Universitat de Lleida

Dissecting genetic changes on wheat adaptation, yield formation and grain quality in Mediterranean environments

Joan Subirà Fillol

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Universitat de Lleida

TESI DOCTORAL

Dissecting genetic changes on wheat adaptation, yield formation and grain quality in Mediterranean environments

Joan Subirà Fillol

Memòria presentada per optar al grau de Doctor per la Universitat de Lleida
Programa de Doctorat en Ciència i Tecnologia Agrària i Alimentària

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Universitat de Lleida
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Departament de Producció Vegetal i Ciència Forestal

Doctoral Thesis

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Joan Subirà Fillol

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Dissecting genetic changes on wheat adaptation, yield formation and grain quality in Mediterranean environments

Memoria de Tesis Doctoral presentada por:

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Para optar al grado de Doctor en el programa *Doctorat en Ciència i Tecnologia Agrària i Alimentària*

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千里の道も一歩から

sen ri no michi mo ippo kara

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RESUM

ABSTRACT

Wheat, and particularly durum wheat, is a typical Mediterranean crop, and the products derived from it are in the base of the 'Mediterranean Diet' pyramid. The fast growth of the world population requires increasing the productivity of the new wheat varieties, even in areas with the largest expected effects of climate change, like the Mediterranean Basin. In addition, they will have to reach the quality standards requested by the manufacturing industry.

The objective of this PhD Thesis was to increase the current knowledge on wheat yield formation and grain quality, as well as to assess the effect of the meteorological variables on them. With this purpose two sets of germplasm were assembled: an historical series of 24 durum wheat genotypes representatives of the released in Spain and Italy through the 20th century, and a set of 20 bread wheat varieties largely grown in Spain in the last decades and representative of the four groups of industrial quality.

The study of the effect of the dwarfing *Rht-B1b* allele on durum wheat aerial and root biomass and its effects on yield formation showed that the reduction of root biomass was, in relative terms, larger than that of aerial biomass (28% and 7.6%, respectively). Moreover, it revealed that an increase of root biomass in semi dwarf wheats did not contribute to raise neither harvest index nor grain yield.

The analysis of the changes caused by durum wheat breeding on grain quality showed that the quality index of the EU increased by 6.2% through the 20^{th} century (0.13% y^{-1} and 0.06% y^{-1} in Italian and Spanish cultivars, respectively). It was mostly due to the increases in gluten strength (32%, largely due to the incorporation of the combination LMW-GS_{aaa} of low molecular weight glutenin subunits), and the semolina yellow colour (10%), which compensated the 10% of decrease in grain protein content. In bread wheat, the high molecular weight glutenin subunits (HMW-GS) with the greatest positive effect on gluten strength were 1 and 2* at *Glu-A1* locus, 7^{oe}+8 and 17+18 at *Glu-B1* and 5+10 at *Glu-D1*.

The assessment of the environmental effect on durum wheat yield and quality showed that the semi dwarf cultivars, carrying the *Rht-B1b* allele, had a

better response than landraces to water availability during grain filling. The analysis of the effects of variety replacement on yield stability revealed that wheat breeding caused a loss of stability viewed from the static approach, but not from the dynamic point of view. The meteorological variables with the largest effect on durum wheat yield were reference evapotranspiration before anthesis and water input during grain filling.

The analysis of the relationship between yield and quality in bread wheat showed that the high quality varieties (included in groups 1 and 2 of the official classification) tended to have a longer cycle length to anthesis and a lower yield that those of low quality (groups 3 and 4) in high yielding Mediterranean environments. The meteorological variables with the largest effect on yield and quality of bread wheat were reference evapotranspiration before anthesis and the maximum temperature between anthesis and physiological maturity.

RESUMEN

El trigo, particularmente el trigo duro, es una especie típica de la cuenca Mediterránea y los productos de él derivados se encuentran en la base de la pirámide de la ‘dieta mediterránea’. El rápido crecimiento de la población mundial hace necesario incrementar la productividad de las nuevas variedades de trigo, incluso en zonas donde se prevén los mayores efectos del cambio climático, como es el caso de la cuenca mediterránea. Además deberán alcanzar los estándares de calidad requeridos por la industria transformadora.

El objetivo de la presente Tesis Doctoral fue contribuir al conocimiento de la formación del rendimiento del trigo y la calidad del grano, así como del efecto que las variables ambientales ejercen sobre ambos. Para ello se utilizó una serie histórica de 24 genotipos de trigo duro, representativos de los cultivados en España e Italia a lo largo del pasado siglo, así como un conjunto de 20 variedades trigo harinero de entre las más cultivadas en España en las últimas décadas, representativas de los cuatro grupos de calidad industrial.

El estudio del efecto del alelo *Rht-B1b*, causante de enanismo, sobre la biomasa aérea y radicular del trigo duro y sus efectos en la formación del rendimiento demostró que la reducción de la biomasa radicular fue, en términos relativos, mayor que sobre la biomasa aérea (28% y 7,6%, respectivamente). Además reveló que un incremento de la biomasa radicular en variedades semienanas no contribuyó a incrementar ni el índice de cosecha ni el rendimiento.

El análisis de los cambios causados por la mejora genética sobre la calidad del grano mostró que el índice de calidad de la UE para trigo duro creció un 6,2% a lo largo del siglo XX (0,13% año^{-1} y 0,06% año^{-1} en los cultivares italianos y españoles respectivamente), causado fundamentalmente por los incrementos en la fuerza del gluten (32%, debido en gran medida a la incorporación de la combinación LMW-GS$_{aaa}$ de subunidades de gluteninas de bajo peso molecular) y el color amarillo de la sémola (10%), que compensaron una disminución del 10% en el contenido de proteína del grano. En trigo harinero las subunidades de gluteninas de alto peso molecular (HMW-GS) con

un mayor efecto positivo sobre la fuerza del gluten fueron la 1 y 2* en el locus *Glu-A1*, la 7^{oe}+8 y la 17+18 en el *Glu-B1* y la 5+10 en el *Glu-D1*.

El examen del efecto del ambiente sobre la producción y calidad del trigo duro mostró que los cultivares modernos, portadores del alelo *Rht-B1b*, tienen una mejor respuesta que los cultivares antiguos a la disponibilidad de agua durante el llenado del grano. El análisis de los efectos de la sustitución varietal sobre la estabilidad del rendimiento demostró que la mejora varietal causó una pérdida de estabilidad desde el punto de vista estático, pero no desde el punto de vista dinámico. Las variables meteorológicas que más afectaron al rendimiento del trigo duro fueron la evapotranspiración antes de antesis y el aporte hídrico durante el llenado del grano.

El análisis de la interdependencia entre el rendimiento y la calidad del trigo harinero demostró que las variedades de alta calidad (grupos 1 y 2 de la norma de calidad de los trigos) tienden a tener un ciclo más largo hasta antesis y un menor rendimiento que las de baja calidad (grupos 3 y 4) en ambientes de medio y alto potencial productivo. Las variables meteorológicas con un mayor efecto sobre el rendimiento y la calidad del trigo harinero fueron la evapotranspiración de referencia antes de antesis y la temperatura máxima entre antesis y madurez fisiológica.

RESUM

El blat, especialment el blat dur, és un cultiu típicament mediterrani i els productes que se'n deriven es troben en la base de la piràmide de la 'dieta mediterrània'. El ràpid creixement de la població mundial fa necessari incrementar la productivitat de les noves varietats de blat, inclús en les zones on es preveuen els majors efectes del canvi climàtic, com és el cas de la conca mediterrània. A més, és necessari que assoleixin els estàndards de qualitat requerits per la industria transformadora.

L'objectiu de la present Tesi Doctoral ha estat contribuir al coneixement de la formació del rendiment del blat i la qualitat del gra, així com avaluar l'efecte que les variables meteorològiques exerceixen sobre ambdós. Per això es va utilitzar una sèrie històrica de 24 genotips de blat dur, representatius dels cultivats a España i Itàlia al llarg del segle XX, així com un conjunt de 20 varietats de blat fariner triades entre les més cultivades a España durant les últimes dècades i representatives dels quatre grups de qualitat industrial.

L'estudi de l'efecte de l'al·lel *Rht-B1b*, causant de nanisme, sobre la biomassa aèria i radicular del blat dur i els seus efectes en la formació del rendiment va demostrar que la reducció de la biomassa radicular va ser, en termes relatius, major que sobre la biomassa aèria (28% i 7,6%, respectivament). A més revelà que un increment de la biomassa radicular en las varietats seminanes no va contribuir a incrementar l'índex de collita ni el rendiment.

L'anàlisi dels canvis causats per la millora genètica sobre la qualitat del gra de blat dur va demostrar que l'índex de qualitat de la UE va créixer un 6,2% al llarg del segle XX (0,13% any^{-1} i 0,06% any^{-1} en els cultivars italians i espanyols respectivament), causat fonamentalment pels increments en la força del gluten (32%, degut majoritàriament a la incorporació de la combinació LMW-GS$_{aaa}$ de subunitats de glutenines de baix pes molecular) i el color groc de la sèmola (10%), que van compensar una disminució del 10% en el contingut de proteïna del gra. En blat fariner les subunitats de glutenines d'alt pes molecular (HMW-GS) amb un major efecte positiu sobre la força del gluten

van ser la 1 i la 2* en el locus *Glu-A1*, la 7^{oe}+8 i la 17+18 en el *Glu-B*1 i la 5+10 en el *Glu-D1*.

L'examen de l'efecte de l'ambient sobre la producció i la qualitat del blat dur va mostrar que els cultivars moderns, portadors de l'al·lel *Rht-B1b*, tenen una millor resposta que els cultivars antics a la disponibilitat d'aigua durant l'ompliment del gra. L'anàlisi dels efectes de la substitució varietal sobre l'estabilitat del rendiment va demostrar que la millora varietal va causar una pèrdua d'estabilitat des del punt de vista estàtic, però no des del punt de vista dinàmic. Les variables meteorològiques que més van afectar al rendiment del blat dur van ser l'evapotranspiració abans d'antesi i l'aportació hídrica durant l'ompliment del gra.

L'anàlisi de la interdependència entre el rendiment i la qualitat del blat fariner va demostrar que les varietats d'alta qualitat (grups 1 i 2 de la norma de qualitat dels blats) tendeixen a tenir un cicle més llarg fins antesi i un menor rendiment que les de baixa qualitat (grups 3 i 4) en ambients de mig i alt potencial productiu. Les variables meteorològiques amb un major efecte sobre el rendiment i la qualitat del blat fariner van ser l'evapotranspiració de referència abans de l'antesi i la temperatura màxima entre antesi i maduresa fisiològica.

INTRODUCCIÓN GENERAL

INTRODUCCIÓN GENERAL

Importancia del trigo en el contexto mundial

El trigo (*Triticum* spp.) es, junto con el arroz y el maíz, uno de los tres principales cultivos a escala mundial que en su conjunto proporcionan el 60% de las calorías para la alimentación humana. Desde su domesticación en el Creciente Fértil hace unos 10.000 años, el trigo ha sido un alimento esencial para las civilizaciones del sur de Europa, oeste de Asia y norte de África (región conocida como SEWANA, por sus siglas en inglés).

Las principales especies cultivadas del género *Triticum* son, siguiendo la nomenclatura de MacKey (MacKey, 2005), el trigo harinero (*T. aestivum* L. ssp. *aestivum* '*vulgare*' [Vill.] MK), especie hexaploide (2n=42), portadora de los genomas AABBDD y el trigo duro (*T*. *turgidum* (L.) Thell. ssp. *Durum* (Desf.) Husn.), especie tetraploide (2n = 28), portadora de los genomas AABB (Carrillo, 2012; Acquaah, 2007). Ambas especies alcanzaron en 2016 una superficie global de 222 Mha, con una producción de 735 Mt (FAS USDA, 2016), de las cuales 36 Mt correspondieron a trigo duro (IGG, 2016).

Los principales productores de trigo en el mundo son China, India, Estados Unidos y Rusia, que en conjunto produjeron 336 Mt en 2014 (FAOSTAT, 2016). El trigo harinero es uno de los cultivos más importantes de la Unión Europea (UE) donde anualmente se cultivan alrededor de 24 Mha (EUROSTAT, 2016). Los principales productores europeos son Francia, Alemania, Polonia, Rumania y España, que en conjunto suponen cerca del 60% de la producción total (EUROSTAT, 2016). Durante el periodo 2000-2016, la superficie anual media dedicada a este cultivo en España fue de 1,52 Mha, obteniéndose 4,88 Mt, con un rendimiento medio de 3,22 t por ha. La Unión Europea es además el principal productor de trigo duro dado que existe una larga tradición de este cultivo en la cuenca Mediterránea. En orden decreciente de producción siguen Canadá, Turquía, Estados Unidos y México (IGG, 2016). La superficie dedicada al trigo duro en la UE durante el año 2015 fue de 2,40 Mha con una producción total de 8,52 Mt (EUROSTAT, 2016), siendo Italia el primer productor seguido de Grecia, Francia y España. En Italia, el área dedicada al cultivo experimentó un gran aumento desde los años 30 del siglo pasado (Bozzini *et al*., 1998) y actualmente la producción de trigo duro se concentra en las regiones del sur del país (ISTAT, 2016), cuyo clima se caracteriza por las elevadas temperaturas y escasez de lluvia típicas del ambiente Mediterráneo. Durante el periodo 2000-2016, la producción media y el área dedicada al trigo duro en Italia fue de 4,23 Mt y 1,47 Mha respectivamente (ISTAT, 2016), lo cual supuso un rendimiento medio de 2,88 t por ha. El cultivo del trigo duro en España se concentra fundamentalmente en zonas de secano, principalmente en las comunidades autónomas de Andalucía y Aragón (Royo y Briceño-Félix, 2011) en las que se obtiene el 95% de la producción total. Durante el periodo 2000-2016, la producción media y el área de cultivo de trigo duro fueron de 1,35 Mt y 0,6 Mha respectivamente (AETC, 2016; MAAMA, 2016), dando lugar a un rendimiento medio de 2,24 t por ha, un 22% inferior al que se obtuvo en Italia durante el mismo periodo. La gran importancia del trigo duro en la cuenca mediterránea pone de manifiesto la necesidad de obtener cultivares bien adaptados a las condiciones Mediterráneas del sur de Europa y el norte de África, caracterizadas por lluvias irregulares y altas temperaturas durante el llenado del grano, al efecto de poder continuar aumentando los niveles de producción y calidad en el futuro.

El rendimiento y sus componentes

Los componentes del rendimiento del trigo se establecen de forma secuencial a lo largo del desarrollo de la planta, bien antes de la antesis (número de espigas), durante la misma (número de granos por espiga) o durante el llenado del grano (peso del grano) (Hobbs y Sayre, 2001; García del Moral *et al*., 2003, 2005).

El número final de espigas depende de la combinación de dos variables, el número de tallos producidos y la proporción de los mismos que dan lugar a espiga (García del Moral *et al*., 2003, 2005). La iniciación y supervivencia de los hijuelos depende del genotipo, el hábito de crecimiento del trigo (invernal o primaveral), la presencia de genes de enanismo, las prácticas agronómicas como la fecha de siembra o la fertilización nitrogenada y las condiciones ambientales durante la fase de ahijamiento (Duggan *et al*., 2000).

El número potencial de granos por espiga se determina durante el desarrollo de los primordios florales antes de la antesis, pero el número final de granos queda fijado cuando parte de las flores son fecundadas dando lugar a granos que inician su desarrollo. Aunque se trata de un carácter bajo control genético, el número de granos por espiga depende mucho de las condiciones ambientales durante la formación de la espiga y la antesis. Las heladas tardías después de la emergencia de la espiga pueden causar el aborto de muchas espiguillas, sobre todo las de la parte apical de la espiga. La formación de granos por espiga puede verse afectada por prácticas agronómicas como la fertilización nitrogenada, la cual afecta positivamente a este proceso (Ewert y Honermeier, 1999).

El peso final de los granos es el último componente del rendimiento que se forma en el trigo. Éste es el resultado del transporte y depósito de asimilados desde las zonas de síntesis (básicamente la fotosíntesis de los órganos verdes por encima del nudo de la hoja bandera) y de almacenamiento provisional (nudos del tallo y pedúnculo de la espiga, principalmente). Se trata de un componente relativamente estable (Royo *et al*., 2006) debido a la compensación que se produce entre fotosíntesis y translocación de reservas acumuladas en preantesis en el tallo y otros órganos vegetativos sobre todo en ambientes secos y calurosos (Blum, 2011).

La calidad del grano de trigo

La mejora de la calidad es indispensable para satisfacer las demandas de la industria transformadora, así como los gustos cambiantes de los consumidores en lo que respecta a la pasta y al pan (Bushuk, 1998). La calidad del trigo viene determinada por características tanto del grano cómo de la sémola o harina que se obtiene en el proceso de molturación. Muchos de los atributos tecnológicos del grano cómo el color amarillo de la sémola y la calidad de la proteína y, en menor cantidad, el contenido de proteína, están bajo control genético aunque se pueden ver modificados por el manejo del cultivo y los factores agroclimáticos que le afecten durante la campaña (Peña y Pfeiffer, 2005). Además, estos factores ambientales tienen un gran impacto en la acumulación de biomasa durante el llenado del grano y afectan al almacenamiento de almidón en el grano (Jenner *et al*., 1991).

El trigo duro se consume en Europa principalmente como pasta, a pesar de que en la cuenca Mediterránea son comunes otros productos derivados del mismo, cómo el cuscús, el bulgur y ciertos panes planos (Peña, 2002), destacando la gran variedad existente de panes tradicionales en Italia que requieren características específicas de calidad de la sémola (Pasqualone, 2012). Para poder garantizar la aptitud tecnológica de la materia prima para la elaboración de todos estos productos el grano de trigo duro debe cumplir ciertos requisitos en lo que refiere al peso específico, la dureza y vitrosidad del grano, el tamaño de la partícula tras la molienda, la intensidad del color amarillo y el contenido y calidad de la proteína (Peña *et al*., 2002; Peña y Pfeiffer, 2005; Sissons, 2008). La UE reglamentó en 2003 el uso del índice de calidad del trigo duro que se basa en el contenido de proteína, la fuerza del gluten, el índice de color amarillo y el peso del grano. Este índice se expresa como porcentaje de la calidad general de cada variedad en referencia a variedades testigos seleccionadas entre las más cultivadas en cada país y se utiliza de forma generalizada para caracterizar las variedades según su calidad global (Nazco *et al*., 2012).

El peso específico del grano y por lo tanto el rendimiento de molturación, están determinados principalmente por la dureza y el peso del grano. En el caso del trigo duro, éste se ve afectado además por la falta de vitrosidad debida a la presencia de manchas blancas y de aspecto almidonoso en el grano (berrendeo). El color de la sémola y la harina es debido a las xantofilas presentes en el grano, lo cual viene determinado por múltiples genes con efectos aditivos siendo además una característica altamente heredable (Pagnotta *et al*., 2005; Ruiz *et al*., 2005). Sin embargo, la vitrosidad del grano, carácter de relevancia para determinar la calidad del trigo duro, está fuertemente afectada por el ambiente (Carrillo *et al*., 2006) y en caso que ésta no sea suficientemente elevada, puede perjudicar la extracción de sémola así como su color y uniformidad (Zarco-Hernández *et al*., 1999; Ruiz *et al*., 2005).

El trigo harinero se destina principalmente a la elaboración de distintos tipos de pan, aunque también se emplea en una amplia gama de productos como pizzas, galletas y pasteles (Peña *et al*., 2002). Con el fin de poder obtener productos de alta calidad resulta esencial conseguir ciertos niveles de proteína en grano, fuerza del gluten así como garantizar unos niveles reducidos de actividad enzimática para evitar la degradación del almidón, con lo que se pueden garantizar unas propiedades reológicas de la masa y una alta calidad del producto final (Trethowan, 2001; Carrillo *et al*., 2006; Shewry, 2009).

El contenido en proteína del grano de trigo suele oscilar entre el 8% y el 17%. La proteína del grano está constituida por albúminas y globulinas, las cuales llevan a cabo principalmente actividades enzimáticas en el citoplasma celular, y por gliadinas y gluteninas, almacenadas en el endospermo. Estos dos tipos de proteínas se conocen con el nombre de prolaminas y son las que forman el gluten, representando alrededor del 80% de las proteínas del endospermo del grano (Peña, 2002; Carrillo *et al*., 2006, Sissons, 2008). La composición y proporción de gluteninas y gliadinas son los principales determinantes de las características viscoelásticas del gluten y por tanto de su calidad culinaria (Weegels *et al.,* 1996), tanto en trigo duro como en harinero. Las gluteninas confieren elasticidad al gluten mientras que las gliadinas son las responsables de su viscosidad y extensibilidad.

La fracción de gluteninas es la principal responsable de la fuerza del gluten (Peña, 2002) y las diferentes subunidades presentes pueden ser separadas según su movilidad mediante electroforesis en gel de poliacrilamida con dodecil-sulfato sódico (SDS-PAGE) (Singh *et al*., 1991), obteniéndose con ello las subunidades de gluteninas de alto peso molecular (*High Molecular Weight*, HMW-GS) y las de bajo peso molecular (*Low Molecular Weight*, LMW-GS), siendo estas últimas las que representan el 80% del total de gluteninas

(Peña *et al*., 2002; Ruiz *et al*., 2005; Sissons, 2008). El perfil electroforético de estas proteínas ha sido utilizado para la identificación de variedades, análisis genealógico y caracterización de poblaciones (Ruiz *et al*., 2012, 2013) ya que ofrecen una medida de la diversidad genética entre poblaciones y dentro de las mismas (Nevo y Payne, 1987).

En el trigo duro la síntesis de HMW-GS está controlada por el complejo de genes *Glu-A1* y *Glu-B1*, localizados en los brazos largos del grupo 1 de cromosomas homólogos (Singh y Shepherd, 1988; Shewry *et al.*, 1992). Los loci responsables de las LMW-GS son el *Glu-A3*, *Glu-B3* y el *Glu-B2* (Carrillo *et al*., 2000), localizados en los brazos cortos del grupo 1 de cromosomas homólogos. En trigo harinero, además de los loci anteriores, también se encuentra el locus *Glu-D1* de HMW-GS y el *Glu-D3* en lugar del *Glu-B2* en el caso de las LMW-GS (Carrillo *et al*., 2006). Mientras que en el caso del trigo harinero las HMW-GS son las principales determinantes de las características cualitativas deseadas, para el trigo duro las LMW-GS tienen una mayor influencia en la calidad final (Peña, 2000; Ruiz *et al*., 2005; Appelbee, 2006), pero en ambos casos es necesaria la combinación óptima de subunidades para la obtención de cultivares de alta calidad (Carrillo *et al*., 2006).

La fuerza del gluten (resultado de la composición y proporción de las prolaminas), es un carácter deseado para la calidad del trigo duro cuya determinación suele realizarse mediante el test de sedimentación SDS (Axford *et al*., 1978). Las propiedades reológicas de la masa de trigo harinero están determinadas tanto por la composición y cantidad de la proteína cómo por la calidad del gluten de la misma (Sliwinski *et al*., 2004; Edwards *et al.,* 2007) y resulta indispensable conocer estas características para poder obtener pan de alta calidad. Uno de los métodos más populares para determinar estas propiedades en Europa es el que utiliza el alveógrafo Chopin, que proporciona una simulación del comportamiento de la masa durante la fermentación debido al efecto de las levaduras. El método Chopin permite obtener parámetros de gran interés como la resistencia ante la deformación de la masa o tenacidad (*P*), su capacidad para ser extendida o extensibilidad (*L*) y la energía de deformación (*W*) necesaria durante el proceso (Carrillo *et al*., 2006). Las características de la masa se pueden ver seriamente perjudicadas por la acción de la α–amilasa en granos germinados en campo, enzima que degrada el almidón para dar lugar a azúcares sencillos. Este efecto se puede determinar en laboratorio mediante el método *Falling number* (Perten, 1964), el cual permite identificar harinas que presentan una alta actividad del enzima α–amilasa y tienen una baja calidad panadera, dando lugar a panes con un escaso volumen de hogaza y una mala estructura de la miga (Trethowan, 2001; Carrillo *et al*., 2006).

A lo largo del siglo pasado se han identificado ciertas subunidades como la 6 o 7+8 en el locus *Glu-B1*, la 2+4+15+19 en el *Glu-B3* y la 12 en el *Glu-B2* que son altamente recomendables para obtener trigos duros con un gluten fuerte (Sissons *et al*., 2005), mientras que en el caso de trigo harinero las subunidades 5+10 en el locus *Glu-D1* y la 1 o 2* en el *Glu-A1* han demostrado tener efectos positivos en la fuerza de la masa (Branlard *et al*., 2003).

Influencia del ambiente sobre el rendimiento y la calidad del trigo

El rendimiento es un carácter complejo determinado por factores genéticos dependientes del genotipo de la planta e influenciado por las condiciones agronómicas y ambientales a lo largo del ciclo de cultivo. El ambiente, como por ejemplo la disponibilidad hídrica y su distribución, el estrés térmico o las prácticas agronómicas, afecta tanto al rendimiento final como a sus componentes constitutivos: número de espigas por unidad de superficie, número de granos por espiga y peso del grano (García del Moral *et al*., 2003; Anderson, 2010; De Vita *et al*., 2010).

Por lo que respecta a la calidad del trigo, los efectos del ambiente son también muy importantes, si bien su grado de influencia varía según la característica de calidad considerada (Williams *et al*., 2008; Vázquez *et al*., 2012; Pinheiro *et al*., 2013). Existen numerosos estudios sobre los efectos de las condiciones ambientales y de manejo del cultivo sobre la calidad del trigo (Ames *et al*., 1999; Rharrabti *et al*., 2003b; Carcea *et al*., 2006; Clarke *et al.,* 2009; Flagella *et al*., 2010; Ercoli *et al*., 2011). Sin embargo, dada su especificidad, resulta esencial profundizar en su estudio con el fin de conocer, y así poder evitar, los efectos negativos sobre el grano, la harina y la sémola.

La interacción genotipo x ambiente (GE por sus siglas en inglés) puede definirse desde un punto de vista biológico o estadístico. Para el primero la interacción GE es debida a la diferente contribución o nivel de expresión de los genes que regulan una determinada característica en distintos ambientes (Basford y Cooper,1998). Desde el punto de vista estadístico, la interacción GE se define como el cambio que experimenta la respuesta de un genotipo para un determinado carácter cuando se cultiva en distintos ambientes. La interacción GE es uno de los mayores retos para los programas de mejora ya que reduce el avance genético al disminuir la correspondencia genotipo-fenotipo. Sin embargo, también permite identificar ambientes para los que ciertos genotipos presentan adaptación específica (Romagosa *et al*., 2008). Con el fin de seleccionar de forma eficiente un carácter de interés y evitar errores durante la selección de líneas en un programa de mejora, resulta necesario conocer las contribuciones relativas tanto del genotipo, como del ambiente y de la interacción GE (Becker y Léon, 1988), la cual puede ser estudiada mediante múltiples métodos aparecidos, principalmente, durante la segunda mitad del siglo XX (Flores *et al*., 1998). Se ha demostrado que el impacto de la mejora genética depende en gran medida del nivel de conocimiento de los mejoradores sobre los factores genéticos y ambientales que causan la interacción GE así como de su importancia en el sistema genotipo-ambiente (Basford y Cooper, 1998). La combinación alélica en las HMW-GS y las LMW-GS puede determinar hasta un 50% de la variabilidad fenotípica en la fuerza de la masa y su extensibilidad (Eagles *et al*., 2002), por lo que esta información puede ser una herramienta de gran interés para la selección en un programa de mejora de la calidad. Sin embargo, con el fin de definir una estrategia eficiente para la mejora de la calidad, resulta necesario determinar en qué medida el ambiente interfiere en su expresión y así poder entender las interacciones GE e identificar fuentes de estabilidad.

Un cultivar puede mostrar un desempeño similar independientemente de cualquier variación en las condiciones ambientales o una respuesta predecible que no presente desviación respecto a la respuesta a los ambientes de estudio (Becker y Léon, 1988). Estos conceptos se conocen como estabilidad estática y dinámica respectivamente, y su estudio es de gran importancia para los programas de mejora en las zonas de clima Mediterráneo, ya que éstas presentan una alta variación interanual en el rendimiento y la calidad (Rharrabti *et al*., 2003a). La estabilidad del rendimiento resulta un carácter muy importante para garantizar unos niveles mínimos de producción que aseguren la viabilidad del cultivo de trigo en una determinada zona. Por su parte, una alta calidad y estabilidad de la materia prima en un cultivar de trigo son muy deseadas por las industrias de molienda y harino-panaderas, ya que permiten garantizar una materia prima que se comporte de forma predecible durante los procesos de transformación a que será sometida posteriormente.

Perspectiva histórica de la mejora genética del trigo

La Revolución Verde y los genes de enanismo

Los inicios de la mejora genética vegetal se remontan a los principios de la agricultura, ya que desde la domesticación del cultivo del trigo, hace unos 10.000 años, los agricultores seleccionaban las mejores espigas para sembrar la siguiente campaña. Sin embargo, la investigación sistemática en genética y citogenética no comenzó hasta principios del siglo pasado (Borlaug, 2005).

En Europa las actividades de mejora de trigo harinero y de trigo duro se iniciaron en Italia con mejoradores como Nazareno Strampelli quien realizó el primer cruzamiento intraespecífico en 1900. Durante las primeras décadas del siglo pasado, la mejora vegetal se basó en estos primeros cruzamientos y en programas de selección dentro de las poblaciones autóctonas. En 1916, Strampelli obtuvo la primera variedad de talla reducida a partir de la introgresión de los genes de enanismo *Rht8* y *Rht9* procedentes de la variedad japonesa ‘Akagomughi’ (Borghi, 2001). Sin embargo, la disminución de la talla no fue un objetivo de la mejora hasta que las prácticas agronómicas mejoraron con la generalización del uso de fertilizantes químicos.

Durante la segunda mitad del siglo XX la productividad de los cultivares de trigo experimentó un gran incremento gracias al efecto combinado de la introducción de los genes de enanismo y de la insensibilidad al fotoperiodo. Ello revolucionó la producción de trigo en las décadas de los 60 y 70 dando lugar a la Revolución Verde (Borlaug, 2007). La introgresión de alelos mutantes de una serie de loci agrupados en el complejo *Rht*, con efecto enanizante en trigo permitió intensificar las técnicas de cultivo, fundamentalmente incrementar las dosis de abonado nitrogenado y agua de riego (Hedden, 2003; Borlaug, 2007) y conseguir así mayores rendimientos. Los genes de enanismo utilizados por Norman Borlaug son el *Rht-B1* (denominado inicialmente como *Rht1*) y el *Rht-D1* (conocido en sus inicios como *Rht2*) (Worland y Snape, 2001). La presencia del alelo *b* en estos loci produce insensibilidad al ácido giberélico junto con diversos efectos pleiotrópicos en las plantas, como disminución de la longitud del coleoptilo y del vigor inicial del trigo (Ellis *et al*., 2004), así como cambios en la acumulación y partición de la biomasa, tanto en trigo harinero (Sánchez-García *et al*., 2015b) como en trigo duro (Álvaro *et al*., 2008c). Existen muchos otros genes de enanismo, como los *Rht8* y *Rht9,* introducidos por N. Strampelli y posteriormente usados por N. Borlaug (Salvi *et al*., 2013), o el *Rht12*, que causan enanismo sin afectar a la sensibilidad al ácido giberélico al actuar a través de otros mecanismos fisiológicos (Worland y Snape, 2001). Sin embargo, aparte de los genes *Rht-B1b*, *Rht-D1b* y *Rht8*, pocos se han utilizado en mejora debido a que reducen excesivamente la altura de la planta o todavía no se conoce con precisión su potencial (Ellis *et al*., 2004; Rebetzke *et al*., 2012a,b).

La mejora del trigo en Italia y España

La mejora del trigo duro en Italia y España siguió diferentes trayectorias durante el siglo XX (Di Fonzo *et al*., 2005; Royo*,* 2005; Royo y Briceño-Félix, 2011). En el caso de Italia, las variedades tradicionales locales fueron utilizadas para fines de mejora, con lo que se incorporaron sus alelos en el acervo genético utilizado posteriormente para la obtención de cultivares modernos (Martos *et al*., 2005). Sin embargo, en España, la falta de continuidad en los programas de mejora españoles durante la primera mitad del siglo XX dificultó el uso de germoplasma tradicional para el desarrollo de cultivares modernos (Royo y Briceño-Félix, 2011), lo que originó una entrada masiva de variedades extranjeras. Durante la primera mitad del siglo XX se llevaron a cabo los primeros esfuerzos de mejora del trigo harinero mediante selección masal de variedades tradicionales (Royo y Briceño-Félix, 2011). Posteriormente, mediante intercambios de germoplasma con otros países europeos, principalmente Italia y Francia, se introdujeron variedades exitosas que se extendieron por el territorio, junto con la continua mejora de las variedades tradicionales mediante selección e hibridación. Sin embargo, en los años 70 los cultivares derivados de germoplasma del Centro Internacional de Mejora de Maíz y Trigo (CIMMYT) se establecieron definitivamente, reemplazando así a las variedades tradicionales locales y cultivares antiguos como ‘Aragón 03’ y ‘Pané 247’ que tuvieron un gran éxito en España durante los años 60 y 70 (Gómez *et al*., 2009; Royo y Briceño-Félix, 2011). Desde los años 70 del siglo XX hacía adelante, el germoplasma introducido por el CIMMYT tuvo un gran impacto en el desarrollo de nuevos cultivares de trigo duro y todavía hoy constituye la mayor parte del acervo genético de los cultivares modernos españoles (Martos *et al*., 2005; Royo, 2005).

En Italia durante los primeros años del siglo XX, se produjo un gran apoyo del gobierno a los estudios destinados a aumentar y estabilizar el rendimiento del trigo harinero (Bozzini *et al*., 1998) donde, como ya se ha mencionado, el primer cruzamiento entre variedades fue llevado a cabo en 1900 por el mejorador italiano Nazareno Strampelli, que tuvo un gran impacto en los primeros estadios de la mejora del trigo en el país (Salvi *et al*., 2013). Unos años más tarde, los genes de enanismo de la variedad de trigo harinero japonesa ‘Akagomughi’ fueron introducidos en el germoplasma local, lo que permitió obtener el primer cultivar enano y por tanto el uso de mayores cantidades de fertilizantes nitrogenados, con lo que se aumentó en potencial de rendimiento del cultivo de trigo. Durante los años 70 un mayor interés en las características de calidad panaderas estimuló a los mejoradores italianos a desarrollar cultivares con mejores características de calidad además de un elevado rendimiento. Esta tarea se consiguió gracias a la separación mediante electroforesis de gluteninas y gliadinas, lo cual permitió sustituir las subunidades de baja calidad presentes en los cultivares tradicionales por otras mejores que son las que caracterizan al germoplasma moderno actual (Borghi, 2001).

Evaluación de los efectos de la mejora genética

Uno de los métodos más utilizados para medir el progreso debido a la mejora (ganancia genética) del rendimiento y la calidad, objetivos prioritarios de los programas de mejora (Pagnotta *et al.,* 2005), es el uso de series históricas de cultivares seleccionados para representar el germoplasma obtenido en un país o región en un lapso de tiempo. Estas series históricas han permitido conocer las características agronómicas, morfológicas, fisiológicas y cualitativas responsables de los cambios observados a lo largo del tiempo en trigo duro y harinero en diversos países (Guarda *et al*., 2004; Motzo *et al*., 2004; De Vita *et al*., 2007).

La cuantificación de la ganancia genética alcanzada en España (y en trigo duro también en Italia) para diversos caracteres es una de las líneas de investigación que ha llevado a cabo el equipo de Mejora de Cereales del *Institut de Recerca i Tecnologia Agroalimentàries* (IRTA) en las últimas décadas, utilizando para ello series históricas de cultivares.

En trigo duro se cuantificó la ganancia genética del rendimiento en 16,9 kg ha^{-1} año^{-1} en Italia y en 23,6 kg ha^{-1} año^{-1} en España, además de determinarse los efectos causados por la mejora en los componentes del rendimiento, la producción de biomasa y su distribución, el llenado del grano, así como en diversas características morfofisiológicas del cultivo (Royo *et al*., 2007, 2008; Álvaro *et al*., 2008a,b,c). Los resultados de estudios llevados a cabo en esta especie mostraron que la biomasa en antesis disminuyó un 21% y un 9% en variedades españolas e italianas, respectivamente, por efecto de la presencia del gen *Rht-B1b* (Álvaro *et al*. 2008a). Sin embargo, todavía no se ha determinado la ganancia genética de las variables de calidad ni los efectos de la interacción GE en el rendimiento y sus componentes en trigo duro.

