and daylength (Table 3). The only exception to this trend was minimum temperatures in the anthesis-maturity period, which depended on site and year × site interaction to the same extent. This pattern was also shown by accumulated radiation, with radiation in the sowing-anthesis period. The year factor explained a notable fraction of variability for radiation in the anthesis-maturity period, while the interactions year × genotype and year × site × genotype explained less than 5% of observed variation.

The average minimum and maximum temperatures from sowing to anthesis increased when moving from northern to southern sites, while from anthesis to physiological maturity daylength decreased in the same direction (Table 4). Mexico-south had long days from sowing to anthesis, consistent with the spring planting at this site.

Table I.3. Percentage of the sum of squares of the combined ANOVA for environmental and agronomic variables determined at maturity, for 42 durum wheat inbred-lines grown in 2007 and 2008 at 4 sites. Tmin, mean of daily minimum temperatures; Tmax, mean of daily maximum temperatures; R, accumulated solar radiation DL, mean daylength, including twilight; GDD: growing-degree days; HI, harvest index; TKW, thousand kernel weight. Subscripts: SA, period from sowing to anthesis; AM, period from anthesis to physiological maturity.

Source of variation	d.f.	$Tmin_{SA}$	$Tmin_{AM}$	$Tmax_{SA}$	$Tmax_{AM}$	R_{SA}	R_{AM}	DL_{SA}	DL_{AM}	$Days_{SA}$	GDD_{SA}	$Days_{AM}$	GDD_{AM}	Biomass	HI	Kernels m^{-2}	TKW	Yield
Year	1	0.005 *	5.54 ***	0.09 ***	0.02 *	6.07 ***	17.3 ***	0.00 ns	0.28 ***	0.01 ***	2.93 ***	21.0 ***	24.7 ***	32.4 ***	1.34 **	0.10 ns	3.31 ***	1.38 *
Site	3	98.2 ***	35.8 ***	98.3 ***	85.0 ***	26.9 ***	27.4 ***	97.6 ***	92.4 ***	95.5 ***	42.2 ***	16.8 ***	6.15 ***	3.8 **	20.6 ***	32.6 ***	15.7 ***	41.3 ***
Genotype	41	0.16 ***	11.7 ***	0.17 ***	0.75 ***	32.3 ***	7.73 ***	0.35 ***	0.88 ***	2.83 ***	37.2 ***	11.6 ***	8.78 ***	6.4 *	29.1 ***	21.8 ***	54.8 ***	9.79 ***
Year×Site	3	1.42 ***	30.5 ***	1.27 ***	12.7 ***	20.1 ***	31.1 ***	1.68 ***	5.10 ***	0.30 ***	1.27 ***	30.1 ***	40.7 ***	20.3 ***	1.36 ns	16.1 ***	4.94 ***	12.3 ***
Year×Genotype	41	0.01 ns	0.71 ns	0.00 ns	0.11 ns	0.48 ns	2.02 ns	0.01 ns	0.02 ns	0.03 ns	0.47 ns	1.86 ns	1.95 ns	2.62 ns	1.53 ns	1.11 ns	1.02 ns	1.04 ns
Site×Genotype	123	0.10 ns	9.65 ***	0.09 ***	0.68 ns	11.0 ***	5.46 ns	0.28 ***	1.06 ***	0.90 ***	12.5 ***	7.75 ***	6.08 ***	8.61	16.1 ***	5.79 ***	6.76 ***	9.28 ***
Year×Site×Genotype	123	0.08 ***	4.06 ***	0.02 ***	0.61 ***	1.73 ***	4.58 ***	0.02 ***	0.13 ***	0.15 ***	1.60 ***	5.01 ***	5.00 ***	6.9 ***	7.29 **	4.62 ***	3.52 ***	6.33 ***
Block (Year×Site)	16	0.001 ***	0.23 ***	0.00 ns	0.04 ***	0.05 ns	0.49 ***	0.001 **	0.01 ***	0.005 *	0.06 ns	0.51 ***	0.68 ***	2.99 ***	1.83 ***	3.02 ***	0.42 *	4.75 ***
Residual	656	0.01	1.76	0.01	0.19	1.41	3.91	0.02	0.16	0.32	1.74	5.3	5.92	16.0	20.8	14.8	9.58	13.8
Total	1007																	

*P<0.05; **P<0.01; ***P<0.001

Table I.4. Mean values for each site of environmental and agronomic variables determined at maturity, for 42 durum wheat inbred-lines grown in 2007 and 2008 at 4 sites. Tmin, mean of daily minimum temperatures; Tmax, mean of daily maximum temperatures; R, accumulated solar radiation DL, mean daylength, including twilight; GDD: growing-degree days; HI, harvest index; TKW, thousand kernel weight. Subscripts: SA, period from sowing to anthesis; AM, period from anthesis to physiological maturity.

Site	$Tmin_{SA}$ (° C)	$Tmax_{SA}$ (° C)	$Tmin_{AM}$ (° C)	$Tmax_{AM}$ (° C)	DL_{SA} (h)	DL_{AM} (h)	$Days_{SA}$	$Days_{AM}$	GDD_{SA}	GDD_{AM}	Biomass (g m^{-2})	HI	Kernels m^{-2}	TKW (g)	Yield (g m^{-2})
North Spain	3.3^{d}	14.1^{d}	11.8^{a}	24.8^{b}	11.9^{b}	15.8^{a}	164^{a}	40.9^{c}	1457^{c}	744^{d}	1490^{a}	0.49^{a}	17879^{a}	38.8^{c}	683^{a}
South Spain	6.0^{c}	18.3^{c}	10.9^{b}	23.6^{c}	11.8^{d}	14.6^{b}	124^{b}	48.0^{a}	1499^{b}	825^{b}	1438^{b}	0.45^{c}	14395^{b}	41.1^{b}	587^{b}
North Mexico	7.0^{b}	24.6^{a}	10.2^{d}	30.4^{a}	11.9^{c}	13.4^{c}	99^{c}	40.6^{c}	1573^{a}	832^{a}	1447^{b}	0.46^{b}	13244^{c}	45.4^{a}	586^{b}
South Mexico	10.2^{a}	24.3^{b}	10.5^{c}	23.5^{d}	14.2^{a}	13.3^{d}	75^{d}	45.7^{b}	1296^{d}	778^{c}	1314^{c}	0.43^{d}	12096^{d}	37.4^{d}	444^{c}

Different letters within columns indicate significant differences according to Duncan's test at $P<0.05$

Agronomic performance

The different seed rate used at each country was successful in generating a comparable number of spikes m^{-2}. The two-years average for South Mexico, North Spain and South Spain were 517, 508 and 426 spikes/m^2, respectively. In North Mexico, the spikes were counted only in 2008, with an average of 415 spikes/m^2 (data not shown).

The combined ANOVA across experiments for agronomic traits showed that the site effect explained 96% of the variability for the number of days from sowing to anthesis (SA,Table 3). The variability induced by site was also the most important for explaining variations in thermal time from sowing to anthesis, kernels m^{-2} and ultimately yield. Variation in the duration of the anthesis-maturity (AM) period, biomass, HI and TKW was explained to a greater extent by variance components other than site, although site did significantly affect all these traits to some extent. The year effect was substantial in explaining variation in the duration of the anthesis-maturity period and biomass, although it was significant for all variables except the number of kernels m^{-2}. When second to the site effect, the magnitude of genotype effect was relatively substantial for kernel weight, thermal time from sowing to anthesis, harvest index, kernels m^{-2} and to some extent for yield (Table 3).

Site means across years indicated a decreasing duration of the period from sowing to anthesis measured in days, and kernels m^{-2} with decreasing the site latitude, independently from sowing time (South Mexico did not reverse the trend, Table 4 and Fig. 1). A similar tendency was observed for yield, except that the differences between South Spain and North Mexico sites were not statistically significant. Thermal time of both developmental periods (SA and AM) as well as TKW increased when the site latitude decreased in the fall-sown trials. However, the trend was reversed (decreasing) in the spring-sown South Mexico site (Table 4). Biomass increased with increasing site latitude, with the difference between South Spain and North Mexico not being statistically significant. The lowest values for all variables except for the grain filling duration (anthesis to maturity) were recorded in South Mexico.

Pearson correlation coefficients between yield and yield components are shown in Table 5. Biomass and HI were in all cases positively and significantly correlated with yield. Correlation coefficients with kernels m^{-2} were significant at both Mexican sites, but not in Spain. The relationship between TKW and yield was highly significant in Spain, decreasing in magnitude from north to south. In Mexico, this relationship was significant only in the spring-planted site of Mexico-south.

Table I.5. Pearson correlation coefficients between yield and its components for experiments conducted at 4 sites in 2007 and 2008 involving 42 durum wheat inbred lines (n=42). HI, harvest index; TKW: thousand kernel weight.

Site	Biomass	HI	Kernels m^{-2}	TKW
North Spain	0.74 ***	0.50 ***	-0.27	0.74 ***
South Spain	0.34 *	0.53 ***	0.15	0.36 *
North Mexico	0.32 *	0.50 ***	0.40 **	0.20
South Mexico	0.61 ***	0.59 ***	0.36 *	0.53 ***

*P<0.05; **P<0.01; ***P<0.001

Linear regression models were fitted to the relationships between phenological variables and yield components, and the statistically significant relationships (P<0.05) are shown in Figure 3. The most significant positive associations were found between the number of days from sowing to anthesis and both, the number of kernels m^{-2} (Fig. 3a) and HI (Fig. 3b). Whereas the number of days from sowing to anthesis increased with increasing latitude, the length of this period measured in thermal time did not follow the same pattern (Table 4), but was nevertheless positively related with TKW (Fig.3c). Large differences in sowing dates between years within sites (from 5 to 18 days, Table 2) resulted in small differences in the number of days from sowing to anthesis (from 1 to 5 days, Fig. 3).

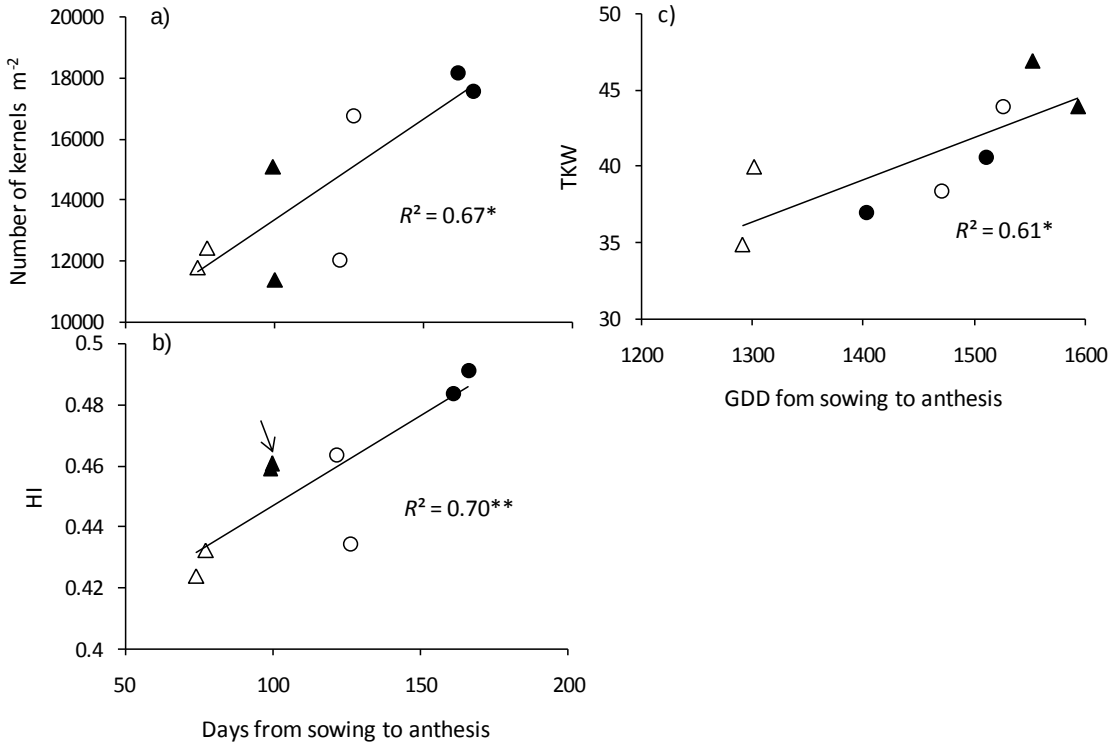

Figure I.3. Statistically significant (* $P<0.05$, ** $P<0.01$) linear regression models fitted to the relationships between phenological and agronomic variables. Each point represents the mean value for one of eight experiments conducted in 2007 and 2008 at 4 sites and involving 42 durum wheat inbred lines. (●) North Spain, (○) South Spain, (▲) North Mexico, and (△) South Mexico. The arrow in Fig. 2b indicates the overlapping of two points.

Relationships between environmental variables and agronomic traits

Regression models fitted to the relationships between environmental variables and agronomic traits revealed that the number of days from sowing to anthesis was strongly and negatively related with the temperature during the same period, particularly the mean of daily minimum temperature (Table 6). However, the duration of the same period expressed in thermal time was mainly explained by daylength −which accounted for 74% of variation− and average daily maximum temperature. Both of these variables had a decreasing effect of the duration of sowing to anthesis. Accumulated radiation and average minimum temperature, on the other hand, had positive moderate increasing effects on the sowing to anthesis duration. No environmental variable could explain variation in the number of days from anthesis to maturity, with the exception of accumulated radiation and mean daylength - jointly explained 96% of the variation of this period -, but only when expressed in thermal time (Table 6). HI and yield were negatively associated with minimum temperatures in the sowing-anthesis period, while mean daylength from anthesis to maturity positively affected the number of kernels m^{-2} (Table 6). None of the environmental variables studied could explain variations in biomass or kernel weight.

Table I.6. Coefficients of the forward regression models fitted to the relationships between agronomic traits as dependent variables, and environmental data as independent variables. Fitted data were means across 42 genotypes and 3 blocks for experiments conducted in 4 sites during 2007 and 2008 (n=8). GDD, growing-degree days; HI, harvest index; TKW, thousand kernel weight; Tmin, mean of daily minimum temperatures; Tmax, mean of daily maximum temperatures; DL, mean daylength, including twilight, R, accumulated solar radiation. Subscripts: SA, period from sowing to anthesis; AM, period from anthesis to physiological maturity.

Dependent variable	Step	Independent variable	Regression coefficient	Partial R^2	Model R^2	
$Days_{SA}$	1	$Tmin_{SA}$	-8.1	0.95	0.95	***
	2	$Tmax_{SA}$	-3	0.04	0.99	**
GDD_{SA}	1	DL_{SA}	-167	0.74	0.74	**
	2	$Tmax_{SA}$	-7.3	0.16	0.9	*
	3	R_{SA}	0.5	0.07	0.97	*
	4	$Tmin_{SA}$	61	0.03	1	**
$Days_{AM}$	-	-	-	-	-	
GDD_{AM}	1	R_{AM}	0.77	0.78	0.78	**
	2	DL_{SA}	58	0.18	0.96	**
Biomass	-	-	-	-	-	
HI	1	$Tmin_{SA}$	-0.01	0.71	0.71	**
Kernels m^{-2}	1	DL_{AM}	2200	0.77	0.77	**
TKW	-	-	-	-	-	
Yield	1	$Tmin_{SA}$	-36	0.81	0.81	**

*P<0.05; **P<0.01; ***P<0.001

The influence of the environmental variables on the relationship between yield and the number of kernels per unit area was examined through linear regression models. Long days and high daily minimum temperatures after anthesis were associated with high kernel number (Fig. 4a, b). For sites with a mean daylength shorter than 14.2 h and average daily minimum temperatures lower than 10.8 ºC, the relationship between yield and kernels m^{-2} became statistically significant (P<0.05) (Fig. 4d,e). On the other hand, low minimum temperatures averaged over the pre-anthesis period were related with high number of kernels per unit area (Fig. 4c) and low correlation coefficients between this yield component and yield (Fig. 4f). Yield became significantly associated with kernels m^{-2} for average daily minimum temperatures before anthesis above 6.9°C (Figure 4b). The boundary value for the number of kernels per unit area from which these relationships became statistically significant, calculated from the equations shown in Fig. 4, was 14,118 kernels m^{-2}.

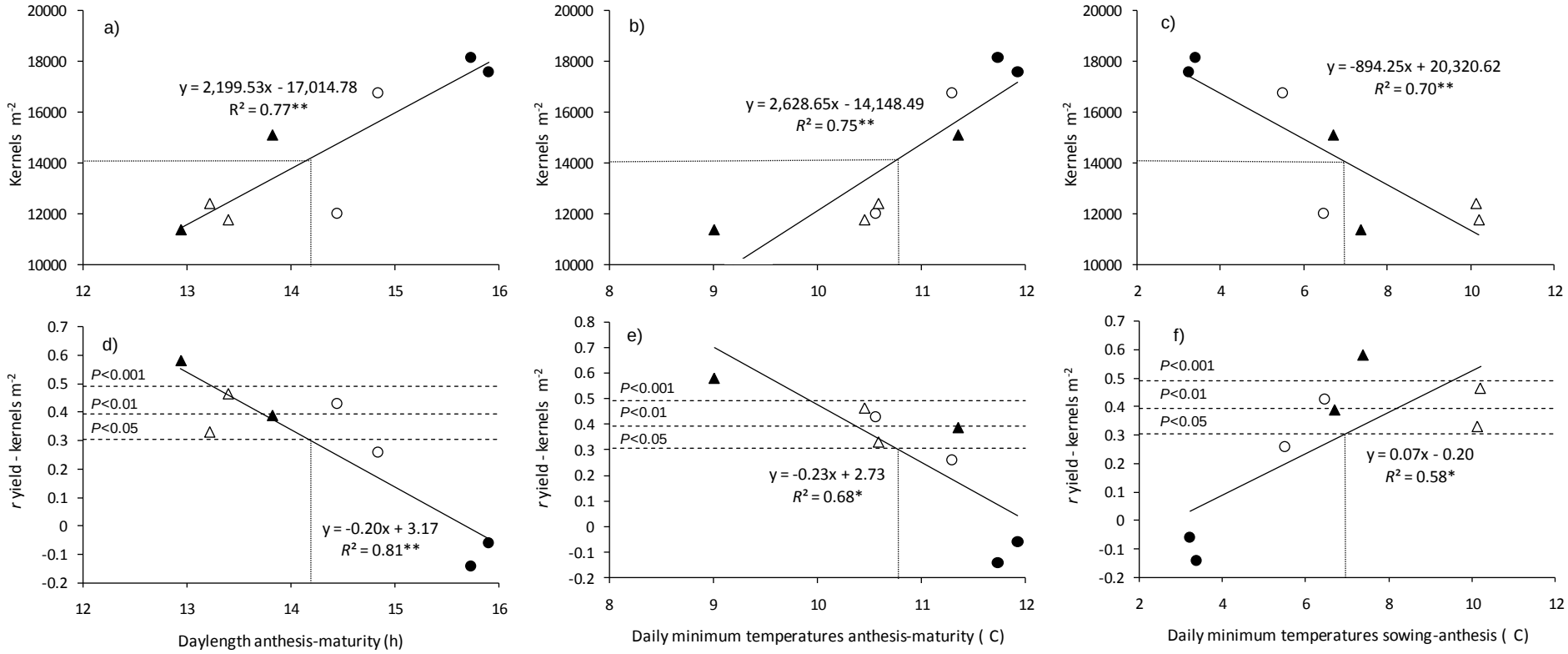

Figure I.4. Statistically significant (* $P<0.05$, ** $P<0.01$) linear regression models fitted to the relationships between environmental variables and the number of kernels m^{-2} (Fig. 4a, 4b and 4c) or the Pearson correlation coefficient (r) between yield and the number of kernels m^{-2} (Fig. 4d, 4e and 4f). Each point represents the mean value for one of eight experiments conducted during 2007 and 2008 in 4 sites and involving 42 durum wheat inbred lines. (●) North Spain, (○) South Spain, (▲) North Mexico, and (△) South Mexico. Solid lines represent the fitted linear regression equations; dotted lines indicate the abscise values from which regression equations become significant in Fig.4d, 4e and 4f, and the corresponding number of kernels m^{-2} in Fig. 4a,4b and 4c. Levels of significance are shown by discontinuous lines in Fig. 4d, 4e and 4f.

None environmental variable was significantly related with kernel weight (Table 6), but a positive and significant relationship was found between thousand kernel weight and the amount of incoming radiation per kernel in the anthesis-maturity period (Fig. 5a). In experiments with radiation values lower than 1.8 kJ per kernel and day, the relationships between kernel weight and yield were statistically significant (P<0.05), but they were not for radiation values greater than this threshold value (Fig. 5b).

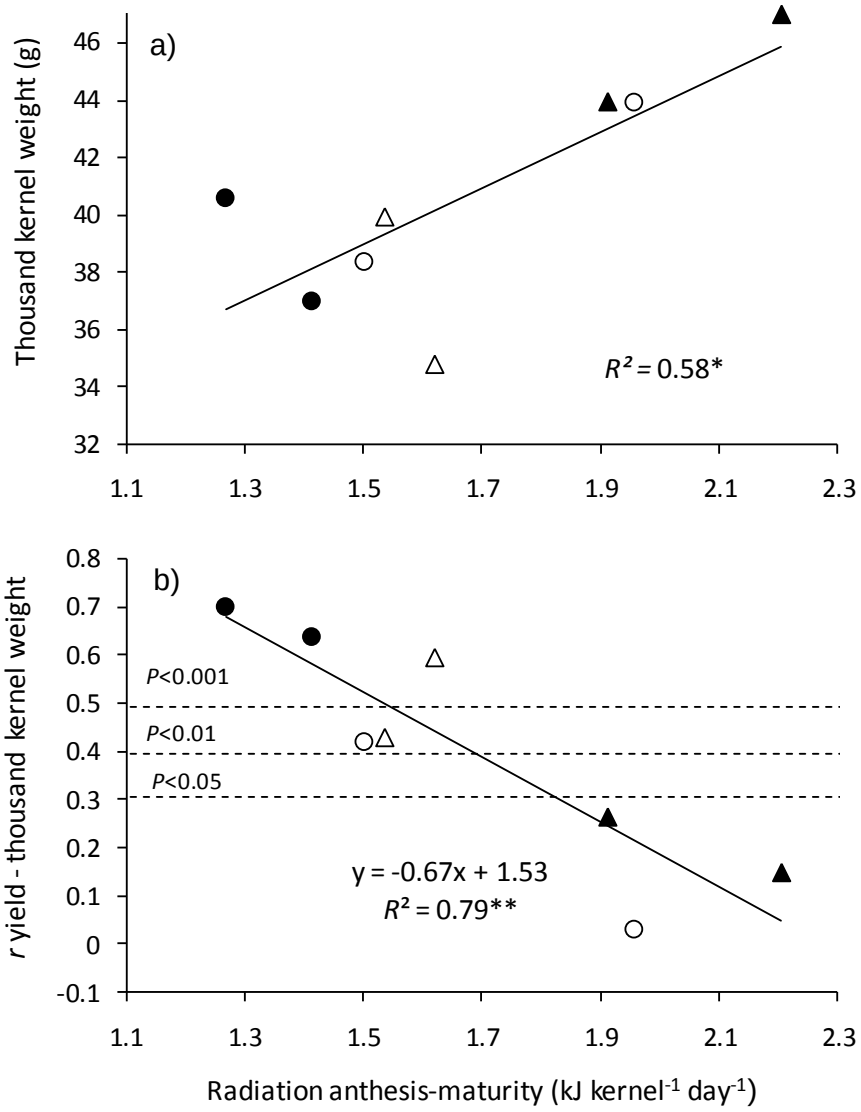

Figure I.5. Statistically significant (* P<0.05, ** P<0.01) linear regression models fitted to the relationships between the mean daily radiation per kernel from anthesis to physiological maturity and, a) thousand kernel weight, and b) Pearson correlation coefficient (r) between yield and kernel weight. Each point represents the mean value for one of eight experiments conducted during 2007 and 2008 in 4 sites and involving 42 durum wheat inbred lines. (●) North Spain, (○) South Spain, (▲) North Mexico, and (△) South Mexico.

DISCUSSION

The global classification and worldwide distribution of wheat into growth habit classes, or groups characterized by various combinations of vernalization requirements and photoperiod sensitivity, is known to be related to a great extent to environmental variables associated with latitude (Casas et al., 2011; Cockram et al., 2007). Differences between the classes are well defined in terms of major "qualitative" genetic variations (*Vrn* and *Ppd* genes) that determine a genotype's adaptation to different "Mega-environments". However, the environmental variation, more "quantitative" in its nature, that exist within growth habit classes, is less well characterized, particularly in the case of fall-sown, spring habit durum wheat.

