Utilidad de los recursos genéticos en mejora: potencial de las variedades tradicionales de trigo duro de la cuenca mediterránea para la mejora de la calidad y el valor agronómico en relación a su origen geográfico

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Utilidad de los recursos genéticos en mejora: potencial de las variedades tradicionales de trigo duro de la cuenca mediterránea para la mejora de la calidad y el valor agronómico en relación a su origen geográfico.

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Cho Juan Perenal tiene un arenal,
con grano de trigo lo quiere sembrar,
lo siembra en la cumbre, lo coge en la mar.

Ansina barbecha Cho Juan Perenal,
ansina lo siembra Cho Juan Perenal,

Estribillo:
ansina ponía sus pies en la mar
y ansina aprende Cho Juan a danzar.

Ansina lo labra Cho Juan Perenal,
ansina lo escarda Cho Juan Perenal.
[Estribillo]

Ansina lo siega Cho Juan Perenal,
ansina lo enmanilla Cho Juan Perenal.
[Estribillo]

Ansina lo engavilla Cho Juan Perenal,
ansina lo enfeja Cho Juan Perenal.
[Estribillo]

Ansina lo carga Cho Juan Perenal,
ansina lo enfresca Cho Juan Perenal.
[Estribillo]

Lo bota en la era Cho Juan Perenal,
ansina lo espeja Cho Juan Perenal.
[Estribillo]

Ansina lo trilla Cho Juan Perenal,
ansina lo vira Cho Juan Perenal.
[Estribillo]

Ansina lo empilona Cho Juan Perenal,
ansina lo despajona Cho Juan Perenal.
[Estribillo]

Ansina lo aventa Cho Juan Perenal,
ansina lo ensaca Cho Juan Perenal.
[Estribillo]

Ansina lo tuesta Cho Juan Perenal,
ansina lo muele Cho Juan Perenal.
[Estribillo]

Ansina lo cierne Cho Juan Perenal,
ansina lo amasa Cho Juan Perenal.
[Estribillo]

Ansina lo come Cho Juan Perenal,
ansina lo caga Cho Juan Perenal.
[Estribillo]

Cho Juan Perenal tiene un arenal,
con grano de trigo lo quiere sembrar,
lo siembra en la cumbre, lo coge en la mar.

Danza del trigo.

Folclore de las Islas Canarias.

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ÍNDICE DE TABLAS

Capítulo 1

Capítulo 2

Capítulo 3

Capítulo 4

ÍNDICE DE FIGURAS

Capítulo 1

Capítulo 2

Capítulo 3

Capítulo 4

RESUMEN

El trigo duro es un cultivo típicamente mediterráneo. La cuenca mediterránea es la mayor zona productora y consumidora de trigo duro del mundo, así como el mercado de importación más significativo. El desplazamiento de las variedades tradicionales que tuvo lugar a partir de la Revolución Verde, como consecuencia de la introducción de variedades semi-enanas, más productivas y homogéneas, fue causa de una importante erosión genética. Se considera que las variedades tradicionales de la cuenca mediterránea constituyen una importante fuente de variabilidad genética que puede ser utilizada en los programas de mejora para la obtención de variedades con características superiores.

En esta Memoria de Tesis Doctoral se estudió el potencial de variedades tradicionales de trigo duro para la mejora de la calidad y el valor agronómico en base a una colección de 172 accesiones procedentes de 21 países mediterráneos. Como referencia se utilizó un conjunto de 20 variedades modernas representativas de la variabilidad cultivada en la zona. Los ensayos de campo se llevaron a cabo en condiciones de secano durante tres años en la localidad de Gimenells (Lérida). Se estudió la variabilidad existente en la colección para las principales características agronómicas y de calidad, la presencia de estructuras geográficas y el efecto del clima prevalente en los países de origen de las variedades sobre su comportamiento agronómico. Se analizó la relación existente entre la fuerza del gluten y la composición alélica en cinco loci de gluteninas de alto (HMW) y bajo (LMW) peso molecular y se identificaron nuevas fuentes de alelos potencialmente interesantes para la mejora de la calidad.

En base a los resultados de rendimiento y calidad, las variedades tradicionales se agruparon en función de su región de origen en tres zonas geográficas: Mediterráneo oriental, Mediterráneo occidental y norte de la península de los Balcanes. Las primeras mostraron las mayores calidad global y variabilidad, pero dieron lugar a granos de poco peso. Las del Mediterráneo occidental tuvieron mayor tasa de llenado de los granos y granos mas pesados, mientras que las de las de los países del norte de la península de los Balcanes mostraron bajas calidad y variabilidad. Las variedades modernas fueron las

más productivas y de mayor calidad global, pero sus granos tuvieron el menor contenido de proteína.

En la colección se identificaron 114 alelos/patrones de bandas y 131 combinaciones distintas de gluteninas. Los alelos *Glu-B1e, Glu-A3a, Glu-A3d*, *Glu-B3a* y *Glu-B2a* tuvieron un efecto positivo sobre la fuerza del gluten, mientras que *Glu-A3b* y *Glu-B2b* tuvieron efecto negativo. Se identificaron en las variedades tradicionales tres patrones de bandas, considerados raros por su baja frecuencia en la población, que afectaron positivamente la fuerza del gluten, dos de ellos con efectos positivo y uno con efecto negativo. La interacción entre loci de gluteninas de alto y bajo peso molecular dio lugar a 126 combinaciones en variedades tradicionales y 9 en variedades modernas, 5 de ellas exclusivas de este último grupo. Dos combinaciones conocidas –incluyendo los modelos LMW-2 (aaa) y LMW-2^- (haa)– y tres inéditas detectadas en un reducido número de genotipos, tuvieron un efecto positivo y significativo sobre la fuerza del gluten en las variedades tradicionales. Se identificaron las variedades ‘PI-366109’, ‘Lobeiro de Grao Escuro’, ‘Trigo Glutinoso’, ‘Raspinegro de Alcalá’ y ‘Enano de Andújar’ como potenciales parentales en programas de mejora de la calidad.

La climatología de la zona de origen de las variedades tradicionales influyó en su fenología, crecimiento, rendimiento y componentes del rendimiento cuando se cultivaron en el noreste de España. Variedades procedentes de países con elevadas radiación solar, evapotranspiración, y temperatura fueron más precoces, con menores tasas de llenado de grano y granos ligeros, mientras que la presencia de lluvias en primavera en la zona de origen dio lugar a genotipos de características opuestas a estas.

ABSTRACT

Durum wheat is a major crop in the Mediterranean Basin which is the largest durum producing area worldwide, the most significant durum import market and the largest consumer of durum wheat products. The displacement of landraces from farmer fields since the Green Revolution, due to the introduction of more productive and homogeneous semi-dwarf varieties, caused an important genetic erosion. Landraces can be considered as likely sources of putatively lost variability and may provide new favorable genes/alleles to be introgressed into modern cultivars.

A collection of 172 durum wheat landraces from 21 Mediterranean countries was assembled and used to examine their value for quality and field performance improvement in breeding programs. A set of 20 representative modern varieties were used for comparison. Field experiments were conducted during 3 years under rainfed conditions in northeastern Spain. The existing variability for the main quality and agronomic traits, the presence of geographic structures as well as the effect of the climate in the countries of origin on the agronomic performance were investigated. The relationship between gluten strength and the allelic composition at five glutenin loci was assessed, and donors of potentially novel alleles for quality improvement were identified.

Based on yield and quality traits, landraces were clustered according to their region of origin in eastern Mediterranean, western Mediterranean, and North-Balkan Peninsula. Landraces from the eastern Mediterranean countries had the highest global quality and the widest variability for quality traits, but were characterized by relatively small grains. Landraces from the western Mediterranean countries had greater grain filling rates and heavier grains, while landraces from the north Balkan Peninsula had low quality and variability. Modern varieties were the most productive and with the highest global quality, but they had the lowest grain protein content and phenotypic variability.

A total number of 114 alleles/banding patterns and 131 different combinations of glutenin were recorded in the whole germplasm collection. Alleles *Glu-B1e, Glu-A3a, Glu-A3d*, *Glu-B3a* and *Glu-B2a* had a positive effect on gluten strength, while *Glu-A3b*

and *Glu-B2b* had a negative one. Three rare banding patterns were identified in landraces affecting significantly gluten strength, two of them with positive effect and one with negative effect. The interaction between HMW- and LMW-GS loci resulted in 126 combinations in landraces and 9 in modern varieties, 5 of them specific of the modern genotypes. Two known combinations −one including LMW-2 (aaa) and another LMW-2$^-$ (haa) − and three new ones, found in a few number genotypes, had a positive and significant effect on the gluten strength of the landraces. ‘PI-366109’, ‘Lobeiro de Grao Escuro’, ‘Trigo Glutinoso’, ‘Raspinegro de Alcalá’ and ‘Enano de Andújar’ were identified as useful donors for quality improvement.

The climate of the zone of origin of the landraces affected their phenology, growth, yield and yield components when grown in northeastern Spain. Landraces from countries with high solar radiation, evapotranspiration and temperature had the shortest cycle length, lowest grain filling rates and lightest grains, while abundant rainfalls during the spring caused the opposite effects.

RESUM

El blat dur és un cultiu típicament mediterrani. La conca mediterrània és la major zona productora i consumidora de blat dur del món, i el mercat d'importació més significatiu. El desplaçament de les varietats tradicionals que va tenir lloc a partir de la Revolució Verda, com a conseqüència de la introducció de varietats semi-nanes, més productives i homogènies, va ser la causa de una important erosió genètica. Es considera que les varietats tradicionals de la conca mediterrània constitueixen una important font de variabilitat genètica que pot ser utilitzada en els programes de millora per a l'obtenció de varietats amb característiques superiors.

En aquesta Memòria de Tesi Doctoral es va estudiar el potencial de varietats tradicionals de blat dur per a la millora de la qualitat i el valor agronòmic en base a una col·lecció de 172 accessions procedents de 21 països mediterranis. Com a referència es va utilitzar un conjunt de 20 varietats modernes representatives de la variabilitat cultivada a la zona. Els assaigs de camp es van dur a terme en condicions de secà durant tres anys a Gimenells (Lleida). Es va estudiar la variabilitat existent a la col·lecció per a les principals característiques agronòmiques i de qualitat, la presència d'estructures geogràfiques i l'efecte del clima prevalent als països d'origen de les varietats sobre llur comportament agronòmic. Es va analitzar la relació existent entre la força del gluten i la composició al·lèlica en cinc loci de glutenines d'alt (HMW) i baix (LMW) pes molecular i es van identificar noves fonts d'al·lels potencialment interessants per la millora de la qualitat.

En base al seu rendiment i qualitat, les varietats tradicionals es van agrupar en funció de la seva regió d'origen en tres zones geogràfiques: Mediterrani oriental, Mediterrani occidental i nord de la península dels Balcans. Les primeres van mostrar la major qualitat global i variabilitat, però els grans foren petits. Les del Mediterrani occidental van tenir major taxa d'ompliment dels grans i grans de més pes, mentre que les dels països del nord de la península dels Balcans van mostrar baixes qualitat i variabilitat. Les varietats modernes foren les més productives i de major qualitat global, però els seus grans van tenir el menor contingut de proteïna.

A la col·lecció es van identificar 114 al·lels/patrons de bandes i 131 combinacions de glutenines diferents. Els al·lels *Glu-B1e, Glu-A3a, Glu-A3d, Glu-B3a* i *Glu-B2a* van tenir un efecte positiu sobre la força del gluten, mentre que *Glu-A3b* i *Glu-B2b* van tenir un efecte negatiu. A les varietats tradicionals es van identificar tres patrons de bandes, considerat rars per la seva baixa freqüència en la població, que van afectar positivament la força del gluten, dos d'ells amb efectes positius i un amb efecte negatiu. La interacció entre loci de glutenines d'alt i baix pes molecular va donar lloc a 126 combinacions en varietats tradicionals i 9 en varietats modernes, 5 d'elles exclusives d'aquest darrer grup. Dues combinacions conegudes –incloent els models LMW-2 (aaa) i LMW-2^- (haa)– i tres inèdites detectades en un reduït nombre de genotips, van tenir un efecte positiu i significatiu sobre la força del gluten en les varietats tradicional. Es van identificar les varietats 'PI-366109', 'Lobeiro de Grao Escuro', 'Trigo Glutinoso', 'Raspinegro de Alcalá' i 'Enano de Andújar' com a parentals interessants per programes de millora de la qualitat.

La climatologia de la zona d'origen de les varietats tradicionals va influir en la seva fenologia, creixement, rendiment i components del rendiment quan es van cultivar al nord-est d'Espanya. Les varietats procedents de països amb elevades radiació solar, evapotranspiració, i temperatura van ser més precoces, amb menors taxes d'ompliment del gra i grans poc pesats, mentre que la presència de pluges en primavera a la zona d'origen va donar lloc a genotips de característiques oposades a aquestes.

INTRODUCCIÓN

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Clasificación, evolución y domesticación del trigo

El trigo es un cultivo perteneciente a la familia *Poaceae*, subfamilia *Pooideae*, tribu *Triticeae*, género *Triticum*. Las especies de este género se clasifican en función de su número de cromosomas en: diploides (2n=2x=14), tetraploides (2n=4x=28) y hexaploides (2n=6x=42), siendo el número básico de cromosomas (x) igual a siete y sus genomas AA, AABB y AABBDD, respectivamente.

El genoma A deriva del diploide salvaje *Triticum urartu* y el B de la especie *Aegilops speltoides;* la hibridación de ambos produjo un trigo tetraploide salvaje *emmer*. De éste surgió una forma cultivada que espontáneamente hibridó con *Aegilops tauschii*, portador del genoma D, dando lugar al hexaploide *Triticum spelta* (Matsuoka y Nasuda, 2004; Peng *et al.*, 2011).

Dentro de los trigos tetraploides, el trigo duro (*Triticum turgidum* L. var. *durum*) es una de las especies de cereal más antiguas e importantes cultivadas en el mundo. Pronto constituyó la principal fuente de grano en la agricultura neolítica, siendo el principal responsable del éxito y desarrollo de ésta. La historia de este cultivo surgió a partir de la domesticación de las especies *einkorn* (*Triticum monococcum* ssp. *monococcum*) y *emmer* (*Triticum turgidum* ssp. *dicoccon*). El primero de ellos evolucionó a partir del diploide salvaje *T. boeticum* mientras que el segundo lo hizo a partir del tetraploide T. *dicoccoides* hace unos 10.000 años (Heun *et al.*, 1997) en la región denominada Creciente Fértil (Harlan, 1981). Esta zona geográfica engloba Israel, Jordania, Líbano y oeste de Siria junto con el sureste de Turquía y, a través de los ríos Tigris y Eufrates, llega hasta Iraq e Irán (Lev-Yadun, 2000). Dentro de esta zona geográfica, en una pequeña región del sureste de Turquía se ha destacado a las Montañas Karacadağ̂, como centro de origen de la domesticación de la *eikorn* (Heun *et al.*, 1997) y *emmer* (Lev-Yadun, 2000, Özkan *et al.*, 2011). Sin embargo, la hipótesis de una única área de origen es actualmente discutida por otros autores que resaltan otras áreas dentro del Creciente Fértil en donde se pudieron haber llevado a cabo los procesos de domesticación de forma simultánea (Fuller *et al.*, 2012).

Los estudios arqueológicos no han determinado con exactitud cuál ha sido la expansión del cultivo desde su zona endémica (Feldman, 2001). La difusión hacia ambos lados del Creciente Fértil de las dos especies domesticadas de trigo, junto a otros cereales como la cebada, estuvo necesariamente ligada a las necesidades propias de las poblaciones humanas de aquel momento. Mejoras en la domesticación equivalieron a mejoras genéticas, como el engrosamiento de tallos, pérdida de glumas y fundamentalmente la obtención de semillas de mayor tamaño, en un lento proceso de especialización ecológica. La primera especie en expandirse desde la zona de origen fue *eikorn*, hacia el noroeste. Este hecho se produjo a través de la península de los Balcanes y Grecia (hace unos 8.000 años). Desde allí, siguió a lo largo de los ríos Danubio (hace 7.000 años) y Rin hasta alcanzar los Países Bajos (hace 6.000 años), Inglaterra y Escandinavia (hace 5.000 años). El transporte marítimo permitió su extensión por la costa del mar Mediterráneo y su posterior cultivo en Italia, España (hace 7.000 años) y sur de Gibraltar (Mac Key, 2005). Además, también el norte de África fue utilizado como ruta de dispersión, permitiendo la entrada del trigo en la península ibérica, durante la Edad Media (Moragues *et al.*, 2006).

A lo largo de todo este proceso de dispersión *eikorn* convivió con cultivos de *emmer* y cebada, estos últimos con mayores rendimientos y mejor adaptación a la domesticación (Mac Key, 2005), por lo que probablemente el proceso de dispersión de *emmer* puede ajustarse bien al sufrido por *eikorn*, tal y como sugieren Özkan *et al.* (2011). Sin embargo sólo llegó hasta Egipto (Mac Key, 2005) desde donde se extendió hacia el sur (Feldman, 2001). En la actualidad, el cultivo de *eikorn* es un relicto, creciendo sólo a pequeña escala en algunas zonas del oeste de Turquía, países balcánicos, Alemania, Suiza, España y el Cáucaso, mientras que *emmer* sólo se encuentra en Jordania, Siria e Israel, la parte central del sureste de Turquía y en áreas montañosas del este de Iraq y oeste de Iran (Özkan *et al.*, 2011).

El cultivo del trigo moderno está referido fundamentalmente a dos especies: el trigo harinero, hexaploide, *Triticum aestivum* ($2n=6x=42$, AABBDD) y el trigo duro, tetraploide, *Triticum turgidum* var. *durum* ($2n=4x=28$, AABB). Ambos han ido reemplazando en los últimos 5.000 años al primitivo *eikorn* (Peng *et al.*, 2011).

La mejora genética del trigo

La necesidad de garantizar los suministros alimenticios humanos, en continuo aumento a consecuencia de los rápidos y fuertes crecimientos demográficos, y la demanda por parte de la agricultura moderna de una mayor homogenización de los procesos productivos, han sido la causa de que durante el siglo XX, y especialmente después de la revolución verde de finales de los años 60, los programas de mejora de trigo hayan tenido efectos importantes. Entre los más significativos destacan: el incremento del rendimiento grano (De Vita *et al.*, 2007; Royo *et al.*, 2008), acortamiento de los ciclos, insensibilidad al fotoperiodo (Ortiz Ferrara *et al.*, 1998), reducción en el tamaño de la planta (Royo *et al.*, 2007), aumento del número de granos como resultado del mayor número de espigas por unidad de área o el incremento del número de granos por espiga, mayor eficiencia en la producción de biomasa y llenado del grano (Álvaro *et al.*, 2008b) o la mejora en la calidad del mismo (Blum *et al.*, 1987; Koç *et al.*, 2000; Motzo *et al.*, 2004; De Vita *et al.*, 2007). Todo ello ha propiciado la obtención de cultivares modernos agronómicamente superiores.

Una consecuencia inevitable de estos procesos de mejora es la implantación de los modernos cultivares mejorados, semi-enanos y genéticamente homogéneos, en detrimento de los tradicionales, a pesar de que las variedades modernas presentan mayor sensibilidad a los cambios en la disponibilidad de los recursos, en comparación con las antiguas variedades, que tienen más capacidad para amortiguar las condiciones adversas (Calderini y Slafer, 1999). Por esta razón, la mejora ha sido muy exitosa en ambientes favorables pero no tanto en ambientes sub-óptimos y en sistemas de agricultura ecológica, en los que la interacción genotipo por ambiente es muy significativa (Newton *et al.*, 2010).

