Genetic gains and changes in the pattern of adaptation of bread wheat varieties grown in Spain during the 20th century

Miguel Sánchez García

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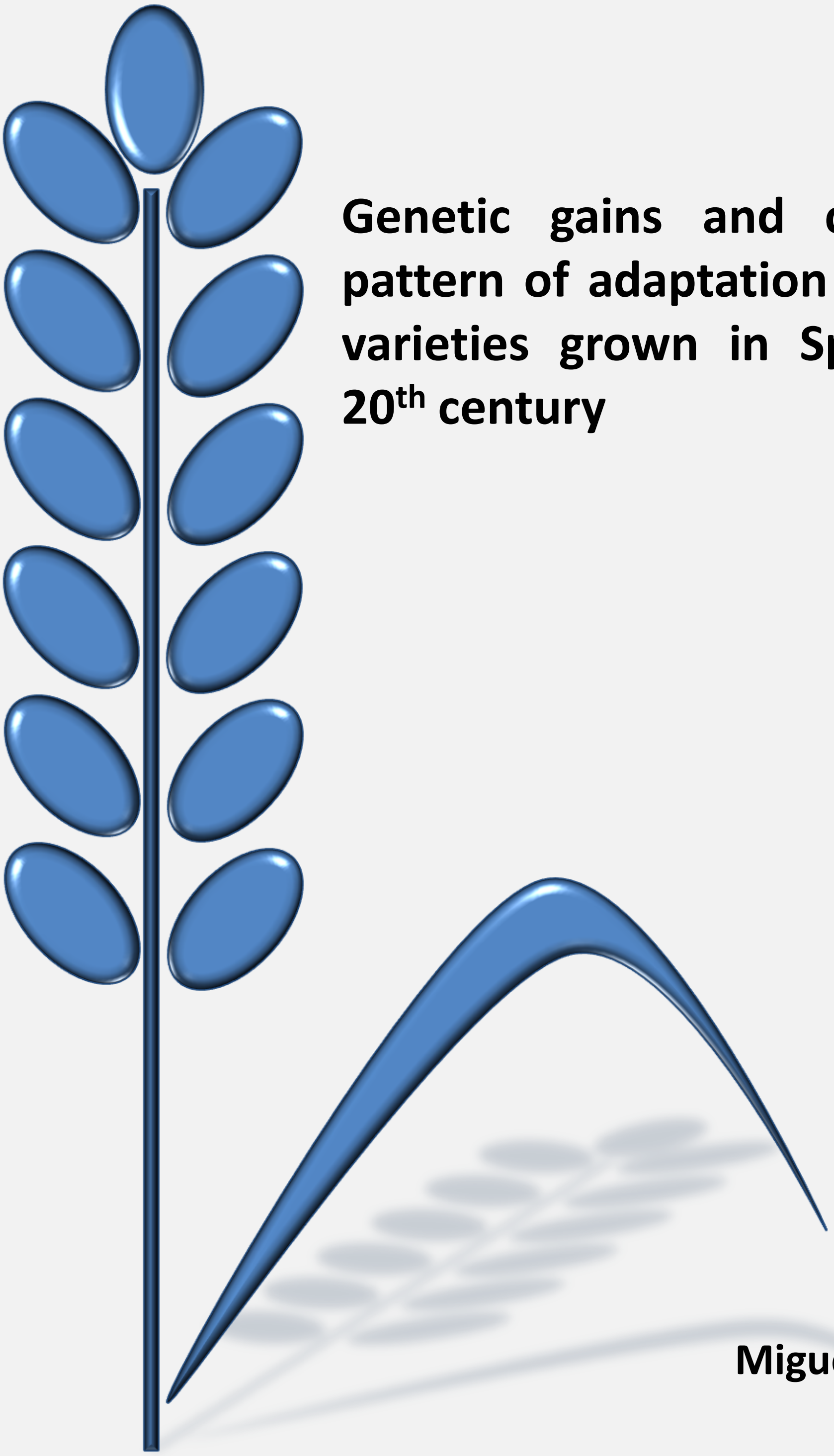

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Miguel Sánchez García
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RECERCA I TECNOLOGIA
AGROALIMENTÀRIES

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Genetic gains and changes in the pattern of adaptation of bread wheat varieties grown in Spain during the 20th century

Memoria de Tesis Doctoral presentada por:

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Para optar al grado de Doctor

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A mis amigos
A mi familia
A mis padres
A Elsa

**Science offers the possibility of far
greater well-being for the human race
than has ever been known before.**
Bertrand Russell

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DISCUSIÓN GENERAL.

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Abstract

Changes caused by breeding on yield, adaptation and bread-making quality of bread wheat grown in Spain during the 20th century were assessed through a historical series of 29 varieties representative of the most widely cultivated in the country. Changes on yield and adaptation were evaluated on eight field experiments conducted in 4 sites, representative of the main wheat growing areas in Spain, during 2006 and 2007. For a deeper study, which included quality assessment, a subset of 16 genotypes representative of the entire collection were selected and tested in four field experiments conducted in Lleida (NE Spain) from 2008 to 2010.

The results indicated that yield improvement in Spain from 1930 to 2000 occurred in two steps. The first one coincided with the cultivation of the first improved varieties from the 1950s, and the second arose from the introduction of foreign varieties, mostly derived from CIMMYT germplasm, from the 1970s. The varieties released during the last decade of the century did not significantly contribute to yield gains. Yield genetic gain was estimated on 35.1 kg ha^{-1} y^{-1} or, in relative terms, 0.88% y^{-1}. It was mainly due to increases in the number of grains per spike (0.60% y^{-1}), and spikes per unit area (0.30% y^{-1}), while grain weight remained unchanged.

The improvement of fertile tillering, the shortening of the time to anthesis, first, and the introduction of major dwarfing alleles that reduced plant height increasing the partitioning of assimilates to the spikes, resulted in yield increases by enhancing harvest index. Above-ground biomass at maturity remained unchanged.

Changes on phenology fitting affected the adaptation of varieties. The first improved varieties were more adapted, when compared with the local landraces, to environments with high daily minimum temperatures and thermal-time before heading. The oldest improved varieties and the winter types among the most recent were more widely adapted than the modern varieties of spring habit. The genetic gains estimated were environment-dependent. Average daily minimum temperature was also the meteorological variable that better explained the differences between environments in the relative genetic gains calculated.

The introduction of the *Rht* major dwarfing alleles – firstly *Rht8c* and *Rht9*, and after the Green Revolution from the 1970's, the GA insensitive *Rht-B1b* and *Rht-D1b*– contributed to reduce plant height by 26.3% in the modern varieties compared with local landraces. These genes, particularly the GA insensitive ones, showed, a pleiotropic effect on leaf area index (LAI) at the beginning jointing, which was reduced by 30% in modern varieties when compared with unimproved cultivars. The higher LAI and leaf area duration (LAD) of modern varieties allowed them gaining and allocating more biomass in the filling grains than the oldest types, resulting in heavier spikes and superior yields. Genetic gains on LAI and LAD during the century (0.83% y^{-1} and 0.79% y^{-1}, respectively) were equivalent to the estimated for yield.

In spite of a decrease on grain protein content (-0.21% y^{-1}), bread-making quality attributes were improved during the 20th century. The introduction of high molecular weight glutenin subunits (HMW-GS) suitable to improve dough strength – such as 1 y 2* at locus *Glu-A1*, 7+8 at *Glu-B1* y 5+10 at *Glu-D1–,* resulted on gluten quality enhancement. The 5+10 subunit also influenced the variety by environment interaction, since the varieties that carry it were more adapted in terms of dough extensibility to irrigated environments. Rheological properties, such as dough strength (W), tenacity (P) and the configuration ratio (P/L) showed important improvements (1.39% y^{-1}, 0.99% y^{-1} and 1.45% y^{-1}, respectively). As consequence, most modern varieties overcame the limit of W ≥ 159 $J \cdot 10^{-4}$ and P/L ≥ 0.56 mm H_2O mm^{-1}, identified as being the minimum required to ensure dough stability during fermentation, avoiding premature alveolus collapsing. However, the reduction of dough extensibility (-0.46% y^{-1}) caused that some modern varieties had unbalanced and extremely tenacious doughs, which produced high dough porosity during fermentation and resulted in low dough maximum heights.

Resumen

Los cambios causados por la mejora en el rendimiento, la adaptación y la calidad harino-panadera del trigo cultivado en España durante el siglo XX fueron evaluados mediante una serie histórica de 29 variedades representativas de las más cultivadas en el país. Los cambios en el rendimiento y la adaptación se abordaron mediante ocho experimentos de campo realizados en 4 localidades, representativas de las principales áreas de cultivo de trigo en España, durante 2006 y 2007. Para un estudio más en profundidad, que incluyó la evaluación de la calidad, un subconjunto de 16 genotipos representativos de toda la colección se seleccionaron y ensayaron en cuatro experimentos de campo realizados en Lleida desde 2008 a 2010.

Los resultados indicaron que la mejora del rendimiento en España desde 1930 hasta 2000 se produjo en dos etapas. La primera coincidió con el cultivo de las primeras variedades mejoradas en la década de 1950, y la segunda surgió de la introducción de variedades foráneas, en su mayoría derivadas de germoplasma del CIMMYT, a partir de la década de 1970. Las variedades liberadas durante la última década del siglo no contribuyeron significativamente al aumento del rendimiento La ganancia genética del rendimiento se estimó en 35,1 kg ha^{-1} año^{-1} o, en términos relativos, 0,88% año^{-1}. Esta ganancia se debió principalmente a aumentos en el número de granos por espiga (0,60% año^{-1}), y espigas por unidad de superficie (0,30% año^{-1}), mientras que el peso del grano se mantuvo sin cambios.

La mejora de la fertilidad de los hijuelos, el acortamiento del tiempo hasta la floración y la introducción de los genes enanismo que promovieron la disminución de la altura de planta favoreciendo el aumento de la partición de los asimilados a las espigas, se tradujo en aumentos en el rendimiento gracias a la mejora del índice de cosecha. La biomasa del cultivo en madurez se mantuvo sin cambios.

Los cambios en la fenología de las variedades influyeron en su adaptación. Las primeras variedades mejoradas se adaptaron mejor, en comparación a las variedades tradicionales, a los ambientes con altas temperaturas mínimas diarias y tiempo térmico antes del espigado. Las variedades antiguas mejoradas y las de hábito invernal mostraron una adaptación más amplia que las variedades modernas de hábito primaveral. La ganancia genética estimada

varió según el ambiente en que se ensayaran las variedades y la temperatura media mínima diaria fue, también, la variable meteorológica que mejor explicó estas diferencias.

La introducción de los alelos de enanismo *Rht,* en primer lugar el *Rht8c* y *Rht9*, y después de la Revolución Verde a partir de la década de 1970, de los alelos *Rht-B1b* y *Rht-D1b* que causan insensibilidad al ácido giberélico, contribuyó a disminuir un 26,3% la altura de planta en las variedades modernas en comparación con las variedades tradicionales. Estos genes, en particular los insensibles al ácido giberélico mostraron un efecto pleiotrópico negativo sobre el índice de área foliar (LAI) al inicio del encañado, que se redujo en un 30% en las variedades modernas en comparación con los cultivares tradicionales. El mayor LAI y duración de área foliar (LAD) de las variedades modernas les permitió acumular y translocar más biomasa a los granos durante el llenado de los mismos que las variedades más antiguas, lo que resultó en espigas más pesadas y rendimientos superiores. Las ganancias genéticas de LAI y LAD durante el siglo (el 0,83% año^{-1} y 0.79% año^{-1}, respectivamente) fueron equivalentes a la estimada para el rendimiento.

A pesar de que el contenido de proteína del grano disminuyó (-0,21% año^{-1}), los atributos de calidad se mejoraron durante el siglo XX. La introducción de gluteninas de alto peso molecular (HMW-GS) más adecuadas para incrementar la fuerza de la masa, como las 1 y 2* codificadas en el locus *Glu-A1*, la 7+8 en el *Glu-B1* y la 5+10 en *Glu-D1*, dieron como resultado un mejora de la calidad del gluten. La subunidad 5+10 influyó también en la interacción variedad x ambiente, ya que las variedades portadoras se adaptaron mejor en términos de extensibilidad de la masa (L) a los ambientes de regadío que el resto de variedades. Propiedades reológicas tales como la fuerza de la masa (W), la tenacidad (P) y la relación entre tenacidad y extensibilidad (P/L) mostraron mejoras importantes (1,39% año^{-1}, 0,99% año^{-1} y 1,45% año^{-1}, respectivamente). Como consecuencia, las variedades más modernas superaron el límite de $W \geq 159$ J·10^{-4} y $P/L \geq 0{,}56$ mm H_2O mm^{-1}, identificado como el mínimo necesario para garantizar la estabilidad de la masa durante la fermentación y evitar, así, el colapso de los alveolos. Sin embargo, la reducción de la extensibilidad de la masa (-0,46% año^{-1}) hizo que algunas variedades modernas produjeran masas desequilibradas altamente tenaces, lo que favoreció el aumento de la porosidad durante la fermentación y dio lugar a bajas alturas máximas de masa.

Resum

Els canvis causats per la millora en el rendiment, l'adaptació i la qualitat harino-fornera del blat conreat a Espanya durant el segle XX van ser avaluats mitjançant una sèrie històrica de 29 varietats representatives de les més conreades al país. Els canvis en el rendiment i l'adaptació van ser avaluats en vuit experiments de camp realitzats en 4 localitats, representatives de les principals àrees de cultiu de blat a Espanya, durant 2006 i 2007. Per a un estudi més en profunditat, que va incloure l'avaluació de la qualitat, es va seleccionar un subconjunt de 16 genotips representatius de tota la col·lecció que es va assajar en quatre experiments de camp realitzats a Lleida (nord-est d'Espanya) des de 2008 a 2010.

Els resultats van indicar que la millora del rendiment a Espanya des de 1930 fins 2000 es va produir en dues etapes. La primera va coincidir amb el cultiu de les primeres varietats millorades en la dècada de 1950, i la segona va sorgir de la introducció de varietats foranies, majoritàriament derivades de germoplasma del CIMMYT, a partir de la dècada de 1970. Les varietats alliberades durant l'última dècada del segle no van contribuir significativament a l'augment del rendiment. El guany genètic del rendiment es va estimar en 35,1 kg ha^{-1} any^{-1} o, en termes relatius, 0,88% any^{-1}. Aquest guany va ser degut a augments en el nombre de grans per espiga (0,60% any^{-1}), i d'espigues per unitat de superfície (0,30% any^{-1}), mentre que el pes del gra es va mantenir sense canvis.

La millora de la fertilitat dels fillols, l'escurçament del temps fins a la floració, en un primer moment, i la introducció dels gens de nanisme que van promoure la disminució de l'alçada de planta afavorint l'augment de la partició dels assimilats a les espigues, es va traduir en augments en el rendiment gràcies a la millora de l'índex de collita. La biomassa del cultiu en maduresa es va mantenir sense canvis.

Els canvis en la fenologia de les varietats van influir en l'adaptació de les varietats. Les primeres varietats millorades es van adaptar millor, en comparació amb les varietats tradicionals, als ambients amb altes temperatures mínimes diàries i temps tèrmic abans del espigat. Les varietats antigues millorades i les d'hàbit hivernal van mostrar una adaptació més àmplia que les varietats modernes d'hàbit primaveral. El guany genètic estimat va variar segons l'ambient en què s'assajaren les varietats i la temperatura mitjana mínima diària va ser, també, la variable meteorològica que millor va explicar aquestes diferències.

La introducció dels al·lels de nanisme *Rht*, en primer lloc, el *Rht8c* i *Rht9*, i després de la Revolució Verda, a partir de la dècada de 1970, dels al·lels *Rht-B1b* i *Rht-D1b* insensibles a l'àcid giberèl·lic, va contribuir a la disminució, en un 26,3%, de l'alçada de la planta en les varietats modernes en comparació amb les varietats tradicionals. Aquests gens, en particular els insensibles a l'àcid giberèl·lic van mostrar un efecte pleiotròpic negatiu sobre l'índex d'àrea foliar (LAI) a l'inici de l'encanyat, que es va reduir en un 30% en les varietats modernes en comparació amb els cultivars tradicionals. El major LAI i durada d'àrea foliar (LAD) de les varietats modernes els va permetre acumular i translocar més biomassa als grans durant l'ompliment que les varietats més antigues, el que va resultar en espigues més pesades i rendiments superiors. Els guanys genètics de LAI i LAD durant el segle (el 0,83% any^{-1} i 0,79% any^{-1}, respectivament) van ser equivalents a l'estimat per al rendiment.

Malgrat una disminució en el contingut de proteïna del gra (-0,21% any^{-1}), els atributs de qualitat van millorar durant el segle XX. La introducció de subunitats d'alt pes molecular de les glutenines (HMW-GS) més adequades per millorar la força de la massa, com les 1 i 2* codificades en el locus *Glu-A1*, la 7+8 en el *Glu-B1* i la 5+10 en el *Glu-D1*, van resultar en un millora de la qualitat del gluten. La subunitat 5+10 va influir també en la interacció varietat x ambient, ja que les varietats que la porten van mostrar millor adaptació que la resta, en termes d'extensibilitat de la massa (L), als ambients de regadiu. Propietats reològiques com la força de la massa (W), la tenacitat (P) i la relació entre tenacitat i extensibilitat (P/L) van mostrar millores importants (1,39% any^{-1}, 0,99% any^{-1} i 1,45% any^{-1}, respectivament). En conseqüència, les varietats més modernes van superar el límit de $W \geq 159\ J \cdot 10^{-4}$ i $P/L \geq 0,56$ mm H_2O mm^{-1}, identificat com el mínim necessari per garantir l'estabilitat de la massa durant la fermentació, evitant el col·lapse dels alvèols. No obstant això, la reducció de l'extensibilitat de la massa (-0,46% any^{-1}) va fer que algunes varietats modernes produïssin masses desequilibrades altament tenaces, el que va afavorir l'augment de la porositat durant la fermentació i va donar lloc a baixes altures màximes de massa.

INTRODUCCIÓN GENERAL

Introducción general

El cultivo del trigo en el mundo

El trigo harinero (*Triticum aestivum* L.) es uno de los principales cultivos a escala mundial. Su importancia como fuente de hidratos de carbono, tanto para alimentación humana como animal y su versatilidad a la hora de permitir la elaboración de una gran gama de productos derivados, explican su interés económico y social. Durante los últimos años el consumo de trigo ha excedido su producción (McFall y Fowler, 2009) poniendo de manifiesto la necesidad de incrementar dicha producción para poder hacer frente a la creciente demanda. En 2011 la superficie sembrada a nivel mundial fue de más de 217 Mha, con una producción global estimada de 654 Mt y un rendimiento medio de 3,0 t ha^{-1} (FAOSTAT 2011). Como la población mundial aumenta exponencialmente (se prevé que en el año 2050 se alcancen los 9191 millones de habitantes) (ONU, 2007) se ha estimado que para poder abastecerla y evitar hambrunas, en 2020 será necesario producir 1000 Mt de trigo. Como está previsto que la superficie cultivable no se incremente más de un 5% en los próximos años, se estima que el 80% de la producción futura de trigo habrá de provenir de terrenos actualmente en cultivo, es decir de incrementos en el rendimiento. Para satisfacer la demanda prevista, será necesario un rendimiento medio de 4 t ha^{-1}, lo que supondrá un aumento del 1,6% anual en términos relativos. La creciente limitación en el uso del agua para riego, el incremento del precio de los insumos y las restricciones al uso de productos fitosanitarios, hacen que no se pueda confiar en que se consigan mayores productividades como fruto de una mayor intensificación de las prácticas agronómicas. Por este motivo se espera que la mejora genética tenga un papel preponderante en los futuros aumentos de la productividad.

La filosofía que subyace en la actualidad es diferente a la que se aplicó durante el siglo XX. En aquel momento, como consecuencia de la Revolución Verde, para aumentar la producción se procedió a modificar el ambiente mediante el empleo de fertilizantes, riegos, etc. Actualmente se pretende obtener variedades que se adapten al ambiente, causando el menor impacto ecológico posible para integrarse en sistemas de producción sostenible.

Situación actual del cultivo de trigo en España

El trigo harinero es un cultivo importante en España con una producción de casi 6 Mt en el año 2011 y una superficie sembrada de aproximadamente 1,61 Mha (MAGRAMA, 2011), lo que supone un 27% de la superficie total dedicada a los cereales en el país, siendo el segundo cereal más importante por superficie de cultivo, después de la cebada. España es además el quinto país en superficie sembrada de trigo harinero en la UE25 con más del 7% de la superficie total (EUROSTAT, 2010). El rendimiento entre 1934 y 2010 ha pasado de una media de 1,3 t ha^{-1} (Nagore, 1934) a 3,69 t ha^{-1} en 2010 (EUROSTAT, 2010) aunque se desconocen las causas específicas que han permitido este incremento y el impacto que ha tenido cada una de ellas.

La mejora genética del trigo harinero en España

La mejora del rendimiento en España parte de los programas de selección dentro de las poblaciones locales exitosas, como los llevados a cabo, entre otros, por J. M. Soler y Coll y J. Pané ya desde las primeras décadas del siglo XX (Royo y Briceño-Félix, 2011). Posteriormente, mediante intercambios de germoplasma con otros países europeos, se introdujeron variedades exitosas provenientes, principalmente de Italia y Francia, como *Libero* y, más tarde, *Estrella* e *Impeto*. De los cruzamientos realizados en los programas de mejora nacionales entre las variedades extranjeras y las variedades locales más productivas resultaron variedades ampliamente cultivadas, como la variedad *Pané 247*, que aún se continúa sembrando en ciertas zonas. La importancia de estas variedades decreció a partir de 1970 con la introducción de variedades semi-enanas provenientes del *Centro Internacional de Mejoramiento de Maíz y Trigo* (CIMMYT; México) como *Yécora*, *Cajeme* o *Siete-Cerros* y en las décadas de 1980 y 1990 de variedades francesas como *Marius* y *Soissons* (Royo y Briceño-Félix, 2011).

La importancia de las variedades extranjeras en el cultivo de trigo en España es innegable. Durante la campaña 2004/2005, de las 10 variedades más utilizadas en base al uso de semilla certificada, 7 no provinieron de programas de mejora españoles, lo que supuso más del 40% del total de semilla certificada vendida en este país (Royo y Briceño-Félix, 2011).

En la actualidad está en marcha un programa de mejora de trigo a escala nacional financiado parcialmente por el *Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria* (INIA), que tiene como objetivo proporcionar variedades adaptadas a las diferentes zonas de cultivo de España. Existen además programas regionales desarrollados por organismos de investigación autonómicos como el *Institut de Recerca i Tecnologia Agroalimentària* (IRTA) de la Generalitat de Cataluña.

Bases morfo-fisiológicas del rendimiento

El rendimiento de un cultivo es la expresión última de la adaptación del mismo a un ambiente dado. Es un carácter cuantitativo de gran complejidad. Al estudiar los factores que lo determinan es común descomponerlo en fracciones que permitan una mejor comprensión de las bases fisiológicas que se encuentran detrás de los incrementos productivos. Una de las formas más comunes de abordar la mejora del rendimiento es el marco conceptual propuesto por Donald y Hamblin (1974), que consiste en considerarlo como el producto de la biomasa de la planta y el índice de cosecha:

Rendimiento = Biomasa de la planta en madurez x *Proporción de la biomasa en madurez dedicada a los granos (índice de cosecha)*

Existen varios trabajos dirigidos a estudiar las causas genéticas que explican los incrementos productivos ocurridos en el pasado mediante el estudio de series históricas de variedades. Este tipo de estudios se han llevado a cabo en países que abarcan un amplio rango de ambientes: Canadá (Hucl y Baker 1997), Estados Unidos (Donmez *et al.*, 2001), México (Waddington *et al.*, 1986), Reino Unido (Austin *et al.*, 1989) y Argentina (Calderini *et al.*, 1995) entre otros. Hay un consenso entre todos ellos por el que se atribuye principalmente el aumento de la productividad a un incremento en el índice de cosecha, favoreciendo la partición de recursos de la parte vegetativa de la planta hacia la reproductiva (Austin, 1980, Slafer *et al.*, 1989, Sayre *et al.*, 1997; Royo *et al.*, 2007). Las conclusiones alcanzadas respecto a los cambios en la biomasa no siempre coinciden. Mientras que unos afirman que la biomasa del cultivo en el estadio de cosecha no ha variado (Austin *et al.*, 1980; Waddington *et al.*, 1986) otros reportan aumentos de biomasa paralelos a la mejora de la productividad (Perry y D'Antuono, 1989; Siddique *et al.*, 1989; Shearman *et al.*, 2005), especialmente durante las últimas décadas del siglo XX. El aumento del índice de cosecha se ha plasmado, generalmente, en aumentos en el número de granos por unidad de superficie (Siddique *et al.*, 1989; Sayre *et al.*, 1997; Royo *et al.*, 2007) y, más concretamente, en el aumento del número de granos por espiga, aunque durante el siglo pasado se observaron en ciertos trabajos ligeros incrementos en el peso de los granos (Perry y D'Antuono, 1989; Calderini *et al.*, 1995).

Los grandes avances en el rendimiento, la adaptación y las características de interés económico del trigo harinero logrados durante el siglo XX en todo el mundo se debieron a la identificación e introgresión de una serie de genes con efectos mayores sobre la fisiología de la planta que han permitido mejoras globales del cultivo (Worland y Snape 2001). Los incrementos observados en el índice de cosecha en todo el mundo fueron debidos en gran medida a la introducción de los genes de enanismo (*Rht*) que disminuyeron la altura de la planta sin alterar su biomasa final (Austin *et al.*, 1980). Estos genes, al reducir la longitud de los entrenudos, redujeron la competencia entre los mismos y la espiga en formación, incrementándose el número de granos por unidad de superficie y el índice de cosecha (Fischer y Stockman 1986). La introducción por parte del mejorador Nazareno Strampelli de los genes *Rht9* y, principalmente, *Rht8c,* provenientes de la variedad japonesa *Akakomugi* (Worland *et al.*, 1998) en el germoplasma italiano trajo consigo una importante reducción de la altura de la planta y un aumento del reparto de biomasa a los órganos reproductivos (Worland y Snape 2001). Estos genes se extendieron más tarde al germoplasma del resto de Europa, por cruzamientos con variedades desarrolladas por N. Strampelli. La introducción de los genes *Rht-B1b* y *Rht-D1b*, provenientes del cruce *Norin 10* / *Brevor* que disminuyen adicionalmente la altura de la planta en un 18% (Flintham *et al.*, 1997) inhibiendo su sensibilidad a las giberelinas, en las variedades liberadas principalmente a partir de la década 1970 después de la Revolución Verde, produjo un aumento del rendimiento estimado en un 15% (Worland y Snape 2001).

Se ha sugerido que el índice de cosecha puede tener un límite fisiológico alrededor de 0.64 (Foulkes *et al.*, 2011). Estudios recientes han puesto de manifiesto la poca o nula mejora en el índice de cosecha lograda durante las últimas décadas del siglo XX, que parece haberse estancado en valores cercanos a 0,50-0,55 en los ambientes más óptimos (Sayre *et al.*, 1997; Shearman *et al.*, 2005). A pesar del límite fisiológico teórico, desarrollar variedades que superen un índice de cosecha de 0,50 sigue siendo difícil en muchos ambientes (Fischer y Edmeades 2010). Estas aparentes limitaciones contribuyen a considerar el incremento de la biomasa del cultivo en madurez, sin alterar el índice de cosecha, como una de las estrategias principales a potenciar en los futuros programas de mejora (Parry *et al.*, 2011).

Ante la disparidad de resultados obtenidos bajo diferentes condiciones ambientales, y en países con trayectorias distintas, en lo concerniente a la mejora del trigo harinero, determinar la extensión de la ganancia genética del rendimiento del trigo harinero en España y comprender los mecanismos a través de los cuales se ha producido esta ganancia, puede suponer una herramienta útil para determinar los futuros pasos a seguir para la obtención de variedades más adaptadas y exitosas.

Producción y distribución de asimilados

El principal factor que determina la biomasa de un cultivo es la tasa fotosintética acumulada durante el ciclo de cultivo (Parry *et al.*, 2011). Una aproximación para estudiar el rendimiento de un cultivo consiste en considerar la biomasa de éste en madurez como el producto de la radiación interceptada por el cultivo y la eficiencia del cultivo en el uso de la radiación. A pesar de que en ambientes mediterráneos, la translocación de asimilados sintetizados antes de la floración y acumulados en los tallos puede desempeñar un papel mayor que en ambientes con condiciones no limitantes (López-Castañeda y Richards 1994; Ruuska *et al.*, 2006), esta aproximación indica la importancia que tiene el mantenimiento y la efectividad de los órganos fotosintéticos sobre el rendimiento potencial. Una de las estrategias sugeridas para lograr incrementar los rendimientos de las futuras variedades, es incrementar la captura de luz por parte del dosel, ya sea favoreciendo un rápido desarrollo del área foliar y/o retrasando la senescencia (Parry *et al.*, 2011)

Un rápido desarrollo foliar permite aumentar la capacidad fotosintética de la planta desde los primeros estadíos del cultivo. Además permite acelerar la formación de un dosel capaz de evitar la evapotranspiración en los momentos iniciales del ciclo de cultivo, cuando la demanda de agua por parte de la planta es reducida. En ambientes mediterráneos, donde la disponibilidad de agua en el suelo durante el llenado del grano es a menudo un factor limitante que puede determinar el rendimiento de un cultivo, el mantenimiento de una mayor cantidad de agua en el suelo durante el desarrollo vegetativo para ser utilizada durante el llenado, puede contribuir a incrementar el rendimiento de una determinada variedad (Condon *et al.*, 2004).

La duración del área foliar (Leaf Area Duration; LAD) es también un mecanismo fisiológico que permite incrementar la capacidad fotosintética total del cultivo y de esta manera la síntesis de biomasa que puede translocarse hacia los granos (Richards 2000). Los incrementos en la duración del área verde han sido recientemente relacionados con aumentos del rendimiento en ambientes de tipo mediterráneo no limitados por la disponibilidad de agua (Christopher *et al.*, 2008), indicando la importancia de este carácter para la mejora genética del trigo.

Adaptación al ambiente

España dispone de una gran variedad climática tanto geográfica como temporal debido a su latitud, a su orografía y a su localización en una península entre el océano Atlántico y el mar Mediterráneo. Las grandes variaciones climáticas interanuales, propias de los países mediterráneos, donde los estreses abióticos constituyen el principal factor limitante del rendimiento, hacen que el éxito de la mejora esté en gran parte determinado por la respuesta de las variedades a los factores climáticos (Cooper y Byth, 1996). Entender los mecanismos que intervienen en la adaptación de las variedades al ambiente, así como identificar las principales variables climáticas que determinan la sensibilidad de las variedades al mismo y el efecto que tienen sobre los principales componentes del rendimiento, es crucial para el desarrollo de nuevas variedades más adaptadas y con un rendimiento mayor y más estable.

La mayoría de los estudios publicados hasta la fecha en todo el mundo acerca de la mejora genética del trigo harinero, han centrado su atención en el rendimiento y sus componentes. Sin embargo, son escasos los trabajos que abordan el estudio de los cambios producidos por la mejora sobre los patrones de adaptación del germoplasma.

Los mejoradores han seguido varias estrategias para favorecer la adaptación de las variedades al ambiente. En el CIMMYT la selección se lleva a cabo en ambientes de alto potencial con el objetivo de obtener variedades con amplia adaptación, lo que hasta la fecha dando lugar a importantes mejoras del rendimiento (Braun *et al.*, 1997). Sin embargo, estudios recientes han demostrado que en ambientes limitantes, la mejora del rendimiento ha sido debida a la selección de variedades con baja interacción con el ambiente y alto rendimiento potencial (Cattivelli *et al.*, 2008), mientras que en ambientes de alto potencial, las variedades más exitosas de cereales han mostrado una tendencia hacia una adaptación más específica (Pswarayi *et al.*, 2008; Royo *et al.*, 2008).

Calidad harino-panadera y atributos relacionados

o Calidad de los trigos españoles

Los usos del trigo en España son variados pero una parte importante del total dedicado a la alimentación humana, concretamente 1,6 Mt (un 74% del total) se dedica a la elaboración de pan en sus diferentes formas, (MAGRAMA 2011a). Más del 38,5% del trigo consumido en España es importado (MAGRAMA 2011b). Una de las razones que se encuentran detrás de este hecho es la necesidad de compensar la baja calidad de los trigos producidos en España con la importación de trigos foráneos de alta calidad. Mientras que en Francia en 2011 el 61% del trigo producido pudo clasificarse entre las dos mejores categorías de la clasificación nacional de la calidad de los trigos (clases E y 1; ARVALIS 2011), en

España, en la zona noroeste, que engloba casi el 55% de la producción de trigo del país, el 94,7% de la producción perteneció a las dos peores clases (clases 4 y 5; AETC 2011) de la reciente clasificación oficial de la calidad de los trigos españoles (BOE 2010). Incluso en la zona sur, que representa tan sólo el 8,7% de la producción total de España y donde se producen los trigos de mayor calidad, sólo un 39.9% del trigo producido perteneció a las tres primeras clases (clases 1 a 3; AETC 2011).

Las causas de esta aparente baja calidad de la producción pueden residir en la falta de variedades de alta calidad, una pobre adaptación de estas a los ambientes españoles, debido al origen extranjero de muchas de las variedades sembradas en España y/o a la falta de incentivos para los agricultores compensen la siembra de variedades de alta calidad, pero quizás menos productivas.