The present study is an attempt to provide such missing information by looking at important environmental variables, some of them strongly linked to latitude, and their general effects on developmental phases (pre- and post-anthesis) duration, yield and main yield components of wheat. Genotypes used were adapted to latitudes roughly up to 40 degrees, which represent the majority of the durum wheat grown worldwide. This excludes the types grown at high latitudes, above 45 degrees (Canada, North-Dakota, Kazakhstan and Russia) where photoperiod sensitivity is either required or highly beneficial, and the winter or facultative types sown in northern and central Europe and parts of central Asia, where a combination of significant vernalization needs and photoperiod sensitivity are required. The present study is relevant to wheat genotypes characterized by weak or no vernalization requirements and reduced to no photoperiod sensitivity. As represented in 3 of the 4 testing sites used in our experiments, these genotypes are mostly fall-sown. The collection evaluated was purposely selected to include genotypes with the widest possible range of flowering dates, including the earliest types (exemplified by the check Mexa) and very late types (similar to the check Anton) that may have adaptation only to the highest latitudes (northern Spain) within the range of latitudes under study.

The main environmental variables differentiating between sites were temperature and daylength, as stated by ANOVA (Table 3) and PCA (Fig. 2). However, differences between sites were much better explained by differences in average daily temperatures from sowing to anthesis (that increased from the north to the south) than from anthesis to maturity. The dependence of daylength on sites was expected given the wide range of site latitudes and the existing relationship between daylength and latitude (Forsythe et al. 1995). On the other hand, solar radiation showed an important year-to-year difference within each site, in accordance with the variations in solar activity (Fröhlich and Lean, 2004) and the yearly variations in cloudiness (Stanhill and Cohen, 2001). Despite the fact that some models use minimum and maximum temperatures to estimate solar radiation, Trnka et al. (2005) showed that the deviation of such estimations may be substantial, that is, radiation and temperature do not follow the same pattern, as observed in this study.

The ANOVA of phenological traits revealed that the latitude effect explained 96% of variations in the number of days from sowing to anthesis, suggesting a cause-effect between site and pre-anthesis duration, in calendar terms (Table 3). Low temperatures from sowing to anthesis, particularly the average daily minimum temperatures during this period, characteristic of northern latitudes, increased the number of days needed for flowering. It is well known that development has a universal response to temperature, and so the relationship between temperature and development is a common part of the phenology prediction models (White, 2003). Our results support previous studies demonstrating that high average temperatures (minimum or maximum) reduce the duration of the pre-anthesis phase (McMaster, 2008; Siebert and Ewert, 2012; White, 2003), and go further in linking this result primarily to site, with year-to-year variation being much less significant.

However, in this study no relationship was found between the number of days pre-anthesis and thermal time during the same period (Table 4), probably because thermal time from sowing to anthesis was almost equally affected by genotype as well as by site, while days from sowing to anthesis was hardly affected by genotype (Table 3). Our results showed that the effect of site on the thermal time from sowing to anthesis was more associated to daylength than to temperature (Table 6). The long daylength during the sowing-anthesis period due to spring planting in the south of Mexico (14.2 h in average) explains the short duration of this phase at this site, for wheat types that do not require any vernalization like the ones used in this study. The effect of daylength on development has been previously demonstrated (Giunta et al., 2001; Wang et al., 2009) and delayed development and late dates to anthesis have been associated to short daylength during the sowing-anthesis phase (Kirby et al., 1999). The long photoperiod recorded in the south of Mexico caused an acceleration of development which resulted in low biomass, lower kernel number per unit area, and lower HI and overall yield (Table 4).

The duration of the anthesis-maturity period was environmentally controlled by the interaction year by site and to a lesser extent by the two main factors of this interaction (Table 3). Nevertheless, none of the environmental variables studied could be associated with the length of the anthesis-maturity period measured in days (Table 6). However, as expected, thermal time from anthesis to maturity was positively and significantly associated with the accumulated radiation in the same period (R^2 = 0.78, P<0.01, Table 6), which depended much more on the year than on the latitude, as shown in Table 2.

The effect of environmental variables associated with latitude on yield formation was elucidated by PCA and regression analysis. We dissected yield formation through two alternative approaches extensively used in simulation models. Both consider yield as the product of: i) biomass and HI, and ii) number of kernels per unit area and TKW. Biomass accumulation and harvest index play a central role in yield calculation in some

models such as Sirius (Jamieson et al., 1998) or CROPSYST (Stöckle et al., 2003), among others. Some simulation models include kernels m^{-2} in their calculations, such as CERES-Wheat (Pecetti and Hollington, 1997)–, DSSAT (Jones et al., 2003) or STICS (Brisson et al., 1998). Our results showed that the components of the second approach could be better explained in terms of environmental variables than the first ones.

In the present study, variation in aboveground biomass at maturity was mostly explained by year and the year x site interaction, with site alone explaining less than 4% of its variation even when it was statistically significant (Table 3). Biomass was positively correlated with yield in all sites, but it was not associated with any of the environmental variables studied (Table 6). These results suggest that biomass did not follow a pattern directly associated with the site latitude, but was strongly affected by year-to-year environmental variations at a same site. The quantitative nature of biomass production (Quarrie et al., 2006) makes it strongly influenced by other factors affecting growing conditions, including crop management practices such as water supply and nitrogen fertilization (Villegas et al., 2001).

The site effect accounted for 15% of the variance in HI (Table 3), but the pattern of variation of HI according to site was not straightforward (Table 4). HI was negatively related to both average minimum temperatures in pre-anthesis (Table 6) and the duration of the pre-anthesis period measured in days (Fig. 3). These results indicate that the influence of environmental variables on HI mostly resulted from the effect of minimum temperatures on the duration of the pre-anthesis phase. Since biomass was not associated with environmental variables, the impact of minimum temperature on the pre-anthesis phase is likely to affect HI through an effect of the number of kernels m^{-2} by increasing the opportunity for floret formation.

Environment strongly influenced the number of kernels m^{-2}, due to the effect of daylength in the anthesis-maturity period and minimum temperatures before and after anthesis (Table 6 and Fig. 4). The decreasing daylength and average minimum daily temperatures after anthesis when moving from northern to southern latitudes, as well as increases in the same temperatures before anthesis, resulted in a reduction in the number of kernels per unit area. It has been demonstrated that final kernel number is affected by environmental conditions both before and after anthesis (Abbate et al., 1995; Fischer, 2011) and that high temperatures in pre-anthesis cause a reduction in the number of kernels per unit area (Ugarte et al., 2007). Our results suggest that average minimum temperatures lower than 6.9ºC pre-anthesis contributed to develop a high potential kernel number, while average minimum temperatures above 10.8 ºC and daylength superior to 14.2 h post-anthesis also played a positive role in grain setting. These boundary values in environmental variables corresponded to about 14,000 kernels m^{-2} that was the critical value below which the number of kernels per unit area became a limiting factor for yield, as revealed by the

significant correlations found between the number of kernels m^{-2} and yield for values below this level (Fig. 4).

Thousand kernel weight (TKW) was the agronomic trait that showed the greatest genetic control since genotype effects accounted for 55% of its sum of squares, with latitude effect nevertheless explaining 16% of the same statistical parameter (Table 3). In the present study none significant relationship appeared between TKW and the environmental variables studied, a result that disagrees with the conclusions of Ugarte et al. (2007) which indicated that kernel weight responds to temperature variations in pre-anthesis. Nevertheless, our results revealed that, for fall-sown experiments, TKW increased when moving southward (Table 4), but its relationship with yield decreased in the same direction (Table 5), and both effects were associated with the radiation per kernel and day during the period anthesis-maturity (Fig. 5). These results suggest that the low radiation per kernel in the northern latitudes constrained TKW, thereby making it a limiting factor for yield formation in those environments. The location in Fig. 5b of the points corresponding to the spring planting in southern Mexico confirmed that the relationship between kernel weight and yield depended on the available radiation per kernel during the anthesis-maturity period, this relationship being associated with the site latitude for similar planting dates. This study revealed that the minimum radiation needed by a kernel to fill properly and avoid kernel weight becoming yield-limiting was ca. 1.8 kJ day^{-1} (Fig. 5). It may be hypothesized that low kernel weights were the result of the number of kernels m^{-2} being too high to be filled with the available incoming radiation. Similar results were found in barley by Bingham et al. (2007) in a study conducted at high latitudes (52-57°N), but in the latter case the positive relationship between radiation per single kernel and kernel weight was limited to a short period around anthesis and not to the whole grain filling period as in the present study. Takahashi and Kanazawa (1996), in a study conducted also at high latitude (45°N), found that a reduction of radiation at the end of grain filling caused lower kernel weights due to smaller starch granules. On the other hand, recent studies conducted at lower latitudes (around 32°N) have reported that great reductions of radiation during grain filling caused only small reductions in yield (Li et al., 2010; Mu et al., 2010) and kernel weight (Li et al., 2010). All these results suggest that the limitation that radiation exerts on yield formation increases at sites located at higher latitudes.

The site effect was the most important in explaining yield variations (Table 3) through the effect of minimum temperatures in pre-anthesis. The negative effects of high temperatures before heading on durum wheat yield (Royo et al., 2010), and the implication of minimum temperatures in pre-anthesis on the genotype x environment interaction for wheat yield (Sanchez-Garcia et al., 2012) have been recently reported. Yield reductions are mostly related to lower number of kernels m^{-2} under high pre-anthesis temperatures, mainly associated with an acceleration of the spike growth (Fischer, 1985; 2011). This acceleration results in a reduction in the potential number of florets (González et al., 2011) and consequently of kernels m^{-2}, which is not fully compensated later by kernel weight (Foulkes et al., 2011; Peltonen-Sainio et al., 2007). In fact, it has been postulated the lengthening of the stem elongation phase as a strategy to increase yields in the future (González et al., 2011; Reynolds et al., 2009) as

has happened in the past (Isidro et al., 2011). Higher temperatures during grain filling have been also associated with lower yields (Asseng et al., 2011; Majoul-Haddad et al., 2013), which was not the case in the present study, probably because the anthesis-maturity period in all experiments occurred at ranges of temperatures that were not considered to result in significant heat-stress induced yield reduction. However, at higher temperatures predicted by some climate change models (Gouache et al., 2012), this variable can become yield limiting and the interaction of environmental variables during the grain-filling period may become more critical for yield formation.

CONCLUSIONS

The results of this research support and give scientific explanations, based on environmental factors, to the findings of previous studies concluding that grain yield of durum wheat is mostly determined by kernel weight in the cooler conditions of northern environments, while the number of spikes per unit area predominantly influences grain production in the warmer southern environments (García del Moral et al., 2003). An additional evidence supporting this statement came from the study of Moragues et al. (2006), who reported that grain yield of durum wheat Mediterranean landraces was mainly related to variations in kernel size among those from the northern shore, while spikes per unit area was the most important determinant of yield among those from the southern part of the same basin. Our results indicate that low minimum temperatures during the pre-anthesis period, typical of autumn planting in northern environments, favored the formation of a large kernel number, but the insufficient radiation per grain during grain filling in these environments limited the realization of genetic potential in terms of kernel weights to maximize yield potential. Accordingly, kernel weight became a limiting factor for yield, as shown by the significant correlation coefficients between yield and kernel weight in northern environments. In contrast, the high minimum temperatures in pre-anthesis registered in southern latitudes resulted in a reduced number of kernels per unit area that, below certain value, became a limiting factor for yield. However, the high level of solar radiation per grain during the grain filling period favored the formation of heavy grains that contributed to the achievement of high yields.

In quantitative terms, our empirical results suggest that, for the range of environments dealt with in this study, the number of kernels became a yield limiting factor for values lower than ca. 14,000 kernels m^{-2} which occurred in environments with average daily minimum temperatures greater than 6.9°C from sowing to anthesis and above to 10.8°C from anthesis to maturity, with average daylength during this period of more than 14.2 h. Moreover, kernel weight was a limiting factor for yield when radiation values from anthesis to maturity were lower than ca. 1.8 kJ day^{-1}. These data may be useful for growth models trying to estimate the effect of global change on the productivity of spring growth-habit durum wheat.

From an adaptive breeding standpoint, the results of the present study suggest that, even within the typical fall-sown, spring habit durum wheat class, different strategies

may be needed to address latitude-related differences in important yield-limiting factors under optimal growing conditions. Whereas improving yield through inheritable increases in kernel size is the most logical approach for the northern latitudes, a focus on kernel per unit area is most likely to result in yield progress at the southern latitudes. It is important to note that these different latitude-related directions in breeding strategies should be viewed as different “emphases”, not in an exclusive manner, as both yield components are inter-related in forming yield and a balance between both has to be ultimately reached for optimal yield formation at any latitude.

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Chapter II

EFFECT OF *PPD-1* GENES ON DURUM WHEAT FLOWERING TIME AND GRAIN FILLING DURATION IN A WIDE RANGE OF LATITUDES

ABSTRACT

Understanding the effect of genetic factors controlling flowering time is essential to fine-tune crop development to each target environment in order to maximize yield and end-use quality. A set of 35 durum wheat (*Triticum turgidum* L. var. *durum*) genotypes of spring growth-habit involving different allelic combinations for *Ppd-A1* and *Ppd-B1* genes was grown during two years at four sites on a range of latitudes from 41°38'N to 19°31'N. The period emergence-flowering was reduced when moving from the north to the south. Genotypes that flowered earlier due to the presence of alleles causing photoperiod insensitivity enlarged their grain filling period, but less than the shortening until flowering. All possible allelic combinations for the alleles previously reported at *Ppd-A1* and *Ppd-B1* were present in the collection, in which the frequency of the insensitive allele GS105 was greater (34%) than that of allele GS-100 (20%). Differences between allelic combinations in the duration from emergence to flowering accounted for ca. 65% of the variability induced by the genotype effect, with the remaining 35% being explained by genes controlling earliness *per se*. Allelic combinations *Ppd-A1b*/*Ppd-B1b* and *Ppd-A1b*/*Ppd-B1a* led to the same cycle length until flowering and maturity in the four sites, either measured in days or GDD. The shortest flowering time across sites corresponded to the allelic combination GS100/*Ppd-B1a*. The effect of the allele conferring photoperiod sensitivity at *Ppd-A1* was stronger than that at *Ppd-B1* (*Ppd-A1b*>*Ppd-B1b*). According to their effect on flowering date, the effect of photoperiod insensitivity alleles was classified as GS-100>GS-105>*Ppd-B1a*. The phenotypic expression of the alleles at *Ppd-B1,* hided when allele *Ppd-A1b* was present, increased when allele GS-105 was at *Ppd-A1,* denoting interaction between genes *Ppd-A1* and *Ppd-B1*. The phenotypic expression of alleles conferring photoperiod insensitivity at *Ppd-A1* increased in sites with average daylength from emergence to flowering lower than 12h. A large effect of earliness *per se* was recorded between the genotypes with the same allelic variants at *Ppd-A1* and *Ppd-B1* loci. The present study marks a further step towards the elucidation of the phenotypic expression of genes regulating photoperiod sensitivity and the environmental effects on them.

Abbreviations

ACP Accumulated photoperiod, Anthesis, *Eps* Earliness per se, GDD growing degree-days, GS Genotype x site interaction,

Keywords: Photoperiod, phenology, earliness *per se*, environment, allelic combinations

INTRODUCTION

Maximizing yield potential in any given environment requires optimizing the use by the plant of resources such as water, nutrients or radiation, and to avoid negative effects from any type of stress during the vegetative and grain filling periods. This can only be achieved by growing varieties with adjusted flowering time and life cycle duration for the targeted environmental conditions. Flowering time is a critical stage of wheat development as it defines the duration of spike formation and therefore the allocation of resources to seed production and marks the beginning of the grain filling period. The trade-off between resource allocation and stress avoidance is also of primary importance, e.g. brief episodes of high temperatures (>32-36°C) coinciding with a critical period of only 1-3 days around anthesis can greatly reduce seed set and yield (Wheeler *et al.,* 2000). Therefore, setting the optimum flowering time for a target environment is essential, not only for enhanced grain yield, but also to permit full expression of end-use quality genetic potential. Manipulation of flowering time has always been a major objective in wheat breeding programs. Understanding its underlying genetic control and the environmental effect on its expression is crucial to fine-tune phenology for a particular set of environmental conditions for optimum and stable performance.
Wheat flowering time is mainly controlled by three groups of loci, two of which interact with environmental factors, namely photoperiod sensitivity genes (*Ppd*) and vernalization requirement genes (*Vrn*) (Distelfeld *et al*., 2009). The third group of loci, controlling 'narrow-sense earliness' or 'earliness *per se*', acts on the developmental rate independently of vernalization and photoperiod (Scarth and Law, 1984).

Vernalization is the acquisition or acceleration of a plant's ability to flower by exposure to cold (Chouard, 1960). According to the vernalization requirements wheat is classified as having winter or spring growth-habit. Winter wheat has a considerable vernalization requirement but spring wheat may be insensitive or partly sensitive to vernalization. Vernalization requirement is mainly controlled by the *Vrn-1* genes. Durum wheat contains a homoelogous copy of *Vrn-1*, designated *Vrn-A1* and *Vrn-B1* located on the long arms of chromosome 5A and 5B, respectively (Yan *et al.,* 2004, Fu *et al.,* 2005). As compared to hexaploid wheat, the major elite durum wheat gene pools show no major vernalization requirements (spring wheat), while functionally variant alleles are present at main loci for the photoperiod-sensitive response (Clarke *et al.,* 1998).

Photoperiod sensitive wheat is stimulated to flower only in long-days and flowering is delayed under short days provided that any requirement for vernalization is met. In spring habit wheat, photoperiod sensitive types cannot be grown as an overwinter crop in tropical or low latitude-areas, since the daylength requirement would not be satisfied in a short enough time-frame to produce a commercially viable crop (Worland and Snape, 2001). Photoperiod insensitive wheat flowers independently of day length and can be grown to maturity in long or short day environments. This is of particular advantage in warmer and dry climates as early flowering varieties are able to fill their grains prior to the onset of high temperatures and drought stress occurring late in the season (Worland and Snape, 2001).

The intensive selection for photoperiod insensitivity in modern wheat was an important factor in the success of the 'Green Revolution' cultivars with the "shuttle breeding" strategy (selecting plants in segregating populations shuttling generations between two highly contrasting environments but both of short cycle duration) resulting in the selection of early types, most of them with little to no photoperiod sensitivity, with adaptation to a broad range of temperate agricultural environments. This permitted the wide spread of the Mexican semi-dwarf wheats to millions of hectares around the world (Borlaug, 1995). First implemented by N. E. Borlaug, this shuttle-breeding approach still represents the cornerstone of the wide-adaptation breeding strategy used by the International Maize and Wheat Improvement Center (CIMMYT) in Mexico. Yield advantages resulting from photoperiod insensitivity have been estimated to over 35% in Southern European environments and to 15% in Central Europe (Wordland, 1996). The effects of breeding on flowering time of durum wheat have been described (Motzo and Giunta, 2007; Giunta *et al.,* 2007; Álvaro *et al.,* 2008). A wide variation in flowering dates exists among durum wheat landraces, which is associated with their area of origin. A recent study demonstrated that the number of days to heading and flowering of Mediterranean landraces steadily increased when moving from the warmest and driest zone of origin to the coldest and wettest one (Royo *et al.,* 2014).

In durum wheat, photoperiod sensitivity is controlled by genes at the *Ppd-A1* and *Ppd-B1* loci (collectively designated as *Ppd-1*), located on chromosomes 2AS and 2BS, respectively (Laurie, 1997). Insensitivity results from mutations in the *Ppd-1* genes on the A or B genomes. By convention, alleles conferring photoperiod insensitivity are assigned by an "*a"* suffix (e.g. *Ppd-A1a,* McIntosh *et al.,* 2003), whilst wild-type alleles are given a "*b"* suffix.

The basis and degrees of photoperiod insensitivity have been insufficiently characterized in durum wheat. Wilhelm *et al.,* (2009) found two large deletions (1027 and 1117bp deletion designated as allele 'GS100' and 'GS105, respectively) in the promotor the *Ppd-A1* gene in durum wheat which remove a common region from the wild-type sequence. The presence of either deletion accelerated flowering, which led to the conclusion that these deletions are the likely causal basis of photoperiod insensitivity in tetraploid wheat (Wilhelm *et al.,* 2009). A quantitative trait locus (QTL) associated with *Ppd-A1a* significantly reducing heading date was detected by Maccaferri *et al.,* (2008) in a recombinant inbred line (RIL) population derived from the cross 'Kofa' ('GS-100' allele) x 'Svevo' ('GS-105' allele), suggesting that these alleles decrease photoperiod sensitivity to different degrees. As both mutations predominate in modern durum wheat, but are absent from wild tetraploid wheat, it has been suggested that the *Ppd-A1* insensitive alleles arose by mutation during domestication (Bentley *et al.,* 2011). The *Ppd-B1* locus has been originally mapped by by Hanocq *et al.,* (2004) and Mohler *et al.,* (2004) in bread wheat and was confirmed by Maccaferri *et al.,* (2008) in durum wheat. Beales *et al.,* (2007) found several polymorphisms within *Ppd-B1* genes of hexaploid wheat, including five SNPs and a retrotransposon insertion. However, none corresponds with photoperiodic response, implying that the critical mutation causing the allelic difference of the *Ppd-B1* gene has not been found. Diaz *et al.,* (2012) investigated the changes in flowering time to be associated with increased copy number and not with specific sequence polymorphism in bread wheat. Nishida *et*

al., (2013) reported a novel mutation in the 5' upstream region of *Ppd-B1* suggesting that an allelic series of photoperiod insensitive mutation exists in hexaploid wheat.

The third component regulating flowering time, earliness *per se,* is characterized by a polygenic inheritance and 20 meta-QTLs associated to it have been identified on chromosomes 1B, 3A, 3B, 4A, 4B, 5B, 6A and 6B (Griffiths *et al.,* 2009; Kamran *et al.,* 2013). Although the effects of earliness *per se* are considered relatively small, it can cause measurable variations in flowering date independently from the effect of major genes such as *Ppd* or *Vrn* (Van Beem *et al.,* 2005).
The objective of this study was to examine the effect of allelic variants at *Ppd-A1* and *Ppd-B1* genes on the length of durum wheat's developmental periods, namely, emergence to flowering and flowering-physiological maturity, across a wide range of low latitudes. The relative effect of putative earliness *per se* is also evaluated.

MATERIALS AND METHODS

Plant Material

Thirty-five spring durum wheat (*Triticum turgidum* L. var. *durum*) genotypes were used in the study. Thirty resulted from a divergent selection process within the offspring of crosses between parents with contrasting flowering time. Five late-flowering genotypes (Durabon, Megadur, 2716-25.94.01, 2805-49.94.02, 2905-13.93.04), from the breeding program of the University of Hohenheim (Germany) were crossed with five early-flowering advanced lines (Sooty_9//Rascon_37, Cado/Boomer_33, Dukem_12/ 2 * Rascon_21, Guanay and Snitan) from the CIMMYT-Mexico program. F_1, F_2, and F_3 populations were advanced in bulk at CIMMYT. From each F_4 population, an early-flowering and a late-flowering plant were selected with the objective of capturing the maximum range for time to flowering. From F_5 to F_7 generations, selected lines were selfed, purified and increased at the Institute for Food and Agricultural Research and Technology (IRTA) in Spain. At F_8 and F_9 generations the seed of fixed lines with contrasting flowering dates was used in field experiments. The collection also included two sister lines derived from the cross CF4-JS40/3/Stot//Altar84/Ald, and 3 well known commercial cultivars with varying flowering dates that were used as checks: Mexa (early-flowering in Mexico and Spain), Simeto (late-flowering in Mexico and medium-late-flowering in Spain) and Anton (late-flowering in both countries).

Molecular characterization

The selected genotypes were analyzed with a set of molecular markers. STS, SSR and SNP markers tracing identified polymorphisms in durum wheat were initially utilized. Subsequently, additional markers for known bread wheat alleles were tested.
The genotypes were first characterized for the *Vrn-1* and *Vrn-3* loci (*Vrn-A1, Vrn-B1* and *Vrn-B3*) to determine the spring or winter growth habit. Dominant spring alleles due to variation in the promoter and intron-1 region of the *Vrn-A1* locus were identified utilizing the gene-specific STS markers described by Yan *et al.,* (2004) and Fu *et al.,* (2005). In addition, we tested for the presence of a SNP in Exon 4 of *Vrn-A1* identified so far only in bread wheat (Diaz *et al.,* 2012). Deletion alleles affecting vernalisation response in intron-1 of the *Vrn-B1 and in Vrn-B3* were detected as described in Fu *et al.,* (2005), Chu *et al.,* (2011), and Yan *et al.,* (2006).