El desplazamiento de los cultivares tradicionales por variedades modernas mejoradas ha hecho peligrar la conservación de los mismos y la de sus ancestros salvajes, con la consiguiente reducción de la diversidad genética, ampliamente documentada en numerosos estudios sobre los efectos de la mejora en cereales como el trigo duro (Martos *et al.*, 2005; Thuillet *et al.*, 2005; van de Wouw *et al.*, 2010). Este proceso ha sido denominado erosión genética. La pérdida de diversidad es una consecuencia directa de la utilización de un reducido número de individuos donadores de genes asociados a características deseables en las nuevas variedades, fenómeno que algunos han denominado como ‘cuello de botella’. En aquellas regiones genéticas que contienen los genes que codifican para rasgos mejorantes, la pérdida de variabilidad es mucho mayor que en caso de la selección natural.

A pesar de que las expectativas futuras mantienen la tendencia alcista de la demanda de alimentos, por los crecimientos poblacionales y la expansión de nuevos mercados económicos, durante los últimos años las tasas de crecimiento del rendimiento grano del trigo son mucho menores que los experimentados durante la segunda parte del siglo XX. Dado que la extensión de las áreas potencialmente cultivables está muy limitada, las necesidades futuras obligan a la obtención de incrementos adicionales en el rendimiento de grano (Slafer y Calderini, 2005) y en la calidad del mismo.

Variedades tradicionales y recursos genéticos

El primitivo proceso de domesticación del trigo en la zona del Creciente Fértil y la posterior dispersión de este cultivo a través de la cuenca mediterránea, favoreció a lo largo del tiempo el desarrollo de múltiples variedades tradicionales o *landraces*. Todas ellas son portadoras de una gran diversidad genética, por la continua adaptación a un gran número de condiciones ambientales y micro-hábitats como consecuencia de la presión de selección, tanto natural como inducida por la acción del hombre.

Camacho Villa *et al.* (2005) definieron el término *landrace* como una población dinámica de un cultivo, con un origen histórico, identidad diferenciada y que carece de un mejoramiento formal, siendo a menudo genéticamente diversa, localmente adaptada y asociada a sistemas de cultivo tradicionales. La heterogeneidad entre plantas hace que las poblaciones que conforman tengan una gran competitividad interna, justamente lo opuesto a la homogeneidad genética de las variedades modernas.

Por todo ello, el alto grado de polimorfismo genético de las variedades tradicionales de trigo duro detectado en muchos estudios (Ruiz *et al.*, 2002; Aguiriano *et al.*, 2006; Nazco *et al.*, en prensa) es muy apreciado en los programas de mejora, siendo importante conocer qué características de dichas variedades favorecen la adaptación a ambientes limitantes (Araus *et al.*, 2002) y pueden utilizarse para aumentar, tanto la producción como la calidad del grano de las variedades comerciales actuales. La introgresión de los genes portadores de esa información está permitiendo aumentar la diversidad genética perdida en el pasado, garantizando así el éxito de los programas de mejora actuales.

Las variedades tradicionales constituyen la principal fuente biológica capaz de proporcionar la variabilidad necesaria a los cultivos para garantizar en el futuro las necesidades alimentarias de la población mundial. Una de las estrategias de conservación de la heterogeneidad genética de estas variedades es la creación de colecciones nucleares. Estas colecciones concentran la mayor parte de la variabilidad genética de una extensa base de germoplasma inicial en un reducido conjunto de accesiones capaz de representar a la colección inicial en su totalidad. En la actualidad también se está trabajando en el desarrollo de colecciones nucleares dirigidas, en las que se pretende generar una colección que recopile la diversidad genética que afecta a un aspecto muy concreto de la mejora, por ejemplo, la calidad del grano. Por esta razón, el uso efectivo de los recursos genéticos en los programas de mejora requiere la evaluación y caracterización del germoplasma disponible con el objetivo de detectar material potencialmente interesante para los mejoradores. En este sentido, la cuenca del mar Mediterráneo constituye un centro geográfico de primer orden para la obtención de germoplasma procedente de variedades antiguas de trigo, siendo especialmente importante el Creciente Fértil por ser área de origen de éste y otros cultivos.

Efecto del ambiente sobre el comportamiento agronómico del trigo

En la cuenca Mediterránea el cultivo de trigo duro está frecuentemente sometido a numerosos estreses, tanto de tipo biótico como abiótico. Entre los últimos destaca el estrés ocasionado por la sequía y las altas temperaturas durante la primavera, fenómenos característicos del clima mediterráneo. Las condiciones adversas afectan de forma diferente al cereal en función del momento, duración e intensidad del estrés, habiéndose demostrado que las mayores reducciones del rendimiento aparecen cuando el estrés tiene lugar en la fase vegetativa tardía y durante el periodo de llenado del grano (Simane *et al.*, 1993b), momento en el que se suele producirse una reducción de las precipitaciones y la evapotranspiración del cultivo supera la disponibilidad de agua en el suelo. El efecto del estrés hídrico sobre el número de granos es mucho mayor si tiene lugar en torno a la floración, mientras que el estrés post-antesis afecta directamente al peso y tamaño del grano (Rebetzke *et al.*, 2009). En ambos casos disminuye el rendimiento final del cultivo.

Bajo estas condiciones, el ajuste fenológico es el principal mecanismo de adaptación de los trigos, ya que puede evitar mermas en el rendimiento y reducir las oscilaciones de un año a otro. La obtención de variedades con ciclos más cortos es uno de los objetivos de algunos programas de mejora, debido a que permiten a la planta escapar de las condiciones climáticas adversas y de determinadas enfermedades. El mecanismo de escape permite que cuando tienen lugar las condiciones más desfavorables, el cultivo se encuentre en una fase suficientemente avanzada del ciclo, de manera que el efecto de los estreses sea nulo o mucho menor.

Muchos estudios han demostrado la capacidad de las plantas para detectar y adaptarse ante los cambios de fotoperiodo y temperatura, siendo el ajuste fenológico una estrategia para maximizar la supervivencia (Worland y Snape, 2001). El trigo no es una excepción, por lo que genotipos de floración precoz son adecuados en zonas más cálidas y secas por su mayor adaptación a estas condiciones, mientras que los tardíos aprovechan mejor los recursos en las zonas más frías y húmedas (Kato *et al.*, 1998; Goldringer *et al.*, 2006). La adaptación de los cultivares mejorados a un amplio rango de ambientes se ha explicado por su insensibilidad al fotoperiodo (Ortiz Ferrara *et al.*, 1998).

El desarrollo del cultivo es el resultado de la interacción entre factores ambientales y genéticos, que definen la adaptabilidad del trigo a un ambiente determinado (Slafer y Whitechurch, 2001). De esta interacción entre genotipo y ambiente también derivan cambios en la producción total de biomasa a lo largo del ciclo de desarrollo y en el rendimiento final (Van den Boogaard *et al.*, 1996). Existe una relación significativa entre el rendimiento del grano y la biomasa en el estadio de antesis y durante el llenado del grano, descrita en trigo harinero, cebada y trigo duro (Boggini *et al.*, 1990; Villegas *et al.*, 2001), lo que denota la importancia del estudio de la acumulación de biomasa en trigo.

En condiciones mediterráneas, un alto vigor inicial del cultivo se ha asociado a una mayor biomasa en los restantes estadios de desarrollo y con un mayor rendimiento grano (Richard, 1996). Sin embargo, estas relaciones dependen en gran medida de las condiciones ambientales durante el desarrollo del cultivo, tales como la cantidad y distribución de las lluvias, o las propiedades del suelo (Botwright *et al.*, 2002). El número de hijuelos por planta también está influenciado por las condiciones de humedad, frío y nutrición del cultivo después de la emergencia (Rebetzke *et al.*, 2009) y la supervivencia de los mismos es función de características genotípicas y ambientales. Un menor número de hijuelos favorece un mayor peso del grano (Duggan *et al.*, 2005), por lo que pueden tener una fuerte influencia en el índice de cosecha y el rendimiento final.

En condiciones no limitantes para el crecimiento, la mayoría de los fotoasimilados que se acumulan en el grano provienen de la actividad fotosintética de la hoja bandera, así como de la penúltima hoja de la planta y de su espiga. Sin embargo, las condiciones de estrés hídrico y térmico en las que se produce el llenado del grano en condiciones mediterráneas limitan la fotosíntesis (Shepherd *et al.*, 1987; Papakosta y Gagianas, 1991). La capacidad que posee el trigo para completar el llenado del grano con la removilización de reservas provenientes del tallo y otros órganos vegetativos (Frederik y Bauer, 1999; Royo *et al.*, 1999; Davies *et al.*, 2000), hace que bajo condiciones de estrés hídrico parte del llenado del grano se produzca a expensas de la translocación de asimilados acumulados en la planta antes de la antesis (Simane *et al.*, 1993a; Royo *et al.*, 1999; Tahir y Nakata, 2005; Álvaro *et al.*, 2008b*)*.

El contenido de clorofila de las hojas juega un papel muy importante en la actividad fotosintética del trigo y, por lo tanto, en el rendimiento final. Puesto que en condiciones mediterráneas el cultivo suele estar expuesto a altos niveles de radiación, una mayor concentración de clorofila puede contribuir de forma positiva a la obtención de altas tasas fotosintéticas (Pierre *et al.*, 2008). Mediciones rápidas y simples del contenido de clorofila pueden realizarse a través de los valores de SPAD (*Soil Plant Analysis Development*) y son de gran utilidad en el estudio del cultivo. Algunos factores ambientales como la radiación, estado hídrico de la planta o fertilidad del suelo, condicionan en gran medida el contenido en clorofila de las hojas. Sin embargo, en algunos estudios se ha encontrado mayor variabilidad genética que ambiental para el contenido en clorofila (Giunta *et al.*, 2002), indicando que es posible la selección por este carácter.

Frente a los efectos que los factores ambientales ocasionan en las características de crecimiento, desarrollo y productividad de las plantas, existen características genéticas con mecanismos de compensación para lograr la mejor adaptación. El componente genético de una determinada variedad fue adquirido en unas condiciones ambientales determinadas y marcará el tipo de estrategia para el que la variedad está preparada.

La calidad del trigo

La gran importancia de la cuenca mediterránea en la producción de trigo duro ha estado históricamente asociada al elevado consumo de productos derivados de este cereal. La molturación del grano proporciona sémola y harina, productos básicos a partir de los cuales se han elaborado alimentos tradicionales de vital importancia en el sustento de la población. La pasta es el principal producto elaborado a partir del trigo duro y el alimento local que mayor expansión ha tenido fuera de la cuenca mediterránea. El couscous es otro destacado alimento fabricado a partir de este cereal, y es ampliamente consumido en los países del norte de África. El pan obtenido a partir de harina de trigo duro y el bulgur son también alimentos de primera necesidad en países del Oriente Medio, Balcanes y norte de África.

Los criterios de calidad del trigo duro vienen determinados por el producto final que se pretende obtener a partir de este cereal, siendo la calidad para pasta la más demandada en la actualidad. La obtención de variedades que garanticen óptimos valores de calidad es uno de los principales objetivos de los programas de mejora y una de las vías para conseguir productos diferenciados con mayor valor añadido. En el ámbito de la Unión Europea se favorece el cultivo de estas variedades con incentivos económicos y se fijan parámetros evaluadores como el índice de calidad (QI), que se calcula a partir de los siguientes atributos de calidad: contenido de proteína, fuerza del gluten, índice de color amarillo y el peso específico del grano o el peso de mil granos (Reglamento (CE) nº 2237/2003, publicado en el DOUE de diciembre de 2003).

Las proteínas son los componentes más destacados a la hora de definir la calidad del grano y dentro de ellas las gluteninas y gliadinas son las más importantes. Ambos tipos, que forman las prolaminas, constituyen el gluten y son proteínas de reserva acumuladas durante el periodo de llenado del grano y utilizadas posteriormente como fuente de nitrógeno por la semilla, durante la fase germinativa (Shewry y Halford, 2002). El contenido de proteína está muy influenciado por las condiciones ambientales (Shewry *et al.*, 2003; Labuschagne *et al.*, 2009), pero la calidad de la proteína tiene un alto componente genético (Carrillo *et al.*, 2006), que se traduce en una gran variabilidad entre genotipos.

Además de la cantidad de proteína es importante su composición, pues de ella depende la viscosidad y elasticidad del gluten. Como indicador de estas dos propiedades se utiliza la fuerza del gluten, siendo las prolaminas los principales componentes que la afectan. Las gluteninas son proteínas poliméricas, constituidas por cadenas de múltiples polipéptidos unidos por puentes disulfuros, mientras que las gliadinas son monoméricas, constituidas por polipéptidos de cadenas simples (Wrigley *et al.*, 2009). El análisis de la composición de gluteninas se lleva a cabo normalmente por electroforesis en gel de poliacrilamida (PAGE) con dodecil sulfato sódico (SDS), ya que éste agente reductor rompe los enlaces disulfuro que las unen, originando subunidades de alto peso molecular (HMW) y de bajo peso molecular (LMW) que muestran diferente movilidad en los geles. En el caso de las gliadinas, la electroforesis se realiza en geles de poliacrilamida a pH ácido, pudiendo diferenciarse en base a su movilidad las α, β, γ y ω-gliadinas.

En trigo duro, las subunidades de gluteninas HMW son codificadas por los loci *Glu-A1* y *Glu-B1* del complejo *Glu-1*, localizado en el brazo largo de los cromosomas del grupo de homeología 1 (Carrillo *et al.*, 2006). En el caso de las subunidades de gluteninas LMW, la codificación es producida en los loci *Glu-A3, Glu-B3* y *Glu-B2* (Ruiz y Carrillo, 1995; Vázquez *et al.*, 1996), situados en el brazo corto de los cromosomas del grupo 1.

Numerosas investigaciones han relacionado la calidad del trigo duro con la presencia de las subunidades de gluteninas LMW debido a su efecto sobre la viscoelasticidad del gluten, siendo especialmente importantes para la obtención de firmeza en la pasta (Ruiz y Carrillo, 1995; Vázquez *et al.*, 1996; Carrillo *et al.*, 2006), mientras que las HMW tienen menos influencia, siendo lo contrario en el caso del trigo harinero. A pesar de que la valoración del efecto de las subunidades HMW sobre el trigo duro ha dado resultados contradictorios, parece que los efectos positivos son destacados cuando el producto final a obtener a partir de la masa es pan, tanto en trigo duro como harinero, y especialmente en el caso de las variedades tradicionales (Tarekegne y Labuschagne, 2005).

Se ha observado también la importancia de las relaciones entre los componentes proteicos del gluten, de tal forma que la mayor proporción de gluteninas con respecto a las gliadinas generalmente ofrece mayor calidad de cocción en la pasta (Walsh y Gilles, 1971; Wasik y Bushuk, 1975) y mayor fuerza en la masa (Sissons *et al.*, 2007).

Estudios realizados sobre la influencia de las subunidades de gluteninas LMW sobre la fuerza del gluten de trigo duro (Payne *et al.*, 1984), identificaron dos modelos de combinaciones de estas subunidades con un claro efecto sobre la calidad. Estos modelos o patrones fueron denominados como: LMW-1, asociado con poca fuerza y mala calidad y LMW-2, asociado con buenos valores de fuerza. Además ambos modelos están estrechamente ligados con las gliadinas γ-42, en el caso del LMW-1, y las γ-45, en el caso del LMW-2, pues dichas variantes alélicas de gliadinas se heredan ligadas a ambos modelos, aunque son éstos últimos los que mayor efecto tienen sobre la calidad del gluten. Posteriormente, se han detectados otros modelos adicionales tales como LMW-1−, LMW-2− y LMW-2* (Carrillo *et al.*, 1990; Vázquez *et al.*, 1996; Nieto-Taladriz *et al.*, 1997) con distintos efectos sobre la calidad pues, mientras unos están relacionados con poca fuerza, como el LMW-1−, otros lo están con alta fuerza, como el LMW-2−.

Debido al carácter genético de estas proteínas y su influencia sobre los parámetros de calidad del trigo, la caracterización de las mismas en colecciones de variedades tradicionales suministra una importante valoración de la diversidad genética dentro y entre poblaciones (Nevo y Payne, 1987; Igrejas *et al.*, 1999), además de ser una fuente de variabilidad importante para los programas de mejora.

Otro factor que afecta a la calidad del grano es el contenido en carotenoides en el endospermo. Los carotenoides son pigmentos orgánicos que desarrollan funciones variadas en las plantas, entre las que destacan las siguientes: intervienen en la actividad fotosintética como captadores de luz, actúan como compuestos foto-protectores y antioxidantes, y son precursores del ácido abscísico. El color final de los productos derivados del trigo es el resultado tanto de la coloración inicial que estos pigmentos le confieren a los granos como de las condiciones posteriores de molturación, almacenamiento y procesado a las que estos son sometidos, ya que todas ellas producen pérdidas y modificaciones de la coloración inicial (Hidalgo y Brandolini, 2008a, 2008b). Dado que el color de los alimentos juega un papel muy importante en las características organolépticas de los mismos, la valoración del contenido de carotenos del grano es un indicio de la calidad del mismo como materia prima y su estimación resulta muy útil en los programas de mejora por la variabilidad existente entre especies y variedades (Digesù *et al.*, 2009).

A escala nutricional, los carotenoides también juegan un papel destacado en la dieta humana, estando relacionados con abundantes beneficios para la salud, siendo uno de los factores más notables dentro de la dieta con mayor reconocimiento a nivel internacional, la dieta mediterránea, en la que el trigo es uno de los alimentos básicos. Los carotenoides más representativos en la dieta humana son: *α*-caroteno, *β*-caroteno, *β*-criptoxantina, licopeno, luteína y zeaxantina. Entre los beneficios más destacados se cuentan el ser precursores de la vitamina A (*β*-caroteno), aumentar la absorción de hierro, favorecer la disminución de enfermedades cardiovasculares y de cánceres, y proteger de la degeneración macular y las cataratas. En el endospermo del trigo duro, el carotenoide más abundante es la luteína (Panfili *et al.*, 2004), y la cantidad de estos pigmentos presentes en esta especie es superior a la existente en el trigo harinero.

OBJETIVOS

OBJETIVOS

Esta Memoria de Tesis Doctoral se enmarca en una línea de investigación cuya finalidad es la generación de conocimiento científico útil para la mejora del trigo duro en condiciones mediterráneas.

Los objetivos generales de esta Memoria de Tesis Doctoral han sido los siguientes:

1. Analizar el potencial de variedades tradicionales y antiguas de trigo duro de la cuenca Mediterránea como parentales para la mejora de la calidad en relación a variedades modernas mejoradas. Este objetivo general se concreta en los siguientes objetivos específicos:

i. Investigar si existe una estructura geográfica en la cuenca mediterránea en base al rendimiento y la calidad del trigo duro.

ii. Estudiar la variabilidad fenotípica existente para las principales características cualitativas.

iii. Identificar genotipos útiles como parentales para la mejora de la calidad en programas de mejora.

2.- Estudiar la composición alélica para gluteninas de alto y bajo peso molecular en una colección de germoplasma de trigo duro de la cuenca mediterránea y su relación con la fuerza del gluten. Para su desarrollo se plantearon los siguientes objetivos específicos:

i. Identificar la composición alélica y las combinaciones existentes en la colección para subunidades de gluteninas de alto y bajo peso molecular en los siguientes cinco loci: *Glu-A1*, *Glu-B1*, *Glu-A3*, *Glu-B3* y *Glu-B2*.

ii. Estudiar la relación entre la composición alélica y la fuerza del gluten.

iii. Estudiar la posible existencia en la colección de una estructura geográfica en base a la configuración genética para subunidades de gluteninas y la fuerza del gluten.

iv. Identificar variedades tradicionales o antiguas útiles para la mejora de la fuerza del gluten.

3.- Estudiar la relación existente entre el clima prevalente en los países de origen de las variedades tradicionales y antiguas de trigo duro y su comportamiento agronómico en las condiciones de cultivo del noreste de España.

Para alcanzar los objetivos propuestos se hizo acopio de una colección de 172 variedades tradicionales y antiguas de trigo duro de 21 países de la cuenca mediterránea y 20 variedades modernas, entre las cuales se seleccionó el germoplasma apropiado para abordar cada uno de los objetivos.