El estudio de la evolución producida por la mejora genética en la calidad harino-panadera en España es un tema de capital importancia dada la influencia de la misma sobre los beneficios económicos de los agricultores y la industria harinera y panadera.

o Efecto de la composición de las prolaminas de reserva del grano sobre la calidad

La calidad harino-panadera del trigo está altamente correlacionada con las propiedades de la matriz proteica, que a su vez depende tanto de la cantidad como de la calidad de las proteínas que la forman (Finney 1943). Estas propiedades se deben particularmente a la composición de las proteínas que conforman el gluten. De estas proteínas, las subunidades de las gluteninas de alto peso molecular (*High Molecular Weight Glutenin Subunits*; HMW-GS), codificadas en el complejo de loci *Glu-1*, son las que mayor efecto tienen sobre la calidad harino-panadera del trigo harinero, especialmente sobre algunas propiedades reológicas de las masas, como la fuerza y la tenacidad (Branlard y Dardevet 1985; Payne *et al.*, 1987; Vawser y Cornish 2004). El descubrimiento del control genético de las proteínas que conforman la matriz proteica del gluten – gluteninas de alto (HMW) y bajo (LMW) peso molecular y gliadinas – favoreció la selección para proteínas de alta calidad, principalmente las HMW al ser más sencilla su identificación (Carrillo et al., 2006). Un estudio llevado a cabo con variedades españolas en 1988 determinó la influencia relativa que la composición alélica en los loci *Glu-1*, especialmente el *Glu-A1* y *Glu-D1*, tuvo sobre las diferencias observadas entre las variedades en la fuerza obtenida mediante el alveograma y el índice de sedimentación Zeleny (Payne *et al.*, 1988).

Como se ha comentado previamente, las HMW-GS son determinantes en la calidad final del trigo harinero. Tanto es así que se han publicado índices de comportamiento reológico de las masas que anticipan de forma bastante certera la fuerza de las mismas, por la composición alélica en los genes *Glu-1* (Payne *et al.*, 1987; Branlard *et al.*, 1992). A pesar de que existen diferencias, estos índices coinciden en señalar algunas subunidades como de claro efecto positivo, como la 1 y 2* codificadas en el *Glu-A1* y la 5+10 en el *Glu-D1*, o negativo, como la Null codificada en el *Glu-A1* o la 20 en el *Glu-B1,* sobre la fuerza de las masas (Payne *et al.*, 1987; Branlard *et al.*, 1992). Incluso se ha descrito la influencia de los alelos codificados en los loci *Glu-1* sobre las interacciones con el ambiente de las variedades para algunas propiedades reológicas como: la extensibilidad o la fuerza (Blumenthal *et al.*, 1995; Johansson *et al.*, 2000; Panozzo y Eagles, 2000; Hristov *et al.*, 2010).

- Propiedades reológicas de la masa

Las propiedades reológicas de la masa caracterizan el comportamiento de la misma cuando está sometida a tensiones de deformación como las que ocurren durante los procesos de amasado y fermentación. Así, características como la fuerza o la extensibilidad de las masas son determinantes para la obtención de un producto de calidad (Dobraszczyk y Morgenstern 2003).

El alveograma obtenido mediante el alveógrafo Chopin es la principal técnica utilizada, tanto en España como en otros países europeos (Carrillo *et al.*, 2006), tales como Italia o Francia, para determinar las propiedades reológicas de extensión biaxial de las masas (Mesdag y Donner 2000). El procedimiento permite determinar de forma relativamente simple una serie de características como la tenacidad (*P*), la extensibilidad (*L*), el cociente entre ambas (*P/L*) que es un indicador del equilibrio de la masa, y la fuerza de la masa (*W*), todos ellos determinantes en el desempeño de las masas durante el amasado, la fermentación y la cocción. Entre otras características de calidad de los granos y la harina, estos atributos son los utilizados en la reciente clasificación de calidad de trigos española publicada por el Ministerio de Agricultura (BOE 2010).

- Efecto del ambiente sobre la calidad de los trigos

Por su influencia en las propiedades relacionadas con la calidad harino-panadera el contenido de proteína acumulada en el grano ha sido un atributo tenido en cuenta en las clasificaciones de calidad de los trigos de prácticamente todos los países (Mesdag y Donner 2000). Por este motivo se han llevado a cabo numerosos estudios con el fin de analizar las variaciones en la composición química de los granos, que han determinado que las diferencias en la composición tienen un origen tanto genotípico como ambiental. Se han descrito una serie de QTL (*Quantitative Trait Loci*) que juegan un papel importante en el control del contenido de proteína en el grano. Sin embargo, estos QTL también han mostrado una importante interacción con el ambiente (Branlard y Marion, 2011). La importancia de la interacción genotipo x ambiente de la concentración de proteína reside en la influencia que esta tiene sobre muchos atributos que determinan la calidad de una harina. Así, los atributos más relacionados con la concentración de proteína, como la extensibilidad de la masa (Cornish *et al.*, 2001), suelen presentar una mayor interacción genotipo por ambiente haciéndolos más inestables (Williams *et al.*, 2008).

La influencia de la proteína del grano sobre la interacción genotipo por ambiente de las propiedades reológicas, entre otros atributos de calidad, no es exclusiva de su concentración. Recientes estudios han encontrado una interacción diferencial con el ambiente de algunas propiedades reológicas o de la propia concentración de proteína en función de la composición alélica de las proteínas que conforman el gluten (Hristov *et al.*, 2010; Johansson *et al.*, 2000; Panozzo and Eagles, 2000).

Cuantificar la interacción genotipo por ambiente de las variedades españolas en lo relativo a los atributos de calidad y el efecto del tipo de proteína del gluten en dicha interacción, con el fin de poder predecir el comportamiento reológico de la masa obtenida de una variedad en un ambiente determinado, debe ser un objetivo de todo programa de mejora. Ello permitirá proveer a los agricultores de variedades con unas características de calidad previsibles con las que cumplir las demandas de la industria harino-panadera.

○ Fermentación

El interés de los mejoradores en las propiedades reológicas de las masas radica en que permiten predecir el comportamiento de las mismas durante los procesos de panificación, como el amasado y la fermentación. Los test que se realizan para determinar las propiedades reológicas se llevan a cabo sin levadura y en condiciones de temperatura ambiente, pudiendo diferir de las obtenidas bajo condiciones reales de fermentación (Dobraszczyk y Morgenstern 2003). Realizar pruebas en tiempo real de la evolución del comportamiento de las masas durante la fermentación puede ayudar a confirmar y completar los datos obtenidos de las pruebas reológicas.

Un equipo que permite determinar el comportamiento de las masas durante la fermentación es el reofermentómetro F3 (Chopin), que monitoriza el desarrollo de la masa y la producción de CO_2 durante la fermentación en condiciones controladas, obteniéndose una serie de variables que están altamente correlacionadas con el volumen final del pan, como la altura máxima alcanzada por la masa durante las 3 horas que dura el proceso de fermentación (DMH de su denominación en inglés: *Dough Maximum Height*) (Ktenioudaki *et al.*, 2010; Ktenioudaki *et al.*, 2011). Además permite conocer la tolerancia de una masa a los procesos de fermentación, lo que influye sobre el volumen final del pan. La tolerancia a la fermentación está relacionada con el colapso de los alveolos de un pan durante su fermentación. Un rápido y acusado colapso de las estructura alveolar de la masa, creada por las burbujas de CO_2 liberadas durante la fermentación, puede reducir drásticamente el volumen del mismo, haciéndolo inservible para la venta. Variables como el tiempo necesario para el comienzo de la liberación de CO_2, es decir el inicio de la porosidad, la altura final de la masa o la proporción de la altura máxima perdida al final del proceso de fermentación, son buenos indicadores de la tolerancia al proceso de fermentación.

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OBJETIVOS

Objetivos

El objetivo general de esta Memoria de Tesis Doctoral es estudiar los cambios debidos a la mejora genética del trigo harinero (*Triticum aestivum* L.) cultivado en España a lo largo del siglo XX. Para ello se estudian un conjunto de características relevantes para la productividad, adaptación y calidad del cultivo, al efecto identificar aquellos atributos que los han hecho posible y con el fin de generar información que permita mejorar la eficiencia de los programas de mejora genética. Este objetivo general se concreta en los siguientes objetivos específicos:

1) Cuantificar la ganancia genética del rendimiento del trigo harinero en España a lo largo del siglo XX e identificar los componentes del rendimiento y caracteres asociados responsables de los cambios ocurridos.

2) Estudiar los cambios originados por la mejora en los patrones de adaptación del trigo a las principales zonas de cultivo del país, mediante el estudio de la interacción genotipo por ambiente (GE).

3) Analizar los cambios en la acumulación de biomasa y su distribución en la planta debidos a la mejora genética y determinar el efecto de la introducción de los alelos de enanismo *Rht8c*, *Rht-B1b* y *Rht-D1b* han tenido sobre dichos cambios.

4) Determinar los cambios en las características de calidad harinera producidos por la mejora genética durante el pasado siglo y cuantificar la influencia que sobre ésta ha tenido la composición de las subunidades de gluteninas de alto peso molecular (HMW-GS) codificadas en el complejo de loci *Glu-1*.

Para alcanzar los objetivos propuestos se reunió una serie histórica de 29 variedades de trigo harinero representativas de las variedades cultivadas en España en diferentes periodos a lo largo del siglo XX. De esta serie histórica se seleccionaron las variedades más adecuadas para lograr a cada objetivo.

La presente Memoria de Tesis Doctoral está formada por cuatro capítulos elaborados como entidades independientes de información, al objeto de que sean 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 *Journal of Agricultural Science*, el **capítulo 2** ha sido publicado en *Field Crops Research* y los **capítulos 3** y **4** están en fase de revisión.

CHAPTER 1

Genetic improvement of bread wheat yield and associated traits in Spain during the 20th century

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CROPS AND SOILS RESEARCH PAPER

Genetic improvement of bread wheat yield and associated traits in Spain during the 20th century

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SUMMARY

A collection of 26 wheat genotypes widely grown in Spain during the 20th century was evaluated in eight contrasting environments in order to quantify breeding achievements in yield and associated traits. From 1930 to 2000, yield increased at a rate of 35·1 kg/ha/yr or 0·88%/yr, but estimations of relative genetic gain (RGG) were environment-dependent. RGG estimated for yield were positively associated with the average minimum daily temperatures from sowing to heading in the testing environments ($R^2 = 0{\cdot}81$; $P < 0{\cdot}01$). The number of grains/spike and the number of spikes/m^2 increased at a rate of 0·60%/yr and 0·30%/yr, respectively, while grain weight remained unchanged. The present study detected two main episodes of yield improvement during the century. The first one coincided with the introduction, during the 1950s, of the first improved cultivars derived from intra-specific crosses, which increased the yield of landraces by 30% due to an increase of *c*. 58% in the number of grains/spike, accompanied by a 16% reduction in grain weight. These initial cultivars (termed 'old-bred' in a previous study by Sanchez-Garcia *et al*. 2012) exhibited a higher harvest index (HI), increased from 0·25 to 0·40, but maintained the same aboveground biomass at maturity as the landraces (despite reducing both plant height and the number of tillers/plant) due to increases in the proportion of tillers bearing spikes. The second yield gain occurred after the introduction, in the early 1970s, of semi-dwarf germplasm from CIMMYT (International Maize and Wheat Improvement Centre) and some French cultivars. This new germplasm further reduced plant height, improved HI up to 0·45 and increased the number of tillers/plant while maintaining their rate of fertility, thus resulting in a yield gain of *c*. 37%. The cultivars released during the last decade of the century did not contribute to significant yield improvements.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is a traditional crop in Spain, which is the fifth wheat producer of the European Union, devoting 1·4 million hectares to the species in 2010 (EUROSTAT 2011). However, during the 20th century investments in wheat breeding in Spain were irregular and genotype replacement relied mostly on the introduction of foreign germplasm.

Wheat breeding activities in Spain started in the 1920s, mainly with the classification of local landraces and bulk selection within them (Royo & Briceño-Félix 2011). However, the early breeding programmes were interrupted by the Spanish Civil War (1936–39), which severely damaged the incipient efforts of cereal researchers to produce improved wheat genotypes. During the two decades after the war, breeding activities were devoted to further improving traditional wheat genotypes through selection and hybridization, and foreign improved cultivars, mainly from Italy and France, were successfully introduced. Some of the cultivars released during the 1950s (such as the Italian cultivars Mara and Impeto and the French cultivar Estrella) carried the *Rht8c* dwarfing gene and probably the photoperiod-insensitive gene *PpD1* (Worland *et al.* 1998), which has been reported to affect plant

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height, cycle length and crop productivity (Borojevic & Borojevic 2005). The advent of CIMMYT semi-dwarf germplasm in the late 1960s had a great impact on Spanish agriculture. It was rapidly adopted in southern Spain, but the French germplasm continued to be grown in northern Spain (Royo & Briceño-Félix 2011). The major dwarfing genes *Rht1B* and *Rht1D* were introduced with CIMMYT germplasm, and have been reported to result in yield increases of *c*. 15% in near-optimal conditions (Worland & Snape 2001). A study conducted in durum wheat, whose history is parallel to that of bread wheat, indicated that new sources of alleles were used in the crosses that led to the cultivars released in Spain since 1990 (Martos *et al.* 2005).

Understanding the crucial characteristics of the crop associated with the genetic gains achieved through breeding is an essential step for improving the current knowledge of yield-limiting factors and the design of future breeding strategies. Historical series of genotypes have been deployed and used in several countries to assess the genetic gains achieved during a period of time through selection and breeding (Austin *et al.* 1989; Donmez *et al.* 2001; Morgounov *et al.* 2010). However, information about gains in yield and related traits in Spain is scarce.

The number of grains/m^2 and, more precisely, the number of grains/spike, have been reported to be the main traits contributing to yield rises under a wide range of environmental conditions (Perry & D'Antuono 1989; Donmez *et al.* 2001; Royo *et al.* 2007), whereas changes in grain weight have proven to be null (Austin *et al.* 1989; Brancourt-Hulmel *et al.* 2003) or even negative (Siddique *et al.* 1989; Royo *et al.* 2007). Increases in the number of spikes/m^2 of bread and durum wheat during the 20th century have also been detected, mainly under Mediterranean conditions (Perry & D'Antuono 1989; Canevara *et al.* 1994; Royo *et al.* 2007), and they have been attributed to increases in the number of spikes/plant (Royo *et al.* 2007).

From the point of view of biomass production and allocation, past genetic gains in bread wheat yield have been associated widely with increases in harvest index (HI) and decreases in plant height (Canevara *et al.* 1994; Brancourt-Hulmel *et al.* 2003). The introduction of dwarfing genes reduced the size of the vegetative organs, thus enhancing the availability of assimilates to the reproductive organs of the plant, expressed as a higher number of grains/spike (Álvaro *et al.* 2008*a*). Austin *et al.* (1980) proposed 0·62 as a theoretical upper limit for HI, but HI rises beyond 0·50 are difficult to attain (Fischer & Edmeades 2010). However, in some countries current levels are close to this upper limit and further yield improvements must therefore be based on increasing biomass while maintaining HI (Pfeiffer *et al.* 2000; Shearman *et al.* 2005).

The uneven history of bread wheat introduction in Spain during the last century may have led to the boosting of different plant attributes in different phases, as has been reported in other countries where the paths followed to achieve yield gains during the last century differed across periods (Austin *et al.* 1989; Canevara *et al.* 1994). Through a study of the main periods of release of bread wheat genotypes in Spain, the objective of the present work was to quantify yield breeding progress during the 20th century and to identify the key traits associated with it.

MATERIALS AND METHODS

Plant material

A collection of 28 bread wheat genotypes representative of those most widely grown in Spain during the 20th century was assembled. The genotypes were grouped into four breeding periods: (i) Spanish landraces grown before 1940; (ii) initial cultivars derived from crosses, including those improved and grown from the mid-1940s until the Green Revolution (termed 'old-bred' in a previously published paper, Sanchez-Garcia *et al.* 2012); (iii) intermediate cultivars introduced in Spain from 1970 and mostly derived from semi-dwarf germplasm of CIMMYT and French cultivars; and (iv) modern cultivars, released during the last decade of the 20th century (Table 1).

Experimental setup

Twenty-six genotypes of the collection were tested in multi-location experiments harvested in 2006 and 2007, but sown in November or December of the previous year (Table 1). Experiments were conducted at four sites representative of the main bread wheat growing areas in Spain: Burgos, in the north, with cold winters and mild summers; Lleida in the north-east, where terminal stress was moderate and the plots were irrigated; Albacete in the south-east, with cold winters and very hot and dry summers; and Cordoba in the south-west, with warm winters and very hot summers (Table 2).

For a more detailed study, a subset of 16 genotypes representative of the entire collection (Table 1) was

Table 1. *Description of the genotypes used in the study and the breeding periods to which they belong*

Genotype	Pedigree	Origin	Year of release in Spain	Experiments*
Local landraces				
Aragon 03	Selection of the landrace 'Catalan de Monte.'	Spain	<1940	Multi-location (ML) and Lleida (LL)
Barbilla	Landrace	Spain	<1940	ML and LL
Candeal	Landrace	Spain	<1940	ML and LL
Chamorro	Landrace	Spain	<1940	ML
Negrillo	Landrace	Spain	<1940	ML
Initial				
Mara	Autonomia/Aquila	Italy	1947	LL
Impeto	Frassineto-405/Villa-glori	Italy	1950	ML and LL
Estrella	Mon-desir/Ardito//Mouton-a-epi-rouge/k-3/3/Mouton-epi-rouge	France	1952	ML and LL
Pané 247	L-4/Mentana	Spain	1955	ML and LL
Intermediate				
Siete Cerros	Penjamo-62/Gabo-55	CIMMYT	1969	ML
Yecora	Ciano-67(sib)//Sonora-64/Klein-rendidor/3/Siete-cerros-66	CIMMYT	1972	LL
Cajeme	Ciano-67(sib)//Sonora-64/Klein-rendidor/3/Siete-cerros-66	CIMMYT	1972	ML and LL
Anza	Lerma-rojo-64//Norin-10/Brevor/3/3*Andes-enano	CIMMYT/USA	1974	ML and LL
Astral	Fortunato/YGA/3/Florence/Aurore//G-4	France	1974	ML
Marius	Cadet//Thatcher/Vilmorin-27/3/Ariana/Fundulea	France	1980	ML and LL
Rinconada	Unknown	Spain	1981	ML and LL
Alcalá	Unknown	Spain	1984	ML
Cartaya	Kuz/Buho//Kal/BB	CIMMYT	1984	ML
Soissons	Iena/hn-35	France	1990	ML and LL
Modern				
Bancal	Unknown	Spain	1991	ML
Pinzón	Unknown	Spain	1992	ML
Gazul	Unknown	Spain	1992	ML and LL
Etecho	VPM/Talent//Fidel/3/Declic	France	1995	ML
Babui	Unknown	Spain	1997	ML
Berdun	Unknown	France	1998	ML
Isengrain	Apollo/Soissons	France	1998	ML and LL
Craklin	Unknown	France	1999	ML
Califa Sur	Unknown	Spain	2001	ML and LL

* ML = genotypes included in eight multi-location experiments in 2006 and 2007; LL = genotypes included in experiments conducted in Lleida in 2008 and 2009.

selected and further evaluated in four field experiments in Lleida during two additional crop seasons (2008 and 2009) under two contrasting water regimes: irrigated and rainfed. Irrigated experiments received supplementary water inputs of 150 mm in 2008 and 96 mm in 2009 (see details in Table 2).

All plots were sown in a randomized complete block design with three replications. Plots consisted of eight rows 5 m long and 0·15 m apart. Sowing density was 350 seeds/m^2 in the multi-location experiments conducted in 2006 and 2007 and 450 seeds/m^2 in the experiments conducted in Lleida in 2008 and 2009. Experimental details are given in Table 2. Plots were mechanically harvested at ripening.

Data recording

All plots were mechanically harvested at ripening and yield was expressed as 120 mg/g of grain moisture.

Table 2. *Experimental sites, description of the environmental conditions and management practices*

	Multi-location experiments (ML)								Lleida experiments (LL)			
Site	Burgos (NW)		Lleida (NE)		Albacete (SE)		Cordoba (SW)		Lleida (NE)			
Coordinates	42°5′N, 3°39′W		41°40′N, 0°20′E		39°03′N, 02°05′W		37°51′N, 04°48′W		41°40′N, 0°20′E			
Altitude (m asl)	864		200		700		93		200			
Soil texture	Sandy loam		Fine loam		Clay loam		Clay loam		Fine loam			
Soil classification*	Gleyic Cambisol		Calcic Cambisol		Calcic Cambisol		Eutric Fluvisol		Calcic Cambisol			
Harvest years	2006	2007	2006	2007	2006	2007	2006	2007	2008	2008	2009	2009
Water regime	Rainfed	Rainfed	Irrigated	Irrigated	Rainfed	Rainfed	Rainfed	Rainfed	Rainfed	Irrigated	Rainfed	Irrigated
Experiment code	B06	B07	L06	L07	A06	A07	C06	C07	L08R	L08I	L09R	L09I
Environmental conditions from:	*Sowing to heading*								*Sowing to anthesis*			
Thermal time (GDD; °C)	940	986	1170	1399	965	1183	1349	1239	1342	1299	1144	1014
ET_0 (mm)	282	263	347	247	303	344	259	228	273	259	243	239
T_{min} (°C)	0·5	0·4	2·1	3·5	1·0	1·0	6·2	4·8	3·1	3·0	2·3	2·7
T_{max} (°C)	12·1	11·9	12·8	13·8	13·8	14·1	17·0	16·6	14·1	14·2	13·4	12·9
Water input (rainfall + irrigation, mm)	212	172	345	282	106	165	274	174	102	252	183	234
Environmental conditions from:	*Heading to maturity*								*Anthesis to maturity*			
ET_0 (mm)	240	240	262	345	476	454	394	437	170	155	113	160
T_{min} (°C)	9·2	7·7	12·5	13·3	12·4	11·7	14·6	13·2	11·5	11·3	11·8	12·7
T_{max} (°C)	26·5	22·5	28·1	27·4	28·2	29·4	30·1	27·3	23·1	23·0	27·0	27·3
Water input (rainfall + irrigation, mm)	67	145	8·0	194	84	21	33	162	133	133	8·6	54
Weed control	Bromoxinil + Ioxinil + MCPP		Linuron + Trifluralin and Bromoxinil + Ioxinil + MCPP		MCPP and MCPA		Clodinafop-propargyl and Tribenuron-methyl		Linuron + Trifluralin and Bromoxinil + Ioxinil + MCPP			
N applied (kg N/ha)												
Seed bed	24	24	80	0	12	12	45	45	0	0	0	0
Top dressing	85	85	173	78	40	40	129	129	100	90	30	40
P applied (kg P_2O_5/ha)	45	45	150	110	36	36	115	115	128	120	68	113
K applied (kg K_2O/ha)	45	45	150	183	12	12	0	0	213	200	113	188
Sowing date	Dec 12	Dec 1	Nov 19	Nov 15	Dec 20	Dec 6	Dec 22	Dec 12	Nov 20	Nov 19	Nov 24	Nov 27
Harvest date	Jul 4	Jul 23	Jun 23	Jul 12	Jul 17	Jul 27	Jun 30	Jul 12	Jul 10	Jul 14	Jul 13	Jul 1
Mean yield (kg/ha)	2909	5362	4472	6492	1473	2771	3397	4845	4207	5416	5434	7215

GDD=growing degree days.

T_{min} and T_{max} are average minimum and maximum daily temperatures, respectively.

ET_0 is the reference evapotranspiration computed by the Penman–FAO methodology (Allen *et al.* 1998).

* Soil-type classification according to FAO (1998).

Yield components (number of spikes/m^2, number of grains/spike and mean grain weight (MGW)) and the number of plants/m^2 and stems/plant in Lleida experiments, were determined from the whole plants pulled up from a 1 m long sample taken from a central row of each plot before harvesting.

In the experiments conducted in Lleida in 2008 and 2009, 10 plants were randomly selected from the sample in the laboratory for further determinations. Plant height was measured in 10 main tillers from the tillering node to the top of the spike excluding the awns, number of spikelets/spike, number of grains/spikelet and spike length were assessed for main stems and tillers separately. Spikes and grains were weighed after being oven-dried at 70 °C for 48 h. The rest of the sample was used to determine the total number of stems/plant and the proportion of them bearing spikes (fertile tillering) and, after drying, aboveground biomass and grain weight, which were used to calculate HI.

Statistical analysis

Combined ANOVA were performed for all the studied traits. In the model used for the ANOVA of multi-location experiments (2006 and 2007), the genotype effect was partitioned into breeding period considered as a fixed effect and genotype within breeding period considered as a random effect. All analyses were carried out with the SAS-STAT (SAS Institute Inc. 2009) and GENSTAT (Payne *et al.* 2006) statistical packages.

Absolute genetic gain (AGG) and relative genetic gain (RGG) during the 20th century were computed as the slope of the linear regression between the absolute or relative value of the trait and the year of genotype release. Relative values were computed for each genotype as a percentage with regard to the average value of all genotypes.

Stepwise regression analysis was conducted with the RGG for yield in each experiment as the dependent variable and the climatic features depicted in Table 2 as the independent variables. Regression models (linear or bilinear when appropriate, $Y = BX + A$ $(X </> C) + BC$ $(X </> C)$, with the slope of the second segment equal to zero) were also fitted to the relationships between variables and those between variables and the year of release of the genotypes, using the Tablecurve 2D v2.03 software (Jandel 1991).

RESULTS

Yield and yield components

The combined ANOVA of yield data from multi-location experiments revealed a significant effect of most factors (data not shown). The mean yield of the experiments ranged from 1473 kg/ha in Albacete in 2006 (A06) to 6492 kg/ha in Lleida in 2007 (L07, Table 2). When the whole set of experiments was considered together, yield was estimated to increase significantly at a rate of 35·1 kg/ha/yr, which represents an RGG of 0·88%/yr (Table 3, Fig. 1), whereas the regression equation fitted to the relationship between year of release and yield for intermediate and modern genotypes was not significant (Fig. 1). Given the statistical significance of the site × year × genotype (breeding period) interaction, genetic gains in yield and yield components were computed for each experiment. The results indicated great variability between experiments, with AGG for yield ranging from 7·7 kg/ha/yr in A06 to 66·5 kg/ha/yr in C07, and RGG ranging from 0·37 in B07 to 1·59%/yr in C06 (Table 3). The coefficient of determination of the relationship between the mean yield of each experiment and the estimated AGG was $R^2 = 0{\cdot}37$ ($P = 0{\cdot}106$), and that of the relationship between the mean yield of each experiment and the RGG was $R^2 = 0{\cdot}02$ ($P = 0{\cdot}738$). The average daily minimum temperature from sowing to heading was the only variable entered in the stepwise regression model built to explain variations in yield RGG using climatic features as independent variables (Fig. 2).

The number of grains/spike and, to a lesser extent, the number of spikes/m^2 were the yield components most affected by breeding activities during the 20th century, as they increased at a relative rate of 0·60%/yr and 0·30%/yr, respectively, while no significant changes were observed for MGW when data were averaged across experiments (Table 3). However, the estimated genetic gains for yield components also differed greatly between experiments. The number of spikes/m^2 showed significant increases in B06, L06, and C06, while no significant changes were detected in the remaining experiments. Similarly to the findings for yield, increases in the number of grains/spike were significant in all the experiments, with RGG ranging from 0·37%/yr in B07 to 1·08%/yr in C07. Changes in MGW were only significant in three experiments, with RGG ranging from −0·22 to −0·40%/yr (Table 3). Although the genetic gains for yield and yield components depended on the experiment, differences

Table 3. *AGG and RGG in yield and yield components of bread wheat determined in eight multi-location experiments* (D.F.: 25), *with standard deviation* (S.D.) *of yield and yield components AGG and RGG values across environments*

	Yield				Spikes/m^2				Grains/spike				MGW			
Experiment code*	AGG (kg/ ha/yr)	RGG (%/yr)	R^2	P	AGG (spikes/ m^2/yr)	RGG (%/yr)	R^2	P	AGG (grains/ spike/ yr)	RGG (%/yr)	R^2	P	AGG (10^{-3} g/ grain/yr)	RGG (%/yr)	R^2	P
B06	25	0·86	0·48	<0·001	2·27	0·47	0·40	<0·001	0·22	0·87	0·58	<0·001	−0·112	−0·40	0·44	<0·001
B07	20	0·37	0·32	0·003	NS	NS	0·00	0·776	0·11	0·37	0·20	0·020	NS	NS	0·04	0·320
L06	42	0·93	0·70	<0·001	2·14	0·48	0·34	0·002	0·18	0·41	0·37	<0·001	−0·081	−0·22	0·23	0·013
L07	54	0·82	0·70	<0·001	NS	NS	0·09	0·151	0·19	0·54	0·38	<0·001	NS	NS	0·11	0·089
A06	8	0·52	0·29	0·004	NS	NS	0·05	0·284	0·11	0·38	0·15	0·047	−0·110	−0·37	0·40	<0·001
A07	13	0·45	0·15	0·048	–	–	–	–	0·17	0·46	0·21	0·018	NS	NS	0·03	0·394
C06	54	1·59	0·69	<0·001	1·89	0·38	0·24	0·011	0·27	0·78	0·62	<0·001	NS	NS	0·12	0·074
C07	67	1·37	0·80	<0·001	NS	NS	0·08	0·158	0·36	1·08	0·79	<0·001	NS	NS	0·05	0·268
SD	21·8	0·435			0·193	0·055			0·083	0·266			0·0173	0·096		
Overall	35·1	0·88	0·82	<0·001	1·36	0·30	0·28	0·006	0·20	0·60	0·55	<0·001	−0·046	−0·14	0·12	0·079

* Experiment codes: A, Albacete; B, Burgos; C, Cordoba; L, Lleida; 06, 2006; 07, 2007.

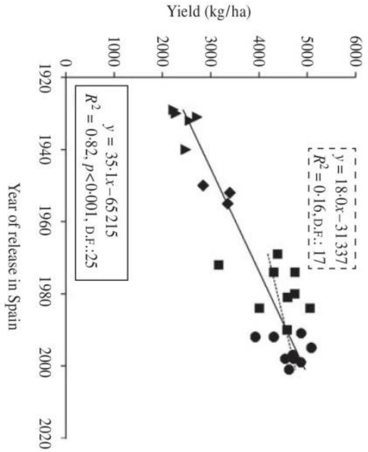

Fig. 1. AGGs in grain yield of 26 bread wheat genotypes released in Spain during the 20th century. Each point was obtained by averaging data of eight environments and three blocks per environment. Breeding periods are identified with triangles for local landraces, diamonds for initial cultivars, squares for intermediate cultivars and circles for modern cultivars. The broken line indicates the gain between intermediate and modern cultivars.

between experiments were of a quantitative nature because all significant rates of change were positive for spikes/m^2 and grains/spike, but negative for MGW. None of the climatic features considered contributed significantly to explaining genetic changes in yield components.

The results showed that differences between breeding periods were significant for yield, grains/spike and MGW, but not for number of spikes/m^2 (data not shown). The breeding period was the factor that explained the largest proportion of variability for grains/spike (data not shown).

Grain yield during the 20th century increased by 88%, but the largest rises occurred before the end of the intermediate period, since no significant differences in yield and yield components appeared between intermediate and modern cultivars (Table 4). The initial cultivars out-yielded the local landraces by 30%, while an additional yield increase of 37% was attained by the intermediate cultivars. Yield gains were due to increases of 20% in the number of spikes/m^2 and of 65% in the number of grains/spike, but a reduction of 10% in MGW. The improved yield of initial cultivars in comparison with the landraces was due to their greater number of grains/spike (which increased by 58%), despite the fact that the number of spikes/m^2 was similar and MGW was 16% lower (Table 4). Changes in yield components between

Table 4. *Mean values of yield and yield components for 26 bread wheat genotypes released in different periods in Spain and grown in eight multi-location experiments. Numbers in parentheses indicate the percentage of change with respect to the previous period. The standard error of the differences (S.E.D.) of the means of yield and yield components are presented*

Breeding period	D.F.	Yield (kg/ha)	Spikes/m^2 *	Grains/spike	MGW (10^{-3} g)
Landraces	4	2457	401	22·3	36·7
Initial	2	3198 (30)	432 (7·7)	35·2 (58)	30·7 (− 16)
Intermediate	8	4397 (37)	459 (6·2)	36·8 (4·5)	33·4 (8·8)
Modern	8	4628 (5·2)	483 (5·2)	36·7 (− 0·3)	33·0 (− 1·2)
S.E.D.		274	39·5	2·45	1·92

* The number of spikes/m^2 could not be determined in A07.

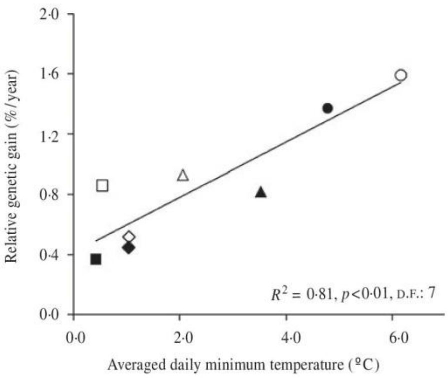

Fig. 2. Relationship between the RGG for yield and the average daily minimum temperatures from sowing to heading. Each point corresponds to one of eight experiments identified as follows: squares, Burgos; diamonds, Albacete; triangles, Lleida and circles, Cordoba. Open and solid symbols correspond to experiments conducted in 2006 and 2007, respectively.

initial and intermediate cultivars ranged from 4·5% for grains/spike to 8·8% for MGW, but none of them were significant.

Biomass and its partitioning

Data from four experiments conducted in Lleida during 2 years under two water regimes (L08R, L08I, L09R and L09I) with a subset of 16 representative genotypes were used to assess the changes caused by breeding on biomass production and allocation. The results indicate that yield gains were due to increases in HI, with aboveground biomass being unchanged (Fig. 3*a*–*d*). HI increased from 0·24 for the landrace Aragon 03 to 0·48 for the newest cultivar, Califa Sur. However, improvements in HI were not linear across the century, as a plateau in HI was reached after 1961 (Fig. 3*b*). Although no significant relationship was found between yield or year of release and biomass when the 16 genotypes were considered together, positive and significant relationships between biomass and yield and between biomass and year of genotype release appeared when landraces were removed from the analyses (broken lines in Fig. 3*c*, *d*). Crop biomass increased significantly and linearly from initial cultivars to modern ones, but the great variability within periods for this trait prevented differences between breeding periods from being significant in the ANOVA (data not shown).

The average number of plants/m^2 at maturity was 380 in 2008 and 416 in 2009, without significant differences between genotypes (data not shown). The introduction of initial cultivars reduced the number of stems/plant at maturity (Fig. 3*f*), but caused a rise in the proportion of stems bearing spikes (Fig. 3*g*, *h*), which thereafter remained unchanged. Initial cultivars were also a first step in the reduction of plant height, which decreased steadily until the end of the century (Fig. 3*i*, *j*). The increase in fertile tillering was associated with increases in HI of up to 0·32 (Fig. 4*a*), and with a decrease in the total number of stems/plant that started with the introduction of the first improved cultivars (Fig. 4*c*). In addition, increases in HI beyond 0·36 were associated with a reduction in plant height that started with the release of initial cultivars (Fig. 4*b*).