For *Ppd-A1*, two SNP KASP assays were applied to detect the 1,027 bp 'GS-100' type and 1,117 bp 'GS-105' type deletions in durum wheat (Wilhelm *et al.,* 2009). Furthermore, the genotypes were tested for the presence of the bread wheat 1.2kb insertion (cv. Chinese Spring as check) and 306 bp deletion (cv. Cappelle-Desprez as check) at *Ppd-A1*, respectively (Beales *et al.,* 2007). For *Ppd-B1*, linked SSR markers *gwm148* and *gwm257* as described in Hanocq *et al.,* (2004) were first utilized. Gene-specific KASP assays determining the junction between intact *Ppd-B1* copies in bread wheat cv. 'Sonora64' (containing three copies of *Ppd-B1*) and cv. 'Chinese Spring' (carrying four copies of *Ppd-B1*) were tested to assess if similar copy number variation exist in durum wheat (Diaz *et al.,* 2012). Quantitative techniques to identify on-copy and two-copy *Ppd-B1* alleles of bread wheat were not considered. Following Beales *et al.,* (2007), we designated the photoperiod insensitive allele as *Ppd-1a*. The alternative allele, which we have assumed to infer some photoperiod sensitivity, was arbitrarily designated *Ppd-1b*.

PCR assay reaction mixture in single 10 µl reactions used to amplify all primers contained final concentrations of 1x Buffer with Green Dye (Promega Corp., US), 200 µM dNTPs, 1.2 mM $MgCl_2$, 0.25 µM of each primer, 50ng of DNA, 1U of DNA polymerase (Promega) and 50ng of DNA template. The PCR profile was 94°C for 2 min followed by 30 cycles of 94°C for 1 min, 54 to 60°C for 2 min (dependent on the primer), 72°C for 2 min. Products were separated on 1.2% agarose gels in TAE buffer. SNP polymorphisms were scored using LGC Genomics KASP reagents (www.lgcgenomics.com) in reactions containing 2.5ml water, 2.5 ml 2xKASPar Reaction mix, 0.07 ml Assay mix and 50ng of dried DNA with a PCR profile of 94°C for 15 min activation time followed by 20 cycles of 94°C for 10 sec, 57°C for 5 sec, 72°C for 10 sec and followed by 18 cycles of 94°C for 10 sec, 57°C for 20 sec, and 72°C for 40 sec. Fluorescence was read as an end point reading at 25°C.

Experimental field setup

Field experiments were conducted in 2007 and 2008 at two sites in Spain: Lleida in the north (Spain-North 41°38'N), and Jerez de la Frontera in the south (Spain-South 37°0'N), and two locations in Mexico: Ciudad Obregon in the north (Mexico-North 27°21'N) and El Batan (Texcoco) in the Central Mexican Highlands (Mexico-South 19°31'N). The experiments were arranged in randomized complete block designs with three replications, and plots of 12 m². 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. Three experiments were autumn-planted (from Nov 19 to Dec 22) and the fourth, the one established in Mexico-South, was spring planted (from 18 to 28 May) for a summer crop cycle. Irrigation was provided during the whole cycle in the Mexico-North site (full irrigation) and when necessary to avoid water stress (Fig. 1) in the other 3, mostly rainfed, sites (Spain North and South, Mexico-South).

Data recording

The following developmental stages were determined on the central part of each plot according to the Zadoks' scale (Zadoks *et al*., 1974): 10 (emergence), 65 (flowering or anthesis) and 87 (physiological maturity, indicated by the lost of green color in the spikes peduncles). A plot was considered to have reached a given developmental stage when at least 50% of the plants exhibited the stage-specific phenotypic characteristics. Daily maximum and minimum temperatures and rainfall were obtained from weather stations located in the experimental fields or at a distance shorter than 3 km from these. The duration of the periods from emergence to flowering and from flowering to physiological maturity were expressed in calendar days and thermal time (GDD, growing-degree days). The latter was calculated by summing the daily values obtained as GDD = $[(T_{max} + T_{min})/2]-T_{base}$, where T_{max} and T_{min} are daily maximum and minimum air temperature, respectively. A base temperature of 0°C was used (Gallagher, 1979), as when Tmin<0°C it was considered Tmin=0°C (McMaster and Wilhelm, 1997). Photoperiod was calculated with the model proposed by Forsythe *et al*., (1995), as a function of latitude, Julian day, and including the civil twilight (when the center of the sun is six degrees below the horizon). Accumulated photoperiod (ACP, hours) from emergence to flowering and from flowering to maturity was calculated by summing the daily photoperiod during each development period. According to Ortiz Ferrara *et al.,* (1998), to estimate earliness per se for each genotype, the period emergence-flowering (both in days or GDD) in South Mexico was considered, after substracting the main effect of the corresponding allelic combination of *Ppd-1* at this site. The earliest allelic combination was considered as a reference, *i.e.* the flowering time at South Mexico for the genotypes of the earliest allelic combination was considered earliness *per se*.

Statistical analyses

Analyses of variance were carried out using the GLM procedure of the SAS statistical package (SAS Institute Inc., 2009), considering a fixed factors model. The sum of squares of the genotype and its interactions were partitioned into differences between the allelic combinations identified at *Ppd-A1* and *Ppd-B1* loci*,* and differences within each of them. Means were compared according to the Duncan's multiple range test at *P*=0.05. A linear regression model was fitted to the relationship between ACP between emergence and flowering and the number of days of the same period. The relationship between the length of the periods emergence-flowering and flowering-maturity was assessed through the calculation of the Pearson correlation coefficients using the mean data of genotypes across environments (n=34).

RESULTS

Molecular characterization

The molecular characterization revealed that all of the 35 genotypes were spring types carrying the dominant allele *Vrn-A1c* with a deletion in intron-1 of *Vrn-A1* (Yan *et al.*, 2004), and the recessive alleles *vrn-B1* and *vrn-B3* (Fu *et al.,* 2005, Yan *et al.*, 2006).

Three alleles were identified at *Ppd-A1*. Sixteen out of the 35 genotypes carried the allele *Ppd-A1b* conferring photoperiod sensitivity, while the alleles 'GS-105' and 'GS-100' were identified in 12 and 7 genotypes, respectively. For *Ppd-B1*, the wild-type allele conferring photoperiod sensitivity (*Ppd-B1b*) was detected in 14 genotypes, whilst the mutation conferring photoperiod insensitivity (*Ppd-B1a*) was identified in 21 genotypes using the linked SSR markers.

The additional polymorphisms that were identified only in bread wheat (the SNP in Exon 4 of *Vrn-A1*, Indels in *Ppd-A1* and three and four copies number variations in *Ppd-B1*) were not observed in this set of durum wheat. The genotypes were classified in *Ppd-A1* – *Ppd-B1* allelic combinations (Table 1). Given the low frequency of the allelic combination GS-100 *Ppd-A1* and *Ppd-B1b* –identified only in the check variety Mexa– this combination was removed from the statistical analyses. The checks Simeto and Anton had the allelic combination identified as SI in Table 1.

Table II.1. Allelic combinations for *Ppd-A1* and *Ppd-B1* genes present in a collection of 35 durum wheat genotypes produced through a divergent selection process for flowering time, acronyms used and frequencies within the collection.

Ppd-A1		*Ppd-B1*		Allelic combination acronym	Number of lines
Allele*	Photoperiod response	Allele	Photoperiod response		
Ppd-A1b	Sensitive	*Ppd-B1b*	Sensitive	SS	7
Ppd-A1b	Sensitive	*Ppd-B1a*	Insensitive	SI	9
GS-105 *Ppd-A1a*	Insensitive	*Ppd-B1b*	Sensitive	I5S	6
GS-105 *Ppd-A1a*	Insensitive	*Ppd-B1a*	Insensitive	I5I	6
GS-100 *Ppd-A1a***	Insensitive	*Ppd-B1b*	Sensitive	I0S	1
GS-100 *Ppd-A1a*	Insensitive	*Ppd-B1a*	Insensitive	I0I	6

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

** Discarded from statistical analyses due to uniqueness in present collection

Environmental and genetic effects on crop development

The four experimental sites have been previously characterized in terms of their environmental variables (Chapter I) and the growing conditions during the two evaluation years are summarized in Fig. 1. Yearly average temperatures increased when moving from the north to the south. Total water input ranged from 294 to 583 mm, including irrigation, but did not result in any measurable water stress in any of the experiments. The largest variation in photoperiod amplitude during the growth cycle, calculated as the difference between days of maximum and minimum photoperiod, corresponded to Spain-North with 6.05 h. This amplitude decreased sharply with decreasing latitude, to a minimum of 1.15 h in the Mexico-South site. This latter site was the only one with decreasing photoperiod, due to the spring planting, while in the remaining sites the photoperiod increased during the growth cycle (Fig. 1).

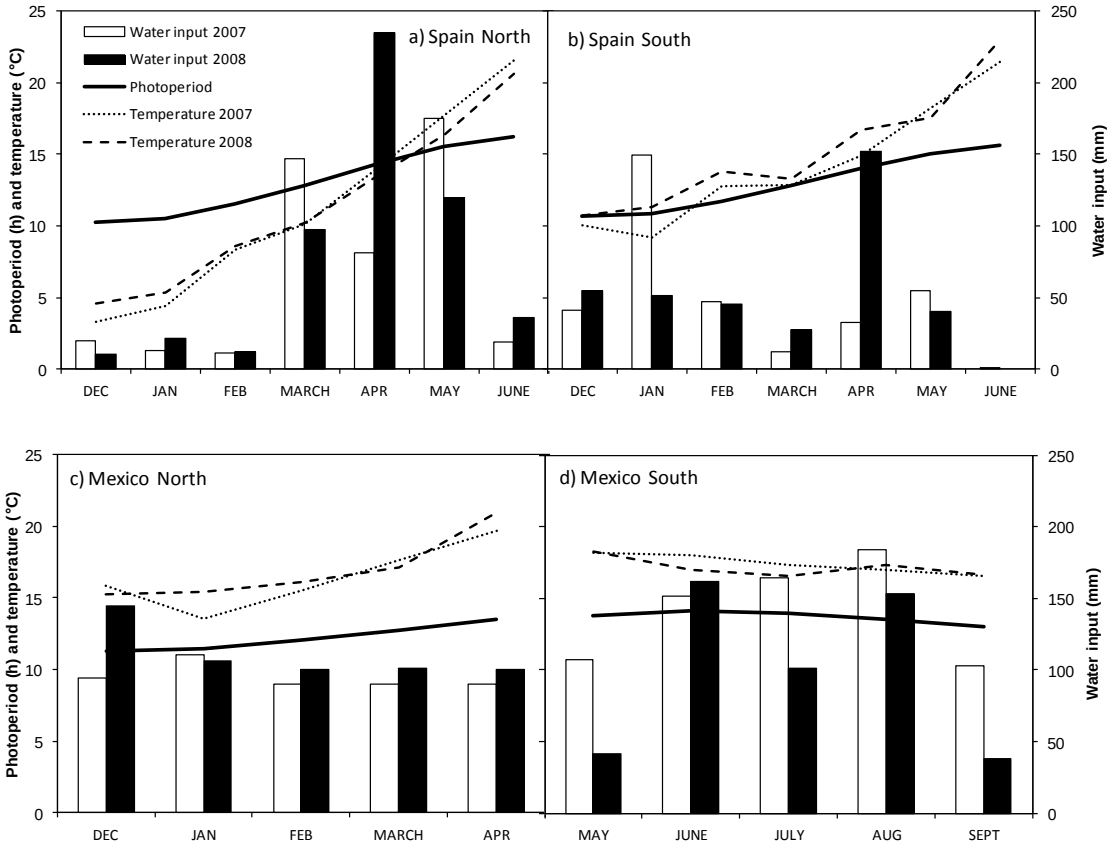

Figure II.1. Environmental conditions prevailing during the experiments conducted in 2007 and 2008 at two contrasting sites in Spain and Mexico.

The results of ANOVA are presented in Table 2. For time to emergence differences between genotypes were not statistically significant (P>0.05) in any experiment (data not shown), indicating a high degree of synchrony in the emergence of all genotypes. The environmental effects (site, year and site x year) accounted for the largest proportion of the variability in crop phenology (Table 2). The site effect was the most important in explaining the length of the periods emergence-flowering and emergence-maturity. However, the duration of the period flowering-maturity (grain filling period) greatly depended also on the year effect and the site x year interaction. The genotype effect had a much greater influence on the length of the periods emergence-flowering and emergence-maturity when expressed in GDD than when it was expressed in days (Table 2). For the duration emergence-flowering, regardless of the measurement unit, differences between allelic combinations accounted for ca. 65% of the variability due to genotype, with the remaining 35% being explained by differences within combinations (deduced from Table 2). The genotype effect accounted for ca. 10% of variation in the flowering-maturity period, either measured in days or GDD. Differences between allelic combinations accounted for ca. 51% and 40% of the variation induced by the genotype effect for the duration of this period in days and GDD, respectively. For the total cycle length, the period of emergence-maturity, allelic combinations accounted for ca. 44% of the genotype effect (deduced from Table 2).

The genotype x site (GS) interaction accounted for the largest portion of the genotype x environment interaction for all the variables measured. For the periods emergence-flowering and emergence-maturity the contribution of the GS interaction to explain the variance of the model was greater for GDD than for the number of days (Table 2). The site x between allelic combinations interaction was significant for all the variables and accounted from ca. 21% to 51% of the variance explained by the GS interaction, and from 0.19% to 4.32% of the total variance of the model. The interactions between site and the variability within allelic combinations were significant in all cases and explained between 0.08% and 3.27% of the total variance of the models (Table 2).

Table II.2. Percentage of the sum of squares (% SS) of the ANOVA for the number of days and thermal time (GDD, growing degree-days) for developmental periods of 34 durum wheat genotypes grown at two contrasting sites in Spain and Mexico in 2007 and 2008. The genotype effect and the environment × genotype interaction are partitioned into differences between allelic combinations and differences within each of them (see Table 1 for allelic combination acronyms).

Source of variation	df	Emergence-flowering		Flowering-maturity		Emergence-maturity	
		Days	GDD	Days	GDD	Days	GDD
		%SS	% SS	% SS	% SS	%SS	% SS
Site	3	86.77 ***	55.76 ***	16.55 ***	5.89 ***	91.30 ***	51.90 ***
Year	1	1.96 ***	0.00 ns	21.16 ***	23.82 ***	0.00 ns	9.91 ***
Site x Year	3	4.89 ***	0.71 ***	30.72 ***	39.83 ***	4.41 ***	17.22 ***
Genotype	33	4.36 ***	29.55 ***	11.47 ***	9.77 ***	2.38 ***	11.60 ***
Between allelic combinations	4	2.89 ***	19.13 ***	5.90 ***	3.88 ***	1.08 ***	5.12 ***
Within SS	6	0.45 ***	3.39 ***	0.53 ***	0.44 ***	0.25 ***	1.27 ***
Within SI	8	0.43 ***	3.01 ***	1.44 ***	1.21 ***	0.19 ***	0.98 ***
Within I5S	5	0.35 ***	2.51 ***	1.32 ***	1.15 ***	0.11 ***	0.65 ***
Within I5I	5	0.05 ***	0.31 ***	1.24 ***	1.43 ***	0.18 ***	0.85 ***
Within I0I	5	0.19 ***	1.20 ***	1.04 ***	1.66 ***	0.57 ***	2.73 ***
Genotype x Site	99	1.54 ***	10.97 ***	8.44 ***	7.24 ***	0.89 ***	3.99 ***
Site x Between allelic combinations	12	0.49 ***	3.56 ***	4.32 ***	2.89 ***	0.19 ***	0.95 ***
Site x Within SS	18	0.14 ***	1.05 ***	0.51 ***	0.55 ***	0.11 ***	0.46 ***
Site x Within SI	24	0.46 ***	3.27 ***	1.03 ***	1.03 ***	0.29 ***	1.12 ***
Site x Within I5S	15	0.12 ***	0.89 ***	0.62 ***	0.63 ***	0.09 ***	0.47 ***
Site x Within I5I	15	0.13 ***	0.80 ***	0.71 ***	0.68 ***	0.08 ***	0.34 ***
Site x Within I0I	15	0.20 ***	1.40 ***	1.25 ***	1.46 ***	0.13 ***	0.65 ***
Genotype x Year	33	0.05 **	0.34 ***	1.77 ***	1.96 ***	0.15 ***	0.81 ***
Genotype x Site x Year	99	0.24 ***	1.31 ***	4.72 ***	5.34 ***	0.42 ***	2.21 ***
Rep (Site x Year)	16	0.01 **	0.08 *	0.54 ***	0.75 ***	0.08 ***	0.42 ***
Residual	528	0.18	1.28	4.63	5.40	0.37	1.94
Total	815						

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

Main effect of allelic variation at *Ppd-A1*

Genotypes carrying allele *Ppd-A1b* −conferring photoperiod sensitivity− had in average a longer time to flowering than those carrying any of the two alleles conferring photoperiod insensitivity (Fig. 2a and 2b). However, allelic variants at *Ppd-A1a* reduced the duration of the period emergence-flowering in Spain-South or Mexico-North more than in Spain-North or Mexico-South. Emergence-to-flowering time reductions of 9 and 11 days were observed in Spain-South and Mexico-North in the genotypes carrying allele GS-105 compared to those carrying the sensitive allele, while in Spain-North and Mexico-South the reductions were only of 3 and 5 days, respectively (Table 3).

Similarly, the reduction in the duration of the period emergence-flowering caused by allele GS-100 at *Ppd-A1*a when compared with *Ppd-A1b* were of 7, 16, 13 and 6 days in Spain-North, Spain-South, Mexico-North and Mexico-South, respectively, and a similar pattern occurred when comparisons were made in GDD (Table 3). In order to examine the divergences in the expression of *Ppd-A1a* alleles at contrasting sites during emergence-flowering, we investigated the relationship between this period and the environmental variables recorded at each site. The results showed that differences in emergence-flowering caused by alleles at *Ppd-A1*a were larger in sites with a average photoperiod below 12 h, as shown in Fig. 3.

The comparison of the reduction in flowering time associated with the two mutations at *Ppd-A1* causing photoperiod insensitivity showed that allele GS-100 had a generally stronger effect than allele GS-105 (Fig. 2a and 2b). However, at both Mexican sites, while the same trend was observed numerically, differences were not statistically significant (Table 3). The effect of *Ppd-A1* alleles on the duration of the flowering-maturity period was in opposite direction and a lesser extent than the one observed for time to flowering (Fig. 2a and 2b). On average, alleles GS-105 and GS-100 enlarged the flowering-maturity period by 3 days (or 45 GDD) and 4 days (or 63 GDD) respectively, when compared with the wild-type, but their effect was not consistent across sites (Table 3). Given that the effect of *Ppd-A1* alleles conferring photoperiod insensitivity was greater in shortening the time to flowering than enlarging the period between flowering and maturity, their effect amounted to a net reduction of total cycle length (Fig. 2a and 2b). However, the intensity of their effect depended on the site (Table 3).

Main effect of allelic variation at *Ppd-B1*

The comparison of the two allelic variants at *Ppd-B1* revealed that, on average, the allele *Ppd-B1*a −conferring photoperiod insensitivity− reduced the duration of the period emergence-flowering by 2 days or 40 GDD compared with the allele *Ppd-B1*b (Fig. 2c and 2d). This effect was consistent in all sites except in Mexico-South where differences between the two alleles were not statistically significant (Table 3).

Photoperiod insensitivity conferred by *Ppd-B1*a caused a slight lengthening of the mean flowering-maturity period across sites, but this effect was significant when only when the duration was expressed in days, not when expressed in GDD. This tendency to increase the grain filling period as consequence of the presence of allele *Ppd-B1a* was common to all sites, but not always statistically significant (Table 3). Compared with the wild-type, the presence of allele *Ppd-B1a* reduced total cycle length by one day or 33 GDD on average and consistently in all sites except in Mexico-North where differences were not statistically significant.

Table II.3. Main allelic effects at *Ppd-A1* and *Ppd-B1* loci on the number of days and thermal time (GDD, growing degree-days) on the development of 34 durum wheat genotypes grown at four sites of varying latitude. Numbers in parentheses are within each trait and locus the difference with the sensitive allele. Values are means across 2 years.

Allele	Photoperiod response	Spain north	Spain south	Mexico north	Mexico south	Spain north	Spain south	Mexico north	Mexico south
		Days emergence-flowering				GDD emergence-flowering			
Ppd-A1									
Ppd-A1b	Sensitive	134 [a]	118 [a]	99 [a]	69 [a]	1281 [a]	1466 [a]	1577 [a]	1174 [a]
GS-105	Insensitive	131 [b] (-3)	109 [b] (-9)	88 [b] (-11)	64 [b] (-5)	1233 [b] (-48)	1323 [b] (-143)	1366 [b] (-211)	1087 [b] (-87)
GS-100	Insensitive	127 [c] (-7)	102 [c] (-16)	86 [b] (-13)	63 [b] (-6)	1160 [c] (-121)	1236 [c] (-230)	1332 [b] (-245)	1058 [b] (-116)
Ppd-B1									
Ppd-B1b	Sensitive	134 [a]	115 [a]	94 [a]	67 [a]	1272 [a]	1410 [a]	1487 [a]	1129 [a]
Ppd-B1a	Insensitive	131 [b] (-3)	110 [b] (-5)	92 [b] (-2)	66 [a] (-1)	1224 [b] (-48)	1354 [b] (-56)	1442 [b] (-45)	1119 [a] (-10)
		Days flowering-maturity				GDD flowering-maturity			
Ppd-A1									
Ppd-A1b	Sensitive	39 [c]	44 [c]	38 [b]	46 [a]	724 [b]	774 [c]	777 [c]	773 [a]
GS-105	Insensitive	41 [b] (+2)	49 [b] (+5)	43 [a] (+5)	46 [a] 0	739 [b] (+15)	839 [b] (+65)	872 [a] (+95)	780 [a] (+7)
GS-100	Insensitive	45 [a] (+6)	54 [a] (+10)	42 [a] (+4)	45 [a] (-1)	792 [a] (+68)	899 [a] (+125)	835 [b] (+58)	772 [a] (-1)
Ppd-B1									
Ppd-B1b	Sensitive	40 [b]	46 [b]	40 [a]	46 [a]	733 [a]	804 [b]	814 [a]	789 [a]
Ppd-B1a	Insensitive	41 [a] (+1)	49 [a] (+3)	41 [a] (+1)	45 [b] (-1)	746 [a] (+13)	828 [a] (+24)	825 [a] (+11)	767 [b] (-22)
		Days emergence-maturity				GDD emergence-maturity			
Ppd-A1									
Ppd-A1b	Sensitive	174 [a]	162 [a]	137 [a]	115 [a]	2005 [a]	2240 [a]	2354 [a]	1947 [a]
GS-105	Insensitive	172 [b] (-2)	158 [b] (-4)	131 [b] (-6)	110 [b] (-5)	1972 [b] (-33)	2162 [b] (-78)	2238 [b] (-116)	1867 [b] (-80)
GS-100	Insensitive	171 [b] (-3)	156 [c] (-6)	128 [c] (-9)	108 [c] (-7)	1952 [b] (-53)	2135 [c] (-105)	2167 [c] (-187)	1830 [c] (-117)
Ppd-B1									
Ppd-B1b	Sensitive	174 [a]	161 [a]	134 [a]	113 [a]	2005 [a]	2214 [a]	2301 [a]	1918 [a]
Ppd-B1a	Insensitive	172 [b] (-2)	159 [b] (-2)	133 [a] (-1)	111 [b] (-2)	1970 [b] (-35)	2182 [b] (-32)	2267 [a] (-34)	1886 [b] (-32)

Means within columns, periods and loci with the same letters are not significantly different at $P < 0.05$ according to a Duncan's test.

Table II.4. Interaction effects of the allelic combination at *Ppd-A1* and *Ppd-B1* loci on the number of days and thermal time (GDD, growing degree-days) on the development of 34 durum wheat genotypes grown at four sites of varying latitude. For each trait and site numbers in parentheses are the differences with the combination of sensitive alleles at both loci (SS). Values are means across 2 years.