Durante muchos años la mejora ha considerado sólo la parte aérea de la planta, mientras que la selección directa teniendo en cuenta la biomasa radicular se ha llevado a cabo en muy raras ocasiones debido al tiempo necesario y coste de dicha actividad (Muñoz-Romero *et al*., 2010; Blum, 2011). A pesar de la importancia que tiene un sistema radicular vigoroso para aumentar el rendimiento bajo condiciones de sequía en ambientes Mediterráneos (Palta *et al*., 2011), el efecto de los genes de enanismo sobre el sistema radicular ha recibido muy poca atención por parte de la comunidad científica internacional. Hace unas décadas Cholick *et al*. (1977) demostraron que el sistema radicular del trigo puede alcanzar profundidades mayores de 300 cm tanto en el caso de los cultivares tradicionales cómo en los cultivares portadores de genes de enanismo. Más recientemente se ha demostrado que la mejora ha aumentado la distribución de biomasa hacia las raíces y su plasticidad como respuesta a la sequía en el trigo harinero (Song *et al*., 2010), aunque no hay datos contrastados sobre el efecto de la mejora en la biomasa radicular del trigo duro. Uno de los métodos utilizados para determinar la biomasa radicular es el de los tubos de PVC que se utilizó en los ensayos de esta Tesis Doctoral. El método se basa en la utilización de tubos de unos de 100-150 cm de longitud y 10-18 cm diámetro que se pueden colocar en un invernadero, lo cual permite conocer el valor de la biomasa radicular de una planta mediante el lavado y el posterior secado y pesado de la misma (Blum, 2011).

En el caso del trigo harinero se ha determinado una ganancia genética del rendimiento durante el pasado siglo de 35,1 Kg ha^{-1} y año^{-1}, mientras que las del número de espigas por m^{-2}, el número de granos por espiga y el peso de mil granos ha sido de 1,36 espigas m^{-2} año^{-1}, 0,20 granos espiga^{-1} año^{-1} y -0,046·10^{-3} g grano^{-1} año^{-1} (Sanchez-Garcia *et al*., 2013). Por su parte, las variables de calidad *W*, *P* y *L* experimentaron una ganancia genética de 2,74

J·10^{-4} año^{-1}, 0,59 mm H_20 año^{-1} y -0,53 mm año^{-1}, respectivamente (Sanchez-Garcia *et al.,* 2015a). El efecto de la interacción GE en el rendimiento supuso el 14,5% de la suma total de cuadrados del ANOVA, mientras que los valores de sus componentes oscilaron entre el 17,6% y el 27,7% (Sanchez-Garcia *et al.,* 2012). En la misma línea de investigación se llevaron a cabo diversos estudios sobre el efecto de la introducción de los genes de enanismo en el crecimiento del cultivo y la distribución de la biomasa en los órganos aéreos de la planta. Así, Sánchez-García *et al*. (2015b) asociaron los patrones de acumulación y distribución de biomasa al número de alelos de enanismo presentes en trigo harinero, demostrando que la presencia de un gen de enanismo disminuyó un 20% la biomasa y el índice de área foliar (LAI) al inicio del encañado, mientras que la reducción fue del 40% en variedades portadoras de dos genes de enanismo.

Los capítulos que componen esta Tesis Doctoral contribuyen a incrementar el conocimiento existente sobre los efectos de la mejora genética sobre caracteres de calidad y desarrollo radicular del trigo duro, así como sobre la interacción GE en trigo duro y harinero, todos ellos temas no abordados por el grupo hasta la fecha.

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OBJETIVOS

OBJETIVOS

El objetivo general de la presente Memoria de Tesis Doctoral es contribuir a incrementar el conocimiento existente sobre la formación del rendimiento y la calidad industrial del grano de trigo utilizando para ello dos enfoques de gran relevancia para la mejora genética: el histórico y el medioambiental.

Los objetivos específicos de la misma son los siguientes:

1. Cuantificar el efecto del alelo *Rht-B1b*, causante del enanismo de los trigos de la Revolución Verde, sobre la biomasa aérea y radicular del trigo duro y sus consecuencias sobre la formación del rendimiento.

2. Determinar los cambios causados por la mejora genética sobre la calidad del grano de trigo. Este objetivo se concreta en los dos sub-objetivos siguientes:

   a) Cuantificar la ganancia genética de las características de calidad del trigo duro a lo largo del siglo XX en España e Italia.

   b) Estudiar los cambios ocurridos a lo largo del tiempo en la composición de las subunidades de gluteninas de bajo (LMW-GS) y alto (HMW-GS) peso molecular y su efecto sobre la calidad del gluten en trigos duro y harinero.

3. Evaluar el efecto del ambiente sobre la producción y calidad del trigo, en concreto:

   a) Estudiar los efectos de la mejora genética del trigo duro sobre la interacción genotipo x ambiente para el rendimiento y sus componentes y la estabilidad de los mismos.

   b) Evaluar la interdependencia entre el rendimiento y la calidad del trigo harinero y su relación con la clasificación oficial de los cultivares de acuerdo a los grupos de la norma de calidad.

   c) Identificar las principales variables meteorológicas implicadas en la respuesta diferencial de los genotipos de trigo harinero y duro en cuanto a rendimiento y calidad.

Estos objetivos se abordan en los capítulos de la presente Memoria de Tesis Doctoral de acuerdo a la siguiente relación:

	Capítulo 1	**Capítulo 2**	**Capítulo 3**	**Capítulo 4**
Objetivo 1	X			
Objetivo 2a		X		
Objetivo 2b		X	X	
Objetivo 3a				X
Objetivo 3b			X	
Objetivo 3c			X	X

Para alcanzar los objetivos propuestos se constituyó una serie histórica de 24 cultivares de trigo duro representativos de los cultivados en España e Italia a lo largo del pasado siglo, y un conjunto de 20 cultivares trigo harinero cultivados en España en las últimas décadas y representativos de los cuatro grupos de calidad industrial de la norma oficial contenida en los RD1615/2010 y RD 190/2013.

La presente Memoria de Tesis Doctoral está formada por cuatro capítulos, elaborados como entidades independientes de información, al objeto de ser publicados como artículos científicos en revistas de impacto. En el momento de la redacción de esta Memoria de Tesis Doctoral el **Capítulo 1** ha sido publicado en *Plant & Soil*, el **Capítulo 2** en *Crop & Pasture Science*, el **Capítulo 3** está en fase de evaluación y el **Capítulo 4** se ha publicado en el *European Journal of Agronomy*.

CHAPTER 1

Changes in durum wheat root and aerial biomass caused by the introduction of the *Rht-B1b* dwarfing allele and their effects on yield formation

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REGULAR ARTICLE

Changes in durum wheat root and aerial biomass caused by the introduction of the *Rht-B1b* dwarfing allele and their effects on yield formation

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Abstract
Aims This study aimed to quantify the changes in root and aerial biomass of durum wheat brought about by the introduction of the *Rht-B1b* dwarfing allele and their effects on yield formation.
Methods A historical series of 24 Mediterranean cultivars with allelic variants *a* (tall) and *b* (semi-dwarf) at *Rht-B1* locus was tested in tubes in three greenhouse experiments and six field experiments.
Results The dwarfing allele reduced the aerial biomass of each plant at anthesis by 7.6 % and the root by 28.1 % (25.4 %, 26.7 % and 36.0 % in the upper, middle and lower root sections, respectively). Aerial and root biomass were reduced by 27.0 g y^{-1} and 7 g y^{-1} respectively, but the relative rate of change was much greater for roots (−0.73 % y^{-1}) than for aerial organs (−0.17 % y^{-1}). Aerial biomass at anthesis was negatively associated with spike number, harvest index and yield in tall cultivars, but no significant relationship was found for semi-dwarf ones.
Conclusions The root/aerial biomass ratio was 29 % lower in semi-dwarf than in tall cultivars. In tall cultivars large aerial biomass at anthesis was detrimental to yield formation, while in semi-dwarf cultivars high aerial biomass at anthesis had no effect on yield formation.

Keywords Breeding effects · Genetic gain · Historical series · PVC tubes · Root section

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Introduction

The root system of wheat is essential for firmly anchoring the plant to the soil and taking up water and nutrients from it. In reduced-input agricultural systems, root traits affecting the acquisition of mineral elements often determine yield (Ehdaie et al. 2010; White et al. 2013). In the Mediterranean Basin, one of the largest durum wheat producers in the world, more than 50 % of the total grain of durum wheat is produced in arid and semi-arid conditions, with severe drought most years (Loss and Siddique 1994; Araus et al. 2003; García del Moral et al. 2005). In dryland agricultural systems, a large root system that promotes access to soil water and nutrients is regarded as beneficial for plant growth (Richards 2008), although under terminal drought a greater investment in fine roots at depth would improve yield due to the better access to water and nitrogen (King et al. 2003). Accordingly, root dry weight at depth has been related to drought adaptation (Lopes and Reynolds 2010).

The introduction of semi-dwarf cultivars with greater resistance to lodging during the Green Revolution in the second half of the 20th century led to major yield gains (Borlaug 2007). Dwarfness in most durum wheat cultivars is controlled by the *Rht-B1b* (formerly *Rht1*) allele. In order to incorporate in durum wheat the dwarfing genes already identified and used in bread wheat, crosses between tall durums and semi-dwarf bread wheats were intensively carried out by Dr. Borlaug's program at CIMMYT from the late 1950's. The varieties 'Langdon enano' and 'Barrigon Yaqui enano' (whose pedigree is YAKTANA-54//NORIN-10/BREVOR/3/2*BARRIGON-YAQUI) were the first semi-dwarf durum lines resulting from that crosses (Julio Huerta-Espino, pers. Comm.) The objective was to transfer the short and stiff straw character in order to allow the intensification of agronomic practices (increased sowing rate, fertilizer applications and water supply). The *Rht-B1b* allele induces insensitiveness to gibberellic acid, thus preventing stem elongation (Peng et al. 1999; Hedden 2003). In addition to the reduced height of the plant, the *Rht-B1b* allele has well-known pleiotropic effects on plant growth, and morphological and physiological characters (Gale and Youssefian 1985; Li et al. 2011; Rebetzke et al. 2012). It increases yield, mostly under favourable conditions, by altering the proportion of dry matter allocated to grain (De Vita et al. 2007; Royo et al. 2007, 2008; Álvaro et al. 2008a). However, the effect of the *Rht-B1b* allele on the root system has been poorly explored (Waines and Ehdaie 2007). Previous studies found no consistent association between height genes and root growth and function (Blum 2011). Though the *Rht-B1b* allele is known to inhibit stem growth, thus making available a surplus of assimilates that are used for thickening the roots (Miralles et al. 1997) without reducing the stem diameter (Calderini et al. 1996), it has been proposed that the enlargement of the root system and its penetration ability might not be under the control of dwarfing genes (Miralles et al. 1997; Kubo et al. 2005). Vigorous shoot growth has been related to vigorous root growth under a wide range of conditions (Mian et al. 1993). However, negative relationships have been identified between root dry weight and plant height in bread wheat (Miralles et al. 1997), and a lower shoot:root ratio has been found in dwarf cultivars (Siddique et al. 1990), seeming to suggest that the selection of cultivars with higher yield and other suitable traits during the 20th century also led to an unintentional selection of those with a reduced investment in root biomass (Zhu and Zhang 2013). Furthermore, modern cultivars containing the *Rht-B1b* allele have shown an increased capacity to take up water in irrigated environments (Pask and Reynolds 2013), thus demonstrating greater efficiency in the use of water after anthesis than the old tall cultivars (Subira et al. 2015).

Quantifying root characteristics in field experiments is a labour-intensive task because the root crop zone is difficult to access (Motzo et al. 1993; Waines and Ehdaie 2007; Izzi et al. 2008; Botwright Acuña and Wade 2012). Therefore, little attention has been devoted to root systems in breeding programmes in the past, most of which have dealt with the above-ground plant organs (Waines and Ehdaie 2007). A number of root observation methods have been proposed, but due to their cost or slowness for routine screening work in breeding programmes, the most widespread among breeders has been the tube method (Blum 2011). The assessment of the root system is essential because water availability is the most limiting factor for the correct expression of yield potential even in irrigated environments, and projections indicate that less usable water will be available in the future (Pask and Reynolds 2013). The tube method is therefore useful for boosting knowledge of the most appropriate root systems for the drought environments that will be more common in the future.

The effect of breeding activities during the 20th century and the introduction of dwarfing alleles on yield, yield components, biomass production and allocation was previously studied by our team on a historical series of 24 Italian and Spanish durum wheat cultivars released in different periods and with different allelic compositions in the *Rht-B1* gene (Royo et al. 2007, 2008; Álvaro et al. 2008a, 2008b, 2008c; Subira et al. 2014). The same set of cultivars was used in the present study with the following aims: (i) to quantify the changes occurring in root biomass due to the introduction of the *Rht-B1b* dwarfing allele, (ii) to determine the relationship between the changes in root biomass and the changes in aerial biomass, and (iii) to study the relationship between biomass and yield formation in tall and semi-dwarf cultivars. Objectives (i) and (ii) were addressed through experiments in PVC tubes under greenhouse conditions, while objective (iii) was addressed using data of field experiments and relating them to those obtained in the greenhouse experiments.

Materials and methods

Plant material

The plant material used in this study consisted of a historical series of 24 durum wheat (*Triticum turgidum* L. var. *durum*) cultivars selected to represent the germplasm grown in Italy and Spain during the last century (Table 1). The set included tall landraces cultivated before 1945, early semi-dwarf cultivars derived from CIMMYT germplasm such as 'Mexa', landmark early European cultivars such as 'Creso', and cultivars released by local breeding programmes during 1990s in both countries. PCR-based markers specific for the base pair responsible for the semi-dwarf phenotype were used to identify specifically wild-type (*Rht-B1a*) and mutant (*Rht-B1b*) allelic variants at the *Rht-B1* locus following the methodology described by Ellis et al. (2002), thus allowing classifying the cultivars into two groups: tall, carrying the *Rht-B1a* allele, and semi-dwarf, with the *Rht-B1b* allele. This methodology confirmed the presence of the *Rht-B1b* allele in the genome of the Italian cultivar 'Adamello', which had previously been considered not to carry any dwarfing allele (Isidro et al. 2011; Álvaro et al. 2008a, 2008b, 2008c; Royo et al. 2007), according to the results of the test for sensitivity to gibberellic acid (Gale and Gregory 1977).

Experiments in tubes in the greenhouse

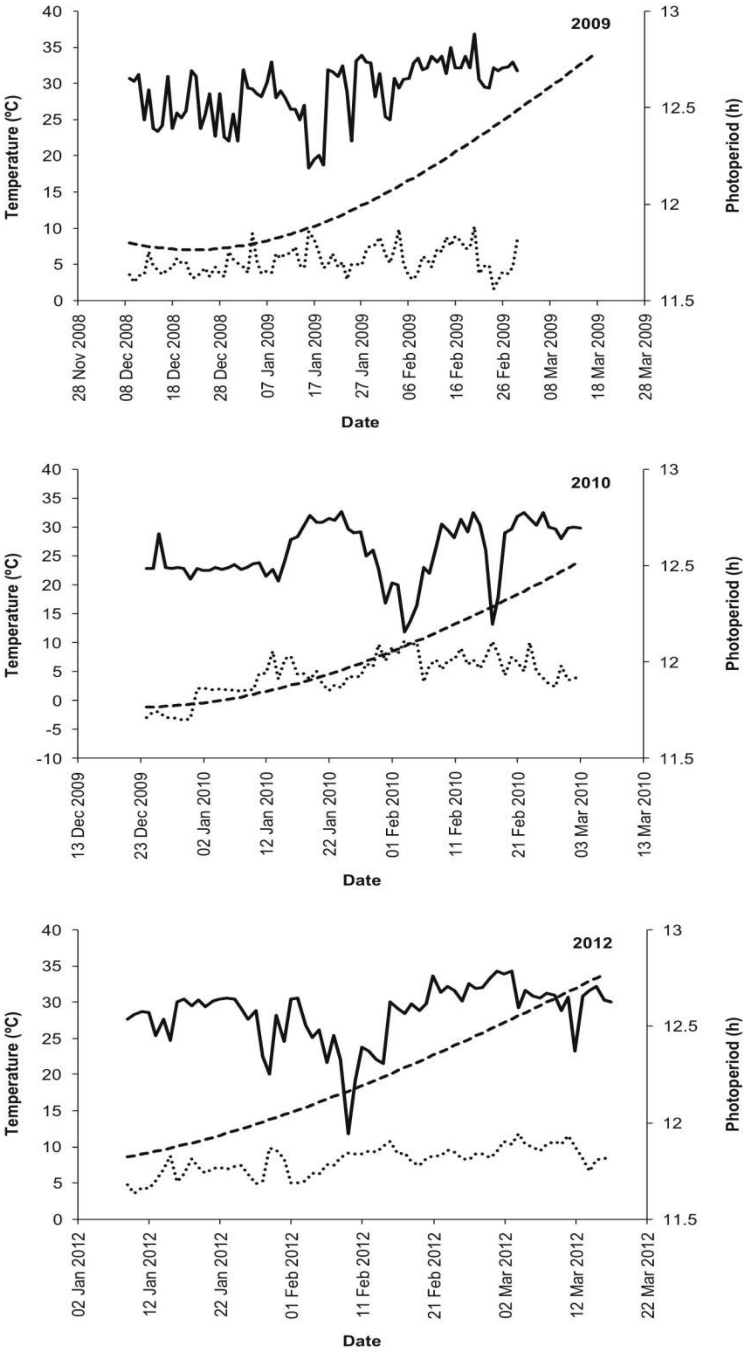
Three experiments were conducted under greenhouse conditions during three growing seasons at CIMMYT research station in Toluca, State of Mexico (19º16'N, 99º34'W). Temperatures and daily photoperiod are shown in Fig. 1. Plants were grown in PVC tubes of 120 cm height and 10.2 cm width filled with a soil/sand mixture at a 1:3 ratio and sealed at the bottom with a hole for drainage. Each tube was fertilized with 3.0 g of urea and 0.5 g of triple superphospate (TSP), and irrigation was provided periodically to prevent water limitation. Experiments consisted of 72 tubes in 2009 and 2010 and 96 in 2012, arranged in randomized complete block designs with three replications in 2009 and 2010 and four in 2012. Planting dates were 2, 10 and 21 December in 2008, 2009 and 2011, respectively. Three seeds of each cultivar (all of them with a diameter between 2.8 mm and 3.5 mm) were sown in each tube and after seedling emergence only two plants per tube were kept.

At anthesis (Zadoks stage 65, Zadoks et al. 1974) plant height was measured from the soil to the top of the spike excluding the awns, and the entire plants, including the roots, were removed from the tubes (Fig. 2). The roots were carefully washed following the methodology described in Blum (2011) and divided into three sections (upper, middle and lower) of equal length. The above-ground biomass and the three root sections obtained

Table 1 Allelic variant at *Rht-B1* locus, name, year of release and country of origin for a historical series of 24 Italian (I) and Spanish (S) durum wheat cultivars

Tall cultivars (*Rht-B1a*)			Semi-dwarf cultivars (*Rht-B1b*)		
Name	Year of release	Country of origin	Name	Year of release	Country of origin
Balilla Falso	<1930	I	Creso	1974	I
Razza 208	<1930	I	Adamello	1985	I
Senatore Cappelli	1930	I	Simeto	1988	I
Carlojucci	1945	I	Cirillo	1992	I
Capeiti 8	1955	I	Flavio	1992	I
Trinakria	1970	I	Zenit	1992	I
Blanco Verdeal	<1930	S	Camacho	1975	S
Clarofino	<1930	S	Esquilache	1976	S
Pinet	<1930	S	Mexa	1980	S
Rubio de Belalcázar	<1930	S	Ariesol	1992	S
Bidi 17	1950	S	Senadur	1995	S
			Astigi	1999	S
			Boabdil	2000	S

Fig. 1 Daily photoperiod (*dashed line*) and indoor minimum (*dotted line*) and maximum (*solid line) daily* temperatures during the three years of greenhouse tube experiments at Toluca's research station, CIMMYT (Mexico)



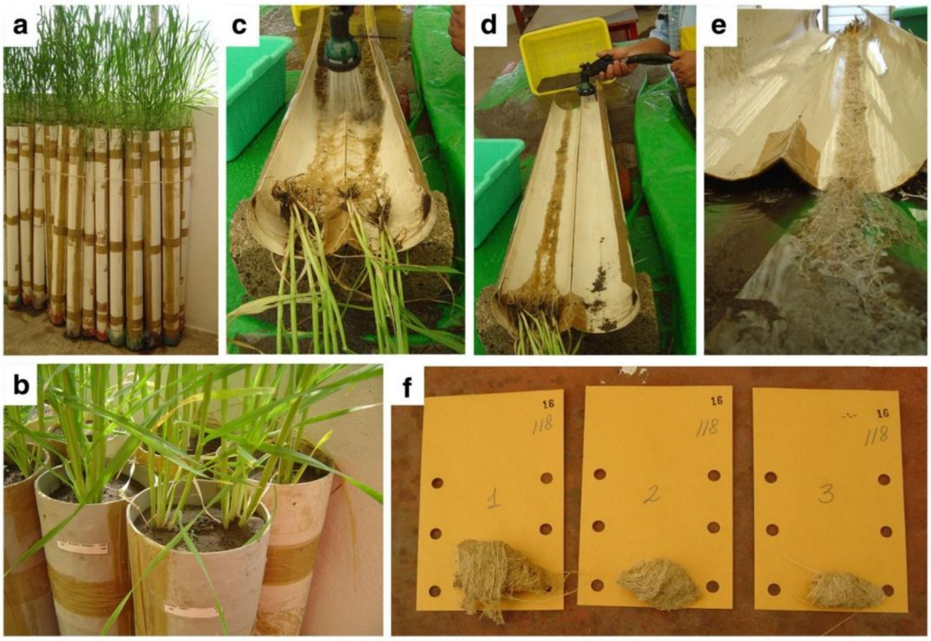


Fig. 2 Methodology used in experiments with tubes in the greenhouse. **a** PVC tubes with growing wheat plants; **b** detail of the growing plants; **c-e** process of slowly pulling out the roots by laying down the tubes and carefully washing off all the soil; **f** the three sections of the roots of an individual tube before being oven-dried

from each tube were oven-dried at 70 °C for 48 h and weighed. Dry weight of plant parts was expressed on a single-plant basis.

Field experiments

Six field experiments were conducted with the same set of cultivars in 2003, 2004 and 2005 at two contrasting sites in Spain: Gimenells in the north-east and Chimeneas in the south (Table 2). Experiments followed randomized complete block designs with three replications and plots of 12 m^2 (8 rows, 0.15 m apart). Sowing rate was adjusted to 400 and 350 fully viable seeds per m^2 at Gimenells and Chimeneas, respectively. Plots were fertilised following the recommendations for maximizing yields while preventing lodging, and were kept disease- and insect- free with preventive pesticide applications. Anthesis date was recorded for each plot when 50 % of the plants reached this stage and plants within a 50 cm-long row per plot were pulled up at this stage. In the laboratory the number of plants in each sample was recorded and the plants were oven-dried at 70 °C for 48 h to obtain the crop dry weight (CDW, g per m^2) and the aerial biomass per plant as the ratio between CDW and the number of plants per m^2. A second 50-cm-long row was randomly sampled at ripening (Zadoks stage 92) on each plot and used to determine the number of spikes per m^2 and the number of grains per spike. Harvest index (HI) was obtained on a dry weight basis as the ratio between total grain weight and above-ground biomass of the same sample. The harvest was conducted mechanically at commercial maturity and grain yield was adjusted at 12 % moisture level. Thousand kernel weight was calculated using a sub-sample of the grain obtained.

Statistical analyses

Combined ANOVAs were performed across greenhouse experiments considering the experiment, the

Table 2 Site and description of the six field experiments conducted in this study

Site	Gimenells (North of Spain)			Chimeneas (South of Spain)		
Coordinates	41°40'N, 0°20'E			37°08'N, 3°49'W		
Altitude (m asl)	200			684		
Soil texture	Fine loamy			Silty clay		
Soil classification	Mesic calcixerolic xerochrept			Loamy calcixerolic xerochrept		
Harvest year	2003	2004	2005	2003	2004	2005
Sowing date	26 Nov 2002	16 Dec 2003	26 Nov 2004	23 Dec 2002	13 Nov 2003	10 Dec 2004
Environmental conditions from sowing to anthesis (SA)						
T_m(°C)	8.64	8.13	7.24	10.7	9.81	9.43
ETo (mm)	244	206	226	309	290	348
RH (%)	80.3	95.6	91.4	68.6	71.2	56.5
WI (rainfall + irrigation, mm)	170 + 150	202 + 85	62.7 + 150	249 + 40	334 + 40	89.8 + 120
Environmental conditions from anthesis to maturity (AM)						
T_m(°C)	17.6	20.0	18.8	21.3	16.2	21.1
ETo (mm)	117	140	140	118	139	182
RH (%)	78.9	87.9	85.2	45.6	66.9	38.8
WI (rainfall + irrigation, mm)	62.5 + 0	6.03 + 65	52.9 + 0	0	79.8 + 0	6.00 + 0
Mean yield (kg ha^{-1})	5378	6495	6670	2571	4327	1425

T_m: average mean daily temperature; ETo: accumulated reference evapotranspiration computed by the Penman-FAO methodology (Allen et al. 1998); RH: average mean daily relative humidity; WI: water input (rainfall + irrigation)

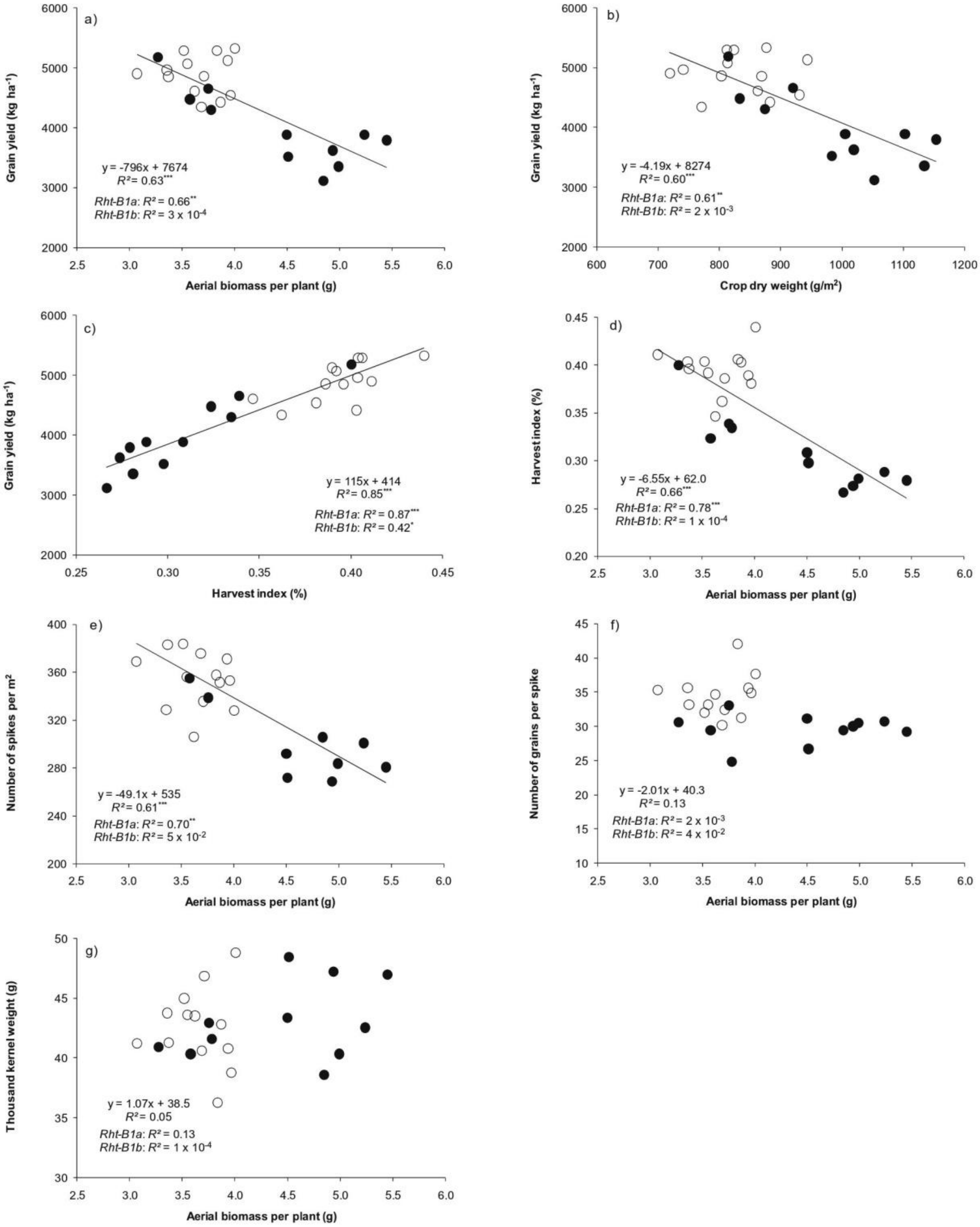
cultivar and their interaction as fixed factors in the model. The sum of squares of the cultivar effect and its interaction were partitioned into differences between alleles at the *Rht-B1* locus and differences within each of them. Means were compared with the Student *t* test ($P = 0.05$). Absolute (AGG) and relative (RGG) genetic gains were computed as the slope of the linear regression line fitted to the relationship between the absolute or relative value of the trait and the year of cultivar release. Relative values were computed for each cultivar as percentages irrespective of the average value of all cultivars. Linear regression models were fitted to the relationships between: i) traits assessed in field experiments, ii) traits assessed in the greenhouse, and iii) biomass assessed in tubes and yield and biomass related traits determined under field conditions. In all cases mean cultivar data across replications and experiments were used. All analyses were performed with the JMP V.8.0 (SAS Institute Inc. 2009) and Enterprise Guide 4.2 statistical software (SAS Institute Inc. 2006).

Results

Field experiments

Results of the field experiments showing genetic changes in yield, yield components, biomass and HI have been published elsewhere (Royo et al. 2007, 2008; Álvaro et al. 2008a, 2008b). In this study, the examination of the relationships between yield and its components in terms of biomass production and allocation revealed that, for the whole set of cultivars, grain yield under field conditions

Fig. 3 Relationships between traits assessed in field experiments. ▶ Each point corresponds to the mean data of a durum wheat cultivar across six Mediterranean environments and three replications per environment. Relationships between grain yield (y axis) and **a** aerial biomass per plant at anthesis, **b** crop dry weight at anthesis, **c** harvest index. Relationships between aerial biomass per plant (x axis) and **d** harvest index, **e** number of spikes per m^2, **f** number of grains per spike, and **g** thousand kernel weight. *Black circle* Tall cultivars (*Rht-B1a*), *White circle* Semi-dwarf cultivars (*Rht-B1b*). Regression equations refer to the models fitted to all cultivars ($n = 24$). Coefficients of determination of linear models fitted to tall ($n = 11$) and semi-dwarf ($n = 13$) cultivars are also shown in each figure. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$


a)
Grain yield (kg ha-1)
6000
5000
4000
3000
2000
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
Aerial biomass per plant (g)
y = -796x + 7674
R² = 0.63***
Rht-B1a: R² = 0.66**
Rht-B1b: R² = 3 x 10-4
b)
Grain yield (kg ha-1)
6000
5000
4000
3000
2000
600
700
800
900
1000
1100
1200
Crop dry weight (g/m²)
y = -4.19x + 8274
R² = 0.60***
Rht-B1a: R² = 0.61**
Rht-B1b: R² = 2 x 10-3
c)
Grain yield (kg ha-1)
6000
5000
4000
3000
2000
0.25
0.30
0.35
0.40
0.45
Harvest index (%)
y = 115x + 414
R² = 0.85***
Rht-B1a: R² = 0.87***
Rht-B1b: R² = 0.42*
d)
Harvest index (%)
0.45
0.40
0.35
0.30
0.25
0.20
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
Aerial biomass per plant (g)
y = -6.55x + 62.0
R² = 0.66***
Rht-B1a: R² = 0.78***
Rht-B1b: R² = 1 x 10-4
e)
Number of spikes per m²
400
360
320
280
240
200
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
Aerial biomass per plant (g)
y = -49.1x + 535
R² = 0.61***
Rht-B1a: R² = 0.70**
Rht-B1b: R² = 5 x 10-2
f)
Number of grains per spike
45
40
35
30
25
20
15
10
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
Aerial biomass per plant (g)
y = -2.01x + 40.3
R² = 0.13
Rht-B1a: R² = 2 x 10-3
Rht-B1b: R² = 4 x 10-2
g)
Thousand kernel weight (g)
50
45
40
35
30
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
Aerial biomass per plant (g)
y = 1.07x + 38.5
R² = 0.05
Rht-B1a: R² = 0.13
Rht-B1b: R² = 1 x 10-4

was negatively associated with above-ground biomass at anthesis, determined either at the plant level (Fig. 3a) or at the crop level (Fig. 3b). However, when these relationships were assessed for tall and semi-dwarf cultivars separately they were only significant for the former, which showed greater variability for biomass and also for grain yield (Fig. 3a and 3b). Harvest index was significantly and positively associated with grain yield, and accounted for 85 % of yield variations in the model considering all cultivars (Fig. 3c). This relationship was also significant when semi-dwarf and tall cultivars were measured separately, but its reliability was greater for the latter group. In accordance with these results, aerial biomass per plant at anthesis and HI were negatively associated, particularly for the set of tall cultivars, but not for the semi-dwarf ones (Fig. 3d).

The assessment of the relationship between aerial biomass per plant at anthesis and the three main yield components (number of spikes per m^2, number of grains per spike and grain weight), showed that for the whole set of cultivars the number of spikes per unit area was negatively associated with aerial biomass per plant at anthesis (Fig. 3e), but no significant relationships were observed between the number of grains per spike and grain weight with aerial biomass per plant at anthesis (Fig. 3f and 3g). This pattern was similar for tall cultivars, while for the semi-dwarf cultivars no significant relationship was found between aerial biomass per plant and any of the three yield components.

Greenhouse experiments

The results of the ANOVA showed large significant differences between experiments, but negligible differences between replicates within each of them (Table 3). Differences between cultivars were significant for all the traits assessed and always accounted for more than 21 % of total variation. For plant height the cultivar effect explained 67.1 % of total variation. Differences between cultivars carrying and not carrying the *Rht-B1b* allele were also significant for all traits, but for plant height they accounted for 54.5 % of total variation. Variability within the semi-dwarf cultivars was greater for all traits than the variability within the tall cultivars, except with regard to plant height.

The introduction of the *Rht-B1b* dwarfing allele had a greater effect on root biomass per plant, which decreased by 28.1 %, than on aerial plant biomass, which only decreased by 7.6 % (Table 4). Accordingly, the root/aerial biomass per plant ratio was 28.6 % lower in semi-dwarf cultivars than in tall ones. The lower section of the root, the most distant from the root crown, was the one most altered by the *Rht-B1b* dwarfing allele, as it was reduced by 36 % in the semi-dwarf compared with the tall cultivars, while differences in the upper and middle sections were 25 % and 27 %, respectively (Table 4). The distribution of root weight in the three sections was similar in tall and semi-dwarf cultivars: 52 % and 54 % in the upper section, 26 % and 27 % in the middle section, and 22 % and 19 % in the lower section, respectively (deduced from Table 4).

Among tall cultivars 'Trinakria' showed the lowest values for all the traits assessed in the greenhouse, while 'Blanco Verdeal' had the largest aerial and total biomass per plant and 'Bidi 17' had the largest root biomass per plant and the greatest root/aerial biomass ratio (Supplementary Table 1). The lowest values for root biomass within the semi-dwarf cultivars were recorded for all sections in 'Simeto', which also had the lowest root/aerial biomass ratio. On the other hand, the highest values for all traits except plant height and root biomass in the upper section were recorded in the cultivar 'Camacho'.

Absolute (AGG) and relative (RGG) genetic gains were significant and negative for all traits (Table 5). In absolute terms, the aerial and total biomass per plant decreased at a higher yearly rate than the root biomass. However, in relative terms the decrease was much greater for root biomass (-0.73 g y^{-1}) than for aerial biomass (-0.17 g y^{-1}).

Aerial biomass was positively and significantly associated with root biomass, both for the whole root (Fig. 4a) and for each of its sections (Fig. 4b, 4c and 4d). These relationships were also significant when tall and semi-dwarf cultivars were considered separately, except for the root middle section in tall cultivars (Fig. 4c).