Spike components

The contribution of the main and secondary spikes to yield improvement during the 20th century was investigated in the experiments conducted in Lleida in 2008 and 2009 with 16 genotypes. The weight at ripening of main and secondary spikes increased

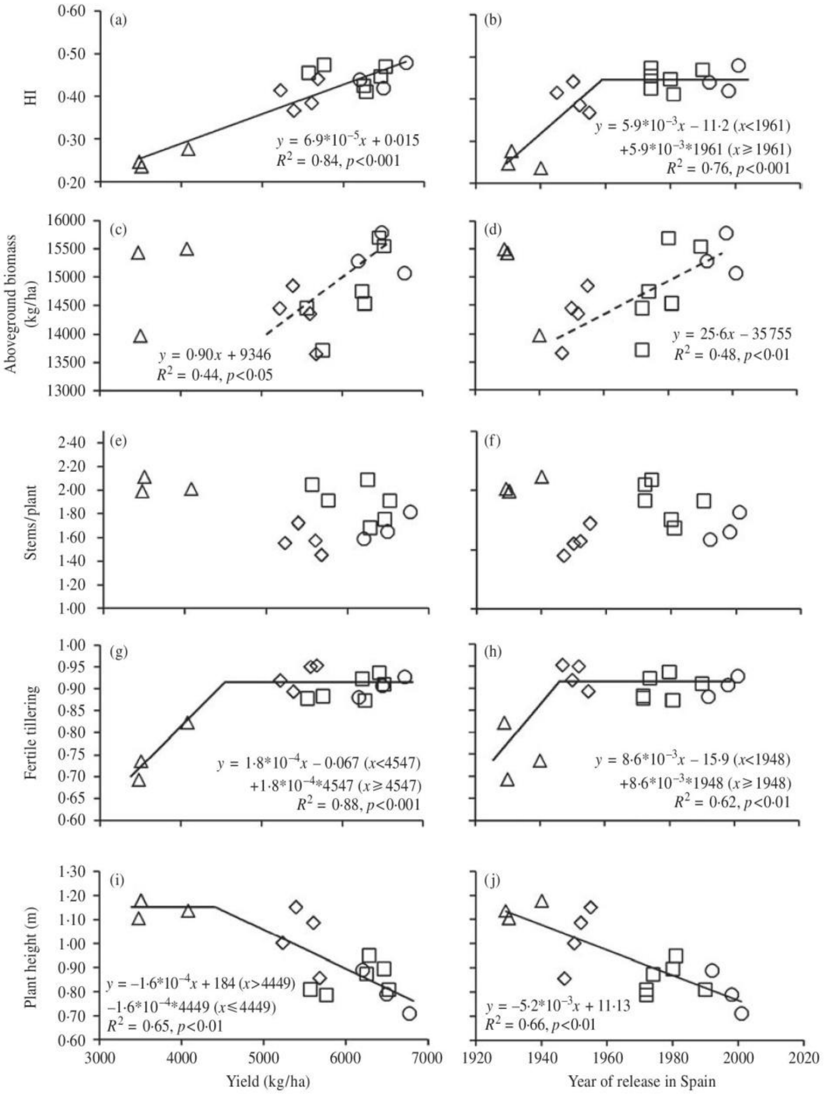

Fig. 3. Relationship between HI, aboveground biomass, fertile tillering, number of stems/plant at maturity and plant height and both yield and year of release of 16 bread wheat genotypes widely grown in Spain during the 20th century. Each point represents the mean value of one genotype across four experiments. Breeding periods are identified with triangles for local landraces, diamonds for initial cultivars, squares for intermediate cultivars and circles for modern cultivars. Broken lines indicate relationships without considering local landraces (D.F.: 15).

significantly during the last century, with RGG of 0·57%/yr and 0·75%/yr, respectively (Fig. 5*a*). These increases were mainly based on a boost in the number of grains/spike, which accounted for RGG of 0·58%/yr and 0·70%/yr in main and secondary spikes, respectively (Fig. 5*b*), while no significant changes in MGW were observed (Fig. 5*c*). From a breeding historical perspective, the largest gain in spike weight occurred with the introduction of initial cultivars, which caused an increase of 31% in main spike weight

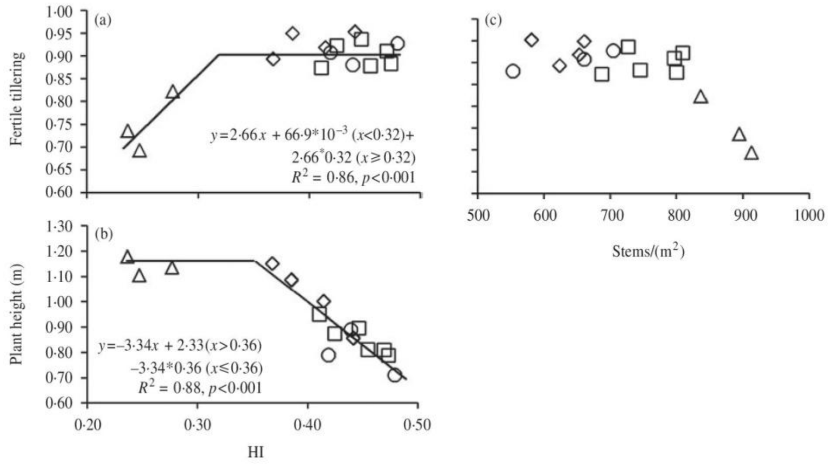

Fig. 4. Relationships between (*a*) HI and fertile tillering, (*b*) HI and plant height, and (*c*) fertile tillering and number of stems/m², of 16 bread wheat genotypes widely grown in Spain during the 20th century. Each point represents the mean value of one genotype across four experiments. Breeding periods are identified with triangles for local landraces, diamonds for initial cultivars, squares for intermediate cultivars and circles for modern cultivars. Equations and R^2 values are only presented for significant relationships at $P<0{\cdot}05$ (D.F.: 15).

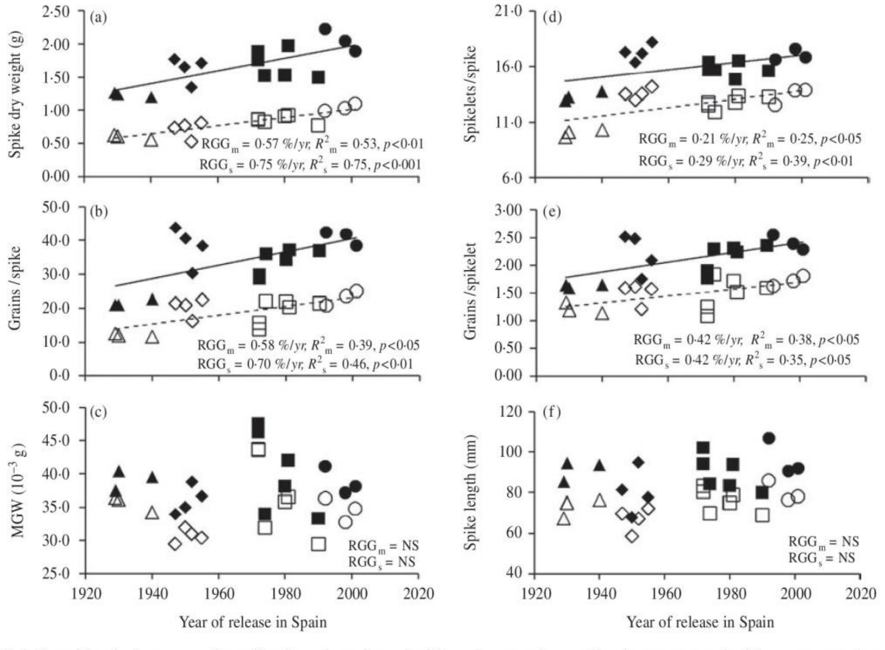

Fig. 5. Relationships between main spike (m, closed symbols) and secondary spike (s, open symbols) components and year of release of 16 bread wheat genotypes widely grown in Spain during the 20th century. Each point represents the mean value of one genotype across four experiments. Breeding periods are identified with triangles for local landraces, diamonds for initial cultivars, squares for intermediate cultivars and circles for modern cultivars (D.F.: 15). RGG = relative genetic gain.

and an increase of 77% in the number of grains/spike. The rise in the weight of secondary spikes was evenly distributed during the century.

The increases in the number of grains/spike were due to improvements in both the number of spikelets/spike, which increased at a rate of 0·21%/yr and 0·29%/yr on main stem and tillers, respectively (Fig. 5*d*), and the number of grains/spikelet, which increased at a rate of 0·42%/yr in both types of spike (Fig. 5*e*). Genetic gains in the number of grains of main spikes contributed by initial cultivars were related to the improvement of both the number of spikelets/spike, which increased by 29%, and the number of grains/spikelet, which rose significantly, by *c*. 37%.

No changes in spike length were recorded (Fig. 5*f*). The slopes of the linear models fitted for each spike component were compared between main and secondary stems, but no significant differences between them were found (data not shown).

DISCUSSION

The evaluation of historical series of genotypes is a common approach used in several countries to quantify the achievements of past breeding efforts (Austin *et al.* 1989; Perry & D'Antuono 1989; Donmez *et al.* 2001; Morgounov *et al.* 2010). The present study examined a set of 26 bread wheat genotypes widely grown in Spain in different periods during the 20th century, in order to estimate the genetic gain in yield and to understand its causes through the identification of crop traits associated with yield improvements. The AGG for yield was 35·1 kg/ha/yr, which equated to an RGG of 0·88%/yr. These results are comparable or slightly higher than those reported by previous studies conducted with bread wheat in other countries and with durum wheat in Spain (Royo *et al.* 2008). RGG for yield has been reported to be of 0·51%/yr in the UK from 1900 to 1985 (Austin *et al.* 1989), 0·66%/yr in France from 1962 to 1988 (Brancourt-Hulmel *et al.* 2003), 0·68%/yr in the United States (Cox *et al.* 1988) and 0·70%/yr in Siberia (Morgounov *et al.* 2010). The fact that some of the genotypes most widely grown in Spain during the last century were derived from CIMMYT germplasm may explain the coincidence between the yield RGG estimated in the present study and the 0·88%/yr reported by CIMMYT for the period 1962–88 (Sayre 1996).

When RGG for yield was calculated for each of the eight environments in which field experiments were conducted, the range of variation was greater than 400%, suggesting a very high dependence of the estimated genetic gains on the environmental conditions. Previous studies have attributed these dissimilarities to the different yield potential of the environments (Brancourt-Hulmel *et al.* 2003), to factors affecting old and modern genotypes in an opposite way, such as the incidence of host-specific pathogens (Cox *et al.* 1988), and to the environmental characteristics that induce genotype × environment interactions (GEI). GEI is a main constraint in assessing genetic gains, especially in countries with great environmental variations and large yield fluctuations between years, as is typical in Mediterranean environments. The bias created by the assessment of yield gains in only one environment was overcome in the present study by conducting experiments under a wide range of environmental conditions involving the main wheat-growing areas of Spain. In the present study, the differences between the mean yield of the eight environments explained 0·37 of the variations in yield AGG. Although this value was not significant, probably due to the limited number of environments involved in the present study, it accounted for a considerable proportion of the observed variability, thus showing a tendency of the mean yield of the environment to partially explain differences in the estimated AGG in yield.

In contrast, variations in yield RGG across environments were largely explained by the average minimum daily temperatures from sowing to heading (0·81 of the total RGG variance). The lowest RGG were recorded in the environments with the lowest minimum temperatures before heading independently of the mean yield of the environment, indicating that the yield differences between landraces and improved cultivars increased as the minimum daily temperatures before heading increased. In view of the results of a previous study conducted with the same set of genotypes (Sanchez-Garcia *et al.* 2012), this result may be interpreted in adaptation terms as: (i) the specific adaptation of Spanish landraces to cold environments, (ii) the wider adaptation of modern cultivars in comparison with local landraces and (iii) the good performance of modern spring genotypes in warm Spanish environments. Average minimum daily temperature has been reported to be a major factor for wheat yield formation under Mediterranean conditions (Siddique *et al.* 1990; Le Houerou 2004) and is among the main variables determining GEI on yield in Spain (Voltas *et al.* 2005; Sanchez-Garcia *et al.* 2012). The importance of temperature before heading in adaptation of wheat to Mediterranean conditions has also been demonstrated in durum wheat (Royo *et al.* 2010).

Of the three main yield components, the number of grains/spike was the one most related to wheat breeding achievements, as it explained up to 0·67 of yield increases in Spain during the last century, the remaining 0·33 being explained by the number of spikes/m^2, while no consistent changes were observed in MGW. The relationship between the number of grains/spike and genetic gains in yield is universally accepted in bread and durum wheat, as it has been identified in many studies involving a wide range of environmental conditions (Waddington *et al.* 1986; Austin *et al.* 1989; Perry & D'Antuono 1989; Canevara *et al.* 1994; Donmez *et al.* 2001; Royo *et al.* 2007). In the present study, increases in yield and number of grains/spike were significant in all the environments, while increases in number of spikes/m^2 were not consistent in all of them. This could be associated with the larger GEI component of the variance recorded for number of spikes/m^2 (0·28 of total variation) in comparison with yield (0·14) and number of grains/spike (0·18). The non-significant differences between breeding periods in the number of spikes/m^2 could also be related to the data missing in one of the experiments. Moreover, increases in the number of spikes/m^2 were only significant in three environments characterized by dry and warm grain-filling periods, which probably caused a reduction in the tiller survival in the landraces, the latest-heading genotypes (Sanchez-Garcia *et al.* 2012), which increased the differences in spikes/m^2 between landraces and improved cultivars. The great environmental effects on the number of spikes/m^2 found in the present study are consistent with the variability of results of previous studies evaluating genetic changes in spikes/m^2 over time, which range from a steady amount (Waddington *et al.* 1986; Calderini *et al.* 1995) to large increases (Austin *et al.* 1989; Donmez *et al.* 2001; Shearman *et al.* 2005), with slight improvements also being reported from experiments conducted under Mediterranean conditions (Perry & D'Antuono 1989; Canevara *et al.* 1994; Royo *et al.* 2007).

The classification of genotypes into historical breeding periods made it possible to identify two main episodes contributing to genetic improvements in yield in Spain during the last century. The first one, which caused a yield increase of 30%, was the transition from traditional landraces to the first cultivars released from the earliest Spanish breeding programmes and the improved cultivars introduced in Spain from foreign countries, mainly Italy and France during the 1950s. The increase estimated for this period matches with the improvement that occurred in 1956 in the wheat yield of Spanish farms (Calderini & Slafer 1998). The reduction in plant height during this period was accompanied by an increase in the proportion of tillers bearing spikes and a huge rise in HI. The yield rise derived from the introduction of initial cultivars was partially caused by an improvement of *c.* 58% in the number of grains/spike despite a reduction in grain weight of *c.* 16% in comparison with local landraces. Three of the four initial cultivars included in the present study (Estrella, originally named Etoile-de-Choisy, Impeto and Mara) carry the *Rht8c* dwarfing gene and probably the photoperiod-insensitive gene *PpD1* (Worland *et al.* 1998), which have been reported to reduce plant height, enhance spike fertility, and consequently improve grain yield (Borojevic & Borojevic 2005). The present results indicate that the great increase in the grain number of initial cultivars was sufficient to compensate for the reduction in grain weight, thus resulting in higher yields. These results evidence the success of the work conducted by wheat breeding pioneers, such as Nazareno Strampelli in Italy (Salvi *et al.* forthcoming) and Josep Pané in Spain.

A second relevant breeding episode occurred with the introduction, in the late 1960s, of cultivars derived from CIMMYT semi-dwarf germplasm, such as the widely grown cvars Siete Cerros, Yecora, Cajeme, Anza and Cartaya, and of European cultivars of winter growth-habit, such as the French cvars Marius and Soissons. This new germplasm further reduced plant height and increased the number of tillers/plant while maintaining their rate of fertility. This transition brought a yield increase of 37% in comparison with the initial cultivars, but it was due to slight increases in all three main yield components rather than significant rises in any of them. The wide adaptation of foreign cultivars released during this period (Sanchez-Garcia *et al.* 2012) may partially explain their great success in terms of both productivity and adoption by farmers. The evidence of a non-significant yield increase during the period 1970–2000 suggests that a plateau was reached for yield gains in Spain during the last 30 years of the century, agreeing with the results reported for other countries where slight or non-significant yield increases were observed (Sener *et al.* 2009; Fischer & Edmeades 2010).

One of the physiological approaches used to understand yield formation consists of considering yield as a function of the biomass per unit area at maturity and the HI or proportion of the aboveground biomass partitioned to grain (yield = aboveground biomass × HI). The introduction of initial cultivars during the 1950s did not significantly modify the crop biomass, but caused a 60% increase in HI, which rose from 0·25 to 0·40. The huge improvement in HI in this first stage could be associated with three factors: (i) a reduction in plant height of *c*. 10%, very probably due to the presence in initial cultivars of *Rht8c* and *PpD1* genes, (ii) a 28% decrease in the number of stems/plant and (iii) an increase from 0·75 to 0·93 in the proportion of stems bearing spikes. Tillering reduction has been described as an advantageous trait under terminal drought stress, as it diminishes soil water use prior to anthesis (Richards 1988; Duggan *et al.* 2005). A decrease in plant height and the number of stems/plant and an increase in the proportion of them bearing spikes probably reduced competition between the growing spikes and the growing stems, enhancing the partitioning of assimilates to the spike (Fischer & Stockman 1986) and thus resulting in an increase in the weight of main spikes. Improvements in the number of spikelets/spike and in grain setting were not accompanied by an enlargement of the spikes, so spike compactness augmented. Increases in spike fertility and decreases in grain weight have been reported to be pleiotropic effects of the *Rht8c* dwarfing gene and the photoperiod insensitive gene *PpD1* (Canevara *et al.* 1994; Borojevic & Borojevic 2005). The efforts made by local breeders during the early decades of the 20th century to increase grain setting probably improved the adaptation of wheat to Mediterranean environments, where abiotic stresses during grain filling are the main cause of floret abortion (Álvaro *et al.* 2008*b*).

The introduction of intermediate cultivars caused a slight, not significant, increase in aboveground biomass, but caused a second, smaller rise in HI, which reached values of *c*. 0·45 that were not further improved by the cultivars released during the 1990s. Rises in HI during this phase could be explained by a further reduction of 17% in plant height and increases of *c*. 55% in the number of tillers/plant, while their rate of fertility was maintained. Given the limited scope for raising HI beyond 0·50 (Fischer & Edmeades 2010), further yield improvements must rely on biomass production, as was reported for the cultivars released in the UK between 1983 and 1995 (Shearman *et al.* 2005). However, this does not seem to be the case in Spain, where neither the yield changes nor the 4·0% increase in biomass during the last decade of the century were significant.

The great differences induced by the environment in the rates of genetic gain found in the present study may have important implications in the current scenario of global climate change. The 1·8 °C rise in average global temperature predicted for 2050 (Meehl *et al.* 2007) will force farmers to grow genotypes which are more tolerant, among other factors, to high temperatures (Ceccarelli *et al.* 2010; Peltonen-Sainio *et al.* 2011). The large effect of the climatic variables, particularly temperature, over the estimated genetic gains of the present study emphasizes the role played by adaptation mechanisms over past breeding achievements, and the need to continue their improvement to face future environmental changes. The highest genetic yield gains observed in environments with greater minimum temperatures before heading suggest that the genotypes released recently in Spain are better adapted than the old ones to high temperatures. Therefore, the adaptation trends related to breeding progress in Spain during the last century appear to be in the right direction to meet the expected temperature increases.

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CHAPTER 2

Breeding affected the pattern of adaptation of bread wheat varieties to Spanish environments

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Breeding effects on the genotype × environment interaction for yield of bread wheat grown in Spain during the 20th century

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ABSTRACT

This study evaluates the changes caused by breeding in the genotype × environment interaction of the bread wheat varieties most widely cultivated in Spain during the 20th century. A set of 27 varieties was tested in 8 environments representative of the bread wheat growing areas in the country. Minimum temperature and thermal time from sowing to heading were the main environmental traits determining genotype × environment (GE) interactions for yield. Landraces were the least productive and showed specific adaptation to environments with low minimum temperatures before heading and high relative air humidity after heading. Yield consistency was low in landraces due to large environmental effects on the number of spikes and grain weight. Old-bred varieties (released between 1945 and 1955) were the first step towards breeding for wide adaptation due to the lower dependence of their final spike number and grain weight on water availability during grain filling. Differences in the adaptation pattern of the varieties introduced since the 1960s were caused by their growth habit. Winter types were more widely adapted than spring ones, mostly because they had a more consistent number of grains per spike. Grain weight of winter varieties was enhanced in environments with high relative humidity during grain filling. Spring varieties had more grains per spike in environments with high minimum temperatures and thermal time before heading. The number of grains per spike was the yield component that most closely followed the pattern of adaptation observed for grain yield.

1. Introduction

Understanding how breeding has affected the pattern of adaptation of introduced cultivars to target regions is an essential step towards improving current knowledge on wheat yield-limiting factors and designing future breeding strategies. The comparison of old cultivars and modern adapted varieties within representative historical series is a common approach for evaluating wheat breeding progress and identifying traits related to yield improvement (Austin et al., 1980; Donmez et al., 2001; Royo et al., 2007, 2008).

Most studies addressing genetic gains in wheat yield have focused on changes in yield components or relevant physiological traits (Álvaro et al., 2008a; Austin et al., 1989; Donmez et al., 2001; Waddington et al., 1986). However, these studies, usually based on multi-environment experiments, have rarely addressed genotype × environment (GE) interactions, so little is known about the relationship between genetic gains in yield and the pattern of adaptation of improved varieties.

In the Mediterranean Basin, where yield fluctuations between seasons are large, and abiotic stresses are the main constraints to yield formation, breeding success is hindered by the different sensitivities of the genotypes to the environmental factors (Cooper and Byth, 1996), expressed as GE interaction. Under these conditions, understanding the adaptation mechanisms and identifying the main plant traits responsible for GE is crucial for the development of new cultivars with improved yield potential and a sustained performance across seasons and environments.

Some authors have suggested that yield gains in cereals, resulting from the increased capacity of modern varieties to respond to non-limiting environments, have been globally followed by a trend towards specific adaptation (Pswarayi et al., 2008; Royo et al., 2008). However, the breeding programs of CIMMYT (International Wheat and Maize Improvement Center) have based their approach for wide adaptation on selection in environments with high potential (Braun et al., 1997), and yield improvements under near-optimal conditions have also brought yield increases in limiting environments (Araus et al., 2002; Trethowan et al., 2002).

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Yield gains under limiting conditions have been attributed to the selection of varieties with both low GE interaction and high yield potential (Cattivelli et al., 2008).

Bread wheat (*Triticum aestivum* L.) is grown on 1.45 Mha in Spain, which represents 7.2% of the area devoted to wheat cultivation in the EU-25 (EUROSTAT, 2010). Due to its latitude (between 36°00′ and 43°50′) and its location in a peninsula between the Atlantic Ocean and the Mediterranean Sea, Spain is a country with a great variety of climatic types, ranging from typical Mediterranean to Atlantic. In general, precipitation is limited and irregularly distributed. From a climatic point of view, two main areas of wheat cultivation may be distinguished: the North and the South. In the North the climate is a blend of Mediterranean and Continental, with cold winters and temperate summers, while in the South typical Mediterranean environments, characterized by warm to hot dry summers and mild winters, predominate (Royo and Briceño-Félix, 2011). Winter varieties are grown in the north, while facultative and spring varieties are grown in the south (Royo and Briceño-Félix, 2011). Consequently, Spanish wheat growing areas cover a wide range of climatic conditions and wheat adaptation patterns, resulting in significant GE interactions (Voltas et al., 2005).

Wheat breeding activities in Spain started in the early 20th century with the selection of local landraces and the initiation of breeding programs (Royo and Briceño-Félix, 2011). Throughout the century, foreign germplasm was introduced. French and Italian varieties arrived in the first half of the century, but the largest rise in overall productivity occurred after 1960, primarily as a consequence of the introduction of CIMMYT semi-dwarf germplasm (Royo and Briceño-Félix, 2011). Even now, varieties introduced from neighboring countries are among the most widely cultivated. However, the physiological bases of yield gains and the environmental variables determining the pattern of adaptation of the modern varieties remain basically unknown.

The objectives of this research were: (i) to ascertain the changes caused by breeding activities on the GE interaction of bread wheat, and (ii) to identify the climatic variables involved in their differential response to environmental conditions. A set of 27 varieties among the most widely cultivated in Spain in different periods during the 20th century was assembled for this study.

2. Material and methods

2.1. Plant material

A set of 27 bread wheat varieties representative of the most widely grown in Spain in different periods of the 20th century was assembled. On the basis of their growth habit and period of cultivation they were classified as: (i) local landraces (LR); (ii) old-bred varieties (OB), including those improved and grown before the Green Revolution; (iii) intermediate spring varieties (IS), mostly derived from semi-dwarf CIMMYT germplasm; (iv) modern spring varieties (MS); and finally (v) and (vi) subpopulations formed by winter varieties of French origin corresponding to intermediate (IW) and modern periods (MW), respectively (Table 1).

2.2. Experimental setup

Eight field experiments were conducted in Spain during two consecutive crop seasons, 2005–2006 and 2006–2007, at four locations representative of the main bread wheat growing areas: Burgos, in the north, with cold winters and mild summers; Lleida in the north-east, under irrigated conditions and moderate terminal stress; Albacete in the south-east, with cold winters and very hot and dry summers; and Cordoba in the south with warm winters and very hot summers (Table 2).

Experiments were sown in a randomized complete block designs with three replications. Plots consisted of eight 5-m-long rows, 0.15 m apart. Sowing density was 350 seeds m^{-2}. All the experiments were fertilized and the pests, weeds and diseases were controlled according to standard cultural practices.

2.3. Environment characterization

Environments were characterized on the basis of agro-climatic variables measured from sowing to heading (Zadoks stage 55; Zadoks et al., 1974) and from heading to maturity (Zadoks stage 92). Environmental variables were daily averages from data recorded at weather stations placed at each site. The following variables were measured from sowing to heading (SH) and from heading to maturity (HM): length of the period, expressed as thermal time (TT, GDD); water input (WI, mm); average daily minimum and maximum temperatures ($T_{\min}$, $T_{\max}$; °C); reference evapotranspiration (ET_0, mm); and average maximum and minimum daily relative air humidity ($Rh_{\max}$, $Rh_{\min}$, %).

2.4. Data recording

Plots were mechanically harvested at ripening and yield was expressed at 12% of grain moisture. Yield components (number of spikes per m^2, number of grains per spike and thousand kernel weight) were determined from the plants contained in a 1-m-long sample taken from a central row of each plot before harvesting.

2.5. Statistical analysis

In order to characterize the environments, principal component analysis (PCA) was conducted with the climatic variables measured in each environment. Combined analyses of variance across environments were conducted for yield and yield components.

The GE interaction was partitioned according to the AMMI model (Gauch and Zobel, 1997). The number of bilinear terms retained in the analysis was determined according to the proportion of the sum of squares (SS) of the GE interaction explained by each IPCA.

Factorial regression analyses were conducted to determine the effect of specific environmental covariables on the phenotypic performance. In order to establish their relative importance, each environmental covariable was implemented in a one-covariable factorial regression model. An F test was carried out for each variable considering the unexplained GE variation as denominator (Voltas et al., 2005). Only variables with $F > 1$ were selected. The best model was fitted from all possible combinations of selected variables. Variables corresponding to the sowing-heading period were included in the model prior to the ones related to the grain filling period.

Analyses were carried out with the SAS-STAT statistical package (SAS Institute Inc., 2009) and GENSTAT (Payne et al., 2006) software.

3. Results

3.1. Environment characterization

The first two axes of the PCA biplot, summarizing the relationships between the environmental variables and the environments, explained 70.8% of total variation (Fig. 1). The first principal component (PC), which explained 46.5% of total variance, separated environments B06 and B07 from the rest. The eigenvalues of the PCA showed that environments B06 and B07 were characterized by lower temperatures during grain filling (HM) and also before heading (SH), as well as lower reference evapotranspiration from heading to maturity, than the remainder environments.

Table 1
Characterization of the 27 genotypes used in this study. Yield, yield components and days to heading are means across environments.

Variety	Pedigree	Origin	Year of release in Spain	Yield ± SE (kg ha^{-1})	Spikes m^{-2} ± SE[a]	Grains spike^{-1} ± SE	TKW ± SE (g)	Days to heading ± SE
Landraces (LR)								
Aragon 03	Selection of the landrace "Catalan de Monte"	Spain	<1940	2486 ± 221	413 ± 24.9	23.4 ± 1.23	33.1 ± 1.39	144 ± 5.85
Barbilla	Landrace	Spain	<1940	2231 ± 286	353 ± 17.0	23.3 ± 1.55	37.8 ± 1.60	144 ± 5.72
Candeal	Landrace	Spain	<1940	2281 ± 275	371 ± 23.7	21.5 ± 1.26	36.8 ± 1.71	142 ± 5.58
Chamorro	Landrace	Spain	<1940	2721 ± 352	425 ± 36.6	23.2 ± 1.35	38.6 ± 1.29	145 ± 6.17
Negrillo	Landrace	Spain	<1940	2567 ± 288	444 ± 32.9	20.3 ± 1.07	37.1 ± 1.5	146 ± 5.93
Old bred (OB)								
Impeto	Frassineto-405/Villa-glori	Italy	1950	2845 ± 268	444 ± 30.3	35.6 ± 2.21	27.2 ± 1.22	133 ± 6.01
Estrella	Mon-desir/Ardito//Mouton-a-epi-rouge/k-3/3/Mouton-epi-rouge	France	1952	3395 ± 335	459 ± 25.6	30.8 ± 1.55	34.2 ± 1.36	136 ± 5.94
Pané 247	L-4/Mentana	Spain	1955	3352 ± 309	394 ± 24.6	39.3 ± 2.32	30.5 ± 1.28	136 ± 6.49
Intermediate spring (IS)								
Siete Cerros	Penjamo-62/Gabo-55	CIMMYT	1969	4385 ± 375	423 ± 25.7	42.8 ± 1.72	30.5 ± 1.12	134 ± 5.77
Cajeme	Ciano-67(sib)//Sonora-64/Klein-rendidor/3/Siete-cerros-66	CIMMYT	1972	3166 ± 387	402 ± 30.6	28.7 ± 1.83	39.0 ± 1.62	132 ± 5.09
Anza	Lerma-rojo-64//Norin-10/Brevor/3/3*Andes-enano	CIMMYT/USA	1974	4743 ± 403	545 ± 33.6	35.1 ± 1.41	31.2 ± 1.19	131 ± 5.42
Rinconada	Unknown	Spain	1981	4591 ± 394	417 ± 22.5	34.3 ± 1.48	37.1 ± 1.35	129 ± 5.57
Alcalá	Unknown	Spain	1984	5055 ± 418	465 ± 24.9	40.5 ± 1.40	34.7 ± 1.49	132 ± 5.94
Cartaya	Kavkaz/Buho(sib)//Kalyansona/Bluebird	CIMMYT	1984	4009 ± 402	386 ± 25.2	43.8 ± 1.24	31.8 ± 1.31	132 ± 5.58
Modern spring (MS)								
Bancal	Unknown	Spain	1991	4874 ± 428	445 ± 23.3	34.8 ± 1.43	37.5 ± 1.36	132 ± 5.54
Pinzón	Unknown	Spain	1992	3923 ± 366	383 ± 15.1	37.8 ± 1.92	34.7 ± 1.48	132 ± 5.47
Gazul	Unknown	Spain	1992	4305 ± 343	411 ± 16.5	39.3 ± 1.61	34.2 ± 1.17	131 ± 5.45
Babui	Unknown	Spain	1997	4706 ± 420	520 ± 36.1	34.6 ± 1.56	33.2 ± 1.28	132 ± 5.60
Califa Sur	Unknown	Spain	2001	4623 ± 395	468 ± 21.9	37.0 ± 1.62	33.1 ± 1.28	131 ± 5.15
Intermediate winter (IW)								
Astral	Fortunato/YGA/3/Florence/Aurore//G-4	France	1974	4302 ± 381	482 ± 34.3	36.2 ± 1.40	32.8 ± 1.54	136 ± 5.75
Marius	Cadet//Thatcher/Vilmorin-27/3/Ariana/Fundulea	France	1980	4742 ± 374	479 ± 29.8	31.5 ± 1.15	34.7 ± 1.36	134 ± 5.97
Soissons	Iena/HN35	France	1990	4580 ± 433	536 ± 32.2	38.1 ± 1.13	28.9 ± 1.04	139 ± 5.61
Modern winter (MW)								
Etecho	VPM/Talent//Fidel/3/Declic	France	1995	5087 ± 368	468 ± 33.0	36.0 ± 1.13	34.3 ± 1.39	136 ± 5.99
Berdun	Unknown	France	1998	4719 ± 484	585 ± 43.7	38.9 ± 1.62	28.8 ± 1.49	136 ± 5.67
Isengrain	Apollo/Soissons	France	1998	4544 ± 352	463 ± 28.5	39.4 ± 1.48	30.2 ± 1.03	138 ± 5.58
Craklin	Unknown	France	1999	4867 ± 494	602 ± 47.3	32.1 ± 1.24	31.2 ± 0.91	137 ± 5.53
Cezanne	Thesee/87B29	France	2000	4938 ± 416	–	–	–	136 ± 6.10

[a] The number of spikes per m^2 could not be determined in A07 and so data are means of seven environments.

The second PC, explaining 24.3% of total variation, was mostly related to water input and minimum relative humidity from heading to maturity in the positive direction and to reference evapotranspiration from sowing to heading in the negative direction. PC2 separated environments B07, L07 and C07 – mainly characterized by high water input during grain filling – from B06, L06, A06 and A07, which underwent high evapotranspiration before heading (Fig. 1).