Allelic combination	Acronym	Spain north		Spain south		Mexico north		Mexico south		Spain north		Spain south		Mexico north		Mexico south	
		Days emergence-flowering								GDD emergence-flowering							
Ppd-A1b/Ppd-B1b	SS	134 ^{a}		117 ^{a}		98 ^{a}		69 ^{a}		1280 ^{a}		1457 ^{a}		1554 ^{a}		1160 ^{a}	
Ppd-A1b/Ppd-B1a	SI	134 ^{a}	(0)	118 ^{a}	(+1)	100 ^{a}	(+2)	70 ^{a}	(+1)	1282 ^{a}	(+2)	1473 ^{a}	(+16)	1595 ^{a}	(+41)	1185 ^{a}	(+25)
GS-105/Ppd-B1b	I5S	133 ^{a}	(-1)	111 ^{b}	(-6)	90 ^{b}	(-8)	65 ^{b}	(-4)	1264 ^{a}	(-16)	1354 ^{b}	(-103)	1409 ^{b}	(-145)	1094 ^{b}	(-66)
GS105/Ppd-B1a	I5I	130 ^{b}	(-4)	107 ^{c}	(-10)	86 ^{c}	(-12)	64 ^{b}	(-5)	1201 ^{b}	(-79)	1292 ^{c}	(-165)	1322 ^{c}	(-232)	1080 ^{b}	(-80)
GS100/Ppd-B1a	I0I	127 ^{c}	(-7)	102 ^{d}	(-15)	86 ^{c}	(-12)	63 ^{b}	(-6)	1160 ^{c}	(-120)	1236 ^{d}	(-221)	1332 ^{c}	(-222)	1058 ^{b}	(-102)
		Days flowering-maturity								GDD flowering-maturity							
Ppd-A1b/Ppd-B1b	SS	40 ^{c}		45 ^{d}		38 ^{c}		47 ^{a}		730 ^{bc}		788 ^{d}		774 ^{c}		790 ^{a}	
Ppd-A1b/Ppd-B1a	SI	39 ^{c}	(-1)	44 ^{d}	(-1)	38 ^{c}	0	45 ^{b}	(-2)	719 ^{c}	(-11)	763 ^{d}	(-25)	779 ^{c}	(+5)	760 ^{b}	(-30)
GS-105/Ppd-B1b	I5S	40 ^{c}	(0)	48 ^{c}	(+3)	42 ^{ab}	(+4)	46 ^{ab}	(-1)	736 ^{bc}	(+6)	822 ^{c}	(+34)	859 ^{ab}	(+85)	788 ^{a}	(-2)
GS105/Ppd-B1a	I5I	42 ^{b}	(+2)	50 ^{b}	(+5)	44 ^{a}	(+6)	45 ^{ab}	(-2)	741 ^{b}	(+11)	855 ^{b}	(+67)	885 ^{a}	(+111)	772 ^{ab}	(-18)
GS100/Ppd-B1a	I0I	44 ^{a}	(+4)	54 ^{a}	(+9)	42 ^{b}	(+4)	45 ^{ab}	(-2)	792 ^{a}	(+62)	899 ^{a}	(+111)	835 ^{b}	(+61)	772 ^{ab}	(-18)
		Days emergence-maturity								GDD emergence-maturity							
Ppd-A1b/Ppd-B1b	SS	174 ^{a}		163 ^{a}		135 ^{a}		115 ^{a}		2010 ^{a}		2246 ^{a}		2328 ^{a}		1949 ^{a}	
Ppd-A1b/Ppd-B1a	SI	174 ^{a}	(0)	162 ^{a}	(-1)	138 ^{a}	(+3)	115 ^{a}	(0)	2001 ^{a}	(-9)	2236 ^{a}	(-10)	2374 ^{a}	(+46)	1944 ^{a}	(-5)
GS-105/Ppd-B1b	I5S	174 ^{a}	(0)	159 ^{b}	(-4)	133 ^{b}	(-2)	111 ^{b}	(-4)	2000 ^{a}	(-10)	2177 ^{b}	(-69)	2268 ^{b}	(-60)	1881 ^{b}	(-68)
GS105/Ppd-B1a	I5I	171 ^{b}	(-3)	157 ^{bc}	(-6)	130 ^{c}	(-5)	109 ^{bc}	(-6)	1943 ^{b}	(-67)	2148 ^{bc}	(-98)	2208 ^{c}	(-120)	1852 ^{bc}	(-97)
GS100/Ppd-B1a	I0I	171 ^{b}	(-3)	156 ^{c}	(-7)	128 ^{c}	(-7)	108 ^{c}	(-7)	1952 ^{b}	(-58)	2135 ^{c}	(-111)	2167 ^{c}	(-161)	1830 ^{c}	(-119)

Means within columns and periods with the same letters are not significantly different at P <0.05 according to a Duncan's test.

Allelic combinations at *Ppd-A1* and *Ppd-B1*

The comparison of the mean values of cycle length across sites showed that the genotypes carrying the allelic combination *Ppd-A1b*/*Ppd-B1a* (SI) had the longest duration from emergence-flowering and the shortest flowering-maturity, whether these were measured in days (Fig. 2e) or GDD (Fig. 2f). However, when individual sites are considered, differences between combination SI and the wild-type (SS) were not statistically significant at any site for the length of the period emergence-flowering or in total cycle length (Table 4), except for the period flowering-maturity in Mexico-South.

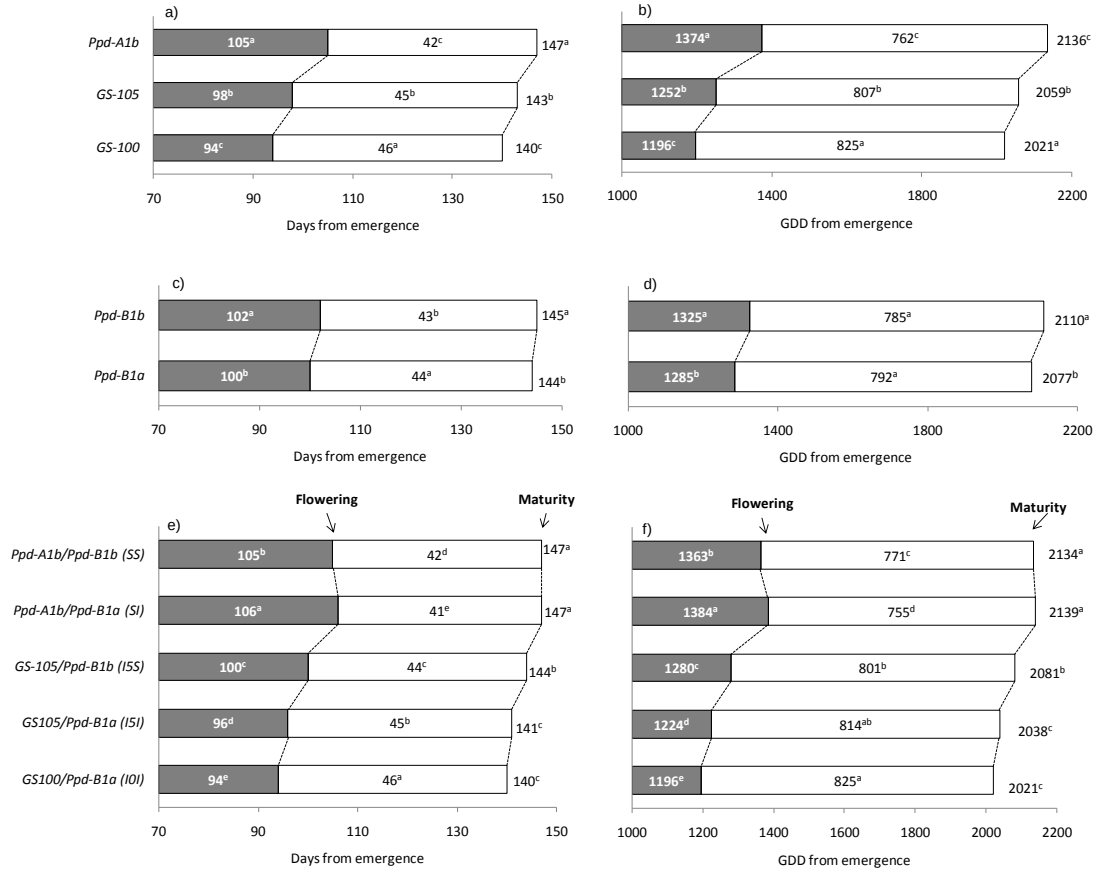

Figure II.2. Average number of days and thermal time (GDD, growing degree-days) corresponding to different developmental stages of 34 durum wheat genotypes grown for two years at 4 sites of different latitudes: a,b) contrast based on allelic composition at *Ppd-A1*, c,d) contrast based on allelic composition at *Ppd-B1*, and e,f) contrast based on allelic composition at both loci. Diferent letters indicate significant differences according to Duncan`s test $P<0.05$.

Combinations involving the non-wild type at *Ppd-A1* (I5S, I5I,I0I), resulted in a significant reduction of the period emergence-flowering, lengthening of the duration from flowering to maturity and a net shortening of the total cycle length, to extents that were dependent on the allelic composition at *Ppd-B1* (Fig. 2e and 2f). However, in Mexico-south the length of the period flowering-maturity did not differ between combinations I5S, I5I, I0I and SS (Table 4). The effect of allele GS-105 depended on the allele present at *Ppd-B1* as the genotypes carrying the sensitive allele at *Ppd-B1* (I5S)

were on average four days or 56 GDD delayed in their flowering compared to those carrying the insensitive allele (I5I) (Fig. 2e and 2f). This effect was consistent in all sites except the Mexico-South (Table 4). On the other hand, genotypes carrying the combination I5I slight lengthened their grain filling period on average by one day or 13 GDD when compared with those carrying combination I5S. The divergence between both combinations was maximal in Spain-South. The tendency was the same in Spain-North and Mexico-North, but in the latest site differences were not large enough to be statistically significant (Table 4). As a result of these differences, net total cycle length was increased on average by 3 days or 43 GDD in genotypes carrying the combination I5I in relation to those carrying combination I5S (Fig. 2e and 2f).

The shortest time to flowering corresponded to the allelic combination I0I (Fig. 2e and 2f), but differences with combination I5I were statistically significant only in the two Spanish sites (Table 4). In addition, combination I0I resulted in slightly longer grain filling period than combination I5I in all sites except in the Mexico-South, but compensation effects led to similar total cycle length in genotypes carrying these two combinations.

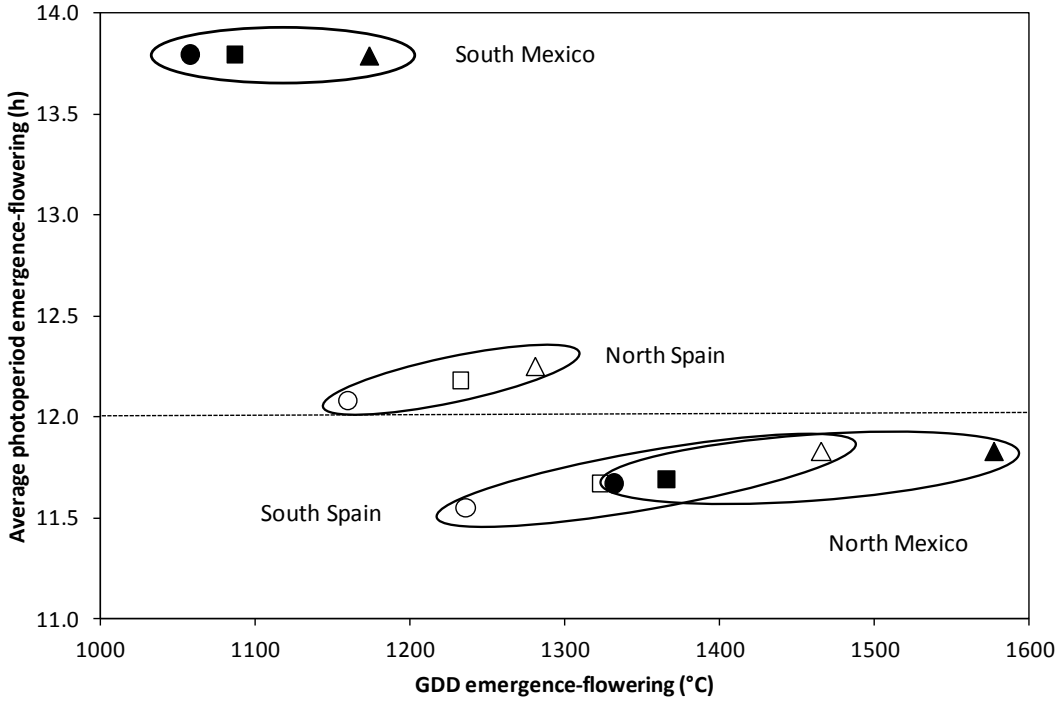

Figure II.3. Relationship between thermal time (GDD, growing degree-days) from emergence to flowering and average daily photoperiod in the same period. Each point represents the mean value of a set of durum wheat genotypes carrying the same allele at the *Ppd-A1* locus grown in field experiments in 2007 and 2008 at four sites. Triangle=*Ppd-A1b* conferring photoperiod sensitivity (wild-type); Square=GS-105; Circle=GS-100. Open and solid symbols correspond to Spanish and Mexican sites, respectively.

The relationship between accumulated photoperiod and the number of days before and after flowering is shown in Fig. 4. For the duration of the period emergence-flowering the interaction between photoperiod and allelic combination was quantitative in nature, with all the site clusters showing points corresponding to the allelic combinations SS and SI in the upper part and allelic combination I0I always in the bottom part, with the combinations I5S and I5I having, in most cases, intermediate positions. As shown in Table 4, the spring sowing-time in Mexico-South resulted in reduced differences in time to flowering between the allelic combinations, showing significant differences only between combinations having the sensitive and insensitive alleles at *Ppd-A1*. A linear model ($y = 0.0904x - 10.374$; $R^2 = 0.97$; $P<0.001$) could be properly fitted to the relationship between accumulated photoperiod and number of days from emergence to flowering (n=20, points shown on Fig. 4a). Fig. 4b shows a substantial interaction between the period flowering-maturity and allelic combination groups at different sites. A negative relationship existed between the duration of the periods emergence-flowering and flowering-maturity either expressed in days (r=-0.77, $P<0.0001$) or in GDD (r=-0.65, $P<0.001$).

Earliness *per se*

In the ANOVA (Table 2), the SS for variation within allelic combinations were highly significant, indicating an important source of variation in the duration of all developmental phases independently of the allelic composition at the *Ppd-1* loci, and *Vrn* loci since the latter were fixed. This suggests the existence of earliness *per se* effects. The effect of *Eps* in the Mexico-South site, where there should be minimal effect of allelic combinations at the *Ppd-1* loci due to the day length theoretically exceeding the photoperiod requirements for any spring wheat. At this site the average daylength between emergence and flowering across genotypes was 13.79±0.01h, and for the day of flowering it was 13.71±0.10 h. Under these conditions, a wide range of flowering time was observed within each allelic combination. Differences in flowering date between the earliest and the latest genotype were of 17d (or 298GDD), 14d (or 241 GDD), 8d (or 145 GDD), 14d (or 237 GDD), and 18d (or 309 GDD), for allelic combinations SS, SI, I5S, I5I and I0I, respectively (Fig. 5).

DISCUSSION

This study focused on the phenotypic expression of allelic variants of photoperiod response genes *Ppd-A1* and *Ppd-B1* in a set of 34 genotypes. All genotypes revealed the same spring growth-habit at *Vrn-1* based on published molecular markers. Interactions between *Ppd-1* and *Vrn-1* alleles, frequently mentioned in the literature (Casao *et al.,* 2011; Turner *et al.,* 2013) should not be expected in the present set of genotypes. Furthermore, the high synchrony in time to full emergence between the genotypes used provides confidence that the differences in phenology observed in the present study were not affected by variations in germination or crop establishment capacities.

The frequency of the insensitive allele GS-105 in the population was greater than that of allele GS-100 (34% and 20%, respectively), in agreement with the preponderance of

allele GS-105 in advanced germplasm from ICARDA and CIMMYT reported by Bentley *et al.,* (2011). It has been speculated that the mutation resulting in allele GS-105 preceded the one that originated allele GS-100, with the first detected in old landraces, while GS-100 was found only in few modern varieties to date (Bentley *et al*., 2011). Mutations within the gene sequence of *Ppd-B1* have been reported (Beales *et al.,* 2007; Shaw *et al.,* 2012), but none corresponded with photoperiodic response implying that the critical mutation causing the allelic difference of the *Ppd-B1* gene has not been found. The role of copy number variation has been recently described in bread wheat (Díaz *et al.,* 2012) and Nishida *et al.,* (2013) reported a novel mutation in the 5' upstream region of *Ppd-B1*.

Mutations by Nishida *et al.,* 2013 and three- and four-copies number variants found in bread wheat varieties Chinese Spring and Capelle-Deprez were not found in this study using durum wheat elite germplasm. However, the overall number of parents in this study was low. A more extensive allele mining study in durum wheat of *Ppd-A1* and *Ppd-B1* is therefore warranted. The marker *gwm148* linked to *Ppd-B1* in several studies including durum wheat (Hanocq *et al*., 2004, Mohler *et al.,* 2004, Maccaferri *et al.,* 2008) showed polymorphism in our study. Cane *et al.,* (2013) reported the relationship of *gwm148* with a two-copy allele in bread wheat. Quantitative techniques to identify on-copy and two-copy *Ppd-B1* alleles were not considered in this study but will be investigated further.

The previously published allelic combinations in durum wheat at *Ppd-A1* and *Ppd-B1* were present in our collection. The relatively balanced representation of alleles conferring photoperiod sensitivity (46% at *Ppd-A1* and 40% and *Ppd-B1*) and their variants conferring photoperiod insensitivity, confirm the suitability of the present germplasm collection for addressing the objectives of the study.

Photoperiod, or period of daylight every 24 hours, is dependent on latitude and season (Lee, 1970). Previous papers have shown that latitude integrates a number of variables affecting wheat development, among which the most relevant are photoperiod (Laurie *et al*., 1995), temperature (Craufurd and Wheeler, 2009), and their interaction (Hemming *et al*., 2012). With the aim of testing our germplasm under field conditions and a wide range of photoperiods, we conducted experiments in four sites with latitudes ranging from 19° to 41° N, and average daylength from sowing to flowering between 11.8h and 14.2h, and from flowering to maturity from 13.3h to 15.8h (Chapter I). Although each site corresponded to a different latitude, the site effect in this study involved not only daylength differences, but also other environmental (e.g. temperature, water input, soil type) and agronomic (e.g. dose and type of fertilizers, sowing dates) variations, which should provide more robustness to the average differences in phenology observed between *Ppd-1* groups and combinations.

The results of the ANOVA showed that the site effect was the most important factor explaining variability in time to flowering and total cycle length. The year effect −which included seasonal differences in environmental variables and small variations in agronomic conditions−, and the interaction site x year, had much lesser effect than the site itself. This result suggests that differences between sites in photoperiod and temperature (associated to the latitude), were probably the most important variables in explaining phenological differences between sites. This statement is supported by

the results of a recent study involving the same experiment, which showed that photoperiod and temperature jointly explained by ca. 77% of environmental variation between sites, and that daylength from sowing to flowering steadily increased from the north to the south, while daylength from flowering to maturity followed the opposite trend (Chapter I). The spring-planting in Mexico-South had important implications in the results obtained, as discussed below.

The results of the ANOVA showed that grain filling duration largely depended on the year and the site x year interaction. This observation is in accordance with the reported relatively low heritability of grain filling duration, and the large environmental influence on this trait (Egli, 2004; Royo *et al.,* 2006). The environmental effects accounted for more than 90% of the variance for the period emergence-flowering when it was expressed in days, with the genotype effect accounting for less than 5% of total variation. However, when this period was expressed in GDD, the environment and the genotype explained ca. 56% and 29% of total variance, respectively. The decrease in the fraction of total variance accounted for by the environment when the period emergence-flowering was expressed in thermal time is a consequence of the involvement of temperature −a major determinant of the rate of plant development (Craufurd and Wheeler, 2009)− in the calculation of GDD. As thermal time integrates the effect of temperature and the number of days, our results suggest that the differences observed in cycle length until flowering when expressed in number of days and GDD were largely due to the contrasting temperatures between experimental sites. Chmielewski and Rötzer (2002) showed that plant phenology is delayed by 2.3 days every 100 km when moving from south to north, although the absolute values are highly dependent on the temperatures during the spring (Siebert and Ewert, 2012).

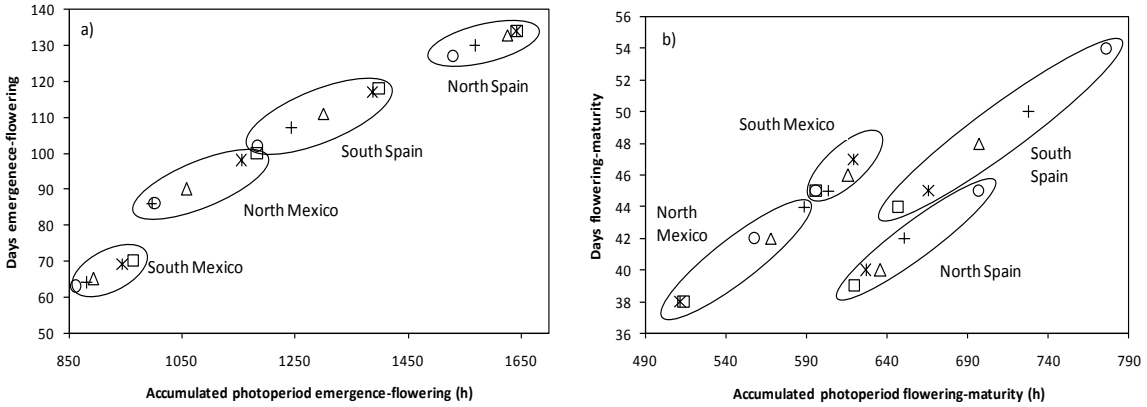

Figure II.4. Relationships between accumulated photoperiod and: a) number of days from emergence to flowering, b) number of days from flowering to maturity. Each point represents the mean value of a set of durum wheat genotypes carrying the same allelic combination for the *Ppd-A1* and *Ppd-B1* genes grown in field experiments in 2007 and 2008 at four sites with contrasting latitude.✳=SS, □=SI, △=I5S, ＋=I5I, ○=I0I, see Table 1 for allelic combination acronyms.

The strong linear relationship existing between accumulated photoperiod and number of days from emergence to flowering shown in Fig. 4a demonstrated a consistent trend in flowering time regulation associated with the different allelic combinations. Within the range of latitudes and with the genotypes used in this study, time from emergence to flowering ranged from 63 to 134d and accumulated photoperiod until flowering from 863 and 1642h.

The fraction of the genotype effect explained by differences between allelic combinations was about 65% both expressed in days or GDD. As all genotypes studied had spring growth habit and no interaction with vernalization requirement is expected, this result provides a quantitative estimate of the relative effect of allelic variation at *Ppd-1* loci in explaining genotypic variance for time to flowering. However, it also suggests that other genetic factors determined about 35% of the genotypic variance, factors which also significantly interacted with the site. This non *Ppd-1* related variation is supported by the fact that there were statistically significant differences in all traits within all allelic combinations groups, suggesting the involvement of *Eps*, assumption which is in accordance with previous studies reporting the influence of minor genes on bread wheat flowering time (Griffiths *et al*., 2009; García *et al*., 2011). However, Diaz *et al.,* (2012) recently reported that copy number of the *Ppd-1* genes may play a role in producing variation in flowering time within a same allelic combination group. Such effect of copy number has not been determined in durum wheat but cannot be discounted, so both bases for explaining the residual genotypic variation (not related to differences in allelic combinations at *Ppd-1* loci) remain possible.

Within allelic combinations, genotype variability for the emergence-flowering period was greater in the presence of *Ppd-A1b* allele. It may be hypothesized that within genotypes carrying the sensitive allele, other genes implied in regulation of flowering time have a higher phenotypic expression than when they are present in combination with the mutant allele. Shaw *et al.,* (2012) suggested that the accumulation of *Ppd-1* mutant alleles in bread wheat caused a maximum rate of earliness. In their study, the genotypes with three mutations had an effect on phenology similar to genotypes with the two mutations with the strongest effect. We hypothesize that, in our study, the *Ppd-A1* gene is powerful enough to cause a rate of earliness close to the maximum in durum wheat. This could explain the small differences observed between genotypes with mutant alleles in *Ppd-A1*. The percentage of total variance for the period flowering-maturity explained by differences between allelic combinations was lower than 1% even when this period was expressed in GDD. Moreover, the percentage of total variance explained by differences between allelic combinations was around 15% lower for this period than for the length of the period emergence-flowering suggesting a more limited effect of *Ppd* genes on cycle length after flowering.