La presente Memoria de tesis Doctoral se compone de 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 Euphytica, el capítulo 2 ha sido aceptado en el Journal of Cereal Science y los capítulos 3 y 4 están en fase de revisión.

CAPÍTULO 1

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Can Mediterranean durum wheat landraces contribute to improved grain quality attributes in modern cultivars?

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Abstract The variability for quality attributes existing in a collection of 154 durum landraces from 20 Mediterranean countries and 18 modern cultivars was determined with the ultimate goal of identifying potential quality-enhancing genotypes for use in breeding programs. Field experiments were conducted during 3 years under rainfed conditions in northeastern Spain. Environmental effects were the most important in determining protein content, grain yield and yellow color index of the endosperm (grain flour), and the least important in determining EU quality index (QI), gluten strength and grain filling rate. QI is a weighed composite index determined from protein content, gluten strength, yellow color index and thousand kernel weight. Multivariate analysis detected four groups; three including landraces and one comprising modern cultivars. Landraces from the eastern Mediterranean countries had the highest mean QI and the widest variability for individual quality traits, but were characterized by relatively small grains. Landraces from the western Mediterranean countries had greater grain filling rates and heavier grains. Protein content, gluten strength and yellow color index were similar between eastern and western groups. The low QI and reduced variability characterizing the landrace group from the north Balkan Peninsula support the hypothesis of a different origin for this group. Modern cultivars, as a group, were the most productive and showed high QI, but they had the lowest grain protein content and phenotypic variability. Landraces that could be used as sources of quality-improving attributes and/or those that could be used in breeding programs without substantial quality handicaps were identified from different groups. Landraces can be particularly useful in breeding programs to improve gluten strength, grain weight and accelerate grain filling rate.

Keywords Kernel weight · Test weight · Mixed model · Sedimentation index · Modern varieties

Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is cultivated on around 17 million hectares worldwide.

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It is a major crop in the Mediterranean basin which is the largest durum producing area worldwide, the most significant durum import market and the largest consumer of durum wheat products.

In the Mediterranean Basin, durum is cultivated mostly in rainfed environments, where crops are often exposed to environmental stresses. High temperatures and drought during grain filling frequently constrain full expression of yield potential, but they do not usually have negative effects on grain quality. The release of durums with high quality standards is one of the major goals of breeding programs in the region. The EU offers economic incentives for the production of high quality durum grain. A quality index (QI) was established by an EU Commission Regulation in 2003 based on protein content, gluten quality, yellow color index and test weight (or thousand kernel weight). The QI is expressed as a percentage of the overall quality of each variety with reference to check varieties selected among the most widely cultivated cultivars in each country. On the southern and eastern shores of the Mediterranean Basin, the emerging industrialization and modernization of durum processing industries is pressuring national programs to release cultivars with improved quality attributes in order to maintain the competitiveness of national production *vis* a *vis* that of the European producers and to reduce the need for "quality-driven" imports.

Archaeological evidence shows that the earliest domesticated wheats from the Fertile Crescent dated to approximately 10,000 years BP, spread to the west of the Mediterranean Basin, reaching the Iberian Peninsula around 7,000 years BP (Feldman 2001; MacKey 2005). This process of migration and both natural and human selection resulted in the establishment of local landraces specifically adapted to a diversity of agro-ecological zones. Landraces and pure line cultivars obtained through mass selection from them during the first decades of the twentieth Century were widely grown until the early 1970s, when they were rapidly displaced by homogeneous and more productive semi-dwarf cultivars. While this displacement resulted in dramatic productivity increases all around the Mediterranean basin and was critical to raising wheat production enough to mitigate the effect of rapid demographic growth, it undeniably resulted in a decrease of the cultivar diversity in the region and the near-extinction of on-farm genetic variability (Skovmand et al. 2005). It has been suggested that the level of genetic diversity underlying successful modern varieties may have fallen due to reduced numbers of ancestors, the pursuit of specific ideotypes (Pecetti and Annicchiarico 1998), the high selection pressure applied in breeding programs, and the relatively small number of varieties currently cultivated (Skovmand et al. 2005). The genetic erosion caused by the displacement of landraces is a concern for plant breeders because these genetic resources may be the most valuable sources for broadening the genetic bases for many traits in current breeding programs. While the problem is globally addressed by international strategies trying to coordinate the identification, characterization, description and accessibility to genetic resources (Newton et al. 2010), specific efforts to identify potentially new genetic variability for immediate use in breeding programs are still needed.

Local landraces may provide new alleles for the improvement of commercially valuable traits. Introgression of these alleles into modern cultivars can be very useful, especially when breeding for suboptimal environments (Ceccarelli et al. 1991). Mediterranean durum landraces represent a particularly important group of genetic resources because of their extensive genetic variability and their documented resilience to pests, resistance to diseases and tolerance to abiotic stresses (Pecetti et al. 1994). However, their plant height, general lateness and low harvest index have restricted their current cultivation to a few marginal areas or to the framework of organic farming, and have discouraged small breeding programs from evaluating and using them extensively as parents in crossing.

Breeding activities carried out in durum wheat in the Mediterranean Basin during the twentieth century resulted in increased grain yields (De Vita et al. 2007; Royo et al. 2008), earliness, shorter plants (Royo et al. 2007) and improved biomass partitioning to the grain (Álvaro et al. 2008). Various studies also addressed the effects of genetic improvement on grain quality traits (Blum et al. 1987; Koç et al. 2000; Motzo et al. 2004, De Vita et al. 2007) and evaluated the end-use quality of local landraces (Pecetti and Annicchiarico 1993; Boggini et al. 1997). Effective use of genetic resources in breeding programs requires evaluation of the diversity in the genepool and characterization of available accessions in order to detect the presence of variants of potential interest for breeding purposes. An increase in the available genetic variation through the use of landraces in breeding programs seems possible in terms of end-product quality, given the high level of polymorphism of key quality genes and the large genetic diversity found for quality traits between and within landrace subpopulations (Ruiz et al. 2002; Aguiriano et al. 2006; Moragues et al. 2006). A study conducted with a set of 63 Mediterranean durum landraces indicated that genetic diversity for glutenin protein subunits was structured according to geographical patterns (Moragues et al. 2006).

The present study was conducted with a set of 154 durum landraces from 20 Mediterranean countries and 18 modern cultivars with the following objectives: (i) to assess whether a geographic structure exists in the region on the basis of yield and quality traits, (ii) if so, to study the variability existing between and within groups, and (iii) to identify donors of potentially novel alleles for quality improvement in durum breeding programs.

Materials and methods

Experimental setup

A collection of 154 durum landraces from 20 Mediterranean countries, including the major durum producers/users (Fig. 1; Table 1), and 18 modern cultivars (Table 1) were grown during 3 years in Gimenells (41°40 ′N, 0°20′ E, and 200 m a.s.l.) in Lleida province (north-eastern Spain). Landraces were selected from a larger collection comprising 231 accessions of different origin on the basis of genetic variability determined by 33 SSR markers in order to represent the genetic diversity of ancient local durums from the Mediterranean basin (Royo et al. unpublished). Accessions provided by public gene banks (Centro de Recursos Fitogenéticos INIA—Spain, ICARDA Germplasm Bank and USDA Germplasm Bank) were increased in bulk and selected for uniformity. Increase plots were planted in the same field in years previous to each experiment to ensure a common origin for seeds of all lines. The modern set included Spanish, Italian, and French cultivars, as well as the U.S. desert durum cultivar Ocotillo. Experiments were performed in a non-replicated modified augmented design with three replicated checks (cv. Claudio, Simeto and Vitron). Individual plots were 6 m^2 and comprised eight 5 m rows, spaced 0.15 m apart. Sowing density was adjusted to 250 viable seeds m^{-2}. Climatic data were recorded by a weather station placed in the same field (Table 2). Experiments were conducted under rainfed conditions, but water scarcity after sowing in 2007 made it necessary to irrigate once

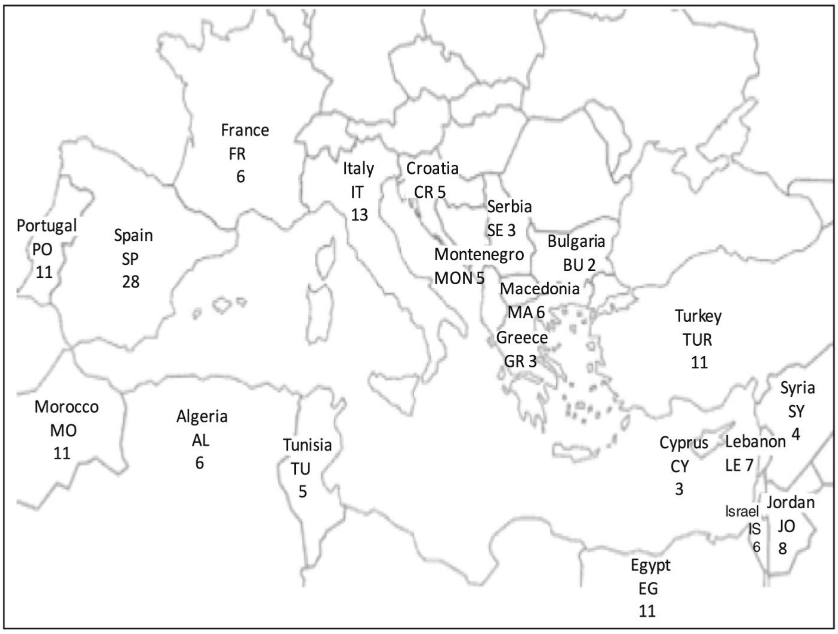


Fig. 1 Countries (and country codes) of origin of 154 landraces included in the study. The number of landraces from each country is also indicated

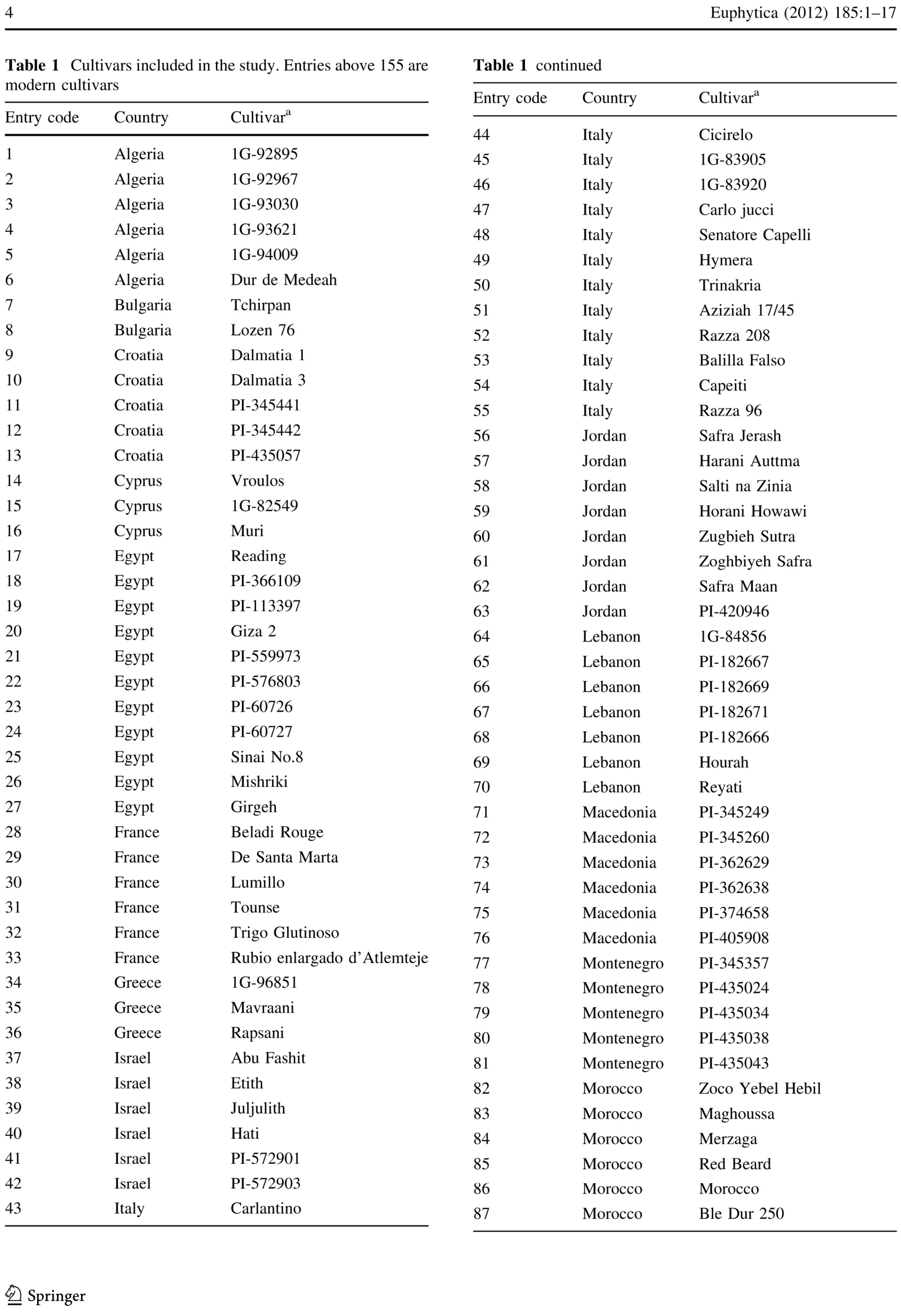


Table 1 Cultivars included in the study. Entries above 155 are modern cultivars

Entry code	Country	Cultivar[a]
1	Algeria	1G-92895
2	Algeria	1G-92967
3	Algeria	1G-93030
4	Algeria	1G-93621
5	Algeria	1G-94009
6	Algeria	Dur de Medeah
7	Bulgaria	Tchirpan
8	Bulgaria	Lozen 76
9	Croatia	Dalmatia 1
10	Croatia	Dalmatia 3
11	Croatia	PI-345441
12	Croatia	PI-345442
13	Croatia	PI-435057
14	Cyprus	Vroulos
15	Cyprus	1G-82549
16	Cyprus	Muri
17	Egypt	Reading
18	Egypt	PI-366109
19	Egypt	PI-113397
20	Egypt	Giza 2
21	Egypt	PI-559973
22	Egypt	PI-576803
23	Egypt	PI-60726
24	Egypt	PI-60727
25	Egypt	Sinai No.8
26	Egypt	Mishriki
27	Egypt	Girgeh
28	France	Beladi Rouge
29	France	De Santa Marta
30	France	Lumillo
31	France	Tounse
32	France	Trigo Glutinoso
33	France	Rubio enlargado d'Atlemteje
34	Greece	1G-96851
35	Greece	Mavraani
36	Greece	Rapsani
37	Israel	Abu Fashit
38	Israel	Etith
39	Israel	Juljulith
40	Israel	Hati
41	Israel	PI-572901
42	Israel	PI-572903
43	Italy	Carlantino
44	Italy	Cicirelo
45	Italy	1G-83905
46	Italy	1G-83920
47	Italy	Carlo jucci
48	Italy	Senatore Capelli
49	Italy	Hymera
50	Italy	Trinakria
51	Italy	Aziziah 17/45
52	Italy	Razza 208
53	Italy	Balilla Falso
54	Italy	Capeiti
55	Italy	Razza 96
56	Jordan	Safra Jerash
57	Jordan	Harani Auttma
58	Jordan	Salti na Zinia
59	Jordan	Horani Howawi
60	Jordan	Zugbieh Sutra
61	Jordan	Zoghbiyeh Safra
62	Jordan	Safra Maan
63	Jordan	PI-420946
64	Lebanon	1G-84856
65	Lebanon	PI-182667
66	Lebanon	PI-182669
67	Lebanon	PI-182671
68	Lebanon	PI-182666
69	Lebanon	Hourah
70	Lebanon	Reyati
71	Macedonia	PI-345249
72	Macedonia	PI-345260
73	Macedonia	PI-362629
74	Macedonia	PI-362638
75	Macedonia	PI-374658
76	Macedonia	PI-405908
77	Montenegro	PI-345357
78	Montenegro	PI-435024
79	Montenegro	PI-435034
80	Montenegro	PI-435038
81	Montenegro	PI-435043
82	Morocco	Zoco Yebel Hebil
83	Morocco	Maghoussa
84	Morocco	Merzaga
85	Morocco	Red Beard
86	Morocco	Morocco
87	Morocco	Ble Dur 250

Table 1 continued

Entry code	Country	Cultivar[a]
88	Morocco	Oned Zenati
89	Morocco	Mahmoudi C
90	Morocco	Maghoussa Amizmiz
91	Morocco	Cobras
92	Morocco	Haj Mouline
93	Portugal	Marques
94	Portugal	Raposinho
95	Portugal	Raspinegro
96	Portugal	Anafil
97	Portugal	Espanhol
98	Portugal	Dezassete
99	Portugal	Durazio Rijo Glabro
100	Portugal	Amarelo Barba Preta
101	Portugal	Alentejo
102	Portugal	Tremes rijo
103	Portugal	Lobeiro de grao escuro
104	Serbia	Belgrade 9
105	Serbia	PI-378303
106	Serbia	PI-585195
107	Spain	Arisnegro de Tenerife
108	Spain	Basto Dura
109	Spain	Blanco de Corella
110	Spain	Blanquillo
111	Spain	Candeal de Salamanca
112	Spain	Colorado de Jerez
113	Spain	Enano de Andújar
114	Spain	Fartó
115	Spain	Griego de Baleares
116	Spain	Gros de Cerdaña
117	Spain	Heraldo del Rhin
118	Spain	Pinet
119	Spain	Pisana cañihueca
120	Spain	Raspinegro Canario
121	Spain	Raspinegro de Alcalá
122	Spain	Recio de Almería
123	Spain	Verdial
124	Spain	Alonso
125	Spain	Azulejo de Villa del Río
126	Spain	Blancal
127	Spain	Blanquillón de Boñar
128	Spain	Claro de Balazote
129	Spain	Entrelargo de Montijo
130	Spain	Farto cañifino
131	Spain	Rubio de Miajadas
132	Spain	Rubio de Montijo
133	Spain	Ruso
134	Spain	Recio de Cañete
135	Syria	1G-95812
136	Syria	1G-95841
137	Syria	1G-95847
138	Syria	1G-95931
139	Tunisia	Louri AP 5
140	Tunisia	Souri
141	Tunisia	Realforte
142	Tunisia	Biskri
143	Tunisia	Hamira
144	Turkey	Mindium
145	Turkey	BGE−018192
146	Turkey	BGE−018351
147	Turkey	BGE−018353
148	Turkey	BGE−018354
149	Turkey	BGE−019262
150	Turkey	BGE−019263
151	Turkey	BGE−019264
152	Turkey	BGE−019265
153	Turkey	B6E−019266
154	Turkey	BGE−019270
155		Amilcar
156		Ancalei
157		Arment
158		Astigi
159		Boabdil
160		Bolo
161		Claudio
162		Gallareta
163		Hispasano
164		Jupare
165		Meridiano
166		Ocotillo
167		Senadur
168		Simeto
169		Sula
170		Svevo
171		Vitron
172		Vitronero

[a] BGE-numbers are codes from the Centro de Recursos Fitogenéticos (I.N.I.A., Madrid), IG-numbers are codes from ICARDA Germplasm Bank. PI-numbers are codes from USDA Germplasm Bank

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

	2007	2008	2009
Sowing date	21 Nov 2006	20 Nov 2007	20 Nov 2008
Average monthly temperatures during growth cycle			
T_{min} (°C)	−6.9	−8.1	−5.9
T_{max}(°C)	33.2	34.8	36.7
T_{mean} (°C)	11.4	11.2	11.9
ET_0 daily mean (mm)	2.38	2.4	2.62
Water input from sowing to harvesting			
Rainfall (mm)	208	258	237
Irrigation (mm)	0	50	0
Soil characteristics			
Texture	Clay-loamy	Loamy	Sandy-clay-loamy
pH	8.1	8.3	8
P (mg kg^{-1})	25	27	117
K (mg kg^{-1})	163	118	590
Organic matter (%)	2.18	2.2	3.11
CE at 25°C (dS m^{-1})	0.42	0.29	0.34
Fertilization (kg ha^{-1})			
N (top dressing)	32	30	20
P_2O_5	110	128	68
K_2O	183	213	113
Harvest date	2 July 2007	2 July 2008	15 July 2009

to allow germination. Plots were mechanically harvested at ripening and yield was expressed at a 12% moisture level. Weeds and diseases were controlled according to standard cultural practices.