Relationship between traits assessed in tubes in the greenhouse and yield formation

The analysis of the relationship between aerial biomass per plant assessed in tubes and aerial biomass per plant (Fig. 5a) and per unit area (Fig. 5b) in field experiments showed positive and significant associations in all cases when all cultivars were taken. Moreover, the relationship between aerial biomass per plant in tubes and grain yield (Fig. 5c) showed a similar trend to that observed when both traits were assessed in the field (Fig. 3a). Although aerial biomass per plant in tubes was more than three times that recorded in field experiments, both models showed the same tendency and were equally

Table 3 Percentage of the sum of squares (type III) of the ANOVA for the aboveground and root traits of the 24 durum wheat cultivars of the historical series assessed in three greenhouse experiments

Source of variation	d.f.	Plant height	Aerial biomass per plant (*a*)	Root biomass per plant				Total biomass per plant (*a* + *b*)	Root/aerial biomass per plant (*b*/*a*)
				Total (*b*)	Upper section	Middle section	Lower section		
Experiment	2	13.2 ***	30.2 ***	24.9 ***	23.9 ***	23.4 ***	19.4 ***	30.1 ***	22.1 ***
Rep (Experiment)	7	0.09	1.96	1.53	2.04	1.42	1.41	1.96	1.50
Cultivar	23	67.1 ***	21.2 ***	25.3 ***	23.6 ***	21.8 ***	26.0 ***	22.1 ***	25.8 ***
Between *Rht-B1*	1	54.5 ***	10.8 ***	11.3 ***	8.54 ***	10.3 ***	12.4 ***	11.1 ***	9.97 ***
Within *Rht-B1b*	12	5.03 ***	6.02 **	11.6 ***	11.8 ***	9.81 ***	9.71	6.95 ***	13.5 ***
Within *Rht-B1a*	10	8.14 ***	5.00 **	3.05	3.76 *	2.15	4.50	4.48 **	7.29
Exp x Cultivar	46	10.7 ***	13.7 *	17.6 ***	19.9 ***	16.5 *	17.9 **	14.1 **	18.5 ***
Exp x Between *Rht-B1*	2	3.12 ***	1.99 **	3.64 ***	3.77 ***	2.93 **	4.05	2.42 **	3.14 ***
Exp x Within *Rht-B1b*	24	3.71 ***	4.30	5.23	6.51	6.71	5.07	4.11	2.75
Exp x Within *Rht-B1a*	20	4.16 ***	7.30 *	8.99 ***	9.80 ***	6.97	9.08	7.51 **	8.19 **
Residual	161	1.98	28.2	25.6	25.1	33.2	31.7	26.9	28.1
Total	239								

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

significant for the whole set of cultivars. Similarly, the model fitted to the relationship between aerial biomass per plant measured in tubes and HI determined in the field (Fig. 5d) was similar to that obtained when both traits were determined under field conditions (Fig. 3d). The consistency of all these results led us to examine the relationships between root biomass and both yield (Fig. 5e) and HI (Fig. 5f), which, as expected, were negative in both cases, as occurred for aerial biomass.

Discussion

Aerial and root biomass in the greenhouse

The great disparity between the data obtained in the three greenhouse experiments was not unexpected considering the differences in the range of temperatures recorded in them. Previous studies demonstrated great experimental variability in root traits under both

Table 4 Mean values across three greenhouse experiments for the aboveground and root traits of tall (*Rht-B1a*) and semi-dwarf (*Rht-B1b*) durum wheat cultivars of the historical series

Trait	*Rht-B1a*		*Rht-B1b*		Difference (%)
	Mean	Range	Mean	Range	
Plant height (cm)	88.8^{a}	73.5–108	60.7 ^{b}	54.0–75.5	*−31.6*
Aerial biomass per plant (g) (*a*)	17.0 ^{a}	15.4–17.9	15.7 ^{b}	15.1–17.2	*−7.60*
Total root biomass per plant (g) (*b*)	1.14 ^{a}	0.85–1.33	0.82 ^{b}	0.56–1.28	*−28.1*
Root biomass per plant upper section (g)	0.59 ^{a}	0.43–0.77	0.44 ^{b}	0.31–0.66	*−25.4*
Root biomass per plant middle section (g)	0.30 ^{a}	0.25–0.37	0.22 ^{b}	0.15–0.34	*−26.7*
Root biomass per plant lower section (g)	0.25 ^{a}	0.17–0.31	0.16 ^{b}	0.10–0.29	*−36.0*
Total biomass per plant (g) (*a* + *b*)	18.1 ^{a}	16.3–19.2	16.6 ^{b}	15.6–18.5	*−8.29*
Root/aerial biomass per plant (*b*/*a*)	0.07 ^{a}	0.055–0.079	0.05 ^{b}	0.036–0.074	*−28.6*

Means within rows followed by the same letter are not significantly different according to the Student's t test at $P = 0.05$

Table 5 Absolute (AGG) and relative (RGG, % y^{-1}) genetic changes for the aboveground and root traits of the 24 durum wheat cultivars of the historical series assessed in three greenhouse experiments

Trait	R^2	AGG	RGG	AGG units
Aerial biomass per plant (g) (*a*)	0.66^{***}	−27.0	−0.17	g · y^{-1}
Total root biomass per plant (g) (*b*)	0.54^{***}	−6.99	−0.73	g · y^{-1}
Root upper section biomass per plant (g)	0.47^{***}	−3.42	−0.67	g · y^{-1}
Root middle section biomass per plant (g)	0.51^{***}	−1.68	−0.67	g · y^{-1}
Root lower section biomass per plant (g)	0.61^{***}	−1.83	−0.90	g · y^{-1}
Total biomass per plant (g) (*a* + *b*)	0.65^{***}	−34.0	−0.20	g · y^{-1}
Root/aerial biomass per plant (*b*/*a*)	0.48^{***}	−0.32	−0.56	y^{-1}

***$P < 0.001$

greenhouse conditions (Botwright Acuña et al. 2007) and field conditions (Botwright Acuña and Wade 2012). However, the results of the three replicates of each experiment were consistent, as shown by the lack of statistical significance for all traits of the replicate effect (nested to the experiment) of the ANOVA. The

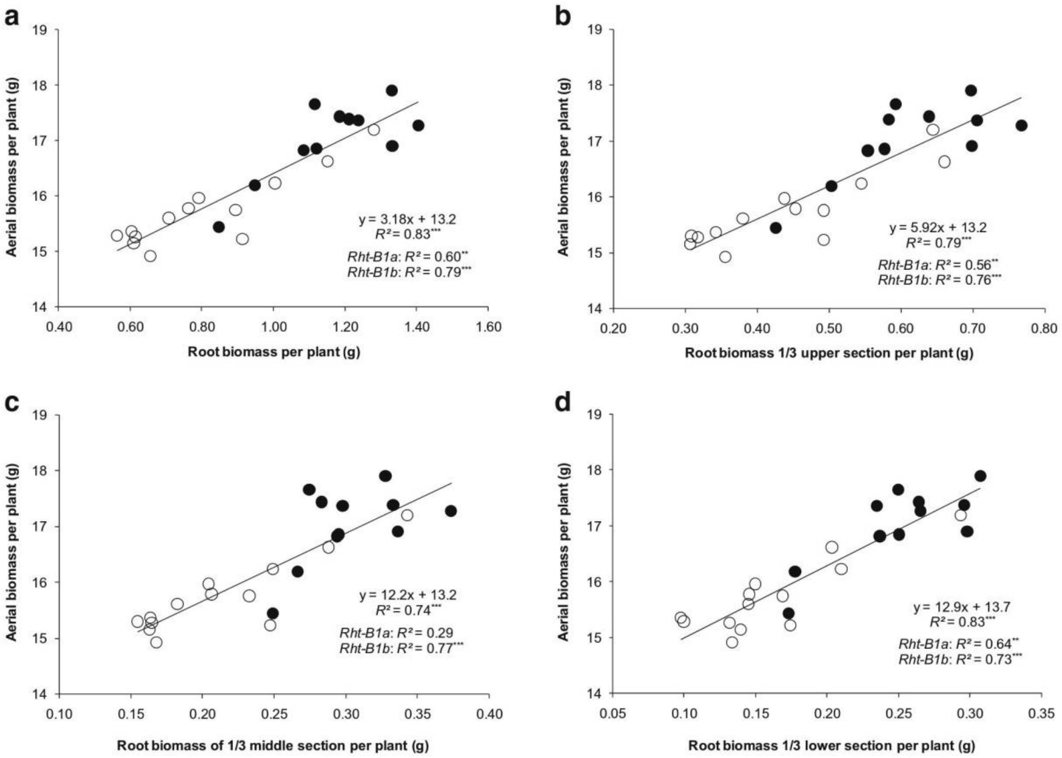


Fig. 4 Relationship between traits assessed for tubes in the greenhouse. Each point corresponds to the mean data of a durum wheat cultivar across three experiments and three (2009 and 2010) or four (in 2012) replications per experiment. Relationships between aerial biomass per plant and **a** total root biomass, **b** upper 1/3 root section biomass, **c** middle 1/3 root section biomass, and **d** lower 1/3 root section biomass. *Black circle* Tall cultivars (*Rht-B1a*), *White circle* Semi-dwarf cultivars (*Rht-B1b*). Regression equations refer to the models fitted to all cultivars ($n = 24$). Coefficients of determination of linear models fitted to tall ($n = 11$) and semi-dwarf ($n = 13$) cultivars are also shown on each figure. **$P < 0.01$; ***$P < 0.001$

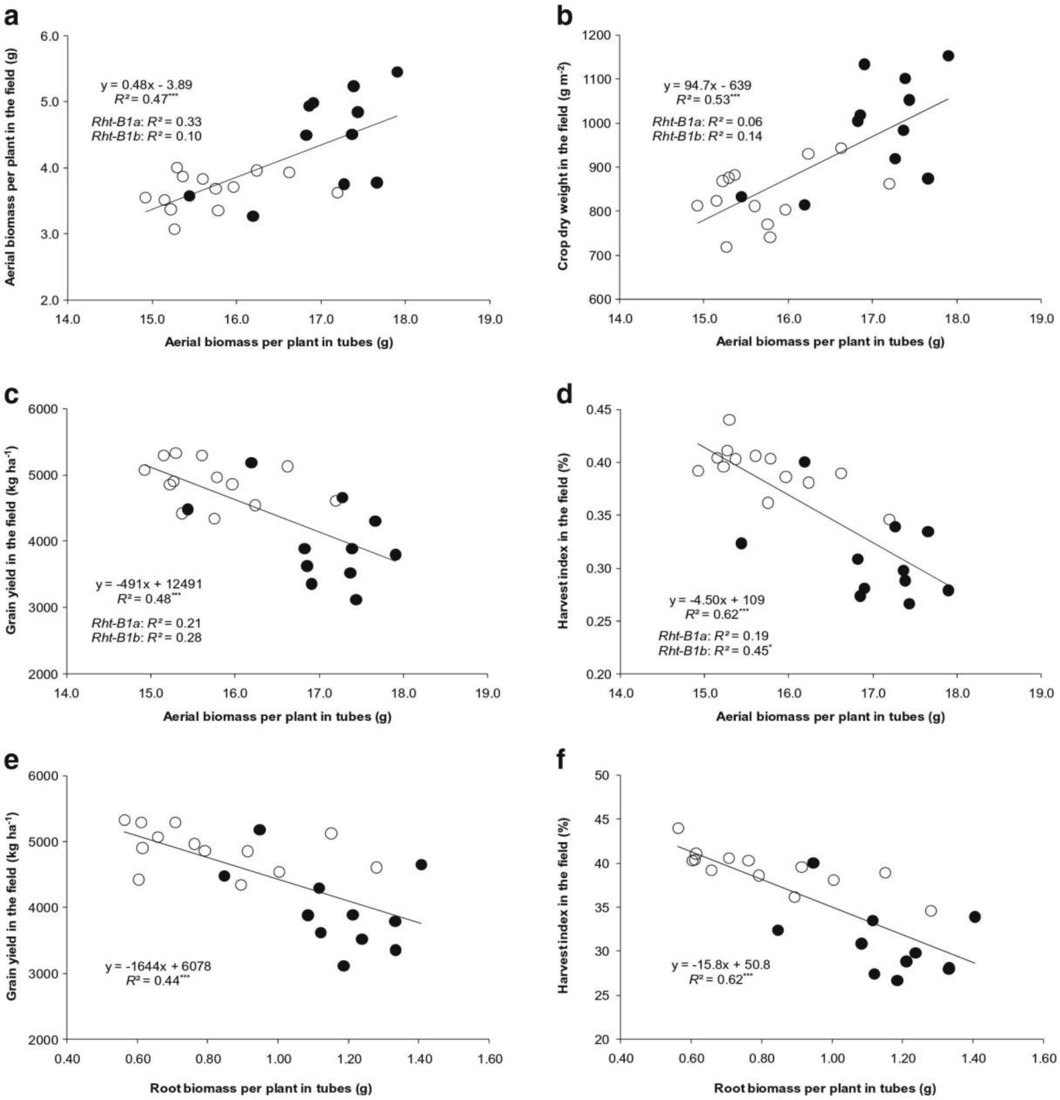


Fig. 5 Relationships between traits assessed for tubes in the greenhouse (*x* axis) and traits assessed in the field (*y* axis). Relationships between aerial biomass per plant in tubes and **a** aerial biomass, **b** crop dry weight, **c** yield and **d** harvest index. Relationships between root biomass per plant and **e** yield and **f** harvest index. Each point corresponds to one of 24 durum wheat cultivars. *Black circle* Tall cultivars (*Rht-B1a*), *White circle* Semi-dwarf cultivars (*Rht-B1b*). Regression equations refer to the models fitted to all cultivars (n = 24). Coefficients of determination of linear models fitted to tall (n = 11) and semi-dwarf (n = 13) cultivars are also shown on each figure. ***$P < 0.001$

significance of the cultivar effect for all traits and the high percentage of total variability explained by its sum of squares denoted the wide genetic diversity in the set of cultivars used in the current study. Particularly for plant height, the cultivar effect accounted for ca. 67 % of total variation, and differences between *Rht-B1* alleles explained more than 81 % of genotypic variability, thus supporting the importance of plant height in differentiating durum cultivars released before and after the Green Revolution (Royo et al. 2007, 2008; Graybosch and Peterson 2010). The greater variability of aerial and root biomass found within semi-dwarf cultivars when compared with tall ones supports the statement that genetic variability was not reduced in modern Mediterranean durum wheat cultivars as a consequence of the breeding activities conducted during the 20th century (Martos et al. 2005).

The significant differences observed in the greenhouse between cultivars carrying the *Rht-B1a* (tall) and *Rht-B1b* (semi-dwarf) allele for aerial and root biomass indicated that the dwarfing allele not only reduced above-ground biomass, as widely reported by previous studies (Brancourt-Hulmel et al. 2003; Royo et al. 2007; Álvaro et al. 2008a), but also caused a decrease in total root biomass and in each root section. Moreover, the reduction caused by the dwarfing allele in root biomass (ca. 28 %) was much greater than that observed in aerial biomass (ca. 8 %), as shown by the relative rate of change, which was more than four times higher for the roots than for the aerial organs (-0.73 % y^{-1} and -0.17 % y^{-1}, respectively). Nevertheless, as the aerial fraction of the plant was much heavier than the root fraction, when expressed in absolute terms the genetic change was about four times greater for the above-ground biomass (-27.0 g y^{-1}) than for the roots (-7 g y^{-1}). The relative change in the root/aerial biomass ratio was more than twice that recorded for aerial or total biomass, showing that in relative terms the dwarfing allele had a greater effect on reducing the dry matter of roots than on reducing that of aerial organs. The root/aerial biomass ratio was 29 % lower in semi-dwarf cultivars than in tall ones. Considering the higher grain yield of the former (Canevara et al. 1994; De Vita et al. 2007; Motzo et al. 2004; Royo et al. 2008), this finding may suggest that cultivars carrying the *Rht-B1b* allele have greater root efficiency for input capture than those carrying the *Rht-B1a* allele, which is in agreement with the recent proposal of Subira et al. (2015) that semi-dwarf cultivars have an improved capacity to respond to water availability after flowering. In addition, the larger biomass at depth of tall cultivars may denote greater transpiration under drought stress (Blum 2011).

The strong relationship found between root and aerial biomass assessed in tubes was consistent with the reduction caused in both of them by the *Rht-B1b* dwarfing allele. However, the slope of the linear regression model fitted to the relationship between aerial biomass and biomass of the lower root-section was more than twice the slope of the model fitted to the upper root-section. This result may indicate that differences between cultivars in root biomass are related to greater differences in aerial biomass when the divergence is due to the lower rather than the upper part of the root.

Relationship between aerial biomass and yield in the field

The results of the study of the relationships between aerial biomass and grain yield under field conditions depended on the specific set of cultivars that were considered when the models were fitted. For the whole set of cultivars, around 60 % of yield variations were explained by differences in the aerial biomass, considered either on a plant or a crop basis, which were negatively associated with yield. Moreover, for the whole set of cultivars 85 % of yield variations were explained by genotypic differences in HI, which had a positive relationship with yield and a negative association with aerial biomass. However, when semi-dwarf and tall cultivars were analysed separately, these relationships were statistically significant only for tall cultivars, suggesting that for this sub-set large biomass at anthesis resulted in low yields, probably due to its negative association with HI. These results indicate that, for the set of cultivars carrying the *Rht-B1a* allele used in the current study, the maintenance of a large crop biomass competed with the allocation of dry matter in the grain or, in other words, large biomass at anthesis was detrimental for achieving high yields. In contrast, for the set of semi-dwarf cultivars, yield did not depend on the crop biomass at anthesis but was positively associated with HI. These results suggest that, for the set of cultivars carrying the *Rht-B1b* studied here, yield relied on the capacity of the plant to accumulate photosynthates in the grain, independently of the size of the plant canopy at anthesis. It has been demonstrated that one of the greatest effects of the dwarfing allele was the increase in HI, related to the increase in the number of grains per spike and spikelet and an improved translocation efficiency of pre-anthesis assimilates to grains (Royo et al. 2007, 2008; Álvaro et al. 2008b, 2008c).

Differences between tall and semi-dwarf cultivars were also detected when the relationships between aerial biomass and the three main yield components were examined in field experiments. Although both number of grains per spike and grain weight were independent of the plant biomass at anthesis regardless of the set of cultivars considered, for genotypes carrying the *Rht-B1a* allele the number of spikes per unit area was negatively associated with aerial biomass, while the two traits were independent for semi-dwarf cultivars. These results may suggest competition in tall cultivars between the investment of resources in enlarging the canopy and the development of reproductive organs. This competition did not seem to exist within semi-dwarf cultivars, probably because their lower plant biomass allowed them to develop a larger number of spikes, as reported by Royo et al. (2007).

Aerial biomass assessed in tubes was positively and significantly associated with that obtained in the field when all cultivars were included in the model, but not for each set independently. These results demonstrate that, although plant development in tubes was much greater than that recorded under field conditions at commercial plant densities, the results obtained in tubes and in the field were properly correlated, but only when the model included a wide range of variability. This assumption was confirmed when similar trends were found in the relationships between yield and aerial biomass assessed in both the field and in tubes, and similar patterns were also obtained for the relationships between HI and aerial biomass determined by field and tube experiments when all cultivars were included in the model. In order to predict the relationship between root biomass and yield, it was hypothesized that if a significant association existed between the aerial biomass assessed in tubes and in the field for the whole set of cultivars, a relationship would also most likely exist between root biomass in tubes and in field experiments. In accordance with this assumption, the negative associations that appeared between root biomass and both yield and HI when all cultivars were considered in the model confirm the findings of previous studies regarding the lower efficiency of the biomass of tall varieties in terms of yield formation (Álvaro et al. 2008a, 2008c).

Conclusions

The introduction of the *Rht-B1b* allele in modern durum wheat cultivars resulted in a reduction in aerial biomass of the plant and an even greater reduction in root biomass along its whole length. A strong and significant positive relationship was found between the aerial and root biomass in the three sections, thus showing that the reduction of the root biomass due to the introduction of the *Rht-B1b* allele occurred in both the upper and the lower parts of the root. Within the tall cultivars, those with large aerial biomass had a lower number of spikes per unit area and also a lower HI, which resulted in yield reductions. On the other hand, within the semi-dwarf cultivars a greater aerial biomass was neither detrimental nor beneficial in terms of yield formation.

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CHAPTER 2

Breeding progress in the pasta-making quality of durum wheat cultivars released in Italy and Spain during the 20th century

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Breeding progress in the pasta-making quality of durum wheat cultivars released in Italy and Spain during the 20th Century

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Abstract. Genetic improvement of quality traits of durum wheat achieved in Italy and Spain during the 20th Century was investigated using an historical series of 12 cultivars from each country. The European Union durum wheat quality index increased by 6.25% (0.13% year^{-1} in Italian and 0.06% year^{-1} in Spanish cultivars). Protein content decreased by ~10% (–0.14% year^{-1} in Italian and –0.19% year^{-1} in Spanish cultivars) but protein per ha increased at a rate of 0.35% year^{-1} (0.41% year^{-1} in Spanish and 0.26% year^{-1} in Italian cultivars). Yellow colour index increased by 9.9% (0.15% year^{-1} in Italian and 0.10% year^{-1} in Spanish cultivars). Test weight and vitreousness did not suffer significant changes over time. Gluten strength increased by 32.1% or 0.54% year^{-1} in Italian, and 27.9% or 0.33% year^{-1} in Spanish cultivars. Much larger genetic control on gluten strength was found in Italian than in Spanish cultivars. Changes in sedimentation index (41.1% or 0.64% year^{-1} in Italy, and 41.6% or 0.49% year^{-1} in Spain) were the consequence of the progressive incorporation into recent cultivars of favourable low molecular weight glutenin subunits (LMW-GS). Breeding increased the frequency of the LMW-GS combination aaa, which was present in 75% of all intermediate cultivars and in 100% of the modern Italian cultivars. A LMW-GS combination not previously reported (d?b) was identified in two modern Spanish cultivars. Breeding programs were also successful in increasing the stability of gluten strength and the sedimentation index.

Additional keywords: genetic gain, genetic improvement, glutenin subunits, historical series, old to modern cultivars, quality stability.

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Introduction

Italy and Spain are among the main durum wheat (*Triticum turgidum* L. var. *durum*) producers of the European Union (EU), where most of the grain is devoted to pasta manufacturing (Di Fonzo *et al*. 2005; Royo 2005). In Mediterranean environments, durum wheat is mostly grown under rainfed conditions, where the crop is frequently exposed to environmental stresses, with high temperatures and water scarcity common during the grain-filling period. This usually limits the achievement of high yields, but in most years has low or nil negative effects on grain attributes determining pasta-making quality. The release of durum cultivars with high quality standards has been a major breeding concern during the last half of the past century (Pagnotta *et al*. 2005) and is still one of the main goals of breeding programs in the region.

Pasta cooking properties are mostly related to high grain protein content and to the quality of its gluten protein. These traits, together with vitreousness and yellow semolina colour, are of great importance for durum wheat quality (Di Fonzo *et al*. 2005). Gluten, which constitutes around the 80% of the endosperm protein, is composed of gliadins and glutenins (Peña *et al*. 2002; Sissons 2008) and its properties depend on its protein subunit composition and resulting polymeric structure. It is generally accepted that glutenins confer elasticity and gliadins are responsible for the viscosity and extensibility of the gluten, with the interactions between the two protein fractions determining the ultimate gluten quality of one cultivar (Weegels *et al*. 1996). It is also accepted that, of the two fractions, the glutenin is still the main one responsible of the gluten strength (Peña *et al*. 2002).

Glutenin subunits can be separated according to their relative mobility into high molecular weight (HMW-GS) and low molecular weight (LMW-GS) using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Singh *et al*. 1991). The LMW-GS comprise 80% of the total glutenin (Peña *et al*. 2002; Ruiz *et al*. 2005; Sissons 2008). In durum wheat the synthesis of the HMW-GS is controlled by the *Glu-A1* and *Glu-B1* loci, whereas for the LMW-GS, the *Glu-A3*, *Glu-B3* and *Glu-B2* loci are responsible (Carrillo *et al*. 2000). Both HMW-GS and LMW-GS, but mainly the latter, have a great influence on gluten strength and on the pasta-making quality of durum wheat (Peña 2000; Ruiz *et al*. 2005), stressing the need to obtain cultivars with the optimal HMW-GS and LMW-GS combinations to boost gluten quality.

Contrasting breeding strategies were reportedly adopted in Italy and Spain during the 20th Century. Whereas ancient Italian cultivars were incorporated and their alleles recombined during the process of genetic improvement and modern cultivar development, Spanish breeding efforts did not involve the use of traditional local germplasm (Martos *et al.* 2005; Royo *et al.* 2007). Breeding programs conducted in Italy since the beginning of the 20th Century (Maliani 1979) are considered as pioneering in the world of durum wheat and, as a result, the Italian durum wheat pool currently represents the most outstanding and differentiated pool in the Mediterranean Basin compared with the dominating CIMMYT (International Maize and Wheat Improvement Center) derived germplasm (Royo *et al.* 2009). These early programs largely relied on local landraces. Old cultivars, such as 'Senatore Cappelli', were widely cultivated until the end of the 1960s and were broadly used as parents in Italian breeding programs, being present in the genetic background of many modern cultivars (Martos *et al.* 2005). Italian cultivars released from the late 1970s and the 1980s originated from crosses between local accessions and CIMMYT germplasm, with more recent ones, such as 'Simeto' and 'Zenit', still broadly used by many Italian farmers (Di Fonzo *et al.* 2005). On the other hand, the lack of continuity in Spanish local breeding programs during the first half of the 20th Century became a constraint for the use of the genetic background of local landraces into modern cultivar development (Royo and Briceño-Félix 2011). From the 1970s onwards, introduced CIMMYT germplasm had a great impact on cultivar releases in the country, where 'Mexa' (a cultivar derived from the CIMMYT hallmark 'Mexicali 75') covered ~90% of the durum wheat area during the mid-1980s (Martos *et al.* 2005; Royo 2005). Similar widespread impact was achieved with the release and extensive adoption, during the mid-1980s and 1990s and until very recently, of the 'Yavaros'-derived lines such as 'Yavaros 79' itself, 'Vitron', 'Nuño' and others.

References to the genetic gains in durum wheat quality during the last Century in Mediterranean countries are scarce. De Vita *et al.* (2007) showed an increase in pasta-making quality during the 20th Century in durum wheat cultivars released in Italy, due to the incorporation of favourable alleles in modern cultivars, such as the 7+8 subunit of the *Glu-B1* locus, which increased gluten strength. However, no similar studies have been conducted to assess the breeding progress in quality during the 20th Century in Spain.

The present study was conducted using an historical series of 24 durum wheat cultivars released in different periods during the 20th Century in Italy and Spain to: (*i*) ascertain the changes achieved by breeding on the most relevant grain quality traits, and (*ii*) to assess the relationship between allelic variations associated with HMW-GS and LMW-GS composition and the changes in gluten strength observed during the same period.

Materials and methods

Plant material

Twenty-four durum wheat (*Triticum turgidum* L. var. *durum*) cultivars, 12 Italian and 12 Spanish, were selected to represent the germplasm grown in Italy and Spain during the 20th Century. Based on the year of release, the cultivars were assigned to three periods: old (mainly landraces, released before 1945), intermediate (released between 1950 and 1985) and modern (released from 1988 to 2000) (Table 1). The intermediate group included early semi-dwarf cultivars derived from CIMMYT germplasm, such as cv. Mexa, and landmark early European cultivars such as cv. Creso. The modern set included cultivars released by local breeding programs during the last decade of the Century in both countries. In the selection of Spanish modern cultivars we avoided the inclusion of cultivars of foreign origin (derived from Italian, French or CIMMYT germplasm), despite the fact that these are dominant in the Spanish market. This was done to enable assessment of the impact of modern efforts conducted locally in Spain. Phylogenetic relationships have been previously ascertained in this historical series (Martos *et al.* 2005), which has also been used to assess changes in yield formation (Royo *et al.* 2007, 2008), and biomass production and allocation (Álvaro *et al.* 2008*a*, 2008*b*, 2008*c*).

Experimental

Five experiments were conducted in Lleida (north-eastern Spain) involving four growing seasons (2001, 2002, 2004 and 2005) and two locations: Gimenells (41°40′N, 0°20′E) under irrigated conditions (2001, 2002, 2004, 2005); and Foradada (41°88′N, 0°76′E), a rainfed site (2002). Soils were mesic Calcixerolic Xerochrept in Gimenells and Xerofluvent Oxiaquic in Foradada (Soil Survey Staff 1999), both with a fine-loamy texture. The experimental design was a randomised complete block with four replicates and plots of 12 m^2 (8 rows 0.15 m apart). Planting time was between 31 October and 16 December in all cases, at a sowing rate of 400 fully viable seeds m^{-2}. Plots were fertilised following the recommendations for maximising yields while preventing lodging, and were kept disease- and insect- free with preventive pesticide applications. Weather stations located near the experimental sites provided daily meteorological data. Water input (rainfall + irrigation) ranged from 275 to 322 mm from sowing to anthesis and from 36.1 to 137 mm from anthesis to maturity. Average daily mean temperatures ranged from 7.12 to 9.18°C from sowing to anthesis, and from 16.9 to 21.1°C from anthesis to maturity. Mean length of the grain filling period, expressed as thermal time (growing degree-days, GDD), ranged from 311 to 390 GDD.

Quality analyses

Plots were mechanically harvested at commercial maturity and grain yield was expressed at 12% moisture basis. A sample of ~250 g of whole grain from each plot was cleaned and used for quality analysis. Grain protein content (%) was determined by a near-infrared spectroscope (NIT, Infratec® 1241 grain analyser; Foss, Hilleroed, Denmark) previously calibrated for protein content against the standard Kjeldahl method. Whole-grain flour samples were obtained with a whole-meal grinder; fine particle size was ensured by attaching a 0.5-mm screen to the grinder. Gluten strength was determined on 1 g of whole-grain flour samples using the SDS sedimentation test, following the methodology of Axford *et al.* (1978) as modified by Peña *et al.* (1990), and using stoppered, 25-mL graduated cylinders. The sedimentation index was computed as the ratio between gluten

Table 1. Origin, year of release and allelic/banding pattern combinations for high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits (GS) identified in the 24 durum wheat cultivars of the historical series included in this study

Period	Cultivar	Year of release	HMW-GS		LMW-GS			
			Glu-A1	*Glu-B1*	*Glu-A3*	*Glu-B3*	*Glu-B2*	Combination
			Italian					
Old	Balilla Falso	Before 1930	1	20	null	2+4+15+18	null	h?b
	Razza 208	Before 1930	null	20	null	2+4+15+19	12	haa
	Senatore Cappelli	1930	null	20	6	2+4+15+19	12	aaa
	Carlojucci	1945	null	20	null	2+4+15+19	12	haa
Intermediate	Capeiti 8	1955	null	20	6	2+4+15+19	12	aaa
	Trinakria	1970	null	20	null	2+4+15+19	12	haa
	Creso	1974	null	6+8	6	2+4+15+19	12	aaa
	Adamello	1985	null	7+8	6	2+4+15+19	12	aaa
Modern	Simeto	1988	null	7+8	6	2+4+15+19	12	aaa
	Cirillo	1992	null	20	6	2+4+15+19	12	aaa
	Flavio	1992	null	15+16	6	2+4+15+19	12	aaa
	Zenit	1992	null	6+8	6	2+4+15+19	12	aaa
			Spanish					
Old	Blanco Verdeal	Before 1930	null	6+8	11	2+4+15+17	null	efb
	Clarofino	Before 1930	null	13+16	6	2+4+15+19	12	aaa
	Pinet	Before 1930	null	14^{+}+18	null	2+4+16+17	12	h?a
	Rubio de Belalcázar	Before 1930	1	13+16	6	2+4+15+19	12	aaa
Intermediate	Bidi 17	1950	null	20	6	2+4+15+19	12	aaa
	Camacho	1975	null	6+8	null	2+4+15+19	null	hab
	Esquilache	1976	null	7+8	6	2+4+15+19	12	aaa
	Mexa	1980	null	7+8	6	2+4+15+19	12	aaa
Modern	Ariesol	1992	null	7+8	6	2+4+15+19	12	aaa
	Senadur	1995	null	6+8	6	2+4+15+19	12	aaa
	Astigi	1999	null	20	6+11	4+15+19	null	d?b
	Boabdil	2000	null	7+17	6+11	4+15+19	null	d?b

strength and protein content, expressed as mL per % protein unit. Yellow colour index (b, CIE L*a*b* colour system) was estimated on whole-grain flour using a portable reflectance colourimeter (CR-400; Konica-Minolta Sensing, Inc., Tokyo) equipped with a filter tri-stimulate system. Test weight (TW, kg hL^{-1}) was determined by the GAC2100 analyser (Dickey-John Co., Auburn, IL, USA). These four quality traits were used to calculate the EU quality index (QI) for durum (European Commission Regulation No. 2237/2003, 23 December 2003), using cv. Simeto as reference check. Each quality trait was expressed for each cultivar as a percentage of the mean value of cv. Simeto (assumed to be 100%), and the QI was calculated by weighting each trait according to the following percentages: protein content (40%), gluten strength (30%), yellow index (20%), TW (10%). Grain vitreousness (%) was determined by counting the number of vitreous grains after cutting a random sample of 100 grains per plot.

Allelic composition of HMW-GS and LMW-GS

Electrophoretic analyses were performed to identify HMW- and LMW-GS composition at five loci: *Glu-A1*, *Glu-B1*, *Glu-A3*, *Glu-B3*, *Glu-B2*. Electrophoresis was run on a bulk of 10 seeds from each cultivar. For the old cultivars, a spike of the dominant type was previously selected and its seeds were planted in the subsequent growing season on an individual row. This row was harvested at ripening, and 10 seeds from the bulk were taken for electrophoresis. Electrophoretic analysis (1D SDS-PAGE) was conducted according to the protein extraction process of Singh *et al.* (1991) and the protocols implemented at CIMMYT by Peña *et al.* (2004). The nomenclature followed was that proposed by Payne and Lawrence (1983) for the HMW-GS and that of Nieto-Taladriz *et al.* (1997) for the LMW-GS.

Statistical analyses

Combined analysis of variance (ANOVA), in which the cultivar effect was partitioned into its components (period, country, period × country interaction, and cultivar within period and country), were performed for all quality traits. Additional ANOVAs were conducted for the sedimentation index considering as factors the HMW-GS and LMW-GS allelic combinations identified in the cultivars from each country. Means were compared by Tukey test at $P = 0.05$. Absolute (AGG, trait unit $year^{-1}$) and relative (RGG, % $year^{-1}$) genetic gains were computed for each quality trait as the slope of the linear regression model fitted to the relationship between the absolute or relative value of the trait and the year of cultivar release. Relative values were computed for each cultivar as percentage irrespective of the average value of all the cultivars for a given country. The stability of each quality trait was determined for each cultivar as the slope (*b*) of the joint regression analysis (Finlay and Wilkinson 1963), and slopes were compared by using PROC GLM of SAS statistical package (SAS Institute Inc. 2009*a*). In order to assess the changes produced in the stability of the quality traits across time, the relationship between *b* and the year of release was studied for those traits in which the regression slopes differed significantly between cultivars. All analyses were performed with the JMP ver. 8 software (SAS Institute Inc. 2009*b*) and Genstat ver. 13 (Genstat 2010).

Results

Genetic changes on grain quality

The combined ANOVA for grain quality traits revealed that the percentage of total variance explained by the cultivar effect was very large for yellow index and quality index (48% and 33%, respectively), somewhat lower but still substantial for protein per ha, gluten strength and sedimentation index (21–24%), and low for test weight, protein content and vitreousness (<12%) (Table 2). The partitioning of the cultivar effect into its components showed that differences between breeding periods explained 0.45–23.4% of total variance corresponding to 3.8% (for test weight) to 49.2% (for yellow index) of the cultivar effect. The country effect was significant for all traits except for quality index and vitreousness. Even in the case of statistical significance of the country effect, the percentage of variation explained was always ≤1%.

Comparison of the mean values of grain quality traits for the cultivars released in different periods in Italy and Spain showed a steady increase in all but protein content, test weight and vitreousness (Table 3). Positive changes in the quality index over time were due to substantial increases in gluten strength and yellow index, compensating for the significant decrease in protein content and test weight. Albeit significant statistically, this decrease in protein content was relative and did not result in values below 14% in any of the historical or country groups considered. Test weight did not suffer significant changes over time. The largest improvements from old to modern cultivars occurred in protein per unit area, gluten strength and, consequently, in the sedimentation index. Vitreousness suffered a significant decrease in cultivars from the intermediate period compared with the old cultivars, but this reduction was partially compensated for in the modern cultivars (Table 3).

The overall rate of genetic change in the quality index was 0.09% year^{-1}, and the improvement was more than double for Italian than for Spanish cultivars (Table 4). Relative genetic gains for gluten strength, yellow index and sedimentation index were also greater in the Italian germplasm. Protein content decreased at a higher rate in Spanish than in Italian cultivars, but the protein yield per ha increased from old to modern cultivars by 0.41% year^{-1} in Spanish and 0.26% year^{-1} in Italian cultivars. The rates of genetic change in test weight and vitreousness were not significant for either country (Table 4).