In this study the longest time to flowering was consistently recorded in allelic combinations carrying the wild-type allele *Ppd-A1b* which resulted in an average delay of flowering of 3 days or 49 GDD compared to allelic combinations involving the wild-type allele at the other locus, *Ppd-B1b*. These results suggest a stronger effect of the allele conferring photoperiod sensitivity at *Ppd-A1* than that at *Ppd-B1 (Ppd-A1b > Ppd-B1b)*. Similarly, mutant alleles causing insensitivity at *Ppd-A1* (GS-105 and GS-100) had

a stronger effect in reducing flowering time than the mutant allele at *Ppd-B1 (Ppd-A1a* > *Ppd-B1a)*. Our results are in agreement with those recently reported in hexaploid wheat by Shaw *et al*., (2012), who classified the photoperiod insensitivity alleles according to their effect on flowering date as follows: *Ppd-D1a* > *Ppd-A1a* > *Ppd-B1a*, when *Ppd-A1a* is the GS-100 durum wheat variant. Other studies in bread wheat ranked differently the relative strength of these genes. Scarth and Law (1984) speculated that *Ppd-B1a* may have a stronger effect than *Ppd-A1a*, and this in turn would be stronger than *Ppd-D1*. Worland *et al.,* (1998) suggested that *Ppd-D1* confers greater precocity, followed *Ppd-B1*, with the *Ppd-A1* gene having a smaller effect. A greater effect of *Ppd-B1a* than *Ppd-D1a* of hexaploid wheat was reported by Tanio and Kato (2007), who suggested that there may be different alleles conferring insensitivity in the B genome, with different effects on earliness. A recent study suggests that copy number in addition of diverse mutations have different effects on the date of anthesis (Díaz *et al*., 2012).

Our results suggest that the effect of allele *Ppd-B1a* depended on the allele present at *Ppd-A1*. In presence of allele *Ppd-A1b* the effect of *Ppd-B1a* gene was minor. However, in the absence of *Ppd-A1b*, *Ppd-B1a* reduced flowering time by 4 days or 56 GDD in comparison to the wild-type allele at the same locus. This tendency was similar in all sites except Mexico-south where differences were not statistically significant. These results point out the interaction existing between genes *Ppd-A1* and *Ppd-B1*. Tanio and Kato (2007) described an incomplete dominance and interaction between genes *Ppd-B1* and *Ppd-D1* similar to that observed in this study between genes *Ppd-A1* and *Ppd-B1*.

Among the two allelic combinations causing photoperiod insensitivity at *Ppd-A1*, GS105/*Ppd-B1a* and GS100/*Ppd-B1a,* the latter resulted in on average two days or 28 GDD earlier flowering genotypes. Moreover, genotypes carrying allele GS-100 independent of the allele at *Ppd-B1* flowered 4 days before the ones carrying allele GS-105. However, the latter group included different alleles at *Ppd-B1* and the former only the ones carrying allele *Ppd-B1a*, the tendency was consistent across all sites, suggesting that allele GS-100 had a stronger effect than GS-105. These results are in agreement with those reported by Bentley *et al.,* (2011) who found that allele GS-100 conferred earlier flowering than GS-105, and are also consistent with previous observations in durum wheat (Clarke *et al.,* 1998; Maccaferri *et al.,* 2008; Wilhelm *et al.,* 2009). Flowering time of the genotypes carrying *Ppd-B1b* or *Ppd-B1a* alleles showed that the effect of the mutation at this locus was lower than that of the mutations at *Ppd-A1.*

Allelic variants at *Ppd-1* genes causing photoperiod insensitivity had an opposite, but more modest effect on grain filling period when compared with their effect on flowering time. Consequently, a consistent negative relationship was found between time to flowering and grain filling duration. It could be attributed to the environmental conditions prevailing at the end of the growth cycle (acceleration of senescence due to heat), but also to the presence of genetic factors inducing earlier flowering in combination with elongating grain filling duration, as the QTL regulating *Eps* (*QFlt.dms-5B.1*) recently identified by Kamram *et al.,* (2013). Bogard *et al.,* (2011) also found co-location of QTLs responsible of grain filling duration and anthesis date in bread wheat.

As a result, total cycle length was slightly reduced following the same general trends observed for time to flowering. Early-flowering genotypes have their spike growth phase under lower temperature, resulting in a higher grain number per unit land (Ratjen *et al.*, 2012; Villegas *et al.*, submitted). This represents a stronger sink force, which has been associated to accelerated senescence (Gan, 2007; Fois *et al.,* 2009), as could be the case of the earliest genotypes in our study.

Our results showed that photoperiod affected the expression of alleles at *Ppd-A1*. In the Spain-North site, where mean photoperiod from emergence to flowering was 12.2h, differences in flowering time between genotypes carrying different allelic combinations were statistically significant but small. In Spain-South or Mexico-North, where photoperiod from emergence to flowering averaged 11.7h, those differences increased. In Mexico-South, with decreasing photoperiod from emergence to flowering and 13.79h of average day-length, total cycle length was strongly shortened, and average differences between allelic combinations groups were reduced. Based on our results, it appears that the phenotypic expression of photoperiod sensitivity genes increases in sites with average pre-flowering daylength lower than 12 h, which is in agreement with the results of by Kumar *et al.,* (2012), who reported a critical photoperiod of 12h for bread wheat phenotypes to manifest the genetic contribution of *Ppd-D1*.

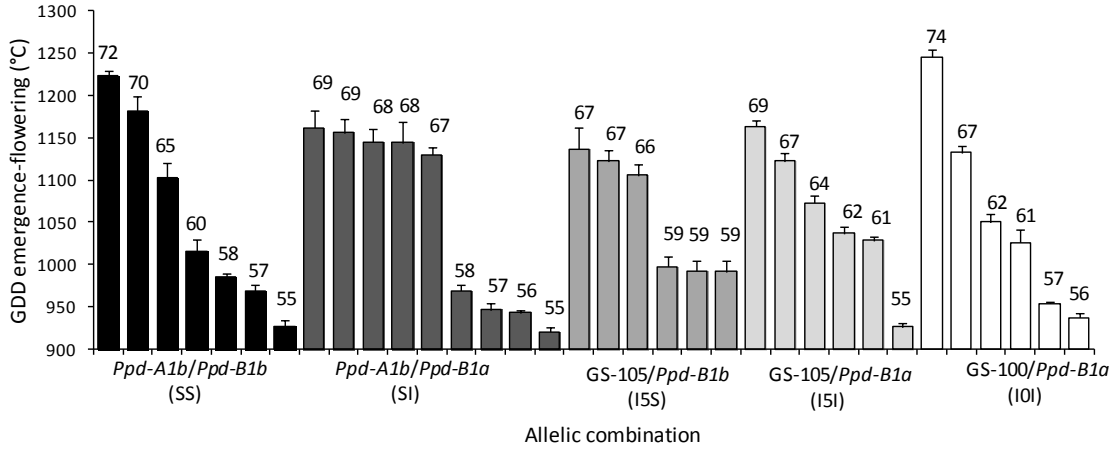

Figure II.5. Variability for the length of the period emergence-flowering within the genotypes carrying different allelic combinations for *Ppd-A1* and *Ppd-B1* genes in experiments conducted in Southern-Mexico under average daylengths from emergence to flowering of 13.79 h. The number of days is indicated over the bars.

Earliness *per se* genes have been reported to be strong enough to induce earlier flowering, even in the presence of *Vrn* and *Ppd* genes (van Beem *et al.,* 2005). It can be assessed only in conditions which minimize the effects of *Vrn/Ppd* related variations. Since all the genotypes used in this study had spring growth-habit and the same alleles at *Vrn* loci, their vernalization requirements were considered non-significant and identical, thereby not interfering with any putative earliness per se that may be involved in controlling their flowering time. More importantly, and in order to minimize the effects of *Ppd* genes while assessing earliness *per se*, it is important to

select conditions in which all photoperiod requirements are fulfilled to the greatest possible extent. The Mexico-South site, with its spring planting and 14h day-length during all pre-flowering period provided such an environment and the opportunity to assess earliness *per se* within our collection. Since *Ppd* effects were still significant at this site, they were properly discounted for *Eps* calculations, according to Ortiz Ferrara *et al.,* (1998).

Effectively, a wide range of *Eps* effect was recorded, given that differences between the latest and the earliest genotypes reached 19d or 325 GDD from emergence to flowering. This was a larger effect than the one between allelic combinations recorded in Spain-South, where *Ppd* effects were maximized (15d or 221 GDD of difference in flowering time). These results suggest an important role of *Eps* in the germplasm used in this study. In addition, we did not find any relationship between allelic combination and the effect of *Eps*, as all the allelic combination groups showed a wide range of time to flowering. These results are consistent with those reported by Gomez *et al.,* (2014) in Argentinean bread wheat cultivars, and those for heading time reported by Gawronski and Schnurbusch (2012) describing the effect of the *Eps-3A*^{m} under glasshouse conditions. Miura and Worland (1994) found that the effect of *Eps* in reducing heading date was independent of environmental stimuli, but Lewis *et al.,* (2008) reported significant interactions between *Eps-A*^{m}*1* alleles and temperature, suggesting that the effect of some *Eps* genes may vary depending on the environment. The findings of this study open further approaches for the identification and study of new genes regulating *Eps* in durum wheat.

Conclusions

The results obtained in this study allowed us to conclude that in durum wheat: i) *Ppd-A1a* had a stronger effect conferring photoperiod in-sensitivity than *Ppd-B1a*, ii) Allele GS-100 had a stronger effect that GS-105 so that according to their effect on flowering date the effect of photoperiod insensitivity alleles could be classified as *Ppd-A1a* (GS-100) > *Ppd-A1a* (GS-105) >*Ppd-B1a,* iii) interaction existed between genes *Ppd-A1* and *Ppd-B1*, iv) the phenotypic expression of photoperiod genes increased in sites with mean daylength from emergence to flowering lower than 12h, v) the effect of alleles causing photoperiod insensitivity on flowering date was much greater than the subsequent reduction of grain filling period, and vi) the effect of earliness *per se* in shortening time to flowering were comparable to those of the major genes causing photoperiod insensitivity.

This study is the first one showing the phenotypic effect of *Ppd-1* genes in durum wheat under field conditions on a range of latitudes, thus being a significant contribution to the elucidation of the phenotypic expression of genetic factors controlling flowering time in durum wheat.

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Chapter III

Allelic variants at *Ppd-A1* in durum wheat affect yield formation strategies in environments with contrasting patterns of photoperiod and temperature

ABSTRACT

Yield formation in wheat is strongly affected by flowering date. In this study path-coefficient analysis was applied to determine how allelic variants at *Ppd-A1* in the presence of allele *Ppd-B1a*, affect yield formation strategies of spring durum wheat. Twenty-five genotypes, 10 carrying allele *Ppd-A1b* causing photoperiod sensitivity, and 8 and 7 carrying the mutant alleles *Ppd-A1a* 'GS-105' and 'GS-100', respectively, causing photoperiod insensitivity, were used. Experiments were conducted in 2007 and 2008 at three sites: Spain-north, Spain-south and Mexico. In Spain, experiments were planted in autumn, and growth developed under increasing temperature and photoperiod. In contrast, the spring-planting of Mexico resulted in a slight decrease of both environmental variables from crop emergence. Allele *Ppd-A1b* significantly delayed flowering time (VP) at the three sites, thus shortening the duration of the grain filling period (GFP). Allele 'GS-105' reduced VP less than allele 'GS-100'. While genotypes carrying allele *Ppd-A1b* had consistently lower yields as a result of a lower number of spikes per m^2 (NSm^2) and lower kernel weight (KW) than those carrying any of the mutant alleles, they had a superior number of kernels per spike (NKS). Allele 'GS-100' led to a greater yield and NKS than allele 'GS-105'. The yield component that exerted the most direct influence on grain yield variations in Spain-north was KW, while in Spain-south it was NKS, particularly in early-flowering genotypes. In Spain-north the greater number of spikes significantly reduced KW, mainly in those genotypes with a longer GFP. The competition between NKS and KW was greater in Spain-south than at the other two sites. The combination of large and constant photoperiods and smooth temperatures in Mexico significantly reduced VP and the competition between the NSm^2 and NKS. At this site the yield of genotypes carrying allele 'GS-100' was dependent mostly on KW, while a high NSm^2 impaired high yield in genotypes carrying allele *Ppd-A1b*, the latest to flower. In these genotypes a long GFP was favourable for obtaining a higher NKS and heavier kernels. Compensatory effects among yield components were lowest in the most favorable environment of northern Spain. Path-coefficient analysis was useful in detecting relationships between yield components indiscernible by simple correlation coefficients.

INTRODUCTION

Durum wheat (*Triticum turgidum* L. var. *durum*) is grown in temperate zones worldwide between latitudes 55°N and 40°S (Palamarchuk, 2005). The growing regions correspond mostly to the Mediterranean Basin, the North American Great Plains, India, and the former USSR (International Wheat Council, 2001). The contrasting environments of these areas require wide genetic variability in adaptation traits for the crop to match its development within favorable environmental conditions. One of the most important components of wheat adaptation is flowering time as it affects the reproductive success of the crop and is critical for yield formation.

Time to flowering within a season is largely determined by crop responses to temperature and photoperiod. Flowering time in wheat is a complex character controlled by three genetic systems, namely vernalization (*Vrn*), photoperiod (*Ppd*), and earliness per se (*Eps*). The adaptability and yield potential of wheat in different environments are determined mainly by these three genes systems and their interactions with growing temperatures (Gororo *et al*., 2001). It is well known that high temperatures accelerate plant development and in many cases responses to photoperiod and temperature are not linear (Angus *et al*., 1981a,b). Durum wheat has both, winter and a spring growth habits, although spring types are prevalent. Winter wheats need to be exposed to a continuous chilling treatment (vernalization) to transit to reproductive growth, whereas spring wheats do not have vernalization requirements (Kamran *et al*., 2014).

The response to photoperiod is genetically controlled in wheat by *Ppd* genes (Worland and Snape, 2001). Photoperiod sensitivity in durum wheat is determined at the *Ppd-A1* and *Ppd-B1* loci, located on chromosomes 2AS and 2BS, respectively (Laurie, 1997). Photoperiod insensitivity results from mutations at one or more of the collinear (homeoeoallelic) *Ppd-1* gene, with individual mutations conferring different degrees of earliness. In durum wheat Wilhelm *et al.,* (2009) found two large deletions within the *Ppd-A1* gene, designated as allele 'GS-100' and 'GS-105', which accelerated flowering, leading to the conclusion that these deletions are the likely cause of photoperiod insensitivity in tetraploid wheat. As these two mutations predominate in modern durum wheat but are absent from wild tetraploid wheat, it has been suggested that *Ppd-A1* insensitive alleles arose by mutation during domestication (Bentley *et al.,* 2011). The *Ppd-B1* locus was originally mapped by Hanocq *et al.,* (2004) and Mohler *et al.,* (2004) in bread wheat, and confirmed by Maccaferri *et al.,* (2008) in durum wheat. Photoperiod insensitive alleles are designated by the suffix 'a' and sensitive by the suffix 'b', *Ppd-A1a* and *Ppd-B1a* indicate insensitive, whereas *Ppd-A1b* and *Ppd-B1b* indicate sensitive alleles at the two loci (McIntosh *et al*., 2007). Earliness *per se* is the difference in flowering time of varieties whose vernalization and photoperiod requirements have been fulfilled (Kato *et al*., 2001). Although sensitivity to photoperiod is a characteristic determined by genotype (Masle *et al*., 1989), the individual response is strongly influenced by allelic composition and pleiotropic effects between genes that regulate the photoperiodic response (Guo *et al*., 2010). A comprehensive review of the factors controlling flowering time in wheat may be found in Kamran *et al*. (2014).

Durum wheat is ancestrally a photoperiod sensitive plant that flowers rapidly in long days but is late flowering in short days. A lack of fulfilment of photoperiod requirements in sensitive varieties delays flowering, with magnitude varying with the presence of specific photoperiod response genes and the latitude of the growing region (Kamran *et al*., 2014). However, many growing environments require day neutral (photoperiod insensitive) varieties that flower rapidly in short or long days. Photoperiod insensitivity allows production in environments where appropriate temperature and rainfall coincide with short day conditions or where early flowering allows grain filling to escape from high summer temperatures, thereby avoiding drought stress (Worland and Snape, 2001), as occurs in the Mediterranean Basin.

Photoperiod insensitivity was an important component of the 'Green Revolution' and continues to be widely used globally by durum wheat breeders (Royo *et al*., 2009).

Understanding the effect of flowering time on yield formation and its interaction with the patterns of temperature and photoperiod could significantly contribute to the development of more stable and productive cultivars. Grain yield in wheat can be analyzed in terms of three yield components: number of spikes per m^2, number of kernels per spike and kernel weight. These three components appear sequentially, with the later developing components under control of earlier-developing ones, and therefore they may interact in compensatory patterns during plant development (Gibson and Paulsen, 1999). Thus, simple correlation coefficients may not provide a clear picture of the importance of each component in determining grain yield. Path-coefficient analysis divides the correlation coefficients into direct and indirect effects, thus allowing the separation of the direct influence of each yield component on grain yield from the indirect effects caused by the mutual relationships among the yield components themselves.

The importance of photoperiod in determining flowering time in wheat has been widely studied. Moreover, the literature is relatively abundant on the use of path-coefficient analysis to evaluate yield relationships in wheat. However, we are not aware of any study applying such analysis to determine the effect of allelic variants at *Ppd-1* genes on yield formation strategies in durum or bread wheat. The rationale behind the present study is that allelic variants at *Ppd-A1* affect flowering time, thereby influencing the relationship between yield and its components in a distinct manner depending on the pattern of photoperiod and temperature of the growing environment. The objectives of this study were (i) to investigate the influence of different allelic variants at *Ppd-A1*, in presence of the *Ppd-B1a* allele, on grain-yield formation strategy in durum wheat grown in three environments with contrasting regimes of temperature and photoperiod, by using an ontogenetic diagram; and (ii) to evaluate the usefulness of path-coefficient analysis to elucidate the reciprocal relationships that different *Ppd-A1* alleles induce on plant phenology, yield components, and grain yield under different photoperiods.

MATERIALS AND METHODS

Plant material

Twenty-three durum wheat (*Triticum turgidum* L. var. *durum*) inbred lines and two commercial cultivars were used in this study. The lines resulted from divergent selection within the offspring of crosses between late-flowering genotypes (Durabon, Megadur, 2716-25.94.01, 2805-49.94.02, 2905-13.93.04), from the University of Hohenheim (Germany), and early-flowering advanced lines (Sooty_9//Rascon_37, Cado/Boomer_33, Dukem_12/2*Rascon_21, Guanay and Snitan) from the CIMMYT (International Centre for Wheat and Maize Improvement, Mexico) durum wheat breeding program. Two CIMMYT unrelated sister lines, derived from the cross CF4-JS

40/3/Stot//Altar84/Ald, were also included in the collection. The commercial cultivars used as checks were 'Simeto' (medium-late-flowering) and 'Anton' (late-flowering).

The whole set had spring growth-habit, as revealed by the molecular characterization for *Vrn-A1*, *Vrn-B1* and *Vrn-B3* loci conducted in a previous study (Chapter II). The analysis of the allelic composition for *Ppd-1* (see Chapter II, for methods), showed that the whole set was monomorphic for *Ppd-B1* as all the genotypes carried allele *Ppd-B1a*, which confers photoperiod insensitivity. For *Ppd-A1*, three sub-sets of 10, 8, and 7 genotypes carried one of the following alleles respectively: allele '*b'* (wild type) conferring photoperiod sensitivity, allele 'GS-105', and allele 'GS-100', the last two conferring photoperiod insensitivity (Table 1).

Table III.1. Acronyms of the allelic combinations tested and number of genotypes of each of them.

Ppd-A1		*Ppd-B1*		Allelic combination acronym	Number of lines
Allele*	Photoperiod response	Allele	Photoperiod response		
Ppd-A1b	Sensitive	*Ppd-B1a*	Insensitive	SI	10
GS-105 *Ppd-A1a*	Insensitive	*Ppd-B1a*	Insensitive	I5I	8
GS-100 *Ppd-A1a*	Insensitive	*Ppd-B1a*	Insensitive	I0I	7

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

Experimental set up

Six field experiments were carried out in 2007 and 2008 at three sites: Gimenells in the north of Spain (Spain-north 41°38'N), Jerez de la Frontera in the south of Spain (Spain-south 37°0'N), and El Batan (Texcoco) in the Central Mexican Highlands (Mexico 19°31'N) (Table 2). Each experiment consisted of 12-m^2 plots arranged in a randomized complete block design with three replications. The sowing density was the currently used at each site in order to obtain around 450 spikes m^{-2} at all sites. Experiments were autumn-planted in Spain, and spring-planted in Mexico to fit within the usual summer crop cycle at this site (Table 2). Plot management was implemented to maximize yield at each location, to the extent allowed by local conditions. Soil analyses were performed and fertilization was provided accordingly (Table 2). Plots were maintained free of weeds, diseases and pests and were irrigated when necessary to prevent any significant water deficit. Plots were kept disease- and insect-free with preventive pesticide applications. Lodging was prevented, when needed, using networks of strings to support lodging-prone or tall genotypes.

Table III.2. Site location and agronomic details

	Spain-north		Spain-south		Mexico	
Site	Gimenells		Jerez de la Frontera		El Batán	
Coordinates	41°38'N, 0°23' E		37°0'N, 3°40' W		19°31'N, 98° 50'W	
Altitude, m.a.s.l.	200		30		2249	
Soil Characteristics						
Classification	Mesic Calcixerolic Xerochrept		Versiol Uderts		Cumulic Hapludoll	
Texture	Fine loamy		Fine		Clay	
pH	8.1		7.7		5.9	
P, mg kg^{-1}	16		40		65	
K, mg kg^{-1}	134		155		312	
Organic matter, %	2.4		2.5		5.0	
Year	2007	2008	2007	2008	2007	2008
Seasonal rainfall + irrigation, mı	447	502	297	341	583	411
Mean temperatures, °C						
Vegetative period	7.9	10.0	11.7	12.4	17.6	16.8
Grain filling period	18.2	17.0	17.8	17.0	17.0	17.3
Average daylength, h						
Vegetative period	12.0	12.5	12.0	11.6	14.0	14.0
Grain filling period	15.7	15.9	14.8	14.4	13.4	13.2
Sowing date	24/11/2006	19/11/2007	12/12/2006	30/11/2007	18/05/2007	28/05/2008

Data recording

Dates of flowering and physiological maturity were recorded in each experimental plot when approximately 50% of the main spikes reached Zadoks stages 65 and 87, respectively (Zadoks *et al*., 1974). Daily maximum and minimum temperatures were obtained from weather stations placed in the experimental fields or at a distance of less than 3 km away. Thermal time (GDD, growing-degree days) was calculated by summing the daily values obtained as GDD = $[(T_{max} + T_{min})/2]$, where T_{max} and T_{min} are the daily maximum and minimum air temperatures, respectively. A base temperature of 0°C was used (Gallagher, 1979), as when T_{min}<0°C it was considered T_{min}=0°C (McMaster and Wilhelm, 1997). Thermal time from emergence to flowering and from flowering to physiological maturity will be further referred to as vegetative period (VP) and grain filling period (GFP), respectively. Plots were divided in two sections of 6 m^2, one used for destructive sampling and the other left untouched for bulk harvest and estimation of grain yield at ripening (g m^{-2}), subsequently adjusted to a 10% moisture basis. At maturity, a 0.5-m row length of a central representative section was uprooted in each plot, and thespikes were counted. Kernel weight (KW, g) was obtained by weighing a randomly drawn sample of 200 kernels from harvested grain of each plot previously oven-dried at 70°C for 48 h. The number of kernels m^{-2} was computed as the ratio between grain yield and TKW.

Statistical and path-coefficient analyses

Analyses of variance were carried out using the GLM procedure of the SAS statistical package (SAS Institute Inc., 2009), considering a fix factor model. Means were compared according to the Duncan's multiple range test at *P*=0.05.