Quality analyses

A sample of about 250 g of grain was randomly drawn from each plot, cleaned and used for quality determinations. Grain protein content (%) was determined by a near infrared spectroscope (NIT, Infratec® 1,241-grain analyzer, Foss Tecator AB, Sweden) calibrated against the standard Kjeldahl method. Gluten strength was determined on 1 g of whole grain flour samples by the SDS (sodium dodecyl sulfate) sedimentation test, using the method of Axford et al. (1978) as modified by CIMMYT, using stoppered 25 ml graduated cylinders. The sedimentation index (SI) was calculated as the quotient between gluten strength and protein content, expressed as ml/protein unit. Yellow color index was estimated on whole grain flour by means of a reflectance colorimeter (CR-400, Konica-Minolta) equipped with a filter tri-stimulate system. Thousand kernel weight (TKW) was estimated as the mean weight of three sets of 100 grains per plot. These four quality traits were used to calculate the EU quality index (QI) for durum (European Commission Regulation No 2237/2003, 23 Dec, 2003), using the varieties Simeto, Gallareta and Vitron as reference checks. Each quality trait was expressed for each cultivar as a percentage of the mean value of the three quality checks (assumed to be 100%), and the QI was calculated by weighting each trait according to the following percentages: protein content (40%), gluten strength (30%), yellow index (20%), and TKW (10%). Test weight (TW) was determined by the GAC2100 Dickey-John equipment, and expressed as kg hl^{-1}. Grain filling rate (GFR) was obtained as the quotient between grain dry weight and grain filling duration—considered to be the period between anthesis and physiological maturity, and expressed as thermal time (growing degree-days, GDD). Thermal time was calculated from daily average temperatures, considering 37°C as the upper limit of temperature according to Angus et al. (1981).

Statistical analysis

Raw data were fitted to a linear mixed model with the check cultivars as fixed effects, and the row number, column number and variety as random effects (Littell et al. 1996). Variance components and best linear unbiased predictors (BLUPs) were estimated for the quality data of each cultivar each year by Restricted Maximum Likelihood (REML). These calculations were performed with the MIXED procedure of the SAS-STAT statistical package (SAS Institute Inc. 2009).

The geographic structure of the population was studied through a principal component analysis (PCA) conducted with yield and quality data, and performed on the correlation matrix calculated with the mean country data across years for landraces and the mean data across years for modern cultivars. Analyses of variance were used to assess the variation between the groups obtained in the PCA analysis. Group means were compared by the Student–Newman–Keuls (SNK) test at $P = 0.05$. Mean values across years of the quality traits for each cultivar were used to conduct a hierarchical cluster analysis by the Ward method of the JMP V.8 software (SAS Institute Inc. 2009).

Results

Geographic structure

The first two PC axes of the PCA shown in Fig. 2, accounted for ca. 62.5% of the total variance with an even distribution between both axes (axis 1, 34.0%; axis 2, 28.5%). The eigenvectors of the various components, represented in Fig. 2a, reflect the extent to which each variable weighs on the two components. Principal component 1 was related to QI and gluten strength and, to a lesser extent, to yellow index and protein content in the positive direction, and to the variables defining grain weight (TKW and TW) in the negative direction. Increases on principal component 2 were related to yield in the positive direction, and to protein content in the negative direction. The cosine of the angles between vectors indicated a strong positive correlation between QI, gluten strength and yellow index and, similarly, between TKW and TW. On the contrary, the angle between the vectors representing yield and protein content, at close to 180° indicated a negative correlation between the two variables.

Figure 2b shows the landscape of countries of origin and modern varieties based on yield and quality traits. This analysis clearly showed four distinct groups. Modern varieties were separated from the landraces based on yield performance, landraces being less productive. The landraces were classified into three groups with distinct quality patterns. Landraces from Cyprus, Turkey, Syria, Egypt, Lebanon, Jordan and Israel in the right part of the figure indicated that landraces from the eastern Mediterranean Basin had the best overall grain quality on the basis of their high gluten strength and yellow index, and for Israeli landraces, also high protein content. Landraces from the north-Balkan countries (Croatia, Montenegro, Macedonia, Bulgaria and Serbia) were located on the opposite side of Fig. 2b, revealing their high grain weight and low overall grain quality and gluten strength. Landraces from the western Mediterranean countries, both from the north (Italy, Spain, France, Portugal and Greece) and the south (Algeria, Morocco and Tunisia) plotted between the north-Balkan and eastern Mediterranean countries. Despite its geographic location in the Balkan Peninsula, Greece clustered with the western Mediterranean countries. The location of modern cultivars in the upper part of the figure indicated high grain yield, gluten strength and good overall quality.

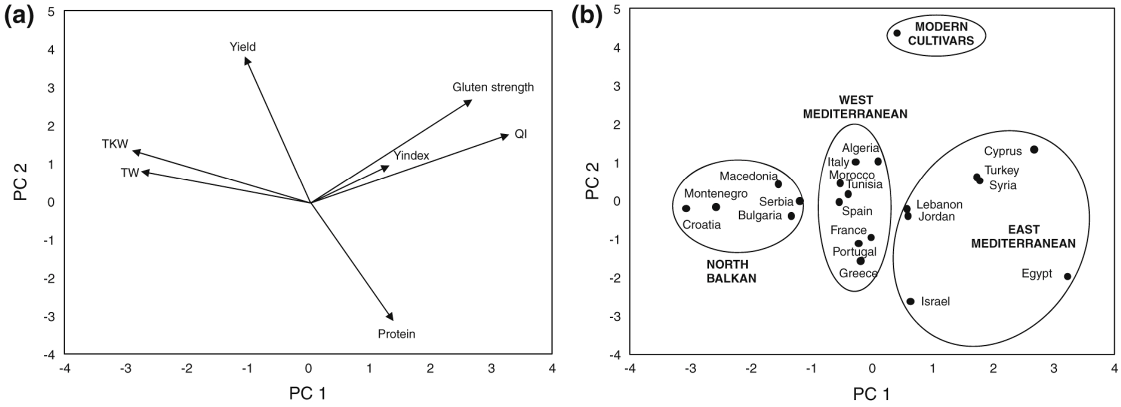


Fig. 2 Biplot of principal component analysis. **a** Eigenvalues of the correlation matrix symbolized as vectors representing yield and quality traits. *QI* quality index, *YI* yellow index, *TW* test weight, *TKW* thousand kernel weight. **b** Points representing the modern cultivars and the 20 countries of origin of the landraces are plotted on the plane determined by the first two axes

Variability between and within groups

Analyses of variance (ANOVAs) were conducted on all traits. The partitioning of cultivar effect according to the results of the PCA allowed quantification of the percentage of genotypic variance explained by differences between and differences within groups (Table 3). The results indicated low environmental, but a large genetic, effects on QI, gluten strength, grain filling rate and, to a lesser extent, TW and SI. On the other hand, protein content, TKW, grain yield and yellow index showed larger year than cultivar effects. The percentages of genotypic variation explained by differences between groups ranged between 12% (for yellow index) and 36% (for yield and protein content) and were significant for all traits. Differences within groups were not significant for yield. However, except for protein content, variability within some groups was greater than that observed between them (Table 3). North-Balkan and modern cultivars were much less variable in terms of quality and grain filling rate than the groups from the east and west of the Mediterranean Basin.

Mean values for each group are shown in Table 4. Yield, gluten strength, yellow index and SI of modern cultivars were significantly greater, and the protein content significantly lower, than those of landraces. High protein content was recorded in the eastern and western groups, which only differed in yield, TW, TKW and grain filling rate. The north Balkan group had the lowest quality, but a high grain filling capacity, which resulted in the heaviest grains (Table 4). Pearson correlation coefficients between TKW and grain filling rate were $r = 0.90$ ($P < 0.001$) for modern cultivars and $r = 0.92$, $P < 0.001$ for landraces.

Comparison of landrace means by countries within groups indicated significant differences for all studied

Table 3 Analyses of variance for yield, quality traits and grain filling rate (GFR) for the 172 cultivars. Cultivar effect is partitioned according to the groups identified by PCA

	Yield (kg ha^{-1})			QI (%)		Protein content (%)		Gluten strength (ml)	
	d.f.	SS (10^5)	%SS	SS	%SS	SS	%SS	SS	%SS
Year	2	523	58***	269	1***	1,726	79***	161	9***
Cultivar	171	170	19***	14,841	73***	277	13***	1,264	73***
Between groups	3	62	36***	2,819	19***	99	36***	302	24***
Within eastern	49	36	21 NS	4,151	28***	49	18***	359	28***
Within north Balkan	20	15	9 NS	1,164	8***	19	7*	84	7***
Within western	82	51	30 NS	6,314	42***	94	34***	503	40***
Within modern	17	7	4 NS	393	3 NS	16	6*	16	1 NS
Error	342	214	23	5,364	26	180	8	310	18
Total	515	907		20,474		2,183		1,735	

	Yellow index		TKW (g)		TW (kg hl^{-1})		SI (ml prot^{-1})		GFR (mg GDD^{-1})		
	SS	%SS	SS	%SS	SS	%SS	SS	%SS	d.f.	SS	%SS
Year	923	56***	37,053	67***	502	16***	3.62	36***	2	0.007	11 NS
Cultivar	587	36***	13,733	25***	1,701	55***	5.38	53***	171	0.038	60***
Between groups	69	12***	2,759	20***	294	17***	1.82	34***	3	0.010	26***
Within Eastern	186	32***	3,443	25***	834	49***	1.33	25***	49	0.010	26***
Within north Balkan	113	19***	636	5**	93	5*	0.35	7***	20	0.002	5*
Within western	184	31***	6,391	46***	401	24***	1.83	34***	82	0.015	39***
Within modern	35	6***	504	4**	79	5*	0.05	1 NS	17	0.001	3 NS
Error	132	8	4,764	8	875	29	1.16	11	340	0.018	29
Total	1,642		55,550		3,078		10.2		513	0.063	

QI quality index, *TKW* thousand kernel weight, *TW* test weight, *SI* sedimentation index

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

Table 4 Mean values and ranges of yield, quality traits and grain filling rate (GFR) for the four groups identified by PCA

	Yield (kg ha^{-1})		QI (%)		Protein content (%)		Gluten strength (ml)		Yellow index	
	Mean[a]	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Eastern	3,435^{c}	(3,083–3,715)	100^{ab}	(90–113)	16.6^{a}	(15.3–17.8)	8.7^{b}	(5.6–11.7)	15.3^{b}	(11.5–17.6)
North Balkan	3,570^{b}	(3,265–3,973)	93^{c}	(87–105)	16.1^{b}	(15.2–17.1)	7.2^{c}	(5.3–9.5)	14.6^{c}	(12.7–16.6)
Western	3,521^{b}	(3,157–3,780)	98^{b}	(87–108)	16.6^{a}	(15.1–18.3)	8.3^{b}	(5.4–11.6)	14.8^{bc}	(12.8–17.0)
Modern	3,824^{a}	(3,629–4,060)	102^{a}	(97–107)	15.2^{c}	(14.2–16.4)	10.3^{a}	(9.5–11.2)	15.9^{a}	(14.4–18.0)

	TKW (g)		TW (Kg hl^{-1})		SI (ml prot^{-1})		GFR (mg GDD^{-1})	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Eastern	44.6^{b}	(28.9–54.9)	77.4^{b}	(71.3–81.1)	0.53^{b}	(0.34–0.70)	0.069^{c}	(0.048–0.085)
North Balkan	50.7^{a}	(40.8–55.0)	78.8^{a}	(76.3–80.4)	0.46^{c}	(0.34–0.60)	0.080^{a}	(0.062–0.087)
Western	49.4^{a}	(35.8–64.2)	79^{a}	(73.2–82.1)	0.51^{b}	(0.31–0.71)	0.076^{b}	(0.049–0.093)
Modern	46.7^{b}	(41.2–52.0)	79.3^{a}	(75.2–80.9)	0.69^{a}	(0.63–0.75)	0.066^{c}	(0.058–0.076)

QI quality index, *TKW* thousand kernel weight, *TW* test weight, *SI* sedimentation index

[a] Values with different letters within column differ at $P = 0.05$

traits between countries of the Eastern Mediterranean Basin (Table 5). The greatest QI was recorded in Cypriot landraces, which also showed the best gluten strength and SI values. However, they had lower yellow indices than Jordanian landraces, which in turn showed the lowest gluten strengths of the Eastern group. Egyptian landraces had very low TKW and TW, probably due to their low grain filling rates. Differences between north-Balkan countries were significant only for yellow index, TKW and grain filling rate. The mean yellow index of landraces from Serbia, Macedonia and Bulgaria were significantly higher than those of landraces from Croatia and Montenegro. The heaviest grains belonged to Croatian landraces, but without differences from those of neighboring countries except Bulgaria, whose landraces had the lowest grain filling rate and TKW of the group. The western Mediterranean countries were less variable in terms of QI, protein content, TW and grain filling rate. Yellow index and TKW were the only traits for which differences between country means were significant within the three groups.

Potential sources of germplasm for improvement of grain quality attributes

A bi-dimensional cluster obtained from the mean quality data of each cultivar across years is shown in Fig. 3. The horizontal axis groups genotypes according to phenotypic similarity with regard to the quality traits considered in the analysis, arranged in the vertical cluster. Red, black and green colors in the figure indicate high, medium and low values, respectively, for the traits shown at the bottom of each column, the intensity of the color indicating the level of each attribute within each class. The two main branches of the horizontal cluster separate the cultivars for which mostly green color appears in the columns of gluten strength, SI, QI and SI (upper branch), from those with predominant red color in the same columns (lower branch). This suggests that the upper horizontal branch includes cultivars with poor overall quality, whereas, with the exception of entries 45, 21, 92, 19 and 22 (placed close to the bottom of the cluster), the lower branch mostly contains cultivars with good overall quality. All modern cultivars and 31 of the 50 landraces from countries of the eastern Mediterranean basin clustered in the lower branch, whereas 20 of the 21 landraces of the north-Balkan group and 48 of the 83 landraces of the western Mediterranean Basin clustered in the upper branch.

Whereas this clustering does not match the geographic structure resulting from PCA, it illustrates the phenotypic variability and similarity between genotypes on the basis of quality traits. Landraces from each country were in general unevenly distributed along the branches, but in some cases they tended to cluster together. This was the case for the Egyptian landraces, with 9 of the 11 genotypes located together in branch F of Fig. 3, and for the Turkish landraces with all six clustering in branch C. Similarly, the 5 Croatian entries clustered closely in branch B, together with other north-Balkan landraces characterized by high grain weight. Moreover, 5 of the 8 Jordanian entries clustered together in branch A, characterized by high yellow pigment and low gluten strength. The use of colors allows a fast visual identification of cultivars with the best quality, that is, red color in all columns.

Fourteen of the 18 modern cultivars clustered together in branch E, suggesting a high degree of similarities in grain quality among them. The green color of the column corresponding to protein content in this branch confirms the generally lower protein content within this group, whereas some variation existed for TKW, TW and yellow pigment. Cultivars Arment (No. 157), Svevo (170), Astigi (158) and Ocotillo (166), clustered apart from the rest. Svevo, among modern cultivars, had the best overall quality. Some landraces with very good quality clustered in branch D, such as the Spanish landraces Recio de Cañete (134), Recio de Almería (122), and Blancal (126), Tunisian landrace Biskri (142) and Italian cultivar Senatore Capelli (48).

Fig. 3 Bi-dimensional clustering showing the relationships ▶ between cultivars according to grain quality traits shown in the *bottom* part of the figure. Cultivars are indicated by their number and country of origin (Table 1; Fig. 1). *Red* and *green* colors in the columns denote high and low values, respectively, for the quality traits. The level of the trait is indicated by *color* intensity. *Black color* denotes intermediate values. Cultivar codes are colored according to the groups defined by PCA (Fig. 2), *red* for the eastern Mediterranean countries, *blue* for the north Balkan, *green* for the western Mediterranean countries and *black* for modern cultivars

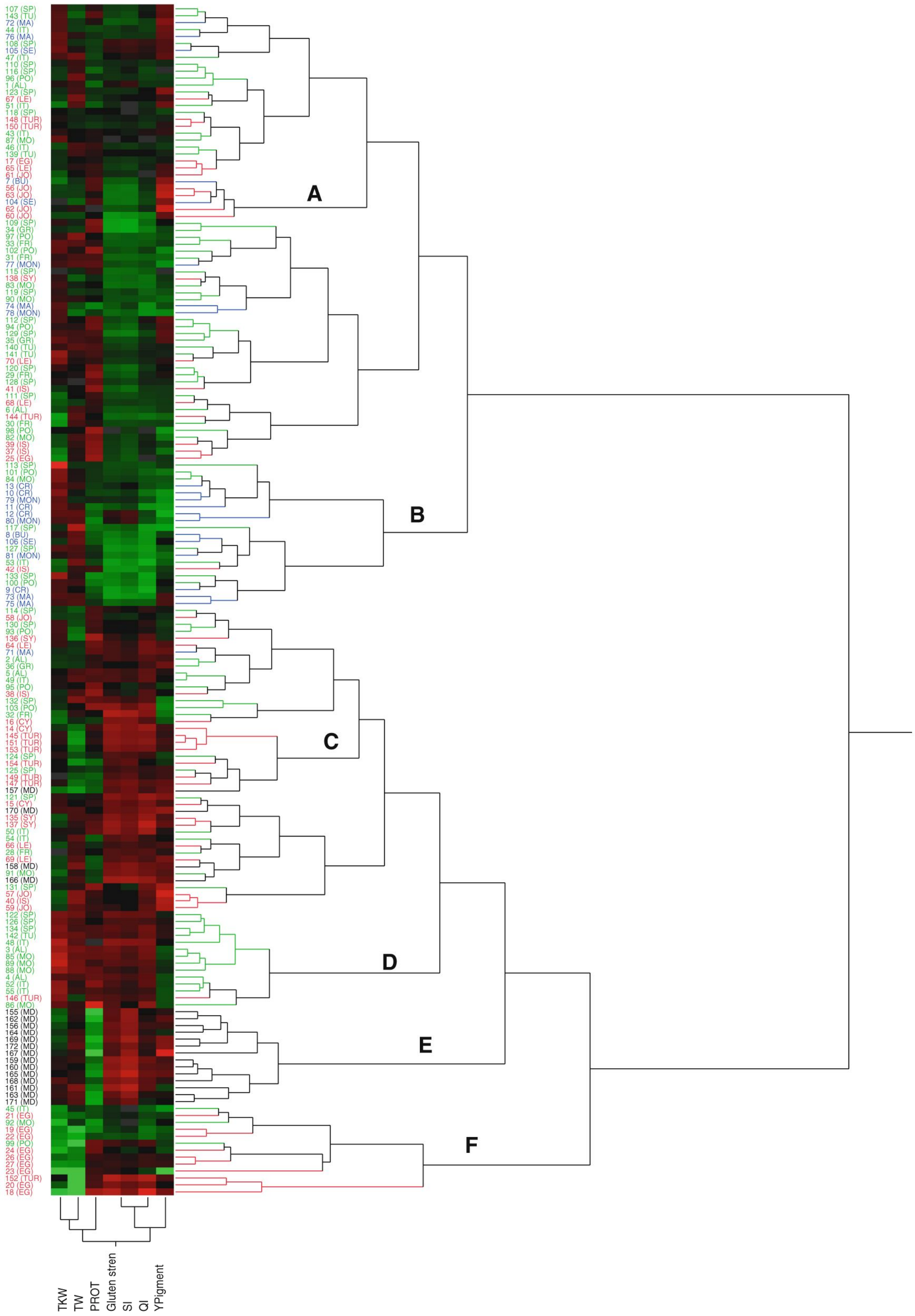
In order to help identify cultivars with the best quality attributes for use as donors for quality