The stability of the quality traits was assessed by comparing the cultivar slopes of the regression models (*b*) fitted to the relationship between the genotype and the environmental means. The results revealed differences between slopes ($P < 0.05$) only for gluten strength, yellow index, sedimentation index and vitreousness (data not shown). For these traits the slopes of the joint regression analyses were plotted against the year of cultivar release, with the results showing that for gluten strength and sedimentation index, the

Table 2. Percentage of the sum of squares of ANOVA for the quality traits measured in the historical series of 24 durum wheat cultivars released in different periods in Italy and Spain and tested in five environments

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; n.s., not significant

Source of variation	d.f.	Quality index	Protein content	Gluten strength	Yellow index	Test weight	Protein per ha	Sedimentation index	Vitreousness
Environment	4	22.9 ***	78.3 ***	53.6 ***	35.3 ***	73.8 ***	43.5 ***	63.2 ***	64.6 ***
Block (environment)	15	3.50 ***	1.66 ***	1.67 ***	0.88 ***	1.10 ***	3.28 ***	1.20 ***	1.77 ***
Cultivar		32.9 ***	10.8 ***	23.4 ***	47.6 ***	11.8 ***	24.2 ***	21.6 ***	9.17 ***
Period	2	8.70 ***	5.78 ***	10.7 ***	23.4 ***	0.45 ***	14.2 ***	12.7 ***	2.71 ***
Country	1	1×10^{-3} n.s.	0.19 ***	0.16 **	0.52 ***	0.16 ***	0.93 ***	0.34 ***	0.10 n.s.
Period × country	2	0.24 n.s.	0.16 **	0.09 n.s.	0.67 ***	0.25 ***	1.30 ***	0.11 *	0.02 n.s.
Cultivar (period × country)	18	23.7 ***	4.72 ***	12.4 ***	23.9 ***	10.5 ***	7.60 ***	8.40 ***	6.50 ***
Cultivar × environment		23.8 ***	4.01 ***	15.2 ***	11.5 ***	10.1 ***	17.5 ***	8.74 ***	13.2 ***
Period × environment	8	7.21 ***	1.03 ***	4.89 ***	1.36 ***	4.19 ***	5.91 ***	1.16 ***	1.22 ***
Country × environment	4	1.34 ***	0.30 ***	0.55 ***	0.14 *	0.24 ***	1.21 ***	0.58 ***	0.11 n.s.
Period × country × environment	8	2.92 ***	0.16 n.s.	1.75 ***	2.42 ***	0.63 ***	0.59 *	0.93 ***	2.13 ***
Cultivar (period × country) × environment	72	12.3 ***	2.53 ***	8.18 ***	7.63 ***	4.73 ***	9.68 ***	6.13 ***	9.59 ***
Residual	345	15.8	4.45	6.40	4.92	4.50	11.1	5.00	11.4
Total	479								

Table 3. Mean values across five environments of the quality traits for 24 durum wheat cultivars released in different periods in Italy and Spain

Values in italics indicate the percentage change from old to modern cultivars. Means within a column and country followed by the same letter are not significantly different according to Tukey's Studentised range test at $P = 0.05$

Period	Quality index		Protein content (%)		Gluten strength (mL)		Yellow index		Test weight (kg hL^{-1})		Protein per ha (kg ha^{-1})		Sedimentation index (mL per % protein)		Vitreousness (%)	
								Italian								
Old	99.0c		16.0a		5.61c		12.9c		79.7ab		666b		0.36c		89.9a	
Intermediate	103b	*4.21*	15.1b	*–5.74*	6.90b	*23.0*	13.4b	*3.68*	80.0a	*0.37*	797a	*19.7*	0.48b	*30.8*	83.6b	*–7.05*
Modern	106a	*7.13*	14.7c	*–8.04*	7.40a	*32.1*	14.4a	*11.4*	79.6b	*–0.20*	777a	*16.6*	0.51a	*41.1*	87.7a	*–2.52*
								Spanish								
Old	100c		16.0a		5.94c		13.3b		78.9b		592c		0.39c		91.6a	
Intermediate	103b	*2.29*	14.8b	*–7.63*	6.90b	*16.2*	13.4b	*0.31*	79.9a	*1.22*	739b	*24.8*	0.48b	*24.4*	84.2b	*–8.04*
Modern	106a	*5.37*	14.2c	*–11.3*	7.59a	*27.9*	14.5a	*8.41*	79.6a	*0.94*	801a	*35.2*	0.55a	*41.6*	88.6a	*–3.28*

Table 4. Absolute (AGG) and relative (RGG, % year^{-1}) genetic changes in quality traits of Italian and Spanish durum wheat cultivars released during the 20th Century

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

Trait	Italian and Spanish ($n = 24$)			Italian ($n = 12$)			Spanish ($n = 12$)			AGG units
	R^2	AGG	RGG	R^2	AGG	RGG	R^2	AGG	RGG	
Quality index	0.29**	0.10	0.09	0.31	0.14	0.13	0.40*	0.07	0.06	year^{-1}
Protein content (%)	0.63***	–0.03	–0.17	0.52**	–0.02	–0.14	0.73***	–0.03	–0.19	% year^{-1}
Gluten strength (mL)	0.54***	0.03	0.42	0.49*	0.04	0.54	0.81***	0.02	0.33	mL year^{-1}
Yellow index	0.32**	0.02	0.12	0.33*	0.02	0.15	0.35*	0.01	0.10	year^{-1}
Test weight (kg hL^{-1})	0.01	4.70×10^{-3}	0.01	3×10^{-3}	-2.60×10^{-3}	-3.26×10^{-3}	0.05	9.64×10^{-3}	0.01	kg hL^{-1} year^{-1}
Protein/ha (kg/ha)	0.57***	2.54	0.35	0.04*	1.96	0.26	0.70***	2.92	0.41	kg ha^{-1} year^{-1}
Sedimentation index (mL per % protein)	0.69***	2.52×10^{-3}	0.55	0.61**	2.86×10^{-3}	0.64	0.89***	2.29×10^{-3}	0.49	mL per % protein year^{-1}
Vitreousness (%)	0.10	–0.06	–0.07	0.05	–0.05	–0.05	0.18	–0.07	–0.08	% year^{-1}

b values decreased over the 20th Century, at a rate of 0.01 year^{-1} and 0.004 year^{-1}, respectively (Fig. 1*a, c*), but for yellow index and vitreousness changes were not statistically significant (Fig. 1*b*, *d*).

Allelic composition of HMW-GS and LMW-GS

Twelve and 18 alleles, encoded by *Glu-1*, *Glu-3* or *Glu-2*, were identified in the Italian and Spanish cultivars, respectively (Table 1). The null allele was the most frequent at *Glu-A1*, with only two old cultivars—Italian cv. Balilla Falso and Spanish cv. Rubio de Belalcázar— having the alternative allele 1 at this locus (Table 1). Four and six different alleles were found at *Glu-B1* in Italian and Spanish cultivars, respectively, with the old Italian cultivars included in this study being monomorphic for allele 20 at this locus. Greater allelic diversity was found at *Glu-B1* in the modern cultivars of both countries than in the groups of intermediate or old cultivars. Band 6 was the most frequent at *Glu-A3*, but the null allele at this locus was present in some old and intermediate cultivars from both countries (Table 1). The most frequent banding pattern at *Glu-B3* was 2+4+15+19 (*Glu-B3a*), which was common to 11 Italian and eight Spanish cultivars, in all three breeding periods considered together. The two alleles described previously at *Glu-B2*, *Glu-B2b* (null) and *Glu-B2a* (band 12) were present in the collection, but band 12 predominated, especially within the Italian germplasm.

Five and seven allelic combinations at the *Glu-1* loci and three and five combinations specific to *Glu-2*/*Glu-3* loci were detected in Italian and Spanish cultivars, respectively (Table 1). Four previously described LMW-GS combinations associated with the LMW models—LMW-2 (combination aaa), LMW-2^{-} (combination haa), LMW-1 (efb) and LMW-1 (hab)—were identified in the historical series. Combination aaa at LMW-2 (allele 6 at *Glu-A3*, bands 2+4+15+19 at *Glu-B3*, and band 12 at *Glu-B2*) was found in eight Italian and seven Spanish cultivars (Table 1). Combination haa (null allele at *Glu-A3*, bands 2+4+15+19 at *Glu-B3*, and band 12 at *Glu-B2*) was not present in Spanish germplasm, but was in three Italian cultivars. Combinations efb (allele 11 at *Glu-A3*, bands 2+4+15+17 at *Glu-B3*, and the null allele at *Glu-B2*) and hab (null allele at *Glu-A3*, bands 2+4+15+19 at *Glu-B3*, and the null allele at *Glu-B2*) were found in old and intermediate Spanish cvv. Blanco Verdeal and Camacho, respectively. In addition, three allelic combinations, so far unclassified, were found in Italian cv. Balilla Falso and Spanish cvv. Pinet, Astigi and Boabdil (Table 1). The total number of different allelic combinations for HMW/LMW-GS loci found in the whole collection was 13, i.e. six in Italian and 10 in Spanish cultivars, only three of them being shared by both sets of genotypes.

In order to identify the environmental and genetic effects on gluten quality associated with specific allelic combinations, ANOVAs were conducted separately for Italian and Spanish cultivars with the mean sedimentation index values of the cultivars sharing a common HMW or LMW allelic combination, by considering these combinations as factors of the ANOVA. The results revealed much greater genetic control and less environmental effect on sedimentation index in Italian than in Spanish cultivars for both loci (Table 5). Comparison of the sedimentation index values of the cultivars from each country sharing a common allelic combination for HMW- or LMW-GS loci showed a larger range of variation within Italian than Spanish germplasm. The most favourable combination at HMW-GS loci was that identified in modern Spanish cv.

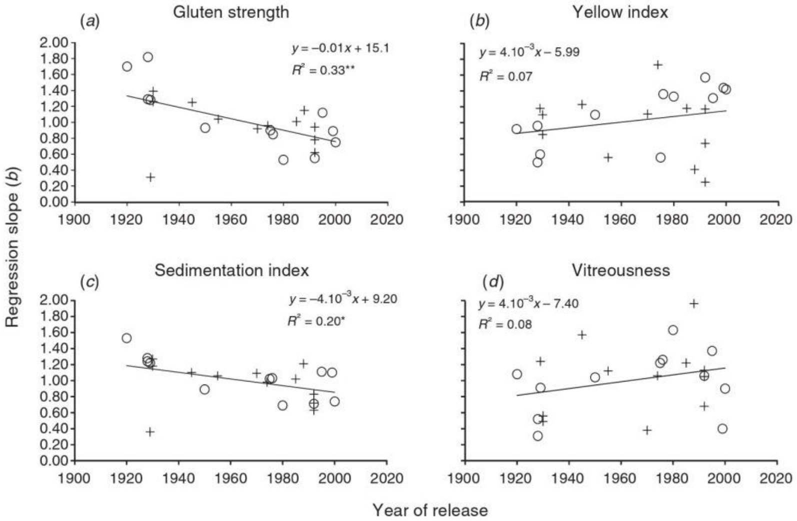


Fig. 1. Relationship between the slope (*b*) of the joint regression analysis (Finlay and Wilkinson 1963) and the year of release of the 24 cultivars of the historical series for: (*a*) gluten strength, (*b*) yellow index, (*c*) sedimentation index, and (*d*) vitreousness. +, Italian cultivars; ○, Spanish cultivars. $^{*}P < 0.05$; $^{**}P < 0.01$.

Boabdil (null allele at *Glu-A1* and bands 7+17 at *Glu-B1*) (Table 1) with a mean sedimentation index of 0.53 (Table 6). In the Spanish germplasm, cultivars carrying the LMW models hab and the unclassified d?b—none of them found in Italian cultivars—had the greatest sedimentation index values, 0.50 and 0.52, respectively. In the Italian germplasm, combination LMW-2 aaa was the most favourable for a high sedimentation index, with an average value of 0.49. Two unclassified LMW combinations (h?a and h?b), identified in Spanish and Italian germplasm, respectively, led to the poorest sedimentation index values, 0.37 and 0.22, respectively (Table 6).

Table 6 shows the mean sedimentation index values of each of the 13 allelic combinations found in the collection for HMW- and LMW-GS loci, and the number of cultivars carrying each of them. The combination formed by the null allele at *Glu-A1*, bands 6+8 at *Glu-B1*, and LMW-2 combination aaa, shared by Italian cvv. Creso and Zenit and Spanish cv. Senadur, resulted in the highest sedimentation index (Table 6). However, combination aaa of the LMW-2 model was present not only in cultivars with high gluten quality, but also in some with low sedimentation index (0.36 in Table 6), such as Spanish cv. Rubio de Belalcázar, which suggests a large interaction between HMW-GS and LMW-GS loci. Combination aaa of model LMW-2 (band 6 at *Glu-A3*, bands 2+4+15+19 at *Glu-B3*, and band 12 at *Glu-B2*) resulted in a high sedimentation index when the null allele was present at *Glu-A1*, interacting with bands 6+8, 7+8 or 15+16 at *Glu-B1*, but it resulted in reduced sedimentation index when combined with bands 20 or 13+16 at *Glu-B1* (Table 6).

The distribution of HMW- and LMW-GS combinations in cultivars from different historical periods, shown in the right columns of Table 6, demonstrates that those resulting in low sedimentation index values were more frequent in the old cultivars, whereas the highest sedimentation index was associated with specific combinations with a high frequency in the intermediate and, particularly, in the modern cultivars. The most successful HMW- and LMW-GS combinations found in cultivars from both countries included combination aaa of LMW-2, but the presence at LMW locus of band 6+11 at *Glu-A3*, bands 4+15+19 at *Glu-B3*, and the null allele at *Glu-B2* in modern Spanish cvv. Boabdil and Astigi also resulted in high sedimentation index values (Tables 1 and 6).

Discussion

Genetic gains in grain quality traits

Quality is one of the primary goals of durum wheat breeding programs in the EU, where premiums have been established to promote the cultivation of high-quality cultivars (Royo and Briceño-Félix 2011). With the aim of regulating these premiums, the quality index (QI) was defined to consider in an integrative way the most important durum wheat grain characteristics for pasta making. The relatively low environmental effect and large

Table 5. Percentage of the sum of squares of ANOVA for the sedimentation index considering the allelic combinations found in Italian and Spanish cultivars for the high molecular weight (HMW) and low molecular weight (LMW) glutenin subunit loci

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

Source of variation	HMW				LMW			
	d.f.	Italy	d.f.	Spain	d.f.	Italy	d.f.	Spain
Environment	4	42.7***	4	73.9***	4	45.7***	4	79.3***
Block (environment)	15	1.00**	15	1.29*	15	1.09	15	1.88***
Allelic combination	4	48.2***	6	13.2***	2	43.7***	4	11.5***
Env. × allelic combinations	16	7.34***	24	6.10***	8	7.68***	16	5.50***
Residual	60		90		30		60	
Total	99		139		59		99	

Table 6. Mean values of the sedimentation index (mL per % protein ± standard error) for each high molecular weight (HMW) and low molecular weight (LMW) glutenin allelic combination found in 24 durum wheat cultivars from the historical series and number of cultivars of each period carrying them

HMW		LMW			Sedimentation index	Number of cultivars		
Glu-A1	*Glu-B1*	*Glu-A3*	*Glu-B3*	*Glu-B2*		Old	Intermediate	Modern
null	6+8	6	2+4+15+19	12	0.56 ± 0.02	0	1	2
null	7+17	6+11	4+15+19	null	0.53 ± 0.03	0	0	1
null	7+8	6	2+4+15+19	12	0.52 ± 0.02	0	3	2
null	20	6+11	4+15+19	null	0.51 ± 0.04	0	0	1
null	15+16	6	2+4+15+19	12	0.51 ± 0.03	0	0	1
null	6+8	null	2+4+15+19	null	0.50 ± 0.04	0	1	0
null	20	6	2+4+15+19	12	0.43 ± 0.02	1	2	1
null	6+8	11	2+4+15+17	null	0.42 ± 0.04	1	0	0
null	20	null	2+4+15+19	12	0.41 ± 0.02	2	1	0
null	13+16	6	2+4+15+19	12	0.37 ± 0.04	1	0	0
null	14^{+}+18	null	2+4+16+17	12	0.37 ± 0.05	1	0	0
1	13+16	6	2+4+15+19	12	0.36 ± 0.05	1	0	0
1	20	null	2+4+15+18	null	0.22 ± 0.01	1	0	0

genetic control of this index observed in this study confirmed the value of QI for the quality classification of EU durum cultivars, as recently stated by Nazco *et al*. (2012). This study demonstrated a steady QI increase during the 20th Century in both Italian and Spanish durum wheat cultivars, with a larger genetic gain recorded in the former (7.13% from old to modern cultivars and a RGG of 0.13% $year^{-1}$ in Italy, 5.37% with a RGG of 0.06% $year^{-1}$ in Spain). However, the larger genetic gain obtained for Italian cultivars was due to the slightly lower quality of the old Italian genotypes compared with the old Spanish ones, since modern cultivars from both countries reached a similar values for QI. The greater efforts made by Italian breeders to improve the global quality of their durum wheats may be a consequence of the longer breeding tradition for this crop in this country (Royo *et al*. 2009), likely linked to the economic importance of durum wheat in Italy regarding its production and the land surface devoted to it (MPAAF 2011).

Protein content is the quality trait with a largest weight in the balanced QI. The large environmental effect obtained in this study for protein content confirms the findings of previous studies conducted with durum wheat in Mediterranean environments (Rharrabti *et al*. 2003). Nevertheless, our results suggest that cultivars of different periods had a similar response to environmental variations in terms of protein content, thus suggesting that breeding has not contributed to the stability of this trait. Despite the global decrease recorded in this study in grain protein content (0.17% $year^{-1}$), total protein production per ha increased at a rate of 0.35% $year^{-1}$, which is likely a consequence of large grain yield increases accompanied by increased nitrogen uptake and/or translocation capacity. A previous study with the same historical series used here reported an average yield gain of 0.61% $year^{-1}$ (Royo *et al*. 2008), which indicates that the yield increases achieved during the 20th Century largely compensated for the decreases in the protein content of the newly released cultivars. Several studies have reported increases in grain yield accompanied by significant decreases in grain protein content (Motzo *et al*. 2004; De Vita *et al*. 2007; Dotlačil *et al*. 2010; Nazco *et al*. 2012). The negative relationship between yield and protein content (Rharrabti *et al*. 2001) has been associated with a dilution effect of nitrogen compounds when carbohydrate deposition increases through photosynthesis (Lawlor 2002; Martre *et al*. 2003). Even with the loss of grain protein content resulting from past breeding activities, the grain of modern durum wheats from Italy and Spain contained, on average, 14.7% and 14.2% protein, respectively, levels that exceed the minimum values required by the pasta-processing industry set at ~12.5% (Peña *et al*. 2002).

Of the four grain quality traits considered in the calculation of the QI index, gluten strength was the most important for explaining global quality increases in both countries, as it increased across periods by 32.1% and 27.9% in Italy and Spain, respectively, with RGG of 0.54% $year^{-1}$ and 0.33% $year^{-1}$. Gluten strength improvements largely compensated for the decreases in protein content, thus leading to gains in sedimentation index of ~41% on average. However, the rate of increase in sedimentation index in Italy was superior to that in Spain due to the larger rate of improvement of gluten strength and the lower rate of protein content decrease. Consequently, as sedimentation index increased (0.64% $year^{-1}$ in Italy and 0.49% $year^{-1}$ in Spain), as explained by the changes in the allelic composition of the glutenins, the quality of grain proteins was improved through breeding. Moreover, breeding programs were also successful in increasing the stability of gluten strength, and consequently, but with a lower intensity, that of the sedimentation index.

Yellow pigment concentration is mandatory to attain the bright yellow coloured pasta products demanded by the consumers, thus becoming an important goal in the EU (Di Fonzo *et al*. 2005). As expected, genotypic effects accounted for a large percentage (47.6%) of the phenotypic variability for yellow colour index. However, despite the large heritability of this trait (Clarke *et al*. 2006), the attained RGGs in both countries were less than one-third of those recorded for gluten strength. The largest changes in yellow colour index occurred between intermediate and modern cultivars. Rapid methods for semolina colour assessment, such as reflectance colourimeters, were already documented in the mid 20th Century (Matz and Larsen 1954; Walsh *et al*. 1969), but the largest changes in yellow colour were not recorded in that period because yellow colour has only been considered a relevant criterion for pasta making in recent decades (Digesù *et al*. 2009). Our results revealed that the stability of yellow colour was not improved in modern cultivars, which showed a wide range of response to environmental changes.

In agreement with the findings of other authors (Rharrabti *et al*. 2003; Taghouti *et al*. 2010), test weight and vitreousness were two of the traits with larger environmental effect, and any significant rate of variation over time was not observed. The lack of improvement of test weight may be related to the positive phenotypic and genotypic correlation between test weight and plant height reported in durum wheat (Clarke *et al*. 2009). As the old cultivars of this historical series do not carry dwarfing genes, the plant height reduction observed in some intermediate and all modern cultivars due to the introduction of dwarfing genes (Álvaro *et al*. 2008*a*) probably limited the attainment of grains with improved test weight. The fact that the cultivar slopes of the regression models (*b*) fitted to the relationship between the genotype and the environmental means did not differ between genotypes for test weight, and were maintained across time, is in agreement with this statement. Vitreousness showed a distinct behaviour; despite remaining unchanged through time, the stability of vitreousness differed between cultivars, but it remained unchanged over years, partially due to the wide variability detected within the most recently released cultivars.

Changes in glutenin allelic composition and its effects on gluten strength

Spanish cultivars showed high allelic variability in both their HMW- and LMW-GS loci, with 18 alleles encoded by *Glu-1*, *Glu-3* or *Glu-2* loci, compared with the 12 observed in the Italian germplasm. A previous study dealing with the phylogenetic relationships of the same historical series used here demonstrated closer genetic relationships within the Italian cultivars than within the Spanish ones (Martos *et al*. 2005), in agreement with the breeding histories of this crop in both countries. Durum wheat germplasm usually grown in Italy until 1970 seemed to be structured around a few, well-identified breeding groups with a relatively narrow genetic basis, which was dominated by a few hallmark, founder genotypes (Bozzini *et al.* 1998; Pecetti and Annicchiarico 1998; Di Fonzo *et al.* 2005; De Vita *et al.* 2007; Royo *et al.* 2010), whereas breeding in Spain largely relied on the introduction of germplasm of foreign origin (Royo and Briceño-Félix 2011). Despite the different breeding histories prevailing in both countries, the largest improvements in gluten strength were produced with the introduction and release of the first improved cultivars in both cases. Gluten strength increased 23.0% and 16.2% between old and intermediate cultivars in Italy and Spain, respectively, whereas increments between intermediate and modern cultivars were smaller at 7.2% and 10.0%.

Within our set of germplasm, changes in the allelic composition at the *Glu-A1* locus during the whole period were characterised by the loss of the subunit 1—which was only identified in one old cultivar of each country—replaced by the null. The null allele has been found in very high frequencies in other durum collections (Vallega 1988; Branlard *et al.* 1989; Nazco *et al.* 2013), and it has been found practically fixed in modern germplasm worldwide. Despite studies reporting the positive effect on durum wheat quality of allele 1 at the *Glu-A1* locus (Martínez *et al.* 2005), the two old cultivars carrying it in this study had weak gluten.

The largest number of alleles in the set of cultivars from both countries was found at the *Glu-B1* locus. Subunit 20, which was monomorphic in locus *Glu-B1* in Italian old cultivars seems to have been progressively replaced by subunits 6+8 and 7+8, which have been widely associated with strong gluten and, therefore, good pasta-making quality (Liu and Rathjen 1996; Sissons *et al.* 2005; Gregová *et al.* 2012). On the other hand, subunit 20 was not present in the old Spanish cultivars, one of which already had subunit 6+8 at *Glu-B1*, thus probably causing a slightly stronger gluten strength in old Spanish cultivars than in the Italian ones (Table 3). Previous studies have reported a high frequency of allele 6+8 in landraces from the Iberian Peninsula (Moragues *et al.* 2006). Subunit 7+17 at *Glu-B1*, found in modern cv. Boabdil, seems to have positively affected gluten strength.

Only two allelic variants (null and band 6 or *Glu-A3a*) were found in Italian germplasm at *Glu-A3* locus, while subunits 11 and 6+11 were also found in Spanish germplasm. In cultivars from both countries the null allele, common in old and in a few intermediate cultivars, was replaced by band 6 (*Glu-A3a*) in the modern cultivars. Band 6 has been found to be the most frequent both in landraces and modern cultivars (Nieto-Taladriz *et al.* 1997; Carrillo *et al.* 2000; Moragues *et al.* 2006; Nazco *et al.* 2013). The two modern Spanish cultivars carrying subunit 6+11 at *Glu-A3* had good gluten strength, but this result could not be attributed to this band exclusively, rather it is more likely due to the interaction with the alleles present at other loci.

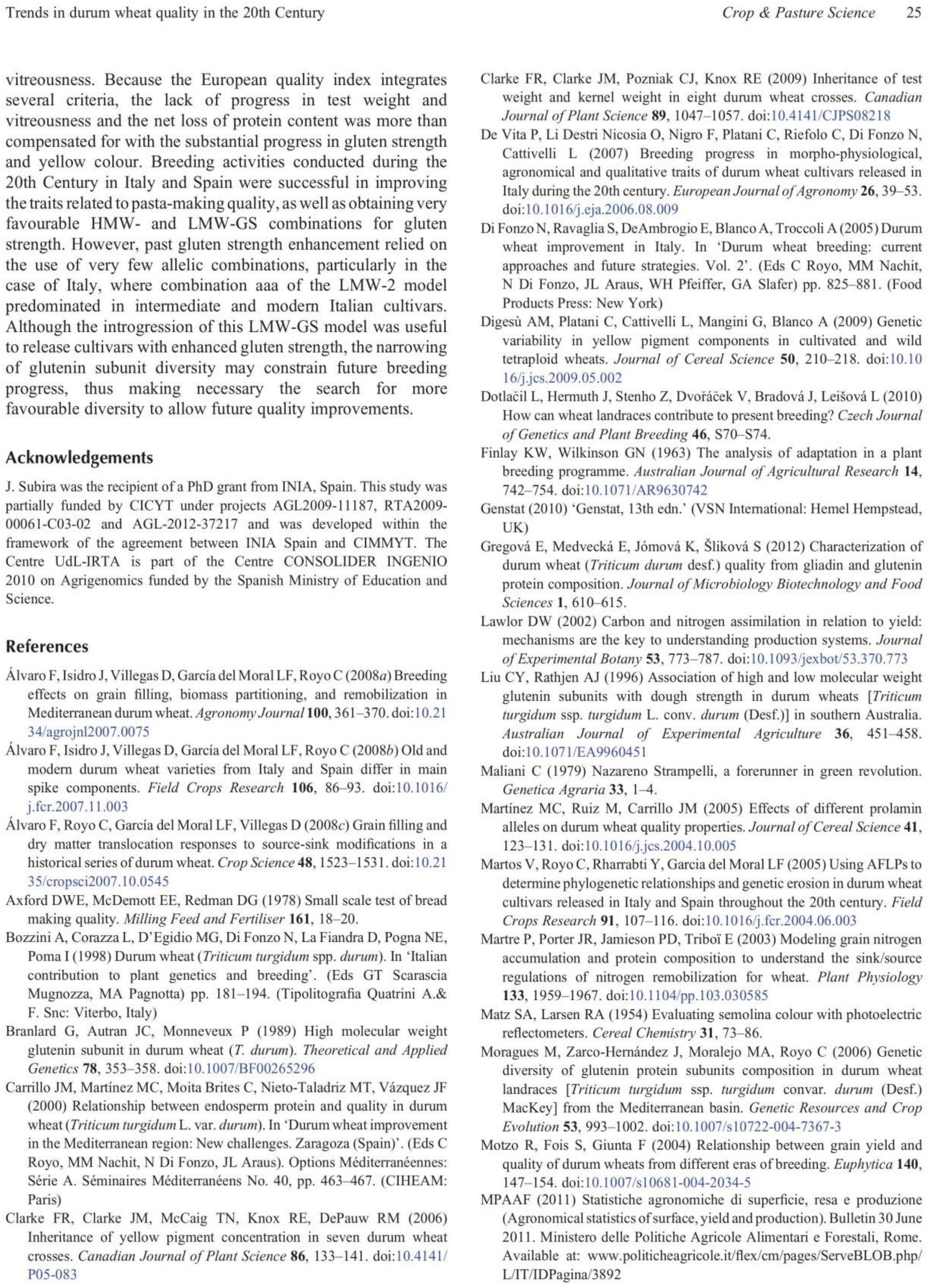
Only two allelic variants were found at the *Glu-B3* locus in both Italian and Spanish cultivars. One of the most important changes observed between old and intermediate cultivars in the allelic composition of LMW-GS in cultivars from both countries was the loss of diversity at *Glu-B3*, which became monomorphic for the banding pattern 2+4+15+19 in intermediate cultivars of both countries. This locus has a large influence on durum wheat gluten strength, but its effect may depend on its interaction with other LMW loci (Martínez *et al.* 2005). In this context, combination aaa of model LMW-2 (subunit 6 at *Glu-A3*, subunit 2+4+15+19 at *Glu-B3*, and subunit 12 at *Glu-B2*) has been largely recommended to be used in breeding programs (Sissons *et al.* 2005). Breeding increased the frequency of this combination, which was present in 75% of the intermediate cultivars of both countries and in 100% of the modern Italian ones. Greater allelic variability was observed within Spanish modern cultivars, with a previously unreported LMW-GS combination (d?b) identified, in addition to the combination aaa of the LMW-2 model. The uniformity in the banding pattern of LMW-GS of Italian intermediate and modern germplasm may have resulted from a breeding strategy based on few founder cultivars such as cv. Senatore Cappelli. This cultivar, an 'africanum' type selected from the North African population 'Jennah Khortifa', carries the combination aaa, and has probably given it to its descendants, cv. Capeiti 8 (derived from the cross Cappelli/Eiti), cv. Simeto (derived from the cross Capeiti 8/Valnova) and cv. Flavio (derived from the cross Latino/Cappelli), among others. However, combination aaa of model LMW-2 was present not only in cultivars with high gluten strength, such as cvv. Creso, Zenit and Senadur, but also in others with very weak gluten, such as cv. Clarofino. In addition, the two most successful allelic combinations in terms of gluten strength found in this study (resulting in sedimentation index values of 0.56 and 0.53, and SDS-sedimentation test values of ~7.86) had in common the null allele at the *Glu-A1* locus. All of these results confirm the important interaction between HMW- and LMW-GS on gluten strength, as reported by previous studies (Payne *et al.* 1984; Pogna *et al.* 1990; Ruiz and Carrillo 1995; Nazco *et al.* 2013).

The results of the ANOVA conducted to quantify the effect of the allelic combinations at the HMW- and LMW-GS loci on gluten quality showed that the portion of the variance for the sedimentation index explained by these combinations was about four times higher in Italian than in Spanish cultivars. These results reveal much larger genetic control on gluten strength in Italian than in Spanish cultivars, probably an outcome of breeding efforts devoted in Italy to pyramid favourable alleles and allelic combinations that enhance gluten quality and its stability.

None of the six most favourable allelic combinations in terms sedimentation index—all resulting in average SDS-sedimentation values >0.50 (Table 6)—was present in either Italian or Spanish old cultivars, whereas they were more frequent in modern (87.5%) than in intermediate (62.5%) sets of germplasm. These results demonstrate that improvements in gluten quality were a consequence of the replacement of alleles and allelic combinations at HMW- and LMW-GS loci, which could also have provided a higher stability for gluten quality in recent cultivars.

Conclusions

The results of this study showed the significant improvements achieved in durum wheat quality in Italy and Spain during the 20th Century. Despite the fact that protein content was reduced, protein yield per ha increased very significantly. Yellow colour index and gluten strength also increased significantly. However, no significant changes were observed in test weight and



vitreousness. Because the European quality index integrates several criteria, the lack of progress in test weight and vitreousness and the net loss of protein content was more than compensated for with the substantial progress in gluten strength and yellow colour. Breeding activities conducted during the 20th Century in Italy and Spain were successful in improving the traits related to pasta-making quality, as well as obtaining very favourable HMW- and LMW-GS combinations for gluten strength. However, past gluten strength enhancement relied on the use of very few allelic combinations, particularly in the case of Italy, where combination aaa of the LMW-2 model predominated in intermediate and modern Italian cultivars. Although the introgression of this LMW-GS model was useful to release cultivars with enhanced gluten strength, the narrowing of glutenin subunit diversity may constrain future breeding progress, thus making necessary the search for more favourable diversity to allow future quality improvements.

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CHAPTER 3

On the relationships between wheat yield and bread-making quality related traits in high yielding Mediterranean environments

Chapter under revision

On the relationships between wheat yield and bread-making quality related traits in high yielding Mediterranean environments

Abstract

Twenty bread wheat cultivars selected among the most widely grown in Spain during the last decades, and representative of the four bread-making quality groups established by Spanish official regulations, were grown in eight Mediterranean environments with yields ranging from 6785 to 9722 kg ha^{-1}. The official classification in four quality groups disagreed with the clustering formed with data across environments that consistently classified the cultivars in two quality types: high and low, with the latter type showing superior yields and longer time to anthesis. A putative antagonism appeared between cultivars showing high yield and long cycle length to anthesis, and those with high quality grains. The negative correlation coefficient between yield and grain protein content (GPC) was significant for the low-quality cultivars, but not for the high-quality ones, whose grain consistently had more GPC. The number of days from sowing to anthesis showed the largest environmental influence. Reference evapotranspiration from sowing to anthesis reduced kernel weight and yield, but increased GPC, while high average maximum temperatures during grain filling reduced grain weight and had a positive effect on GPC. The advantage of the 2+12 banding pattern at *Glu-D1* on gluten extensibility depended on the protein content induced by environmental conditions. Assessment of HMW-GS composition revealed that the subunits with the strongest positive effect on gluten strength (*W*) at each locus were 1 and 2* at *Glu-A1*, 7^{oe}+8 and 17+18 at *Glu-B1* and 5+10 at *Glu-D1*. All combinations containing the null allele at *Glu-A1* locus or subunits 2+12 or 4+12 at *Glu-D1* negatively affected *W.* However, due to epistatic effects, subunits 2* at *Glu-A1* and 5+10 at *Glu-D1* increased or reduced gluten strength respectively when combined with subunits 7*+8 or 6+8 at *Glu-B1*. Our results suggest that the selection for appropriate combinations for HMW-GS may result in gluten strength increases independently of the yield potential of the cultivar.

Introduction

Bread wheat (*Triticum aestivum* L.) provides 19% of the daily calories intake worldwide since it is the second most important staple crop in the world (FAOSTAT, 2010), and an essential component of the Mediterranean diet as a source of complex carbohydrates, proteins and dietary fibre (Peña *et al*., 2002). The predominance of wheat in human diets requires that cultivars meet specific quality criteria for the manufacture of the wide range of food products derived from it (Belderok, 2000).

For bread making, dough rheological properties during mixing and fermentation, as well as their response to possible constraints during these processes, are determinant for product functionality (Caballero *et al*., 2007; Wang *et al*., 2015). Baking quality is strongly related to the viscoelastic properties of the gluten matrix, which are mostly determined by the quantity and quality of glutenins and gliadins, the reserve proteins of the grain endosperm that form gluten (Finney, 1943). Although grain protein content (GPC) has a major genetic control (Peterson *et al*., 1992; van Lili *et al*., 1995; Grausgruber *et al*., 2000), it is generally assumed that it may be strongly affected by environmental factors such as rainfall and temperature, soil fertility and nutrient availability (Mariani *et al*,. 1995; Williams *et al*., 2008).

In contrast, variability in protein quality has been largely attributed to genotype (Branlard and Dardevet., 1985; Peña *et al*., 2002; Butow *et al*., 2003), as it mostly depends on the composition of gluten proteins (Pannozzo and Eagles, 2000; Vawser and Cornish, 2004). Gluten quality in bread wheat is mainly determined by high molecular weight glutenin subunits (HMW-GS), coded at the *Glu-1* loci of the homeologous chromosomes. Although HMW-GS are less abundant in the grain than low molecular weight glutenin subunits (LMW-GS), they have a greater impact on the rheological behaviour of bread wheat dough during fermentation (Weegels *et al*., 1996; León *et al*., 2010). High molecular weight glutenin subunits 5+10 at the *Glu-D1* locus, and subunits 1 and 2* at the *Glu-A1* locus have been reported to increase dough strength, while the null allele at *Glu-A1* and subunit 20 at *Glu-B1* locus are considered to be detrimental (Payne *et al*., 1987; Branlard *et al*., 2003). Despite its strong genetic control, gluten strength may also be affected by environmental conditions particularly when large environmental variability is involved. A study comprising six American countries revealed larger environmental than genotypic effects on gluten strength (Vazquez *et al*., 2012).