Pearson correlation coefficients were computed from the mean values across years, sites, and blocks for all the studied traits: (1) length of the period from plant emergence until flowering, (2) number of spikes m^{-2}, (3) duration of grain filling period (from flowering to physiological maturity), (4) number of kernels $spike^{-1}$, (5) kernel weight, and (6) grain yield. Path-coefficient analysis was carried out to partition the correlation coefficient, r_{ij}, into direct and indirect effects. The following four sets of simultaneous equations were solved to determine the path-coefficients, P_{ij} (with subscripts indicating the six traits), as described in García del Moral *et al*. (2003):

$$r_{26} = P_{26} + r_{24} P_{46} + r_{25} P_{56}$$
$$r_{46} = r_{24} P_{26} + P_{46} + r_{45} P_{56}$$
$$r_{56} = r_{25} P_{26} + r_{45} P_{46} + P_{56}$$

$$r_{25} = P_{25} + r_{23} P_{35} + r_{24} P_{45}$$
$$r_{35} = r_{23} P_{25} + P_{35} + r_{34} P_{45}$$
$$r_{45} = r_{24} P_{25} + r_{34} P_{35} + P_{45}$$

$$r_{14} = P_{14} + r_{12} P_{24} + r_{13} P_{34}$$
$$r_{24} = r_{12} P_{14} + P_{24} + r_{23} P_{34}$$
$$r_{34} = r_{13} P_{14} + r_{23} P_{24} + P_{34}$$

$$r_{13} = P_{13} + r_{12} P_{23}$$
$$r_{23} = r_{12} P_{13} + P_{23}$$

In the equation $r_{13} = P_{13} + r_{12} P_{23}$, P_{13} is the direct effect of trait 1 on 3 (the path-coefficient), while $r_{12} P_{23}$ is the indirect effect of trait 1 on 3 via 2. Similar definitions apply to the other equations.

RESULTS

Environmental

Wide differences in the pattern of changes of photoperiod and temperature during growth cycle were detected between sites. Although photoperiod and mean temperature increased from emergence to maturity at the Spanish sites, a decreasing trend was observed in the spring-planting in Mexico (Fig. 1). The lowest temperatures before flowering and the longest daylength during GFP were recorded in Spain-north, while the opposite was observed for the spring planting in Mexico, with intermediate values recorded in Spain-south (Fig. 1 and Table 2).

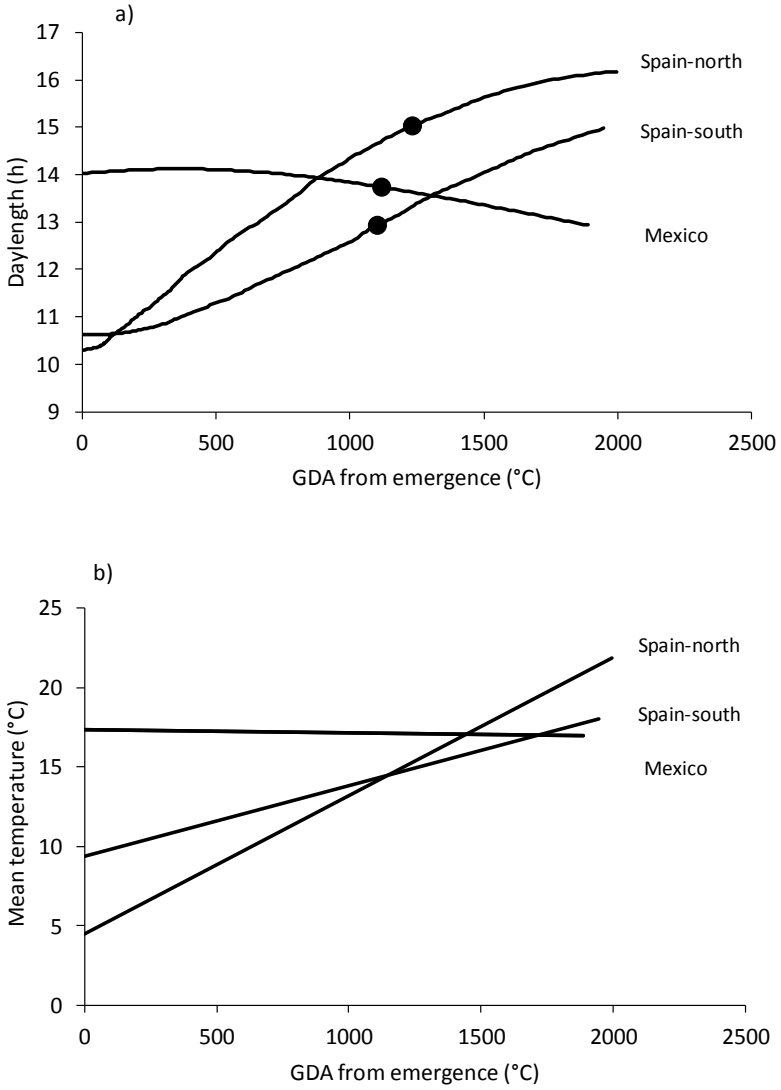


Fig. III.1. Environmental conditions at the testing sites. a) Mean daylength (points indicate average flowering time at each site), and b) lines fitted to mean daily temperatures. Data are means of experiments conducted in 2007 and 2008 and involving 25 durum wheat inbred lines.

Effect of site and allelic combination on yield and related traits

The ANOVA revealed that differences between sites were statistically significant for all traits except for the number of kernels per spike (Table 3). Time to flowering was longer in Spain-north, without significant differences between the other two sites, while the longest GFP was recorded in Spain-south. When expressed in calendar terms, the number of days of the VP was 131, 110, and 66 d in Spain-north, Spain-south and Mexico, respectively, while the GFP was 41, 49 and 45 d at the same sites, respectively. Grain yield decreased when moving from the north to the south, but this pattern was not followed by any of the yield components. A similar number of spikes per m^2 was reached in Spain-north and Mexico, while in Spain-south this yield component was

significantly lower. The heaviest grains were obtained in Spain-south and the lightest in Mexico.

Table III.3. Site and allelic combination means for the length from emergence to flowering (vegetative period), length from flowering to maturity (grain filling period), yield components and yield of 25 durum wheat genotypes grown during two years at three contrasting latitudes. Genotypes carried different alleles at *Ppd-A1* and were monomorphic for allele *a* (causing photoperiod insensitivity) at *Ppd-B1*. GDD: growing-degree days.

	Vegetative period (GDD)	Grain filling period (GDD)	Spikes m^{-2}	Kernels $spike^{-1}$	Thousand kernel weight (g)	Grain yield ($g\ m^{-2}$)
Site						
North Spain	1232 ^{a}	749 ^{c}	509 ^{a}	40.2 ^{a}	40.0 ^{b}	690.2 ^{a}
South Spain	1101 ^{b}	832 ^{a}	416 ^{b}	41.1 ^{a}	42.3 ^{a}	585.7 ^{b}
Mexico	1117 ^{b}	767 ^{b}	508 ^{a}	37.8 ^{a}	38.2 ^{c}	442.6 ^{c}
Allelic combination (acronym)						
Ppd-A1b/Ppd-B1a (SI)	1229 ^{a}	756 ^{c}	460 ^{b}	42.4 ^{a}	38.0 ^{b}	548.6 ^{c}
GS105/Ppd-B1a (I5I)	1122 ^{b}	785 ^{b}	491 ^{a}	36.7 ^{c}	42.1 ^{a}	579.7 ^{b}
GS100/Ppd-B1a (I0I)	1068 ^{c}	819 ^{a}	488 ^{a}	39.2 ^{b}	41.2 ^{a}	599.7 ^{a}

Means within columns for sites or allelic combinations with the same letters are not significantly different at $P<0.05$ according to a Duncan's test.

The comparison of cycle duration of genotypes carrying one of the three alleles at *Ppd-A1* −with allele *Ppd-B1a* causing photoperiod insensitivity being common to all genotypes− revealed that allele *Ppd-A1b* significantly delayed flowering time and reduced the duration of the GFP (Table 3). Allele 'GS-100' had the greatest effect on reducing VP and enlarging the GFP, while allele 'GS-105' caused intermediate effects. Genotypes carrying allele *Ppd-A1b* had a lower yield, due to a low number of spikes per m^2 and kernel weight, despite having a greater number of kernels per spike than those carrying any of the mutant alleles at this locus (Table 3). Among the latter, allele 'GS-100' led to greater grain yield and kernels per spike than allele 'GS-105'.

The site x allelic combination interaction was statistically significant for the length of the VP and GFP; however, it had a quantitative nature as the rank of thermal time for the three allelic combinations was identical in the three sites with the exception of the length of the GFP in Mexico, which was similar for the three allelic combinations (Table 4). The site x allelic combination interaction was not significant for yield or for yield components.

Table III.4. Means of the site x allelic combination interaction for the length from emergence to flowering (vegetative period), from flowering to maturity (grain filling period), yield components and yield of 25 durum wheat genotypes grown during two years at three contrasting latitudes. Genotypes carried different alleles at *Ppd-A1* and were monomorphic for allele a (causing photoperiod insensitivity) at *Ppd-B1*. GDD: growing-degree days.

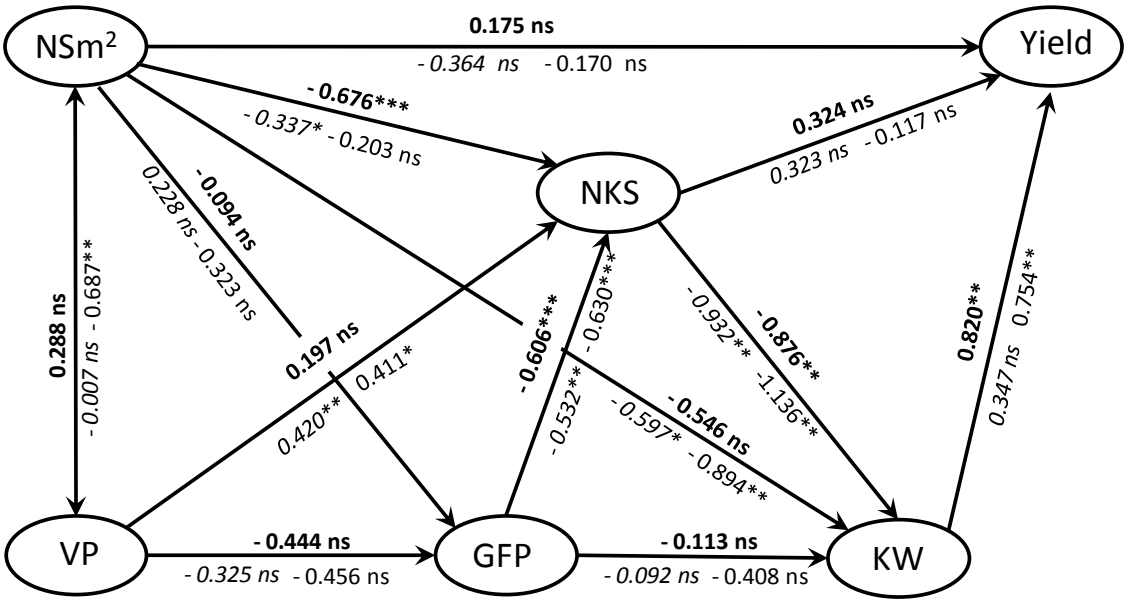
Site	*Ppd-A1b/ Ppd-B1a* (SI)	*GS105/ Ppd-B1a* (I5I)	*GS100/ Ppd-B1a* (I0I)
	Vegetative period (GDD)		
North Spain	1289 [a]	1220 [b]	1162 [c]
South Spain	1221 [a]	1056 [b]	980 [c]
Mexico	1177 [a]	1089 [b]	1060 [b]
	Grain filling period (GDD)		
North Spain	726 [b]	739 [b]	792 [a]
South Spain	778 [c]	847 [b]	893 [a]
Mexico	764 [a]	770 [a]	768 [a]

Means within rows for allelic combinations with the same letters are not significantly different at $P<0.05$ according to a Duncan's test.

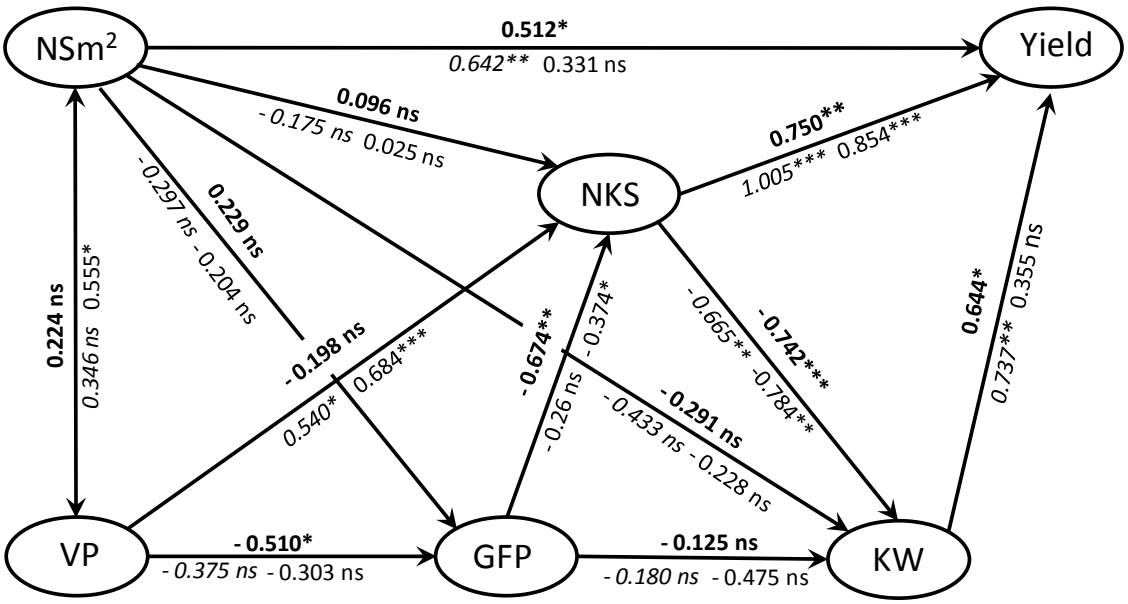
Path-Coefficient Analysis

Path-coefficient analysis was performed to dissect the interrelationships among traits and their indirect effects on grain-yield formation. This analysis provides an effective means of partitioning correlation coefficients into unidirectional pathway (direct effects) and alternate pathways (indirect effects). For this purpose, we used the cause-effect system based on the ontogeny of the durum wheat plant as described in García del Moral *et al*. (2003). The analysis was performed separately for each of the three sites, and for each of the three sets of genotypes carrying a common allelic combination regarding photoperiod sensitivity (Table 1). Diagrams of the path-coefficient analyses are shown in Fig. 2, and the correlation coefficients showing direct and indirect effects for the traits studied in Tables 5 to 8.

a) Spain-north



b) Spain-south



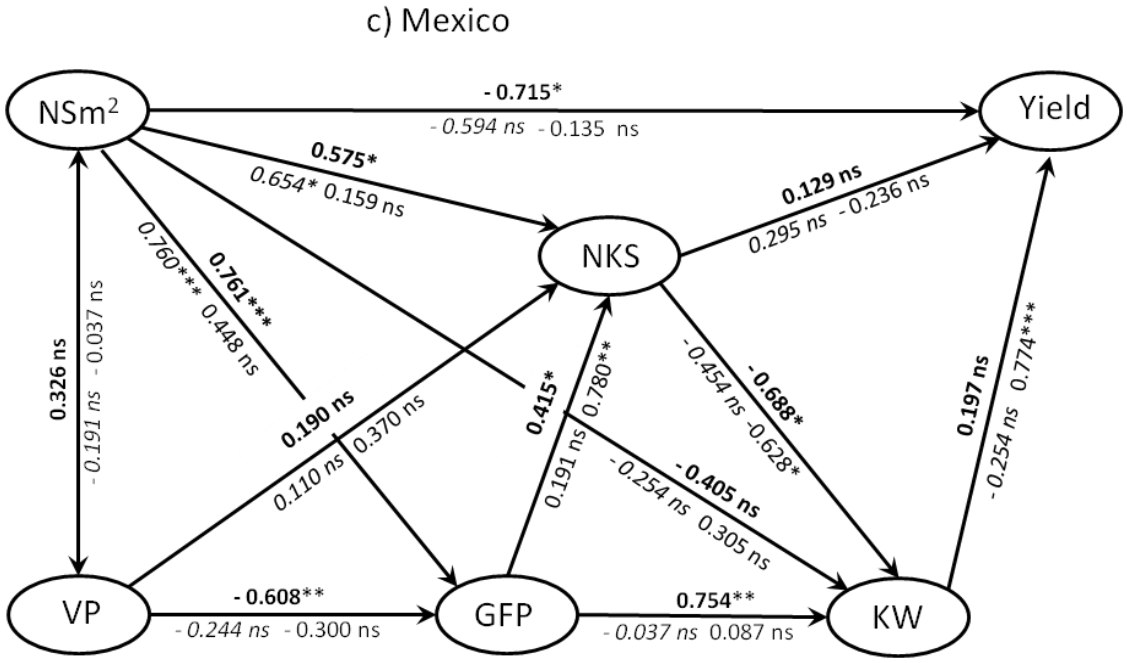


Fig. III.2. Path coefficient diagrams showing the direct effects between yield and number of spikes per m^2 (NSm^2), number of kernels per spike (NKS), kernel weight (KW), length of the vegetative period (VP, growing degree-days from emergence to flowering), and length of the grain filling period (GFP, growing degree-days from flowering to maturity) in: a) North of Spain, b) South of Spain, and c) Mexico (spring-planting). Single-headed arrows indicate path coefficients, and the double-headed arrows simple correlation coefficients. Values for the three allelic variants at *Ppd-A1* are indicated with different letter type as follows: *Ppd-A1b* (sensitive) bold, *Ppd-A1a* GS-105 (insensitive) italics, and *Ppd-A1a* GS-100 (insensitive) standard. In all cases the allele at *Ppd-B1* was *Ppd-B1a* (insensitive). *,**,*** significant at 0.05, 0.01, and 0.001 probability level, respectively.

In Spain-north grain yield of genotypes carrying alleles *Ppd-A1b* or 'GS-100' depended mainly on kernel weight, while for genotypes carrying allele 'GS-105' the influence of the three yield components was similar, although not significant (Fig. 2a). However, in Spain-south, the most important yield components in determining the variations in grain yield of genotypes carrying allele *Ppd-A1b* or 'GS-105' were the number of kernels per spike and kernel weight, followed by a somewhat smaller influence of the number of spikes per square meter (Fig. 2b). For genotypes carrying allele 'GS-100', grain production depended mainly on variations in the number of kernels per spike, without any significant influence from the other two yield components. In the spring planting of Mexico, the yield of genotypes carrying allele *Ppd-A1b* was negatively and significantly affected by the increase in the number of spikes per square meter, while the other two yield components exerted a positive but much smaller and not significant influence (Fig. 2c). At this site, yield components showed no significant effect on the yield of genotypes carrying allele 'GS-105', while kernel weight positively affected grain yield when allele 'GS-100' was present (Fig. 2c). The analysis of the direct and indirect effects for yield and its components indicated that, in most cases, the path analysis confirmed the results obtained by the simple correlation analysis,

given that the indirect effects were in general quite small (Table 5). However, when analyzing the relationship of some components with grain yield in genotypes carrying alleles *Ppd-A1b* or 'GS-105', the influence of indirect effects was important in Spain-south. In this case, the presence of a number of strong indirect effects caused certain direct effects to become statistically significant, while their corresponding correlation coefficients were not significant (Table 5). On the other hand, the direct effects of kernels per spike and kernel weight on grain yield were not significant in Mexico for the genotypes carrying allele *Ppd-A1b*, while the corresponding correlation coefficients were significant (Table 5).

The number of kernels per spike exerted a strong negative direct influence on kernel weight in all sites and for all three allelic combinations, independently of the length of the GFP, except for the genotypes carrying *Ppd-A1b* in Mexico for which the duration of the GFP exerted a positive direct effect on kernel weight (Fig. 2 and Table 6). The number of spikes per m^2 tended to negatively affect kernel weight, but this effect was significant only in Spain-north for genotypes carrying allelic variants conferring photoperiod insensitivity at *Ppd-A1*. Although not resulting in statistical significance, the effect of the number of spikes per square meter on kernel weight turned to be positive in Mexico for genotypes carrying allele 'GS-100' (Fig. 2c and Table 6). This effect appeared to be caused by an important indirect effect via kernels per spike at this site for this set of genotypes (Table 6). The analysis of indirect effects revealed that the effect of the number of spikes on kernel weight was affected by indirect effects on a distintc manner in Spain-north, where a positive indirect effect occurred through the number of kernels per spike, than in Mexico, where the indirect effect of the number of kernels per spike was negative. Moreover, in Spain-south, the sign and intensity of this indirect relationship varied with the allele present at *Ppd-A1* (Table 6). The length of the GFP determined a direct negative effect on kernel weight, although this effect was not significant, with the exception of genotypes carrying allele *Ppd-A1b* in Mexico, for which this influence was positive and highly significant (Fig. 2c and Table 6). The influence of indirect effects on kernel weight was relevant in several cases as they altered either the degree of significance or the sign of some correlation coefficients again due to a positive indirect effect of the number of kernels per spike in Spain, but a negative indirect effect in Mexico (Table 6).

The direct influence of spikes per square meter on the formation of the number of kernels per spike was negative and significant in Spain-north for genotypes carrying alleles *Ppd-A1b* or 'GS-105' (Fig. 2a), while it was not significant in Spain-south for any allelic combination (Fig. 2b) and resulted positive and significant in Mexico (Fig. 2c). The length of the GFP negatively and significantly influenced the number of kernels per spike for the three allelic combinations both in Spain-north and Spain-south, while this influence was positive under the environmental conditions of Mexico, although at this site it was not significant for genotypes carrying allele 'GS-105' (Fig. 2c and Table 7). For genotypes carrying allele *Ppd-A1b*, the length of the VP did not exert any significant influence on the number of kernels per spike in any of the three sites, while its effect was positive and significant for genotypes carrying alleles conferring photoperiod insensitivity at *Ppd-A1* when grown in Spain (Fig. 2a, b). However, in Mexico the direct effect of VP was not significant (Fig. 2c). On the basis of our

observations, it can be deduced that in almost all cases the magnitude of the indirect effects exerted on the number of kernels per spike was small, and therefore the path analyses for this yield component tended to confirm the results of the simple correlation study (Table 7). The only exception were the genotypes carrying allele 'GS-105' in Mexico, where a noticeable effect via indirect changes in spikes per square meter caused the lost of significance of the direct effect (Table 7).

The number of spikes per square meter did not exert any significant direct effect on the length of GFP in Spain, although this influence was positive and significant under the environmental conditions of the spring planting in Mexico (Fig. 2c). The examination of indirect effects (Table 8) revealed again that the indirect effects caused by the reciprocal interaction between these variables were of low magnitude, thus confirming the results from the simple correlation study.

Table III.5. Path-coefficient analysis for grain yield of 25 of durum wheat lines carrying different alleles at *Ppd-A1* and allele *a* causing photoperiod insensitivity at *Ppd-B1*, grown during two years at three contrasting latitudes.

Pathway	*Ppd-A1b/Ppd-B1a* (SI)			*GS105/Ppd-B1a* (I5I)			*GS100/Ppd-B1a* (I0I)		
	Spain-north	Spain-south	Mexico	Spain-north	Spain-south	Mexico	Spain-north	Spain-south	Mexico
Spikes per m^2 *vs* grain yield									
Direct effect, P_{26}	0.175 ns	0.512*	-0.715*	–0.364 ns	0.642**	-0.594 ns	–0.170 ns	0.331 ns	-0.135 ns
Indirect effect *via*									
kernels per spike, $r_{24}P_{46}$	–0.157	–0.019	0.112	0.149	0.126	0.232	0.056	0.463	-0.121
kernel weight, $r_{25}P_{56}$	–0.079	–0.184	-0.114	–0.065	–0.324	0.163	–0.260	–0.169	0.016
Correlation, *r26*	–0.061 ns	0.309 ns	-0.717***	–0.280 ns	0.444 ns	-0.199 ns	–0.374 ns	0.625**	-0.240 ns
yield									
Direct effect, P_{46}	0.324 ns	0.750**	0.129 ns	0.323 ns	1.005***	0.295 ns	–0.117 ns	0.854***	-0.236 ns
Indirect effect *via*									
spikes per m^2, $r_{24}P_{26}$	–0.085	–0.013	-0.623	0.168	0.081	-0.467	0.082	0.180	-0.069
kernel weight, $r_{45}P_{56}$	–0.450	–0.427	-0.106	–0.204	–0.471	0.173	–0.311	–0.210	-0.315
Correlation, *r46*	–0.211 ns	0.310 ns	-0.600**	–0.359 ns	0.615**	0.001 ns	–0.346 ns	0.824***	-0.620*
Kernel weight *vs* grain yield									
Direct effect, P_{56}	0.820**	0.644*	0.197 ns	0.347 ns	0.737**	-0.254 ns	0.754**	0.355 ns	0.774***
Indirect effect *via*									
spikes per m^2, $r_{25}P_{26}$	–0.017	–0.146	0.415	0.068	–0.283	0.381	0.059	–0.158	-0.003
kernel per spike, $r_{45}P_{46}$	–0.178	–0.497	-0.069	0.190	–0.641	-0.201	0.047	–0.504	0.096
Correlation, *r56*	0.625**	0.001 ns	0.543*	0.605**	–0.187 ns	-0.074 ns	0.860***	–0.307 ns	0.867***
Residual, *U*	0.752	0.780	0.676	0.756	0.484	0.929	0.498	0.445	0.388

*,**,*** significant at 0.05, 0.01, and 0.001 probability level, respectively.