Table 5 Mean values of yield, quality traits and grain filling rate (GFR) for the twenty countries of origin of landraces

Subpopulation	Country	Yield^{a} (kg ha^{-1})	QI (%)	Protein content (%)	Gluten strength (ml)	Yellow index	TKW (g)	TW (Kg hl^{-1})	SI (ml prot^{-1})	GFR (mg GDD^{-1})
Eastern	Cyprus	3,531^{a}	105^{a}	16.5^{ab}	10.60^{a}	15.1^{b}	45.8^{a}	77.6^{ab}	0.65^{a}	0.071^{ab}
	Egypt	3,357^{ab}	99^{b}	16.6^{ab}	8.71^{bc}	14.6^{b}	38.9^{b}	74.9^{c}	0.53^{b}	0.061^{c}
	Israel	3,272^{b}	98^{b}	17.2^{a}	7.52 ^{cd}	14.9^{b}	43.9^{a}	79.5^{a}	0.44^{c}	0.070^{ab}
	Jordan	3,518^{a}	98^{b}	16.6^{ab}	7.35^{d}	16.7^{a}	44.7^{a}	78.6^{ab}	0.45^{c}	0.066^{bc}
	Lebanon	3,409^{ab}	99^{b}	16.5^{ab}	8.52^{bcd}	15.6^{b}	46.8^{a}	79.1^{a}	0.53^{b}	0.070^{ab}
	Syria	3,571^{a}	103^{ab}	16.9^{ab}	9.37^{b}	15.3^{b}	47.2^{a}	77.9^{ab}	0.56^{b}	0.069^{b}
	Turkey	3,480^{a}	101^{ab}	16.3^{b}	9.59^{b}	15.2^{b}	48.1^{a}	76.6^{bc}	0.60^{ab}	0.077^{a}
North Balkan	Bulgaria	3,619^{a}	93^{a}	16.3^{a}	6.54^{a}	15.5^{a}	45.0^{b}	79.1^{a}	0.41^{a}	0.068^{b}
	Croatia	3,477^{a}	91^{a}	15.7^{a}	7.38^{a}	13.5^{b}	52.9^{a}	79.2^{a}	0.48^{a}	0.082^{a}
	Macedonia	3,603^{a}	94^{a}	16.2^{a}	7.13^{a}	15.8^{a}	51.2^{a}	78.6^{a}	0.45^{a}	0.083^{a}
	Montenegro	3,612^{a}	92^{a}	16.2^{a}	7.30^{a}	13.3^{b}	51.0^{a}	78.8^{a}	0.46^{a}	0.080^{a}
	Serbia	3,556^{a}	94^{a}	16.2^{a}	7.07^{a}	15.8^{a}	49.2^{a}	78.6^{a}	0.45^{a}	0.080^{a}
Western	Algeria	3,614^{a}	101^{a}	16.7^{a}	9.14^{a}	14.8^{abc}	49.2^{ab}	79.5^{a}	0.56^{a}	0.076^{a}
	France	3,411^{b}	98^{a}	16.7^{a}	8.23^{ab}	14.5^{bc}	46.4^{b}	79.5^{a}	0.50^{ab}	0.072^{a}
	Greece	3,404^{b}	96^{a}	16.9^{a}	7.12^{b}	15.5^{a}	48.3^{ab}	78.3^{a}	0.43^{b}	0.078^{a}
	Italy	3,602^{ab}	99^{a}	16.4^{a}	8.74^{a}	15.0^{abc}	48.7^{ab}	79.5^{a}	0.54^{a}	0.072^{a}
	Morocco	3,561^{ab}	99^{a}	16.7^{a}	8.62^{a}	14.5^{bc}	50.8^{ab}	79.3^{a}	0.53^{a}	0.076^{a}
	Portugal	3,451^{ab}	97^{a}	16.9^{a}	7.97^{ab}	14.2^{c}	48.5^{ab}	78.5^{a}	0.48^{ab}	0.076^{a}
	Spain	3,509^{ab}	97^{a}	16.5^{a}	8.03^{ab}	15.1^{abc}	50.0^{ab}	78.8^{a}	0.49^{ab}	0.077^{a}
	Tunisia	3,537^{ab}	99^{a}	16.9^{a}	8.26^{ab}	15.2^{ab}	51.8^{a}	79.2^{a}	0.50^{ab}	0.079^{a}

QI quality index, *TKW* thousand kernel weight, *TW* test weight, *SI* sedimentation index

[a] Values within each column and group with different letters differ at $P = 0.05$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improvement in breeding programs, entries within the upper 10th percentile for each trait are shown in Table 6. Except for yellow color index and TKW, the values of the best landraces did not differ from those of the best modern cultivars. The greatest QI was recorded in landrace No. 18 from Egypt, whose QI was 13% superior to the average quality index of the three modern cultivars used as quality checks. Landraces with high QI generally had high gluten strength, e.g. 135 and 137 from Syria, 151 and 152 from Turkey, Trinakria (50) from Italy and Vroulos (14) from Cyprus. Less common was the same landrace among the upper 10th percentile for both QI and protein content; the exception was Morocco (86). Only two landraces, No. 18 from Egypt and 103 (Lobeiro de Grao Escuro) from Portugal were among the upper 10th percentile for QI, protein content and gluten strength simultaneously. The Spanish landrace Raspinegro de Alcalá (121) was among the best for QI, gluten strength, yellow index and SI taken together. Among the modern cultivars, Svevo (170) and Ocotillo (166) had the greatest protein content. Even though differences were not statistically significant, the highest value for yellow index obtained among the landraces was 0.4 colorimeter points below that of Senadur (167), a modern cultivar with the highest yellow pigment content. The Israeli landrace Hati (40) was among the best entries for yellow index and TW simultaneously. Simeto and Svevo were among modern cultivars with heavier grains, but neither reached the grain weight of the Spanish landrace Enano de Andújar (113), whose grains weighed an average 64.2 mg grain^{-1} and had the highest grain filling rate.

Discussion

Principal component analysis grouped the collection into four clusters, having one including exclusively modern cultivars, and three landrace groups based on major geographical regions within the Mediterranean basin in the direction east to west (Figs. 1, 2). ANOVA showed that differences between the four groups were statistically significant for all the studied traits, validating the classification produced by PCA.

The ANOVA showed that the component of variation due to years was larger than the component due to cultivar for protein content, TKW, yield and yellow index, suggesting a large environmental influence on these traits. These results are in agreement with those of previous studies conducted in similar environments reporting high environmental effects on grain protein content in durum wheat (Mariani et al. 1995; Ames et al. 1999; Koç et al. 2000; Rharrabti et al. 2001, 2003; De Vita et al. 2007; Taghouti et al. 2010). However, it is generally accepted that genotypic effects are more important than environmental effects for yellow color index. Our differing results may be partially explained by the strong environmental effect on TKW, which could also affect the ratio of endosperm to bran and, consequently the yellowness of the wholemeal samples. Moreover, the small range of yellow color index values between groups could also contribute in explaining the results of the ANOVA. In contrast, QI and gluten strength results showed that the percentage of total variance explained by the genotypic effect was 73%, much higher than the fraction explained by the year effect, suggesting a greater genetic than environmental control for these two traits. The very large genetic effect of QI justifies its use by the EU for quality classification of durum varieties. In addition, the component of variation due to cultivar effect was also greater than the year effect for grain filling rate, test weight and SI, suggesting less environmental impact on these traits than on protein content, yield or yellow index. These results confirm the predominant genetic control of gluten strength and test weight recently reported by Taghouti et al. (2010) for Moroccan durum cultivars. The percentage of total variance explained by differences between groups was higher for SI (18%), gluten strength (17%), grain filling rate (16%), and QI (14%), than for test weight (9%), grain yield (7%), TKW (5%), protein content (5%) and yellow index (4%), suggesting that the classification in groups was more useful in explaining phenotypic variability in traits with high genetic control than for those largely affected by environment.

Eastern Mediterranean landraces collected in the area closest to the origin of tetraploid wheat (Feldman 2001), had the best overall quality of the three regions, but were generally characterized by lighter grains, probably as a consequence of their low grain filling rate. According to Feldman (2001) and MacKey (2005), the spread of durum occurred across the Mediterranean Basin from the Fertile Crescent occurred via Turkey (8,500 B.P.), the Balkan Peninsula, Greece and Italy (8,000 B.P.), and from there to

Table 6 Mean values of cultivars in the upper 10th percentile for yield, quality traits and grain filling rate (GFR)

Yield		QI		Protein		Gluten strength	
Entry No.	Value (kg ha^{-1})	Entry No.	Value (%)	Entry No.	Value (%)	Entry No.	Value (ml)
Landraces							
8	3,974	18	113	86	18.3	18	11.7
77	3,788	137	110	18	17.8	152	11.7
73	3,781	152	109	136	17.7	32	11.6
54	3,780	50	108	98	17.7	137	11.1
3	3,779	14	107	25	17.6	14	11.0
50	3,746	135	107	102	17.6	50	10.9
133	3,740	121	107	38	17.6	151	10.8
116	3,734	151	106	103	17.5	103	10.8
5	3,733	86	106	41	17.5	48	10.8
43	3,718	103	106	131	17.5	145	10.8
80	3,717	32	106	37	17.5	135	10.7
62	3,715	145	106	29	17.5	121	10.7
85	3,709	48	106	39	17.4	153	10.6
Modern							
161	4,060	166	107	170	16.4	166	11.2
165	3,956	170	106	166	15.9	161	11.2

Yellow index		TKW		TW		SI		GFR	
Entry No.	Value	Entry No.	Value (g)	Entry No.	Value (Kg hl^{-1})	Entry No.	Value (ml prot^{-1})	Entry No.	Value (mg GDD^{-1})
Landraces									
57	17.6^{ab}	113	64.2^{a}	117	82.1	32	0.71	113	0.093
62	17.5^{ab}	89	59.3^{ab}	97	81.1	152	0.70	89	0.091
63	17.4^{ab}	85	58.4^{ab}	40	81.1	48	0.67	55	0.090
56	17.2^{ab}	48	58.2^{ab}	57	81.0	14	0.67	48	0.088
40	17.1^{ab}	88	56.9^{ab}	132	80.9	91	0.67	85	0.087
59	17.1^{ab}	133	56.8^{ab}	67	80.8	16	0.66	72	0.087
131	17.0^{ab}	3	56.7^{ab}	3	80.8	151	0.66	133	0.087
7	16.6^{b}	141	56.3^{ab}	88	80.8	145	0.66	105	0.087
123	16.6^{b}	55	55.9^{ab}	89	80.7	18	0.66	12	0.087
72	16.5^{b}	52	55.7^{ab}	96	80.7	137	0.65	88	0.087
121	16.5^{b}	10	55.0^{ab}	53	80.6	147	0.65	78	0.086
104	16.5^{b}	134	54.9^{ab}	47	80.6	153	0.65	35	0.086
69	16.4^{b}	146	54.9^{ab}	122	80.6	121	0.65	10	0.085
Modern									
167	18.0^{a}	168	52.0^{b}	161	80.9	165	0.75	170	0.076
170	16.8^{ab}	170	50.9^{b}	158	80.8	161	0.74	168	0.072

QI quality index, *TKW* thousand kernel weight, *TW* test weight, *SI* sedimentation index

[a] Only significant differences ($P = 0.05$) between cultivars within each column are indicated

North Africa and the Iberian Peninsula (7,000 B.P.). Our results show that eastern and western Mediterranean groups are very similar for the majority of quality traits, since they differ only in grain weight and grain filling rate. Larger differences in gluten strength among eastern and western groups would be expected given the likely effects of the growing environment and end-use on the allelic composition of cultivars (Stachel et al. 2000; Li et al. 2000), and the different allelic frequencies of glutenin loci reported for landraces of south-west Asia and south-west Europe (Moragues et al. 2006). The heaviest grains of the landraces from the western Mediterranean countries may be interpreted as a consequence of diversification that occurred in situ during the expansion of durum from the east to the west across the Mediterranean Basin.

Landraces from the north Balkan group were very different in their quality traits when compared with landraces from eastern and western Mediterranean countries and Greek landraces, suggesting that they could have a different origin. This possibility is supported by two lines of evidence reporting that wheats from Bulgaria and the former Yugoslavia may have their origin in the steppes of south Russia and the Volga region. One such line of evidence consists of similarities between the gliadin compositions of Bulgarian durum landraces and Russian and Ukrainian durums (Melnikova et al. 2010), and explained in ethnic terms by population migrations. The other, comes from the findings of Dedkova et al. (2009), who demonstrated that T. *dicoccum* accessions from former Yugoslavia, Bulgaria and Russia do not carry the 7A:6B translocation that is common in the *dicoccum* accessions from western Mediterranean countries; they proposed a division of European T. *dicoccum* into two groups: West European and Volga-Balkan. Our hypothesis that the north-Balkan group could have a different origin than the other three groups would also explain the clustering of Greek landraces with those from the western Mediterranean countries, matching with the accepted expansion pattern of durum wheat within the Mediterranean Basin (MacKey 2005; Moragues et al. 2007).

The second axis of the PCA, accounting for 28.5% of the total variance, separated modern cultivars from landraces based on greater yield and lower grain protein content. Although the experiments were conducted under relatively dry conditions to provide appropriate growing environments for the landraces (thus avoiding lodging of tall genotypes) modern cultivars out-yielded the landraces by an average 9%, which was expected at the yield levels observed in this study (3–4 t/ha). Our results show a boost of durum wheat yield in Mediterranean countries resulting from the use of improved varieties, in agreement with previous studies (Koç et al. 2000; De Vita et al. 2007; Royo et al. 2008). This yield increase was accompanied by a significant decrease in grain protein content, agreeing with Dotlačil et al. (2010). The known negative relationship between yield and protein content (Rharrabti et al. 2001) is associated with dilution of nitrogen compounds when carbohydrate deposition increases through photosynthesis (Lawlor 2002; Martre et al. 2003). However, all groups, including those with the lowest average protein contents, were characterized by levels in excess of the minimum limits required by industry.

The mean grain filling rate of modern cultivars was 12% lower than that of landraces overall, and significantly lower than that of landraces from the western Mediterranean Basin. A previous study comparing the maximum grain filling rate of landraces and modern varieties from Italy and Spain (Álvaro et al. 2008) concluded that breeding activities had not modified this trait. Disagreement between the results may be due to the fact that the study of Álvaro et al. compared maximum grain filling rates, resulting in values of around 0.22 mg GDD^{-1}, whereas in the current study comparisons were made between mean grain filling rates, which gave much lower values (about 0.073 mg GDD^{-1}). Our results show that the lighter grains of modern durum cultivars when compared with landraces from western Mediterranean countries, as reported in previous studies (Royo et al. 2007), was strongly associated with lower grain filling rates.

Landraces from the north Balkan and the western Mediterranean countries out-yielded landraces from the eastern Mediterranean, but variability for yield within groups was very low. Nevertheless, the ANOVA's for quality traits indicated that the percentage of genotypic variability explained by differences within groups was much higher for landraces than for modern cultivars, exceeding in some cases the variability explained by differences between groups. Among the landraces the most uniform group in terms of quality was the north-Balkan, probably due to greater climatic and geographic uniformity within the region and/or the low number of landraces from the region included in the study (21) when compared with the eastern (50 landraces) or western (83 landraces) groups. The eastern Mediterranean group conserved the greatest variability of all, consistent with this region being the geographic center of genetic variation for wheat (Skovmand et al. 2005). The comparison of country means indicated that the best overall quality was reached by Cypriot landraces given their high QI, gluten strength and SI.

The bi-dimensional cluster grouped all entries according to quality traits, and was useful in detecting phenotypic similarities among cultivars, countries and regions. Given that yield was included within the variables used to perform the PCA, but was removed from the bi-dimensional cluster analysis both classifications do not completely match. The bi-dimensional cluster had two main branches, one of them including cultivars with higher overall quality, gluten strength and SI, and the other comprising cultivars with corresponding low values for the same traits. Modern cultivars were the only group with all entries located in the same branch, because of the general homogeneity of their quality attributes, in spite of the significant variability existing among them for TKW, test weight and yellow index. Within this group, four of 18 modern cultivars clustered apart from the rest. The Italian variety Svevo (170) and the desert durum cultivar Ocotillo (166), reached the greatest quality standards. Both cultivars had the highest protein content among modern cultivars, but Ocotillo also had strong gluten and Svevo had a very high yellow color index and high grain weight. Previous studies reported the high gluten quality of modern cultivars when compared with old varieties (Motzo et al. 2004).

On the other hand, 95% of the north Balkan landraces were placed in the branch associated with low quality, showing weak gluten and low QI and SI values. The large variability existing within eastern and western groups for quality traits, revealed by ANOVA, was graphically validated by the distribution of entries from both groups along the two main branches of the bi-dimensional cluster. Only 42% of landraces from the western Mediterranean countries clustered in the branch related to good quality, whereas for the eastern Mediterranean countries the corresponding percentage was higher (62%). These results confirm the superior mean quality of landraces closest to the centre of origin of the species, but also their large diversity in terms of quality traits. Despite the variability existing within Egyptian landraces for QI, gluten strength and SI, 9 of 11 landraces from this country clustered together due to the very low grain weight characterizing all of them. However, Egyptian landrace No. 18 reached the highest QI and gluten strength and very high protein content. The Italian cultivar Senatore Capelli (48) identified in this study as one of the cultivars with the highest grain quality, clustered close to some other Italian cultivars such as Razza 208 (52), Razza 96 (55), Trinakria (50), Capeiti (54) and the modern cultivar Simeto (168), confirming the close genetic relationship between these cultivars reported in previous studies (Martos et al. 2005), and in agreement with the extensive use of Senatore Capelli as a quality donor in Italian breeding programs (Boggini et al. 1997).

Concluding remarks

The results of this study underline the substantial variability in grain quality attributes among Mediterranean durum germplasm, displaying trends followed by breeding in the region in terms of productivity- and quality-related traits, and allowing the identification of wheat landraces useful for breeding purposes.

The largest variability for quality traits was recorded in landraces from the eastern Mediterranean basin in agreement with its proximity to the Fertile Crescent where the domestication of wheat initiated (Harlan 1981). A second very diverse group was that of the landraces from the western Mediterranean basin, whose largest difference from the eastern group was related to grain weight. North-Balkan and modern groups were the most uniform in terms of quality, and this may be explained by the different geographic origins in the case of the first, and by the impact of market-related breeding pressure in the case of modern cultivars. As in previous studies (Motzo et al. 2004; De Vita et al. 2007; Royo et al. 2007) our work indicated that modern cultivars had greater yields and lower protein contents than landraces, but with higher protein quality as demonstrated by their improved gluten strength.

Our results identified landraces with improved quality traits for use in durum breeding programs, particularly for gluten strength and grain weight. Further studies identifying genes and QTLs associated to these traits will make their introgression to productive genetic backgrounds easier and more effective.