3.2. GE interaction for yield

Mean environmental yields ranged from 1491 kg ha^{-1} (A06) to 6557 kg ha^{-1} (L07) (Table 2), while variety yields across environments ranged from the 2231 kg ha^{-1} of the landrace Barbilla to the 5087 kg ha^{-1} of the modern winter variety Etecho (Table 1). Cycle length until heading was greater for the landraces than for the other subpopulations (Table 1). Days to heading were negatively related to yield ($r = -0.65$, $P < 0.0001$).

The ANOVA (Table 3A) showed that the effects of environment, variety and GE interaction explained 57.1%, 21.2% and 14.5% of yield variance, respectively. Differences between subpopulations accounted for more than 85% of the SS of the variety effect, while the between subpopulations × environment (SPE) interaction explained 60% of the GE variance (Table 3A).

The first two interaction principal component axes (IPCAs) of the AMMI model explained 75% of the GE interaction, while the third one explained an additional 9.2% (Table 3B). The biplot of the first two IPCAs of the AMMI model (Fig. 2) shows that IPCA1, which accounted for 54.7% of the GE variation, separated the varieties into four groups corresponding to local landraces (LR), old-bred Spanish varieties (OB) and two partially overlapping clusters, corresponding to intermediate and modern winter and spring varieties. The second IPCA explained 20.3% of the SS of the GE interaction and contributed most to separating some winter varieties from the spring ones. Variability among winter varieties appeared to be greater for modern than for intermediate genotypes, while no differences in variability were found among spring varieties of different periods (Fig. 2).

The position of the environments within the biplot of the AMMI analysis indicates that the landraces performed better in A06, A07 and B07 than in the remainder of the environments, while the old-bred varieties, and particularly *cv.* Impeto (located in the upper part of Fig. 2), seemed to be favored by environment B06 (Fig. 2). Both intermediate and modern spring varieties were well adapted to L06 and C06, while winter modern germplasm had larger yields in L07. The location of the winter intermediate subpopulation close to the axis origin in Fig. 2 indicates that it was the most widely adapted.

Table 2
Localization and description of the 8 Spanish environments included in this study.

Site	Burgos (N)		Lleida (NE)		Albacete (SE)		Cordoba (S)	
Coordinates	42°05′N, 3°39′W		41°40′N, 00°20′E		39°03′N, 02°05′W		37°51′N, 04°48′W	
Altitude (m.a.s.l.)	864		200		700		93	
Soil texture	Sandy loam		Fine loam		Clay loam		Clay loam	
	2006	2007	2006	2007	2006	2007	2006	2007
Trial identification	B06	B07	L06	L07	A06	A07	C06	C07
Environmental conditions from sowing to heading (SH)								
Thermal time (°C)	940	986	1170	1399	965	1183	1349	1239
ET_0 (mm)	282	263	347	247	303	344	259	228
T_{min} (°C)	0.5	0.4	2.1	3.5	1.0	1.0	6.2	4.8
T_{max} (°C)	12.1	11.9	12.8	13.8	13.8	14.1	17.0	16.6
Rh_{min} (%)	49.0	52.7	56.1	57.7	46.6	48.6	51.5	49.2
Rh_{max} (%)	89.9	90.8	92.8	92.6	92.6	92.4	93.5	91.8
Water input (rainfall + irrigation, mm)	212	172	345	282	106	165	274	174
Environmental conditions from heading to maturity (HM)								
ET_0 (mm)	240	240	262	345	476	454	394	437
T_{min} (°C)	9.2	7.7	12.5	13.3	12.4	11.7	14.6	13.2
T_{max} (°C)	26.5	22.5	28.1	27.4	28.2	29.4	30.1	27.3
Rh_{min} (%)	25.5	36.9	26.0	30.1	26.7	24.4	28.3	31.1
Rh_{max} (%)	86.8	90.7	81.8	82.6	84.2	84.5	81.7	84.7
Water input (rainfall + irrigation, mm)	67	145	8	194	84	21	33	162
Sowing date	December 12	December 1	November 19	November 15	December 20	December 6	December 22	December 12
Mean yield (kg ha^{-1})	2893	5403	4504	6557	1491	2815	3493	4855

T_{min} and T_{max} are average minimum and maximum daily temperatures, respectively.
Rh_{min} and Rh_{max} are the average minimum and maximum daily relative humidity, respectively.
ET_0 is the reference evapotranspiration computed by the Penman-FAO methodology (Allen et al., 1998).

According to the factorial regression model (Table 3C), the environmental covariables significantly affecting the adaptation of the varieties to the tested environments were average minimum daily temperature from sowing to heading [$T_{min(SH)}$], thermal time during the same period (TT_{SH}), and average maximum daily relative humidity from heading to maturity [$Rh_{max(HM)}$], which jointly retained 68.4% of the SS of the GE interaction with 43% of its df. The same three covariables explained 78.7% of the SPE interaction. Therefore, the interaction between these three environmental covariables and the subpopulations explained 47.1% of the total GE interaction variance with only 8.2% of its df. The environmental covariables instead of environmental variables $T_{min(SH)}$ and TT_{SH} accounted for 59.7% of the GE variance and 74.3% of the SPE interaction, with $T_{min(SH)}$ representing 44.6% and 64.3% of the SS of the GE and the SPE interactions, respectively (Table 3C).

The vectors of the covariables retained in the model are depicted in the AMMI biplot shown in Fig. 2. Local landraces and old-bred

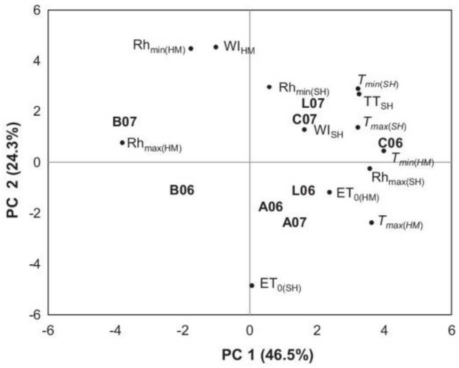


Fig. 1. Biplot of the first two axes of the PCA analysis summarizing the relationships between the environmental covariables and the environments. Environments are represented in bold (see Table 2 for environment identification). Points correspond to the eigenvalues of the following environmental variables: T_{min} (average minimum daily temperature), T_{max} (average maximum daily temperature), Rh_{min} (average minimum relative humidity), Rh_{max} (average maximum daily relative humidity), TT (thermal time), WI (water input), and ET_0 (reference evapotranspiration). Subscripts SH and HM stand for the periods from sowing to heading and from heading to maturity, respectively.

Table 3
Partitioning, according to three models, of the GE interaction for the grain yield of an historical series of 27 bread wheat varieties (Var), grouped in 6 subpopulations (SP), and grown in 8 environments (Env). (A) ANOVA, (B) AMMI, and (C) factorial regression. $T_{min(SH)}$: average minimum daily temperature from sowing to heading, TT_{SH}: average thermal time from sowing to heading, $Rh_{max(HM)}$: average maximum daily relative humidity from heading to maturity.

Source of variation	df	SS (10^6)	SS (%)	*F*	*p*-Value
Total	647	2652			
(A) *ANOVA*					
Env	7	1513	57.1	94.4	<0.001
Block (E)	16	37	1.4	6.1	<0.001
Var	26	562	21.2	57.3	<0.001
Between SP	5	480	85.4	24.7	<0.001
Within SP	21	82	14.6		
Var × Env	182	384	14.5	5.6	<0.001
Between SP × Env	35	230	59.9	6.3	<0.001
Within SP × Env	147	154	40.1		
(B) *AMMI*					
Var × Env	182	384	14.5	5.6	<0.001
IPCA1	32	210	54.7	17.4	<0.001
IPCA2	30	78	20.3	6.9	<0.001
IPCA3	28	35	9.2	3.3	<0.001
Residuals	92	61	15.8		
(C) *Factorial regression*					
Var × Env	182	384	14.5	5.6	<0.001
$T_{min(SH)}$ × Var	26	171	44.6	17.5	<0.001
TT_{SH} × Var	26	58	15.1	5.9	<0.001
$Rh_{max(HM)}$ × Var	26	34	8.7	3.4	<0.001
Deviations	104	121	31.6		
Var × Env	182	384	14.5	5.6	<0.001
SP × Env	35	230	59.9	6.3	<0.001
$T_{min(SH)}$ × SP	5	148	64.3	43.9	<0.001
TT_{SH} × SP	5	23	10.0	6.8	<0.001
$Rh_{max(HM)}$ × SP	5	10	4.4	3.0	0.012
Deviations	20	49	21.3		
Error	416	157	5.9		

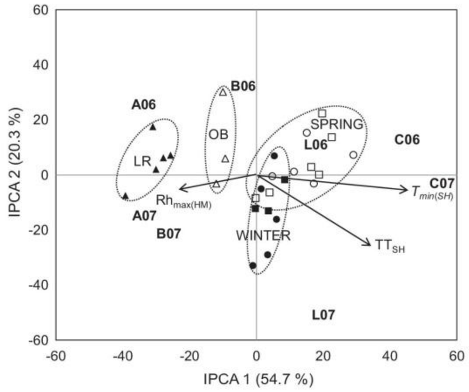


Fig. 2. Biplot of the first two axes of the AMMI model for yield data. Subpopulations are identified by symbols: (▲) local landraces (LR), (△) old-bred varieties (OB), (□) intermediate spring, (○) modern spring, (■) intermediate winter, and (●) modern winter varieties. Environments are represented in bold (see Table 2 for environment identification). $T_{\min(SH)}$, average minimum daily temperature from sowing to heading; TT_{SH}, average thermal time from sowing to heading; $Rh_{\max(HM)}$, average maximum daily relative humidity from heading to maturity.

varieties were located on the opposite side of the vectors representing $T_{\min(SH)}$ and TT_{SH}, but close to the $Rh_{\max(HM)}$ vector. On the other hand, modern spring varieties were positioned farther from the $Rh_{\max(HM)}$ vector than other varieties, being closer than them to the $T_{\min(SH)}$ vector. Most winter intermediate and modern varieties were located between the vectors representing TT_{SH} and $RH_{\max(HM)}$.

3.3. GE interaction for yield components

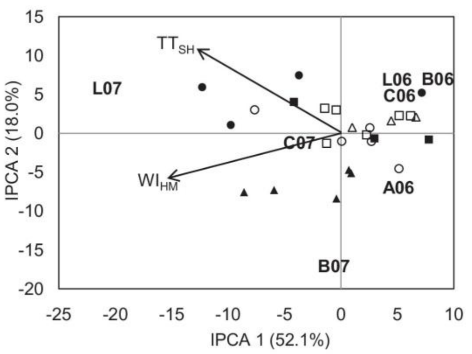
The ANOVA showed that the number of grains per spike was the most stable yield component across environments as the GE interaction for this trait explained only 17.6% of the total variance, while it accounted for 20.8% and 27.7% of total variance for TKW and number of spikes per m^2, respectively (Table 4). Moreover, the variety effect explained 44.4% of the variation in the number of grains per spike, while this value was less than 20% for the other two yield components (Table 4).

The percentage of the sum of squares of the GE interaction explained by differences between subpopulations was similar for the number of grains per spike and TKW (39.9% and 40.7%, respectively), while it was slightly lower for the number of spikes per m^2 (34.3%) (Table 4).

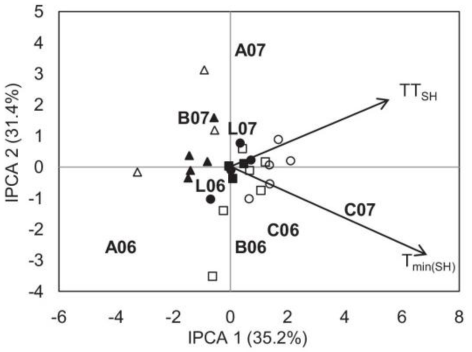
The environmental covariables that explained the GE interaction depended on the yield component. Though they did not match exactly with the ones retained by the factorial regression model for yield, the thermal time until heading (TT_{SH}) and the average minimum daily temperature during the same period [$T_{\min(SH)}$] had a significant effect on the number of grains per spike, and the former also on the number of spikes per m^2.

The GE interaction for the number of spikes per m^2 was mainly due to the different sensitivities of the genotypes to the water input from heading to maturity (WI_{HM}) and TT_{SH} (upper part of Table 4). These two variables jointly explained 41.1% and 60.6% of the GE and SPE interaction variances, respectively. The first two axes of the AMMI model explained 70.1% of the GE variation for the number of spikes per m^2 (Fig. 3a). Though the biplot did not show a clear subpopulation structure for this trait, landraces were clustered in

a) Number of spikes per m^2



b) Number of grains per spike



c) Thousand kernel weight

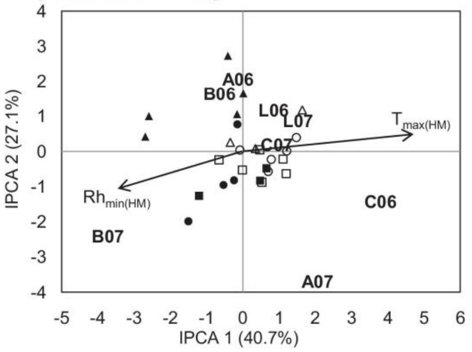


Fig. 3. Biplot of the first two axes of the AMMI model for: (a) number of spikes per m^2, (b) number of grains per spike, and (c) thousand kernel weight. Subpopulations are identified by symbols: (▲) local landraces (LR), (△) old-bred varieties (OB), (□) intermediate spring, (■) intermediate winter, (○) modern spring, and (●) modern winter varieties. Environments are represented in bold (see Table 2 for environment identification). TT_{SH}, average thermal time from sowing to heading; WI_{HM}, water input from heading to maturity; $T_{\min(SH)}$, average minimum daily temperature from sowing to heading; $T_{\max(HM)}$, average maximum daily temperature from heading to maturity; $Rh_{\min(HM)}$, average minimum daily relative humidity from heading to maturity. The number of spikes per m^2 could not be determined in A07.

Table 4
ANOVA and factorial regression models for the partitioning of the GE interaction for the yield components of an historical series of 26 bread wheat varieties[a] (Var) grouped in 6 subpopulations (SP), and grown in 8 environments[b] (Env). TT_{SH}: average thermal time from sowing to heading, WI_{HM}: water input from heading to maturity, $T_{min(SH)}$: average minimum daily temperature from sowing to heading, $T_{max(HM)}$: average maximum daily temperature from heading to maturity, $Rh_{min(HM)}$: average minimum daily relative humidity from heading to maturity. Only post-heading environmental variables are considered for thousand kernel weight.

Source	df	SS (10^3)	SS (%)	*F*	*p*-Value
Number of spikes m^{-2}					
Total	545	11,578			
Env	6	3883	33.5	36.8	<0.001
Var	25	2109	18.2	13.9	<0.001
Between SP	5	965	45.8	3.4	0.023
Var × Env	150	3207	27.7	3.5	<0.001
TT_{SH} × Var	25	795	17.6	5.2	<0.001
$WI_{HM.}$ × Var	25	1062	23.5	7.0	<0.001
Deviations	100	1350	58.9		
SP × Env	30	1101	34.3	3.3	0.003
TT_{SH} × SP	5	315	28.6	5.7	<0.001
WI_{HM} × SP	5	352	32.0	6.4	<0.001
Deviations	20	434	39.4		
Error	350	2132	18.4		
Number of grains spike^{-1}					
Total	623	60.2			
Env	7	14.5	24.1	64.5	<0.001
Var	25	26.7	44.4	54.6	<0.001
Between SP	5	19.8	74.2	11.5	<0.001
Var × Env	175	10.6	17.6	3.1	<0.001
$T_{min(SH)}$ × Var	25	2.2	20.9	4.6	<0.001
$TT_{SH.}$ × Var	25	2.3	21.6	4.7	<0.001
Deviations	125	6.9	65.1		
SP × Env	35	4.3	39.9	2.7	<0.001
$T_{min(SH)}$ × SP	5	1.8	42.4	9.7	<0.001
$TT_{SH.}$ × SP	5	0.7	17.1	3.9	<0.001
Deviations	25	1.8	40.6		
Error	400	7.8	13.0		
Thousand kernel weight (TKW)					
Total	623	32.4			
Env	7	12.0	37.1	20.0	<0.001
Var	25	6.2	19.2	16.4	<0.001
Between SP	5	2.7	42.7	3.0	0.036
Var × Env	175	6.7	20.8	2.6	<0.001
$T_{max(HM)}$ × Var	25	2.5	37.8	6.8	<0.001
$Rh_{min(HM)}$ × Var	25	0.8	12.0	2.1	0.001
Deviations	125	3.5	51.7		
SP × Env	35	2.7	40.7	2.7	<0.001
$T_{max(HM)}$ × SP	5	1.0	38.0	8.6	<0.001
$Rh_{min(HM)}$ × SP	5	0.4	15.5	3.5	0.004
Deviations	25	1.3	46.5		
Error	400	6.0	18.7		

[a] Data of *cv.* Cezanne were missing in C07 and so it was removed from the analysis.
[b] The number of spikes per m^2 could not be determined in A07.

the bottom part of the figure and apart from the other groups, while old-bred and intermediate spring varieties were clustered together and jointly with 3 of the 5 modern spring varieties (Fig. 3a). Three of the four modern winter varieties were located in the negative part of IPCA1, suggesting that this subpopulation tended to develop more spikes in environments with a long pre-flowering period and a better water supply after heading. Water input after heading also favored the production of spikes in the landraces (Fig. 3a). The AMMI analysis also revealed an important year effect on spike formation, as the environments in 2006 were located on the positive side of the first IPCA, whereas the environments in 2007 were located on the negative side (Fig. 3a). The mean number of spikes per m^2 was 422 in 2006 and 495 in 2007.

The two main environmental covariables responsible for GE interaction for yield also significantly affected the number of grains per spike. $T_{min(SH)}$ and TT_{SH} accounted for 42.5% and 59.5% of the GE and the SPE interaction variances, respectively, for this trait (central part of Table 4). The AMMI analysis showed a trend of the landraces and old-bred varieties towards the negative sense of the vectors representing the two environmental covariables, while spring subpopulations were the closest to the positive sense of both vectors (Fig. 3b). Environments B07, L06 and L07 were favorable to the production of a large number of grains per spike in landraces and old-bred varieties, while for spring varieties from both intermediate and modern periods C06, B06 and C07 were more appropriate (Fig. 3b).

The post-heading environmental covariables mostly determining the GE interaction for TKW were the average maximum daily temperature from heading to maturity [$T_{max(HM)}$] and the average minimum daily relative humidity from heading to maturity [$Rh_{min(HM)}$], which explained 49.8% and 53.5% of the GE and SPE interaction sum of squares, respectively (bottom part of Table 4). The AMMI analysis showed a contrasting behavior of the landraces (located at the left upper part of Fig. 3c towards the same sense of the $Rh_{min(HM)}$ vector and the opposite sense of the $T_{max(HM)}$ vector) and the other subpopulations that were more stable for this trait, as proved by their placement close to the origin of the biplot (Fig. 3c).

4. Discussion

4.1. Environmental

The eight environments used in this study differed for some of the most important environmental traits affecting crop growth under Mediterranean conditions, e.g. minimum temperatures to accomplish vernalization requirements, water input, late frost risk and terminal water, and heat stresses (Loss and Siddique, 1994). This wide range of weather conditions resulted in a broad variation of mean yields, which ranged from 1491 kg ha^{-1} in A06, the environment with the lowest water input before heading, to 6557 kg ha^{-1} in L07, the environment with the highest thermal time from sowing to heading and the highest water input during grain filling. However, differences in yield could not be explained solely by weather conditions, since some of the environments clustering together in the environmental PCA biplot differed widely in their final yield (e.g. A06 and A07). Major differences between environments were induced by contrasting temperatures during the whole cycle. Thus, B07 and C06, the most distant environments according to PCA, were characterized by low and high temperatures, respectively. Yields were greater in 2007 than in 2006, mostly because of the greater water input during grain filling in 2007.

4.2. GE interaction for yield

Although almost half of the total yield variance was due to the environment effect, our results revealed the existence of different adaptation patterns among the 27 varieties included in the historical series, as 14.5% of total yield variance was explained by the different sensitivities of the varieties to the environmental conditions or GE interaction. The subpopulation structure, defined on the basis of the period of cultivation and the growth habit of the varieties, explained 18.1% of total variance and 59.9% of the GE variance for yield. This result and that of the AMMI analysis – which grouped the varieties into four clusters corresponding to landraces, old-bred cultivars, winter varieties and spring varieties (without separating in the last two groups those from intermediate and modern periods) – suggest that breeding efforts for yield increases during the last century in Spain affected the adaptation pattern of the cultivated varieties. Landraces were the lowest-yielding varieties across environments and showed specific adaptation to environments with low minimum temperatures and thermal time before heading and high maximum relative humidity during grain filling. This result may be a consequence of their phenology, as landraces had the longest pre-heading period, which very likely resulted in an avoidance of the late frost damage during spike formation at the expense of increasing their susceptibility to terminal stresses in warm environments, so they performed best when humidity was high during grain filling.

Old-bred cultivars tended to be well adapted to cold environments, which was not surprising given that the three were obtained in Mediterranean-continental areas. Winter varieties from the intermediate period were the most widely adapted to the tested environments, but only three of the five winter modern types showed a high degree of yield consistency across environments, while the remaining two showed specific adaptation to the environment with greatest water input during grain filling. Moreover, spring cultivars ranged from those having wide adaptation to those showing better performance in the warmest environments with high temperatures before heading, conditions which are less suitable for the rest of the subpopulations.

Wheat breeding in Spain during the 20th century largely relied on the introduction of foreign germplasm (Royo and Briceño-Félix, 2011). Though most intermediate and modern varieties were not developed to specifically meet Spanish conditions, our results revealed that they showed improved adaptability compared with the old germplasm. Intermediate and modern varieties of winter growth habit introduced from France proved to be the most widely adapted, which explains their success in Spain. The wide adaptation of some of the spring varieties used in this study may be related to their CIMMYT origin, since it is well known that breeding efforts in CIMMYT have focused on developing wide-adaptation genotypes from selections made in environments with high yield potential (Braun et al., 1997).

Average minimum daily temperature and thermal time from sowing to heading were the main environmental covariables determining yield responses to the GE interaction. Both variables have been reported to be responsible for GE in studies conducted with modern bread wheat varieties in Spain (Voltas et al., 2005). Actually, the minimum average temperature and the thermal time before heading are directly involved in the phenological adjustment, one of the main adaptation mechanisms of cereal crops (Motzo and Giunta, 2007; Richards, 1991). The importance of temperature before heading on wheat adaptation to Mediterranean conditions was recently demonstrated by a study involving 191 durum wheat accessions grown in nine Mediterranean environments (Royo et al., 2010).

Our results indicate that bread wheat yield increases in Spain over the last century were related to a reduction in the length of the pre-flowering period, as had been previously reported in durum wheat (Álvaro et al., 2008c). The reduction of the pre-flowering period may allow avoiding terminal stresses characteristic of Mediterranean environments, despite an increased risk of late frost damage.

4.3. GE interaction for yield components

Of the three yield components studied the number of grains per spike was the most constitutive. Differences between varieties explained 44.4% of the variance for the number of grains per spike, but only 18.2% and 19.2% of the total variance for the number of spikes per m^2 and thousand kernel weight, respectively. However, it has been reported that the genetic control of this trait is complex, since it is determined by different events during crop development (Álvaro et al., 2008b; Loss and Siddique, 1994), largely dependent on the environmental conditions. Factorial regression analysis showed that the environmental covariables that mostly affected the number of grains per spike (i.e. minimum daily temperature and thermal time from sowing to heading), were the same as those mainly affecting grain yield. Accordingly, the adaptation pattern of the subpopulations for the number of grains per spike was the most similar to the adaptation pattern previously observed for grain yield, and thus supported the reported relationship between increases in the number of grains per spike and yield genetic gains during the 20th century (Brancourt-Hulmel et al., 2003; Reynolds et al., 1999; Royo et al., 2007). The AMMI analysis demonstrated that, as in the case of yield, the number of grains per spike was favored in landraces and old-bred varieties by low minimum temperatures before heading, the number of grains per spike being less sensitive to the environmental changes in the landraces than in the old-bred varieties. Grain formation of winter germplasm was relatively insensitive to temperature variations, with winter germplasm showing the highest degree of stability for this yield component. On the other hand, high minimum temperatures and thermal time before heading led to a large number of grains per spike in spring varieties.

The AMMI analysis suggested that the larger number of spikes per unit area recorded in 2007 was mainly a consequence of the greater water input after heading and, to a lesser extent, of the greater thermal time before heading in the second year of experiments. The results also indicated that for spike formation, landraces and modern winter varieties had a different pattern of adaptation to that of the other subpopulations. High water input after heading resulted in a great number of spikes mostly in landraces, but also in modern winter varieties. This finding was probably a consequence of the later heading dates of these two subpopulations, which very likely compelled grains to be filled under warmer and drier conditions than those experienced by early-heading genotypes; in these cases water input probably favored the emergence of spikes in late-formed tillers. Moreover, a high thermal time before heading favored spike formation on three of the four winter modern varieties, particularly *cv.* Craklin and Berdun.

The AMMI analysis conducted for thousand kernel weight revealed that high relative humidity during grain filling was a crucial trait for higher kernel weight formation in modern winter varieties and landraces, thus confirming the results obtained by the PCA conducted for yield. High temperatures after heading were detrimental to grain filling (Royo et al., 2006), especially for late-heading subpopulations. Spring varieties were the most stable regarding grain weight, probably because their earliness limited the damage to their grain formation caused by terminal stresses.

5. Concluding remarks

The subpopulation structure used in this study was helpful for explaining GE interactions for yield and yield components. However, the subpopulation × environment interaction was more explanative for yield than for any of the yield components analyzed, probably because of the compensation mechanisms between yield components within subpopulations.

Landraces showed a contrasting pattern of adaptation compared with the other subpopulations. They proved to have specific adaptation (to environments with low minimum temperatures before heading and high relative humidity during grain filling) and low yield consistency across environments owing to large environmental effects on the number of spikes per unit area and grain weight. Old-bred varieties constituted the first step towards breeding for adaptation. Though they were also best adapted to cold environments, their yield was more stable than that of landraces, mostly because their final spike number and grain weight were less dependent on water input during grain filling. This lower dependence was probably due to their earliness. However, the grain setting of old-bred varieties was penalized in areas with high temperatures before heading, which are unusual in the areas of origin of these varieties. A second and final step in breeding for adaptation was reached with the introduction in the 1960s of intermediate varieties, whose adaptation pattern does not differ significantly from that of modern cultivars. The main differences in adaptation between the pool of varieties introduced in recent times stemmed from their growth habit. Winter varieties showed wider adaptation than spring types, primarily because of the great steadiness of their number of grains per spike. The longest cycle of winter varieties, probably associated with their vernalization requirements, favored their adaptation to environments with high relative humidity during grain filling, which enhanced grain weight. On the other hand, GE interaction for yield was greater in spring varieties than in winter types, and most varieties showed good adaptation to environments with warm winters, probably favoring the formation of a high potential number of grains per spike. Compared with that of other subpopulations, the grain weight of spring varieties was very stable across environments.

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CHAPTER 3

Breeding effects on dry matter accumulation and partitioning in Spanish bread wheat during the 20th century

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En revisión.

Breeding effects on dry matter accumulation and partitioning in Spanish bread wheat during the 20th century

Abstract

Changes caused by breeding in the pattern of bread wheat growth were studied in four field experiments involving a historical series of 16 representative varieties. The collection, which included landraces (grown until the 1940's), initial varieties (1947-1955; termed 'old-bred' in a previous study by Sanchez-Garcia et al. 2012a) and modern varieties (1972-2001), was genetically characterized for *Rht8c*, *Rht-B1b* and *Rht-D1b* dwarfing alleles. The patterns of biomass accumulation and partitioning were modified by the introduction of improved varieties, but above ground biomass at physiological maturity was similar for the three periods. Biomass accumulation and yield were strongly dependent on the number of major loci carrying dwarfing alleles present in the genotypes. Biomass and leaf area index (LAI) at the beginning of jointing were reduced by ca. 20% and 40% in varieties carrying one or two dwarfing alleles, respectively. The rates of biomass accumulation from the beginning of jointing to anthesis were similar across wheats from the three historical periods, and also amongst varieties carrying a different number of dwarfing alleles. Although 20.7% of absolute yield gains during the last century were due to increases in the weight of spikes per unit area from anthesis to milk-grain stage, 79.3% occurred from the milk-grain stage to physiological maturity. While initial varieties maintained and modern varieties increased above ground biomass from the milk-grain stage to physiological maturity, landraces lost biomass during this phase. The contribution of photosynthesis during this last phase of the grain filling period was enhanced in modern varieties, mostly due to increases in LAI at milk-grain stage (0.83% y^{-1}) and of LAD from it to physiological maturity (0.79% y^{-1}).

Keywords: crop biomass; historical series; CDW; dwarfing alleles; *Rht*; LAI; LAD; grain filling.

Introduction

Wheat is the second most important staple crop in the world and is essential to global food security. Currently cultivated on 216.8 million ha worldwide, it provides 19% of the daily intake of calories and 21% of protein for humans (FAOSTAT 2011). Yield improvement has been a primary objective of bread wheat (*Triticum aestivum* L.) breeding programs around the world. A conceptual framework frequently used to interpret historical yield gains consists in expressing yield as the product of biomass (crop dry weight, CDW) and harvest index (HI). It is generally accepted that wheat yield gains due to breeding during the 20th century were accomplished through increases in HI, with few or no changes in CDW (Brancourt-Hulmel et al., 2003; Canevara et al., 1994; Donmez et al., 2001; Royo et al., 2007). It has been suggested that increases in HI were a result of the selective pressure put on morphology and assimilate partitioning process when selecting for yield, which was not put on biomass (Blum, 2011). Even though the theoretical maximum HI attainable in bread wheat, set at 0.62 by Austin et al. in 1980, has recently being revised and updated up to 0.64 (Foulkes et al., 2011), HI values above 0.50 have rarely been achieved (Fischer and Edmeades, 2010). Therefore, it has been proposed that future international breeding efforts towards yield improvements should be based on increases in CDW production without decreasing HI (Parry et al., 2011) or, in other words, biomass and yield should be selected for while retaining HI (Blum, 2011).

Yield gains have been principally related to plant height reductions resulting from the introgression of major dwarfing genes (Austin et al., 1980), intended as the mutant alleles of a series of loci grouped in the *Rht* loci complex, that showed a major effect promoting short height (Worland and Snape, 2001). Basically, two types of *Rht* genes have been described, the ones which its mutant alleles promote short height by inducing the plant insensitiveness to gibberellic acid (GA; e.g. *Rht-*

B1b and *Rht-D1b*) and the ones that promote short height by affecting other physiological mechanisms (e.g. *Rht8c*, *Rht9* or *Rht12*) (Worland and Snape, 2001). In spite of the yield raises accomplished, pleiotropic effects, such as decreased early vigor, have been reported with the introduction of the *Rht* dwarfing alleles, particularly with the ones providing GA insensitiveness (Ellis et al., 2004).

Bread wheat yield improvements in Spain during the 20th century took place in two steps. The first consisted in the introduction, from the late 1940's, of foreign varieties mostly issued from early Italian and French breeding programs, and the release of the first improved varieties of local origin. The first *Rht* dwarfing alleles were introduced in the Spanish germplasm by these varieties, particularly in the GA sensitive loci *Rht8* and *Rht9* (Worland *et al*., 1998). Consequently, plant height was reduced and, as a pleiotropic effect, more resources were diverted from vegetative to reproductive organs, contributing to increases in HI (Sanchez-Garcia *et al*., 2012b). The second step started with the introduction, from the 1970’s, of modern semi-dwarf varieties mostly derived from CIMMYT and French breeding programs. These varieties introduced in the Spanish wheat pool the gibberellic acid (GA) insensitive dwarfing alleles *Rht-B1b* and *Rht-D1b* that further reduced plant height and increased again HI (Sanchez-Garcia *et al*., 2012b).

There is a large body of literature that describes the impact of selective breeding on HI and crop biomass,, particularly the impact of dwarfing genes, (Brancourt-Hulmel *et al*., 2003; Royo *et al*., 2007; Clayshulte *et al*., 2007). Less is known about the changes caused by historical breeding, and specially the introgression of dwarfing genes, on the patterns of growth and biomass allocation during specific phases of the growth cycle. For example, the relative impact of dwarfing genes on biomass accumulation during the vegetative phase of the cycle, from jointing to heading date when the plant accumulates and stores the water soluble carbohydrates that will contribute to the filling spike, is unclear. Understanding these effects and their relationship with yield gains could be helpful for designing future breeding strategies based on crop biomass increases.

The partitioning of biomass between vegetative and reproductive organs, principally after anthesis, is a main process determining yield formation. Grain filling in wheat is supported by transient photosynthesis and the translocation of assimilates accumulated prior to anthesis and stored temporary in the vegetative organs (Blum, 1988; Kobata *et al*., 1992). A previous study conducted in durum wheat under Mediterranean conditions demonstrated that the contribution of pre-anthesis assimilates to grain filling increased in modern varieties arriving to values close to 30% (Álvaro *et al*., 2008a). This result shows the importance of photosynthesis during grain filling − mostly by the spike and the flag leaf − on final grain weight, even under Mediterranean environments, where yield greatly depends on translocation of pre-anthesis assimilates to the grain. Increasing canopy light capture by promoting fast early-leaf area growth and/or delaying leaf senescence has been suggested as a potential key trait for future yield raises (Parry *et al*., 2011).

The aim of the present study was to investigate the changes on biomass accumulation and allocation caused by the introduction of improved bread wheat varieties in Spain during the 20th century from a temporal perspective, through the involvement of varieties belonging to different breeding periods, and also from the genetic point of view, by considering the allelic structure of their dwarfing genes.

Material and Methods

Plant material

Plant material consisted in a collection of 16 bread wheat varieties representative of the most widely cultivated in Spain in different periods of the 20th century. The

Table 1. Description of the varieties used in the study grouped according to the breeding period in which they were grown. Data of plant height and harvest index are means of four field experiments.