Table III.6. Path-coefficient analysis for kernel weight of 25 of durum wheat lines carrying different alleles at *Ppd-A1* and allele *a* causing photoperiod insensitivity at *Ppd-B1*, grown during two years at three contrasting latitudes.

Pathway	*Ppd-A1b/Ppd-B1a* (SI)			*GS105/Ppd-B1a* (I5I)			*GS100/Ppd-B1a* (I0I)		
	Spain-north	Spain-south	Mexico	Spain-north	Spain-south	Mexico	Spain-north	Spain-south	Mexico
Spikes per m^2 *vs* kernel weight									
Direct effect, P_{25}	–0.546 ns	–0.291 ns	-0.405 ns	-0.597*	–0.433 ns	-0.254 ns	–0.894**	–0.228 ns	0.305 ns
Indirect effect *via*									
grain filling period, $r_{23}P_{35}$	0.025	–0.014	0.424	-0.021	0.077	-0.029	0.004	0.177	0.038
kernels per spike, $r_{24}P_{45}$	0.424	0.019	-0.600	0.431	–0.084	-0.358	0.544	–0.426	-0.323
Correlation, *r25*	–0.097 ns	–0.286 ns	-0.581*	-0.187 ns	–0.440 ns	-0.641**	–0.346 ns	–0.477 ns	0.020 ns
Grain filling period *vs* kernel weight									
Direct effect, P_{35}	–0.113 ns	–0.125 ns	0.754**	-0.092 ns	–0.180 ns	-0.037 ns	–0.408 ns	–0.475 ns	0.087 ns
Indirect effect *via*									
spikes per m^2, $r_{23}P_{45}$	0.121	–0.033	-0.228	-0.137	0.185	-0.204	0.009	0.085	0.134
kernels per spike, $r_{34}P_{45}$	0.481	0.424	-0.462	0.696	0.299	-0.307	0.823	0.524	-0.468
Correlation, *r35*	0.489 *	0.266 ns	0.064 ns	0.467 ns	0.304 ns	-0.548*	0.424 ns	0.134 ns	-0.247 ns
Kernel per spike *vs* kernel weight									
Direct effect, P_{45}	–0.876 **	–0.742***	-0.688*	-0.932**	–0.665**	-0.454 ns	–1.136**	–0.784**	-0.628*
Indirect effect *via*									
spikes per m^2, $r_{24}P_{25}$	0.265	0.008	-0.353	0.276	–0.054	-0.200	0.428	–0.123	0.157
grain filling period, $r_{34}P_{35}$	0.062	0.071	0.506	0.069	0.081	-0.025	0.296	0.317	0.064
Correlation, *r45*	–0.549 **	–0.663**	-0.536*	-0.587*	–0.638**	-0.679**	–0.412 ns	–0.590*	-0.407 ns
Residual, *U*	0.722	0.677	0.590	0.620	0.663	0.713	0.629	0.701	0.872

*,**,*** significant at 0.05, 0.01, and 0.001 probability level, respectively.

Table III.7. Path-coefficient analysis for kernels per spike of 25 of durum wheat lines carrying different alleles at *Ppd-A1* and allele *a* causing photoperiod insensitivity at *Ppd-B1*, grown during two years at three contrasting latitudes.

Pathway	*Ppd-A1b/Ppd-B1a* (SI)			*GS105/Ppd-B1a* (I5I)			*GS100/Ppd-B1a* (I0I)		
	Spain-north	Spain-south	Mexico	Spain-north	Spain-south	Mexico	Spain-north	Spain-south	Mexico
Vegetative period *vs* kernels per spike									
Direct effect, P_{14}	0.197 ns	–0.198 ns	0.190 ns	0.420**	0.540*	0.110 ns	0.411*	0.684***	0.370 ns
Indirect effect *via*									
spikes per m^2, $r_{12}P_{24}$	–0.195	0.021	0.188	0.002	–0.060	-0.125	0.139	0.014	0.006
grain filling period, $r_{13}P_{34}$	0.286	0.309	-0.149	0.174	0.127	-0.074	0.148	0.156	-0.221
Correlation, *r14*	0.288 ns	0.132 ns	0.229 ns	0.596**	0.607**	-0.089 ns	0.698**	0.854***	0.155 ns
Spikes per m^2 *vs* kernels per spike									
Direct effect, P_{24}	–0.676***	0.096 ns	0.575*	–0.337*	–0.175 ns	0.654*	–0.203 ns	0.025 ns	0.159 ns
Indirect effect *via*									
Vegetative period, $r_{12}P_{14}$	0.057	–0.045	0.062	–0.003	0.187	-0.021	–0.282	0.380	0.014
Grain filling period, $r_{23}P_{34}$	0.135	–0.077	0.234	–0122	0.114	0.154	0.006	0.139	0.341
Correlation, *r24*	–0.484*	–0.026 ns	0.871***	–0.462 ns	0.126 ns	0.787***	–0.479 ns	0.544*	0.514 ns
Grain filling period *vs* kernels per spike									
Direct effect, P_{34}	–0.606***	–0.674 **	0.415*	–0.532	–0.265 ns	0.191 ns	–0.630***	–0.374*	0.780**
Indirect effect *via*									
Vegetative period, $r_{13}P_{14}$	–0.093	0.091	-0.068	–0.137	–0.258	-0.042	–0.096	–0.285	-0.105
Spikes per m^2, $r_{23}P_{24}$	0.150	0.011	0.324	–0.078	0.074	0.527	0.002	–0.009	0.069
Correlation, *r34*	–0.549**	–0.572**	0.671**	–0.747	–0.449 ns	0.676**	–0.724**	–0.668**	0.744**
Residual, *U*	0.532	0.802	0.420	0.443	0.758	0.605	0.400	0.391	0.529

*,**,*** significant at 0.05, 0.01, and 0.001 probability level, respectively.

Table III.8. Path-coefficient analysis for de duration of grain filling period (measured as growing-degree days) of 25 of durum wheat lines carrying different alleles at *Ppd-A1* and allele *a* causing photoperiod insensitivity at *Ppd-B1*, grown during two years at three contrasting latitudes.

Pathway	*Ppd-A1b/Ppd-B1a* (SI)			*GS105/Ppd-B1a* (I5I)			*GS100/Ppd-B1a* (I0I)		
	Spain-north	Spain-south	Mexico	Spain-north	Spain-south	Mexico	Spain-north	Spain-south	Mexico
Vegetative period *vs* grain filling									
Direct effect, P_{13}	–0.444 ns	–0.510*	-0.608**	–0.325 ns	–0.375 ns	-0.244 ns	–0.456 ns	–0.303 ns	-0.300 ns
Indirect effect *via* spikes per m^2, $r_{12}P_{23}$	–0.028	0.051	0.248	–0.001	–0.103	-0.145	0.222	–0.114	0.016
Correlation, r_{13}	–0.472*	–0.459*	-0.360 ns	–0.326 ns	–0.478 ns	-0.389 ns	–0.234 ns	–0.417 ns	-0.284 ns
Spikes per m^2 *vs* grain filling period									
Direct effect, P_{23}	–0.094 ns	0.229 ns	0.761***	0.228 ns	–0.297 ns	0.760***	–0.323 ns	–0.204 ns	0.448 ns
Indirect effect *via* Vegetative period, $r_{12}P_{13}$	–0.128	–0.114	-0.198	0.002	–0.130	0.046	0.313	–0.169	-0.011
Correlation, r_{23}	–0.222 ns	0.115 ns	0.563*	0.230 ns0	–0.427 ns	0.806***	–0.010 ns	–0.373 ns	0.437 ns
Residual, *U*	0.877	0.860	0.594	0.917	0.833	0.541	0.944	0.893	0.848

*,**,*** significant at 0.05, 0.01, and 0.001 probability level, respectively.

DISCUSSION

Environmental

The dissection of the relationships between yield and yield components revealed qualitative differences between sites. Such differences may be interpreted in terms of environmental conditions, particularly photoperiod and temperature. The low temperature and short photoperiod registered in Spain-north before flowering were most likely responsible for genotypes having the longest VP at this site. Although showing the shortest GFP, the greatest yield was recorded in Spain-north, in agreement with the positive effect of VP lengthening the on grain yield reported in previous studies (Royo *et al*., 2000; Villegas *et al*., 2001). The lower yield in Spain-south when compared with Spain-north was due to a lower density of spikes, although the sowing rate was identical at both sites. More spikes per square meter in the north than in the south of Spain have been attributed in a previous study to higher nitrogen availability and more adequate rainfall in the former zone (García del Moral *et al*., 2003). These features may also have contributed to the higher yields observed in this study.

The long photoperiods and high temperatures occurring after the spring-planting in Mexico considerably reduced time to flowering, in agreement with the well known advance of the flowering date in wheat under long photoperiods and high temperatures (Rawson and Richards, 1993; Penrose and Martin, 1997; Ortiz-Ferrara *et al*., 1998; Kirby *et al*., 1999; Giunta *et al*., 2001). The high and steady temperatures recorded in Mexico was the reason for differences in thermal time between Mexico and the two Spanish sites were lower than those in the number of days to flowering. In Mexico, the latter variable was about half than in Spain-north and 40% lower than in Spain-south. The shorter cycle length in Mexico (111 days) compared with those of Spain-north (172 days) and Spain-south (159 days) may explain the small number of kernels per spike and the low kernel weight that resulted in the lowest yield in Mexico.

Allelic effect on yield and yield components

The effect of mutations causing photoperiod insensitivity at *Ppd-A1* on the length of the VP was consistent across sites and within each site, as allele 'GS-105' always reduced time to flowering less than allele 'GS-100' when compared with the wild type, in agreement with the results of previous studies (Maccaferri *et al*., 2008; Wilhelm *et al*., 2009). The positive effect of lengthening the VP on grain yield reported in the literature (Royo *et al*., 2000; Villegas *et al*., 2001) is in agreement with the results of this study for site comparisons, but not when comparing the effect of allelic combinations across sites and within each site, as the combination showing the longest VP (SI) consistently led to the lowest yield. In the three sites, flowering delay caused by allele *Ppd-A1b* resulted in a lower number of spikes per square meter. The low number of kernels per spike recorded in genotypes carrying insensitive alleles at *Ppd-A1* could be attributable to a shortening of the stem elongation phase and also to a reduction in the number of spikelets per spike, in agreement with the reported pleiotropic effects of insensitive *Ppd* alleles in advancing spikelet primordial initiation (Rawson and Richards, 1993) and reducing spikelets per spike (Snape *et al*., 2001; Dyck *et al*., 2004). Nevertheless, although having fewer kernels per unit area (18,020 and 19,130 kernels

per m^2 in allelic combinations I5I and I0I, respectively, compared with the 19,504 kernels per m^2 of allelic combination SI), the heavier kernels of the plants carrying the mutant alleles led to greater yields than the wild type.

Path-coefficient analyses

Regarding the associations among traits and with grain yield, path-coefficient analyses provided a different picture than simple correlations. According to the direct effects (path coefficients), the yield component that exerted the most direct influence on grain yield variations in Spain-north was kernel weight, while in Spain-south the number of kernels per spike and spikes per square meter had also a positive effect, as found in a previous study (García del Moral *et al*., 2003). The relevance of the number of kernels per spike on yield formation in Spain-south was maximized in the earliest genotypes, that is to say, in those carrying allele 'GS-100'. In this case, path analysis confirmed that, although the correlation coefficient showed a significant effect of the number of spikes per square meter, in these genotypes it was due to an indirect effect of the number of kernels per spike which was the yield components mostly affecting grain yield at this site. The significant relationship between kernels per spike and grain yield is in agreement with the findings of previous studies (Simane *et al*., 1993; García del Moral *et al*., 2003; Del Blanco *et al.*, 2001), and has been attributed to a sink limitation (Fischer, 1985). Such limitation may have occurred in Spain-south, where the number of kernels per square meter was 16% and 11% lower than the existing in Spain-north and Mexico, respectively. The greatest direct effect of the number of kernels per spike on yield observed in genotypes carrying insensitive alleles in Spain-south may be related to an increase in floral abortion, as lengthening the phase between booting and flowering has been reported to be associated with increased floret survival and kernels per spike (Isidro *et al*., 2011).

A positive direct effect of kernels per spike on yield was observed in Spain-south, where important indirect effects through both kernels per spike and kernel weight made the corresponding correlation coefficients change from non-significant to highly significant direct effects. The strong negative indirect effect of kernels per spike on kernel weight in Spain-south, observed in the three allelic combinations, indicate greater competition between kernels per spike and kernel weight in Spain-south than in the other two sites. The great importance of kernel weight in determining the variations in grain yield under the conditions of Spain-north coincide with the found by García del Moral *et al*. (2003) in a previous study with 25 durum wheat genotypes. In contrast, the number of spikes per square meter had much more influence on productivity in environments type as Spain-south, as we observed.

At the spring-planting site in Mexico, the direct effect exerted by kernel weight on grain yield was significant and positive only for the earliest-flowering genotypes, that is to say the ones carrying allele 'GS-100'. In this regard, in genotypes carrying alleles *Ppd-A1b* or 'GS-105' this direct effect was not significant despite the statistical significance of the corresponding coefficient of simple correlation for the genotypes carrying allele *Ppd-A1b* (Table 5). This change of significance in genotypes carrying allele *Ppd-A1b* was caused by a powerful indirect and positive effect *via* number of spikes per square meter, which caused the effect observed in the simple correlation

coefficient. This change could be explained by considering that the combination of large and constant photoperiods and smooth temperatures that occurred in Mexico during the VP decreased the competition between the number of spikes and kernels per spike that. This competition was strong in Spain-north for the late-flowering genotypes (those carrying alleles *Ppd-A1b* or 'GS-105') as revealed by the significant and negative direct effects between these two components at this site. These results could be consequence of the effect of the interaction between temperature and photoperiod on the duration of the phases of apical development (Kirby *et al*., 1987).

The results obtained in the spring planting experiment in Mexico revealed a different yield formation strategy in early- and late-flowering genotypes in this site. As mentioned above, the yield of genotypes carrying allele 'GS-100' relied mostly on kernel weight. As temperatures decreased at the end of the cycle in Mexico, the early-flowering genotypes benefited from warmer temperatures during the beginning of the GFP, thus avoiding the frost that may occur at the end of this period. Such a strategy resulted in heavy grains which significantly contributed to greater yields. In contrast, for the late-flowering genotypes carrying allele *Ppd-A1b*, a significant negative relationship was found between the length of VP and GFP. Among these genotypes, those with a longer GFP produced more kernels per spike (with a positive indirect effect of the number of spikes per square meter), and heavier kernels, while a high number of spikes per unit area was detrimental for yield, as reflected both by correlation and direct effect. This was a qualitative difference with the yield formation strategy observed in the Spanish sites in which the direct effect of the number of spikes on yield was not significant or positive.

The negative relationships of number of spikes per square meter on kernels per spike found for all allelic combinations in Spain-north are consistent with the results of Simane *et al*. (1993) in durum wheat and may indicate a compensatory effect between tiller production and apical development, arising from the negative allometry between these two traits during plant development (Hamid and Grafius, 1978). In contrast, the direct positive effect of the number of spikes on kernel number in Mexico indicates that the competition between the two yield components was low in this environment, probably due to the decreasing photoperiod. Moreover, the direct negative effect of the number spikes on yield in Mexico may denote that a large spike density (high sink-size) could not be properly managed by the plant, mostly by the latest flowering genotypes, which probably had a higher proportion of infertile spikes, which were waste of resources for the plants as they did not contribute to yield formation.

The correlation coefficient between spikes per square meter and kernel weight was not significant in Spain-north; however the path analysis demonstrated that it was due to a strong positive effect of the number of kernels per spike, a trait that increased as the genotypes were earlier to flower, thus resulting in negative significant direct effects. These observations may indicate that in Spain-north a greater number of spikes significantly reduced kernel weight, mainly in those genotypes with a longer GFP and a greater density of spikes. This observation contrasted with that in Mexico, in which more spikes per unit area did not affect final kernel weight due to an indirect negative effect of the number of kernels per spike, probably because kernels were very

light in Mexico. The negative influence of the number of kernels per spike on kernel weight found in the three sites and allelic combinations can be interpreted as of competition for a limited supply of resources during grain growth.

In genotypes carrying alleles 'GS-105' or 'GS-100', the length of the VP in Spain had a positive effect on the number of kernels per spike due to a positive indirect effect of the length of the GFP. This observation indicates that in early-flowering genotypes the length of the GFP indirectly contributed to a high number of kernels per spike. In contrast, in Mexico, the length of the VP did not affect the number of kernels per spike. This lack of effect was probably consequence of the short VP, which did not allow sufficient expression of the differences in the number of kernels per spike between allelic combinations. In contrast, a long GFP in Spain was negative for reaching a large number of kernels per spike. This effect has been associated to an increase in the abortion of pollinated florets after flowering due to cold temperatures (García del Moral *et al*., 2003). In contrast, in Mexico, with greater and more stable temperatures, a long GFP resulted in more kernels per spike as a result of an indirect positive effect of the spikes per square meter. In the spring-planting in Mexico a large number of spikes per square meter exerted a positive effect on the duration of the GFP, which in turn affected positively the number of kernels per spike. These results indicate that the number of spikes in Mexico was below the filling capacity and so the crop was sink-limited. Our results illustrate the negative relationship existing between the length of the periods before and after flowering (Knott and Gebeyehou, 1987; Blum, 1996), in addition to very few indirect effects *via* other yield components. In fact, the risk of low temperatures and/or drought during the grain filling period is a critical issue when determining the sowing date on a given environment (Loss and Siddique, 1994) because of the impact of environmental factors in reducing grain weight and yield in the areas of durum wheat production.

From the examination of indirect effects it can be deduced that, in general, the greatest influence of such effects on determining yield and yield components occurred in the most unfavorable environments, *i.e.* Spain-south and Mexico. In contrast, because the less-limiting environment of Spain-north the influence of indirect effects was less intense. The low compensatory effects observed in the most favorable environment are in agreement with a previous study (García del Moral *et al*., 2005), and can be attributed to a greater availability of water and nitrogen during the critical phases of plant development and a longer VP caused by the interaction of short photoperiods and low temperature. In the more stressed environments of Spain-south and Mexico the organogenesis of yield components was probably restricted as a result of increased competition for limited resources.

CONCLUSIONS

This study demonstrates the effect of flowering date, associated with different allelic variants at *Ppd-A1*, on yield formation in three environments with contrasting patterns of temperature and photoperiod, through de dissection of the inter-relationships between the length of pre- and post-flowering period, the main yield components and the yield itself. The intricate relationships between yield and its components could be

properly analyzed though path analyses that in some cases, revealed relationships indiscernible by simple correlation analyses.

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DISCUSIÓN GENERAL

1. Introducción

Esta Tesis Doctoral es fruto de la colaboración entre equipos del IRTA, el IFAPA y la Universidad de Granada en España y el Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) de México. El disponer de un equipo multidisciplinar en diversas localizaciones ha sido fundamental para el desarrollo del germoplasma, la caracterización molecular y el fenotipado en ambientes contrastantes.

El **material vegetal** utilizado consiste en una colección de genotipos de trigo duro (*Triticum turgidum* L. var. *durum*) fruto de la selección divergente llevada a cabo durante varios años en México y España dentro de la progenie de cruzamientos entre parentales con distinta fecha de floración. Los genotipos tardíos procedieron de la Universidad de Hohenheim en Alemania y los precoces del CIMMYT. El proceso seguido para la selección del germoplasma utilizado se detalla en el apartado de materiales y métodos del capítulo II-2.3. A diferencia de las poblaciones diseñadas habitualmente para el mapeo e identificación de QTL (*Recombinant Inbred Lines*, RILs o *Near-Isogenic lines*, NILs) (Anderson *et al.,* 2011), el germoplasma evaluado en esta Tesis Doctoral corresponde a una serie de familias relacionadas que han sido seleccionadas en condiciones naturales en ambientes diversos. Por tanto, cabe esperar una mayor variabilidad genética en este material vegetal que en una población de RILs debido a que procede de un número de parentales mayor. Sin embargo, las líneas de fecha de floración divergente seleccionadas dentro de cada cruzamiento tampoco entrarían en la categoría de NILs por el hecho de que las hermanas fueron separadas en F_4, cuando la homocigosis teórica alcanza un 87,5% (Fehr, 1983).

La **caracterización molecular** del material vegetal utilizado en esta Tesis Doctoral se llevó a cabo en el laboratorio molecular de CIMMYT y consistió en primer lugar en la determinación del hábito de crecimiento (invernal o primaveral), mediante el análisis de loci *Vrn-A1*, *Vrn-B1* y *Vrn-B3*. La composición alélica para genes de respuesta al fotoperíodo en trigo duro (loci *Ppd-A1* y *Ppd-B1*) se determinó en base a diversos tipos de marcadores moleculares, tal como se describe en el capítulo II-2.4. En el germoplasma estudiado se observó que, entre los alelos que confieren insensibilidad al fotoperíodo − ambos mutaciones del alelo original responsable de la sensibilidad

(Wilhelm *et al*., 2009)−, la frecuencia del alelo GS-105 fue mayor que la del alelo GS-100 (34% y 20%, respectivamente). Se ha especulado que el alelo GS-105 podría ser una mutación más antigua que la que dio lugar al alelo GS-100, que solo ha sido identificado en variedades modernas (Bentley *et al*., 2011). Por otra parte, la frecuencia del alelo original en la colección es del 46%, lo que concuerda en términos evolutivos con lo sugerido por Innan y Kim (2004), en cuanto a que los alelos originales tienen una alta frecuencia en comparación con cada una de las mutaciones recientes. Dado que todos los genotipos resultaron ser de hábito de crecimiento primaveral, se establecieron las combinaciones alélicas para *Ppd-1* lo que dio lugar a un desequilibrio en el número de genotipos portadores de cada una de ellas. En particular la combinación GS-100/*Ppd-B1b* apareció en un único genotipo (el testigo 'Mexa') que no fue tenido en cuenta en los análisis de resultados.

El **fenotipado** se llevó a cabo durante los años 2007 y 2008 en experimentos de campo, en un amplio rango de latitudes con diferentes regímenes de fotoperíodo y temperatura. Este enfoque resulta particularmente novedoso y aporta consistencia a los resultados obtenidos, ya que la mayoría de los estudios publicados sobre el efecto del fotoperíodo y la temperatura sobre la fenología del trigo se han realizado bajo condiciones controladas, generalmente en invernaderos con diferentes tratamientos de luz y temperatura. Sin embargo, el enfoque utilizado en esta Tesis Doctoral presenta una gran dificultad en la interpretación de los resultados, mucho más sencilla cuando se trata de condiciones controladas. Los experimentos correspondientes a los capítulos I-1.3 y II-2.3 se llevaron a cabo en localidades situadas en cuatro latitudes: Lleida (Norte de España, 41°38´N), Jerez de la Frontera (Sur de España, 37°0´N), Ciudad Obregón (Norte de México, 27°21´N) y Texcoco (Sur de México, 19°31´N), mientras que para el capítulo III solamente se incluyeron tres de las localidades al no disponer de la totalidad de los datos necesarios en los experimentos de Ciudad Obregón.

En el capítulo I se estudia el efecto de los factores ambientales sobre la fenología del cultivo y el rendimiento y su formación. En el capítulo II se analiza en profundidad el efecto de los genes *Ppd-1* y se estima el efecto de *Eps* sobre la duración del período emergencia-antesis y antesis-madurez en un rango de ambientes. Dado el mayor efecto sobre la fecha de floración de la composición alélica en *Ppd-A1* que en *Ppd-B1*,

en el capítulo III se diseccionan los efectos directos e indirectos de la composición alélica para *Ppd-A1* en genotipos portadores del alelo *Ppd-B1a* sobre el rendimiento y sus componentes.