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CAPÍTULO 2

Known and novel variability in glutenin subunit composition of Mediterranean landraces and modern durum wheat cultivars and its relationship with gluten strength

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Abstract

The allelic composition at five glutenin loci (*Glu-A1*, *Glu-B1*, *Glu-A3*, *Glu-B3*, and *Glu-B2*) was assessed by one-dimensional SDS-PAGE on a set of 155 durum wheat landraces from 21 Mediterranean countries and 18 modern varieties. Gluten strength in durum wheat, the main factor determining pasta cooking properties, is determined primarily by the overall glutenin subunit composition. Gluten strength was determined by SDS-sedimentation on whole meal from grain samples of field experiments conducted during three years in northeastern Spain. One hundred and fourteen alleles, or banding patterns, were identified (25 at *Glu-1* and 89 at *Glu-2*/*Glu-3* loci); 85.1% of the banding patterns found in landraces were considered as rare (frequency <0.05), and 84.5% of them had not been reported before. Global genetic diversity index was 0.71 for landraces and 0.38 for modern varieties. All modern varieties exhibited medium to strong gluten type, and none of the 13 banding patterns identified in this group had a significant effect on strength, while ten banding patterns had significant effect on the gluten strength of the landraces. Alleles *Glu-B1e* (band 20), *Glu-A3a* (band 6) and *Glu-A3d* (bands 6+11), *Glu-B3a* (bands 2+4+15+19) and *Glu-B2a* (band 12) significantly increased the SDS-value of the landraces. On the contrary, *Glu-A3b* (band 5) and *Glu-B2b* (null allele) had a negative effect on gluten strength. Only three, among the rare banding patterns, affected significantly gluten strength. The presence of *Glu-B1a* (band 7) at *Glu-B1* locus, found in 6 landraces, affected negatively gluten strength, while banding patterns 2+4+14+15+18 (present in one Egyptian landrace) and 2+4+15+18+19 (present in two landraces from France and Portugal) at *Glu-B3* had a positive effect on it. The frequency of landraces with outstanding gluten strength was much greater in the eastern than in the western Mediterranean countries. The geographical pattern displayed from the frequencies of *Glu-A1c* is discussed.

1. Introduction

Historically the Mediterranean Basin is the most important production area of durum wheat (*Triticum turgidum* L. var. *durum*), the most significant durum import market, and the largest consumer of products derived from this cereal grain (Royo *et al.*, 2009). Many traditional Mediterranean foods are manufactured from durum wheat. Pasta is the most common durum end product in southern Europe, North Africa as well as in non-Mediterranean regions such as North America and the former Soviet Union. Durum wheat is extensively consumed as couscous in North Africa and flat bread and bulgur are part of the staple diet in Eastern-Mediterranean countries (Elias and Manthey, 2005). Durum wheat is usually cultivated under rainfed conditions in the Mediterranean Basin, which often causes a number of environmental stresses to this crop. Terminal drought stress, a combination of water scarcity and warm temperatures during the grain filling period usually results in yield reductions, but, in most cases allows for the production of high grain quality.

Archaeological evidences show that the earliest domesticated wheats came from the Near East, in the region known as the Fertile Crescent. Dated to approximately 10,000 years BP, they spread to the west of the Mediterranean Basin, reaching the Iberian Peninsula around 7,000 years BP (Feldman, 2001; MacKey, 2005). This migration process and both natural and human selection resulted in the establishment of local landraces along the Mediterranean Basin well, but specifically, adapted to diverse agro-ecological zones. These unimproved landraces, which contain the largest genetic diversity within the species, practically disappeared from farmers' fields as consequence of the introduction of the more productive and homogeneous semi-dwarf varieties released since the Green Revolution. The extreme homogeneity in cultivar structures typical of the modern agriculture caused a dramatic loss of natural variation from the middle of the 20th century in southern Europe and during the 70s-80s in Northern Africa, which resulted in genetic erosion and possibly an increase in genetic vulnerability of wheat crops. Landraces, mostly conserved in germplasm repositories, can be considered as likely sources of putatively lost variability and may provide new favorable genes/alleles to be introgressed into modern cultivars.

Some studies have shown the existence of variability for quality traits in durum wheat landraces (Aguiriano *et al.*, 2008; Moragues *et al.*, 2006; Newton *et al.*, 2010), as well as is related tetraploid species (Sissons and Batey, 2003). The ability to tap into this diversity depends on the identification of accessions containing genes and alleles demonstrated to be useful in breeding programs for quality improvement. Gluten strength is one of the main factors influencing durum wheat quality. It is commonly determined by the SDS-sedimentation test and depends on the composition of the storage proteins. They are comprised of gliadins (monomeric proteins) and glutenins (polymeric proteins), the major components of gluten with the latter reported to be the most influential on gluten strength. Based on their mobility in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), single polypeptides from the glutenin fraction are separated after di-sulfide bond reduction, into high molecular weight- (HMW-GS) and low molecular weight (LMW-GS) subunits (Lindsay and Skerritt, 1999; Veraverbeke and Delcour, 2002). The HMW-GS are encoded by the complex at the *Glu-1* loci (*Glu-A1* and *Glu-B1*), located on the long arm of group 1 homologous chromosomes (Shewry et at., 1992; Singh and Sheperd, 1988). LMW-GS are encoded by genes at *Glu-A3*, *Glu-B3* and *Glu-B2* loci (Ruiz and Carrillo, 1995; Vázquez *et al.*, 1996).

The allelic polymorphism controlling the HMW-GS and the LMW-GS has been used in diversity studies and cultivar identification (Edwards *et al.*, 2007; Ribeiro *et al.*, 2011). However, the difficulty in scoring alleles of the LMW-GS has limited their use in research (Lerner *et al.*, 2009) and hindered the selection for specific LMW-GS alleles in wheat breeding programs (Zhang *et al.*, 2004). Recent improvements in uni-dimensional SDS-PAGE separation protocols implemented at CIMMYT (International Maize and Wheat Improvement Center) have allowed to better distinguish LMW-GS alleles and opened the possibility of effectively using them in diversity studies (Liu *et al.*, 2010; Peña *et al.*, 2004).

The present study examined the SDS-PAGE glutenin protein banding patterns in a set of 155 Mediterranean durum wheat landraces from 21 countries and a set of 18 representative modern varieties, with the main purpose of determining the diversity of banding patterns as related to known allelic variability for HMW-GS and LMW-GS. The relationship between glutenin composition and gluten strength and the possible existence of a geographic structure in the collection, based on both glutenin composition and gluten strength, were also investigated.

2. Material and methods

2.1. Plant material

The study was conducted on a collection of 155 durum wheat landraces from 21 Mediterranean countries including the major durum producers/users (Table 1) and 18 representative modern cultivars. Seeds provided by gene banks were increased in bulk and purified by eliminating off types. Landraces were selected ─from a larger collection of 231 accessions─ on the basis of their genetic variability determined by 33 SSR-markers, in order to represent the genetic diversity of ancient local durum populations from the Mediterranean Basin (Nazco *et al.*, 2012). The modern set included Spanish, Italian, and French cultivars, as well as one U.S. desert durum cultivar. Several of these, particularly those from Spain, are derived from CIMMYT and have been released as major cultivars also in North Africa and elsewhere around the Mediterranean Basin. The landraces received from the Germplasm Banks in 2005 were bulk purified selecting only the dominant type (usually with a frequency above 80% on the bulk) and increased in 2006. Seed used for the field experiments from 2007 to 2009 came from purified bulks grown during each previous year. A spike of the dominant type was selected in 2006 and grown as a head-row. From this purified material, 10 seeds were taken for the electrophoretic analysis.

Table 1. Country of origin of the 155 landraces included in the study and number of locus-specific banding pattern detected by SDS-PAGE at the HMW- and LMW-glutenin loci.

Country of origin	Number of entries
Algeria	6
Bulgaria	2
Croatia	5
Cyprus	3
Egypt	11
France	6
Greece	3
Israel	6
Italy	13
Jordan	8
Lebanon	7
Libya	1
Macedonia	6
Montenegro	5
Morocco	11
Portugal	11
Serbia	3
Spain	28
Syria	4
Tunisia	5
Turkey	11

Loci	Landraces	Modern
HMW glutenin:		
Glu-A1	5	1
Glu-B1	20	4
LMW glutenin:		
Glu-A3	15	3
Glu-B3	72	3
Glu-B2	2	2

Grain samples were collected from field trials established under rainfed conditions during three years at Gimenells (41°40 'N latitude, 0°20' E longitude, and 200 m of altitude a.s.l.) in the Lleida province (north-eastern Spain). Experiments consisted of non-replicated entries sown in plots of 6 m^2 comprising 5 m rows, spaced 0.15 m apart, and arranged in a modified augmented design with three replicated checks (cultivars Claudio, Simeto and Vitron). Common seed source (from increase plots) was used in all field trials. Sowing density was adjusted to 250 viable seeds m^{-2} and plots were kept free of weeds and diseases according to standard cultural practices. A sample of grain, mechanically harvested at full maturity, was randomly drawn from each plot, cleaned and used for gluten strength determination.

2.2. HMW-GS and LMW-GS allelic composition

Electrophoresis was run on a bulk of ten seeds from a purified head-row of the dominant type. Electrophoretic analysis (1D SDS-PAGE) was conducted following the protocols implemented at CIMMYT by Peña *et al.* (2004), following the nomenclature of Nieto-Taladriz *et al.* (1997) and Martinez *et al.* (2004), to identify high and low molecular weight glutenin subunit composition at 5 loci (*Glu-A1*, *Glu-B1*, *Glu-A3*, *Glu-B3*, and *Glu-B2*). The banding patterns/alleles with a frequency below 0.05 were classified as rare (Russell *et al.*, 2000).

2.3. Gluten strength determination

Gluten strength was determined on a sample of 1 g of whole wheat flour using the SDS- (sodium dodecyl sulfate) sedimentation test, of Axford *et al.* (1978), as modified by Peña *et al.* (1990).

2.4. Statistical analysis

SDS-sedimentation data were fitted to a linear mixed model with the check cultivars as fixed effects, and the row number, column number and genotype as random effects (Littell *et al.*, 1996). Restricted Maximum Likelihood (REML) was used to estimate the variance components and to produce the best linear unbiased predictors (BLUPs) for data of each cultivar/year combination. The calculations were done through the MIXED procedure of the SAS-STAT statistical package (SAS Institute Inc. 2000), which was used for all the analyses. A standard analysis of variance (ANOVA) was conducted with the BLUPs of gluten strength data. Genotypes were classified according to the mean value of SDS-sedimentation across experiments in outstanding ($SDS \geq 11$), very high ($10 < SDS < 11$), high ($9 \leq SDS \leq 10$), medium ($7 \leq SDS < 9$) and low ($SDS < 7$) gluten-strength groups and the genotype effect was partitioned in the ANOVA according to this classification. Means were compared by the Student-Newman-Keuls (SNK) test at $P=0.05$. Genetic diversity was calculated with the D index (Weir, 1996), according to the following expression:

$$D_j = 1 - \Sigma\, p^2_{ij}$$

where p is the frequency of the ith allele at locus jth. The allelic effect on gluten strength was computed as the difference between the mean SDS-sedimentation values across years of genotypes carrying or not-carrying a given allele/banding pattern. The allelic frequency of the allele *Glu-A1c* (null) for each country was used to conduct hierarchical cluster analyses by the Ward method of the JMP V.8 software (SAS Institute Inc. 2000).

3. Results

One-hundred and fourteen banding patterns, potentially *Glu-1*/*Glu-3* allele-specific, were identified by SDS-PAGE (bottom part of Table 1), 97 of which appeared only in the landraces with frequencies below 0.05, thus being considered herein as corresponding to rare patterns/alleles. All the patterns detected in modern cultivars, which are considered allele-specific according to the *Glu-1*/*Glu-2*/*Glu-3* nomenclature used, were also present in the landraces. For the two loci encoding for HMW-GS, 25 alleles/banding patterns were identified in landraces and only 5 of them were detected in the modern varieties. For the 3 loci encoding the LMW-GS the difference in variability was even higher between the 2 groups, with landraces exhibiting some 89 potentially allele-specific banding patterns with only 8 of them preserved in the modern cultivars. *Glu-B3*, encoding for *LMW-GS*, was the locus showing the largest number of patterns in landraces, while *Glu-B1* (HMW-GS) was the most variable locus in the modern cultivars (bottom part of Table 1).

The allelic/banding pattern frequencies at each presumed locus are shown in Table 2. Only 25.4% of the banding patterns detected have been previously described. The most frequent allele in the collection was the null allele at *Glu-A1*, which was found in 61.94% of the landraces and in all modern varieties, the latter group being monomorphic at this locus. A banding pattern tentatively designated as *2*** (Branlard *et al.*, 1989), was detected in the area corresponding to the size of the subunits expected at this locus. At the *Glu-B1* locus, the second one coding for HMW-GS, modern cultivars exhibited the 3 most frequent banding patterns known to be present in germplasm groups worldwide, namely, 7+8, 6+8 and 20 (Aguiriano *et al.*, 2008; Branlard *et al.*, 1989; Carrillo *et al.*, 1990; Peña *et al.*, 1994), while as many as twenty banding patterns could be distinguished in the landrace group in the mobility area corresponding to these HMW-GS. Subunit 20 was the most common in the landraces, with a frequency of 36.77%, while 7+8 was the most common in modern varieties, with a frequency of 55.55% (Table 2). At *Glu-A3*, of the 15 patterns identified overall, the most frequent was allele *Glu-A3a* (band 6), which was present in 29.68% of the landraces and 66.67% of the modern varieties. The second most prevalent allele in the modern varieties was *Glu-A3d* (bands 6+11) (27.78%), and the null allele in the landraces (19.35%), the latter being absent in modern cultivars. The two alleles reported and described elsewhere at *Glu-B2 (Glu-B2b* or null *and Glu-B2a* or band 12) were observed at high frequencies in both sets of germplasm, with the null allele being the most frequent in the landraces (59%), while *Glu-B2a* found at twice the frequency of the alternative allele in modern varieties (66.67%). At the most variable locus overall, *Glu-B3*, 72 banding pattern/putative alleles were found in landraces but only 3 of them were present in modern varieties and 55 of them were recorded only in a single landrace (Table 2). The most frequent banding pattern at this locus was 2+4+15+19 (*Glu-B3a*), which was present in 77.78 % of the modern varieties and in the 32.9% of landraces. Other patterns with relatively high frequency in the landraces were 2+4+15+18 and 14+17, with a frequency of 7.10% and 5.16%, respectively, and in modern varieties 4+15+19, with a frequency of 16.67% (Table 2). The allele 3+15+19 was only found in the modern French cultivar Arment.

The overall genetic diversity index (D) for landraces was 0.71, significantly higher ($P < 0.035$) than the same index for modern varieties (0.38) (Table 3). The overall genetic diversity was numerically highest in landraces from Spain (0.68), Portugal (0.67), Egypt (0.62) and Croatia (0.61), and lowest in accessions from Algeria and Bulgaria (0.30), Turkey (0.33), France (0.34) and Tunisia (0.35) (Table 3). However, all these values were not statistically different from each other. For the HMW-G subunits locus *Glu-A1,* the highest diversity indices were observed in landraces from Egypt, Portugal and Spain, while landraces from Portugal, Spain, Algeria, Cyprus and Greece had the highest diversity indices for *Glu-B1*. The largest diversity were found in landraces from Spain, Portugal, Macedonia and Croatia at *Glu-A3*, from Spain, Egypt, Macedonia and Lebanon at *Glu-B3* and from Lebanon, Croatia, Tunisia, and Jordan for *Glu-B2* (Table 3).

The ANOVA for gluten strength showed that both year and genotype effect were statistically significant, accounting for 9% and 73% respectively, of total variation (Table 4). The partitioning of the genotype effect into its individual components revealed that differences between groups of accessions, based on their SDS-sedimentation values, accounted for 91.5% of the genotypic variability, which corresponded to 67% of total variation, while differences within each sedimentation group were not statistically significant (Table 4).

The effect on gluten strength of each allele/banding pattern was computed, for landraces and modern varieties separately, as the difference between the mean SDS-sedimentation test value of the accessions carrying and not-carrying the allele/pattern (Table 5). Allelic/banding pattern effect was not significant among the modern cultivars group, with all members showing high to outstanding SDS values. On the other hand, significant main effects of some alleles/banding patterns were detected within the landraces. At the HMW-GS loci, the only allele with a significant effect was *Glu-B1e* (band 20), which increased by 0.50 ml the SDS-sedimentation test value of the landraces carrying it. Four alleles/banding patterns at LMW-GS loci increased significantly the gluten strength of landraces, namely bands 6 (allele *Glu-A3a*) and 6+11 (*Glu-A3d*) at *Glu-A3*, banding pattern 2+4+15+19 (*Glu-B3a*) at *Glu-B3*, and allele 12 (*Glu-B2a*) at *Glu-B2*, while two alleles, namely band 5 (*Glu-A3b*) at *Glu-A3* and the null allele (*Glu-B2b*) at *Glu-B2*, had negative effects on it (Table 5). Only three among the rare banding patterns found in the landraces had a significant effect on gluten strength (data not shown). Putative allele 7 at *Glu-B1*, present in 6 (three Montenegrin, one Croatian and two Spanish) landraces (3.87%), reduced the SDS-sedimentation value by 1.75 ml (*P* <0.01) on average. Banding patterns 2+4+14+15+18 (present in the Egyptian landrace PI-366109) and 2+4+15+18+19 (present in two landraces, Trigo Glutinoso from France and Lobeiro de Grao Escuro from Portugal) at *Glu-B3* increased SDS values by 3.44 ml (*P* <0.05) and 2.96 ml (*P* <0.01), respectively.

Table 2. Allelic/banding pattern frequencies (%) at the HMW and LMW-glutenin subunits loci for landraces and modern varieties.

Locus	Allelic/banding pattern	Landraces	Modern	Locus	Banding pattern	Landraces	Modern	Locus	Banding pattern	Landraces	Modern
Glu-A1	**1**	21.94	-	*Glu-B3*	1+13+14+17	0.65	-	*Glu-B3*	**2+4+15+17+19**	0.65	-
	1 '	0.65	-		1+14+17	0.65	-	(Cont.)	2+4+15+18	7.10	-
	2**	0.65	-		1+15+17	0.65	-		2+4+15+18+19	1.29	-
	2*	14.84	-		1+15+17+18	0.65	-		**2+4+15+19**	32.90	77.78
	null	61.94	100		1+3+13+14+17	0.65	-		2+4+16+17	1.94	-
Glu-B1	**7**	3.87	-		1+3+13+16	0.65	-		2+4+16+17+18	0.65	-
	14	0.65	-		1+3+14+15+17	0.65	-		2+4+16+19	1.29	-
	20	36.77	22.22		1+3+14+15+18	0.65	-		2+4+17	1.29	-
	13+16	3.23	-		**1+3+14+18**	1.29	-		2+4+7+13+17+19	0.65	-
	13+18	0.65	-		1+7+15+16	0.65	-		2+4+9+13+17	0.65	-
	13+19	0.65	-		13+14+16	0.65	-		3+13+15+19	0.65	-
	14+ + 18	1.29	-		13+16	0.65	-		3+13+18	0.65	-
	14+15	1.29	-		13+19	0.65	-		3+14+16	1.29	-
	19+22	1.94	-		14+15+17	0.65	-		3+14+16+18	0.65	-
	19+8	1.94	-		14+15+18+19	0.65	-		3+14+17+19	0.65	-
	20+18	0.65	-		14+16	1.29	-		3+15+17	0.65	-
	6+ + 17	0.65	-		14+16+19	0.65	-		3+15+18+19	0.65	-
	6+17	1.29	-		14+17	5.16	-		3+15+19	0.65	5.55
	6+18	2.58	-		14+17+18	0.65	-		3+7+14+15+19	0.65	-
	6+22	0.65	-		14+18	0.65	-		3+7+16+19	0.65	-
	6+8	22.58	16.67		15+16+17	0.65	-		3+8+14+17	0.65	-
	7+17	4.52	5.56		15+16+18	0.65	-		3+9+14+17+18	0.65	-
	7+22	0.65	-		15+18	1.29	-		3+9+15+18	0.65	-
	7+8	13.55	55.55		16+17+19	0.65	-		4+15+19	0.65	16.67
	7+8 -	0.65	-		16+18	0.65	-		4+16+19	0.65	-
Glu-A3	**5**	8.39	-		16+19	1.29	-		7+13+19	0.65	-
	6	29.68	66.67		2+4+13+15+18	0.65	-		8+13+14+16	0.65	-
	10	3.23	-		2+4+13+16+17	0.65	-		9+13+17	0.65	-
	11	9.03	-		2+4+13+16+19	0.65	-		9+13+17+18	0.65	-
	20	1.29	-		2+4+13+17	0.65	-		9+15+17+18	0.65	-
	10+11	2.58	-		2+4+13+17+18	0.65	-		9+15+17+19	0.65	-
	11+10	0.65	-		2+4+13+19	1.29	-		9+15+18	0.65	-
	11+20	0.65	-		2+4+14+15+16	1.29	-	*Glu-B2*	**12**	41	66.67
	5+10	0.65	-		2+4+14+15+18	0.65	-		**null**	59	33.33
	5+10+11	3.23	-		**2+4+14+15+19**	1.29	-				
	5+11	9.68	5.55		2+4+14+17	0.65	-				
	6+10	1.29	-		2+4+14+17+18	0.65	-				
	6+11	5.81	27.78		2+4+14+18	1.29	-				
	6+20	4.52	-		2+4+14+18+19	0.65	-				
	null	19.35	-		**2+4+15+16**	1.94	-				

* Known banding patterns are indicated in bold type

Table 3. Genetic diversity indices calculated for 5 glutenin loci for 155 landraces and 18 representative modern varieties, and for the landraces per country of origin.