Variety	Pedigree	Origin	Year of release in Spain	*Rht* dwarfing alleles	Plant height (cm)	Harvest index
			Landraces			
Aragon 03	Selection of the landrace "Catalan de Monte"	Spain	<1940	*Absent (rht)*	118	0.24
Barbilla	Landrace	Spain	<1940	*Absent (rht)*	114	0.28
Candeal	Landrace	Spain	<1940	*Absent (rht)*	111	0.25
				Mean	*114*	*0.26*
			Initial varieties			
Mara	Autonomia/Aquila	Italy	1947	*Rht8c + Rht9**	86	0.44
Impeto	Frassineto-405/Villa-glori	Italy	1950	*Rht8c*	100	0.41
Estrella	Mon-desir/Ardito//Mouton-a-epi-rouge/k-3/3/Mouton-epi-rouge	France	1952	*Rht8c*	109	0.38
Pané 247	L-4/Mentana	Spain	1955	*Absent (rht)*	115	0.37
				Mean	*103*	*0.40*
			Modern varieties			
Yecora	Ciano-67/3/Sonora-64/Klein-rendidor//Siete-Cerros-66	CIMMYT	1972	*Rht-B1b + Rht-D1b*	79	0.47
Cajeme	Ciano-67(sib)//Sonora-64/Klein-rendidor/3/Siete-Cerros-66	CIMMYT	1972	*Rht-B1b + Rht-D1b*	81	0.45
Anza	Lerma-rojo-64//Norin-10/Brevor/3/3*Andes-enano	CIMMYT /USA	1974	*Rht-B1b*	87	0.42
Marius	Cadet//Thatcher/Vilmorin-27/3/Ariana/Fundulea	France	1980	*Rht?*	90	0.45
Rinconada	Unknown	CIMMYT	1981	*Rht-D1b*	95	0.41
Soissons	Iena/hn-35	France	1990	*Rht-B1b*	81	0.47
Gazul	Unknown	Spain	1992	*Rht-D1b*	89	0.44
Isengrain	Apollo/Soissons	France	1998	*Rht-B1b*	79	0.42
Califa Sur	Unknown	Spain	2001	*Rht-D1b + Rht8c*	71	0.48
				Mean	*84*	*0.45*
				SED between breeding periods	*5.7*	*0.02*

* *Rht9* locus characterization for Mara was obtained from Korzun *et al.*, 1998

collection included: i) Spanish landraces grown before 1940; ii) initial varieties , released and grown from the mid 1940s until the Green Revolution, and iii) modern varieties , released and grown from 1970 until the end of the 20th century. Most modern varieties had French origin or were derived from semi-dwarf CIMMYT germplasm (Table 1).

Molecular characterization

DNA was extracted from leaf samples obtained from young leaves pooled from three seedlings of each variety following the methodology proposed by Doyle and Doyle (1990).

Table 2. Description of the environments.

Cropping season	2007-08	2007-08	2008-09	2008-09
Water regime	Rainfed	Irrigated	Rainfed	Irrigated
Environmental conditions from sowing to anthesis				
Accumulated GDD	1311	1357	1265	1258
Accumulated ET_0 (mm)	273	259	243	239
Average minimum daily temperature (°C)	3.1	3.0	2.3	2.7
Average maximum daily temperature (°C)	14.1	14.2	13.4	12.9
Water input (rainfall + irrigation, mm)	102	252	183	234†
Environmental conditions from anthesis to physiological maturity				
Accumulated ET_0 (mm)	170	155	113	160
Average minimum daily temperature (°C)	11.5	11.3	11.8	12.7
Average maximum daily temperature (°C)	23.1	23.0	27.0	27.3
Water input (rainfall + irrigation, mm)	133	133	9.0	54†
Sowing date	Nov. 20	Nov. 19	Nov. 24	Nov. 27
Mean yield (kg/ha)	4065	5416	5434	7201

ET_0 is the reference evapotranspiration computed by the Penman-FAO methodology (Allen *et al.*, 1998).
† Sprinkler irrigation was used in 2009.

PCR-based markers were used to identify specifically both wild-type (*Rht-B1a* and *Rht-D1a*) and mutant (*Rht-B1b* and *Rht-D1b*) allelic variants at *Rht-B1* and *Rht-D1* loci. This characterization was carried out in the Plant Industry laboratories (CSIRO, Canberra) following the methodology described by Ellis *et al*. (2002). For the *Rht8* locus, the WMS261 products were obtained following the methodology proposed by Korzun *et al*. (1998) and their size was determined with an ABI PRISM 3130 Genetic Analyzer in the IRTA laboratories (Lleida, Spain).

Experimental setup

Four field experiments were conducted during two crop seasons and under rainfed and irrigated conditions in Lleida (Northeastern Spain, 41° 40’N, 0° 20’E, 200 m a.s.l.) on a fine loam Calcic Cambisol soil. Rainfed experiments received 235 mm and 192 mm in 2007-08 and 2008-09, respectively, and supplementary irrigations provided 150 mm the first year and 96 mm the second (Table 2). Each experiment consisted of a randomized complete block design with three replications and plots of eight 5 m-length rows, 0.15 m apart. Sowing rate was 450 seeds m^{-2}. All the experiments were fertilized appropriately and pests, weeds and diseases were controlled according to standard cultural practices.

Field data recording

Zadoks's growth stage (Zadoks *et al.*, 1974) was periodically determined on a plot basis. Biomass and leaf area were determined following the methodology described in Royo and Tribó (1997), and Villegas *et al.* (2001). Samples of plants within a 50-cm-long section on a central row per plot were pulled up at the first node-detectable stage (GS 31 of the Zadoks' scale), booting (GS 47), anthesis (GS 65) and milk-grain stage (GS 75), and a sample section of 1 m-length was taken at physiological maturity (GS 87). At each sampling occasion not less than 15 cm were left from a previous sampled area in the same row, and care was taken that adjacent rows had intact plant populations. In the laboratory the number of plants from each sample was counted and 10 representative plants per plot were separated into vegetative (leaves+ stems) and reproductive (spikes) organs. Except at GS47, leaf area projection (one side) was measured using a leaf-area meter (LI-3100E, LI-COR Inc., Lincoln, NE) and LAI was computed as the ratio of green leaf area per sample area. Yellow and dry leaves were not considered. Samples were oven-dried at 70°C for 48 h, then the dry weight of vegetative biomass and spikes per plant determined. Crop dry weight (CDW) was expressed on a sample basis as g m^{-2}. Leaf area duration (LAD) was calculated by approximating the area from a trapezium to the curve of LAI against time (Aparicio *et al.*, 2002), and was determined in growing degree-days (GDD). Plant height was measured at physiological maturity in ten main stems per plot, from the tillering node to the top of the spike excluding the awns. Plots were mechanically harvested at ripening and yield was expressed at 12% of grain moisture.

Statistical analysis

Principal component analysis (PCA) was performed on the correlation matrix, calculated on the mean data across environments and replicates of CDW determined at different growth stages, LAD and yield. Standard analyses of variance were used to analyze the data obtained. The sum of squares of the variety effect was partitioned in the ANOVAs in differences between and within breeding periods, and also according to the groups formed by varieties carrying a common number of *Rht* dwarfing alleles (*Rht* DA), arisen from the PCA. All the terms in the model were considered fixed excepting the genotypic residual (genotypic variance retained within groups), used to test the differences between groups. Least-square means were computed and compared by Duncan multiple range tests. In order to ascertain whether the rate of biomass accumulation from jointing to anthesis changed across periods, three different linear regression models with increasing complexity, were fitted to the relationships between CDW and thermal time. Firstly, the same regression line was used for the three breeding periods to fit the mean variety data across years. A second model forced a common slope for the three periods, and finally, three regression lines with different slopes were used. The models were compared through ANOVA. Slopes were only assumed to be different if the most complex model was significantly better ($P<0.05$) than the simplest ones.

Stepwise linear regression analyses were conducted to explain the relationships between variables. Absolute and relative genetic gain estimations were computed as the slope of the linear regression between the absolute or relative value of the trait and the year of genotype release. Relative values were computed for each genotype as a percentage with regard to the average value of all genotypes. All analyses were carried out with the SAS-STAT (SAS Institute Inc., 2003) and R (R Development Core Team, 2011) statistical packages.

Results

Molecular characterization for major dwarfing genes

The genotypes of the major dwarfing genes showed a trend across the different breeding periods (Table 1). The three landraces and the initial variety Pané 247, with plant height ranging from 111 cm to 118 cm, carried wildtype alleles of the dwarfing genes, which are typically associated with tall stature (Table 1). Other initial varieties, with plant heights between 86 and 109 cm, carried mutant alleles of *Rht8* and *Rht9* that have previously been linked to semi-dwarf phenotypes. Modern Spanish wheat varieties carried alleles of *Rht-B1* and *Rht-D1* that typically result in strong reductions in plant height, consistent with the reduced stature of modern varieties (Table 1). Interestingly, the modern semi-dwarf variety Marius did not appear to carry the mutant alleles *Rht-B1b*, *Rht-D1b* or *Rht8c* that have previously been linked to reduced plant height. This variety might carry novel alleles of the known dwarfing genes or could carry variation at alternative dwarfing genes.

Multivariate analysis

Multivariate analysis was used to examine the relationship between varieties for a set of growth traits and productivity, and the relationships between these variables. The first two axes of the PCA shown in Fig. 1 accounted for 81.4% of the total principal component analysis variance (axis 1, 56.8%; axis 2, 24.6%), suggesting that most of the information contained in the data could be summarized by projecting the points in the plane determined by the first two axes. Principal component 1 was related to CDW at different stages, from jointing to milk-grain, on the positive direction, and to yield and LAD on the negative one. The location of the points corresponding to each variety on the plane determined by the first two axes showed that PC1 grouped the varieties according to the number of major *Rht* loci carrying the dwarfing allele (*Rht* DA). The four tall varieties (*Rht* DA-0) were located in the positive extreme of PC1, while the four ones carrying

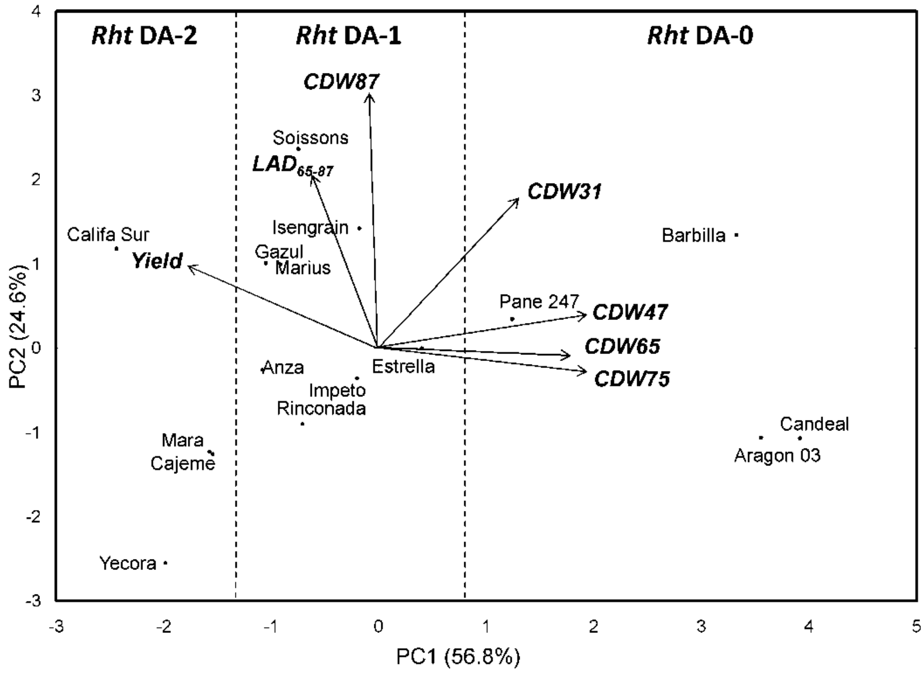

Figure 1. Biplot of the first two axes of the PCA analysis summarizing the relationships between crop dry weight (CDW) at five developmental stages according to Zadoks' scale (jointing, 31; booting, 47; anthesis, 65; milk-grain, 75; and physiological maturity, 87), leaf area duration from anthesis to physiological maturity ($LAD_{65\text{-}87}$) and grain yield. Discontinue lines separate the varieties according to the number of *Rht* dwarfing alleles (*Rht* DA) they carry.

two *Rht* dwarfing alleles (*Rht* DA-2) were placed in the negative side. The intermediate position between these two groups was occupied by the varieties carrying one *Rht* dwarfing allele (*Rht* DA-1) (Fig. 1). These results suggest that tall varieties accumulated more biomass from the first node detectable stage (CDW_{31}) to milk-grain stage (CDW_{75}) than the others. Despite greater biomass accumulation during early growth stages, these tall varieties achieved the lowest yields. Maximum yields, but less biomass, were recorded by the varieties carrying 2 *Rht* dwarfing alleles (*Rht* DA-2).

Principal component 2 was related with CDW at physiological maturity (CDW_{87}), leaf area duration after anthesis (LAD_{65-87}), CDW at the first-node detectable stage (CDW_{31}) and, to a lesser extent, grain yield. The four French varieties (Soissons, Isengrain, Marius and Estrella), and two Spanish grown varieties derived from French breeding programs (Gazul and Califa Sur), were located from the edge to the positive side of PC2, whilst Italian and CIMMYT derived varieties were placed on its negative sense. These results suggest that crop dry weight at physiological maturity (CDW_{87}), LAD, early biomass and yield tended to be greatest in French derived varieties.

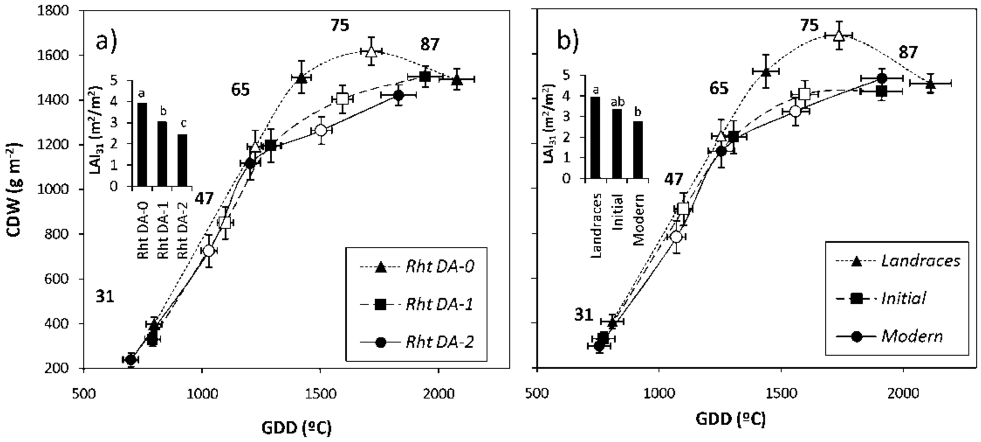

Figure 2. Changes in the crop dry weight of bread wheat varieties grouped according to: a) the number of *Rht* dwarfing alleles (*Rht* DA) they carry, and b) the breeding period (BP) in which they were released/grown. Points are means of 4 experiments. Numbers correspond to growth stages according to Zadok's scale: 31, first node detectable; 47, booting; 65, anthesis; 75, milk grain; 87, physiological maturity. Closed symbols correspond to stages 31, 65 and 87 and open symbols represent stages 47 and 75. Inset are depicted the differences in leaf area index (LAI) between *Rht* DA and BP at the first node detectable stage (31). Error bars indicate distances between groups within growth stages. Bars in inset figures followed by the same letter are not significantly different. Means were compared using Duncan multiple range tests at P=0.05.

Biomass production and partitioning

The variety effect explained ca. 40% of the total variance for CDW at the first node detectable stage (GS31), but this value decreased in successive growth stages as

Table 3. Percentage of the sum of squares of the ANOVA explained by the different factors of the ANOVA for biomass traits determined at five growth stages. The variety factor (Var) was partitioned in differences between and within (data not shown) breeding periods (BP) or the groups formed according to the number of *Rht* dwarfing alleles (*Rht* DA) present in the varieties. Numbers in parentheses for the ANOVA factors are df. GS stands for growth stage and the following number corresponds to the Zadoks scale (Zadoks et al., 1974).

Growth trait	Growth stages	Environment	Block(Env)	Variety	Variety partitioning		Var x Env	Residual
					Between Rht DA _(2)_	_Between BP(2)_		
		(3)	(8)	(15)			(45)	(120)
Crop dry weight (CDW)	First node (GS31)	1.1	2.9	40.9***	_67.4**_	_34.4_	31.5***	23.6
	Booting (GS47)	48.1***	1.3	24.5***	_79.1***_	_74.4***_	11.3**	14.8
	Anthesis (GS65)	52.5***	0.9	12.5***	_69.7***_	_62.1**_	12.2	21.9
	Milk grain (GS75)	27.8***	4.4	14.0***	_72.5***_	_71.7***_	19.8***	34.0
	P. maturity (GS87)	49.1***	1.6	4.3	_26.3_	_17.4_	16.0*	29.0
Aboveground Biomass	Anthesis (GS65)	50.1***	0.9	18.4***	_74.2***_	_71.7***_	10.3	20.2
	Milk grain (GS75)	29.5***	3.1	31.1***	_75.3***_	_86.7***_	12.4	23.9
	P. maturity (GS87)	42.4***	0.9	34.5***	_82.1***_	_72.2***_	6.8	15.4
Spikes weight	Anthesis (GS65)	37.2***	1.1	16.9***	_7.0_	_57.6**_	21.4***	23.4
	Milk grain (GS75)	7.9***	3.9	15.4***	_29.1_	_59.6**_	36.3***	36.5
	P. maturity (GS87)	16.3***	1.1	42.5***	_61.1**_	_86.2***_	18.5***	21.6

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

the environmental effect became more important (Table 3). Except at GS31 in most cases the variety x environment interaction accounted less than the environment in explaining the variability of the experimental data. Varieties differed in the biomass allocated in vegetative organs at all growth stages, but at maturity differences in CDW between them were not statistically significant.

The partitioning of the variety effect allowed comparison of the variability induced by the general genotypic effect associated with different breeding periods *versus* the specific impact of the number of *Rht* dwarfing alleles present in the genotypes. Differences between the three groups of varieties with different number of *Rht* dwarfing alleles generally explained a greater percentage of the variety variance for CDW than more general differences across the breeding periods. Differences in the number of major dwarfing alleles explained 67.4% of CDW variety variance at GS31, while no significant differences were found between breeding periods at this stage (Table 3). The number of *Rht* dwarfing alleles present in the varieties significantly affected LAI at GS31 (inset in Fig. 2a). Similarly, a decreasing tendency in LAI was apparent when breeding periods were compared (Fig. 2b).

From the first node detectable stage to milk-grain stage, the CDW of the varieties with the dwarfing allele at one or two *Rht* loci was lower than that of the tall varieties (*Rht* DA-0) (Table 4). When compared with the landraces, the reduction at GS31 was 17.5% for the varieties with a mutant allele at one *Rht* locus and 40.4% for the varieties carrying two *Rht* dwarfing alleles (Table 4). Changes in CDW from GS31 to anthesis followed the same increasing trend for the three groups of *Rht* DA genotypes (wild type, single or double dwarf genotypes) (Fig. 2a), and the differences between the slopes of the three curves were not statistically significant (data not shown). The reason for the tall varieties having more CDW at anthesis than the other groups was due to their greater biomass at GS31 and the longer thermal time needed by this group to reach anthesis. Actually, tall varieties

Table 4. Mean values (g m^{-2}) for biomass and its fractions at different growth stages for each of the groups formed by the varieties carrying the same number of *Rht* dwarfing alleles (DA) and for each breeding period. Values are means of 4 experiments. Numbers in parentheses indicate the percentage of change relative to the tall varieties (*Rht* DA-0) for the dwarfing genes partition, and to the landraces for the breeding period partition. GS stands for growth stage and the following number corresponds to the Zadoks scale (Zadoks et al., 1974).

Growth trait	Zadoks stage	Number of Rht dwarfing alleles (*Rht* DA) present					Breeding periods				
		Rht DA-0	*Rht* DA-1		*Rht* DA-2		*Landraces*	*Initial*		*Modern*	
Crop dry weight (CDW)	First node (GS31)	401^a	331^b	(-17.5)	239^c	(-40.4)	406^a	330^a	(-18.7)	297^a	(-26.8)
	Booting (GS47)	1186^a	844^b	(-28.8)	722^b	(-39.1)	1234^a	908^b	(-26.4)	783^b	(-36.5)
	Anthesis (GS65)	1486^a	1196^b	(-19.5)	1117^b	(-24.8)	1522^a	1229^b	(-19.3)	1166^b	(-23.4)
	Milk grain (GS75)	1628^a	1404^b	(-13.8)	1265^c	(-22.3)	1682^a	1419^b	(-15.6)	1343^b	(-20.2)
	P. maturity (GS87)	1473^a	1497^a	(+1.6)	1422^a	(-3.5)	1469^a	1433^a	(-2.5)	1491^a	(+1.5)
Aboveground biomass	Anthesis (GS65)	1262^a	973^b	(-25.4)	866^b	(-34.6)	1295^a	1054^b	(-19.9)	910^b	(-32.7)
	Milk grain (GS75)	1187^a	887^b	(-27.4)	766^b	(-37.8)	1249^a	968^b	(-24.6)	810^c	(-37.7)
	P. maturity (GS87)	950^a	706^b	(-21.0)	636^c	(-35.4)	936^a	763^b	(-18.5)	652^c	(-30.3)
Spikes dry weight	Anthesis (GS65)	224^a	223^a	(-0.4)	251^a	(+12.1)	227^a	175^b	(-22.9)	256^a	(+12.8)
	Milk grain (GS75)	440^a	519^a	(+18.0)	505^a	(+14.8)	433^b	451^b	(+4.2)	537^a	(+24.0)
	P. maturity (GS87)	574^b	782^a	(+36.2)	836^a	(+45.6)	546^c	670^b	(+22.7)	842^a	(+54.2)

Means within a row and *Rht* DA group or breeding period followed by the same letter are not significantly different at 5% probability level.

reached GS65 after 622 GDD from GS31, while the varieties carrying one mutant allele at the *Rht* loci needed 501 GDD, and those carrying two 505 GDD, the differences between *Rht* DA-0 and the two other groups being statistically significant ($P<0.001$). Growth rate beyond anthesis decreased in the three groups. Tall varieties achieved the maximum CDW at milk-grain stage, while for varieties carrying dwarfing alleles the maximum CDW was recorded at physiological maturity (Table 4 and Fig. 2a). Except at physiological maturity, the number of *Rht* DA present in the studied varieties did not contribute to significantly explain in the ANOVA the variability in spikes weight per unit area due to the variety effect (Table 3).

Differences in the CDW of varieties from distinct breeding periods were significant at booting, anthesis and milk grain stages (Table 4, Fig. 2b). In all these growth stages landraces accumulated more biomass than initial and modern varieties. Differences between the three groups were maximized at booting, when the CDW of initial and modern varieties were 26.4% and 36.5%, respectively, lower than that of landraces (Table 4). Initial and modern varieties had similar CDW at all growth stages. The breeding period accounted for a large proportion of the variability in spikes weight per unit area due to the variety effect (Table 3). Spikes weight per unit area was greater in modern varieties than in landraces and initial varieties, being the maximum differences recorded at physiological maturity (Table 4).

Biomass accumulated in the spikes from anthesis to milk-grain stage was significantly greater in initial and modern varieties than in landraces (Fig. 3a). Increases in total CDW resulted from the difference between gains in spikes weight and losses in vegetative biomass. Although differences between breeding periods in the decrease of vegetative biomass from anthesis to GS75 were not statistically significant, a tendency was observed to greater losses in improved varieties (Fig. 3a). Increase in spikes weight from milk-grain stage to physiological maturity was almost half of the amount accumulated in the preceding period for the landraces, but similar to previous gains for initial and modern varieties (Fig. 3b). While initial varieties maintained and modern varieties increased total CDW from milk-grain stage to maturity, landraces lost biomass during this period. Given that the weight of spikes per unit area increased, the loss of above ground biomass in landraces during the second part of the grain filling period could only be due to a decrease of biomass in vegetative organs, as shown in Table 4.

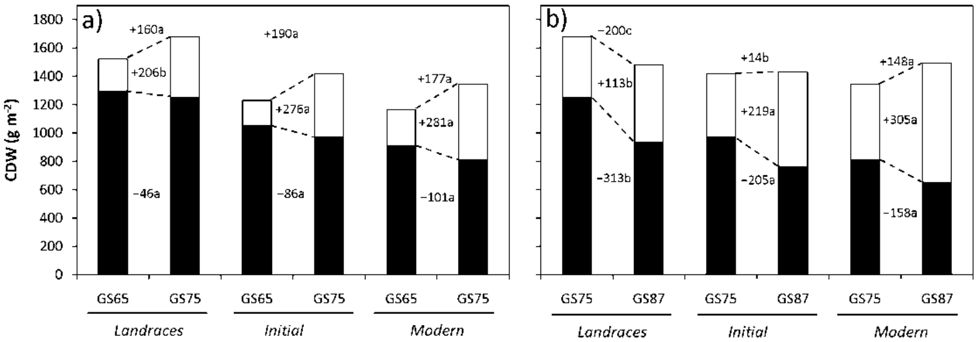

Figure 3. Changes in crop dry weight and its components (vegetative biomass in dark and spike weight in white), for bread wheat varieties belonging to different breeding periods. a) Changes between anthesis (GS65) and milk-grain stage (GS75), and b) changes between milk-grain stage (GS75) and physiological maturity (GS87). Numbers within a component for different breeding periods followed by the same letter are not significantly different at P=0.05.

Growth traits contribution to spike weight gains

In order to study the relationship between yield gains during the 20th century and changes in spikes weight through grain filling, a stepwise linear regression model was fitted, with yield as dependent variable and spike weight at anthesis (SW_{65}), and the gains in spikes weight during the two phases in which grain filling was divided, as independent variables (Table 5a). The results indicated that the increases in the weight of spikes per unit area from milk grain stage to maturity ($\Delta SW_{75\text{-}87}$) was the first variable entering in the model, explaining 55% of yield

Table 5. Regression equations for the relationships between: a) Yield as a dependent variable and spike weight per unit area at anthesis (SW_{65}), spike weight gain between anthesis and milk-grain stage ($\Delta SW_{65\text{-}75}$), and spike weight gain between milk grain and physiological maturity ($\Delta SW_{75\text{-}87}$) as independent variables. b) Spike weight gain between milk-grain stage and physiological maturity ($\Delta SW_{75\text{-}87}$) as a dependent variable, and vegetative biomass at anthesis (VDW_{65}), leaf area duration from anthesis to physiological maturity ($LAD_{65\text{-}87}$) and leaf area duration between milk-grain stage and physiological maturity ($LAD_{75\text{-}87}$) as independent variables. c) Leaf area duration between milk-grain stage and physiological maturity ($LAD_{75\text{-}87}$) as a dependent variable and leaf area index at anthesis (LAI_{65}), milk-grain (LAI_{75}) and physiological maturity (LAI_{87}), thermal time from sowing to milk-grain (GDD_{75}), thermal time from sowing to physiological maturity (GDD_{87}), and from milk grain to physiological maturity ($GDD_{75\text{-}87}$) as independent variables.

Dependent variable	Independent variables	Regression equation	Variable	Partial R^2	Model R^2	*P*
a)						
Yield	SW_{65}	$Y = 0.675 + 74.7\ 10^{-4}\ \Delta SW_{75\text{-}87} + 11.6\ 10^{-3}\ \Delta SW_{65\text{-}75}$	$\Delta SW_{75\text{-}87}$	0.55	0.55	<0.001
	$\Delta SW_{65\text{-}75}$		$\Delta SW_{65\text{-}75}$	0.32	0.87	<0.001
	$\Delta SW_{75\text{-}87}$					
b)						
$\Delta SW_{75\text{-}87}$	VDW_{65}	$Y = -1.80 + 1.78\ 10^{-5} * LAD_{75\text{-}87}$	$LAD_{75\text{-}87}$	0.58	0.58	<0.001
	$LAD_{65\text{-}87}$					
	$LAD_{75\text{-}87}$					
c)						
$LAD_{75\text{-}87}$	LAI_{65}	$Y = -78.1 + 172\ LAI_{75} + 0.249\ GDD_{75\text{-}87}$	LAI_{75}	0.88	0.88	<0.001
	LAI_{75}		$GDD_{75\text{-}87}$	0.08	0.96	<0.001
	GDD_{75}					
	GDD_{87}					
	$GDD_{75\text{-}87}$					

Only variables that met the 0.05 significance level entered into the model.

variations, while the increases from anthesis to milk-grain stage ($\Delta SW_{65\text{-}75}$) explained an additional 32%.

With the aim of determining whether the observed differences in $\Delta SW_{75\text{-}87}$ were mostly attributable to the vegetative biomass at anthesis –source of assimilates to be remobilized to the growing grains during grain filling– or to the duration of leaf area after anthesis –associated to the current photosynthesis capacity during grain filling–, a second stepwise regression model was fitted. In this model $\Delta SW_{75\text{-}87}$ was the dependent variable and vegetative biomass at anthesis (VDW_{65}), and leaf area duration, from anthesis to milk-grain stage ($LAD_{65\text{-}87}$), and from milk-grain stage to physiological maturity ($LAD_{75\text{-}87}$) were the independent variables. The results indicated that $LAD_{75\text{-}87}$ was the only variable entered in the model explaining 58% of the variability for $\Delta SW_{75\text{-}87}$ (Table 5b).

A third regression analysis was conducted to study the relationship between $LAD_{75\text{-}87}$ and its associated variables LAI_{65}, LAI_{75}, and the thermal-time, from sowing to milk-grain stage (GDD_{75}), from sowing to physiological maturity (GDD_{87}), and between these two growth stages ($GDD_{75\text{-}87}$). The results showed that the most important variable determining $LAD_{75\text{-}87}$ was LAI_{75}, which accounted for 88% of the observed variability, which increased until 96% when $GDD_{75\text{-}87}$ was added to the model (Table 5c).

Gains in spike weight from milk-grain stage to maturity and LAI_{75}, $LAD_{75\text{-}87}$ and the year of release of the varieties were strongly related with the breeding period (Fig. 4a to 4c). Modern varieties had the greatest LAI at milk-grain stage, which increased from landraces during the 20th century at a rate of 0.0055 y^{-1} (Fig. 4d) or, in relative terms, 0.83% y^{-1} (data not shown). Similarly, LAD from milk-grain stage to maturity increased from landraces to modern varieties (Fig. 4b), with a relative gain of 0.79% y^{-1} (data not shown). The increase in spikes weight per unit area was

3.14 g m^{-2} y^{-1} (Fig. 4c), and yield increases were estimated in 3.96 g m^{-2} y^{-1} (data not shown).

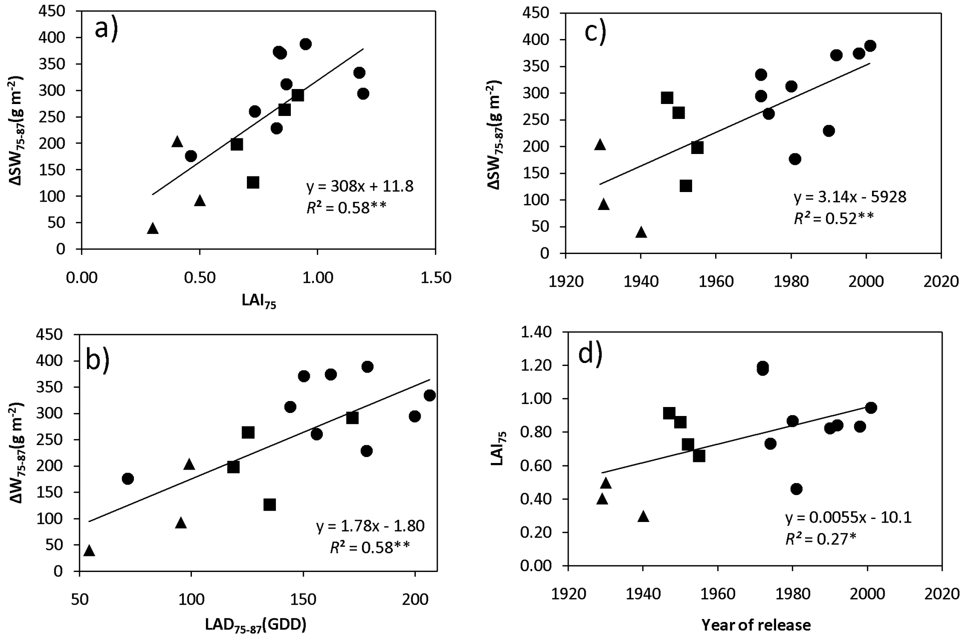

Figure 4. Relationship between increases in spike weight per unit area from milk-grain stage to physiological maturity (ΔSW_{75-87}) and: a) leaf area index at milk-grain stage (LAI_{75}), b) leaf area duration from milk-grain stage to physiological maturity (LAD_{75-87}), and c) year of release. d) Relationship between LAI at milk-grain stage (LAI_{75}) and the year of release. Each point corresponds to the mean data of a variety across four experiments. Breeding periods are identified with triangles for local landraces, squares for initial varieties and circles for modern varieties. * $P<0.05$; ** $P<0.01$.

Discussion

The results of the present study indicate that, at maturity, total above ground biomass of bread wheat varieties grown in Spain was not significantly modified by the introduction of improved varieties during the 20th century. However, significant changes occurred in dry matter accumulation and partitioning through the crop cycle.

The set of varieties used in this study reflected the history of introduction of *Rht* dwarfing alleles in the wheats grown in Spain in the last century. Great differences in plant height existed between the unimproved landraces −characterized by the absence of reduced height alleles of the major dwarfing genes, compared to the initial varieties from the early Italian and French breeding programs that carried the first reduce height alleles at *Rht* loci. These early improved varieties showed a great plant height variability that, in this study, was noticed by the differences existing between the Italian GA sensitive variety ‘Mara’ (86 cm height), carrying *Rht8c* and *Rht9* dwarfing alleles (Worland *et al.*, 2001), and the Spanish variety ‘Pané 247’ (115 cm height), the only improved variety in which *Rht* dwarfing alleles were missing. This points out the importance that the pioneer breeders, especially Nazareno Strampelli, gave to reductions in plant height to improve the agronomic performance of wheat varieties (Salvi et al., 2012). Further height decreases occurred after the introduction of modern varieties from the Green Revolution, most of them derived from CIMMYT germplasm, thus carrying the Norin-10/Brevor major dwarfing GA insensitive alleles *Rht-B1b* and/or *Rht-D1b* (Worland and Snape, 2001).