2. Caracterización de los ambientes

Dado el rango de latitudes utilizado en esta Tesis Doctoral para el fenotipado de la colección de genotipos, uno de los primeros aspectos que se estudió fue la identificación de las variables ambientales que más diferían entre localidades. Los resultados del capítulo I-1.4 muestran que las principales variables ambientales que distinguieron entre las mismas fueron las temperaturas máxima y mínima entre siembra y antesis −de las que el efecto localidad explicó más del 98% de su variabilidad− y la duración del día hasta floración. Es conocido que la latitud integra una serie de variables, entre las cuales las más relevantes para el desarrollo del trigo son la temperatura (Craufurd y Wheeler, 2009) y el fotoperíodo (Laurie *et al*., 1995). Si bien el efecto de la radiación fue significativo, su relevancia fue mucho menor para explicar las diferencias entre localidades. La temperatura media registrada en el periodo previo a floración aumentó en dirección norte-sur lo que dio lugar a un acortamiento del ciclo en las localidades de menor latitud.

Un aspecto fundamental que debe tenerse en cuenta en la interpretación de los resultados experimentales es que, a diferencia del resto de localidades, el cultivo en el Batán se llevó a cabo en ciclo de verano, por lo que tanto el fotoperíodo como la temperatura fueron decreciendo en esta localidad a lo largo del ciclo de cultivo. Las localidades España-Norte y México-Sur fueron las más contrastantes en términos ambientales caracterizándose la primera por una mayor longitud del día y superior temperatura mínima durante el llenado y la última por su mayor temperatura y duración del día hasta floración. La localidad México-Norte se caracterizó por las altas temperaturas después de la floración.

3. Efecto del ambiente sobre el desarrollo fenológico

Los resultados contenidos en los distintos capítulos muestran que el ambiente (como adición de la suma de cuadrados de los efectos año, localidad y año x localidad) explicó consistentemente alrededor del 95% de la variabilidad existente en el número de días hasta floración. Sin embargo, cuando la medición se realizó en grados-día se comprobó que el ambiente solamente explicaba el 42% de la variabilidad observada en la fecha de floración. El hecho de que el tiempo térmico integre tanto el efecto de la temperatura como el del número de días confirma que las divergencias observadas en el efecto del ambiente sobre el ciclo hasta floración expresado en número de días o en tiempo térmico fueron debidas en gran parte al contraste de temperaturas entre las localidades de ensayo, como corroboran los resultados del análisis de componentes principales que se presenta en el capítulo I-1.4. La relación entre temperatura y velocidad de desarrollo se conoce desde hace mucho tiempo y está ampliamente documentada en la literatura (Halse y Weir, 1970; Warrington *et al*., 1977; Porter y Gawith, 1999). Este resultado concuerda con lo planteado por McMaster *et al.* (2008; McMaster, 2009) que demostraron que la temperatura tiene un fuerte impacto sobre el ciclo celular y las tasas de crecimiento y desarrollo a lo largo del ciclo de vida de la planta de trigo. Por otra parte, el hecho de que las temperaturas máxima y mínima expliquen una buena parte de las diferencias entre localidades, coincide con los estudios de Chmielewski y Rötzer (2002), quienes demostraron que en Europa la floración se retrasa proporcionalmente hacia el Norte, dependiendo en gran medida de las temperaturas primaverales (Siebert y Ewert, 2012).

El segundo factor ambiental que permitió diferenciar entre localidades fue el distinto patrón de fotoperíodo observado entre las mismas. En la figura II-3, se demuestra que las diferencias en la duración hasta floración entre los alelos en *Ppd-A1* fueron mayores en las localidades donde el fotoperíodo medio es menor a 12 h. Klaimi y Qualset (1973), trabajando con trigo de primavera e invernal, observaron que a partir de alrededor de 14 h-luz las diferencias en fecha de floración entre genotipos sensibles e insensibles al fotoperíodo se minimizaban, maximizándose por debajo de 11h. Diversos estudios más recientes utilizan un rango de 14-16 h-luz para definir el día largo (Allard *et al*., 2012; Bentley *et al*., 2013; Turner *et al*., 2013), en contraposición a

8h como día corto. Wilhelm *et al*. (2009) también encontraron que la expresión de *Ppd-A1a* aumenta en días cortos.

4. Relación genotipo–fenotipo

El control genético de la fecha de floración ha sido ampliamente estudiado en la especie modelo *Arabidopsis thaliana* (Greenup *et al*., 2009; Wellmer y Riechmann, 2010), en la que se han identificado alrededor de 60 genes involucrados en el proceso de floración (Ehrenreich *et al*., 2009). La mayoría de ellos han sido detectados como QTL bajo condiciones controladas (Ehrenreich *et al*., 2009) y en algunos casos no han podido verificarse en condiciones de campo (Weining *et al*., 2002; Brachi *et al*., 2010; Anderson *et al*., 2011). Sin embargo, ha sido recientemente cuando se han intensificado los estudios dedicados la identificación de genes asociados al control de la fecha de floración en cultivos agrícolas como trigo, arroz o cebada (Jung y Müller, 2009; Casao, 2011). En trigo duro se han publicado muchos menos estudios que en trigo harinero (*Triticum aestivum* L.) y entre ellos destaca el publicado recientemente por Sanna *et al*. (2014) en el que estudiaron la interacción de diferentes tratamientos de vernalización y fotoperíodo en condiciones controladas, llegando a identificar 15 QTLs involucrados en la duración de las distintas fases de pre-floración.

El efecto de los genes *Ppd-1* sobre el desarrollo fenológico del cultivo se estudió en el capítulo II. Los resultados muestran que el porcentaje de la variabilidad debida al efecto genotipo explicada por las diferencias entre combinaciones alélicas para la duración del período emergencia-floración fue de 65%. Este resultado sumado al hecho de que el material vegetal es de hábito primaveral, sugiere que el resto de variabilidad puede atribuirse a los genes *Eps*.

La floración más tardía obtenida en las combinaciones alélicas portadoras del alelo *Ppd-A1b* en comparación con las portadoras del alelo *Ppd-B1b* sugiere un mayor efecto de los alelos que confieren sensibilidad al fotoperíodo en *Ppd-A1* que en *Ppd-B1 (Ppd-A1b > Ppd-B1b)*. De igual forma se observó que los alelos responsables de la insensibilidad al fotoperíodo en *Ppd-A1* (GS-105 y GS-100) tuvieron un efecto mayor sobre la fecha de floración que el alelo *Ppd-B1a.* Ello permitió establecer que en lo referente a sus efectos: *Ppd-A1a* > *Ppd-B1a*, lo cual está en línea con lo publicado por

Shaw *et al*. (2012) en trigo hexaploide. En esta especie diversos estudios han evaluado los efectos de los genes *Ppd-A1a* y *Ppd-B1a* con los de *Ppd-D1a* y su interacción (Scarth y Law, 1984; Worland *et al.,* 1998; Tanio y Kato, 2007; Díaz *et al*., 2012), lo cual no es directamente comparable con los resultados obtenidos en trigo duro.

El alelo *Ppd-A1b* fue el que confirió una mayor variabilidad en la fecha de floración lo que condujo a la hipótesis de que en este estudio el efecto del gen *Ppd-A1* fue suficientemente potente como para causar una precocidad a floración cercana al máximo alcanzable en la especie como consecuencia de un efecto evolutivo basado en la poliploidización, lo cual podría explicar las menores diferencias observadas entre los genotipos portadores de *Ppd-A1a*. Los resultados de Shaw *et al.* (2012) en trigo harinero van en la línea de esta interpretación.

La combinación alélica que dio lugar a una fecha de floración más temprana fue GS-100/*Ppd-B1a* y el alelo GS-100 fue entre los causantes de insensibilidad al fotoperíodo el de mayor efecto (GS-100>GS-105>*Ppd-B1a*), en concordancia con lo publicado anteriormente en trigo duro (Clarke *et al.,* 1998; Maccaferri *et al.,* 2008; Wilhelm *et al.,* 2009; Bentley *et al.,* 2011).

El acortamiento observado hasta antesis causado por la presencia de genes *Ppd-1* responsables de la insensibilidad al fotoperíodo tuvo un efecto inverso, si bien de menor magnitud, sobre la duración del llenado del grano, observándose una correlación negativa entre la duración de ambos periodos. Ello puede interpretarse en términos fisiológicos debido a que en ambientes donde las temperaturas al final del ciclo son muy elevadas (como las registradas en los ambientes de siembra otoñal de esta Tesis Doctoral), en muchos casos acompañados de déficit hídrico (como en que ocurre en las dos localidades españolas), la finalización del ciclo viene marcada por las condiciones ambientales que aceleran la senescencia del cultivo (Royo *et al*., 2009). Sin embargo, diversos estudios (Bogard *et al.,* 2011; Kamran *et al.,* 2013) han detectado QTLs que afectando a la fecha de floración del trigo tienen a su vez un efecto sobre la duración del período de llenado del grano.

Las diferencias en fenología entre genotipos portadores de la misma combinación alélica podrían atribuirse a efectos de los genes de *Eps*. Estudios previos han

demostrado que los genes *Eps* pueden afectar significativamente la fecha de floración incluso en presencia de genes mayores de vernalización y fotoperíodo (van Beem *et al.,* 2005). En el capítulo II se hace un intento de estimar su efecto utilizando para ello los resultados de la localidad de El Batán donde la duración del día fue aproximadamente de 14 h, lo cual puede indicar que las necesidades de fotoperíodo estuvieron cubiertas. La significación observada para los genes *Ppd* en esta localidad no son sorprendentes en vista de las recientes conclusiones de un estudio publicado recientemente (Chen *et al.*, 2014) en el que plantas portadoras del alelo GS-100 resultaron ser sensibles al fotoperíodo. El efecto estimado de los genes *Eps* fue incluso mayor que el causado por los genes mayores de respuesta al fotoperíodo en la localidad del sur de España en la que la expresión de los mismos fue máxima, lo que pone de manifiesto la existencia de genes de *Eps* en el germoplasma utilizado en esta tesis Doctoral.

5. Efecto del ambiente y la dotación alélica para *Ppd-1* sobre el rendimiento y sus componentes

Los efectos del ambiente y el genotipo sobre el rendimiento y su formación se analizan en esta Tesis Doctoral en los capítulos I y III. En el primero de ellos el enfoque es meramente ambiental, mientras que en el tercero se evalúa la interacción entre las variantes alélicas en *Ppd-A1* en presencia del alelo *Ppd-B1a*.

Los resultados del capítulo I-1.4 permitieron identificar de forma empírica y dentro de los ambientes estudiados las condiciones de fotoperíodo y temperatura que permitieron alcanzar un número de granos por unidad de superficie que no limitase el rendimiento grano. Los ambientes en los que las temperaturas mínimas medias diarias antes de floración fueron inferiores a 6,9°C favorecieron la formación de un gran número de granos, que también se vio beneficiado cuando estas fueron superiores a 10,8°C y acompañadas de fotoperíodos superiores a 14 h-luz diarias durante el llenado del grano. Un número de granos por unidad de superficie inferior a 14.000 granos/m^2 limitó el rendimiento en los ambientes de estudio. La relevancia de este resultado, si bien únicamente aplicable en los ambientes de estudio, queda puesta de manifiesto por la mayor importancia que tiene el número de granos que el tamaño de los mismos en la determinación del rendimiento (Peltonen-Sainio *et al*., 2007), si bien se conocen

mecanismos de compensación entre ambos componentes (García del Moral *et al*., 2003). De los resultados presentados en el capítulo III puede deducirse que el mayor rendimiento obtenido en España-Norte se debió fundamentalmente a un mayor número de granos por unidad de superficie; sin embargo en España-Sur el peso del grano fue el componente de mayor peso sobre el rendimiento. Sin embargo en el sur de México, donde el número de granos por unidad de superficie fue 12% superior al obtenido en el sur de España, el rendimiento fue un 8% inferior debido a un bajo peso del grano. La compensación entre el número de granos y el peso de los mismos se observó también entre las combinaciones alélicas, ya que la combinación GS-105/*Ppd-B1a*, que dio lugar al menor número de granos (18.020 granos/m^2) fue la que dio lugar a su vez a los granos de mayor peso (42,1 mg), mientras que la combinación *Ppd-A1b*/*Ppd-B1a* que produjo un 8,2% más de granos por unidad de superficie, resultó en granos con un peso un 9,7% inferior a la anterior. Sin embargo, el número de granos por unidad de superficie es producto a su vez de otros dos componentes: el número de espigas por unidad de superficie y el número de granos por espiga. Los resultados de la tabla III-3 muestran que también hubo una compensación entre ambos, tal como han demostrado diversos estudios (Hewstone, 1997; García del Moral, *et al*., 2003; Laurila *et al*., 2012).

Algunos trabajos previos han demostrado la importancia del número de granos sobre el rendimiento y señalado la importancia que tienen las condiciones ambientales antes y después de la floración sobre el mismo (Abbate *et al*., 1995; Ugarte *et al*., 2007; Fisher, 2011). Sin embargo, a diferencia de trabajos previos, este estudio llegó a identificar dentro de los ambientes objeto del mismo, las condiciones de temperatura y fotoperíodo que dieron lugar a un menor número de granos por unidad de superficie y el umbral que hizo de éste componente un factor limitante para el rendimiento. Además en el capítulo I se demuestra que niveles de radiación durante el llenado del grano inferiores a 1,8 kJ $grano^{-1}$y $día^{-1}$ limitaron el peso del grano y, a su vez, el rendimiento. Este resultado explica la mayor influencia del peso del grano en la determinación del rendimiento en el norte de España que en el sur, resultado del análisis por coeficientes de sendero que se presenta en el capítulo III y ya descrito en estudios previos (García del Moral *et al*., 2003; Moragues *et al*., 2006).

En el capítulo III se analizan los efectos directos e indirectos que sobre el rendimiento grano ejercen sus componentes y la duración del ciclo hasta floración y después de esta, intermediados por las variantes alélicas en *Ppd-A1* en presencia del alelo *Ppd-B1a*. Por coherencia con estudios previos sobre el mismo tema (García del Moral *et al*., 2003; 2005) la duración del período emergencia-floración se designa en ese capítulo como periodo vegetativo (VP). Los resultados indicaron que los genotipos portadores del alelo que confiere sensibilidad al fotoperíodo en *Ppd-A1* tuvieron un mayor ciclo hasta floración y, consecuentemente una menor duración del llenado del grano. La consecuencia observada en los componentes del rendimiento fue un menor número de espigas por unidad de superficie y peso del grano pero un mayor número de granos por espiga. Un estudio previo mostró que la relación entre la duración del ciclo hasta floración y el rendimiento grano en trigo duro dependió en gran medida de las condiciones ambientales, ya que en ambientes fríos los genotipos tardíos tendieron a producir un menor número de espigas, mientras que en los ambientes cálidos el efecto fue el contrario (García del Moral *et al*., 2003). El menor número de granos por espiga registrado en los genotipos portadores de alelos de insensibilidad al fotoperíodo en *Ppd-A1* se atribuyó a los efectos pleiotrópicos de dichos alelos sobre la iniciación de los primordios de espiguilla y el número de espiguillas por espiga (Rawson y Richards, 1993; Snape *et al*., 2001; Dyck *et al*., 2004). El menor peso del grano de los genotipos portadores del alelo *Ppd-A1b* puede atribuirse a la menor duración del llenado de su grano, ya que el peso del grano puede expresarse como producto de la tasa media de llenado y la duración del mismo, por lo que una menor duración afecta negativamente al peso final del grano (Royo *et al*., 2000; Àlvaro *et al*., 2008).

Los resultados del análisis por coeficientes de sendero indicaron que los efectos indirectos y lo efectos compensatorios entre componentes del rendimiento fueron menos significativos en ambientes menos limitantes como los del norte de España. Este resultado puede atribuirse a que en esta localidad existen mejores condiciones de humedad y disponibilidad de nutrientes en períodos críticos (García del Moral *et al*., 2003). Estas variaciones de efectos directos e indirectos entre localidades también han sido identificadas en trigo de invierno (Akanda y Mundt, 1996) y trigo sintético (Cooper *et al*., 2012).

La siguiente figura resume las relaciones existentes en cada localidad entre el número de días transcurridos entre emergencia y floración y el rendimiento de grano como promedio de los ensayos llevados a cabo en 2007 y 2008 con los 34 genotipos estudiados en el capítulo II para las combinaciones alélicas *Ppd-A1b/Ppd-B1b* (●), *Ppd-A1b/Ppd-B1a* (○), GS-105/*Ppd-B1b* (▲), GS-105/*Ppd-B1a* (△) y GS-100/*Ppd-B1a* (+):

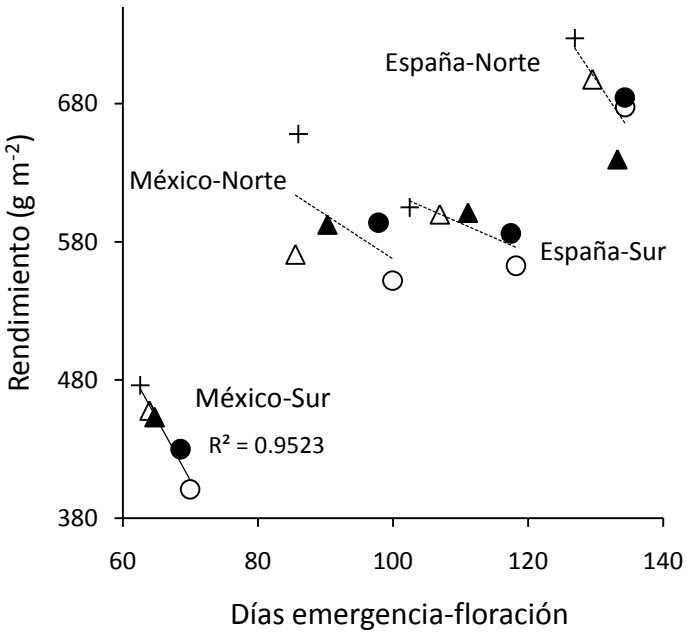

Figura 1. Relación entre el número de días para el período emergencia-floración y el rendimiento de grano de 34 genotipos de trigo duro. Cada punto representa el valor medio de los ensayos realizados los años 2007 y 2008 de las combinaciones alélicas identificadas para los genes *Ppd-1* (*Ppd-A1b/Ppd-B1b* (●), *Ppd-A1b/Ppd-B1a* (○), GS-105/*Ppd-B1b* (▲), GS-105/*Ppd-B1a* (△) y GS-100/*Ppd-B1a* (+), en el Norte-Sur de España y México respectivamente.

Los resultados muestran que la precocidad a floración fue favorable para la consecución de elevados rendimientos en todos los ambientes de estudio en los que la combinación alélica que dio lugar a mayor rendimiento fue en todos los casos GS-100/*Ppd-B1a* y la que dio lugar a menor rendimiento fue *Ppd-A1b/Ppd-B1a* en todas las localidades excepto en el norte de España donde la combinación GS-105/*Ppd-B1b* dio lugar a los menores rendimientos. Estos resultados explican el éxito de la introducción de genes de insensibilidad al fotoperíodo en el germoplasma CIMMYT en un gran rango de latitudes y ha permitido identificar las variantes alélicas que dan lugar a un ajuste fenológico óptimo en cada de las localidades de este estudio.

6. Aplicaciones a los programas de mejora de trigo duro

A partir de la identificación de los alelos de insensibilidad al fotoperíodo en *Ppd-A1* publicada por Wilhelm *et al*. en 2009 se han realizado diferentes estudios para profundizar en el conocimiento de su efecto en condiciones controladas. Sin embargo, no se han encontrado en la bibliografía referencias a estudios en condiciones de campo que incluyan el efecto combinado de los genes *Ppd-1* en trigo duro. Esto pone en valor al trabajo realizado en esta Tesis Doctoral y permite una mayor aplicabilidad de los resultados obtenidos en los programas de mejora.

La identificación de 12 horas-luz como longitud media del día antes de la floración por debajo de la cual aumenta la expresión de las variantes alélicas GS-105 y GS-100, puede ser de utilidad de cara a la introducción del trigo duro en latitudes donde hasta ahora no ha sido cultivado. Otro de los resultados de gran interés para la mejora es la clasificación en función del efecto de cada alelo sobre la fecha de floración (GS-100>GS-105>*Ppd-B1a*). Ambos resultados pueden ser de utilidad como herramientas que permitan a los programas de mejora el diseño de genotipos portadores de las variantes alélicas más adecuadas para cada ambiente objetivo, de manera que adelantando o retrasando la floración puedan evitarse en parte los diferentes tipos de estrés a los que se enfrenta el cultivo de trigo duro.

Desde un punto de vista adaptativo, los resultados de esta Tesis Doctoral sugieren que, incluso en las siembras otoñales y en trigos duros de hábito de crecimiento primaveral, pueden ser necesarias diversas estrategias de mejora para gestionar las diferencias asociadas a la latitud en los factores que limitan el rendimiento del trigo. Los resultados obtenidos sugieren que el rendimiento del trigo duro puede aumentarse de manera eficiente basando la selección en el peso del grano en ambientes objetivo situados en latitudes norte, pero focalizando en el número de granos por unidad de superficie en las situadas más al sur. Sin embargo, es importante destacar que esta divergencia en la estrategia de mejora asociada a la latitud no debe entenderse de manera exclusiva sino como orientación sobre los componentes en los que hacer mayor énfasis en la selección, ya que ambos componentes del rendimiento

están interrelacionados en la formación del mismo y la optimización del rendimiento en cada latitud pasa por que exista un balance entre los mismos.

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CONCLUSIONES

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De los resultados obtenidos en la presente Tesis Doctoral pueden obtenerse las siguientes conclusiones:

1. Las principales variables ambientales que permitieron distinguir entre localidades fueron la temperatura hasta floración, que incrementó en dirección norte-sur, y la duración del día durante el período de llenado del grano que disminuyó en la misma dirección.
2. La duración del período comprendido entre emergencia y antesis se redujo en dirección norte-sur.
3. La expresión fenotípica de la insensibilidad en *Ppd-A1* aumentó en localidades en las que el fotoperíodo medio hasta floración fue inferior a 12 h.
4. Los genotipos que florecieron antes debido a la presencia de alelos confiriendo insensibilidad al fotoperíodo alargaron la duración del llenado del grano pero en menor medida que la disminución causada en la fecha de floración.
5. Las combinaciones alélicas para genes *Ppd-1* explicaron el 65% de la variabilidad inducida por el efecto del genotipo en la fecha de floración, atribuyéndose el 35% restante al efecto de genes de precocidad intrínseca (*Eps*).
6. La combinación alélica causante de la mayor precocidad a floración fue consistentemente la *Ppd-A1* GS-100/*Ppd-B1a*.
7. El efecto del alelo que confiere sensibilidad al fotoperíodo en *Ppd-A1* fue mayor que el de *Ppd-B1* (*Ppd-A1b*>*Ppd-B1b*).
8. De acuerdo a su efecto sobre la fecha de floración, los alelos que confieren insensibilidad al fotoperíodo se clasificaron como GS-100>GS-105>*Ppd-B1a*.
9. Se detectó interacción entre los genes *Ppd-A1* y *Ppd-B1*.
10. Para el rango de ambientes considerados el número de granos fue limitante para el rendimiento cuando su valor fue inferior a 14.000 granos m^{-2}, lo que ocurrió en ambientes con temperaturas medias de las mínimas diarias por encima de 6,9°C antes de floración e inferiores a 10,8°C durante el llenado del grano, acompañadas de fotoperíodos inferiores a 14,2 h.

11. Niveles de radiación durante el llenado del grano inferiores a 1,8 kJ grano^{-1} día^{-1} limitaron el peso del grano, proporcionando una explicación a la mayor influencia del peso del grano en la determinación del rendimiento en latitudes altas en comparación con otras más bajas.
12. Entre los genotipos portadores del alelo de insensibilidad en *Ppd-B1* (*Ppd-B1a*), los portadores del *Ppd-A1b* dieron lugar a menores rendimientos en todos los ambientes debido a un menor número de espigas por unidad de superficie y un menor peso del grano a pesar de tener un mayor número de granos por espiga.
13. En la siembra de primavera de la localidad de Batán el rendimiento de los genotipos portadores de la combinación alélica GS-100/*Ppd-B1a* dependió fundamentalmente del peso del grano mientras que un elevado número de espigas por unidad de superficie fue negativo para el rendimiento de los genotipos portadores de la combinación *Ppd-A1b/Ppd-B1a*.
14. Los efectos compensatorios entre componentes del rendimiento fueron menores en ambientes favorables que en los más limitantes.