	HMW		LMW			
	$D_{GLU\text{-}A1}$	$D_{GLU\text{-}B1}$	$D_{GLU\text{-}A3}$	$D_{GLU\text{-}B3}$	$D_{GLU\text{-}B2}$	D_{mean}
Modern	0.00	0.61	0.48	0.36	0.44	0.38
Landraces	0.55	0.79	0.84	0.88	0.49	0.71
Algeria	0.00	0.67	0.28	0.28	0.28	0.30
Bulgaria	0.50	0.00	0.50	0.50	0.00	0.30
Croatia	0.48	0.56	0.80	0.72	0.48	0.61
Cyprus	0.44	0.67	0.44	0.44	0.44	0.49
Egypt	0.61	0.64	0.78	0.89	0.17	0.62
France	0.00	0.28	0.67	0.78	0.00	0.34
Greece	0.44	0.67	0.44	0.44	0.00	0.40
Israel	0.00	0.50	0.78	0.78	0.00	0.41
Italy	0.14	0.57	0.62	0.63	0.36	0.46
Jordan	0.22	0.53	0.59	0.78	0.47	0.52
Lebanon	0.00	0.61	0.61	0.82	0.49	0.51
Libya	0.00	0.00	0.00	0.00	0.00	0.00
Macedonia	0.28	0.50	0.83	0.83	0.28	0.54
Montenegro	0.32	0.48	0.64	0.72	0.00	0.43
Morocco	0.17	0.56	0.73	0.66	0.40	0.50
Portugal	0.60	0.74	0.83	0.81	0.40	0.67
Serbia	0.44	0.00	0.44	0.67	0.44	0.40
Spain	0.57	0.72	0.87	0.90	0.34	0.68
Syria	0.38	0.63	0.63	0.75	0.38	0.55
Tunisia	0.00	0.48	0.48	0.32	0.48	0.35
Turkey	0.40	0.17	0.74	0.17	0.17	0.33

Table 4. Analysis of variance for gluten strength of 173 entries (155 landraces + 18 modern varieties). Genotype effect was partitioned according to five levels of gluten strength (SDS values): outstanding (SDS ≥ 11), very high (10 < SDS < 11), high (9 ≤ SDS ≤ 10), medium (7 ≤ SDS < 9) and low (SDS < 7).

Source of variation	df	SS	% SS	Pr > F
Year	2	163	9	<0.0001
Genotype	172	1264	73	<0.0001
Between groups	4	1157	91.5	<0.0001
Within outstanding	7	2	0.1	0.9753
Within very high	20	4	0.3	1.0000
Within high	38	9	0.7	1.0000
Within medium	70	63	5.0	0.4898
Within low	33	30	2.4	0.4700
Genotype x year (Error)	344	310	18	
Total	518	1738		

The frequency of alleles/banding patterns significantly affecting gluten strength was calculated for each of the gluten-strength groups considered in the ANOVA. The rare (for *T. turgidum* var. durum) allele *Glu-B1a* (band 7) was not present in any of the 50 landraces with stronger gluten (SDS ≥ 9 ml), but it was detected in 2.8% of the 71 landraces with medium gluten strength, and in 11.8% of the 34 landraces with low gluten strength (Table 6). For the landraces, allele *Glu-B1b* (band 20) was present with a high frequency in the five gluten-strength groups, particularly in about 60% of the genotypes with high and very high gluten strength. It was also detected in modern varieties, but with lower frequencies and not in the outstanding gluten-strength group (Table 6). Band 5 (*Glu-A3b*) at locus *Glu-A3* was absent in landraces with SDS values > 10, but its frequency increased in landraces as gluten strength decreased. In contrast, bands 6 (*Glu-A3a*) and 6+11 (*Glu-A3d*) at *Glu-A3* and 2+4+15+19 (*Glu-B3a*) at *Glu-B3* were detected in higher frequencies in accessions with SDS-values ≥ 9, both in landraces and modern varieties. The rare patterns 2+4+14+15+18 and 2+4+15+18+19 were only present in the landraces with the highest SDS-values (Table 6). Alleles at *Glu-B2* were uniformly distributed among all the gluten strength groups for the landraces, but they were only present in modern varieties with SDS-values ≥ 9. Band 12 (*Glu-B2a*) was present in more than 50% of genotypes with SDS-values ≥ 9, while the alternative null (*Glu-B2b*) allele was highly frequent in landraces with medium and low SDS-values.

Table 5. Effect of known allelic/banding patterns and their effect on SDS-sedimentation volume (ml) in the collection of 155 landraces and 18 modern cultivars.

Allelic/banding pattern	Landraces					Modern			
	Frequency (%)	Present (ml) (a)	Absent (ml) (b)	Main effect (ml) (a-b)		Frequency (%)	Present (ml) (a)	Absent (ml) (b)	Main effect (ml) (a-b)
Glu-A1 a (1)	21.94	8.43	8.22	0.21					
Glu-A1 b (2*)	14.84	7.96	8.32	-0.36					
Glu-A1 c (null)	61.94	8.27	8.26	0.01		100.00			
Glu-B1 e (20)	36.77	8.58	8.08	0.50	*	22.22	10.24	10.31	-0.07
Glu-B1 d (6+8)	22.58	7.92	8.36	-0.44		16.67	10.15	10.32	-0.17
Glu-B1 ao (7+17)	4.52	9.08	8.23	0.85		5.55	10.92	10.26	0.66
Glu-B1 b (7+8)	13.55	7.67	8.36	-0.68		55.56	10.29	10.29	0.00
Glu-A3 b (5)	8.39	7.08	8.37	-1.29	**				
Glu-A3 a (6)	29.68	8.75	8.06	0.69	**	66.67	10.37	10.14	0.23
Glu-A3 e (11)	9.03	8.15	8.28	-0.13					
Glu-A3 d (6+11)	5.81	9.41	8.19	1.22	*	27.78	10.20	10.33	-0.12
Glu-A3 h (null)	19.35	8.17	8.29	-0.12					
Glu-B3 a (2+4+15+19)	32.90	8.93	7.94	0.99	***	77.78	10.28	10.33	-0.05
Glu-B2 a (12)	41.00	8.70	7.97	0.73	**	66.67	10.37	10.14	0.23
Glu-B2 b (null)	59.00	7.97	8.68	-0.71	**	33.33	10.14	10.37	-0.23

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

Only 8 genotypes in the whole collection, five landraces and 3 modern varieties, had SDS-values ≥ 11. None of them carried the alleles/banding patterns clearly associated with negative main effects such as band 5 (*GluA-3b*) at *Glu-A3* or the rare allele 7 at *Glu-B1.* On the other hand, alleles/patterns found to significantly increase gluten strength were detected in all of them. This was the case of band 6 (*Glu-A3a*) at *Glu-A3* and band 12 (*Glu-B2a*) at *Glu-B2*, which were present in three of the five landraces and in the three modern varieties with the highest SDS-values, and the pattern 2+4+15+19 (*Glu-B3a*) at *Glu-B3*, which was also carried by the three modern varieties and one of the landraces with outstanding gluten strength.

The overall genetic diversity was much higher in landraces than in modern varieties, as previously noted. This tendency was also observed when considering diversity within the different gluten strength groups (bottom part of Table 6). Modern varieties with SDS-sedimentation values ≥ 11 were essentially monomorphic at *Glu-A3*, *Glu-B3* and *Glu-B2*, all exhibiting the *Glu-B3* pattern commonly known as LMW-2 type (Pogna *et al.*, 1988), which is associated with strong gluten type and high pasta-cooking quality (Peña and Pfeiffer, 2005; Pogna *et al.*, 1988). Modern varieties with high and very high gluten strength had intermediate to low diversity index values for all loci. The diversity indices for the five SDS-sedimentation groups of the landraces ranged from 0.58 to 0.71, these values not being significantly different from each other.

The geographic distribution of the five gluten strength groups along the Mediterranean Basin is shown in Fig. 1. The highest percentages of accessions with strong to very strong gluten were found in Cyprus (100%), Syria (75%) and Turkey (73%), while 100% of the accessions from Jordan, Israel, Bulgaria, Serbia, Montenegro and Greece had medium to low gluten strength. All accessions from Algeria, Tunisia and Libya had SDS values that were in the intermediate classes, with none belonging to either outstanding or low SDS-values groups. All the western Mediterranean countries had a high percentage of accessions with medium gluten-strength and, with the exception of France, none of the western Mediterranean accessions had SDS-values ≥ 11.

The clustering of countries based on the frequencies of all known alleles/banding patterns did not result in a clear geographical pattern except for the null allele (*Glu-A1c*) at *Glu-A1*, the most frequent in the present collection. Fig. 2 shows that the allelic frequencies for the null allele clusters countries with high (75-100%, branch A) and low (0-60% branch B) frequencies of this allele. Branch C clustered modern varieties and countries in which all the accessions were monomorphic for this locus, while branch D grouped countries in which this allele was present in very high frequency (75 - 92 %). In the lower part of Fig. 2, branch E grouped countries with low to medium frequencies (27 – 60 %), while Croatia, Macedonia and Montenegro, with accessions lacking this allele, were clustered in branch F.

4. Discussion

While the number of modern cultivars (18) included for comparison in the study is lower than that of landraces (155), it can be considered as reliably representative of the current modern Mediterranean germplasm in terms of its variability for glutenin loci, in particular for *Glu-A1*, *Glu-B1* and *Glu-B3*. Because the genotypes are not only major cultivars in the northern-Mediterranean countries where they were released, but several are extensively grown under different names in North Africa and elsewhere, alleles or banding patterns that would be present in the overall modern Mediterranean germplasm are highly likely to be represented within the group of 18 cultivars included in this study. In this context, the present collection of 155 landraces clearly showed much more variability in glutenin composition than modern varieties with only 13 of the 114 alleles/banding patterns identified in the landraces being also present in modern varieties. This indicates an overall loss of 88.6% in allelic variability going from landraces to modern cultivars. Eighty five per cent of the alleles/banding patterns identified in the landraces had a frequency lower than 5%, therefore considered as rare forms, and 84.5% of them had not been previously described. This clearly confirms that local/native durum germplasm resources from the region indeed represent a rich reservoir of untapped diversity for glutenin composition and could be used towards widening the variability within modern germplasm, should some alleles or banding patterns prove to be useful for enhancing/diversifying gluten characteristics.

The largest diversity for HMW-GS was found at the *Glu-B1* locus, both in landraces and modern varieties. However, at the *Glu-A1* locus, modern cultivars were monomorphic, all exhibiting the null allele whereas 4 alternative, non-null alleles were expressed in close to 40% of the landraces. The null allele has been found in very high frequencies in other durum collections (Branlard *et al.*, 1989; Tarekegne and Labuschagne, 2005; Vallega, 1988), and practically fixed in modern germplasm worldwide. The same order in allelic frequencies at *Glu-A1*, namely null *>1 >2**, has been previously reported in durum wheat (Aguiriano *et al.*, 2008; Carrillo, 1995; Cherdouh *et al.*, 2005; Kaan *et al.*, 1993; Moragues *et al.*, 2006). The maximum genetic diversity for this locus was found in the Iberian Peninsula and Egypt, while it was more homogeneous in the Maghreb countries, Lebanon and Israel.

The genetic variability found at *Glu-B1* in this study was much greater than that reported by previous studies conducted with Mediterranean landraces (Moragues *et al.*, 2006) or with durum world collections (Kaan *et al.*, 1993). The four banding patterns identified at the *Glu-B1* locus in modern varieties represented only 77.42% of the allelic frequency in the landraces group, in which 16 additional rare banding patterns were detected. However, the most frequent banding pattern in the landraces (20) did not coincide with the prevalent in modern varieties (7+8). Previous studies have reported a wide presence of band 20 in landraces from North Africa and South West Asia (Cherdouh *et al.*, 2005; Moragues *et al.*, 2006; Zarkti *et al.*, 2010). The high frequency of the 7+8 banding pattern in modern varieties may have resulted from selection in breeding programs, since it has been widely associated with strong gluten and therefore good pasta making quality (Du Cros, 1987; Sissons *et al.*, 2005), although its positive effect could not be evidenced in the present set of genotypes. The rare band 7 at *Glu-B1* locus had been previously detected at very low frequencies in landraces from the Iberian Peninsula and South West Asia (Moragues *et al.*, 2006).

The variability detected for LMW-GS was even wider than the one characterizing HMW-GS as found in other studies (Brites and Carrillo, 2001; Du Cros, 1987; Sissons *et al.*, 2005). However, the number of banding patterns identified at LMW-GS loci in the present collection was much higher than that reported in previous studies, confirming the wide genetic diversity of the germplasm used in this particular study, especially for the *Glu-B3* locus. Nine of the 15 banding patterns detected at *Glu-A3* locus were rare ones. The most frequent banding pattern at this locus, either in landraces and modern varieties, was *Glu-A3a* (band 6), in agreement with the results obtained by Nieto-Taladriz *et al.* (1997), Carrillo *et al.* (2000) and Moragues *et al.* (2006). The 2+4+15+19 banding pattern (*Glu-B3a* allele) at *Glu-B3* was the most prevalent in landraces (32.90%) as well as in modern varieties (77.78%), in accordance with the results obtained by Nieto-Taladriz *et al.* (1997), Carrillo *et al.* (2000) and Moragues *et al.* (2006).

There was no clear sub-regional or geographic trend in the distribution of diversity within the set of landraces studied. Among the most diverse landrace groups were those of the Iberian Peninsula. It is worth noting that in spite of the Spanish landraces being about three times more represented than the Portuguese, the genetic diversity indices of both countries were similar (0.68 and 0.67 respectively), and also similar to the that previously reported by Moragues *et al.* (2006) who found an index of 0.62 in a set of 25 landraces from the Iberian Peninsula. The lowest genetic diversity indices were found for the Algerian, Tunisian, Bulgarian, French and Turkish sub-groups. This is in agreement with the results reported by Hamdi *et al.* (2010) in a study with 856 accessions of Algerian durum wheat, and by Moragues *et al.* (2006) which reported little diversity in Turkish and Bulgarian landraces. As suggested by Ganeva *et al.* (2010), this could be attributed either to agro-ecological factors or to the efficient and consistent selection done by farmers through time, with the aim of improving the uniformity and yield of local populations.

The clustering based on the frequency of the null allele at *Glu-A1*, the most frequent either in modern varieties and landraces, displayed a clear geographical pattern. A very high frequency of this allele was detected in the modern varieties, the Middle East and North African countries, France and Italy. With the exception of Egypt, in which this allele was found at a medium frequency (28%), this geographic distribution is consistent with the dispersal route of durum wheat from the Fertile Crescent to North Africa through the south side of the Mediterranean Basin, as suggested by Moragues *et al.* (2007). The close relationships between France and its

Table 6. Frequency (%) of known and rare allelic/banding patterns with statistical significant effect on gluten strength in genotypes with outstanding, very high, high, medium and low SDS-sedimentation test values (ml) and genetic diversity indices for the same groups. The number of genotypes in each strength group is shown between parenthesis.

Allelic/banding pattern	Landraces					Modern				
	Outstanding SDS≥11 (5)	Very high 10<SDS<11 (14)	High 9≤SDS≤10 (31)	Medium 7≤SDS<9 (71)	Low SDS<7 (34)	Outstanding SDS≥11 (3)	Very high 10<SDS<11 (7)	High 9≤SDS≤10 (8)	Medium 7≤SDS<9 (0)	Low SDS<7 (0)
Glu-B1 a (7)†	0.0	0.0	0.0	2.8	11.8	*0.0*	*0.0*	*0.0*	-	-
Glu-B1 e (20)	40.0	57.1	61.3	19.7	41.2	*0.0*	*28.6*	*25.0*	-	-
Glu-A3 b (5)	0.0	0.0	3.2	9.9	14.7	0.0	0.0	0.0	-	-
Glu-A3 a (6)	60.0	28.6	41.9	31.0	11.8	100.0	57.1	62.5	-	-
Glu-A3 d (6+11)	0.0	28.6	6.5	4.2	0.0	0.0	42.9	25.0	-	-
Glu-B3 a (2+4+15+19)	20.0	57.1	58.1	26.8	14.7	100.0	57.1	87.5	-	-
Glu-B3 2+4+14+15+18†	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
Glu-B3 2+4+15+18+19†	20.0	7.1	0.0	0.0	0.0	0.0	0.0	0.0	-	-
Glu-B2 a (12)	60.0	50.0	61.3	33.8	29.4	100.0	57.1	62.5	-	-
Glu-B2 b (null)	40.0	50.0	38.7	66.2	70.6	0.0	42.9	37.5	-	-
Genetic Diversity Indices										
$D_{Glu\text{-}A1}$	0.64	0.62	0.45	0.51	0.59	0.00	0.00	0.00	-	-
$D_{Glu\text{-}B1}$	0.72	0.64	0.59	0.83	0.74	0.44	0.57	0.63	-	-
$D_{Glu\text{-}A3}$	0.56	0.80	0.76	0.82	0.88	0.00	0.49	0.53	-	-
$D_{Glu\text{-}B3}$	0.80	0.63	0.65	0.91	0.95	0.00	0.49	0.22	-	-
$D_{Glu\text{-}B2}$	0.48	0.50	0.47	0.45	0.42	0.00	0.49	0.47	-	-
D_{mean}	0.64	0.64	0.58	0.70	0.71	0.09	0.41	0.37	-	-

† Allelic/banding patterns with frequencies <0.05

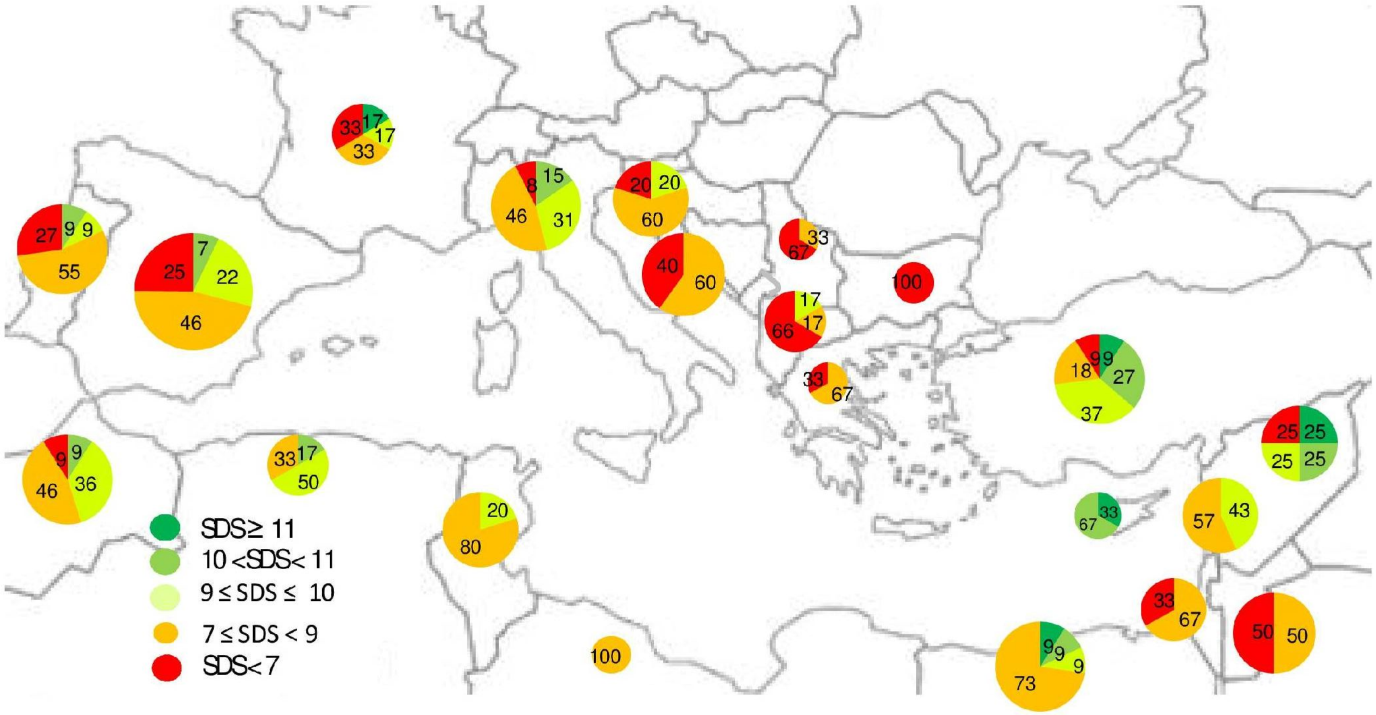

Figure 1. Geographic distribution of gluten strength groups in landraces from the Mediterranean Basin. The colours inside the circles indicate the percentage of entries with outstanding (SDS ≥ 11), very high (10 < SDS < 11), high (9 ≤ SDS ≤ 10), medium (7 ≤ SDS < 9) and low (SDS < 7) SDS values. The size of the circles is proportional to the number of entries from each country according to Table 1.