It is well known that the introduction of dwarfing genes helped boosting wheat HI worldwide during the 20th century (Austin et al., 1989; Worland and Snape, 2001) and particularly in Spain (Royo et al., 2007; Sanchez-Garcia et al., 2012b). The present study revealed that within the set of modern varieties, a consistent trend of HI increases did not exist through the period elapsed from the introduction of Yecora, in 1972, with a HI of 0.47 until the release of Califa Sur, in 2001, with a HI of 0.48. Seeking further improvements in HI through new reductions in plant height may be difficult to achieve, or even undesirable, since the height of the newest variety of the historical series, Califa Sur (71 cm), was close to the lower limit of the optimum plant height range stated by Flintham *et al*. (1997) in 70-100 cm. Since shorter heights may be detrimental for optimum canopy architecture and, therefore, for an efficient photosynthetic performance (Foulkes *et al*., 2011), future yield gains seem to be better attained by increasing CDW without modifying the HI, as proposed by Parry *et al*. (2011) and Blum (2011).

The results of the multivariate analysis indicated that the relationship between dwarfing genes, biomass and yield depended more on the number of dwarfing alleles at the *Rht* loci than on the presence of any specific allele or genotypic combination. In addition, the distribution of varieties along PC1 was more related to the number of dwarfing alleles they carry than to their growth habit. Thus, varieties carrying a mutant allele at one *Rht* gene, such as the French winter cultivars Soissons, Isengrain, Marius and Estrella, and the spring types Anza, Rinconada, Impeto and Gazul (Sanchez-Garcia et al. 2012a), were clustered together according to PC1. These results justified the study of the pattern of biomass accumulation and partitioning on the basis of the number of *Rht* dwarfing alleles (*Rht* DA) present in the historical series.

Multivariate analysis and the differences observed in CDW and LAI between *Rht* DA groups revealed a detrimental effect of dwarfing alleles on biomass and leaf area at the beginning of jointing. This detrimental effect was larger in varieties carrying two of them. Previous studies have reported a negative effect of the GA insensitive dwarfing alleles *Rht-B1b* and *Rht-D1b* –the most common in our set of genotypes– on early vigor. These effects involve shortening of the coleoptile length (Ellis *et al*., 2004) and decreases of vegetative organs size and weight due to reductions on cells length (Youssefian *et al*., 1992; Miralles *et al*., 1998). Despite differences between breeding periods in CDW at the beginning of jointing were not significant, a decrease trend was observed on this trait, which was further confirmed by the progressive reduction of LAI in initial and modern varieties when compared with the landraces. The fact that varieties with a different number of *Rht* DA were included in the initial, and particularly in the modern group of varieties was probably the reason that the changes in CDW at the beginning of jointing across periods were not significant. It has been demonstrated that early crop development may be determinant for adaptation to some Mediterranean-like environments, since a faster rate of seedling development can accelerate soil coverage and prevent soil moisture loses (Condon *et al*., 2004). We offer here the speculation that the reduction of biomass at the beginning of jointing caused by the introduction of *Rht* dwarfing alleles could be behind the worse adaptation of initial and, specially, Spanish modern varieties to poor and dry environments when compared with the landraces, reported in a previous study including most of the varieties used here (Sanchez-Garcia *et al*., 2012a).

Similar rates of dry matter accumulation from the beginning of jointing to anthesis were observed in the three *Rht* DA groups and the three breeding periods. Therefore, differences in CDW between *Rht* DA or breeding periods at anthesis and booting relied upon the initial differences in CDW at the beginning of jointing and the thermal time needed to attain the anthesis. A similar conclusion was obtained in previous studies carried out in Mediterranean-like environments with bread (Richards 1992) and durum wheat (Álvaro *et al*., 2008). Fitting phenology, one of the main adaptation mechanisms, has been related to wheat yield gains in Spain during the 20th century (Sanchez-Garcia *et al*., 2012a). The earliness of initial and modern varieties when compared with the landraces, was the main cause of their lower biomass accumulated prior to anthesis, but it also most likely allowed grains to fill under better environmental conditions avoiding, at least in part, the typical terminal stresses of Mediterranean environments.

Biomass at anthesis was significantly greater in landraces (*Rht* DA-0) than in improved varieties, independently of the breeding period they belong or the number of *Rht* DA they carry. However, these differences disappeared at physiological maturity, when all groups had similar CDW. These results suggest contrasting strategies in landraces and improved varieties for biomass formation and allocation during grain filling. Despite biomass accumulation was better explained by the number of *Rht* DA than by breeding periods, they were not useful to understand the partitioning of biomass to grains during grain filling.

The study of biomass allocation from anthesis to maturity revealed that initial and modern varieties accumulated 176 g m^{-2} (55%) and 267 g m^{-2} (84%), respectively, more biomass in the spikes during this period than the landraces. On the other hand, relative losses of vegetative biomass during this period were -68 g m^{-2} (-19%) and -100 g m^{-2} (-27%) in initial and modern varieties, respectively, when compared with the landraces. Since grain growth is supported by transient photosynthesis and translocation of stored reserves accumulated prior to anthesis in vegetative organs (Blum, 1988), our results suggest that the introduction of the first improved varieties increased the contribution of photosynthesis to grain filling.

Changes in biomass partitioning were studied by fractioning grain filling in two phases, one from anthesis to milk-grain stage, and the other from it to physiological maturity. Increases in spikes weight from anthesis to milk-grain stage were greater in initial and modern varieties than in the landraces. However, in spite of the observed tendency towards a higher remobilization of vegetative biomass and, also an increase in current photosynthesis resulting in higher CDW accumulation, differences between breeding periods were not statistically significant. Thus, we could not attribute the gains in spikes weight per unit area during this phase to a specific increase in photosynthesis or translocation, because they were likely due to both of them.

Changes in biomass accumulation and partitioning from milk-grain stage to physiological maturity revealed contrasting strategies in landraces and improved varieties of different periods. Landraces lost above ground biomass during the second phase of the grain filling, due to the senescence of leaves and the loss of stems weight (313 g m^{-2}) that was much greater than the increases in spikes weight. Thus, part of the decreases of stems weight was not attributable to translocation, but most probably to the dead of secondary tillers. It has been reported that the number of unfertile tillers is much greater in landraces than in improved varieties (Sanchez-Garcia et al., 2012b), and probably the warm and dry environmental conditions at the end of the grain filling period accelerated their senescence. Initial varieties maintained total above ground biomass after milk-grain stage, and the gains in spikes weight during this phase corresponded to the loss of vegetative biomass. This result suggests that translocation was in this phase the main contributor to fill the grains of the first improved varieties grown in Spain. In contrast with the patterns followed by previous cultivars, modern varieties increased total above ground biomass from milk-grain stage to maturity. In addition, their decrease of vegetative biomass was much lower than the increases in the weight of the spikes, thus indicating that the contribution of photosynthesis to grain filling in this last phase was enhanced in modern varieties when compared with the precedent ones. This was probably the cause that the gain in spikes weight from milk-grain stage to physiological maturity was the most relevant to explain yield genetic gains along the century. Actually, our results showed that 79.3% of absolute yield gains of bread wheat in Spain during the last century were due to the increases in spikes weight from milk-grain stage to physiological maturity, while the remainder 20.7% corresponded to the gains in the anthesis to milk-grain phase.

The study of the relationship between growth traits and the increases in spikes weight after milk-grain stage revealed that they were strongly linked to the greater LAD of modern varieties. Leaf area duration has been considered an important mechanism for increasing total crop photosynthesis and, thereby, crop biomass (Richards 2000) and yield performance in non water-limited Mediterranean-like environments (Christopher *et al.*, 2008). When compared with the landraces, the higher LAD of improved varieties could be attributed to their early heading, as it has been previously described (Naruoka *et al*., 2012). However, the higher LAD of modern varieties in relation to the initial cultivars could not be explained in terms of phenological differences, since both sets of genotypes had similar developmental patterns. The reason of the superior LAD of the most recent cultivars was their greater leaf area available at milk-grain stage. These results, and the positive and significant relationship found from milk-grain stage to physiological maturity between the year of release and both, LAI at milk-grain stage and increases in spikes weight, support the previous assumption that enhanced current photosynthesis helped boosting the gains on spikes weight during the last phase of grain filling in modern varieties.

Concluding remarks

Genetic gains in bread wheat yield in Spain during the 20th century were closely related to changes in biomass accumulation and partitioning over the crop cycle. These changes were directly associated to the introduction of mutant alleles at the *Rht* major dwarfing genes, which caused a strong decrease in crop biomass and LAI at the beginning of jointing. Plant height reductions and early anthesis of improved varieties increased biomass partitioning to the spikes and HI.

Although the number of *Rht* DA explained changes in CDW during all the growth cycle better than the breeding periods, the last were more useful to interpret biomass allocation from anthesis to maturity. The reason may be that the introduction of dwarfing genes was only one of the strategies used by breeders to improve yields, but increases in LAI during grain filling and LAD also significantly contributed to productivity increases during the last decades of the century. The maintenance of more green leaf area from milk-grain stage to maturity – the main period determining yield gains during the past century – appeared to be the main contributor to gains on spikes weight. Since no evidence was found that increases on delayed senescence reached a plateau, enhancing LAI at milk-grain stage could be a strategy for further boosting yield gains.

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CHAPTER 4

Changes in bread-making quality attributes of bread Wheat varieties cultivated in Spain during the 20th century

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En revisión

Changes in bread-making quality attributes of bread wheat varieties cultivated in Spain during the 20th century

Abstract

Genetic gains on quality were assessed on grain samples from 4 field experiments involving 16 bread wheat varieties representative of the most widely cultivated in Spain during the 20th century. The allelic composition at three glutenin loci (*Glu-A1*, *Glu-B1* and *Glu-D1*) was obtained by PCR-based DNA markers and published references. From 1930 to 2000 grain protein content decreased at a rate of -0.030% y^{-1}, or in relative terms -0.21% y^{-1}, but the protein produced per hectare increased by 0.39% y^{-1}. Alveographic tests revealed significant changes on dough rheological properties. Dough strength (W) and tenacity (P) increased at relative rates of 1.38% y^{-1} and 0.99% y^{-1}, respectively, while dough extensibility (L) decreased a -0.46% y^{-1}, resulting on an increase of 1.45% y^{-1} of dough equilibrium (P/L). Protein quality improvement could be related to the replacement of the null allele by subunits 1 or 2*at *Glu-A1* and the prevalence of subunits 7+8 and 5+10 at *Glu-B1* and *Glu-D1* loci, respectively, in the most recent varieties. The relationship between dough extensibility and water input during growth cycle was partially explained by the presence of the 5+10 HMW-GS. Fermentation tolerance was improved in the most modern varieties. Dough collapse during fermentation was only avoided in doughs with $W \geq 159$ J·10^{-4} and $P/L \geq 0.56$ mm H_2O mm^{-1}, levels achieved by most modern varieties. The over-strong and unbalanced rheological properties of some modern varieties resulted in highly porous doughs, and no clear advances in dough maximum height during fermentation were attained.

Keywords: wheat; bread-making quality; historical series; rheological properties; *Glu-1*; HMW glutenin subunits; fermentation.

Introduction

Bread wheat is the second most important staple crop in the world providing 18% of the daily calories intake worldwide in 2009 (FAOSTAT, 2009). Its role on human diet makes determinant that cultivated wheat will meet the specific quality criteria needed for the manufacturing of the wide range of food products derived from it.

During the last century in many countries, Spain among them (Royo and Briceño-Félix, 2011), wheat breeding efforts were concentrated on yield increases, being grain quality improvement a secondary breeding objective. Besides, studies evaluating genetic gains frequently refer to yield and associated traits, while references to progress on grain quality are scarce. These studies, which usually involve historical series of genotypes, allow breeders to evaluate selection efficiency and to identify traits associated with genetic gains. Studies on wheat genetic gains have been conducted in several European countries such as Italy (Canevara *et al*., 1994), France (Brancourt-Hulmel *et al*., 2003), UK (Austin et al., 1989), and more recently Spain (Royo *et al*., 2007; Sanchez-Garcia *et al*., 2012). When addressed, wheat quality in these kind of studies is mainly tackled through attributes such as test weight or protein content, but the evaluation of important end-use quality traits such as dough rheological properties or fermentation performance is frequently neglected.

Wheat end-use quality is strongly related to the properties of the gluten matrix that are mainly determined by the quantity and quality of the gluten proteins (Finney, 1943). High molecular weight glutenin subunits (HMW-GS) are of particular interest for bread wheat due to their large influence on dough rheological properties (Branlard and Dardevet, 1985; Payne *et al*., 1987; Vawser and Cornish, 2004), and their role on their genotype by environment interaction, particularly regarding gluten strength and extensibility (Blumenthal *et al*., 1995; Johansson *et al*., 2000; Panozzo and Eagles, 2000; Hristov *et al*., 2010). Subunits 1 and 2* encoded at *Glu-A1* loci, and subunit 5+10 at *Glu-D1* are considered suitable for promoting dough strength, and have played a role in bread wheat quality enhancement in a number of countries such as France and Italy (Mesdag and Donner, 2000). On the other hand, other subunits, as the Null allele at *Glu-A1* are considered to have a negative effect (Payne *et al*. 1987). A study aiming to ascertain the relationship between both dough strength (W) and Zeleny sedimentation volume and the allelic composition at the *Glu-1* loci was conducted with Spanish varieties (Payne *et al*., 1988), but information regarding changes induced by breeding in the HMW-GS composition and their effect on quality properties is still lacking.

In southwestern European countries bread wheat is mainly consumed as baguette bread, and the dough properties during mixing and fermentation are amongst the quality criteria addressed by breeding programs. The study of the response of dough to common constraints occurring during baking processes is of special interest to predict the end-use quality (Dobraszczyk and Morgenstern 2003) since dough's rheological properties are determinant for product functionality (Dobraszczyk and Morgenstern 2003). Biaxial extension parameters, such as dough strength or extensibility, usually obtained through Chopin's alveograph procedures, are among the bread-making quality attributes used to characterize wheat samples according to the official quality classifications (Mesdag and Donner, 2000). All the rheological tests performed on dough aim to predict their behavior under bread-making processes, such as mixing or fermentation. During fermentation, gas cells within the dough expand causing the final loaf volume, critical for bread appeal (Dobraszczyk *et al*., 2000). In spite of this, most fermentation tests are preformed on doughs without yeast at room temperatures, conditions in which the dough rheological properties may differ from the existing under real fermentation conditions (Dobraszczyk and Morgenstern, 2003). For a reliable determination of the fermentation behavior of the varieties, recent studies have proposed the use of fermentation monitoring techniques, particularly through the Rheofermentometer F3 (Ktenoudaki *et al*., 2010, 2011).

The cataloging of bread wheat varieties according to their quality standards is critical, not only for establishing their potential use according to the industry requirements, but also to ascertain the success of the strategies used by breeding programs to address quality improvement. The first classification for bread wheat quality in Spain was implemented in 1945 (Mesdag and Donner, 2000), but it did not include the alveographic parameters that were already used for quality classification in other European countries (Mesdag and Donner, 2000). Alveographic parameters were firstly introduced in Spain for wheat quality characterization in the 1970s (Mesdag and Donner, 2000), and it was not until 2010 when an official classification, similar to the existing in neighboring countries, and mainly based on these parameters, was proposed. This lack of regulation may have contributed to the traditional large imports of wheat grain, mainly of high quality standards, destined to the local milling and baking industries, which in 2011 accounted for up to 38.5% of total wheat consumption in the country (MAGRAMA, 2011). Efforts on the improvement of bread wheat quality in Spain appear, therefore, as a main challenge for present and future breeding programs.

The objectives of the present study were: i) to investigate the changes caused by bread wheat breeding in Spain during the 20th century on the quality attributes related to bread-making performance, ii) to assess the relationship between quality improvement and changes in HMW-GS composition, and iii) to analyze whether breeder's selection for good rheological attributes resulted in an improvement of the variety performance during fermentation.

Materials and Methods

Plant material

A collection of 16 bread wheat varieties representative of the most widely cultivated in Spain during the 20th century was assembled. These varieties were grouped in three breeding periods (BP) according to their year of release, or cultivation period, as follows: i) Spanish landraces grown before 1940; ii) initial varieties derived from crosses, including those improved and grown from the mid 1940s until the Green Revolution, and iii) modern varieties, introduced or released in Spain from 1970 to 2001, and most of them derived from semi-dwarf germplasm of CIMMYT and French origin (Table 1).

Field experimental setup

Four field experiments were conducted during two growing seasons, 2008-09 and 2009-10 in Lleida (north-eastern Spain: 41° 40´N, 0° 20´E; 200 m of altitude a. s. l.) under two water regimes: rainfed and irrigated, the last with additional water inputs of 96 mm and 150 mm in 2009 and 2010 respectively (Table 2). According to the FAO soil classification (FAO, 1998) soil type was Calcic Cambisol with fine loam texture.

Experiments were planted in late November and harvested in July, following a randomized complete block design with three replications. Plots consisted of eight 5 m-length rows, 0.15 m apart. Sowing density was 450 seeds/m^2. Nitrogen fertilization was applied in top dressing as ammonium nitrate. Further experimental details and management practices are described in Table 2.

Table 1. Description of the varieties used in the study and their allelic composition at the high molecular weight glutenin subunits (HMW-GS).

Variety	Pedigree	Origin	Year of release in Spain	HMW-GS[†]			References
				Glu-A1	*Glu-B1*	*Glu-D1*	
	Landraces						
Aragón 03	Selection of the landrace "Catalan de Monte"	Spain	<1940	**Null**	20	4+**12**	*Carrillo et al., 1988*
Barbilla	Landrace	Spain	<1940	**?**[‡]	NI	NI+**12**	
Candeal	Landrace	Spain	<1940	**Null/1**	NI	**5+12**	
	Initial varieties						
Impeto	Frassineto-405/Villa-glori	Italy	1950	**1**	7+8	2+**12**	*Pogna et al., 1989*
Mara	Autonomia/Aquila	Italy	1947	**Null**	7	2+**12**	*Dencic and Borojevich, 1991*
Estrella	Mon-desir/Ardito//Mouton-a-epi-rouge/k-3/3/Mouton-epi-rouge	France	1952	**Null**	7+8	2+**12**	*Branlard et al., 2003*
Pané 247	L-4/Mentana	Spain	1955	**Null**	7+8	2+**12**	*Peña, 2004*
	Modern varieties						
Yecora	Ciano-67//Sonora-64/Klein-rendidor/3/Siete-cerros-66	CIMMYT	1972	**1**	17+18	**5+10**	*CRC, 1998*
Cajeme	Ciano-67//Sonora-64/Klein-rendidor/3/Siete-cerros-66	CIMMYT	1972	**1**	17+18	**5+10**	*CRC, 1998*
Anza	Lerma-rojo-64//Norin-10/Brevor/3/3*Andes-enano	CIMMYT/USA	1974	**Null**	7+8*	2+**12**	*Leon et al., 2010*
Marius	Cadet//Thatcher/Vilmorin-27/3/Ariana/Fundulea	France	1980	**Null**	7+9	2+**12**	*Branlard et al., 2003*
Rinconada	Unknown	CIMMYT	1981	**1**	7+8	**5+10**	*Peña, 2004*
Soissons	Iena/hn-35	France	1990	**2***	7+8	**5+10**	*Branlard et al., 2003*
Gazul	Unknown	Spain	1992	**2***	7+8	**5+10**	*Peña, 2004*
Isengrain	Apollo/Soissons	France	1998	**Null**	7+8	**5+10**	*Lemelin et al., 2005*
Califa Sur	Unknown	Spain	2001	**1**	7+8	**5+10**	*Lucas, 2010*

†: In bold-type are identified the HMW-GS characterized in the present study through PCR-based DNA markers.
‡: It is neither the null, the 1 nor the 2* subunit.
NI: Not identified.

HMW-GS allelic composition

PCR-based DNA markers were deployed to determine the HMW-GS composition at two loci (*Glu-A1* and *Glu-D1*). The methodology proposed by De Bustos *et al.* (2000) was followed to discriminate the allele encoding for the 2* subunit from the ones encoding for subunit 1 and the Null allele at *Glu-A1* locus. PCR-based DNA

Table 2. Experimental details of the study conducted in Gimenells (Lleida, northeastern Spain).

Year	2009		2010	
Water regime	Rainfed	Irrigated	Rainfed	Irrigated
Experiment identification	R09	I09	R10	I10
Water input (rainfall + irrigation, mm) from sowing to maturity	191	288†	277	427
Environmental conditions during grain filling				
Average daily temperature (°C)	19.6	19.9	17.2	17.8
Water input (rainfall + irrigation, mm)	9	54†	5	82
Fertilization (kg ha^{-1})				
N (top dressing)	30	40	50	94
P_2O_5	68	113	105	105
K_2O	113	188	175	175
Means across varieites				
Grain protein content (%; mean ± S.D.)	16.5 ± 1.24	15.2 ± 1.56	14.1 ± 1.11	11.4 ± 0.96
Falling Number (s; mean ± S.D.)	479 ± 101	495 ± 106	428 ± 77	354 ± 55
Yield (kg ha^{-1})	5434	7201	4318	7120

†: Sprinkler irrigation.

markers were deployed at the *Glu-D1* locus to detect the alleles encoding for *Dx5*, *Dy10* and *Dy12* glutenin subunits, following the methodology proposed by Ahmad (2000). All fragments obtained were separated in agarose gels. Allelic composition at *Glu-B1* locus was obtained from published references, which were also used to verify and complete the results obtained (Table 1).

Grain quality

All plots were mechanically harvested at ripening. Test weight (TW; kg hl^{-1}) was measured from cleaned grain samples from each plot, and grain protein content (PC, %) was analyzed with a near-infrared transmitance spectrophotometer (Infratec® 1241-grain analyzer, Foss Tecator AB, Sweden). Total amount of protein produced per unit area (GPpHa, kg ha^{-1}) was determined for each plot as the product of PC and grain yield.

Flour attributes

White flour samples were obtained from milled grain of each plot. Before milling, grain samples were tempered overnight to 16% moisture content. The grain was grinded in a Moulin CD1 mill (Chopin S.A., Villeneuve la Garenne, France).

The following traits were assessed in the white flour samples: rheological properties regarding dough biaxial extension: tenacity (P, maximum overpressure), extensibility (L, length of the curve), strength (W, deformation energy), and the configuration ratio (P/L) with the alveograph (Chopin S.A., Villeneuve la Garenne, France) following the ICC standard method No. 122 (ICC, 1992), ash content (%;

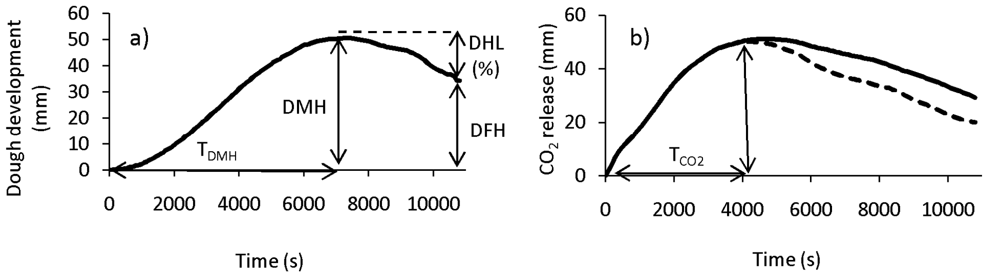

Figure 1: Pattern of changes in: a) dough development, and b) CO_2 production (solid line) and release curves (discontinuous line) samples tested in a rheofermentometer F3. DMH: Dough maximum height; DFH: dough final height; DHL: dough height lost, %; T_{DMH}: time to attain DMH; T_{CO2}: time to CO_2 release.

AACC International Method 08.21.01; AACC, 2000), water absorption using the near-infrared spectrophotometer Inframatic 8600 (Perten instruments, Sweden), and α-amylase activity using Falling Number 1313 (Perten instruments, Sweden) and the AACC International Method 56-81.03 (AACC, 1999). During the second crop season only two replicates per environment were analyzed for the aforementioned attributes.

Fermentation test

In the second year dough samples of 250 g of white flour from each plot of one block of the irrigated experiment were prepared for fermentation test with 3 g (1.2%) of instant active dry yeast (Pante; Puratos, Belgium) and 5 g of salt. The amount of water added was adjusted from the measured water absorption value. The fermentation of each dough sample was monitored using a Chopin rheofermentometer F3 (Chopin S.A., Villeneuve la Garenne, France). The dough was constrained with a piston of 2 kg resistance within the fermentation basin during a 3 hour process with a stable temperature at 28.5°C. The parameters recorded included: dough maximum height (DMH), dough final height at the end of the 3h process (DFH), dough height lost between maximum and final height (DHL, %), time to DMH (T_{DHM}) from the dough development curve and volume of total CO_2 produced (VP_{CO2} or the area behind the gas production curve), volume of CO_2 retained by the dough (VR_{CO2} or the area behind the gas retention curve), the time to CO_2 release (T_{CO2}), and the retention coefficient (RQ or the proportion of total CO_2 volume produced, retained at the end of the process) from the gas production and retention curves. Curves and parameters are represented in Fig. 1.

Statistical analysis

Analyses of variance were carried out for all the traits studied. In the model the genotypic effect was partitioned in differences between breeding periods (BP) and the genotypic residual, that is, the genotypic variance retained within periods. The genotypic residual and its interactions were considered as a random factor. According to the results obtained from the ANOVA analyses, the variety by environment (VxE) interaction of the extensibility (L) parameter of the alveogram was partitioned in an AMMI model (Gauch and Zobel, 1997). The number of bilinear terms retained in the analysis was determined according to the proportion of the sum of squares (SS) of the VxE interaction explained by each IPCA. The effect of the 5+10 HMW-GS encoded at *Glu-D1* locus on VxE interaction was studied by partitioning the variety factor of the ANOVA in differences between varieties carrying and not this HMW-GS and the residual.

Regression models were fitted in order to assess the relationship between variables studied. Bi-linear regression models [$Y = BX+A$ ($X</>C$) + $B*C$ ($X\geq/\leq C$); with the slope of the second segment restricted to 0] were selected when performed better than the linear one. All analyses were carried out with the SAS-STAT (SAS Institute Inc., 2003) and GENSTAT (Payne *et al.* 2006) statistical packages, and Tablecurve 2D v2.03 software (Jandel, 1991) was used for fitting the bi-linear models.

Results

HMW-GS allelic composition

The identification of the HMW-GS composition of the varieties included in the historical series revealed differences between breeding periods (BP). The null allele at *Glu-A1* locus predominated in landraces and old bred varieties, with frequencies greater than 57%, while it was present in only 33% of the modern varieties (Table 1). The *Glu-B1* locus showed the highest number of allelic variants in the historical series studied. Nevertheless, five out of the seven alleles detected at this locus were present in only one variety. Allele 7+8, absent in landraces, was the most frequent in initial and modern varieties, with frequencies of 75% and 44%, respectively. Two of the alleles present at *Glu-D1* locus (4+12 and 5+12) were exclusive of landraces. For the landrace ‘Barbilla’, the *Dx* subunit encoded at *Glu-D1* could not be identified, although our results demonstrated that it was not the *Dx5* subunit. All the initial varieties were monomorphic for the 2+12 allele at *Glu-D1*, while in the most recent cultivars allele 5+10 predominated (77%, Table 1).

Grain quality

The analyses of variance showed that the water regime was, among the environmental factors, the only significantly affecting TW, which mostly depended on the variety effect (Table 3). However, despite the tendency of a greater TW on modern varieties (Table 4), differences between breeding periods were not statistically significant for this trait. Grain protein content and GPpHa were largely affected by the environmental conditions, but differences between breeding periods accounted for most of the variability induced by the genotypic effect (Table 3). With the introduction of initial varieties, PC decreased by 11.3%, but no

Table 3: Percentage of the sum of squares of the ANOVA for grain and bread-making quality attributes. Variety effect and its interactions are partitioned in differences between breeding periods and within them (residual not shown).

	df	Test weight	Grain protein content	Grain protein per ha	Water absorption	Ash content	Biaxial extension parameters			
							W	P	L	P/L
Year	1	<0.01	47.3***	37.7***	13.7***	33.6***	2.74***	0.58*	1.87***	0.07
Water regime	1	21.3***	20.9***	18.6***	2.53**	14.1***	4.05***	2.87***	0.99*	1.53***
Year x Water regime	1	0.56	2.43***	0.01	1.04***	1.97*	3.24***	1.08*	6.76***	0.1
Block (Year x Water regime)	8	1.51	0.63*	1.79**	1.04	1.22	0.31	0.65	1.36	0.78
Variety	15	44.7***	18.6**	18.2**	73.8***	22.5***	80.3***	85.5***	44.9***	76.2***
Between Breeding Periods (BP)	2	19.1	77.2***	75.0***	42.6*	27.2	43.6*	30.4	16.9	30.7
Variety x Year	15	6.34***	3.83***	10.7***	1.49***	7.96***	1.77***	3.18***	9.93***	5.86***
Between BP x Year	2	2.59	28.5	55.57**	1.69	3.23	25.2	10.8	15.1	15.29
Variety x Water regime	15	6.06***	0.76	3.16***	0.36	2.72	1.41***	1.28**	13.4***	3.04**
Between BP x Water regime	2	18.96	0.7	47.1*	22.4	9.64	3.95	8.82	64.4**	49.62*
Variety x Year x Water regime	15	6.54***	1.26**	1.76*	1.04	4.81**	3.26***	1.50***	4.59*	4.01***
Between BP x Year x Water Regime	2	24.63	51.38**	5.84	40.1*	44.5*	45.99*	56.5**	18.3	8.96
Error	120	12.9	4.31	8	2.1	11	2.91	3.33	16.3	8.37
Total	191									

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

significant additional reductions occurred with the introduction of modern cultivars (Table 4). In spite of the reductions in PC, significant increases in GPpHa were observed across BP. Thus, although the relative genetic gain was negative for PC (-0.21% y^{-1}), GPpHa increased at a rate of 0.39% y^{-1} (Table 5).

Table 4: Mean values of grain and bread-making quality attributes of 16 bread wheat varieties from 3 breeding periods across 4 environments. The varieties were grouped according to the breeding period they belong. Numbers in parentheses indicate the percentage of change with respect to the previous period.

		Landraces	Initial		Modern		
	df	2	3		8		SED
Test weight	(kg hl^{-1})	76.7	78.7	(2.61)	79.1	(0.51)	1.37
Grain protein content	(%)	16.0	14.2	(-11.3)	13.8	(-2.8)	0.34
Grain protein per ha	(kg ha^{-1})	692	815	(17.8)	908	(11.4)	35
Water absorption	(%)	51.5	50.4	(-2.14)	53.1	(5.36)	0.86
Ash content	(%)	0.654	0.65	(-0.76)	0.63	(-3.39)	0.013
Biaxial extension parameters							
W	($J{\cdot}10^{-4}$)	83.1	164	(97.4)	249	(51.8)	51.9
P	(mm H_2O)	36.2	53.4	(47.5)	70.8	(32.6)	14.51
L	(mm)	134	119	(-11.2)	108	(-9.24)	13.7
P/L	(mm H_2O mm^{-1})	0.28	0.49	(75.0)	0.74	(51.0)	0.181

Flour and dough attributes

The environmental factors year and water regime explained about half of the variance for flour ash content (Table 3). The relative genetic gain of ash content was statistically significant (*P*<0.05), but the resulting rate of change was extremely

Table 5. Absolute and relative genetic changes for grain and bread-making quality attributes of 16 bread wheat varieties from 3 breeding periods across 4 experiments.

	Absolute changes	Relative changes (% y^{-1})	R^2	Absolute changes units
Test weight	–	–	0.14	kg hl^{-1} y^{-1}
Grain protein content	-0.03	-0.21	0.51^{**}	% y^{-1}
Grain protein per ha	0.003	0.39	0.64^{**}	kg ha^{-1} y^{-1}
Water absorption	0.042	0.080	0.30^{*}	% y^{-1}
Ash content	-0.0005	-0.078	0.27^{*}	% y^{-1}
Biaxial extension parameters				
W	2.74	1.39	0.42^{*}	$J{\cdot}10^{-4}$ y^{-1}
P	0.59	0.99	0.32^{*}	mm H2O y^{-1}
L	-0.53	-0.46	0.29^{*}	mm y^{-1}
P/L	0.009	1.45	0.38^{*}	mm H_2O mm^{-1} y^{-1}

**P*< 0.05; ** *P*< 0.01.

low (-0.078% y^{-1}, Table 5) and no significant differences were detected between BP. Water absorption (WA) was strongly genotype-dependent, since the variety effect accounted for 73.8% of total variation (Table 3). Changes in WA between breeding periods were statistically significant due to differences between initial and modern varieties (Table 4). However, the estimated rate of change across period was very low (Table 5).

The alveographic parameters W and P showed a strong variety effect and, accordingly, the environmental contribution to their total variance was very low (Table 3). However, differences between BP were only significant for W, despite the large increases observed in P and P/L across periods (Table 4), which resulted in significant relative genetic gains of 0.99% y^{-1} and 1.45% y^{-1} respectively, while L decreased at a rate of -0.46% y^{-1} (Table 5).

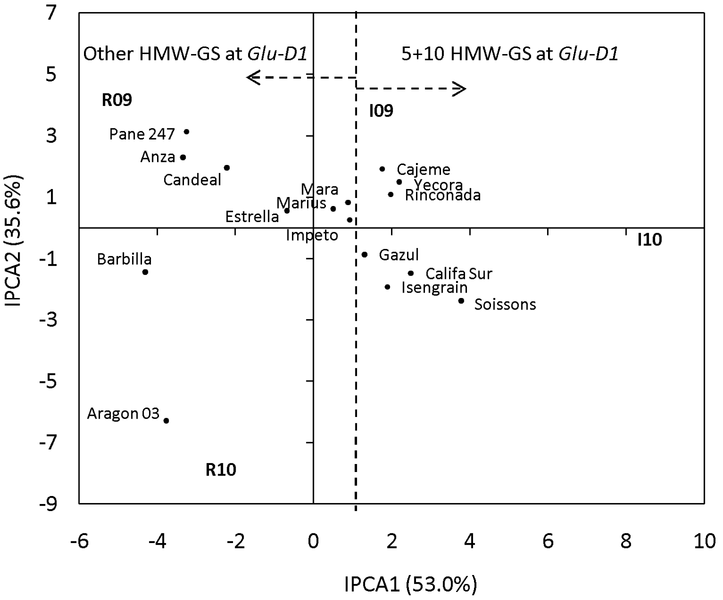

Figure 2. Biplot of the first two axes of the AMMI model for extensibility (*L*). Environments are represented in bold (see Table 2 for environment identification). The discontinue line separates the varieties carrying the 5+10 HMW-GS at *Glu-D1* locus from the remainder.