Dough extensibility, another important rheological property is also determined by the HMW-GS composition. Particularly subunit 2+12 at *Glu-D1*, the locus that accounts for the largest amount of HMW-GS in gluten (Wieser and Zimmermann, 2000), has proved to positively affect dough extensibility (Anderssen *et al*., 2004). However, dough extensibility has proved to be largely influenced by the environment and the genotype x environment interaction (Cornish *et al*., 2001; Sanchez-Garcia *et al*., 2015).

One of the most widespread methods for assessing the rheological properties of dough for baguette production is the Chopin Alveograph (Poblaciones *et al*., 2009). The apparatus simulates the behaviour of dough during fermentation estimating the following dough biaxial extension parameters during this process (Carrillo *et al*., 2006): *W* (gluten strength), *P* (dough tenacity), and *L* (dough extensibility).

Test weight and α-amylase activity are also important traits determining bread-making quality. Test weight is related to flour yield extraction. It has a strong environmental effect (Khalil *et al*., 2002) and has been associated in bread wheat with flour protein content (Schuler *et al*., 1995). The activity of α-amylase enzyme in mature grains is related to the presence of altered starch due to germinated grains that leads to poor loaf volume and crumb structure (Trethowan, 2001). It has an underlying genetic variation but it is also influenced by environmental conditions (Johansson, 2002; Mares and Mrva, 2008).

In order to provide a common frame for breeders, farmers and millers, and to promote the growing of high quality cultivars some countries classify wheat varieties according to quality standards. The Spanish official classification of wheat cultivars by their quality is contained in regulations RD

1615/2010 (7th Dec 2010) and RD 190/2013 (15th March 2013). These directives classify commercial bread wheat cultivars in five quality groups based on GPC, *W*, *P/L* (tenacity/extensibility ratio), Hagberg falling number, and proteolitic degradation. Quality groups 1 to 4 include wheat cultivars appropriate for industrial uses (1 strongest dough, 4 weakest and most extensible dough), while group 5 entails wheats only suitable for animal feeding.

It is well known that high quality wheat can be produced on a wide range of growing conditions in Mediterranean environments (Corbellini *et al*, 1998). However, the unpredictability of the amount and distribution of rains typical of these environments causes large fluctuation on wheat yield (Sanchez-Garcia *et al*., 2012; Subira *et al*., 2015) and bread-making quality attributes (Borghi *et al*., 1997; Hristov *et al*., 2010) across locations and years, thus limiting the suitability of grain for certain end-uses. As adverse conditions for wheat production will become more frequent in a future scenario of global climate change (Trnka *et al*., 2014), the assessment of the extent and influence of meteorological variables on traits responsible for the genotype x environment interaction for yield and those determining bread-making quality becomes of major importance.

This study was conducted with a set of 20 bread wheat cultivars among the most widely grown in Spain during the last decades, and representative of the four official groups of industrial quality, with the aim of: 1) evaluating the quality and yield traits across a range of environments and its relationship with the official quality classification of the cultivar, 2) analysing the effect of environmental traits before and after anthesis on yield and grain quality, and 3) determining the allelic variants and allelic combinations at *Glu-1* with a significant effect on gluten strength.

Material and methods

Plant material

A set of 20 bread wheat (*Triticum aestivum* L.) cultivars were selected among the most widely cultivated in Spain during the last decades, in order to represent the variability among industrial grain-quality groups available for human consumption in the Spanish market. Cultivars included by RD 190/2013 in quality group 5 were not considered in this study due to the heterogeneity of the group that, containing cultivars that do not meet the requirements of any of groups 1 to 4, are mostly devoted to animal feeding. Table 1 shows the quality group of the cultivars used in the current study according to RD 190/2013.

Experimental setup and data acquisition

Eight field experiments were conducted during two growing seasons in four locations representative of the main bread wheat Spanish growing areas: Valladolid (North-West), Lleida (North-East), Córdoba (South-West), and Albacete (South-East) (Table 2). Experiments consisted of non-replicated plots of 6 m^2 (8 rows, 0.15 m apart) arranged according to a modified augmented design with cultivars 'Soissons' and 'Artur Nick' as replicated checks. Seed density was adjusted to 400 viable seeds m^{-2}. Weeds and diseases were prevented with chemical treatments following the standard agricultural practices at each site and year. Nitrogen fertilization was applied in top-dressing as ammonium sulphate at rates between 78 and 140 N units depending on the trial.

Dates of anthesis (GS65 according to Zadoks *et al*., 1974) and physiological maturity (GS87) were recorded on each plot when approximately 50% of the plants reached that stage. Daily meteorological data were obtained from weather stations close to experimental fields. Days from sowing to anthesis (DSA) and from anthesis to maturity (DAM) were calculated for each experiment averaging data across cultivars. The following environmental variables were calculated for each period: average maximum and minimum daily temperatures (T_{max} and T_{min}, respectively, °C); reference evapotranspiration (ETo, mm) computed by the Penman-FAO methodology (Allen *et al*., 1998); mean daily relative air humidity (RH, %) and total water input including irrigation and rainfall (WI, mm). Further experimental details are shown in Table 2. Plots were mechanically harvested at ripening and yield was expressed at a 12% grain moisture basis.

HMW-GS allelic composition

Electrophoretic analyses (1D SDS-PAGE) were performed to identify the high molecular weight glutenin subunit (HMW-GS) composition at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci. Protein extraction was conducted according to Singh *et al*. (1991) and the electrophoretic analyses followed the protocols implemented at CIMMYT (International Maize and Wheat Improvement Centre) by Peña *et al*. (2004). With the aim of discriminating between 7 and 7* *Glu-B1* subunits, additional PCR analyses were performed following Espí *et al*. (2012). The nomenclature proposed by Payne and Lawrence (1983) was used to describe the observed subunits.

Grain and flour quality attributes

A sample of 500 g of harvested grain from each plot was cleaned for quality analyses. Thousand kernel weight (TKW, g) was calculated from the mean weight of two sets of 100 grains per plot. Test weight (TW, kg hl^{-1}) was determined with the GAC2100 (Dickey-John Co) analyzer. Grain protein content (GPC, %) was estimated with a near-infrared transmittance spectrophotometer (Infratec®1241-grain analyzer, Foss Tecator AB, Sweden) previously calibrated for protein content against the standard Kjeldahl method. Grain samples were milled with a Chopin-Dubois CD1 experimental mill (Chopin S.A., Villeneuve la Garenne, France) after being tempered overnight at 16% moisture level. The following rheological properties of dough biaxial extension were determined: strength (*W*, deformation energy; J 10^{-4}), tenacity to extension (*P*, maximum overpressure; mm H_2O), extensibility (*L*, length of the curve; mm) and the configuration ratio (*P/L*). Tests were conducted on white flour samples with the

Alveograph (Chopin, S.A., Villeneuve la Garenne, France) following the ICC Standard Method Number 121 (ICC, 1992). The α-amylase activity was assessed on a 7 g sample of white flour using the Falling Number 1313 (Perten Instruments, Sweden) following the AACC 56-81B (AACC, 1999) Hagberg's falling number method (Perten, 1964).

Statistical analyses

Field and laboratory data were fitted to linear mixed models with the two check cultivars, 'Soissons' and 'Artur Nick', as fixed effects and the row number, column number and cultivar as random effects (Littell *et al*., 1996). Restricted maximum likelihood (REML) was used to estimate the variance components and to produce the best linear unbiased predictors (BLUPs) for the phenotypic data of each cultivar within each environment, using the MIXED procedure of the SAS-STAT statistical package (SAS Institute, 2008). Principal component analysis (PCA) was conducted using mean yield and quality BLUPs data of cultivars across environments. Analyses of variance (ANOVA) for yield and quality data were performed using two approaches for partitioning the cultivar effect: i) differences between official quality groups of the cultivars and differences within them, an ii) differences between the quality types arisen from the PCA and differences within them. Means were compared with the Student's *t* test (P=0.05). The effect of allelic composition at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci were assessed according to an ANOVA model in which the cultivars carrying a specific allelic/banding pattern or allelic/banding pattern combination were tested against the remaining cultivars. Pearson correlation coefficients were calculated for the relationships between yield and quality traits using mean cultivar values across environments (n=20). Linear regression models were used for fitting the relationships between yield and quality traits using the mean values for each environment of cultivars of the high- and low-quality types arisen from the PCA (n=8). Similar models were used to study the relationship between yield and environmental variables using the mean environment values across cultivars for the sowing-anthesis and anthesis-maturity periods. Analyses were performed with GenStat V16 (VSN International Ltd, 2013), Enterprise Guide V4.2 (SAS Institute, 2008) and JMP V8 (SAS Institute, 2009) statistical software.

Results

Multivariate analysis

Principal component analysis (PCA) was performed to explore the relationships between yield, days to anthesis and quality traits across environments. The first two axes of the PCA explained up to 62.8% of the total variance, indicating that most of the information contained in the data could be summarized by projecting the points on the plane determined by the first two axes (Fig. 1). The eigenvectors representing DSA and yield were placed in the negative side of the first axis, while the biaxial extension parameters *W*, *P* and *L*, GPC and falling number were placed in the positive side. The length of each vector's projection on an axis is proportional to its contribution to the principal components of that axis, reflecting the extent to which each variable weights the two components. Dough strength (*W*) was the trait with the highest positive influence on PC1, while yield was the trait with the largest negative effect on that axis. The eigenvectors corresponding to TKW and TW were located in the positive and negative direction of PC2, respectively.

The position on the plane determined by the first two axes of the points representing cultivars showed that those included in quality groups 1 and 2 clustered in the positive direction of PC1, close to the vectors related to quality attributes. In contrast, points representing cultivars of groups 3 and 4 clustered in the negative direction of the same axis, close to the vectors of yield and DSA (Fig. 1). Thus, it may be assumed that the first axis discriminates between high (HQ) and low (LQ) quality cultivars, with the latest showing longer cycle length until anthesis and the highest yields.

According to the position of the eigenvectors of the PCA and considering the mean data of cultivars across environments yield was positively related with

DSA, but negatively associated with GPC, *W* and falling number (Table 3). However, when the analysis was conducted by each quality type independently, the relationship between yield and GPC was only statistically significant for LQ cultivars (Table 3). For HQ cultivars yield was negatively associated with *L* but positively with *P/L*.

Environmental and genetic effects on yield and quality

The ANOVA conducted to quantify the relative effect of the environment and the cultivar in the phenotypic expression of the studied traits revealed that all they were significantly affected by both factors (Table 4). The environment had the largest influence on the number of days from sowing to anthesis, but it had a low influence on *P* and *W,* whose variance was mostly explained by the cultivar effect. Yield was almost equally affected by the environment and the cultivar by environment (GE) interaction. Mean grain yield ranged between 6785 kg ha^{-1} in the south-eastern experiment conducted in 2012 (SE12) to 9722 kg ha^{-1} in the one conducted in the northeast in 2011 (NE11) (Table 2). In all sites, temperatures during grain filling were lower in 2013 than in 2011. Environment SE12, in which the lowest yields were recorded, was the coldest environment at pre-anthesis and the warmest during grain filling. Experiments in the southwest site (SW11 and SW13) were exposed to the warmest conditions before anthesis and the most favourable water status (Table 2).

In order to assess differences between grain quality categories, the sum of squares of the cultivar effect was partitioned into its components (differences between categories and differences within each of them), following two alternative approaches: i) the official quality group of each cultivar, and ii) the quality type of each cultivar arisen from the PCA shown in Fig. 1. The results showed that the differences between quality groups accounted for a greater percentage of total variance than differences between quality types (upper part of Table 4). As expected, variability within categories was larger for the classification in two quality types than for the official classification in four quality groups. With the exception of falling number, the variability explained by differences within categories for the traits used for quality classification (GPC, *W* and *P/L*), was always lower than the one accounted by differences between them.

Mean values comparison revealed that neither quality nor agronomic traits, the official classification arranged the groups according to the values obtained (lower part of Table 4). In addition, differences between quality groups 1 and 2 were not statistically significant for GPC, *P*, *L* and *P/L*, and the values of group 2 were significantly greater for *W* and falling number than those of group 1. However, for *W*, *P* and *P/L* large differences existed between values of groups 1 and 2 and those of groups 3 and 4. On the other hand, in agreement with the results of the PCA, cultivars belonging to the low quality type overyielded those with high quality by 7.8%, and elapsed three more days than them to reach anthesis. Furthermore, cultivars of the high quality type had superior values than the low quality ones for all quality traits (lower part of Table 4).

A positive relationship between GPC and *W* existed for high and low quality types considering mean data across cultivars at each environment, with *W* values being consistently greater for HQ types (Fig. 2a). A strong positive relationship was also found between protein content and *L* for LQ cultivars (Fig. 2b).

In order to ascertain the effect of environmental variables before and after anthesis on yield and grain quality, linear regression models for the periods sowing-anthesis and anthesis-maturity were fitted to the relationships between agronomic and quality traits and the five environmental variables shown in Table 2 by averaging data across cultivars at each environment (n=8). The results showed that only two environmental variables were significantly related with yield and grain quality traits: reference evapotranspiration from sowing to anthesis [$ETo_{(SA)}$] and mean daily maximum temperature from anthesis to physiological maturity [$T_{max(AM)}$]. Reference evapotranspiration from sowing to anthesis was positively associated with the number of days from sowing to anthesis (Fig. 3a), but negatively associated with grain yield (Fig. 3b) and TKW (Fig. 3c). Grain protein content was positively affected by $ETo_{(SA)}$ (Fig.

3d). Relationships between $ETo_{(SA)}$ and falling number (Fig. 3e), *W*, *P* and *L* (data not shown) were not statistically significant.

High average maximum temperatures from anthesis to physiological maturity were negatively associated with TKW (Fig. 3h), but positively related with grain protein content (Fig. 3i) and falling number (Fig. 3j), but none other relationships had significant coefficients of determination (Fig. 3f and 3g). For most traits yearly differences were minimum for the SW site and maximum for the SE one (Fig. 3). Minimum and maximum values for GPC were recorded in the SE site (11.7% in SE11 and 16.4% in SE12). This last environment was the driest before anthesis in view of its lowest water input and relative humidity values as well as the highest reference evapotranspiration of all environments (Table 2).

HMW glutenin subunits composition

Given the importance of gluten quality in identifying the two quality types derived from Fig. 1, and the results of the ANOVA that verified the already well-known strong genetic control of gluten strength, we ascertained the allelic variants and allele-specific combinations at *Glu-A1*/*Glu-B1*/*Glu-D1* loci. The banding pattern of the HMW-GS for these loci is shown for each cultivar in Table 1. Within the *Glu-A1* locus, subunits 1 and 2* were present in 7 and 6 cultivars, respectively, with 86% and 67% of them belonging to the HQ type, respectively (upper part of Table 5). The null allele was present in 7 cultivars, all of them of low quality type. The *Glu-B1* locus showed the largest allelic variability, as 6 allelic variants were identified in the 20 cultivars. Subunits 7^{oe}+8 and 7*+9 were the most frequent, as each of them was present in 25% of the cultivars. Nevertheless, subunit 7^{oe}+8 was more frequent in cultivars of HQ type than subunit 7*+9. Subunit 5+10 at *Glu-D1* locus was present in 55% of the cultivars, 91% of them belonging to the HQ type (upper part of Table 5). The *Glu-D1* subunits 2+12 and 4+12 were present in 35% and 10% of the cultivars, respectively, all them included in the LQ type (upper part of Table 5).

The analysis of the allelic combinations at the *Glu-A1/Glu-B1/Glu-D1* loci revealed 15 different combinations in the set of 20 bread wheat cultivars (bottom part of Table 5). Two of them were the most frequent in genotypes of HQ type: the one that included subunits 2*, 7^{oe}+8 and 5+10 at *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively (present in cultivars ‘Bologna’, ‘Gazul’ and ‘Galera’), and the combination consisting on subunits 1 at *Glu-A1*, 17+18 at *Glu-B1* and 5+10 at *Glu-D1*, identified in cultivars ‘Catedral’, ‘Estero’ and ‘Yecora’ (Table 1).

The analysis of the effect of glutenin subunits on gluten strength revealed that subunits 1 and 2* at *Glu-A1* locus exerted a positive and significant effect on *W*, while the null allele had a negative effect on *W* (upper part of Table 5). At *Glu-B1* locus, only subunits 7^{oe}+8 and 17+18 increased *W,* while subunits 6+8 and 7*+9 were the most detrimental. All HQ cultivars were monomorphic for subunit 5+10 at *Glu-D1*, which was the unique positively affecting *W* at this locus (upper part of Table 5).

Six of the 15 allelic combinations identified at *Glu-A1/Glu-B1/Glu-D1* had a positive and significant effect on *W*, all them containing subunit 5+10 at *Glu-D1* locus (bottom part of Table 5). All cultivars carrying subunit 5+10 at *Glu-D1* belonged to the HQ type except cultivar ‘Camargo’ (Table 1). The most favourable combinations in terms of *W* included subunits 7^{oe}+8 or 7+8 at *Glu-B1* and subunits 1 or 2* at *Glu-A1* (bottom part of Table 5). All combinations containing the null allele at *Glu-A1* locus or subunits 2+12 or 4+12 at *Glu-D1* negatively affected *W*, with all cultivars carrying them belonging to the LQ type (bottom part of Table 5).

Discussion

The current study included cultivars representative of the four bread-making quality groups suitable for industrial use described in the official Spanish regulations. A regulation that classifies cultivars according to the industry end-use standards is of prime usefulness for growers, traders and the seed industry.

However, it has been largely documented that the final grain features depend on the interaction between the genotype of the wheat plant and the environmental conditions, to whom cultivars may show a differential response (Mariani *et al*., 1995; Peña *et al*., 2002; Williams *et al*., 2008; Schulthess *et al*., 2013). Therefore, understanding not only the genetic, but also the environmental factors determining final grain quality and their interaction is of prime importance for maximizing future quality improvements.

Grain quality classification

The multivariate analysis (PCA) conducted in the current study, which included variables of both grain quality and agronomic performance, could not separate the four quality groups as expected *a priori* according to the official classification, as only two clusters (one including groups 1 and 2, the highest-quality ones, and another including groups 3 and 4) were clearly identifiable. The clashing between the values obtained for quality traits across eight environments and the official classification was confirmed by the means comparison after conducting the ANOVAs as quality groups 1 and 2 had similar GPC, *P*, *L* and *P/L* values*,* and group 2 had larger *W* and falling number values than group 1. In addition, GPC of group 4 was significantly higher than that of group 3. On the other hand, the results of the PCA showed that cultivars of quality groups 1 and 2, representing high quality types according to their values for *W*, *P*, protein content and falling number, were consistently separated from groups 3 and 4, which showed low values for these traits, thus representing low quality types. The comparison of means of this two quality types (high and low) confirmed that means of all quality traits were larger for the high quality type. These results suggest that the classification in two quality types was more reliable and appropriate for the results obtained in this study than the official one in four groups.

Grain quality versus agronomic performance

The direction of the eigenvectors for quality and agronomic traits in the PCA indicated that the cultivars with high GPC, *W*, *P* and falling number values, tended to have lower yields and longer cycle length to anthesis than cultivars with low values for these traits. This was confirmed by the positive and significant value of the correlation coefficient between yield and DSA, but the negative relationship of yield with GPS, *W* and falling number. These results, based on data across eight medium to high yielding environments, revealed a putative antagonism between cultivars showing high yield and long cycle length to anthesis, and those with high quality grains. It is generally assumed that yield and GPC are negatively associated (Simmonds, 1995; Oury and Godin, 2007), and this was confirmed in the current study by the strong negative relationship between the two traits. However, our results showed a quantitative difference in this relationship between high and low quality types. Although the tendency was also negative, the correlation coefficient between yield and GPC was significant for the LQ cultivars, but not for the HQ ones, which consistently reached higher GPC. This result indicates a lower slope of the regression line fitted to the relationship between yield and GPC, thus suggesting that some HQ cultivars had a good balance of both yield and GPC, probably resulting from simultaneous selection for yield and GPC in breeding programs. In durum wheat it has been suggested that adequate improvement of protein content in fertile environments may be attained with negligible reductions in grain yield (Rharrabti *et al*., 2001). On the other hand, for cultivars reaching the highest yields, probably consequence of using yield as the primary selection criteria, GPC tended to be penalized.

The positive relationship between GPC and *W* may also explain the negative association between yield and *W* when all cultivars were considered together. However, when this relationship was analysed separately for the two quality types the coefficients of correlation were zero for HQ cultivars and positive, but not significant for LQ cultivars which may indicate that the selection of appropriate combinations for HMW-GS may result in gluten strength increases independently of the yield potential of the cultivar. However, for the high-quality cultivars as yield increased dough extensibility decreased, thus limiting the suitability of flours for bread making due to the increase in the *P/L* ratio that results in unbalanced doughs (Sanchez-Garcia *et al*., 2015 and references herein). This is in agreement with previous studies reporting that the genetic improvement of bread wheat yield in Spain during the 20th century (Sanchez-Garcia *et al.,* 2013) increased *W* and *P*, but led to a decrease in dough extensibility (Sanchez-Garcia *et al*., 2015). However, this negative relationship between yield and *L* was not observed for low-quality cultivars as some of the most productive had *L* values comparable to those of high-quality ones.

Environmental effects on yield and quality

The cultivars used in this study had a huge difference in their sensitivity to the environmental conditions, as revealed by the large percentage of total variance for yield explained by the genotype x environment interaction (40.1%). This value is much larger than the previously reported for bread wheat by Voltas *et al*. (2005) and Sanchez-Garcia *et al*. (2012), in multi-location experiments in Spain (between 2.9% and 14%), and also in comparison with studies conducted in other countries (Brancourt-Hulmel *et al*., 2003; Fufa *et al*., 2005; Li *et al*., 2006; Morgounov *et al*., 2010).

The number of days from sowing to anthesis was the trait that showed the largest environmental influence, with the environment explaining 96.5% of its variability. The results of the current study showed that the main environmental trait affecting cycle length to anthesis was reference evapotranspiration, which exerted a positive effect on it and accounted for 70% of the variation in this trait. The two environments with greatest ETo before anthesis and longest cycle length to this growth stage were SE12 and NW11. On both, ETo largely exceeded water input before anthesis, indicating the occurrence of drought stress (Allen *et al*., 1998). Although it is well known that drought stress before anthesis usually shortens cycle duration (Slafer and Rawson, 1994; Porter and Gawith, 1999), the large ETo observed in these two environments before anthesis could be partially due to the early sowing date in NW11, and also by the low temperatures and water input recorded in SE12 before anthesis. In this study, late anthesis date, mostly resulting from high evapotranspirative demand in pre-anthesis, led to the lowest yields. Previous studies have demonstrated a negative relationship between ETo and either

RUE and NUE (Stöckle *et al*., 2008). Accordingly, elevated ETo values could reduce plant biomass limiting the amount of carbohydrates available for filling the grains and constraining yield. The rapid temperature raise occurring typically in Mediterranean environments during spring compelled late-anthesis cultivars to fill their grains under less favourable conditions in terms of temperature, thus reducing grain weight, as shown by the significant and negative relationship between average daily maximum temperature during grain filling and TKW. It is well known that high temperatures during grain filling limit transient photosynthesis (Allakhverdiev *et al*., 2008; Prasad *et al*., 2011) and have a direct effect on grain filling rate and duration (Wardlaw and Moncur, 1995; Eyshi Rezaei *et al*., 2015).

However, the negative influence of ETo before anthesis on grain weight and yield, and the negative influence of high maximum temperatures during grain filling on grain weight had opposite effects on protein accumulation in the grain. This was probably a result of a concentration of nitrogen compounds in the grain when carbohydrate deposition was reduced due to a photosynthesis constraint (Cox *et al*., 1986; Jenner *et al*., 1991). It has been reported that the highest grain protein contents are usually found under adverse conditions for grain formation, since the production and translocation of compounds such as carbohydrates to the grain is more sensitive to adverse growing conditions than is protein accumulation (Jenner *et al*., 1991; Farooq *et al*., 2011).

The environmental effect on protein quality was low, as variability in *W*, *P* and *P/L* largely depended on the cultivar effect. This is in agreement with previous results (Sanchez-Garcia *et al*., 2015 and references herein) and the general assumption that traits related to protein quality and gluten strength are mostly under genetic control (Williams *et al*., 2008). However, the effect of environment on protein content (Daniel and Triboï, 2002) was obvious in this study where the addition of the environment and the cultivar x environment interaction accounted for 17.4% of total variation for *W*, 15.5% for *P* and 59.7% for *L*, respectively. These values are large enough to make some cultivars fail fulfilling the industry quality standards for rheological properties when grown in certain environments. The large variability existing within quality types for *P/L* was probably induced by the large variability existing within low quality types for *L*.

The environmental control of *W* could be explained in the current study by the positive relationship existing between *W* and GPC, a trait mostly driven by the environment. Gluten strength increased in the environments favouring high GPC, but high quality cultivars (groups 1 and 2) outperformed in terms of *W* low-quality ones (groups 3 and 4) in all environments. Therefore, while genetic selection remains as the main strategy to achieve further quality improvements, our results suggest that there is still scope for protein quality improvement through the selection of the most suitable cultivars for each target environment.

The cultivar was also the factor with a largest effect on dough extensibility (*L*), but the environment and the cultivar x environment interaction explained up to 34.9% and 24.8% of *L* variance, respectively. Cornish *et al*. (2001) observed that the environmental control of dough extensibility was due to its relationship to protein content, while its genetic control was mostly due to the composition of the LMW glutenin subunits, but also, to the HMW ones, mainly at the *Glu-D1* locus and identified the 2+12 allele as favourable for extensibility. The same locus was identified by Sanchez-Garcia *et al*. (2015) as being responsible of 30.4% of the cultivar x environment interaction variance for *L*. Our results confirm the positive relationship between protein content and *L* with a similar tendency for high and low quality types, but the relationship was only significant for low quality cultivars. Low-quality cultivars, mostly carrying the 2+12 allele in the *Glu-D1* locus, exhibited a lower *L* than HQ ones, carrying the 5+10 allele. Therefore, the results of this study allow concluding that the advantage of the 2+12 banding pattern at *Glu-D1* on gluten extensibility depended on the protein content induced by environmental conditions.

Test weight, which determines the bulk density of grains, was strongly affected by the environment, but the high values obtained by all quality categories indicated that grains were well filled and plump (Bayles, 1977). However, the large differences existing between cultivars of low quality demonstrate its usefulness for grain quality classification. Falling number values in this study were high, suggesting low α-amylase activity (Smith and Gooding, 1999), in agreement with the high temperatures and limited water supply during grain filling in the testing environments. The positive and significant relationship between average daily maximum temperature after anthesis and falling number was mostly induced by the highest maximum temperature during grain filling and falling number in SE02. Given that falling number increases as stresses after anthesis increase (Gooding *et al*., 2003), this result is in agreement with the shortest grain filling period, the lowest yield and the high protein content at this site.

HMW-GS banding pattern and gluten strength

The composition of HMW-GS plays a major role on the intrinsic gluten strength of a bread wheat cultivar. In the current study the effect on *W* of the different alleles at each of the *Glu-1* loci and that of the different allelic combinations were quantified. A total number of 12 alleles or subunits were identified among the set of 20 cultivars studied. Three, 6 and 3 alleles were identified at *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively. This diversity is comparable to the observed in a set of 200 French cultivars where 16 alleles were observed by Branlard *et al*. (2003) on the same loci.

The differentiation of subunits 7, 7* and 7^{oe} at *Glu-B1* has been recently elucidated (Espi *et al*., 2012), and therefore many previous studies identified these three subunits as an unique one, thus misclassifying many cultivars carrying a 7 type subunit. In the current study, we were able to identify 5 cultivars carrying the 7^{oe}+8 subunit, 2 carrying the 7+8, and 2 carrying the 7*+8, all them having different, and even contrasting, effects on *W*. While 7^{oe}+8 had a positive effect on *W*, 7*+8 had a negative one, and no significant effect was observed for 7+8. This may be the reason of the lack of coincidence between the results of the present study for the subunit 7+8 and previous studies that reported favourable effects of this subunit on dough properties (Ribeiro *et al*., 2013).

The alleles or subunits with the strongest positive effect on *W* at each locus were 1 and 2* at *Glu-A1*, 7^{oe}+8 and 17+18 at *Glu-B1* and 5+10 at *Glu-D1*. As expected, these were mostly present in the high quality types, and were the ones in a higher proportion within this group of cultivars. The positive effect of these subunits has been previously reported (Butow *et al*., 2003; León *et al*., 2010). However, as their effect on *W* depend on the allelic variants present at the other two loci, it was essential to analyse the effect of combinations. In the current study variations in one single allele even changed the sign of the effect on *W*. This was the case of subunits 2* at *Glu-A1* and 5+10 at *Glu-D1*, both of positive individual effect, which resulted in an increase of gluten strength when combined with the subunit of negative effect 7*+8 at *Glu-B1*, but the combination with 6+8 at the same locus resulted to be detrimental for *W*. Moreover, the additive effect of the subunits varied in different combinations as the combination 1, 7+8 and 5+10 at *Glu-A1*, *Glu-B1* and *Glu-D1*, resulted in a similar positive effect on *W* than 1, 7^{oe}+8, 5+10, despite it was observed that the 7^{oe}+8 subunit has a more favourable effect on *W* than 7+8. Therefore, besides taking advantage of the most favourable alleles and avoiding the most detrimental, quality breeding should better focus on selecting for allelic combinations rather than on single loci.

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Table 1. Year of release, quality group according to the official Spanish classification of wheat quality, and banding pattern of the high molecular weight glutenin subunits (HMW-GS) of the 20 bread wheat cultivars used in this study.

Cultivar	Year of release	Quality group[1]	HMW-GS		
			Glu-A1	*Glu-B1*	*Glu-D1*
Badiel	2008	1	1	7+8	5+10
Bologna	2001	1	2*	7^{oe}+8	5+10
Califa Sur	2001	1	1	7^{oe}+8	5+10
Catedral	2007	1	1	17+18	5+10
Estero	1993	1	1	17+18	5+10
Gazul	1992	1	2*	7^{oe}+8	5+10
Sensas	2006	1	1	7*+9	5+10
Yécora	1974	1	1	17+18	5+10
Galera	1999	2	2*	7^{oe}+8	5+10
Ingenio	2006	2	2*	7*+8	5+10
Anza	1974	3	null	7*+8	2+12
Artur Nick	2001	3	2*	7*+9	2+12
Berdun	1998	3	1	17+18	2+12
Camargo	2009	3	2*	6+8	5+10
Garcia	2006	3	null	7*+9	4+12
Sarina	1995	3	null	7^{oe}+8	2+12
Craklin	1999	4	null	7+8	2+12
Marius	1980	4	null	7*+9	4+12
Odiel	2004	4	null	7*+9	2+12
Paledor	2005	4	null	6+8	2+12

^{oe}: overexpressed subunit.

[1] Classification established by Spanish Regulations 1615/2010 and 190/2013

Table 2. Description of the eight environments included in this study.

Site*	Valladolid (NW)		Lleida (NE)		Córdoba (SW)		Albacete (SE)	
Coordinates	41^{o}41’N, 4^{o}24’W		41^{o}40’N, 0^{o}20’E		37^{o}31’N, 04^{o}53’W		39^{o}02’N, 02^{o}04’W	
Altitude (m asl)	700		200		200		695	
Soil texture	Silt loam		Fine loam		Sandy clay		Clay loam	
Harvest year	2011	2013	2011	2013	2011	2013	2011	2012
Sowing date	19 Nov	3 Dec	25 Nov	29 Nov	26 Nov	30 Nov	24 Nov	23 Nov
Harvest date	6 Jul	25 Jul	15 Jul	8 Jul	6 Jun	25 Jul	26 Jul	18 Jul
Environment code	NW11	NW13	NE11	NE13	SW11	SW13	SE11	SE12
Environmental conditions from sowing to anthesis								
DSA	181	170	155	158	139	108	167	180
T_{max} (ºC)	13.3	12.0	14.0	14.0	16.7	16.0	14.4	10.9
T_{min} (ºC)	2.83	2.13	2.49	2.74	7.02	5.60	2.93	0.72
ETo (mm)	356	286	261	275	250	231	310	393
RH (%)	78.2	80.0	74.8	72.8	78.0	86.2	84.8	70.2
WI (rainfall+irrigation, mm)	300	261	250	286	586	477	337	222
Environmental conditions from anthesis to maturity								
DAM	33	34	39	40	33	32	30	24
T_{max} (ºC)	24.9	22.0	26.0	23.0	25.2	24.7	23.4	29.3
T_{min} (ºC)	10.2	7.18	11.3	9.40	13.0	10.7	9.90	15.8
ETo (mm)	174	155	184	176	147	147	136	150
RH (%)	61.8	64.0	63.0	62.6	65.7	66.9	74.0	44.4
WI (rainfall+irrigation, mm)	46.2	30.1	129	176	85.3	25.6	140	91.3
Mean yield (kg ha^{-1})	7337	8684	9722	7888	8613	8845	8801	6785
Mean grain protein content (%)	15.1	12.7	13.7	12.6	13.2	12.1	11.7	16.4

DSA: days from sowing to anthesis; T_{max} and T_{min}: average maximum and minimum daily mean temperatures; ETo: reference evapotranspiration computed by the Penman-FAO methodology (Allen *et al*., 1998); RH: average relative humidity; WI: water input.

*Geographic location within Spain is indicated in parentheses

Table 3. Pearson correlation coefficients between yield and agronomic and quality traits considering mean cultivar values across environments (n=20) and for high quality (HQ) and low quality (LQ) types separately (n=10) according to the results of the PCA shown in Fig 1. ${}^{*}P< 0.05$; ${}^{**}P< 0.01$; ${}^{***}P< 0.001$.

Trait	All cultivars	HQ	LQ
Days sowing-anthesis (DSA)	0.44*	0.35	0.24
Days anthesis -maturity (DAM)	0.24	0.11	0.26
Thousand kernel weight (TKW)	-0.22	0.05	-0.21
Test weight (TW)	-0.32	-0.16	-0.07
Grain protein content (GPC)	-0.75***	-0.46	-0.73**
W	-0.47*	0.02	0.36
P	-0.40	0.49	0.18
L	-0.42	-0.80**	-0.05
P/L	-0.20	0.67**	0.14
Falling number (FN)	-0.50*	-0.29	-0.29

Table 4. Percentage of the sum of squares (SS) of the ANOVA (upper part) and mean values (lower part) for phenology, yield and quality traits of the 20 bread wheat cultivars assessed in 8 Spanish environments. The SS of the cultivar effect has been partitioned using two alternative approaches: i) according to the groups of Spanish Regulations 1615/2010 and 190/2013, and ii) according to the quality types arisen from the PCA shown in Fig. 1.

Source of variation	df	Days sowing-anthesis	Yield (kg ha^{-1})	Thousand kernel weight (g)	Test weight (kg hl^{-1})	Grain protein content (%)	Biaxial extension parameters				Falling number (s)
							W (J 10^{-4})	*P* (mm H$_2$0)	*L* (mm)	*P/L* (mm H$_2$0 mm^{-1})	
Environment	7	96.5 ***	42.5 ***	51.8 ***	61.2 ***	70.0 ***	9.94 ***	9.54 ***	34.9 ***	21.6 ***	42.0 ***
Cultivar	19	1.85 ***	17.4 ***	33.0 ***	28.6 ***	18.3 ***	82.6 ***	84.5 ***	40.3 ***	57.7 ***	38.3 ***
Fractioning of cultivar effect according to the groups of Official Regulations											
Between quality groups	3	0.44 ***	5.69 ***	6.19 ***	10.2 ***	11.8 ***	78.6 ***	75.1 ***	7.71 ***	30.0 ***	21.5 ***
Within quality group 1	7	0.62	3.67	11.6 *	4.08	2.89	1.10	6.82	10.8	18.6 **	5.75
Within quality group 2	1	0.07	0.82	6.30 **	1.23	0.00	1.61	0.57	0.18	0.07 ns	1.60
Within quality group 3	5	0.46	4.25	5.95	7.26	1.94	1.05	1.47	13.2 **	7.02 ns	9.20 *
Within quality group 4	3	0.27	2.97	2.94	5.87	1.68	0.32	0.59	8.48 *	2.12 ns	0.21
Fractioning of cultivar effect according to the quality types arisen from the PCA											
Between quality type	1	0.31 ***	5.34 ***	3.06 ***	5.68 ***	11.4 ***	75.2 ***	70.5 ***	4.11 ***	29.0 ***	10.9 ***
Within High-quality type	9	0.76	4.75	20.4 **	7.49	2.92	3.25	7.49	11.0	18.6 **	7.96
Within Low-quality type	9	0.78	7.32	9.50	15.4 *	3.96	4.19	6.53	25.2 ***	10.1	19.5 **
Cultivar*Environment (error)	133	1.69	40.1	15.2	10.1	11.7	7.42	5.97	24.8	20.7	19.7
Total	159										
Mean values[1]											
Group 1		156 c	7974 b	40.4 b	84.3 a	14.0 a	332 b	97.1 a	106 a	0.98 a	452 b
Group 2		158 ab	8216 ab	44.0 a	82.3 b	13.9 a	363 a	100 a	108 a	0.98 a	473 a
Group 3		158 b	8697 a	38.4 c	82.8 b	12.7 c	150 c	55.7 b	102 a	0.60 b	434 c
Group 4		159 a	8570 a	39.8 bc	81.2 c	13.0 b	90.6 d	37.8 c	89.7 b	0.48 c	365 d
High quality type		156 b	8022 b	41.1 a	83.9 a	14.0 a	338 a	97.8 a	107 a	0.98 a	456 a
Low quality type		159 a	8646 a	38.9 b	82.1 b	12.8 b	126 b	48.5 b	97.3 b	0.55 b	406 b

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

[1] Means within columns followed by the same letter are not significantly different according to the Student's t test at $P=0.05$

Table 5. Effect of allelic variants and allelic variant combinations of high molecular weight glutenin subunits (HMW-GS) at *Glu-A1*, *Glu-B1* and *Glu-D1* loci on gluten strength (*W*, J 10^{-4}) in the 20 bread wheat cultivars included in this study.