Dough extensibility was the alveographic parameter that showed the lowest stability across environments, with a variety x environment interaction (VxE) that explained 27.9% of total variance (deduced from Table 3). The component that mostly contributed to it was the variety x water regime interaction, which accounted for 13.4% of the total variance, while the variety x year and the variety x year x water regime interactions accounted for lower percentages (Table 3). In order to understand the VxE interaction for L, an AMMI model analysis was performed. The results showed that the first two IPCA explained 88.6% of the VxE variation (Fig. 2). The first IPCA, which accounted for 53.0% of the total variance, separated the rainfed environments, which were located in the negative sense of the axis, from the irrigated ones that were placed in the positive sense, arranging them according to the water input they received. According to this IPCA, the varieties carrying the 5+10 subunit at the *Glu-D1* locus were situated in the right part of the figure. When, in the ANOVA, the variety effect and its interactions were partitioned in differences between varieties carrying or not the 5+10 subunit, the presence or absence of this allele explained 30.4% of the total VxE variance, and 44.1% of the variety x water regime effect (data not shown). These results suggest that the response of L to water input depended on the genotype at the *Glu-D1* locus, particularly the presence/absence of the 5+10 subunit. Moreover, the lowest PC was recorded in the environment with the highest water input, while the highest PC corresponded to the driest environment (Table 2).

Fermentation test

None of the coefficients of determination of the linear regression lines used to calculate absolute and relative genetic gains of dough fermentation parameters were statistically significant. However, a tendency was detected towards a reduction of dough maximum height across periods (DMH), as well as an increase on the time needed to attain it (T_{DMH}) (Table 6). Changes in DMH were mostly due

Table 6: Dough development and characteristics of CO_2 release during fermentation of an historical series of 16 bread wheat varieties from 3 breeding periods determined from samples obtained in the experiment conducted in 2009 under irrigated conditions. DMH: dough maximum height; DFH: dough final height; DHL: dough height lost, %; T_{DMH}: time to DMH; T_{CO2}: time to CO_2 release; VP_{CO2}: total CO_2 volume produced; VR_{CO2}: total CO_2 volume retained; RQ: retention coefficient.

Variety	DMH (mm)	DFH (mm)	DHL (%)	T_{DMH} (s)	T_{CO2} (s)	VP_{CO2} (ml)	VR_{CO2} (ml)	RQ (%)
Landraces								
Aragon 03	48.2	24.7	48.8	7830	4860	1098	1010	91.9
Barbilla	39.3	35.3	10.2	8550	4680	1078	977	90.7
Candeal	50.6	34.3	32.2	7470	4950	1089	1014	93.1
Initial								
Mara	45.8	30.3	33.8	5940	NC	895	870	97.2
Impeto	41.9	41.8	0.24	10710	3600	1135	1012	89.1
Estrella	39.0	31.8	18.4	7290	NC	908	858	94.6
Pane 247	43.4	39.6	8.76	9000	5490	987	927	93.9
Modern								
Cajeme	47.3	46.2	2.33	9630	4050	1234	1087	88.1
Yecora	44.0	41.9	4.77	8190	3150	1317	1112	84.4
Anza	41.6	39.4	5.29	9090	4950	1212	1075	88.7
Marius	420.	19.3	54.1	6210	NC	862	843	97.8
Rinconada	42.1	41.4	1.66	8820	3870	1313	1115	85.0
Soissons	44.2	42.5	3.85	9420	4320	1105	1010	91.4
Gazul	32.7	32.7	0	10800	3870	1091	961	88.1
Isengrain	45.5	45.0	1.10	9360	4230	1150	1033	89.9
Califa Sur	31.8	31.8	0	10800	2880	1218	1032	84.7
Means of the breeding periods (BP)								
Landraces	46.0	31.4	30.4	7950	4830	1088	1000	91.9
Initial	42.5	35.9	15.3	8235	4545	981	917	93.7
Modern	41.2	37.8	8.1	9147	3915	1167	1030	88.7
SEM	3.34	5.05	11.3	967	464	81.1	50.6	2.13

NC: These varieties did not released significant amounts of CO_2 within the 3 hours the test was planned to last.

to the low values attained within the 3 hours process by doughs of the modern varieties ‘Gazul’ and ‘Califa Sur’, which would need more than 3 hours to reach their DMH. Despite differences between BP were not statistically significant, a tendency was observed towards an increase on dough final height (DFH) across time (Table 6). The trend of a DMH decrease and a DFH increase was certainlyy the cause of the significant reduction of 73% observed across periods on the relative dough height loss at the end of the 3 hour process (DHL). No changes appeared on the total CO_2 volume produced and retained, but the time of release decreased significantly in more than 15 min in modern varieties when compared with the landraces (Table 6). In addition, the introduction of modern varieties caused a significant reduction of the retention coefficient (RQ).

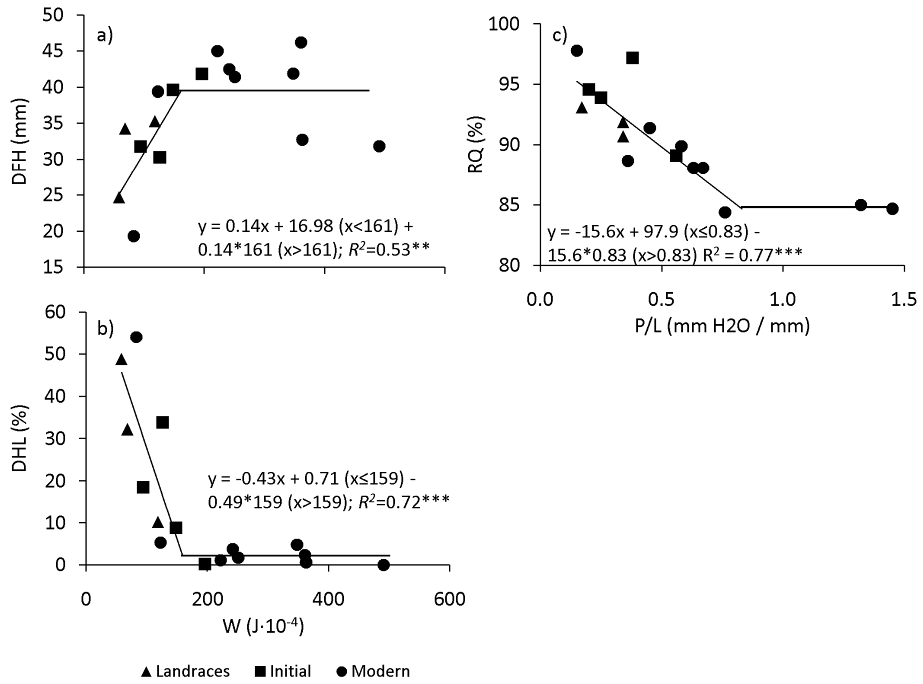

Figure 3. Relationship between dough deformation energy (W) of an historical series of 16 bread wheat varieties from three breeding periods and: a) Dough final height (DFH); and b) Dough height loss (DHL) and c) relationship between the retention coefficient (RQ) and the configuration ratio (P/L). Samples were obtained from one replication of the experiment conducted in 2009 under irrigated conditions.

In order to ascertain whether the genetic improvement of the rheological properties resulted in changes on dough fermentation behavior, correlation coefficients were calculated between dough deformation and fermentation parameters, obtained respectively from the alveograph and the rheofermentometer. The results showed that significant and negative relationships existed between both W and P with DHL and DMH (Table 7). The negative relationship between W and DMH was due to the high W and extremely low DMH values of varieties 'Gazul' and especially 'Califa Sur' which showed the highest W and P (491 $J·10^{-4}$ and 131 mm H_2O, respectively) resulting in the highest P/L value (1.45 mm H_2O mm^{-1}). Actually, when these two cultivars were removed from analysis, the correlation coefficient decreased until r=0.05. Similarly, the

Table 7: Pearson's correlation coefficients for the relationships between the rehofermentometer F3 and the biaxial extension parameters of 16 bread wheat varieties of three breeding periods. Samples were obtained from one replication of the experiment conducted in 2009 under irrigated conditions. DMH: dough maximum height; DFH: dough final height; DHL: dough height lost (%); T_{DMH}: time to DMH; T_{CO2}: time to CO2 release; VP_{CO2}: total CO_2 volume produced; VR_{CO2}: total CO_2 volume retained; RQ: retention coefficient.

	W	P	L	P/L
DMH	-0.52*	-0.53*	0.20	-0.47
DFH	0.40	0.35	-0.27	0.29
DHL	-0.68*	-0.66*	0.38	-0.56*
T_{DMH}	-0.68**	-0.67**	-0.30	-0.55*
T_{CO2}	-0.83***	-0.86***	0.69**	-0.80***
VP_{CO2}	0.61*	0.69**	-0.63*	0.69**
VR_{CO2}	0.49	0.57*	-0.56*	0.57*
RQ	-0.76***	-0.84***	0.65**	-0.81***

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

correlation coefficient between P and DMH was r=0.02 when the data of these two varieties were excluded. Despite the correlation coefficient between W and DFH was not significant, a bilinear model fitted properly to the relationship between these two variables (Fig. 3a). This relationship also improved (R^2=0.73, P<0.01) when the varieties 'Gazul' and 'Califa Sur' were removed of the model. Accordingly, the relationship between W and DHL was negative for W values lower than 159 J·10^{-4} (Fig. 3b) and P/L values lower than 0.56 mm H2O mm^{-1} (data not shown). For W values above 159 J·10^{-4}, and P/L values greater than 0.56 mm H_2O mm^{-1}, DHL was close to zero (Fig. 3b), indicating that DMH and DFH took similar values (Table 6).

The varieties showing higher W, P and P/L values produced and retained more CO_2 (VP_{CO2} and VR_{CO2} values, respectively, Table 7). However, negative associations were also found between W, P, P/L with T_{CO2} and RQ (Table 7). Actually, a significant bi-linear regression model fitted properly to the relationship between P/L and RQ, which showed a negative association between these two variables for P/L values below 0.83 mm of H_2O mm^{-1} (Fig. 3c). The correlation coefficients between T_{CO2} and RQ with L were positive and significant (Table 7). Neither PC nor ash content were significantly related to any fermentation parameter (data not shown).

Discussion

The results of the present study showed that the introduction of improved bread wheat varieties in Spain during the 20th century caused a bread-making quality enhancement, while a reduction of grain protein content was a general trend. The rate of decrease of protein content obtained in this study (-0.21% y^{-1}) was lower than the reported for bread wheat in France from 1946 to 1992 (-0.35% y^{-1}; Brancourt-Hulmel *et al*., 2003) and the recorded in CIMMYT derived varieties after 1950 (*ca*. -0.40% y^{-1}; Ortiz-Monasterio *et al*., 1997), but greater than the described for Italian durum wheat from 1900 to 1990 (-0.11% y^{-1}; De Vita *et al*., 2007). Reductions in grain protein content have been generally associated with yield gains due to a dilution of nitrogen compounds when carbohydrate deposition increases throughout photosynthesis (Jenner *et al*., 1991). Accordingly, the decrease of protein content observed in the wheats grown in Spain during the 20th century was most likely due to the yield increase during this period in the country, which has been recently estimated in 35.1 kg ha^{-1} y^{-1} (Sanchez-Garcia *et al*., 2012). However, changes in protein content did not follow a linear trend, since 82% of the reductions were due to the introduction of the initial varieties, similarly to the reported for Italy (Canevara *et al*., 1994). The cultivation of these first improved varieties –most of them of Italian origin– from the late 1940's resulted in yield increases up to 30% over that of landraces (Sanchez-Garcia *et al.,* 2012), but the 11.3% of decrease in grain protein content limited the rise of the protein production per hectare to a 18%, a much lesser proportion than the achieved by yield gains.

With the introduction of modern varieties from the 1970's, no statistically significant decrease in protein content accompanied the reported yield raises

(Sanchez-Garcia *et al.*, 2012). Modern varieties not only increased grain yield (Sanchez-Garcia *et al.,* 2012), but they also improved the capacity of protein allocation on grains. These results are in accordance with the improvements of nitrogen harvest index reported in France (Brancourt-Hulmel *et al.*, 2003) and Mexico (Ortiz-Monasterio *et al.*, 1997), countries of origin of most Spanish modern varieties. Our results indicate that the negative relationship between yield and protein (Simmonds, 1995) was discontinued with the introduction of the modern varieties, which stabilized the protein content on grains and significantly increased the protein produced per unit area.

Flour attributes also suffered changes during the last century. The results of the ANOVA indicated that despite the 3% decrease recorded on ash content was not statistically significant, the coefficient of determination of the linear regression line fitted to calculate the genetic gain was R^2=0.27 (P<0.05). The divergence between both results can be attributed to computation differences between the two statistical tests. Nevertheless, the discrepancy was minimal since the rate of genetic gain estimated for ash content was extremely low (-0.078% y^{-1}). On the other hand, changes on water absorption capacity were statistically significant, but the rate of increase was also very small (0.080% y^{-1}). All the improvement of water absorption capacity was contributed by modern varieties, probably because its importance for the formulation of different products and its relationship with superior economic returns for the milling industry, were only evident in the last decades of the century (Wrigley *et al.*, 2009).

Although changes on grain and flour attributes were significant, the largest impact of breeding programs on wheat quality corresponded to dough rheological properties, particularly the ones related with protein composition. Dough strength (W) increased during the 20th century at a rate of 2.74 $J{\cdot}10^{-4}$ y^{-1} (1.39% y^{-1} in relative terms), and tenacity (P) augmented 0.59 mm H_2O y^{-1} (0.99% y^{-1}). Changes on both traits were quite evenly distributed in time, since initial and modern varieties showed about twice and thrice the dough W values of the landraces, and about a 50% and 100% greater P than them, respectively. This timing differs from the reported for Italian varieties, in which improvements on gluten strength were only attained during the last three decades of the century (Canevara *et al*., 1994). Considering the Italian origin of many Spanish initial varieties, the lack of progress in Italy could only be attributed to the high quality of Italian landraces, whose W and P values exceeded the obtained in this study for the Spanish landraces by 58% and a 39%, respectively (Canevara *et al*., 1994). However, the pattern of dough W improvement in Spain was similar to the reported in France for the same period (Bonjean *et al*., 2001), which is in accordance with the French origin of most modern Spanish varieties.

The lack of complete information about the allelic composition for HMW-GS of the landraces used in this study prevented to associate changes on allelic variants to the improvement of the dough properties of initial varieties. However, some general trends could be observed along the century. Changes in the frequency of the null allele at *Glu-A1*, which has been reported to have a negative effect on dough strength (Branlard *et al*., 1992; Cornish *et al*., 2001), could partially explain the quality improvement of modern varieties, but not the large W improvement of the initial cultivars, in which this allele predominated. Similarly, the presence on initial varieties of the GS 2+12 at *Glu-D1* did not probably contribute much to dough strength improvement, since its effect on dough strength has been reported to be similar to that of 4+12 subunit (Wrigley *et al*., 2009), which was already present in the landrace Aragon 03 and probably also in Barbilla. Therefore, quality improvement of initial varieties could be mostly attributed to the replacement of the undesirable HMW-GS 20 at the *Glu-B1* locus present in the landrace Aragon 03 – and according to Giraldo *et al*. (2010) predominant in Spanish landraces – by the 7+8 subunit, which has a demonstrated positive effect on dough properties

(Branlard *et al*., 2003; Tohver, 2007). The improvement of dough strength of modern varieties could not only be attributed to the prevalence of 7+8 subunit, but also to the introduction of GS 5+10 at *Glu-D1*, the subunit most determinant at this locus for promoting gluten strength (Branlard *et al*., 2003; Tohver, 2007), and the replacement of the null allele at *Glu-A1* by subunits 1 or 2* that have a proved large positive effect on dough strength (Branlard *et al*., 2003; Tohver 2007; Wrigley *et al*., 2009).

The well-known large effect of HMW-GS composition on dough strength (Payne et al., 1987; Branlard et al., 1992) is in agreement with the great genotypic effect found in this study on both W and P, and explains the success of breeding programs to improve these traits. On the other hand, extensibility appeared to be much more environmentally regulated – the variety x environment interaction accounted for *ca*. 28% of total variance – and so, more difficult to be genetically manipulated. Although it is known that LMW glutenin alleles make a significant genetic contribution to extensibility (Cornish *et al*., 2001), this study showed also a genetic effect of HMW-GS on it, particularly the allelic composition at *Glu-D1* locus. Our results indicate that the presence of the 5+10 subunit explained 30.4% of the variety x environment interaction variance, and 44.1% of the variety x water input interaction for extensibility. The varieties carrying the 5+10 subunit seemed to be more adapted than the rest to environments with high water input, in which the grains obtained showed low protein content, and the resulting dough had low extensibility. It is well known the large effect of the environmental conditions and the protein content in the flour on dough extensibility (Cornish *et al*., 2001), and the traits associated with protein content have been reported to be more affected by the environment and the variety x environment interaction than those related to protein quality and dough rheology (Williams *et al*., 2008).

The study of dough development during fermentation allowed ascertaining the effect of breeding for rheological properties on dough fermentation behavior. The results of the rheofermentometer tests revealed that in baking procedures with long-fermentation, W and P/L should reach a minimum value to ensure that the bubble structure developed during mixing did not collapse during fermentation (Wrigley *et al*., 2009). Our results indicated that doughs with $W \geq 159\ J \cdot 10^{-4}$ and P/L ≥ 0.56 mm H_2O mm^{-1} were needed to bear a 3h fermentation process. Doughs with W and P/L values below this minimum showed progressive alveolus collapsing, resulting in differences between DMH and DFH up to 54% which was the DHL obtained for the modern variety *Marius* that had the lowest P/L value. Our results revealed that most of the modern bread wheat varieties grown in Spain met these requirements. This is in agreement with the reported improvements on mixing time and tolerance, measured with mixograph, reported in several countries (Khalil *et al*., 2002; Fufa *et al*., 2005). From these results we can conclude that gluten quality improvements through breeding allowed modern Spanish varieties to meet the industry requirements in terms of fermentation tolerance and stability.

The present study showed that, for a good fermentation behavior, a balance between P and L values was necessary, given that the higher the P/L ratio, the more dough porosity and its faster start. The strength of the gluten matrix of varieties with high W and P values would prevent alveolus collapsing. However, when dough tenacity was not compensated by an adequate extensibility, dough became too tenacious and too porous, so it could not achieve high DMH, a trait correlated with final loaf volume (Ktenioudaki *et al*., 2010; Ktenioudaki *et al*., 2011). This is in agreement with previous studies that stated the importance of both, extensibility and strength, for an appropriate stability of the gas cells during expansion (Sroan *et al*., 2009; Ktenoudaki *et al*., 2010).

The combination of subunit 7+8 HMW-GS –encoded at the *Glu-B1* locus that over-expresses the *Bx7* HMW-GS, promoting dough strength (Wrigley *et al.*, 2009) and whose effect on dough W and P has shown to be additive to that of the 5+10 subunit at *Glu-D1* (Vawser and Cornish, 2004) – resulted on over-strong and unbalanced doughs, which have been reported to be unsuitable for attaining acceptable loaf volumes (Butow *et al.*, 2003; León *et al.*, 2010). Moreover, in spite of their positive effect on fermentation tolerance, the high W and P/L values of modern varieties probably enhanced the porosity of doughs. However, the interest for the milling and baking industries on varieties with very high dough strength and tenacity would rely on their suitability for mixtures with weaker flours, since varieties with W > 250-300 $J \cdot 10^{-4}$ are usually classified by governments and millers' associations from France, Italy and Spain as "improver wheats" (Mesdag and Donner 2000; BOE 2010).

Modern bread wheat varieties showed a great variability on bread-making quality attributes and DMH values. This could have been the result of farmers' choice of the most productive cultivars, the lack of economic incentives for growing high quality varieties, and the industry demand for wheats with different characteristics for the manufacturing of a large sort of products. The raising importance of frozen doughs may increase the interest for varieties with over-expressed HMW-GS since dough storage under freezing conditions has shown to depolymerize HMW-GS thus affecting bread end-use quality (Ribotta *et al.*, 2001).

From our results it can be concluded that the rates of improvement for W, P and P/L of bread wheat varieties grown in Spain during the 20th century were even greater than the recently reported for yield and associated traits (Sanchez-Garcia *et al.*, 2012), while dough extensibility and grain protein content were significantly reduced. Besides, the improvement of rheological properties was related with changes on fermentation tolerance and stability, probably according to the industry requirements.

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DISCUSIÓN GENERAL

Discusión General

Introducción a la discusión general

Con el fin de responder a los objetivos planteados, se reunió una serie histórica de 29 variedades ampliamente cultivadas en España en diferentes épocas a lo largo del siglo XX. De éstas, se escogieron 27 variedades para dar respuesta a los dos primeros objetivos que se abordan en los **capítulos 1** y **2.** En ellos se estudian los cambios producidos por la mejora genética en el rendimiento y sus principales componentes (número de espigas por unidad de superficie, número de granos por espiga y peso del grano) y la interacción genotipo por ambiente de la serie histórica en cuatro localidades representativas de las principales zonas de cultivo del trigo harinero en España, durante dos campañas consecutivas. De las 27 variedades seleccionadas, la variedad *Cezzane* fue eliminada de los análisis expuestos en el **capítulo 1** debido a la ausencia de datos relativos a los componentes del rendimiento de dicha variedad durante la campaña 2006/2007.

Para dar respuesta a los objetivos *3* y *4*, desarrollados en los capítulos homónimos, y ahondar en la determinación de los caracteres morfo-fisiológicos que han favorecido la ganancia genética, expuestos en el **capítulo 1**, se seleccionó una sub-muestra de 16 variedades que, manteniendo la diversidad de la colección original, permitiera realizar un estudio en mayor profundidad y facilitara su manejo. Esta sub-muestra de variedades se ensayó en la localidad de Gimenells (Lleida) bajo dos regímenes hídricos (secano y regadío) durante dos campañas consecutivas (2007-2008 y 2008-2009 en el caso de los **capítulos 1** y **3** y 2008-2009 y 2009-2010 en el **capítulo 4**).

Las variedades se dividieron a su vez en tres grupos principales definidos por la antigüedad de las mismas y representativos de los principales periodos de la mejora del trigo harinero en España:

1. Variedades tradicionales (*landraces*): Seleccionadas durante miles de años por el ambiente y los agricultores y que fueron ampliamente cultivadas hasta 1950.
2. Variedades Iniciales (*Initial varieties*, denominadas como *old bred varieties en el* **capítulo 2**): Provenientes de los primeros programas de mejora españoles, como la exitosa *Pané 247*, y extranjeros, principalmente italianos y franceses, que alcanzaron gran relevancia a escala mundial, como *Mara* o *Etoile-de-Choisy* (renombrada en España como *Estrella*). Estas variedades fueron sembradas de forma mayoritaria al menos hasta la década de 1970.
3. Variedades modernas (*modern varieties*): Desarrolladas a partir de la Revolución Verde, se introdujeron con gran éxito en España a partir de la década de los 70. Muchas de estas variedades tienen su origen en el Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) en México o son derivadas de cruzamientos con el germoplasma desarrollado en dicho centro. También tienen una gran relevancia las variedades introducidas desde Francia desarrolladas principalmente por distintas empresas. En los **capítulos 1 y 2**, con el fin de investigar el éxito de la mejora durante la última década del siglo pasado, estas variedades fueron divididas a su vez en dos grupos:
    a. Variedades intermedias (*intermediate varieties*), registradas entre 1970 y 1990.
    b. Variedades modernas (*modern varieties*), variedades registradas en España durante la última década del siglo (1991-2001).

La ausencia de diferencias significativas en las características y en los mecanismos fisiológicos entre variedades intermedias y modernas llevó a la fusión de ambos grupos en los **capítulos 3** y **4** de la presente memoria de Tesis Doctoral. En el caso específico del **capítulo 2**, y atendiendo a la naturaleza de los objetivos, las variedades llamadas intermedias y modernas se dividieron a su vez en dos grupos según su hábito de crecimiento, debido a la naturaleza del estudio, para facilitar la interpretación de los resultados. Por estas razones y para facilitar la discusión general de la Tesis, se trataron todas las variedades liberadas después de 1970 como un solo grupo, denominándolas modernas tal y como se hizo en los **capítulos 3** y **4**.

Se realizó la caracterización molecular de los genes de enanismo y las subunidades de alto peso molecular de las proteínas de reserva del grano de las 16 variedades de la sub-muestra, tal como se describe en los **capítulos 3 y 4**.

Los resultados obtenidos en los distintos experimentos realizados permitieron responder a los objetivos fijados, tratándose cada objetivo en su capítulo homónimo. Por otra parte, se obtuvieron nuevas conclusiones del análisis conjunto de los resultados de todos los capítulos.

Variedades utilizadas a principios del siglo XX en España

Antes de la introducción de las primeras variedades obtenidas de programas de mejora genética se cultivaban variedades tradicionales (*landraces*) y su caracterización permitió establecer el punto de partida de la mejora genética del trigo a lo largo del siglo XX. Además permitió cuantificar y determinar los caracteres que influyeron en dicha mejora. El rendimiento medio observado para estas variedades fue de 2.457 kg ha^{-1}, sensiblemente inferior a lo publicado en otros países de nuestro entorno como Italia, donde promediaron 3.640 kg ha^{-1} (Canevara *et al.*, 1994), o Gran Bretaña, donde promediaron 6.327 kg ha^{-1} (Austin *et al.*, 1989). Las variedades tradicionales españolas mostraron gran altura (1,14 m) y baja partición de la biomasa a la espiga, con un índice de cosecha de 0,26, un 24% menos de lo publicado para las variedades tradicionales italianas (Canevara *et al.*, 1994) y 28% menos que en el Reino Unido (Austin *et al.*, 1989). Las variedades españolas, a pesar de mostrar un número relativamente elevado de tallos por planta en madurez, promediaron un índice de fertilidad muy bajo, que resultó en un bajo número de espigas por unidad de superficie.

El estudio de su adaptación a los ambientes españoles mostró que estas variedades están mejor adaptadas que aquellas que las sucedieron a los ambientes con baja temperatura mínima antes del espigado y condiciones menos estresantes durante el llenado del grano. Esto se debió a su fecha de espigado, más tardía, que si bien les beneficia a la hora de evitar los posibles daños ocasionados por las heladas de primavera en el establecimiento de los granos, determina que el llenado del grano se produzca bajo condiciones altamente estresantes de humedad y temperatura, propias del final del ciclo de cultivo en los ambientes mediterráneos. Por esta razón, las variedades tradicionales mostraron granos más pesados en los ambientes con condiciones de alta humedad relativa durante el periodo de llenado.

En cuanto a la calidad harinera, la concentración media de proteína en los granos de las variedades tradicionales fue significativamente superior al de las mejoradas, tanto antiguas como modernas, alcanzando un valor promedio del 16%, superior también al 12% que promediaron las variedades tradicionales italianas (Canevara *et al.*, 1994). Sin embargo, las variedades tradicionales españolas mostraron valores de fuerza de la masa, entendida como la energía necesaria para deformar la masa hasta su ruptura (*W* medida mediante el alveógrafo Chopin) inferiores a los de las italianas, que promediaron un 58% más debido a una mayor tenacidad (Canevara *et al.*, 1994), aunque similares a los de las variedades tradicionales francesas (83 y 75 $J \cdot 10^{-4}$ respectivamente; Bonjean *et al.*, 2001). La baja fuerza de las variedades tradicionales contrastó con una alta extensibilidad (134 mm), un 18% mayor que sus equivalentes italianas (Canevara *et al.*, 1994). La baja tenacidad unido a la alta extensibilidad derivó en un bajo P/L que, si bien, permitió promediar un coeficiente de retención (RT; del inglés retention coefficient) por encima del 90% y alta altura máxima de la masa (DMH; del inglés *Dough Maximum Height*) las hizo intolerantes a procesos de amasado y fermentación largos, produciéndose rápidamente el colapso de los alveolos y obteniendo una altura final de la masa (DFH; del inglés *Dough Final Height*) un 30% inferior a la DMH al cabo de 3 horas de proceso. Mejorar la fuerza de las masas necesaria para los procesos mecanizados pudo ser, junto con la mejora de la productividad, una de las razones que explicaron la rápida y exitosa introducción de las variedades mejoradas extranjeras.

Introducción de las primeras variedades mejoradas

La comercialización de las primeras variedades producto de cruzamientos dirigidos, realizados en programas de mejora pioneros, tanto nacionales como extranjeros, supuso un gran avance en el cultivo del trigo harinero en España. Estas variedades, introducidas a finales de la década de 1940, aportaron un incremento del rendimiento del 30% comparado con las variedades tradicionales cultivadas hasta entonces, posiblemente gracias a un aumento del 58% en el número de granos por espiga, y a pesar de una disminución del 16% en el peso de los mismos.

Por el contrario, las variedades antiguas mejoradas no mostraron diferencias significativas en cuanto a la biomasa en madurez. Por lo tanto, el aumento del rendimiento fue consecuencia de un aumento del índice de cosecha, que se incrementó un 60% con respecto a las variedades tradicionales. Tres fueron los motivos, probablemente interrelacionados entre sí, que explicaron este aumento. El primero consistió en una reducción del número de tallos infértiles, reduciéndose así biomasa vegetativa que no produce espiga, y aumentando la proporción de tallos fértiles de 0,75 a 0,93. Esto, provocó un incremento de los recursos que la planta pudo derivar al establecimiento y llenado del grano y por lo tanto, un incremento del índice de cosecha. La reducción del número de tallos se ha descrito como un carácter de interés en las condiciones de estrés térmico e hídrico durante el llenado del grano típicas del ambiente mediterráneo, ya que permite ahorrar agua durante el desarrollo vegetativo para que pueda ser utilizada durante la fase de llenado, cuando ésta es un factor limitante (Richards *et al.*, 1988; Duggan *et al.*, 2005). Sin embargo, en el presente caso, esta disminución parece estar más relacionada con la optimización de los recursos de la planta, debido a que ésta ya no los invierte en formar tallos infértiles que no contribuyen a incrementar la producción pero que, sin embargo, hacen competencia por dichos recursos a los tallos principales portadores de espiga.

La segunda causa de aumento del índice de cosecha fue la introducción, por primera vez en el germoplasma español, de los genes de enanismo, mayoritariamente el *Rht8c*, pero también el *Rht9*. El alelo *Rht8c* proviene de la variedad japonesa Akakomugi y fue introducido en el germoplasma europeo por el pionero en la mejora genética de trigo, el italiano Nazareno Strampelli. Estos genes favorecen la reducción de la altura de la planta y el incremento de la fertilidad de la espiga (Borojevic y Borojevic 2005). La introducción de estos genes de enanismo provocó un descenso del 10% en la altura de la planta, sin alterar la biomasa final y por tanto un incremento del índice de cosecha. Paralelamente a lo ocurrido con el descenso de los tallos infértiles, la reducción de la altura de la planta conllevó la posibilidad de revertir biomasa vegetativa hacia los órganos reproductivos.

El tercer cambio que favoreció el incremento del índice de cosecha fue el ajuste fenológico. Así, en comparación con las variedades tradicionales, las variedades antiguas mejoradas adelantaron su fecha de antesis en 132 GDD (promedio de los experimentos llevados a cabo en Gimenells durante las campañas 2007-2008 y 2008-2009 en condiciones de secano y regadío), lo que permitió, entre otras cosas, adelantar el inicio de la partición de recursos hacia los granos, favoreciendo el incremento del índice de cosecha y escapar a los momento de mayor estrés. La precocidad en el espigado se debió probablemente a la insensibilidad al fotoperiodo que muestran estas variedades (Worland *et al*., 1998), gracias a la introducción del alelo *Ppd-D1a*, en comparación con las variedades tradicionales mayoritariamente sensibles a la diferencia de horas luz/oscuridad.

Además de favorecer el incremento del índice de cosecha, el adelanto de la floración en las variedades antiguas mejoradas con respecto a las variedades tradicionales conllevó que las primeras estuvieran más ampliamente adaptadas a las condiciones de cultivo españolas que sus predecesoras. El ajuste fenológico, permitió adelantar el periodo de llenado, evitando así en parte, los estreses hídrico y térmico propios del final del ciclo. Esto, junto con la reducción del número de tallos infértiles, favoreció la estabilidad, tanto en el número de espigas por unidad de superficie como en el peso de los granos, los dos componentes que más interactúan con el ambiente de los que conforman el rendimiento, en comparación a las variedades tradicionales. Su amplia adaptación explica, sin duda, el éxito de las variedades antiguas mejoradas como, por ejemplo, la variedad Estrella (*Etoile de Choisy* en origen) sembrada o utilizada como parental en los programas de mejora de muchos países como Polonia, Rumanía, Francia, España, Italia o la antigua Yugoslavia (Bonjean y Angus, 2001).

La reducción del número de tallos infértiles, la disminución de la altura de la planta y el ajuste fenológico favorecieron que las variedades antiguas mejoradas pudieran acumular más biomasa en las espigas incluso desde las primeras fases del llenado. Este incremento se debió tanto a una mayor capacidad fotosintética durante el llenado, sin duda favorecida por el adelantamiento de éste que permitió llevarlo a cabo bajo condiciones climáticas más favorables, como a un incremento en la translocación de fotosintatos acumulados en los tallos antes de la floración. La Figura 1 muestra el efecto paralelo de las tres adaptaciones propias de las variedades antiguas mejoradas que contribuyeron al aumento del índice de cosecha.