Allelic variants			Number of cultivars		Present	Absent	Main effect
Glu-A1	*Glu-B1*	*Glu-D1*	Low quality	High quality	(a)	(b)	(a-b)
1			1	6	309	191	118^{***}
2*			2	4	281	211	71^{***}
null			7		113	296	-183^{***}
	7^{oe}+8		1	4	318	203	115^{***}
	17+18		1	3	281	220	61^{***}
	7+8		1	1	235	232	3
	7*+8		1	1	209	235	-25^{**}
	7*+9		4	1	159	256	-98^{***}
	6+8		2		122	244	-123^{***}
		5+10	1	10	320	124	196^{***}
		4+12	2		127	244	-117^{***}
		2+12	7		124	290	-167^{***}

Allelic variants combination			Number of cultivars		Present	Absent	Main effect
Glu-A1	*Glu-B1*	*Glu-D1*	Low quality	High quality	(a)	(b)	(a-b)
2*	7^{oe}+8	5+10		3	359	210	150^{***}
1	7+8	5+10		1	362	225	137^{***}
1	7^{oe}+8	5+10		1	360	225	135^{***}
1	17+18	5+10		3	317	217	100^{***}
1	7*+9	5+10		1	316	228	88^{***}
2*	7*+8	5+10		1	314	228	87^{***}
1	17+18	2+12	1		173	235	-62^{***}
2*	7*+9	2+12	1		156	236	-80^{***}
null	7^{oe}+8	2+12	1		155	236	-81^{***}
2*	6+8	5+10	1		142	237	-95^{***}
null	7*+9	4+12	2		127	244	-117^{***}
null	7+8	2+12	1		108	239	-130^{***}
null	7*+8	2+12	1		104	239	-135^{***}
null	6+8	2+12	1		102	239	-137^{***}
null	7*+9	2+12	1		68	241	-172^{***}

$^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$

Fig. 1. Principal component analysis (PCA) biplot for phenology, yield and quality traits of the 20 bread wheat cultivars included in the study. Vectors symbolize the eigenvalues of the correlation matrix. *W* gluten strength, *P* tenacity, *L* extensibility. Numbers 1-4 represent the official quality group of each cultivar according to Table 1.

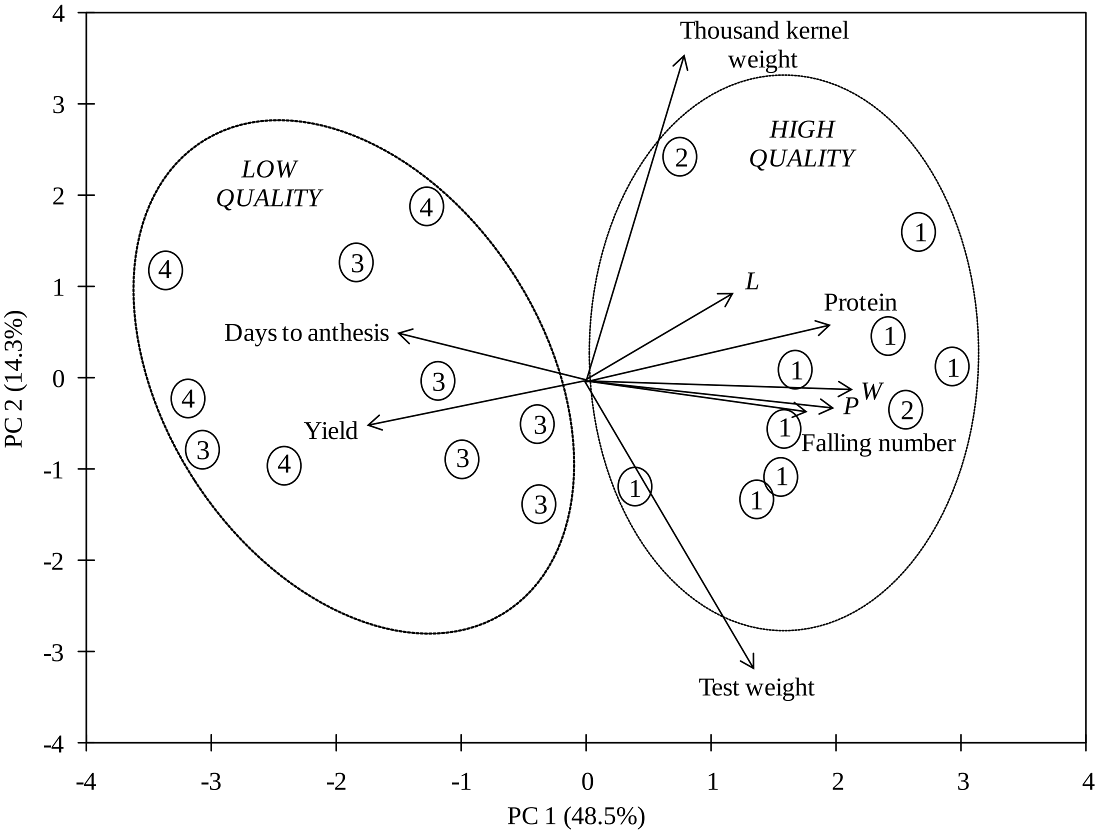


Fig. 2. Relationship between grain protein content and: *W* (dough strength, Fig. 2a), and *L* (dough extensibility, Fig. 2b) across high quality (HQ, ●) and low quality (LQ, ○) cultivars at each of the eight environments of this study. $^{*}P< 0.05$; $^{**}P< 0.01$; $^{***}P< 0.001$.

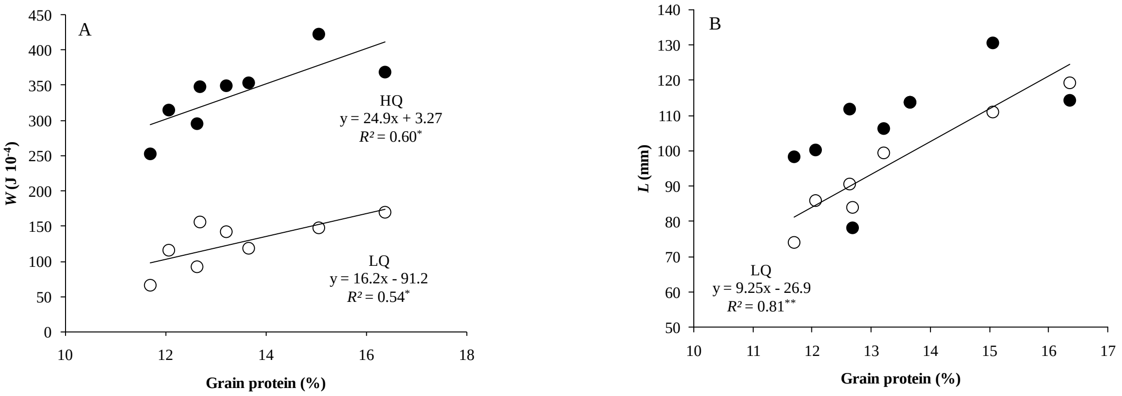
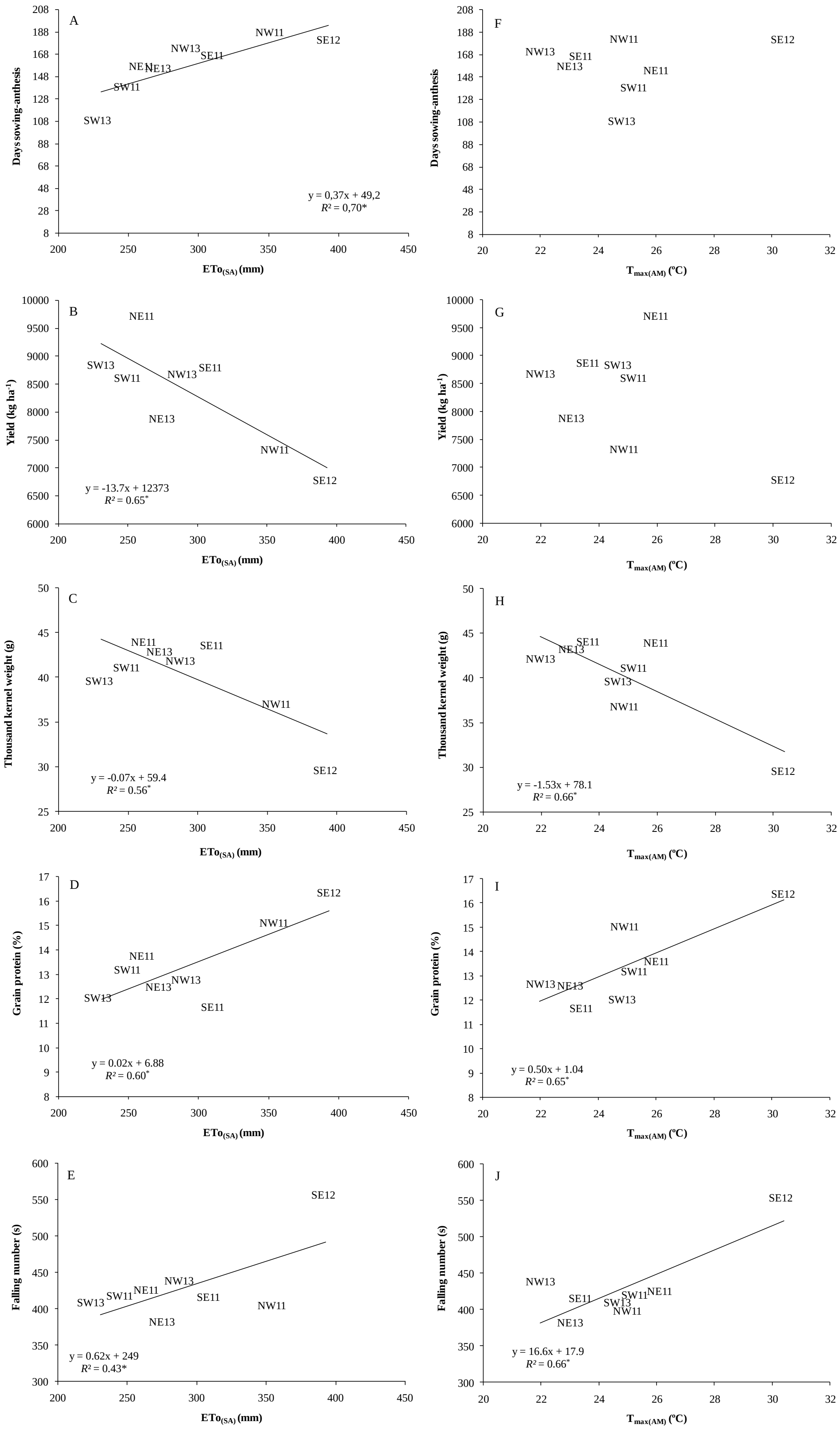


Fig. 3. Relationship between environmental variables and agronomic and quality traits. $ETo_{(SA)}$: reference evapotranspiration from sowing to anthesis; $T_{max(AM)}$: average daily maximum temperature from anthesis to physiological maturity. Each point corresponds to the mean data of an environment (combination of site and year, see Table 2 for acronyms) across 20 bread wheat cultivars. ${}^{*}P< 0.05$; ${}^{**}P< 0.01$; ${}^{***}P< 0.001$.



CHAPTER 4

Breeding effects on the cultivar x environment interaction of durum wheat yield

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Breeding effects on the cultivar × environment interaction of durum wheat yield

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ABSTRACT

Understanding the effect of past durum wheat breeding activities on the cultivar × environment (C × E) interaction of yield and yield components may guide future breeding strategies. A historical series of 24 cultivars released in Italy and Spain during the 20th century was grown in 13 environments with average yields ranging between 1425 and 6670 kg ha^{-1}. The most important environmental factors affecting the C × E interaction for yield were reference evapotranspiration before anthesis and water input during grain filling. The response of cultivars to environmental variables in terms of yield and yield components was associated to the allelic composition for the *Rht-B1* locus. Improved semi-dwarf cultivars (carrying the *Rht-B1b* allele) had the best yield performance in environments with high water input after anthesis, while tall cultivars (carrying allele *Rht-B1a*) were better adapted to environments with high evapotranspirative demand before anthesis and low water input after it. The introduction of the *Rht-B1b* allele improved the capacity of the crop to respond to water availability during grain filling by increasing the number of grains spike^{-1} and grain weight. Yield increases due to breeding caused a loss of stability from the static viewpoint, but not from a dynamic approach based on the superiority measure (P_i). Some semi-dwarf cultivars maintained the levels of yield stability characteristic of the old tall ones. Our results suggest that durum breeding in the 20th century enhanced the response of the crop to environmental improvements.

1. Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is a staple crop in the Mediterranean Basin, where Italy and Spain are among the main European producers (EUROSTAT, 2014). In Mediterranean countries, which represent around 75% of the world durum-growing area, durum wheat is grown mainly under rain-fed conditions. In these environments, yield is generally constrained by water scarcity and heat stress during grain filling, since low and unpredictable seasonal rainfalls and high temperatures are common at the end of the crop cycle (Loss and Siddique, 1994). Moreover, the diversity of thermo-pluviometric patterns results in large spatial and temporal yield fluctuations (Anderson, 2010; De Vita et al., 2010; García del Moral et al., 2003, 2005; Royo et al., 2010). The expected advent of more adverse weather conditions for wheat production in Europe as a consequence of climate change (Trnka et al., 2014; Yang et al., 2014) will require unprecedented efforts towards the release of new cultivars, not only more productive, but also offering improved yield stability across a range of environmental conditions.

The study of historical series formed by cultivars representative of the germplasm developed and cultivated in different periods provide useful information for understanding the agronomical and physiological mechanisms responsible for the yield changes observed over time. Such kind of studies have shown that different approaches were followed in Italy and Spain to release improved durum wheat cultivars (Royo, 2005). Italian breeding programmes, largely relying on local landraces, were conducted from the beginning of the century (Borghi, 2001; Martos et al., 2005). However, in Spain breeding efforts were discontinuous, and foreign germplasm, mainly from CIMMYT, was massively introduced in the country from the 1970s (Royo 2005; Royo et al., 2007). Although different, both approaches resulted in the modification of identical crop characteristics and genetic gains of similar magnitude, 17–20 kg ha^{-1} y^{-1} in Italy (De Vita et al., 2007; Royo et al., 2008), and 24 kg ha^{-1} y^{-1} in Spain (Royo et al., 2008). Compared with unimproved germplasm, modern Italian and Spanish cultivars have early flowering (Isidro et al., 2011), a larger harvest index, more spikes per unit area and more grains per spike (Álvaro et al., 2008b; Royo et al., 2007), while grain weight remained almost unchanged (Álvaro et al., 2008b). The introduction of dwarfing

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genes during the second half of the 20th century produced the greatest advances in durum wheat breeding because they not only strongly affected plant height (Peng et al., 1999), thus, reducing plant lodging, but had also a pleiotropic effect on various agronomic traits (Elias and Manthey, 2005; Singh and Trethowan, 2007). However, the effect of dwarfing genes on the cultivar × environment (C × E) interaction of durum wheat has been poorly explored until now.

Different cultivars may show contrasting responses to a given environment as a consequence of the C × E interaction, a critical issue in cultivar evaluation (Becker and Léon, 1988). The assessment of the relative contributions of the cultivar, the environment and the C × E interaction to cultivar performance is essential to determine the adaptation capacity, which is the cultivar ability to reach its full potential in a specific environment in spite of the constraints imposed on the crop. Some authors have suggested that good performance under severe stress conditions and maximum yield potential in optimal environments are mutually exclusive concepts. They consider the presence in cultivars of constitutive traits for specific adaptation to limiting environments advantageous (Annicchiarico et al., 2005). Nevertheless, breeding programs of the International Maize and Wheat Improvement Center (CIMMYT) that seek wide adaptation and select genotypes at sites of high yield potential (Braun et al., 1996) have also been successful in providing yield increases in limiting environments (Araus et al., 2002; Trethowan et al., 2002).

Yield stability is a property that cultivars need in order to respond to the seasonal fluctuations characteristic of Mediterranean environments. Stability is usually defined as the unaltered performance of a cultivar between years and/or locations and may be considered from two different viewpoints: (i) the static or biological concept, which refers to the lack of response of a cultivar to any variation of the environment; and (ii) the dynamic or agronomic concept, which refers to the fact that a cultivar will respond predictably to improved growing conditions (Becker and Léon, 1988; Cubero and Flores, 2003). The static concept is usually linked to low-yielding cultivars (Becker and Léon, 1988), while the agronomic concept must be accomplished together with improved yield potential to ensure that a cultivar maintains its superiority under a wide range of environments, management practices, and biotic and abiotic stresses (Singh and Trethowan, 2007). A number of statistical methods for elucidating the information contained in a C × E interaction data matrix have been proposed for assessing adaptation and stability (Rharrabti et al., 2003).

The objectives of this study were (I) to assess the changes produced by breeding activities during the last century in the C × E interaction for yield and yield components, (II) to identify the meteorological variables involved in the differential response of the cultivars to the environment, and (III) to ascertain the changes over time in the stability of yield and yield components.

2. Material and methods

2.1. Experimental data

Thirteen field experiments were conducted during six growing seasons, from 2000 to 2005, at three sites: Gimenells (41°40′N, 0°20′E) and Foradada (41°88′N, 0°76′E; only in 2002) in north-east Spain, and Chimeneas (37°08′N, 3°49′E) in southern Spain (Table 1). Sites were chosen to represent contrasting environmental conditions within Spain. The southern area has a Mediterranean climate, with mild winters and hot, dry summers. The northern area has a more continental climate, with lower temperatures at winter and spring and less evenly distributed precipitation.

Each experiment consisted of a randomized complete block design with three replicates and plots of 12 m^2 (8 rows 0.15 m apart) planted at a sowing rate of 400 and 350 viable seeds m^{-2} in the north-eastern and southern experiments, respectively. Plot management was implemented to maximize yield at each location to the extent allowed by local conditions. Fertilization at seed bed and top dressing was adjusted for each experiment according to soil nutrient content and expected crop extractions in order to prevent lodging (Table 1). Weeds were chemically controlled with diclofop-methyl(methyl 2-[2,4-dicholorophenoxy) phenoxy]propanoate at 3 L ha^{-1} and bromoxynil(3,5-dibromo-4-hydroxybenzonitrile) + ioxynil (3,5-diiodino-4-hydroxybenzonitrile) at 3 L ha^{-1}. Pests were controlled with esfenvalerate[(*S*)-α-cyano-3-phenoxybenzyl(*S*)-2-(4-chlorophenyl)-3-methylbutyrate] + phenitrotion(*O*,*O*-dimethyl-*O*-[3-methyl-4-nitro-phenyl]-phosphorotioate) at 3 L ha^{-1}. Diseases were prevented with triadimenol(1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)butan-2-ol) at 0.5 L ha^{-1}.

Daily agro-meteorological variables were recorded in all the environments from weather stations placed at each site. For environment characterization, these data were averaged for two growing periods: from sowing to anthesis (Zadoks stage 65; Zadoks et al., 1974) and from anthesis to physiological maturity (Zadoks stage 87), using as dates of anthesis and physiological maturity the mean of all plots in each environment. The variables calculated from sowing to anthesis (SA) and from anthesis to physiological maturity (AM) were length of the period expressed as thermal time (TT, GDD growing degree-days), average daily mean, maximum and minimum temperatures (T_m, T_{max} and T_{min}, respectively, °C), reference evapotranspiration (ETo, mm), mean daily relative air humidity (RH, %) and total water input (WI, mm) including irrigation and rainfall.

Plots were harvested mechanically at ripening and yield was expressed on a 12% grain moisture basis. From a random sample of the plants contained in a 1-m-long sample taken from a central row of each plot at ripening, the following yield components were determined: number of spikes m^{-2}, number of grains $spike^{-1}$ and thousand kernel weight.

2.2. Plant material

Twenty-four durum wheat (*T. turgidum* L. var. *durum*) cultivars, 12 from Italy and 12 from Spain, were selected to represent the germplasm obtained in both countries during the last century (Table 2). According to their year of release, cultivars were assigned to three periods: old (mainly landraces, cultivated before 1945), intermediate (released between 1950 and 1985) and modern (released from 1988 to 2000). The intermediate group included early semi-dwarf cultivars derived from CIMMYT germplasm, while the modern group included cultivars released by local breeding programmes in both countries during the 1990s. The presence of the *Rht-B1b* dwarfing allele in the cultivars was assessed with a PCR-based marker following Ellis et al. (2002) in DNA samples obtained from young leaf tissues of single plants cultivated under greenhouse conditions. In previous studies, using the test for sensitivity to gibberellic acid conducted following Gale and Gregory (1977), the Italian cultivar 'Adamello' was classified as not carrying dwarfing genes (Isidro et al., 2011; Álvaro et al., 2008a,b,c; Royo et al., 2007), but the PCR-based marker demonstrated that it carries the dwarfing allele *Rht-B1b* (Table 2).

2.3. Statistical analyses

Combined analysis of variance (ANOVA), in which the cultivar and the C × E interaction effects were partitioned into period, origin and period × origin interaction, were performed for yield and

Table 1
Localization and description of the 13 Spanish environments of the study.

Site	Gimenells (North)						Foradada (North)	Chimeneas (South)					
Coordinates	41°40′N, 0°20′E						41°88′N, 0°76′E	37°08′N, 3°49′W					
Altitude (m asl)	200						580	684					
Soil texture	Fine loamy						Fine loamy	Silty clay					
Harvest year	2000	2001	2002	2003	2004	2005	2002	2000	2001	2002	2003	2004	2005
Fertilizer (kg ha^{-1})													
Seed bed ($N-P_2O_5-K_2O$)	106-80-80	80-150-150	80-150-150	75-75-75	75-75-75	75-75-75	60-150-60	52-52-52	52-52-52	52-52-52	45-45-45	45-45-45	45-45-45
Top dressing (N)	79	130	97	35	35	35	104	26	26	26	35	35	35
Sowing date	26 November 1999	17 November 2000	5 December 2001	26 November 2002	16 December 2003	26 November 2004	31 October 2001	9 December 1999	28 November 2000	27 November 2001	23 December 2002	13 November 2003	10 December 2004
Experiment code	NI00	NI01	NI02	NI03	NI04	NI05	NR02	S00	S01	S02	S03	S04	S05
Environmental conditions from sowing to anthesis (SA)													
TT (GDD)	1400	1497	1241	1426	1281	1219	1458	1424	1673	1676	1590	1769	1482
T_m (°C)	8.04	9.14	7.56	8.64	8.13	7.24	6.82	10.0	10.6	9.99	10.7	9.81	9.43
T_{max} (°C)	14.3	14.9	13.3	14.7	13.6	13.2	12.2	17.7	17.6	17.4	17.5	16.9	17.8
T_{min} (°C)	2.63	3.97	2.47	3.22	3.24	1.96	2.21	2.37	4.96	3.85	4.93	4.08	2.30
ETo (mm)	266	236	214	244	206	226	265	299	288	301	309	290	348
RH (%)	78.1	81.4	83.0	80.3	95.6	91.4	83.8	61.3	72.8	69.3	68.6	71.2	56.5
WI (rainfall + irrigation, mm)	101 + 160	125 + 150	94.8 + 182	170 + 150	202 + 85	62.7 + 150	286 + 0	155 + 0	348 + 0	301 + 0	249 + 40	334 + 40	89.8 + 120
Environmental conditions from anthesis to maturity (AM)													
TT (GDD)	432	383	305	521	338	348	296	588	555	291	271	286	388
T_m (°C)	19.5	17.3	16.7	17.6	20.0	18.8	18.2	18.4	16.6	18.1	21.3	16.2	21.1
T_{max} (°C)	26.7	24.5	23.0	25.5	28.2	26.8	25.3	25.8	24.0	25.8	30.0	23.4	30.3
T_{min} (°C)	12.9	10.5	10.2	10.4	12.4	11.7	10.2	10.9	9.68	10.4	12.6	10.1	12.0
ETo (mm)	177	181	155	117	140	140	153	129	139	150	118	139	182
RH (%)	70.1	70.5	65.4	78.9	87.9	85.2	59.4	56.1	57.6	51.4	45.6	66.9	38.8
WI (rainfall + irrigation, mm)	91.1 + 0	116 + 0	87.4 + 0	62.5 + 0	6.03 + 65	52.9 + 0	63.0 + 0	68.6 + 0	48.4 + 0	20.6 + 0	0	79.8 + 0	6.00 + 0
Mean yield (kg ha^{-1})	3424	5241	4541	5378	6495	6670	2879	2222	2113	3131	2571	4327	1425

TT: thermal time calculated as growing degree-days; T_m: mean daily temperature; T_{max} and T_{min}: average daily maximum and minimum daily temperatures; ETo: reference evapotranspiration computed by the Penman–FAO methodology (Allen et al., 1998); RH: average daily relative mean humidity; WI: water input (rainfall + irrigation).

yield components. For each trait the C × E interaction was partitioned according to the AMMI model (Gauch and Zobel, 1997) and the percentage of the sum of squares explained by each interaction principal component axis (IPCA) was calculated. Factorial regression analyses were performed to identify the environmental variables with a major effect on the studied traits, thus keeping the most explanatory model for each of them. Environmental covariables corresponding to the sowing to anthesis and anthesis to maturity periods were considered for yield, number of spikes m^{-2} and number of grains $spike^{-1}$, while for thousand kernel weight only variables from anthesis to maturity were considered.

Four stability indices were calculated for each trait with the whole set of data: the slope of the joint regression analysis (b; Finlay and Wilkinson, 1963), the environmental variance ($S^2{}_{xi}$; Roemer, 1917), the superiority measure (P_i; Lin and Binns, 1988), and the distance of each cultivar from the origin of the axes in the AMMI biplot (v_i; Grausgruber et al., 2000). In order to determine the changes caused by breeding activities on the stability of the studied traits, linear regression models were fitted to the relationship between the stability indices of each cultivar and its year of release. Pearson correlation coefficients were calculated between stability indices with significant changes over time. Statistical analyses were performed with Enterprise Guide v4.2 (SAS Institute, 2008), JMP V8 (SAS Institute, 2009) and GenStat v16 (VSN International, 2013) statistical software.

3. Results

3.1. Environmental conditions

The environmental conditions from sowing to anthesis differed at northern and southern locations. Within each crop season, northern environments had a shorter pre-anthesis period measured in thermal time, and showed lower mean, maximum and minimum temperatures and reference evapotranspiration, and higher relative humidity than southern ones (Table 1). From anthesis to maturity, northern environments were more humid and in general received a greater water input than southern ones, but even so environmental variables largely fluctuated between years in both the north and south. Average yield across cultivars ranged in the north from 2879 kg ha^{-1} in Rainfed-2002 (NR02) to 6670 kg ha^{-1} in Irrigated-2005 (NI05), and in the south from 1425 kg ha^{-1} in 2005 (S05) to 4327 in 2004 (S04).

3.2. Cultivar × environment interaction for yield

The ANOVA revealed that the effects of the environment, cultivar and C × E interaction accounted for 76.4%, 10.4% and 9.4%, respectively, of the total variance for yield (Table 3). The mean yield of cultivars across environments ranged from 2710 kg ha^{-1} (cv. 'Rubio de Belalcázar') to 4759 kg ha^{-1} (cv. 'Boabdil') (Table 2). Differences between periods explained 79.9% of the variance induced by the cultivar effect, while differences between origins only explained 0.34%. Similarly, the period × environment and the origin × environment interactions explained 39.9% and 6.9% of the C × E interaction, respectively (Table 3).

The first two IPCAs of the AMMI model explained 69.3% of the C × E interaction, while IPCA3 explained an additional 11.1% (Table 3). The first IPCA, which explained 52.8% of the C × E sum of squares, classified cultivars according to their period of release, with old and modern cultivars being clearly separated (Fig. 1A). Old cultivars were placed in the positive direction of IPCA1 and the Italian cultivar 'Senatore Cappelli' was the one with the highest value for this axis. Although intermediate cultivars did not form a sep-

Table 2

Origin, year of release, presence of the *Rht-B1b* dwarfing allele determined with SSR, and yield and yield components in the 24 Italian and Spanish durum wheat cultivars of the historical series included in this study. Yield and yield components are means across 13 environments.

Period	Cultivar	Year of release	*Rht-B1b* presence	Yield ± SE (kg ha^{-1})	NS m^{-2} ± SE	NGS ± SE	TKW ± SE (g)
Italian old							
	Balilla Falso	<1930	–	3680 ± 293	378 ± 28.7	26.3 ± 0.69	42.1 ± 1.02
	Razza 208	<1930	–	2942 ± 216	238 ± 15.1	28.3 ± 1.22	49.6 ± 1.22
	Senatore Cappelli	1930	–	3116 ± 236	240 ± 12.7	32.5 ± 1.18	48.0 ± 1.27
	Carlojucci	1945	–	3450 ± 278	276 ± 16.2	32.0 ± 1.37	44.8 ± 0.91
Italian intermediate							
	Capeiti 8	1955	–	4355 ± 339	361 ± 26.9	32.2 ± 1.35	42.2 ± 0.86
	Trinakria	1970	–	3841 ± 282	312 ± 20.5	30.0 ± 0.98	41.9 ± 1.06
	Creso	1974	+	3999 ± 291	349 ± 20.6	30.1 ± 1.18	42.2 ± 1.04
	Adamello	1985	+	4306 ± 315	303 ± 17.7	33.9 ± 1.22	48.2 ± 1.32
Italian modern							
	Simeto	1988	+	4652 ± 346	312 ± 16.8	37.4 ± 1.45	49.2 ± 1.27
	Cirillo	1992	+	4454 ± 335	323 ± 20.4	36.2 ± 1.28	41.7 ± 1.02
	Flavio	1992	+	3899 ± 248	321 ± 16.8	34.8 ± 1.53	40.3 ± 1.19
	Zenit	1992	+	4268 ± 283	345 ± 20.5	32.4 ± 1.10	42.5 ± 1.14
Spanish old							
	Blanco Verdeal	<1930	–	3060 ± 264	267 ± 16.4	27.7 ± 1.18	47.9 ± 1.31
	Clarofino	<1930	–	2849 ± 212	263 ± 14.9	30.0 ± 1.03	41.7 ± 0.78
	Pinet	<1930	–	3332 ± 257	260 ± 17.3	31.8 ± 1.07	45.2 ± 1.17
	Rubio de Belalcázar	<1930	–	2710 ± 205	279 ± 19.9	29.8 ± 1.10	40.2 ± 0.87
Spanish intermediate							
	Bidi 17	1950	–	3734 ± 291	288 ± 19.0	32.2 ± 1.24	45.7 ± 1.22
	Camacho	1975	+	3973 ± 276	265 ± 16.5	36.3 ± 1.35	45.8 ± 1.28
	Esquilache	1976	+	4458 ± 326	345 ± 21.6	33.8 ± 1.30	42.8 ± 1.13
	Mexa	1980	+	3949 ± 288	342 ± 22.2	32.8 ± 1.28	43.8 ± 1.23
Spanish modern							
	Ariesol	1992	+	4380 ± 302	315 ± 18.3	35.9 ± 1.19	43.6 ± 1.21
	Senadur	1995	+	4294 ± 263	344 ± 23.7	34.9 ± 1.07	43.2 ± 1.37
	Astigi	1999	+	4617 ± 354	318 ± 18.1	42.7 ± 1.35	38.2 ± 1.07
	Boabdil	2000	+	4759 ± 324	358 ± 21.9	33.2 ± 1.16	45.2 ± 1.35

NS m^{-2}: number of spikes m^{-2}; NGS: number of grains $spike^{-1}$; TKW: thousand kernel weight.

Table 3
Percentage of the sum of squares (SS) in the ANOVA, AMMI and factorial regression models for the partitioning of the cultivar × environment interaction for yield of the historical series of 24 Italian and Spanish durum wheat cultivars grown in 13 environments. ETo: reference evapotranspiration, WI: water input. SA and AM within parentheses stand for the sowing-anthesis and anthesis-maturity periods, respectively.

Source of variation	df	SS (%)	−log (*P*)
Total	935		
ANOVA			
Environment	12	76.4	20.7
Block (environment)	26	1.00	25.2
Cultivar	23	10.4	183
Period	2	79.9	178
Origin	1	0.34	2.21
Origin × period	2	2.94	13.4
Cultivar(origin × period)	18	16.8	50.5
Cultivar × environment	276	9.41	89.9
Period × environment	24	39.9	93.1
Origin × environment	12	6.95	20.4
Origin × period × environment	24	4.48	7.99
Cultivar(origin × period) × environment	216	48.7	47.4
AMMI			
Cultivar × environment	276	9.41	89.9
IPCA1	34	52.8	109
IPCA2	32	16.5	38.7
IPCA3	30	11.1	25.0
Residuals	180	19.6	
Factorial regression			
Cultivar × environment	276	9.41	89.9
$\mathrm{ETo}_{(SA)}$ × cultivar	23	18.1	46.8
$\mathrm{WI}_{(AM)}$ × cultivar	23	12.1	30.8
Deviations	230	69.8	
Cultivar × environment	276	9.41	89.9
Period × environment	24	39.9	93.1
$\mathrm{ETo}_{(SA)}$ × period	2	19.1	12.4
$\mathrm{WI}_{(AM)}$ × period	2	15.8	10.3
Deviations	20	65.1	
Error	598	2.81	

arate cluster, 'Creso' and 'Camacho' were located in the positive direction of IPCA2, while 'Adamello', 'Esquilache' and 'Mexa' were in the negative direction. 'Adamello' and 'Mexa' were the closest to modern and old cultivars, respectively. Among the intermediate group, cultivars carrying the *Rht-B1b* allele tended to be located close to the modern cultivars, while those that did not carry it were near to the old ones. Southern environments were placed in the positive direction of IPCA2, while the irrigated northern ones were mostly in the negative direction, except North-Irrigated-2002 (NI02), which was located close to South-2004 (S04). The position of the environments within the biplot of the AMMI analysis indicates that the modern cultivars and those carrying the *Rht-B1b* allele performed better in environments South-2004 (S04) and North-Irrigated-2002 (NI02) than in the remainder.

The most explanatory model obtained by factorial regression accounted for 30.2% of the C × E interaction sum of squares, with 16.7% of its degrees of freedom, and included two covariables: reference evapotranspiration from sowing to anthesis [$\mathrm{ETo}_{(SA)}$] and water input from anthesis to maturity [$\mathrm{WI}_{(AM)}$] (Table 3). Both covariables also explained 34.9% of the period × environment interaction. The covariables retained in the model were depicted in the AMMI biplot according to their correlation to the first two IPCAs (Fig. 1A). Water input from anthesis to maturity was strongly and negatively correlated with IPCA1, and was positioned in the same direction as modern cultivars, while $\mathrm{ETo}_{(SA)}$ was located in the positive direction of IPCA1 and IPCA2 close to five of the six southern environments, the old Spanish cultivars 'Rubio de Belalcázar' and 'Clarofino' and the old Italian cultivar 'Razza 208'.