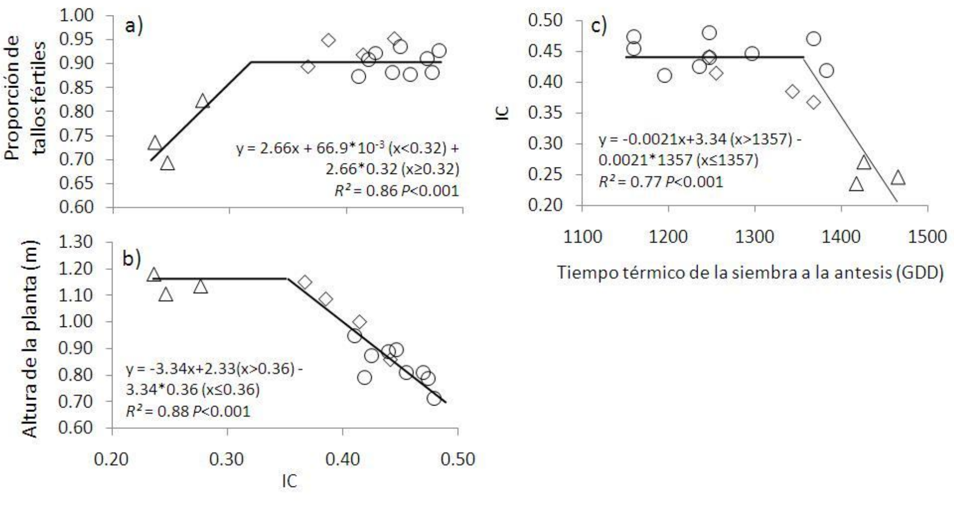

Figura 1. Relaciones entre el índice de cosecha (IC) y a) la proporción de tallos fértiles, b) la altura de la planta y c) el tiempo térmico desde la siembra a la antesis, de 16 variedades de trigo ampliamente sembradas en España durante el siglo XX. Cada punto representa el valor medio de una variedad ensayada en cuatro experimentos. Los periodos de mejora se identificaron de la siguiente manera: los triángulos representan las variedades tradicionales, los rombos las variedades antiguas mejoradas y los círculo las variedades modernas. (g.l.: 15)

El incremento del número de granos por unidad de superficie, derivado de la introducción de las variedades antiguas mejoradas, no se pudo compensar mediante un aumento equivalente en la cantidad de proteína sintetizada por la planta y translocada a los granos. Esto disminuyó significativamente la concentración de proteína en los mismos un 11% con respecto a las variedades tradicionales. A pesar de esta disminución, la introducción de las variedades antiguas mejoradas resultó en un aumento generalizado, aunque no significativo de la calidad, especialmente en lo referido a la tenacidad (*P*) y la fuerza (*W*) de las masas. La sustitución de los alelos de las HMW-GS no deseables para la calidad pero presentes en una alta proporción en las variedades tradicionales, como la subunidad 20 (Giraldo *et al.*, 2010) codificada en el locus *Glu-B1,* por otras con un efecto más positivo sobre las propiedades de la masa, como la 7+8, puede explicar parte de esta mejora.

Las variedades antiguas mejoradas presentaron también una mayor tolerancia a los procesos de fermentación, promediando un descenso del 50% en la proporción de la altura máxima de la masa que se pierde al cabo de 3 horas de fermentación (DHL; del inglés *Dough Height Loss*) con respecto a las variedades del periodo anterior. Sin embargo se observó una gran variabilidad dentro del grupo. Así, la variedad *Impeto*, la primera en expresar la subunidad 1 codificada en el *Glu-A1* que está relacionada con aumentos en la fuerza de las masas (Payne *et al.*, 1987; Branlard *et al.*, 1992; Cornish *et al.*, 2001), mostró gran fuerza de la masa y, por lo tanto, mayor tolerancia a largos procesos de fermentación, con un DHL cercano a 0, mientras que la variedad del mismo grupo *Mara* mostró un DHL del 34%.

Introducción de las variedades modernas, de la Revolución Verde hasta final de siglo.

El segundo gran evento en la mejora del trigo harinero en España fue la comercialización, después de la Revolución Verde en la década de 1970, de las variedades modernas. La introducción de estas variedades dio como resultado un incremento del rendimiento del 37% respecto a las variedades antiguas mejoradas. Este incremento no se debió a un aumento en un componente del rendimiento en particular sino a aumentos similares en número de espigas por m^2, número de granos por espiga y peso de los granos. Al igual que ocurrió con la introducción de las variedades antiguas mejoradas, se observó un aumento de la partición de biomasa hacia el grano, si bien también se observó una tendencia al aumento de la biomasa en madurez.

El incremento de la partición de biomasa estuvo relacionado principalmente con nuevas reducciones de la altura de la planta (17%), gracias a la introducción de los genes de enanismo *Rht-B1b* y *Rht-D1b*. Sin embargo, la introducción de estos genes en el germoplasma español también contribuyó a una disminución del índice de área foliar (LAI; del inglés *Leaf Area Index*) en la etapa de primer nudo detectable, estadio 3.1 de la escala de crecimiento propuesta por Zadoks *et al.* (1974). El efecto pleiotrópico negativo de estos genes sobre el vigor inicial y el tamaño celular se ha descrito en estudios previos (Youssefian *et al.*, 1992; Miralles *et al.*, 1998; Ellis *et al.*, 2004). Sin embargo la presente Memoria de Tesis Doctoral muestra que este efecto se mantiene hasta el principio del encañado y parece afectar tanto al área foliar como a la biomasa del cultivo.

Las variedades modernas acumularon menos biomasa en antesis, pero mostraron una tendencia a acumular más biomasa a final del ciclo, en comparación a las variedades antiguas mejoradas. La capacidad de acumular más biomasa por unidad de superficie en las espigas durante el llenado, principalmente entre los estadios de grano lechoso (7.5 de la escala de Zadoks) y madurez fisiológica (8.7 de la escala de Zadoks), fue determinante para la ganancia de rendimiento ocurrido en este periodo y probablemente contribuyó a aumentar el índice de cosecha. Además del incremento del tamaño del sumidero producido por el aumento del número y peso de las espigas por unidad de superficie en antesis, el incremento en la biomasa sintetizada en la última fase del llenado y translocada a la espiga estuvo relacionado con una mayor capacidad fotosintética. La mayor capacidad fotosintética, producida por un incremento en la proporción de la superficie mantenida verde a final del ciclo, resultó en un aumento paralelo de la duración del área foliar (LAD; del inglés *Leaf Area Duration*) con el rendimiento.

Las variedades modernas, como grupo, no mostraron diferencias en la fecha de espigado con respecto a las variedades antiguas mejoradas. Sin embargo, sí se observaron diferencias en la adaptación a los ambientes en función, principalmente, de su hábito de crecimiento. Así, las variedades modernas de tipo invernal mostraron una adaptación más amplia, es decir, con un rendimiento más estable en todos los ambientes ensayados, que las de tipo primaveral. Esto se debió, principalmente, a una mayor estabilidad en su número de granos por espiga, aunque el peso del grano sí se vio positivamente correlacionado con la humedad relativa del aire durante el llenado. Estas variedades se vieron beneficiadas en ambientes con mayor humedad durante el llenado ya que su fecha de espigado es posterior a las de ciclo primaveral y por lo tanto son probablemente más susceptibles a los estreses terminales. Sin embargo, las variedades de tipo primaveral mostraron una tendencia hacia una mayor adaptación a ambientes con altas temperaturas mínimas antes del espigado. La precocidad de estas variedades las hace más susceptibles a las heladas tardías y por lo tanto se muestran peor adaptadas a ambientes más fríos antes de la antesis, especialmente perjudiciales a la hora de establecer el número de granos por espiga.

Contrariamente a lo ocurrido con la introducción de las variedades antiguas mejoradas, la introducción de las variedades modernas, de mayor rendimiento que sus predecesoras, no conllevó un descenso de la concentración de proteína en los granos. Por lo tanto, estas variedades aumentaron la cantidad de proteína fijada en los granos por unidad de superficie de forma paralela a los incrementos del rendimiento. Este resultado fue consecuente con los incrementos en el índice de cosecha del nitrógeno, publicados para las variedades modernas francesas (Brancourt-Hulmel *et al.*, 2003) y mexicanas (Ortiz-Monasterio *et al.*, 1997), países de origen de muchas de las variedades modernas cultivadas en España. La presencia, de forma mayoritaria, de los alelos que codifican las *subunidades 1 y 2* en* el locus *Glu-A1,* en lugar del alelo que codifica la ausencia de subunidad *Null*, negativamente correlacionada con la fuerza del gluten y especialmente, la introducción del alelo que codifica para la subunidad *5+10* en el *Glu-D1* influyeron de forma significativa en la calidad harinera. Ambos factores contribuyeron a los aumentos en la *W* y la *P* de las masas, un 52% y un 33% respectivamente con relación a las variedades antiguas mejoradas, mientras que la extensibilidad se redujo un 9%.

La mayor *P* y *W* observada en las variedades modernas permitió aumentar de forma generalizada, la tolerancia a los procesos de fermentación, mostrando en promedio una DHL apenas superior al 8%. Sin embargo, en algunos casos, su elevada *W* y *P*, unidas a su baja *L* produjeron un efecto de desequilibrio en las propiedades reológicas de las masas. Este desequilibrio fomentó masas muy tenaces y con una rápida y alta porosidad, lo que probablemente contribuyó negativamente a la DMH que estas variedades alcanzaron durante la fermentación.

Evolución temporal e implicaciones para la mejora

1. Rendimiento y sus componentes

Desde un punto de vista estrictamente temporal, los resultados del **capítulo 1** permitieron establecer en 35,1 kg ha^{-1} año^{-1} la ganancia genética media del rendimiento del trigo harinero en España desde 1930 hasta el año 2001. Esto supuso un incremento relativo medio de 0,88% año^{-1}, siendo esta tasa similar o ligeramente superior a la de otros países europeos (Brancourt-Hulmel *et al.*, 2003; Austin *et al.*, 1989) y de otros continentes (Cox *et al.*, 1988; Morgounov *et al.*, 2010) durante el mismo periodo. Sin embargo, no se observó una ganancia clara en el rendimiento desde la introducción de las variedades modernas en la década de 1970, si bien esto parece ser una constante en varios países (Sener *et al.*, 2009; Fischer y Edmeades 2010).

De forma general, el aumento del rendimiento se debió principalmente a un incremento en el número de granos por espiga (0,60% año^{-1}) y en menor medida, al aumento del número de espigas por unidad de superficie (0,30% año^{-1}). El aumento en el número de granos por espiga es reconocido casi universalmente como la principal causa de la mejora del rendimiento (Brancourt-Hulmel *et al.*, 2003; Austin *et al.*, 1989, Cox *et al.*, 1988; Morgounov *et al.*, 2010). Los incrementos en el número de espiguillas por espiga y, principalmente, del número de granos por espiguilla fueron las principales razones de esta mejora. Sin embargo, el éxito en el incremento del número de espigas por unidad de superficie fue más variable y estuvo determinado por la interacción con el ambiente, como se concluye en el **capítulo 2**. Este resultado estuvo en consonancia con la especificidad geográfica de los aumentos en el número de espigas por unidad de superficie publicados hasta la fecha, siendo, estos incrementos casi exclusivos de países con climas de tipo mediterráneo (Perry y D’Antuono 1989; Canevara *et al.*, 1994; Royo *et al.*, 2007). En cuanto al peso individual del grano, no se observó ninguna tendencia general a lo largo del siglo y la contribución de las espigas provenientes de los hijuelos a la ganancia genética fue similar a la de las espigas provenientes del tallo principal.

2. Biomasa e índice de cosecha

A pesar de la tendencia hacia un incremento de la biomasa a partir de 1950, los incrementos del rendimiento fueron principalmente consecuencia de un aumento de la partición de biomasa hacia los órganos reproductores y no de un aumento de la biomasa de la planta. Esto ha sido una constante en la mayoría de los programas de mejora en todo el mundo (Austin *et al.*, 1989; Perry y D'Antuono 1989; Cox *et al.*, 1988; Canevara *et al.*, 1994; Brancourt-Hulmel *et al.*, 2003; Royo *et al.*, 2007; Morgounov *et al.*, 2010). Los cambios fisiológicos que permitieron el incremento del índice de cosecha fueron básicamente tres: i) el aumento de la proporción de tallos fértiles, gracias a una reducción del número de tallos infértiles, ii) la disminución de la altura de la planta, sin alterar la biomasa de la planta en madurez gracias a la introducción de los genes de enanismo y iii) el ajuste fenológico, que permitió adelantar el desvío de recursos de la parte vegetativa de la planta a los granos.

Sin embargo, la presente Tesis Doctoral demostró que las exitosas estrategias seguidas hasta el momento para incrementar el índice de cosecha de las variedades han alcanzado un punto muy cercano al óptimo y difícilmente podrán ser utilizadas para nuevos incrementos en la partición de biomasa. Así, mientras que nuevas mejoras del rendimiento podrían lograrse fomentando un incremento del número de tallos fértiles por unidad de superficie, un aumento de la proporción de los mismos difícilmente podría alcanzarse ya que, como se vio en el **capítulo 1**, desde 1948, la proporción de tallos fértiles es estable y muy cercana al 100%.

Por otro lado, nuevos incrementos en el índice de cosecha podrían lograrse con nuevas reducciones de la altura de la planta gracias a la introducción y combinación de nuevos genes de enanismo. Sin embargo, a pesar de un teórico incremento del índice de cosecha, reducir más la altura de la planta podría producir un efecto indeseable sobre el rendimiento debido a que la variedad más reciente de la serie histórica, *Califa Sur*, ya promedió una altura (0,71m), muy cercana al límite inferior del rango de altura óptimo establecido por Flintham *et al.* (1997) en 0,70-1,00 m. La reducción de la altura de las plantas por debajo de este rango comprometería la arquitectura óptima del dosel vegetal, reduciendo la efectividad fotosintética del mismo y disminuyendo la biomasa acumulada en madurez (Foulkes *et al.*, 2011). Así, la reducción de la biomasa de las plantas con alturas inferiores a 0,70m no podría ser compensada por los incrementos del índice de cosecha.

En cuanto a un nuevo ajuste fenológico, reducciones adicionales en el tiempo necesario para llegar a la floración podrían lograrse modificando la precocidad *per se* de las variedades, sus requerimientos de vernalización o reduciendo nuevamente los requerimientos de fotoperiodo (Snape *et al.*, 2001). Sin embargo, si bien esta reducción aceleraría probablemente el desvío de asimilados de los órganos vegetativos a los reproductivos, podría ser contraproducente en algunos ambientes, principalmente en aquellos con menores temperaturas antes de la floración, ya que adelantar la fecha de la floración expondría el proceso de formación y establecimiento de los granos a posibles heladas tardías, afectando al número final de granos por unidad de superficie y de esta manera al rendimiento. Por lo tanto, nuevas mejoras en la fenología del trigo harinero deberán, sin duda, depender del ambiente en que la variedad vaya a ser cultivada o proveer a la planta de mecanismos de ajuste que le permitan adaptarse a las condiciones del ambiente en que esté siendo cultivada. Por otro lado, la relación observada entre la acumulación de biomasa en antesis y la duración del ciclo hasta este estadio, implicaría una reducción de la biomasa en antesis como consecuencia del ajuste fenológico, lo que podría comprometer la disponibilidad de asimilados de reserva para el llenado del grano en condiciones de estrés.

Finalmente, existen dudas acerca de la posibilidad de seguir aumentando el índice de cosecha de forma significativa, ya que éste mostró un estancamiento a partir de la introducción, en la década de 1970, de las variedades modernas. Para entonces, el índice de cosecha se estancó en 0,45, lejano del 0,64 establecido como máximo teórico (Foulkes *et al.*, 2011) pero cercano ya a 0,50, un valor que ha demostrado ser difícilmente alcanzable en condiciones de cultivo en muchos ambientes (Fischer y Edmeades 2010). Por lo tanto, los futuros incrementos del rendimiento deberán basarse en aumentos de biomasa o en biomasa e índice de cosecha conjuntamente. De hecho, en el Reino Unido, los incrementos del rendimiento durante las dos últimas décadas del siglo XX ya se basaron en mayor medida en aumentos en la biomasa más que en el índice de cosecha (Shearman *et al.*, 2005).

Los resultados del **capítulo 3** mostraron que en España casi el 80% de la ganancia genética del rendimiento se debió a la mayor capacidad de las variedades mejoradas para acumular más biomasa en las espigas durante las últimas fases del llenado, concretamente después del estadio de grano lechoso. Es más, a lo largo del siglo XX, la duración de la superficie verde en el estadío de grano lechoso se incrementó en un 0,79% año^{-1}, una proporción similar a la ganancia relativa media de rendimiento en España expuesta en el **capítulo 1.** Estos resultados pusieron de manifiesto la importancia que tuvo el incremento de esta fuente de asimilados en los aumentos del rendimiento.

Por lo tanto, junto con el necesario aumento del sumidero de las variedades, recientes experimentos demostraron que el rendimiento del trigo en España está co-limitado por la capacidad de la fuente y del destino (Álvaro *et al.*, 2008; Acreche *et al.*, 2009), aumentar la duración del área foliar se presenta como un carácter a seleccionar para incrementar la capacidad fotosintética del cultivo durante el llenado. Este aumento podría lograrse retrasando la senescencia o incrementando el LAI disponible. Esto permitiría aumentar la capacidad de fuente de las variedades fomentando, a la vez, los incrementos de la biomasa y el índice de cosecha, y de esta manera, el rendimiento.

3. Adaptación

La temperatura media mínima antes del espigado fue la variable que mejor permitió discriminar, junto con el tiempo térmico de siembra a espigado, los cambios en la adaptación del trigo en España producidos por la mejora genética. Estas variables climáticas fueron también determinantes para explicar las diferencias en el número de granos por espiga debidas al ambiente. Así, el número de granos por espiga fue el componente más relacionado con el rendimiento en lo referido al patrón de adaptación, confirmando la preponderancia que tuvo la mejora del número de granos por espiga en los aumentos del rendimiento, incluida su respuesta al ambiente.

A pesar de una mayor especificidad en la adaptación a los ambientes de las variedades más modernas según su hábito de crecimiento, la mejora genética fomentó en España, de forma general, una tendencia hacia una adaptación más amplia de las variedades mejoradas. Esta tendencia se debió, principalmente, a una reducción del tiempo necesario para llegar al espigado, lo que permitió adelantar el periodo de llenado de manera que este se llevara a cabo bajo condiciones menos estresantes de calor y temperatura. Precisamente, la temperatura media máxima entre el espigado y la madurez, junto con la humedad relativa en el mismo periodo, fueron las variables climáticas más determinantes para explicar las diferencias en el peso de los granos debidas al ambiente. Esto indica el papel principal que debe jugar la adaptación del trigo a las condiciones ambientales si se pretende aumentar la producción.

En la presente Memoria de Tesis Doctoral, se observó gran variabilidad en la ganancia genética relativa de rendimiento en función de las características climáticas del ambiente en que se ensayaran las variedades, llegando, estas diferencias a suponer hasta un 400%. Concretamente, las diferencias entre ambientes en temperatura media mínima antes del espigado explicaron el 81% de la variabilidad en las estimaciones de la ganancia relativa de rendimiento. Esto se debió al efecto contrapuesto que tuvo esta variable climática sobre las variedades más antiguas y más modernas, especialmente las más primaverales, perjudicando el rendimiento de las primeras y beneficiando a las segundas.

En el presente escenario de cambio climático, la mejora de la adaptación de las variedades parece más importante que nunca. El presente trabajo mostró, además, que un cambio relativamente pequeño en la temperatura media mínima antes del espigado puede determinar en gran medida el éxito de las variedades. Por ejemplo, en el **capítulo 1** se demostró que una diferencia en la temperatura media mínima diaria de 1,6 ºC como la existente entre los ambientes de Burgos en 2007 y Lleida en 2006, contribuyó a que la diferencia de ganancia relativa de rendimiento entre los dos ambientes fuese de más de 150%. Esta diferencia de temperatura media mínima fue asimismo, un 11% menor que el aumento de la temperatura media previsto en el planeta para 2050 (Meehl *et al.*, 2007). Las mayores ganancias relativas se obtuvieron en los ambientes con mayores temperaturas medias mínimas, indicando que, al menos en lo relativo a esta variable climática, las variedades más modernas parecen estar mejor adaptadas a un aumento de la temperatura que las antiguas.

Una de las estrategias generalmente propuestas para la mejora de la adaptación de las variedades a los futuros cambios ambientales es la sustitución de los genes de enanismo que inducen insensibilidad a las giberelinas por otros, como el *Rht8c*, con los que evitar efectos pleiotrópicos indeseados, pero que producen el mismo efecto en la reducción de la altura de la planta y los incrementos en la partición de asimilados (Rebetzke y Richards, 2000). Los resultados del **capítulo 3** confirmaron el efecto pleiotrópico de los genes de enanismo, principalmente *Rht-B1b* y *Rht-D1b* en las variedades modernas, potencialmente negativo para la adaptación al ambiente Mediterráneo, como fue la disminución de la cobertura foliar del suelo en los primeros estadios del desarrollo, concretamente en el principio del encañado. Una pronta cobertura foliar del suelo puede reducir las pérdidas de agua, producto de la evaporación en los estadios iniciales del cultivo, ahorrando así agua que será determinante durante el llenado del grano en ambientes mediterráneos (Condon *et al.*, 2004). Por lo tanto, se debe estudiar la sustitución en el germoplasma español, de los genes de enanismo que inducen la insensibilidad al ácido giberélico por otros que eviten sus efectos negativos, siempre que se mantengan los beneficios en lo relativo al rendimiento e índice de cosecha.

4. Calidad harino-panadera

La mejora de las variedades cultivadas en España durante el siglo XX conllevó también importantes ganancias en los atributos relacionados con la calidad harino-panadera. Estas mejoras se debieron al incremento de la calidad de las proteínas del gluten y no a su cantidad, ya que se observó un descenso en la concentración de las mismas en el grano, con una tasa anual del -0,21%. Sin embargo este descenso se atenuó a partir de 1970 con el desarrollo de variedades con una mayor capacidad para fijar proteína en los granos por unidad de superficie, igualando prácticamente el ritmo de ganancia relativa del rendimiento y permaneciendo estable, desde esta fecha, la concentración de proteína en grano. Una concentración de proteína mínima es necesaria para la elaboración de productos de panadería, por lo que mantener esta tendencia de incremento paralelo del rendimiento y la síntesis y translocación de proteínas a los granos debería ser un objetivo si se pretende mejorar la calidad de los trigos españoles.

A pesar de la disminución en la concentración de proteína, las propiedades reológicas de la masa, importantes para determinar el comportamiento de las mismas durante los procesos de amasado y fermentación, como la *W* o la *P* mejoraron significativamente durante el siglo XX. Así, la *W* y la *P* se incrementaron a razón de 1,39% y 0,99% año^{-1}, respectivamente, mientras que la extensibilidad (*L*) se redujo un 0,46% año^{-1}, provocando un aumento del cociente entre tenacidad y extensibilidad (*P/L*) de 1,45% año^{-1}. Otras propiedades como la capacidad de absorción de agua, de interés para la industria y que indica indirectamente la calidad de una harina, también se mejoraron durante el siglo XX.

El éxito obtenido en la mejora de la *W* y *P* se debió probablemente a la importancia del control genético sobre estas propiedades ya que el factor genotipo explicó más del 80% de la variabilidad total. La alta influencia del genotipo sobre estas propiedades residió, sin duda, en el gran efecto que tienen las variantes de las HMW-GS sobre dichas propiedades y en el control genético simple que tienen, al codificarse cada HMW-GS en un alelo de solamente tres loci. La introducción de HMW-GS de interés para la calidad harinera (p.ej. *Glu-D1* 5+10 ó *Glu-B1* 7+8) y el abandono de otras con efecto negativo (p.ej. *Glu-A1* Null) contribuyó a esta mejora. Sin embargo, estos alelos comienzan a estar ya fijados en las variedades más modernas indicando que futuras mejoras difícilmente se alcanzarán con nuevas sustituciones de HMW-GS. Por otro lado, otras proteínas como las subunidades de bajo peso molecular de las gluteninas (LMW-GS), o las gliadinas, podrían estar interactuando de forma significativa, por lo que, para diseñar futuras estrategias y obtener nuevas variantes que permitan incrementar la calidad harinera en España, resulta imprescindible la caracterización de estas proteínas.

En cambio, la baja tasa de mejora de la extensibilidad pudo deberse a un menor interés por parte de los mejoradores en la extensibilidad de la masa o a una mayor dificultad en su mejora. Los resultados del **capítulo 4** mostraron una importante interacción con el ambiente, abarcando cerca del 30% de la varianza total, dificultando las labores de mejora por selección. Una parte (cerca del 30%) de la varianza abarcada por la interacción genotipo por ambiente fue debida a la introducción de la HMW-GS 5+10. Así, las variedades que portan esta subunidad mostraron una mayor adaptación a los ambientes con mayor aporte hídrico y menor concentración de proteína. El hecho de que una única subunidad, descrita además como negativa para una buena extensibilidad (*L*) (Cornish *et al*., 2001), influya también en la interacción de las variedades con el ambiente, indica la complejidad de establecer programas de mejora que potencien esta propiedad.

También se observaron mejoras en la tolerancia a procesos de amasado y fermentaciones relacionadas con los incrementos de la *W* y el equilibrio (*P/L*) de las variedades. Los resultados del **capítulo 4** establecieron el umbral de $W \geq 153$ $J \cdot 10^{-4}$ y $P/L \geq 0{,}56$ mm de H_2O mm^{-1} como el mínimo requerido para resistir un proceso de fermentación de 3 horas sin que los alveolos colapsen, indicando que la mayoría de las variedades modernas analizadas cumplirían con estos requisitos. Sin embargo, no hubo una mejora clara en la DMH durante la fermentación, atributo relacionado con el volumen final del pan (Ktenioudaki *et al.*, 2010; Ktenioudaki *et al.*, 2011). Las altas *P* y *W* de algunas variedades modernas, unidas al descenso de la *L*, han fomentado la aparición de masas que, si bien muestran alta estabilidad durante la fermentación, presentan una rápida aparición de porosidad, perjudicando la DMH. La baja extensibilidad de la mayoría de las variedades más modernas y su efecto sobre la porosidad de las masas obliga a los futuros programas de mejora a buscar alternativas genéticas que fomenten la extensibilidad, ya sea mediante la introducción de LMW-GS o gliadinas y a plantear el establecimiento de ambientes recomendados para las futuras variedades liberadas

La alta variabilidad en cuanto a los atributos de calidad, especialmente las propiedades reológicas de las masas, de las variedades modernas españolas se debió, probablemente, a que los agricultores han seleccionado las variedades a sembrar principalmente por su productividad. Esto se debe a la ausencia de incentivos claros por parte de la industria molinera y panadera para la siembra de variedades de alta calidad, aunque la reciente clasificación oficial de la calidad de los trigos españoles (BOE 2010) podría contribuir a cambiar el escenario y poner en valor los trigos de alta calidad. La existencia de variabilidad genética para estas características es un factor positivo para el establecimiento de programas de mejora con el objetivo de incrementar la calidad tecnológica de los trigos cultivados en España, si bien es evidente la necesidad de fijar los objetivos de dichos programas de manera que cumplan con los requisitos específicos del producto o sector objetivo.

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CONCLUSIONS

Conclusions

1. Estimation of genetic gains of bread wheat grown in Spain from 1930 to 2000 depended on the testing environment, particularly minimum daily temperatures before heading. Average yield increases were estimated on 35.1 kg ha^{-1} y^{-1} (0.88% y^{-1}).

2. Yield improvement occurred in two main episodes corresponding to the cultivation of the first improved varieties from the 1950s, and the introduction of foreign germplasm, mostly derived from CIMMYT, from the 1970's. The varieties released during the last decade of the century did not significantly contribute to yield gains.

3. Yield improvement was due to increases in the number of grains per spike (0.60% y^{-1}) and the number of spikes per m^2 (0.30% y^{-1}), while grain weight remained unchanged. The improvement in the number of grains per spike was caused by increases in the number of spikelets per spike (0.25% y^{-1}) and grains per spikelet (0.42% y^{-1}).

4. Aboveground biomass at maturity was maintained, so yield improvements were due to large increases in harvest index associated to:
   i. A reduction of a 26.3% of plant height, due first to the introduction of the GA sensitive dwarfing alleles, *Rht8c* and *Rht9*, and from the 1970's the GA insensitive alleles *Rht-B1b* and *Rht-D1b*,
   ii. A rise of 18% in the fertile tillering, and
   iii. A reduction of about 132 GDD in the cycle length until anthesis.

5. The pattern of adaptation of the bread wheat varieties changed during the 20th century. The first improved varieties had a wider adaptation that the original landraces, and among modern cultivars the winter types showed a wider adaptation than the spring ones.

6. The introduction of improved varieties modified the pattern of biomass accumulation and partitioning, which depends on the number of dwarfing alleles present in the genotypes. Almost 80% of the estimated yield genetic gains are due to aboveground biomass accumulation from milk-grain to maturity.

7. The contribution of photosynthesis to grain filling was improved in modern varieties due to large LAI values at milk-grain stage, and longer leaf area duration (LAD).

8. Grain protein content decreased at a rate of -0.21% y^{-1}, but the grain protein produced per unit area increased at a rate comparable to that of yield (0.39% y^{-1}).

9. Protein quality was improved due to the introduction of HMW-GS with a positive effect on dough strength. Alleles 1 and 2* at *Glu-A1* loci, 7+8 at *Glu-B1,* and 5+10 at *Glu-D1* are the most frequent in modern varieties. The varieties carrying the 5+10 HMW-GS are more adapted than the rest to environments with high water input in which the resulting dough has low extensibility.

10. The rheological properties of doughs, particularly dough strength (W) and tenacity (P) increased during the 20th century improving dough stability and tolerance during fermentation. As extensibility (L) was reduced, the configuration ratio (P/L) increased, causing in some cases unbalanced doughs showing excessive porosity during fermentation.

11. Several of the physiological changes that contributed to yield gains – such as harvest index and fertile tillering – will be no longer useful for future improvements, as they are already close to the theoretical optimum values estimated in non-limiting environments. New strategies for increasing plant biomass and yield should focus on additional traits such as alternative GA-sensitive dwarfing sources, early vigor, stay-green and radiation, water and nitrogen use efficiencies.

Conclusions

1. Estimation of genetic gains of bread wheat grown in Spain from 1930 to 2000 depended on the testing environment, particularly minimum daily temperatures before heading. Average yield increases were estimated on 35.1 kg ha^{-1} y^{-1} (0.88% y^{-1}).

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CONCLUSIONES

Conclusiones

1. El rendimiento de las variedades de trigo harinero sembrado en España desde 1930 a 2000 se incrementó, en promedio, 35.1 kg ha^{-1} año^{-1} (0.88% año^{-1}) aunque la magnitud de la ganancia genética dependió del ambiente en que se ensayaran las variedades y, más concretamente, de la temperatura mínima diaria media antes del espigado

2. El incremento del rendimiento ocurrió principalmente gracias a dos eventos, la introducción de las primeras variedades mejoradas a partir de 1950 y la introducción de germoplasma extranjero desde 1970, en su mayor parte derivado de material de CIMMYT. Las variedades liberadas durante la última década del siglo no contribuyeron a incrementar significativamente el rendimiento.

3. El incremento del rendimiento se debió al aumento del número de granos por espiga (0.60% año^{-1}) y de espigas por unidad de superficie (0.30% año^{-1}), mientras que el peso medio de los granos no varió significativamente. El incremento del número de granos por espiga se debió, a su vez, a aumentos en el número de espiguillas por espiga (0.25% año^{-1}) y de granos por espiguilla (0.42% año^{-1}).

4. No se observaron cambios en la biomasa en madurez y, por lo tanto, el rendimiento se incrementó gracias al aumento del índice de cosecha, debido a:
    i. Una reducción del 26.3% de la altura de la planta, producida en un primer momento por la introducción de los genes de enanismo sensibles a las giberelinas (*Rht8c* y *Rht9*) y a partir de 1970 de los alelos *Rht-B1b* y *Rht-D1b*, insensibles a las giberelinas
    ii. Un incremento del 18% de la fertilidad de los hijuelos
    iii. Una reducción de 132 GDD en la duración del ciclo hasta la antesis

5. Se observó un cambio en el patrón de adaptación de las variedades de trigo durante el siglo XX. Las primeras variedades mejoradas mostraron más amplia adaptación que las variedades tradicionales. Entre las variedades modernas, las de hábito invernal mostraron una adaptación más amplia que las de hábito primaveral.

6. La introducción de las primeras variedades mejoradas modificó el patrón de acumulación de biomasa y su partición en la planta, que dependió del número de alelos de enanismo presente en los genotipos. Casi el 80% de la ganancia genética relativa del rendimiento se debió a aumentos en la acumulación de biomasa desde el estadio de grano lechoso hasta la madurez.

7. La contribución de la fotosíntesis al llenado del granos se incrementó con la introducción de las variedades modernas, principalmente gracias a grandes aumentos en los valores de LAI en el estadio de grano lechoso y en la duración del área foliar (LAD).

8. El contenido de proteína en el grano disminuyó a una tasa de -0.21% año^{-1}, sin embargo, con la introducción de las variedades modernas, la cantidad de proteína producida por unidad de superficie se incrementó en 0.39% año^{-1}, una tasa similar a la que lo hizo el rendimiento.

9. La calidad de las proteínas del gluten se incrementó con la introducción de HMW-GS de efecto positivo sobre la fuerza de la masa. Los alelos 1 y 2* en el locus *Glu-A1*, el 7+8 en el *Glu-B1* y el 5+10 en el *Glu-D1* son los más frecuentes en las variedades modernas. Las variedades que portan el 5+10 mostraron una mayor adaptación a los ambientes con mayor aporte hídrico, aquellos en los que las masas mostraron menores valores de extensibilidad.

10. Las propiedades reológicas de las masas, especialmente la fuerza (W) y la tenacidad (P) se incrementaron durante el siglo XX. Esto contribuyó a incrementar la estabilidad y tolerancia de las masas durante la fermentación. Dado que la extensibilidad se redujo, el cociente entre tenacidad y extensibilidad (P/L) se incrementó, lo que derivó, en los casos más extremos, en masas desequilibradas que mostraron una alta porosidad durante la fermentación.

11. Varios de los cambios fisiológicos que contribuyeron al incremento del rendimiento, el índice de cosecha o el incremento de la fertilidad de los hijuelos, están cerca de los valores óptimos teóricos estimados para ambientes no limitantes. Por lo tanto, las estrategias dirigidas al incremento del rendimiento deberán basarse en el uso de fuentes alternativas de

enanismo sensibles a las giberelinas y mejorar características como la senescencia tardía y la eficiencia en el uso del agua, el nitrógeno y la radiación.