3.3. Cultivar × environment interaction for yield components

The ANOVA showed that the number of spikes m^{-2} was the yield component most affected by the environmental conditions, as the environment factor explained 71.3% of the total variance for this trait, while it accounted for 54.2% and 66.5% of the total variance for the number of grains spike^{-1} and thousand kernel weight, respectively (Table 4). However, the number of spikes m^{-2} was also the yield component that showed the lowest C × E interaction. Cultivar means across environments ranged between 238 and 378 for the number of spikes m^{-2}, and both values were recorded in old Italian cultivars (Table 2). On the other hand, number of grains spike^{-1}, which showed the largest cultivar effect (17.7%), also had the highest C × E interaction, which explained 16.8% of the total variance (Table 4). The number of grains spike^{-1} ranged between 26.3 in 'Balilla Falso' and 42.7 in 'Astigi', and thousand kernel weight was lowest in 'Astigi' and highest in 'Simeto' (Table 2). The percentage of the sum of squares of the C × E interaction accounted by differences between periods ranged from 14.0% for number of spikes m^{-2} to 19.3% for thousand kernel weight (Table 4), all of them lower than the 39.9% for yield, as shown in Table 3.

The first three IPCAs explained 69.4%, 58.8% and 65.9% of the C × E sum of squares for number of spikes m^{-2}, number of grains spike^{-1} and thousand kernel weight, respectively (Table 5 and Fig. 1B–D). One of the two environmental covariables [$\mathrm{ETo}_{(SA)}$] retained in the factorial regression model that dissected the C × E interaction for yield also entered the model for the number of spikes m^{-2}. Reference evapotranspiration from sowing to anthesis jointly with the average daily relative humidity from anthesis to maturity [$\mathrm{RH}_{(AM)}$] and from sowing to anthesis [$\mathrm{RH}_{(SA)}$] explained 52.8% of the C × E sum of squares (Table 5A). These covariables were highly correlated with IPCA1 (Fig. 1B), which tended to separate the environments according to their latitude, with the exception of North-Irrigated-2000 (NI00), which was located in the positive direction of this axis, close to the southern environments due to its high $\mathrm{ETo}_{(SA)}$. The vector of $\mathrm{ETo}_{(SA)}$ was close to southern environments, in accordance with their greatest $\mathrm{ETo}_{(SA)}$, as shown in Table 1. All the remaining northern environments were in the direction of $\mathrm{RH}_{(SA)}$ and $\mathrm{RH}_{(AM)}$. No clear trend was observed in the behaviour of cultivars of different periods regarding these environmental covariables, because old and modern cultivars were mainly separated along IPCA2, but they showed large variability within periods. Among old cultivars, 'Balilla Falso', which was the one with the highest number of spikes m^{-2}, was close to North-Irrigated-2005 (NI05), in Fig. 1B, while 'Senatore Cappelli', which was among the cultivars with the lowest number of spikes m^{-2}, was the closest to the vector of $\mathrm{ETo}_{(SA)}$.

The C × E interaction for the number of grains spike^{-1} was mainly due to the different sensitivities of the cultivars to three post-anthesis environmental variables: thermal time [$\mathrm{TT}_{(AM)}$], water input [$\mathrm{WI}_{(AM)}$] and reference evapotranspiration [$\mathrm{ETo}_{(AM)}$]. These three variables accounted for 32.3% of the C × E sum of squares and $\mathrm{TT}_{(AM)}$ and $\mathrm{ETo}_{(AM)}$ for 37.1% of the period × environment interaction (Table 5). Modern and intermediate cultivars carrying the *Rht-B1b* allele were mainly placed in the negative direction of the IPCA1, indicating that they tended to produce more grains spike^{-1} in environments with low $\mathrm{TT}_{(AM)}$, and high WI and ETo after anthesis (Fig. 1C).

The lowest thousand kernel weight corresponded to 'Astigi' and the highest to 'Simeto' (Table 2). The regression model explaining the C × E interaction for thousand kernel weight included two environmental covariables after anthesis: water input [$\mathrm{WI}_{(AM)}$] and average daily relative humidity [$\mathrm{RH}_{(AM)}$], which jointly accounted for 24.1% of the C × E interaction (Table 5C). The AMMI biplot (Fig. 1D) clustered old and intermediate tall cultivars apart from

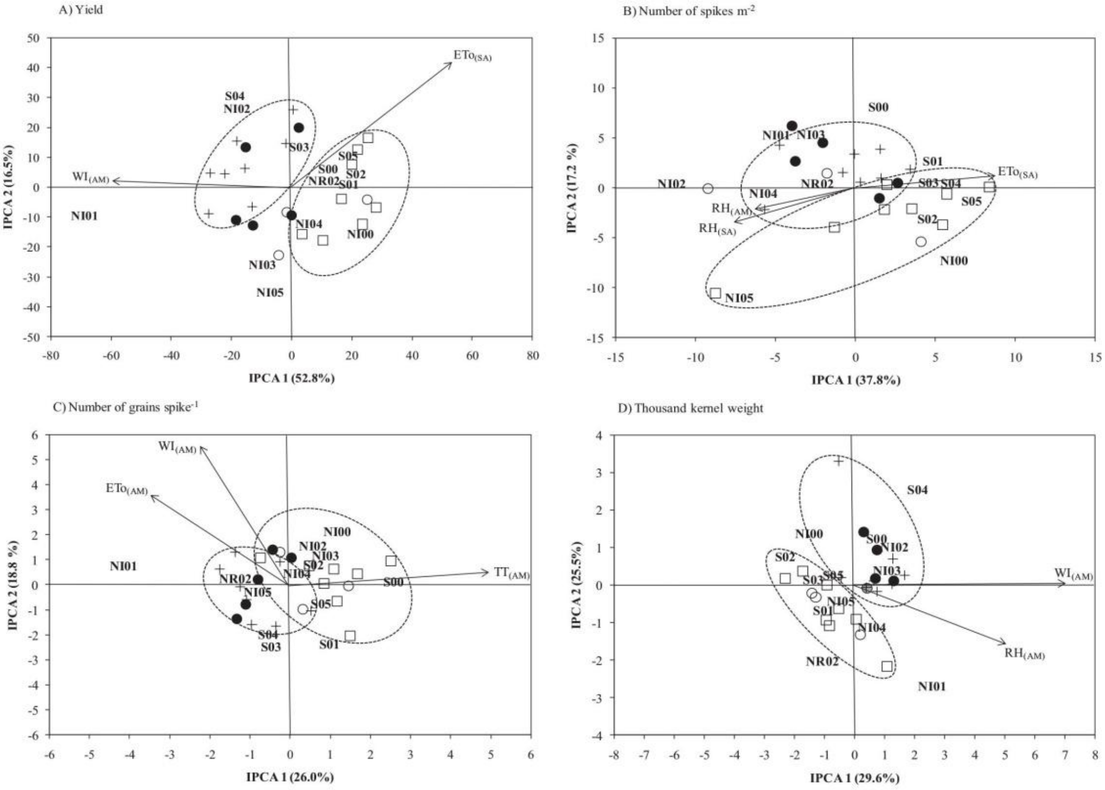

Fig. 1. Biplot of the of the first two axes of the AMMI model for: (A) yield, (B) number of spikes m^{-2}, (C) number of grains spike^{-1}, and (D) thousand kernel weight. Environments are indicated in bold (NI, north irrigated; NR, north rainfed; S, south) followed by the year of the experiment. Cultivars are identified as follows for each period: (□) old, (●) intermediate cultivars carrying the *Rht-B1b* allele, (○) intermediate cultivars not carrying the *Rht-B1b* allele, and (+) modern. ETo: reference evapotranspiration; RH: average daily relative humidity; TT: thermal time; WI: water input. Subscripts SA and AM within parentheses stand for the sowing-anthesis and anthesis-maturity periods, respectively.

the modern and intermediate ones carrying the *Rht-B1b* dwarfing allele. The latter were mostly placed in the positive direction of IPCA1, which was strongly correlated with $WI_{(AM)}$. Large variability existed between tall cultivars regarding their performance in environments with high relative humidity after anthesis. The modern Spanish cultivar 'Senadur' was placed in the upper part of Fig. 1D, suggesting that it showed the highest C × E interaction for thousand kernel weight.

Table 4

Percentage of the sum of squares (SS) in the ANOVA models for the partitioning of the cultivar × environment interaction for yield components of the historical series of 24 Italian and Spanish durum wheat cultivars grown in 13 environments.

		Number of spikes m^{-2}		Number of grains spike^{-1}		Thousand kernel weight	
Source of variation	df	SS (%)	−log (*P*)	SS (%)	−log (*P*)	SS (%)	−log (*P*)
Total	935						
Environment	12	71.3	22.7	54.2	20.1	66.5	19.9
Block (environment)	26	0.65	2.41	0.78	1.77	1	11.6
Cultivar	23	9.61	88.6	17.7	111	14.8	160
Period	2	36.2	48.2	53	82.5	7.52	25.8
Origin	1	1.49	3.02	3.35	7.96	1.8	7.52
Origin × period	2	2.67	4.23	1.72	3.7	5.23	18.5
Cultivar(origin × period)	18	59.6	59.7	42	57	85.4	149
Cultivar × environment	276	10.7	28.6	16.8	36.2	12.7	66.4
Period × environment	24	14	11.9	17.9	20	19.3	36.8
Origin × environment	12	2.83	1.59	2	1.05	4.29	7.95
Origin × period × environment	24	6.52	3.21	7.58	5.59	4.74	5.42
Cultivar(origin × period) × environment	216	76.6	24.3	72.6	28.3	71.7	53.7
Error	598	7.74		10.5		5.05	

Table 5
Percentage of the sum of squares (SS) in the AMMI and factorial regression models for the partitioning of the cultivar × environment interaction for yield components of the historical series of 24 Italian and Spanish durum wheat cultivars grown in 13 environments. ETo: reference evapotranspiration; RH: average daily relative humidity; TT: thermal time; WI: water input. SA and AM within parentheses stand for the sowing-anthesis and anthesis-maturity periods, respectively.

Source of variation	df	SS (%)	−log (*P*)
(A) Number of spikes m^{-2}			
Cultivar × environment	276	10.7	28.6
AMMI			
IPCA1	34	37.8	35.7
IPCA2	32	17.2	13.2
IPCA3	30	14.4	10.6
Residuals	180	30.6	
Factorial regression			
$ETo_{(SA)}$ × cultivar	23	29.9	31.6
$RH_{(AM)}$ × cultivar	23	12.7	10.6
$RH_{(SA)}$ × cultivar	23	10.2	7.55
Deviations	207	47.3	
Period × environment	24	14	12.3
$ETo_{(SA)}$ × period	2	21.8	2.66
Deviations	22	78.2	
(B) Number of grains $spike^{-1}$			
Cultivar × environment	276	16.8	36.2
AMMI			
IPCA1	34	26	27.1
IPCA2	32	18.8	18.3
IPCA3	30	14	12.6
Residuals	180	41.2	
Factorial regression			
$TT_{(AM)}$ × cultivar	23	13	13.4
$WI_{(AM)}$ × cultivar	23	10.9	10.5
$ETo_{(AM)}$ × cultivar	23	8.39	6.92
Deviations	207	67.7	
Period × environment	24	17.9	20
$TT_{(AM)}$ × period	2	28.7	4.93
$ETo_{(AM)}$ × period	2	8.38	1.45
Deviations	20	62.9	
(C) Thousand kernel weight			
Cultivar × environment	276	12.7	66.4
AMMI			
IPCA1	34	29.6	51.5
IPCA2	32	25.6	45.2
IPCA3	30	10.7	16.4
Residuals	180	34.1	
Factorial regression			
$WI_{(AM)}$ × cultivar	23	15.5	29.3
$RH_{(AM)}$ × cultivar	23	8.58	14.1
Deviations	230	75.9	
Period × environment	24	19.3	36.8
$WI_{(AM)}$ × period	2	15.6	2.47
Deviations	22	84.4	

3.4. Trends in stability for yield and yield components

The study of the linear models fitted to the relationships between the year of release of cultivars and the yield stability indices calculated for each cultivar resulted in statistical significance for three of them (Fig. 2). The slope of the joint regression analysis (b) and the environmental variance (S^2_{xi}) increased at a rate of $3.8 \times 10^{-3}\,y^{-1}$ and $2.13 \times 10^{-2}\,y^{-1}$, respectively (Fig. 2A and B), indicating an increase in responsiveness to the environmental conditions over time. The slope of the joint regression analysis for yield ranged between 0.68 (‘Rubio de Belalcázar’) and 1.27 (‘Astigi’), with modern cultivars having the maximum range (from 0.89 to 1.27). A negative trend was observed for the superiority measure (P_i; 10^6), whose rate of change was estimated as $-3.6 \times 10^{-2}\,y^{-1}$ (Fig. 2C). The distance from the origin of AMMI axes (v_i) did not show a significant linear trend for yield for the cultivars released during the 20th century, although the lowest values for this index were recorded in intermediate cultivars (Fig. 2D). ‘Mexa’ and ‘Trinakria’ were the cultivars with the lowest values for v_i, suggesting that their yields had a low C × E interaction. Strong and significant relationships existed between yield stability indices, showing a significant trend over time. For yield, the Pearson correlation coefficient between b and S^2_{xi} was $r = 0.99$ ($P < 0.0001$), between b and P_i it was $r = -0.89$ ($P < 0.0001$), and between S^2_{xi} and P_i it was $r = -0.85$ ($P < 0.0001$).

For the number of spikes m^{-2} significant changes were only observed in P_i, which decreased over years at a rate of $-117\,y^{-1}$ (Table 6). The minimum and maximum values of P_i for the number of spikes m^{-2} were recorded in two old Italian cultivars, ‘Balilla Falso’ ($P_i = 1992$) and Razza 208 ($P_i = 21265$), respectively. The stability indices for the number of grains $spike^{-1}$ followed a similar trend to the ones estimated for yield (Table 6). The trend found for the slope of the joint regression analysis was of the same magnitude as that calculated for yield ($3.1 \times 10^{-3}\,y^{-1}$), but the rates of change for S^2_{xi} ($0.23\,y^{-1}$) and P_i ($-1.06\,y^{-1}$) were lower than those estimated for yield. For the number of grains $spike^{-1}$, the old Italian cultivar ‘Balilla Falso’ showed extreme values for the three stability indices: the lowest for b (0.60) and S^2_{xi} (18.0) and the highest for P_i (164). The extremes for these indices, indicating the lowest stability for the number of grains $spike^{-1}$, were recorded in three modern cultivars: ‘Cirillo’ ($b = 1.63$), ‘Flavio’ ($S^2_{xi} = 81.9$) and ‘Astigi’ ($P_i = 1.81$). No significant linear change was observed in the stability calculated for thousand kernel weight (Table 6).

4. Discussion

The environmental traits affecting the growth of durum wheat showed great variability across the 13 environments included in this study. This variability caused a difference of 5245 kg ha^{-1} between the most- and the least-yielding experiments, which is more than four times the yield obtained in the least-yielding one. The environmental effect explained 76.4% of yield variability, a lower value than the 98% found in a previous study, in which 191 durum wheat accessions were tested at nine sites of four Mediterranean countries (Royo et al., 2010), but higher than the 57% previously reported for bread wheat (Sanchez-Garcia et al., 2012). In the present study the environmental effect accounted for 54.2–71.3% of the observed variance in yield components. These values are much higher than those found in a previous study conducted in the same country that tested a historical series of 27 bread wheat cultivars in eight environments (Sanchez-Garcia et al., 2012). These results suggest larger C × E interaction for durum wheat than for bread wheat. However, in both studies the number of grains $spike^{-1}$ was the yield component least affected by environmental conditions.

In spite of the great effect of environmental conditions on yield, the C × E interaction was highly significant for this trait and explained ca. 10% of its variance. This value is within the range of those reported by previous studies conducted in wheat in the SEWANA (South of Europe, West Asia and North Africa) region (Anderson, 2010; De Vita et al., 2010; Mohammadi and Amri, 2013; Sanchez-Garcia et al., 2012). The partition in the ANOVA of the cultivar effect between periods, origins and their interactions revealed that the period of cultivar release was much more important than the origin of the cultivars for explaining the variability of yield. In addition, as shown by the ANOVA and the biplot of the AMMI model, the contribution of the period to explaining the C × E interaction for yield was much greater than that of the origin, thus leading to the conclusion that the contrasting breeding strategies followed in Italy and Spain during the 20th century evidenced by previous studies (Martos et al., 2005; Royo et al., 2007) resulted in similar adapta-

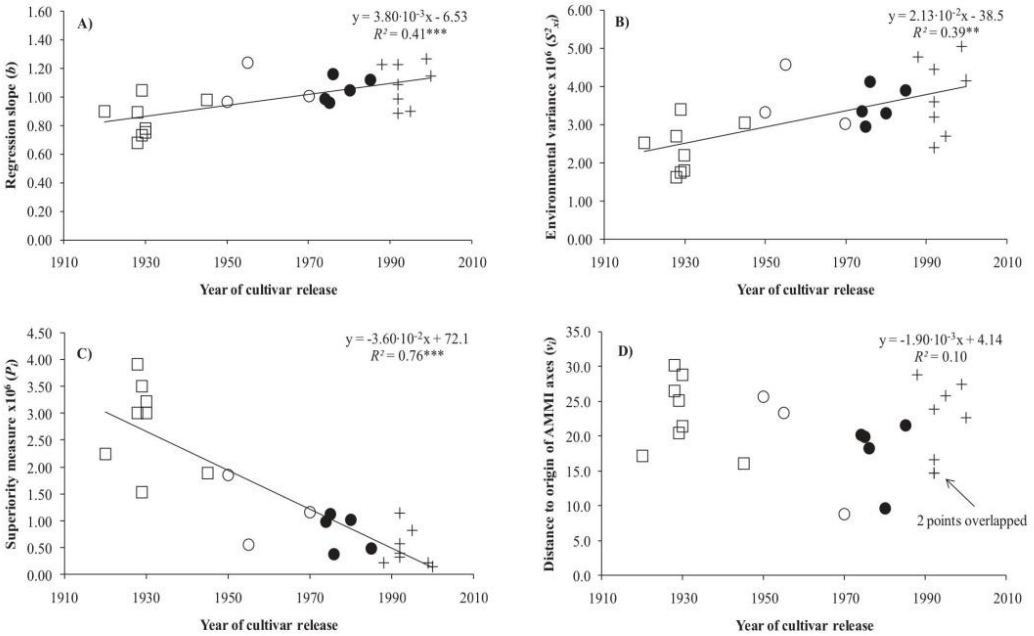

Fig. 2. Relationship between the year of release of the 24 cultivars of the historical series and the stability indices calculated for grain yield: (A) slope of the joint regression analysis (b); (B) environmental variance (S^2_{xi}); (C) superiority measure (P_i); (D) distance from the origin of AMMI axes (v_i). Cultivars are identified as follows for each period: (□) old; (●) intermediate cultivars carrying the *Rht-B1b* allele; (○) intermediate cultivars not carrying the *Rht-B1b* allele; and (+) modern. $^{**}P < 0.01$; $^{***}P < 0.001$.

tion patterns. This result, and the similar ones obtained for yield components, show the presence of different patterns of adaptation to environmental constraints within the set of cultivars of the historical series and justify the identification of the cultivar period but not the origin in Figs. 1 and 2.

The AMMI analysis for yield clustered apart old and modern cultivars, and assigned the intermediate ones to one or the other group according to their allelic composition for the *Rht-B1* locus. Given that the *Rht-B1b* allele was present in all modern cultivars but was absent in the old ones, clusters in the biplot separated tall and semi-dwarf cultivars, thus demonstrating that the introduction and spread of the *Rht-B1b* allele in the early 1970s (Royo and Briceño-Félix, 2011) was critical for explaining the changes observed in the adaptation of durums over time. The location of the point representing the cultivar 'Adamello' in the same cluster as modern and intermediate semi-dwarf cultivars in Fig. 1A suggests a common adaptation pattern, which is in agreement with the presence in this cultivar of the *Rht-B1b* allele, as detected by the PCR-marker.

The most important environmental factors affecting the C × E interaction for yield were reference evapotranspiration before anthesis [$\mathrm{ETo}_{(SA)}$] and water input after this stage [$\mathrm{WI}_{(AM)}$]. The clustering in the biplot for yield of modern and intermediate semi-dwarf cultivars in the direction of the vector representing $\mathrm{WI}_{(AM)}$ indicates that they had the best yield performance in environments with high water input during grain filling, such as North-Irrigated-2002 (NI02) and South-2004 (S04). Moreover, the location of the cluster including old and intermediate tall cultivars on the opposite side and closer to the vector of $\mathrm{ETo}_{(SA)}$ suggested that they adapted better than semi-dwarf cultivars in terms of yield to environments with high evapotranspiration before anthesis and low WI during grain filling. It is well known that the introduction of dwarfing alleles in wheat cultivars during the 20th century reduced plant height and lodging and improved the partitioning of assimilates to the grain increasing the sink capacity of the crop (Addisu et al., 2010; Álvaro et al., 2008a; Rebetzke et al., 2012; Youssefian et al., 1992), and the harvest index (Royo et al., 2007), thus allowing the crop to take advantage of more favourable conditions in terms of nutrients and water availability, and therefore crop intensification. The results of the current study indicate that when water is available after anthesis, semi-dwarf cultivars have a greater potential than the tall ones to use it in benefit of yield increases. Within the old cultivars, 'Senatore Cappelli' (also known as 'Cappelli') showed the

Table 6

Relationships between the year of release of the 24 cultivars of the historical series and the stability indices calculated for yield components. Same data for yield are in Fig. 2.

Trait	Regresssion slope (b)		Environmental variance ($S^2{}_{xi}$)		Superiority measure (P_i)		Distance to the origin of AMMI axes (v_i)	
	Slope	R^2	Slope	R^2	Slope	R^2	Slope	R^2
Number of spikes m^{-2}	1.7×10^{-3}	0.05	28	0.02	−117	0.36^{**}	-4.2×10^{-2}	0.16
Number of grains spike^{-1}	3.1×10^{-3}	0.25^{*}	0.23	0.18^{*}	−1.06	0.56^{***}	−0.01	3×10^{-3}
Thousand kernel weight	1.4×10^{-3}	0.1	0.2	0.15	0.19	0.05	-3.9×10^{-3}	0.03

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

highest score for IPCA1, which was strongly and negative correlated with $WI_{(AM)}$. This result indicates that this cultivar was among the ones least affected by water stress during grain filling, and that it was also the least responsive to water input during grain filling. The position of the point representing 'Senatore Cappelli' close to the vector of $ETo_{(SA)}$ in the biplot for the number of spikes m^{-2} suggests that its adaptation pattern was related to the higher number of spikes m^{-2} in environments with high reference evapotranspiration before anthesis. Though it was the cultivar with the lowest number of spikes m^{-2} across environments, this yield component was less affected by conditions of high $ETo_{(SA)}$ and low $RH_{(SA)}$ and $RH_{(AM)}$ than other old cultivars; however, it took advantage to form heavy grains when relative humidity and water input after anthesis were high, as shown by Fig. 1D. These results reveal a good performance of this cultivar in environments with high evapotranspirative demand before anthesis, and a great efficiency in the use of water during grain filling that resulted in heavy grain formation. These characteristics of adaptation of 'Senatore Cappelli' to stress environments may be behind its large contribution to durum wheat improvement in Italy, where it has been widely used as a parental line and is still present in the background of some of the most successful Italian durum wheat cultivars grown nowadays (Di Fonzo et al., 2005).

The number of spikes m^{-2} was the yield component with the highest environmental effect and the lowest C × E interaction. Old cultivars tended to perform better in southern environments, with high $ETo_{(SA)}$ and low $RH_{(SA)}$ and $RH_{(AM)}$, than modern ones. This effect was due to their lower responsiveness to favourable conditions, as revealed by the significant decrease in the superiority measure (Pi) over the 20th century. The number of spikes $plant^{-1}$ was significantly improved in durum wheat by breeding activities during the last century (Royo et al., 2007), thus increasing the capacity of the crop to revert resources to additional tillers under favourable conditions.

Among yield components the number of grains $spike^{-1}$ had the largest genetic control but the largest C × E interaction. It was also the one most affected by the period of cultivar release, and therefore the yield component most related to changes in crop adaptability over time. Previous studies identified the number of grains $spike^{-1}$ as the most important yield component for raising the number of grains per unit area and yield in durum wheat during the last century (De Vita et al., 2007; Royo et al., 2007). Tough the number of grains $spike^{-1}$ is determined by different events during crop development, only post-anthesis variables explained the observed C × E interaction for this trait, thus showing the importance of floret abortion and grain setting for durum wheat yield determination in Mediterranean environments, as pointed out by previous studies (Álvaro et al., 2008c; Ferrante et al., 2010; Isidro et al., 2011). Modern and semi-dwarf cultivars of the intermediate period tended to produce more grains per spike than tall ones in environments with higher $WI_{(AM)}$ and $ETo_{(AM)}$. The observed improvement in the yield responsiveness was also mainly related to the changes in the performance of this yield component under favourable environments, as the stability indices for this trait showed a similar trend over periods to that estimated for yield. This is in agreement with the reported increase in the number of fertile florets per spike at anthesis that occurred as a pleiotropic effect of the *Rht-B1b* dwarfing allele (Álvaro et al., 2008b). Therefore, cultivars carrying this allele demonstrated a greater capacity to respond to favourable conditions for grain setting around and after anthesis and to produce more grains per spike. However, in terms of number of grains $spike^{-1}$, cultivars not carrying the dwarfing allele performed better than the semi-dwarf ones in environments with a long grain filling period expressed in thermal units. This difference in the adaptation pattern may be explained by the reduction of time to anthesis that resulted from breeding activities in durum wheat during the last century (Isidro et al., 2011; Motzo and Giunta, 2007; Royo et al., 2008), which would favour old cultivars in environments with longer grain filling periods.

The adaptation of modern and semi-dwarf cultivars of the intermediate period to environments with high $WI_{(AM)}$ was also expressed for thousand kernel weight. Although it has been reported that grain weight remained unchanged in durum wheat cultivars grown in different periods during the 20th century (Royo et al., 2007), semi-dwarf cultivars responded positively to water input during grain filling using it to raise the weight of their grains. This result can be explained by the observed increase in potential grain weight (Álvaro et al., 2008a), and grain filling duration (Royo et al., 2008) of modern durum cultivars in comparison with the old ones, and the improvement of the sink capacity caused by the introduction of dwarfing genes, which augmented the constraint to grain filling due to the source of assimilates (Álvaro et al., 2008c; Maydup et al., 2012; Sanchez-Bragado et al., 2014). In consequence, as semi-dwarf cultivars are more source-limited than tall ones and have a longer grain filling period, they could take advantage of favourable water input conditions after anthesis for the production of heavier grains.

The significant changes over time observed for three of the four stability indices calculated for yield are in agreement with the results of the AMMI analysis and indicate that breeding efforts during the last century resulted in an improvement in the response of the crop to high-input environments. The lower values of the regression slope (b) and $S^2{}_{xi}$ obtained for old cultivars in comparison with the modern ones indicate that the yield of the former hardly changed regardless of any variation in the mean yield of the environment, thus revealing that they are more stable than modern cultivars when yield stability is considered from the static point of view. This concept is usually associated with poor yields (Becker and Léon, 1988), as was the case of old cultivars, and is therefore undesirable for breeding purposes. However, a wide range for b and $S^2{}_{xi}$ was observed in modern cultivars, thus indicating that the yield of some of them ('Flavio' and 'Senadur') was as stable as that of some old cultivars, while others ('Astigi', 'Simeto' and 'Cirillo') showed a large response to environmental changes. On the other hand, the low values of the superiority measure of Lin and Bins (P_i) of modern cultivars indicate a short mean square distance between the performance of the cultivar and the maximum response observed across environments. Both results show a proportional increase in the performance of modern cultivars in response to the enhancement of the environmental conditions and, therefore, a trend towards a general adaptation and greater stability if this is considered a 'dynamic' concept (Becker and Léon, 1988). In consequence, as has been reported by previous studies conducted in Italy and Spain (De Vita and Maggio, 2006; De Vita et al., 2010; Royo et al., 2008) and involving yield data of 21 countries (Calderini and Slafer, 1998), modern cultivars not only out-yield their predecessors in relatively poor environments but also respond considerably better to environmental improvements.

Though no significant trend was observed for yield in the distance from the origin of the AMMI axes (v_i), intermediate cultivars tended to have the lowest value for this index. Among them, 'Mexa' and 'Trinakria' were the intermediate cultivars closest to the origin of the AMMI axes, thus indicating the similitude between their yield in each environment and the environmental yield, and therefore a wide adaptation to the environment. The two cultivars have a very different origin but played an important role in durum wheat cultivation and breeding in Spain and Italy, respectively. 'Mexa' is a semi-dwarf cultivar obtained by CIMMYT as a selection from the Mexican cultivar 'Mexicali C75', which was released in 1975 and has been widely cultivated around the world (Huerta-Espino et al., 2011), thanks to its broad adaptation. 'Trinakria' is a tall cultivar from a cross of 'Capeiti 8' that has been extensively used in the development of new Italian cultivars since its release (De Vita et al., 2010).

The only stability index that was significant for the number of spikes m^{-2} was the superiority measure (P_i), which strongly decreased from old to modern cultivars, suggesting a better performance of the latter in terms of spike number. The old Italian cultivar 'Balilla Falso' had the lowest value for this index, in agreement with this cultivar having the largest number of spikes m^{-2} across environments. This cultivar also had the lowest b and $S^2{}_{xi}$ values and the highest P_i value for the number of grains $spike^{-1}$, and was the cultivar with the lowest number of grains $spike^{-1}$ across environments, thus indicating a low grain number in all environments, and very negligible response to improvements in the environmental conditions. On the other hand, 'Cirillo', 'Flavio' and 'Astigi' responded to better environments by increasing their number of grains $spike^{-1}$.

No change over time was observed in the stability of grain weight, which is in agreement with the lack of increases in grain weight as a consequence of the introduction of improved cultivars during the 20th century (Royo et al., 2007).

5. Conclusions

The results of this study have shown that the different breeding strategies followed in Italy and Spain during the last century resulted in analogous adaptation and stability models in terms of yield and yield components. Though the improved cultivars in the two countries had different origins, among the ones analysed herein, those released after 1970 had the common presence of the *Rht-B1b* dwarfing allele, which explained the similarities in the adaptation pattern of cultivars from the two countries and is essential for understanding the changes over time in the C × E interaction of Mediterranean durum wheat cultivars.

Our results showed that cultivars carrying the *Rht-B1b* allele used the water available during grain filling more efficiently than the old tall ones, and this benefited the yield components formed after anthesis, i.e. number of grains $spike^{-1}$ and grain weight. The earlier flowering time of modern cultivars and their longer grain filling period, revealed by previous studies (Royo et al., 2008), are probably related to their better water use during grain filling. Although the number of grains $spike^{-1}$ had the highest genetic control of the three yield components, it also showed the largest C × E interaction and therefore the greatest response to environmental conditions.

Yield increases of modern cultivars have caused a loss of stability from the static viewpoint, but not from a dynamic viewpoint based on the superiority measure (P_i). The current study demonstrated that breeding activities conducted in Spain and Italy during the 20th century were successful not only in improving the durum wheat yield, but also in maintaining in some improved cultivars the levels of yield stability characteristic of the old cultivars.

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DISCUSIÓN GENERAL

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Introducción

La presente Memoria de Tesis Doctoral pretende aportar información útil para los programas de mejora de trigo a través del análisis de los cambios que ha causado la mejora genética en caracteres de importancia comercial y su expresión en diferentes condiciones ambientales. Para ello se han utilizado series históricas de genotipos representativas de las variedades cultivadas por los agricultores en distintas épocas. En los cuatro capítulos de que consta esta Memoria de Tesis Doctoral se abordan los siguientes aspectos: el efecto del gen de enanismo *Rht-B1b*, introducido en trigo duro durante la Revolución Verde, sobre el desarrollo radicular y su relación con la biomasa aérea y el rendimiento (Capítulo 1), la ganancia genética en las principales características de calidad del trigo duro (Capítulo 2), los factores ambientales que afectan a la expresión de la calidad del grano del trigo harinero (Capítulo 3) y la respuesta diferencial de los cultivares de trigo duro a las condiciones ambientales en lo referente a la producción (Capítulo 4).

El enfoque utilizado ha sido doble: el histórico, en el que se ha fijado la atención en la ganancia genética obtenida en caracteres de interés comercial y el impacto de la introducción de los genes de enanismo como hito en la historia de la mejora del trigo; y el medioambiental, en el que se ha abordado el estudio del efecto de las variables ambientales sobre la expresión fenotípica y cómo ésta ha sido modificada por la mejora.

Los estudios previos llevados a cabo por el grupo de investigación del Programa de Cultivos Extensivos del IRTA, en el que se ha realizado esta Tesis Doctoral, han permitido cuantificar la ganancia genética del rendimiento de los trigos duro y harinero a lo largo del tiempo. En trigo duro, la ganancia genética del rendimiento durante el siglo XX en España se estimó en 23,6 kg ha^{-1}año^{-1} (Royo *et al*., 2008), mientras que para el trigo harinero se estimó un incremento de 35 kg ha^{-1}año^{-1} (Sanchez-Garcia *et al*., 2013). A pesar de las diferencias observadas en la magnitud de la ganancia genética del rendimiento entre ambas especies, el componente más relacionado con la misma fue en ambos casos el número de granos por m^{-2} (Royo *et al*., 2008; Sanchez-Garcia *et al*., 2013).

Esta misma línea de investigación ha permitido comprobar que en el caso del trigo duro la mejora genética llevada a cabo durante el siglo XX dio lugar a un aumento del número de plantas por unidad de superficie, del número de espigas por planta, así como a un ciclo más corto hasta antesis, un aumento del índice de área foliar asociado a menor biomasa, un mayor número de flores fértiles en antesis y de granos por espiga, un incremento de la contribución al rendimiento de los carbohidratos solubles acumulados en la planta antes de antesis y un mayor índice de cosecha debido, en parte, a la introducción de los genes de enanismo (Royo *et al*., 2007; Álvaro *et al*., 2008a,b,c). Sin embargo, hasta ahora no se habían estudiado los efectos de la mejora genética sobre la biomasa radicular ni su relación con la biomasa aérea y la formación del rendimiento. Hasta la realización de esta Tesis Doctoral tampoco se habían estudiado los cambios producidos en la respuesta productiva de los genotipos al ambiente (interacción genotipo x ambiente o GE), así como las variables meteorológicas con una mayor influencia sobre dicha interacción.

Por lo que respecta al trigo harinero, estudios previos permitieron determinar que los aumentos de rendimiento se debieron principalmente a un mayor número de granos por espiga y de espigas por unidad de superficie, relacionados con una mayor fertilidad de los hijuelos, a la reducción de la altura de la planta y del ciclo hasta antesis y a un mayor índice de área foliar y duración de la misma (Sanchez-Garcia *et al*., 2013, 2015b). En lo referente a la calidad del grano el estudio concluyó que la mejora del trigo harinero disminuyó el contenido de proteína del grano, pero aumentó la calidad de la misma al mejorar la fuerza del gluten (*W*). Este incremento fue debido a la introducción en el germoplasma de subunidades de gluteninas de alto peso molecular (*High Molecular Weight Glutenin Subunits,* HMW-GS) que favorecen la fuerza del gluten, como la 1 y 2* en el locus *Glu-A1*, la 7+8 en el *Glu-B1* y la 5+10 en el *Glu-D1* (Sanchez-Garcia *et al*., 2015a). No se habían estudiado, sin embargo, la ganancia genética de los principales atributos de calidad para la producción de pasta de trigo duro ni la evolución a lo largo del tiempo de la composición de las subunidades de gluteninas de alto (*High Molecular Weight Glutenin subunits*, HMW-GS) y bajo peso molecular (*Low Molecular Weight Glutenin subunits,* LMW-GS).

Los efectos de la interacción GE en la producción y la calidad del grano del trigo harinero también fueron estudiados por el grupo con anterioridad, observándose que los primeros cultivares mejorados mostraron una adaptación más amplia que los tradicionales y una menor dependencia del número final de espigas y el peso del grano del agua disponible durante el llenado del grano (Sanchez-Garcia *et al*., 2012). En cambio, los cultivares tradicionales mostraron una adaptación específica a ambientes con menores temperaturas mínimas antes de espigado y alta humedad relativa durante el llenado del grano (Sanchez-Garcia *et al*., 2012). En el caso de la calidad de la harina, se comprobó que el ambiente tuvo mucho más efecto en la extensibilidad de la masa (*L)* que en la tenacidad de la misma (*P)* y su fuerza (*W),* mientras que la interacción GE supuso cerca del 28% de la varianza total para *L* (Sanchez-Garcia *et al*., 2015a). Además, los cultivares portadores del alelo que codifica para la subunidad 5+10 del locus *Glu-D1* estuvieron mejor adaptados a ambientes con un alto aporte hídrico durante el ciclo de cultivo y donde los granos presentaron un bajo contenido de proteína y una baja *L* en la masa (Sanchez-Garcia *et al*., 2015a). Los estudios realizados hasta la fecha han permitido cuantificar el efecto positivo causado por la mejora sobre el rendimiento y la calidad del grano. Sin embargo, diversos estudios demuestran que ambas características suelen estar negativamente asociadas. Esta relación se estudió en el tercer Capítulo de esta Memoria de Tesis Doctoral con el fin de proporcionar información útil que permita obtener cultivares de calidad altamente productivos y responder así a los requerimientos del sector.

Durante la ejecución de la presente Tesis Doctoral el doctorando llevó a cabo una estancia de 3 meses en el CIMMYT de México para realizar parte de los experimentos y determinaciones que componen la misma.

Sobre el germoplasma utilizado

La consecución de los objetivos planteados en esta Memoria de Tesis Doctoral requirió el uso de dos series históricas de cultivares, una de trigo duro y otra de trigo harinero, que se utilizaron en experimentos de campo en diferentes ambientes de España. En el caso de la serie histórica de trigo duro también se llevaron a cabo ensayos en invernadero en el centro de investigación de Toluca (México) del Centro Internacional de Mejora de Maíz y Trigo (CIMMYT) con el fin de poder estudiar los cambios producidos por la mejora genética en la biomasa radicular según lo expuesto en el Capítulo 1.

El material vegetal empleado en los Capítulos 1, 2 y 4 consistió en una serie histórica de trigo duro compuesta por 24 cultivares italianos y españoles registrados en diferentes épocas o períodos a lo largo del siglo pasado. Para dar respuesta a los objetivos 2b, 3b y 3c de la presente Memoria de Tesis Doctoral, se reunió un conjunto de 20 cultivares comerciales de trigo harinero, representativos de los más cultivados en España y que fueron registrados en el país entre 1974 y 2009. Estos cultivares son a su vez representativos de los cuatro grupos de calidad adecuados para consumo humano definidos en la clasificación oficial española de calidad de los cultivares de trigo (RD 1615/2010 del 7 de diciembre de 2010 y el RD 190/2013 del 15 de marzo de 2013). A diferencia del conjunto de trigo duro, la selección de cultivares de trigo harinero no tuvo en cuenta el país de origen de los mismos.

La gran variabilidad encontrada en variedades modernas de trigo duro en lo referente a biomasa aérea y radicular (Capítulo 1) apoya la conclusión de Martos *et al*. (2005) de que la mejora genética no ha reducido la variabilidad genética. Esto parece cierto para algunos caracteres de tipo adaptativo, pero en otros casos, como es el de los genes que regulan la calidad del grano, la reducción ha sido evidente tal como ha sido demostrado por Nazco *et al*. (2014a,b).

Efecto del alelo Rht-B1b sobre la biomasa aérea y radicular del trigo duro y sus consecuencias sobre la formación del rendimiento

El efecto de los genes de enanismo (llamados también 'genes de la Revolución Verde') sobre la altura de la planta y la distribución de biomasa en la misma ha sido ampliamente estudiado, tanto en trigo harinero como duro (Austin *et al*., 1980; Berger *et al*., 1990; Hay, 1995; Royo *et al*., 2007; Graybosch and Peterson, 2010; Sanchez-Garcia *et al*. 2015b). Sin embargo, probablemente por la laboriosidad que entraña, no existían hasta la fecha estudios que evaluaran el efecto de dichos genes sobre la biomasa radicular utilizando una serie histórica de cultivares. El primer capítulo de esta Memoria de Tesis Doctoral aborda dicho estudio, llevado a cabo en colaboración con el CIMMYT, mediante el cultivo en tubos en condiciones de invernadero, utilizando para ello la metodología descrita por Cook *et al*. (1997) y Blum (2011).

La introducción del alelo *Rht-B1b*, causante del enanismo de los trigos, disminuyó la biomasa aérea y radicular de la plantas de los cultivares evaluados, aunque el efecto sobre la segunda fue un 21,5% mayor, lo que resultó en una disminución del 29% en la relación biomasa radicular/biomasa aérea. Esto sugiere que la mejora ha revertido parte de los recursos para la producción de biomasa radicular en biomasa aérea y formación del rendimiento. El efecto del alelo *Rht-B1b* sobre la reducción de la biomasa radicular se observó de forma consistente a lo largo de toda la raíz, si bien el decremento fue mayor en la zona radicular más profunda, donde la biomasa radicular se redujo un 36% con respecto a los cultivares altos portadores del alelo *Rht-B1a*. El hecho de que el alelo *Rht-B1b* reduzca en términos relativos mucho más la biomasa radicular que la aérea, aun siendo las variedades semienanas mucho más productivas que las tradicionales, sugiere que uno de los efectos de la mejora genética ha sido incrementar la eficiencia en la absorción (*uptake efficiency*) y/o utilización (*utilization efficiency*) de nutrientes, como también lo sugieren los resultados sobre adaptación que aparecen en el Capítulo 4.

Con el fin de determinar las consecuencias de la introducción del alelo *Rht-B1b* sobre la formación del rendimiento a través de su efecto sobre la producción y distribución de biomasa de la planta, los resultados obtenidos en condiciones de invernadero se relacionaron con los obtenidos previamente en ensayos de campo. Para estudiar la validez de estas asociaciones en un primer paso se determinó la relación existente entre la biomasa aérea en antesis y rendimiento en condiciones de campo. Los resultados mostraron que el aumento del rendimiento del trigo duro ha ido asociado a una disminución de la biomasa del cultivo en antesis y a un incremento del índice de cosecha (IC). Sin embargo, al considerar variedades altas y semienanas por separado, esta relación negativa fue tan solo significativa en los cultivares altos, lo cual sugiere que estos cultivares podrían haber producido más tallos infértiles o mayor número de hojas con menor capacidad para la producción de asimilados en condiciones de cultivo, que no supondría un incremento efectivo del rendimiento ni del índice de cosecha.

A diferencia de lo obtenido en el estudio previo de Villegas *et al*. (2001), los resultados mostraron que la disminución de biomasa aérea causada por la introducción del alelo *Rht-B1b* no tuvo ningún efecto sobre el rendimiento ya que el rendimiento de las variedades semienanas dependió de su capacidad para acumular fotosintatos en el grano, independientemente de la biomasa aérea acumulada en el momento de la floración. Además, en los trigos semienanos utilizados en este estudio no se detectó competencia alguna entre la biomasa aérea y el número de espigas, granos por espiga ni peso del grano, lo cual confirma que el rendimiento se forma en estos trigos de forma independiente a la biomasa aérea existente en antesis. Estos resultados están de acuerdo con el mayor índice de cosecha y eficiencia en la translocación de asimilados acumulados antes de antesis al grano en formación de las variedades semienanas (Royo *et al*., 2007, 2008; Álvaro *et al*. 2008 b,c). Los cambios debidos a la mejora en la biomasa en madurez del cultivo a lo largo del siglo XX han sido estudiados recientemente tanto en trigo harinero (Brancourt-Hulmel *et al*., 2003) como en duro (Royo *et al*., 2007). A pesar de que no se ha detectado un aumento o descenso significativo de la biomasa en madurez a lo largo del tiempo en ninguna de estas especies, sí que se ha observado una mejor distribución de ésta, consiguiéndose así mayores proporciones de grano y por tanto un aumento del IC.

La consistente relación obtenida entre las biomasas aérea y radicular en antesis en condiciones de cultivo en tubos en invernadero, segundo paso de este estudio, justificó el analizar la relación entre la biomasa aérea y radicular medida en invernadero y la distribución de biomasa y el rendimiento en los ensayos de campo. Este tercer paso demostró que futuros aumentos de la biomasa radicular en variedades semienanas no supondrían en sí mismos una mejora del rendimiento ni del índice de cosecha en trigo duro.

La disminución de la biomasa radicular de los cultivares modernos con respecto a los antiguos no disminuyó la eficiencia en el uso de los recursos, tal y como se desprende de los resultados del Capítulo 4, que se comentan más adelante. En el mismo se observa que los cultivares modernos muestran una mayor capacidad para aprovechar el agua disponible durante el llenado del grano y obtener así mayores rendimientos. Estudios previos han sugerido que estos cultivares podrían incorporar características radiculares como una mayor eficiencia en la absorción de nitrógeno y agua del suelo (King *et al*., 2003), mayor capacidad de penetración de las raíces (Kubo *et al*., 2005; Lilley y Kirkegaard, 2011) o un mejor aprovechamiento del agua disponible en el suelo (Wasson *et al*., 2012), que han demostrado tener un efecto significativo sobre la adaptación del trigo al ambiente y su eficiencia en el uso de los recursos. Los resultados obtenidos en el marco de esta Tesis Doctoral sugieren cierta independencia entre estas características y la biomasa radicular.

Se ha sugerido que características radiculares como el ángulo de la raíz seminal están relacionados con el rendimiento en ambientes de disponibilidad hídrica variable (Canè *et al*., 2014). Con el fin de determinar el papel de la raíz en la mejora del rendimiento sería necesario estudiar ésta y otras características radiculares que permitan complementar la información obtenida en este estudio y así establecer futuras estrategias de mejora.

Cambios causados por la mejora sobre la calidad del grano de trigo

Ganancia genética en la calidad del trigo duro para la elaboración de pasta

Tal y como se deriva de los resultados del Capítulo 2, durante la segunda mitad del siglo XX se confirió una importancia creciente a la mejora de la calidad del trigo duro, con la finalidad de garantizar la óptima adecuación de la sémola para la elaboración de pastas alimenticias y derivados, así como de otros productos de interés para los países de la cuenca Mediterránea. A pesar de ello, no fue hasta el año 2003 cuando la Unión Europea estableció el Índice de Calidad del Trigo Duro (Regulación de la Comisión Europea núm. 2237/2003, 23 de diciembre de 2003) con el fin de poder clasificar los cultivares de acuerdo a estándares de calidad. El índice de calidad de la UE para trigo duro (QI) considera el valor ponderado del contenido de proteína del grano, la fuerza del gluten, el color amarillo de la sémola y el peso del grano respecto a testigos de calidad oficiales (Royo y Briceño-Félix, 2011). El estudio que constituye el Capítulo 2 de esta Memoria de Tesis Doctoral pone de manifiesto que la mejora de trigo duro llevada a cabo tanto en Italia como en España, grandes productores de trigo duro de la cuenca Mediterránea, afectó a todas las características que determinan este índice. El contenido en proteína, que constituye el 40% del valor del QI disminuyó un 8% en los trigos cultivados en España y un 11,3% en los Italianos, aunque la calidad del gluten se vio incrementada debido a una mayor presencia de subunidades más favorables para la fuerza del gluten en los loci de gluteninas LMW, como la 6, la 2+4+15+19 y la 12 de los loci *Glu-A3*, *Glu-B3* y *Glu-B2,* respectivamente. A pesar de la disminución de la cantidad de proteína, el contenido de proteína por hectárea aumentó debido al incremento del rendimiento que se ha registrado en los cultivares obtenidos en ambos países a lo largo del siglo XX, que han puesto de manifiesto estudios anteriores (Royo *et al*., 2008).

La mejora no tan sólo ha incrementado la fuerza del gluten, cuyo peso relativo en el QI es del 30%, sino también la estabilidad de este carácter, garantizando así una respuesta más uniforme a ambientes contrastantes y una mejor adecuación a los requisitos de la industria de obtención de pasta y derivados, que requieren unas características físico-químicas y organolépticas constantes en el tiempo y entre lotes. No ha ocurrido lo mismo con el contenido en proteína, atributo que depende en gran medida de los factores ambientales (Nazco *et al*., 2012) y cuya optimización debe ser enfocada no tan sólo desde el punto de vista genético, sino también del manejo del cultivo.

El tercer componente del QI, con un peso relativo del 20% en el mismo, es el índice de amarillo de la sémola, que aumentó tanto en España como en Italia, debido en gran medida a su alta heredabilidad (Schulthess *et al*., 2013). Sin embargo, el peso específico, cuarto y último componente del QI con una importancia relativa en el mismo del 10%, no cambió de manera apreciable, tal y como se ha podido comprobar también con el peso de mil granos en estudios previos utilizando la misma serie histórica (Royo *et al*., 2007). Por esta razón, se puede afirmar que la disminución del contenido de proteína del grano no ha sido debido a un efecto de dilución por la variación del tamaño del grano, sino por un mayor número de granos por unidad de superficie. Como consecuencia de las variaciones producidas en los distintos componentes del QI, se estimó que éste aumentó un 7,13% en las variedades españolas y un 5,4% en las italianas.

Gluteninas de bajo (LMW-GS) y alto (HMW-GS) peso molecular y su efecto sobre la calidad del grano

Las gluteninas LMW son las que ejercen un mayor efecto sobre la calidad del gluten del trigo duro (Ruiz *et al*., 2005). Tal y como se demuestra en el Capítulo 2 de la presente Memoria de Tesis Doctoral, la composición alélica de los loci que controlan su síntesis ha cambiado a lo largo del siglo XX, fijándose en los cultivares modernos algunas de las subunidades más favorables como la 6 y la 6+11 en el locus *Glu-A3* y la 2+4+15+19 en el *Glu-B3*. La selección por este tipo de subunidades ha reducido la variabilidad presente en el germoplasma moderno respecto al tradicional, ya que los alelos observados en los loci *Glu-A3* y *Glu-B3* pasaron de 3 a 2 y de 4 a 2, respectivamente. El hecho de que la combinación LMW-2_{aaa} (unidades 6 en el locus *Glu-A3*, 2+4+15+19 en el *Glu-B3* y 12 en el *Glu-B2*) aparezca como muy estable en los trigos mejorados italianos puede ser una limitación a futuras mejoras de la calidad de los trigos duros.

En el caso de las subunidades de gluteninas de alto peso molecular (HMW), se observó la mayor variabilidad en el locus *Glu-B1*, con un total de 7 subunidades distintas, estando 5 de ellas presentes en el germoplasma moderno de Italia y de España. Este resultado sugiere que la variabilidad de las gluteninas HMW no se ha visto tan afectada, posiblemente, porque su contribución a la fuerza del gluten no es tan importante como en el caso de las LMW, por lo que la mejora no ha puesto tanto énfasis en su selección y muchas de las subunidades presentes en los cultivares antiguos como la 20 o la 6+8 están todavía presentes en el germoplasma moderno de ambos países.

En el Capítulo 3 se estudió la calidad del grano y de la harina de 20 cultivares élite de trigo harinero en España. Se observó un gran efecto de las gluteninas HMW sobre la fuerza del gluten (*W*), siendo las subunidades 1, 7^{oe}+8 y 5+10 en los loci *Glu-A1*, *Glu-B1* y *Glu-D1* respectivamente, las que mostraron un mayor efecto positivo sobre la misma. En particular, la combinación 2*, 7^{oe}+8 y 5+10 en los loci *Glu-A1*, *Glu-B1* y *Glu-D1* respectivamente, fue la mejor al incrementar en 150 unidades los valores de *W* de la masa. Este resultado sugiere que existe cierta interacción entre las subunidades de gluteninas y que puede ser más efectiva la selección de combinaciones que de subunidades. A pesar de que recientemente se han identificado otros genes y/o QTLs relacionados con la calidad panadera del trigo como el *wbm* (Furtado *et al*., 2015), la mejora vegetal puede ser muy efectiva para incrementar la *W* de los nuevos cultivares de trigo harinero, dado que el efecto del genotipo supuso el 83% de la suma de cuadrados del ANOVA para *W*, lo que confirma el reducido efecto ambiental sobre este carácter.

La composición de las gluteninas LMW en trigo duro y de las HMW en trigo harinero está altamente relacionada con la calidad del gluten, y el perfil observado en cada especie a lo largo del tiempo ha ido variando como consecuencia de la mejora genética. Al analizar los loci comunes estudiados en ambas especies, como son el *Glu-A1* y el *Glu-B1*, se observa que el trigo duro presentó una subunidad más que el trigo harinero en los dos casos. Sin embargo, a pesar de que en el trigo duro se estudiaron 5 loci y en trigo harinero solo 3, se observó un mayor número de combinaciones de subunidades en trigo harinero que en trigo duro. Esto sugiere que, a pesar de que en trigo duro se estudiaron cultivares aparecidos a lo largo del siglo XX y en trigo harinero solo los que aparecieron en el mercado español a partir del año 1974, el acervo genético de subunidades de gluteninas en los cultivares estudiados de trigo harinero fue mayor que en trigo duro. Este hecho puede ser debido a que los cultivares de trigo harinero estudiados no fueron de procedencia exclusiva de los programas de mejora autóctonos, mientras que los cultivares de trigo duro procedieron solo de España e Italia, los cuales solo incluyeron una pequeña parte de las subunidades de gluteninas presentes en los cultivares tradicionales de trigo duro de la cuenca Mediterránea (Nazco *et al*., 2014a,b).

Con el fin de evitar la pérdida de diversidad de subunidades de gluteninas en el germoplasma de trigo duro y harinero de la cuenca Mediterránea (Ruiz *et al*., 2002; Moragues *et al*., 2006; Aguiriano *et al*., 2008; Giraldo *et al*., 2010; Nazco *et al*., 2014a,b) y así posibilitar la introducción de subunidades que supongan una mejora de las características del gluten, la mejora deberá considerar el potencial de las variedades tradicionales como recursos fitogenéticos capaces de aportar gran variabilidad a los programas de mejora. Además, la mejora del trigo en el futuro deberá hacer frente a nuevos retos debido al progresivo aumento de las temperaturas y la escasez de lluvias que podrían venir asociadas al cambio climático, el cual va a tener un efecto significativo tanto en la calidad como el rendimiento de los cultivos (Giannakopoulos *et al*., 2009; García-Mozo *et al*., 2011; Olesen *et al*., 2011; Trnka *et al*., 2014).

Efecto del ambiente y su interacción con el genotipo sobre la producción y la calidad del trigo

Efecto sobre el rendimiento y sus componentes y sobre la estrategia de adaptación del trigo duro

En el Capítulo 4 de esta memoria de Tesis Doctoral se han estudiado los efectos de la interacción GE sobre el rendimiento y sus componentes, a saber, número de espigas por m^{-2}, número de granos por espiga y peso del grano, en base a ensayos de campo llevados a cabo en 13 ambientes con una diferencia de rendimiento de 5245 kg ha^{-1} entre los de mayor y menor rendimiento. Los resultados mostraron que los efectos del genotipo y del ambiente representaron el 10% y el 76% de la variabilidad observada para el rendimiento, respectivamente, mientras que la interacción de ambos factores explicó el 9%. En este caso el efecto del genotipo resultó ser aproximadamente la mitad y el del ambiente un 34% superior a los obtenidos en un estudio similar que utilizó una serie histórica de variedades de trigo harinero (Sanchez-Garcia *et al*., 2012). El efecto del ambiente sobre los componentes del rendimiento osciló entre el 54% y el 71% (entre el 24% y el 37% en el caso del citado estudio de trigo harinero) y el del genotipo entre el 10 y el 18% (entre el 18% y el 44% en el caso del trigo harinero), lo que confirma la mayor sensibilidad del trigo duro a los efectos ambientales cuando se compara con el trigo harinero.

Estudios previos han demostrado que en Italia y España se siguieron estrategias divergentes a lo largo del siglo XX para la introducción de variedades de trigo duro mejoradas (Martos *et al*., 2005), que resultaron en cambios genéticos de distinta magnitud en algunos casos (Álvaro *et al*. 2008 a,b,c). Mientras que en Italia la mejora utilizó variedades tradicionales del país en la construcción del acervo genético de las variedades modernas, en España éstas procedieron de la mejora llevada a cabo en otros países. A pesar de ello, el estudio llevado a cabo en el Capítulo 4 de la presente Memoria de Tesis Doctoral demuestra que en ambos países las variedades utilizaron mecanismos similares de adaptación al ambiente y también los mismos modelos de estabilidad en lo referente a rendimiento y sus componentes.

La respuesta del rendimiento estuvo asociada a los cambios observados en el número de granos por espiga, especialmente para los cultivares portadores del alelo *Rht-B1b,* que demostraron una mayor capacidad para aprovechar el agua disponible en el suelo durante el llenado del grano. El biplot de los dos primeros ejes del análisis AMMI para el rendimiento y el peso del grano separó los cultivares modernos portadores del alelo *Rht-B1b* de los antiguos portadores del alelo salvaje *Rht-B1a.* Para ambas variables, la disponibilidad hídrica entre antesis y madurez fisiológica explicó parte de la interacción GE, mostrando los cultivares semienanos una mayor respuesta positiva a este recurso. Esta mayor eficiencia en la captación y/o uso de los recursos hídricos, explica el descenso de la estabilidad estática y el aumento de la estabilidad dinámica de los cultivares de trigo duro a lo largo del siglo XX. Los resultados obtenidos en la presente Tesis Doctoral están de acuerdo con los derivados de estudios previos llevados a cabo con germoplasma italiano aparecido a lo largo del siglo XX (De Vita *et al*., 2010) y con lo observado por Sanchez-Garcia *et al*. (2012) en trigo harinero. Los cultivares modernos mostraron una adaptación más amplia que los tradicionales, los cuales fueron más favorecidos por ambientes con bajas temperaturas mínimas antes del espigado y alta humedad relativa durante el llenado del grano.

Esta mejora en la eficiencia en el uso de los recursos después de la antesis resultó en un incremento en el número de granos por espiga y en el peso del grano y puede estar relacionada con el incremento de la fertilidad floral y, en consecuencia, del número de granos por espiga observados en los trigos a lo largo del siglo pasado (Álvaro *et al*., 2008c). Sin embargo, debido a la insensibilidad a las giberelinas causada por el alelo *Rht-B1b*, el peso del grano de las variedades semienanas no es mayor que el de las altas como se deriva de este estudio y otros previos (Royo *et al*., 2007). La optimización de la relación entre la fuente y el sumidero resulta esencial para maximizar la expresión del rendimiento (Reynolds *et al*., 2012). Trabajos previos han concluido que la mejora ha incrementado la limitación del rendimiento por el tamaño de la fuente de asimilados para el llenado del grano (Álvaro *et al*. 2008c), sin bien en condiciones mediterráneas se manifiesta una co-limitación fuente-sumidero por lo que es posible alcanzar futuros avances mediante la modificación de este último.

Interdependencia entre el rendimiento y la calidad del trigo

La norma española de calidad de los trigos (RD1615/2010 del 7 Diciembre 2010 y RD 190/2013 del 15 de marzo de 2013), establece 4 grupos de variedades de trigo harinero para consumo humano de acuerdo a la calidad de su grano en base a los valores de fuerza del gluten (*W*), contenido de proteína, relación entre tenacidad y extensibilidad de la masa (*P/L*), índice de caída y degradación proteolítica. Los cultivares clasificados como de alta calidad (HQ) en el Capítulo 3 corresponden a los grupos 1 y 2 de la citada norma, mientras que los de baja calidad (LQ) pertenecen a los grupos 3 y 4. El análisis de componentes principales que se llevó a cabo usando datos de rendimiento, días hasta antesis y calidad no permitió la separación de los cultivares en los 4 grupos de la norma de calidad, separando únicamente dos grupos. Ello pone de manifiesto la importancia del estudio de los factores ambientales determinantes de la calidad y la respuesta de los diferentes cultivares a los mismos.

Está generalmente aceptado que los aumentos de contenido de proteína del grano de trigo tienen lugar a expensas del rendimiento (Jenner *et al*., 1991), ya que numerosos estudios han demostrado una relación negativa entre ambas variables (Peterson *et al*., 1992; Simmonds *et al*., 1995, Boggini *et al*., 1997; Pleijel *et al*., 1999; Oury y Godin, 2007). Los resultados del Capítulo 3, basados en ensayos de campo llevados a cabo en 8 ambientes de medio y alto rendimiento potencial, corroboran la existencia de una relación negativa entre el rendimiento (asociado a un ciclo largo hasta antesis) y la calidad del grano en el caso del trigo harinero. Sin embargo, al analizar la relación rendimiento-proteína por separado para cultivares clasificados como de alta calidad (grupos 1 y 2) y baja calidad (grupos 3 y 4), según la clasificación que establece el RD 1615/2010 del 7 de diciembre de 2010 y el RD 190/2013 del 15 de marzo de 2013, se constató que esta relación tan solo fue significativa en el caso de los cultivares de baja calidad, que mostraron un rango de rendimiento más amplio (7697 – 9354 kg ha^{-1}) que los cultivares de alta calidad (7437 – 8603 kg ha^{-1}). Este resultado sugiere que el rendimiento y el contenido de proteína del grano no siempre están negativamente relacionados y determina las condiciones de excepcionalidad a esta regla general. En esta misma línea, un estudio llevado a cabo hace unos años sugirió que en ambientes favorables pueden alcanzarse elevados rendimientos del trigo duro con mínimas reducciones del contenido de proteína (Rharrabti *et al*., 2001). Los resultados del Capítulo 3 demuestran que en algunos cultivares de trigo harinero (como 'Paledor' o 'Berdun') el rendimiento y el contenido en proteína están bien balanceados, ya que dieron lugar a rendimientos por encima de 8600 kg ha^{-1} y contenidos de proteína de alrededor del 13,1%.

En los cultivares de alta calidad, un mayor rendimiento estuvo asociado a una menor extensibilidad de la masa (*L*), dando así lugar a masas con escasa capacidad para producir un volumen de pan adecuado tras la fermentación. Los caracteres de calidad relacionados negativamente con el rendimiento en la colección estudiada fueron la *W*, el índice de caída y el contenido de proteína del grano. La selección de las mejores combinaciones de subunidades de gluteninas de alto peso molecular (HMW) resultó en un aumento de *W* independientemente del rendimiento de la variedad, lo cual puso de manifiesto la importancia de la correcta elección de estas combinaciones en los parentales de los programas de mejora para incrementar la calidad del grano. Los resultados también mostraron que el efecto conjunto del ambiente y de la interacción GE en el caso de *W* y *P* explicaron más del 15% en ambos casos, lo que indica que estas variables, a pesar de estar principalmente influenciadas por el genotipo (que explicó el 83% y 84% de la variabilidad para *W* y *P*, respectivamente), se pueden ver perjudicadas en determinados ambientes de manera que las variedades no alcancen los niveles de los criterios del grupo de calidad en la que han sido clasificadas. El efecto del ambiente sobre la *W*, podría ser debido a la relación positiva observada entre la fuerza del gluten y el contenido de proteína, significativa tanto en los cultivares de alta como de baja calidad. Sin embargo, se sabe que la fuerza del gluten está limitada por el genotipo (Sanchez-Garcia *et al*., 2013), de manera que, con un mismo contenido en proteína, la *W* de dos cultivares puede diferir en más de 200 J 10^{-4}. Para el resto de variables estudiadas tales como el peso de mil granos, el peso específico, el contenido de proteína del grano y el índice de caída, se observó que el efecto del ambiente fue mayor que el del genotipo.

El contenido de proteína del grano estuvo relacionado positivamente con la *L* en los cultivares de baja calidad (LQ, grupos 3 y 4), mayoritariamente portadores de la subunidad 2+12 en el locus *Glu-D1*. Cornish *et al*. (2001) identificaron dicha subunidad como favorable para aumentar la extensibilidad de la masa, si bien los cultivares portadores alcanzaron menores valores de *L* que los cultivares de alta calidad (HQ, grupos 1 y 2) que fueron monomórficos en el locus *Glu-D1* para la subunidad 5+10. Esto indicaría que para los cultivares LQ el efecto del ambiente sobre la *L* viene determinado por el contenido de proteína, por lo que cabría esperar que en ambientes más favorables para el contenido de proteína de grano la *L* de la masa fuera más elevada para este grupo.

Efecto de las variables meteorológicas sobre el rendimiento y la calidad de los trigos harinero y duro

La interacción GE para el rendimiento en trigo harinero estudiada en el Capítulo 3 explicó el 40% de la suma de cuadrados del modelo de ANOVA, valor muy superior al obtenido en la serie histórica de cultivares de trigo duro en que representó el 9% de la variabilidad total. Los resultados mostraron una relación negativa entre la evapotranspiración de referencia entre siembra y antesis [$ETo_{(SA)}$] y el rendimiento del grano. La reducción del rendimiento en ambientes con alta $ETo_{(SA)}$ se debió principalmente al efecto que esta covariable ejerció sobre el peso del grano. Las variables ambientales que tuvieron mayor efecto sobre la calidad del trigo harinero fueron la $ETo_{(SA)}$ y la temperatura máxima entre antesis y madurez fisiológica [$T_{max(AM)}$]. Es conocido que altas temperaturas durante el llenado del grano reducen su duración, el peso del grano y el rendimiento del trigo (Wardlaw y Wrigley, 1994). Se sabe también que temperaturas superiores a 30°C después de antesis afectan a la síntesis de almidón (Jenner, 1994; Panozzo y Eagles, 1998).

El contenido de proteína del grano y el peso de mil granos, las variables más afectadas por el ambiente, mostraron un comportamiento antagónico ante $ETo_{(SA)}$ y $T_{max(AM)}$, ya que en los ambientes con mayores valores de ambas variables se obtuvieron los menores pesos del grano, en consecuencia aumentando en los mismos el contenido relativo de proteína del mismo. Esta relación negativa pudo deberse a un efecto de dilución de los componentes nitrogenados del grano cuando la deposición de carbohidratos aumenta, dando lugar a granos de mayor peso (Cox *et al*. ,1986; Jenner *et al*., 1991).

El 61% de la variabilidad para el peso específico del grano se debió a los efectos ambientales. A pesar de ello, tanto los cultivares HQ y LQ obtuvieron pesos específicos del grano por encima de 82 kg hl^{-1}, si bien los cultivares HQ obtuvieron granos de mayor tamaño y peso específico además de un mayor contenido de proteína. Estos resultados sugieren que el llenado del grano se pudo realizar correctamente en ambos grupos de cultivares independientemente de la calidad de la harina y del rendimiento obtenido, indicando una buena adaptación de ambos grupos de cultivares a los ambientes en los que fueron evaluados en el estudio.

Las variables ambientales responsables de la interacción GE para el rendimiento de los cultivares de trigo duro estudiados en el Capítulo 4 fueron la cantidad de agua disponible durante el llenado del grano [$WI_{(AM)}$] y la $ETo_{(SA)}$, mientras que para los componentes del rendimiento se observó un efecto de la humedad relativa entre siembra y antesis [$RH_{(SA)}$] y entre antesis y madurez [$RH_{(AM)}$] y del tiempo térmico entre antesis y madurez [$TT_{(AM)}$]. Los cultivares portadores del alelo *Rht-B1b* utilizaron mejor el agua disponible tras la antesis que los portadores del alelo *Rht-B1a,* lo cual benefició a los componentes del rendimiento que se establecen tras la misma, a saber, el peso del grano y el número de granos por espiga. El efecto del ambiente y de la interacción GE fue mayor para el número de granos por espiga que para el rendimiento y el resto de sus componentes y por lo que el número de granos por espiga mostró una mayor respuesta a las variables ambientales estudiadas. Sin embargo, al analizar el rendimiento de los cultivares portadores del alelo *Rht-B1a* se comprobó que éstos se adaptaron mejor a los ambientes con alta $ETo_{(SA)}$ y con un bajo aporte hídrico tras la antesis, aunque no dio lugar a una mejora en el rendimiento respecto a los cultivares portadores del alelo *Rht-B1b*. El componente del rendimiento que mejor explicó los aumentos de rendimiento fue el número de granos por unidad de superficie, en la línea de lo reportado por De Vita *et al*. (2007) y Peltonen-Sainio *et al*. (2007), cuyos valores más altos fueron obtenidos en los cultivares modernos italianos y españoles.

Como se ha visto, tanto en el caso de los cultivares de trigo harinero como los pertenecientes al grupo de los modernos en trigo duro, la evapotranspiración de referencia entre siembra y antesis [$ETo_{(SA)}$] fue la covariable que más afectó al rendimiento antes de la floración, y lo hizo de forma negativa. La ETo se define como la cantidad de agua que se puede perder a la atmosfera debido a los procesos de evaporación del agua en una superficie y la transpiración del agua de los tejidos vegetales, y su cálculo resulta sencillo ya que solo son necesarios datos meteorológicos como la radiación, la temperatura del aire, la humedad y la velocidad del viento (Allen *et al*., 1998). Los resultados obtenidos en esta Tesis Doctoral ponen de manifiesto el valor de esta variable para estimar el estrés del trigo en condiciones mediterráneas y demuestran la necesidad de seleccionar en los programas de mejora genotipos de trigo adaptados a la sequía para maximizar su rendimiento en ambientes de tipo mediterráneo.

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CONCLUSIONES

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1. El efecto del alelo *Rht-B1b*, causante del enanismo en trigo duro, sobre la biomasa del cultivo en antesis es mayor en términos relativos sobre la biomasa radicular que sobre la biomasa aérea.

2. En variedades semienananas el rendimiento y su formación son independientes de la biomasa aérea en antesis.

3. Un incremento de la biomasa radicular en variedades semienanas no implicaría una mejora del rendimiento ni del índice de cosecha en trigo duro.

4. La calidad global de los trigos duros españoles e italianos aumentó significativamente a lo largo del siglo XX. El índice de calidad de la UE para trigo duro creció un 6% debido fundamentalmente a los incrementos en la fuerza del gluten (30%) y el color amarillo de la sémola (10%), que compensaron una disminución del 10% en el contenido de proteína del grano.

5. En trigo duro la mejora en la fuerza del gluten se debió a la progresiva incorporación en variedades modernas de subunidades específicas de gluteninas de bajo peso molecular, particularmente la combinación LMW-GS_{aaa}, formada por las subunidades 6 en el locus *Glu-A3*, 2+4+15+19 en el *Glu-B3* y 12 en el *Glu-B2*.

6. En trigo harinero las subunidades de gluteninas de alto peso molecular con un mayor efecto positivo sobre la fuerza del gluten son la 1 y 2* en el locus *Glu-A1*, la 7^{oe}+8 y la 17+18 en el *Glu-B1* y la 5+10 en el *Glu-D1*.

7. Las variedades semienanas de trigo duro, portadoras del alelo *Rht-B1b*, muestran una mayor capacidad de respuesta a la disponibilidad de agua durante el llenado del grano que las variedades tradicionales.

8. La mejora del rendimiento a lo largo del siglo XX causó una pérdida de estabilidad considerada desde una perspectiva estática, pero no desde el punto de vista dinámico.

9. Las variedades de trigo harinero de alta calidad (grupos 1 y 2) tienden a tener un ciclo más corto hasta antesis y un menor rendimiento que las de baja calidad (grupos 3 y 4) en ambientes de medio y alto potencial productivo.

10. Las variables ambientales que tuvieron mayor efecto sobre el rendimiento y la calidad del trigo harinero fueron la evapotranspiración de referencia antes de antesis y la temperatura máxima entre antesis y madurez fisiológica.

11. Las variables ambientales que más afectaron al rendimiento del trigo duro fueron la evapotranspiración de referencia antes de antesis y la disponibilidad hídrica durante el llenado del grano.

12. Las variedades modernas son más eficientes en el uso de recursos, tanto en la captación/utilización de nutrientes absorbidos a través de las raíces como en el uso del agua tras la antesis.

CONCLUSIONS

1. The *Rht-B1b* allele, which causes dwarfism in durum wheat, has a greater effect on root biomass than on aerial biomass at anthesis when assessed in relative terms.

2. In semidwarf varieties, the yield and its formation is independent of aerial biomass at anthesis.

3. An increase of root biomass in semidwarf varieties would not imply an improvement of yield or harvest index in durum wheat.

4. The global quality of Spanish and Italian durum wheats significantly increased during the 20th century. The EU quality index of durum wheat increased a 6% due to improvements of gluten strength (30%) and semolina yellow index (10%), which compensated the 10% decrease in grain protein content.

5. The improvement of durum wheat gluten strength was due to the progressive incorporation of specific low molecular weight glutenin subunits in modern varieties, such as the LMW-GS$_{aaa}$ combination, which is composed by the 6 subunit at the *Glu-A3* locus, the 2+4+15+19 at the *Glu-B3* locus and the 12 at *Glu-B2* locus.

6. In bread wheat, the high molecular weight glutenin subunits with the greatest effect on gluten strength are the 1 and 2* at the *Glu-A1* locus, 7^{oe}+8 and 17+18 at *Glu-B1* and 5+10 at the *Glu-D1*.

7. The semidwarf durum wheat varieties, which carry the *Rht-B1b* allele, showed a better response capacity to water availability during grain filling than landraces.

8. The yield improvement during the 20th century caused a loss of stability from the static viewpoint, but not from the dynamic approach.

9. High quality bread wheat varieties (groups 1 and 2) showed a shorter growing cycle until anthesis and a lower yield than low quality ones (groups 3 and 4) on medium and high productive potential environments.

10. The environmental variables with the largest effect on bread wheat yield and quality are the reference evapotranspiration before anthesis and the maximum daily mean temperature between anthesis and physiological maturity.

11. The environmental variables which mostly affected durum wheat yield were reference evapotranspiration before anthesis and the water availability during grain filling.

12. Modern varieties are more efficient in resources use, both in uptake/utilization of nutrients absorbed through the roots and for water use after anthesis.