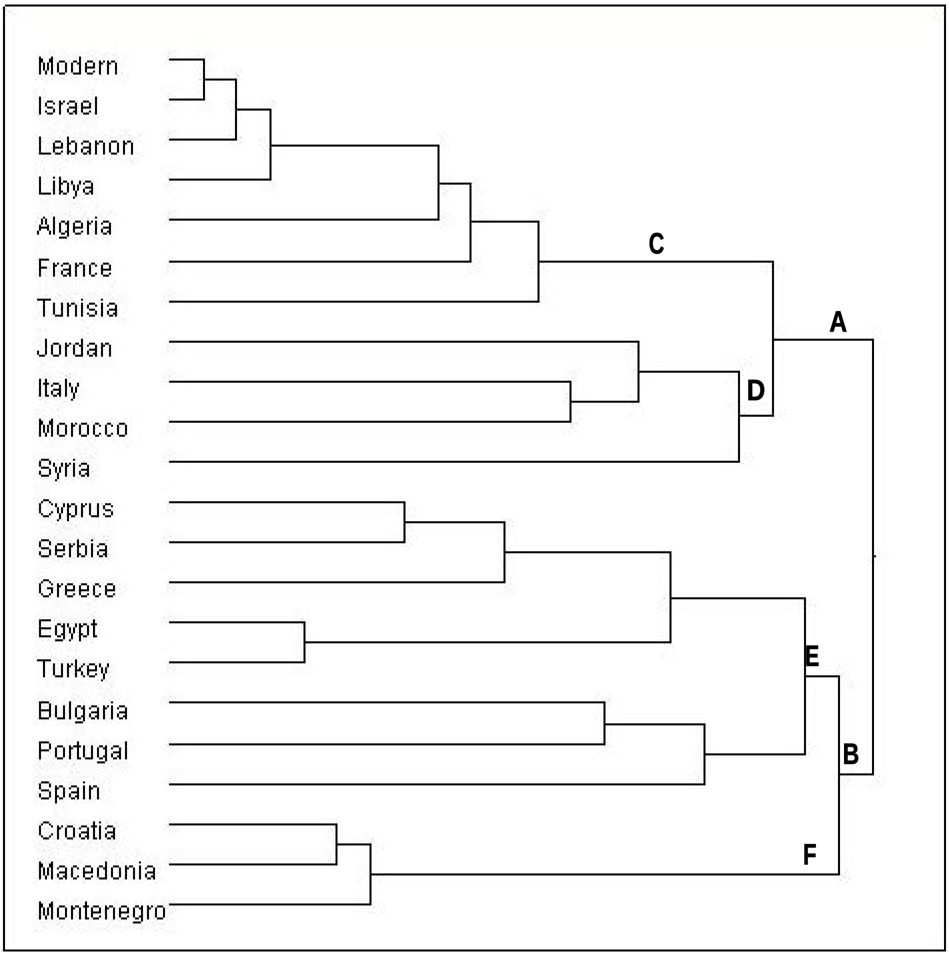

Figure 2. Cluster analysis based on *Glu-A1c* (*null* allele) frequency in modern varieties and by country for landraces.

former colonies in North Africa (Morocco, Algeria and Tunisia) would justify French landraces to be clustered in this group. Moreover, the inclusion of Italian germplasm in this cluster may be consequence of the extensive use of North African landraces by Italian scientist and breeders during the first half of the twentieth century (Di Fonzo *et al.*, 2005). In contrast, all Balkan countries, as well as the Iberian Peninsula countries, Turkey and Egypt were clustered in a branch associated with a low frequency of the null allele at *Glu-A1* that is consistent with a northern dispersal through the Mediterranean Basin.

The ANOVA for gluten strength showed that the component of variation due to genotypes was more than 8 times greater than the component due to the year effect. This result confirms the large genetic control of this trait in durum wheat, as noted by Ames *et al.* (1999) and Taghouti *et al.* (2010). The classification of genotypes in five groups according to their gluten strength determined by the SDS-sedimentation test was appropriate, as demonstrated by the large amount of total variance (67%) explained by differences between groups and the lack of significant differences in gluten strength values within groups.

None of the *Glu-A1* alleles had a significant main effect on gluten strength, as determined by the SDS-sedimentation test. Our results are in line with earlier studies which did not detect any association between strength and allelic composition at *Glu-A1* in durum wheat (Du Cros, 1987). However, other authors did find such a relationship when dough rheological parameters were considered both in durum wheat (Brites and Carrillo, 2001; Ciaffi *et al.*, 1995), and bread wheat. Also, increases in gluten and dough strength have been achieved in durum wheat by transferring HMW-GS coded by *Glu-A1* from *T. dicoccoides* (Ciaffi *et al.*, 1991, 1995). In bread wheat the alleles *Glu-A1*a (1) and *Glu-A1*b (2*) have been shown to improve dough quality and strength compared to the *Glu-A1*c (null) allele (He *et al.*, 2005; Payne, 1987; Payne *et al.*, 1987; Pogna *et al.*, 1992). However, this effect in bread wheat is detected as *Glu-A1* subunits are in interactions with relatively different set of additional HMW-GS and a drastically different set of LMW-GS than those present in durum wheat, which may explain why no effect could be detected in the present set of landraces. It is also important to note that the SDS-sedimentation test is an indirect measure of gluten strength, based on the extent of aggregation/precipitation of the gluten polymer, not a physical measurement of viscolelastic properties of the gluten complex or dough. Further studies using dough rheology parameters may be needed to definitively determine actual strength differences associated with allelic variations at *Glu-A1* in the group of landraces evaluated.

Our results showed that at *Glu-B1*, only banding pattern 20 had a significant main effect on the gluten strength of the landraces - increasing it by about 6% - while its effect in modern varieties was negligible. Previous studies addressing the effect of banding pattern 20 at *Glu-B1* have also reported contrasting results, ranging from a reduction of gluten strength (Ammar *et al.*, 2000; Brites and Carrillo, 2001; Carrillo *et al.*, 1990; Peña *et al.*, 1994; Raciti *et al.*, 2003; Sissons *et al.*, 2005) to a positive effect on bread volume, a trait highly and positively correlated with gluten strength (Boggini and Pogna, 1989), to not significant effects (Vázquez *et al.*, 1996). Again, these divergent results may be due to the fact that there is interaction among HMW-GS and LMW-GS in the co-formation of the gluten complex and main effects of a given allele can be different in different sets of genotypes, especially that, in the case of durum wheat, LMW-GS effect dominates over the effect of the HMW-GS, therefore making any HMW-GS main effect highly dependent on background (Payne *et al.*, 1984; Peña and Pfeiffer, 2005; Ruiz and Carrillo, 1995). In the present study, the presence of subunit 7 at *Glu-B1* had the most detrimental effect on gluten strength.

Two of the three alleles found at *Glu-A3* in modern varieties (*Glu-B3a* and *Glu-A3d*, with bands 6 and 6+11, respectively) had a significant and positive effect on the gluten strength in the landraces. *Glu-A3a* was found at very high frequency in genotypes with outstanding gluten strength (all modern varieties and 60% of the landraces with SDS-values ≥ 11). Despite being positive, its single effect on the gluten strength in modern varieties was not significant, possibly because alleles from other loci with stronger main effects were present and did not allow the detection of the individual effect of *Glu-A3a*. Previous studies have related durum quality with the presence of *Glu-A3a* (Carrillo *et al.*, 2000; Nieto-Taladriz *et al.*, 1997). *Glu-A3d* was more frequent in modern varieties than in landraces (27.78% and 5.81%, respectively), and it had the largest positive effect on the SDS-values of landraces, but its effect in modern varieties was not significant, probably due to additive or complementary effects of individual *Glu-1*/*Glu2*/*Glu3* alleles. On the other hand, allele *Glu-A3b* (band *5)* absent in modern varieties and present in 13 landraces had the worst effect on gluten strength, in agreement with the findings of Carrillo *et al.* (2000) and Martinez *et al.* (2005).

The *Glu-B3a* allele (banding pattern 2+4+15+19) at *Glu-B3* had a positive effect on the gluten strength of the landraces increasing the SDS-value in 0.99 ml in average, while its effect on modern varieties was negligible. The relationship between good quality and the presence of *Glu-B3a* has been observed by other authors (Carrillo *et al.*, 2000; Nieto-Taladriz *et al.*, 1997).

Two rare banding patterns at *Glu-B3* increased significantly the gluten strength of the landraces carrying them: 2+4+14+15+18 and 2+4+15+18+19. The first one was present in only one landrace (PI-366109 from Egypt), which SDS-value was 3.44 ml higher than the mean SDS-value of the remainder landraces. A previous study (Nazco *et al.*, 2012) identified this landrace as having the highest EU quality index, due mostly to its very high gluten strength and protein content. Thus, this landrace could be considered as a valuable donor in durum breeding programs. The banding pattern 2+4+15+18+19 was detected in two landraces, Trigo Glutinoso and Lobeiro de Grao Escuro from France and Portugal, respectively, and its effect on SDS-value was estimated at 2.96 ml, greater than the main effect of any know allele evaluated in this study. Both landraces have a high EU quality index (Nazco *et al.*, 2012), and the high quality of their protein, associated to the presence of the banding pattern 2+4+15+18+19 at *Glu-B3*, supports their use as parents in breeding programs.

The two alleles detected at *Glu-B2* locus (*Glu-B2a* and *Glu-B2b* with band 12 and null, respectively) had significant and opposite effects on the gluten strength of the landraces. *Glu-B2a* was found to exert a positive, but moderate effect on gluten strength. It was more frequent in modern varieties than in the landraces, the opposite being true for the null allele *Glu-B2b*. Very few studies have been conducted on the relationship between *Glu-B2* alleles and gluten strength, and generally the absence or presence of *Glu-B2a* did not result in any significant difference in gluten strength (Martinez *et al.*, 2004, 2005; Ruiz and Carrillo, 1996). This was the case of the set of modern varieties used in this study in which the variation at this locus was not large enough to be statistically significant. However, the results obtained in the landraces, with much wider genetic background, generally weaker gluten overall and more opportunities to measure moderate effects, indicate that these alleles caused moderate and opposite effects on the gluten strength.

The country-specific classification of the frequencies within the five gluten strength-groups offered a picture of the "native" or "pre-green revolution" gluten quality distribution around the Mediterranean Basin. Although a clear pattern was not evident, four of the five landraces with outstanding gluten strength, and seven of the fourteen landraces with very high gluten strength came from eastern Mediterranean countries (Syria, Turkey, Cyprus and Egypt), providing partial indication that the best gluten quality may have been originally concentrated in the Fertile Crescent, where tetraploid wheat originated (Feldman, 2001). On the other hand, none of the landraces from Lebanon, Jordan and Israel, had outstanding or very high gluten strength, but a high percentage of entries with weak gluten. The Balkan Peninsula was characterized by a large number of landraces with poor gluten strength, probably associated to a different geographic origin (Nazco *et al.*, 2012). Western Mediterranean countries showed a wide diversity of gluten types, possibly due in part to their broad representation in the collection. However, landraces with outstanding gluten strength were lacking in this sub-region. These results suggest that during the dispersal of wheat from the east to the west of the Mediterranean Basin the alleles conferring outstanding gluten strength were probably not linked to any variability resulting in increased fitness or adaptation to the new environments and not associated with any local preference.

5. Conclusions

Mediterranean landraces retain a wide genetic diversity for glutenin composition that was mostly lost in modern varieties. Mediterranean landraces are a natural reservoir of alleles potentially useful to enhance and diversify gluten characteristics in durum wheat breeding programs. Landraces from the Iberian Peninsula and Egypt were among the most diverse. The null allele was fixed at the *Glu-A1* locus in the modern varieties studied, while four alternative banding patterns were recognized in the landraces. Only 4 of the 20 banding patterns identified in the landraces at the *Glu-B1* locus were present in modern varieties. Our study confirms previous findings that the variability detected in durum wheat landraces for LMW-GS was wider than for HMW-GS, especially for the *Glu-B3* locus. Only 8 of the 89 banding patterns found in the landraces at *Glu-2*/*Glu3* loci appeared in modern varieties. The clustering of the countries based on the frequency of the null allele at *Glu-A1* was consistent with the proposed routes of durum wheat dispersal from the Fertile Crescent through the West of the Mediterranean Basin.

The medium to high values of gluten strength found in modern varieties reflects the efforts made by breeding programs to improve grain quality. The low variation for gluten strength in modern varieties when compared with landraces could probably explain the lack of significant relationships between the 13 banding patterns identified in this group and gluten strength. Significant increases in the SDS-values of the landraces resulted from the presence of alleles *Glu-B1e* (band 20), *Glu-A3a* (band 6), *Glu-A3d* (bands 6+11), *Glu-B3a* (bands 2+4+15+19) and *Glu-B2a* (band 12) and the two hitherto unpublished banding patterns: 2+4+14+15+18 and 2+4+15+18+19 at *Glu-B3* locus. On the other hand, landraces carrying the *Glu-A3b* (band 5), *Glu-B2b* (null allele) and the rare allele *Glu-B1a* (band 7) showed lower gluten strength values.

Geographic distribution of gluten strength suggests that the best gluten quality was probably originated in the Fertile Crescent and that, during the dispersal of wheat from the east to the west of the Mediterranean Basin, the alleles conferring outstanding gluten strength were frequently lost, likely due to their lack of association with other desirable traits such as yield or local adaptation.

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CAPÍTULO 3

Relationships between allelic combinations at Glu-1/Glu-3 loci and gluten strength in landraces from 21 Mediterranean countries and modern durum wheat

Abstract

A collection of 155 durum wheat landraces and old varieties from 21 Mediterranean countries and 18 representative modern varieties was used to assess the relationship between gluten strength and the allelic combinations at five glutenin loci (*Glu-A1*, *Glu-B1*, *Glu-A3*, *Glu-B3* and *Glu-B2*). Gluten strength was determined by SDS-sedimentation test on grain from field experiments conducted during three years in northeastern Spain. A total number of 131 different allelic/banding pattern combinations were recorded in the whole germplasm collection. The interaction between high HMW-and low LMW-molecular weight glutenin subunit GS (HMW-GS and LMW-GS, respectively) loci resulted in 126 combinations in landraces and 9 in modern varieties, 5 of them specific of the modern genotypes. Two LMW models (LMW-2 and LMW-2-), comprised of contrasting LMW-GS combinations, were identified in the collection. LMW-2 was present in 78% of modern varieties, including the only three with outstanding gluten strength (Ocotillo, Claudio and Meridiano), while 14% of the landraces had LMW-2 and 6% LMW-2-. All modern varieties had strong gluten. Two known combinations −one including LMW-2 (aaa) and another LMW-2-(haa) − and three new ones, found in a few number of genotypes, had a positive and significant effect on the gluten strength of the landraces. Both LMW models were very frequent in landraces from Morocco, Algeria, Tunisia and Italy, less frequent in those from Spain, Portugal, Lebanon, Turkey and Jordan and absent in landraces from France, the Balkan Peninsula, Cyprus, Egypt, Israel, Libya and Syria.

Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is one of the oldest cultivated cereal species in the world. The earliest wheats, dated to approximately 10,000 years BP, were domesticated in the region known as Fertile Crescent, which extends from the coast of Israel up to south-eastern Turkey and westwards through Syria, Iraq and western Iran, (Feldman, 2001). From this region durum wheat spread out to the west of the Mediterranean Basin, reaching the Iberian Peninsula around 7,000 years BP (Feldman 2001; Mac Key 2005). The most widely accepted theory about wheat dispersal in the Mediterranean region suggests that durum wheat entered to North Africa and the Iberian Peninsula from the south of Italy (Mackey 2005). However, recent findings based on the genetic similarities between landraces from the Maghreb countries and those from Spain and Portugal have suggested North Africa as an additional route for wheat introduction in the Iberian Peninsula (Moragues *et al.,* 2006c, 2007).

During the process of migration from the east to the west of the Mediterranean Basin durum wheat suffered a gradual adaptation to a large number of different environments (Moragues *et al.*, 2006 a,b). Natural and human selection resulted in a wide diversity of local landraces well, but specifically adapted to diverse different agro-ecological zones. These dynamic populations, with distinct identity and lacking formal crop improvement, are considered to be genetically diverse, locally adapted and associated with traditional farming systems (Camacho Villa *et al.*, 2005). With the exception of Italy, where breeding programs started at the beginning of the twentieth century (Borghi, 2001), landraces were mostly grown in Mediterranean countries until the advent of the Green Revolution from the middle of the 20th century. The gradual replacement of traditional landraces by improved, more homogeneous and productive semi-dwarf cultivars obtained from breeding programs, resulted in a loss of genetic diversity or genetic erosion from the middle of the 20th century in southern Europe and during the 70s-80s in Northern Africa. Nowadays landraces are considered the natural reservoir of the genetic variation within the species and one of the most important sources for favorable genes/alleles to be used in breeding programs for the development of improved varieties.

Durum wheat is currently one of the crops most largely cultivated in the Mediterranean Basin which is the most significant durum import market and the largest consumer of durum wheat products (Royo *et al.*, 2009). Durum wheat is usually grown under rainfed conditions, in environments with large climate fluctuations in which terminal drought and heat are the most frequent stresses constraining grain yield. However, in most cases, environmental conditions during grain filling allow the production of grains with the high quality standards demanded by the industry.

Durum-made pasta cooking quality is largely affected by gluten strength, which is commonly evaluated using the SDS-sedimentation test. Gluten strength depends on the composition of gliadins and glutenins, proteins stored in the grain endosperm, with glutenins being the more influential. The glutenin subunits (GS), can be identified according to their mobility in sodium dodecyl sulphatepolyacrilamide gel electrophoresis (SDS-PAGE); they can be separated into high (HMW-GS) and low (LMW-GS) molecular weight glutenin subunit (Lindsay and Skerritt, 1999; Veraverbeke and Delcour, 2002). These proteins have been used as a tool for identification of cultivars, pedigree analysis and population characterization (Pogna *et al.*, 1989; Igrejas *et al.*, 1999) because they offer a measure of genetic diversity within and between populations (Nevo and Payne, 1987).

The HMW-GS are encoded by the complex *Glu-1* loci (*Glu-A1* and *Glu-B1*), located on the long arm of group-1 homologous chromosomes (Singh and Sheperd, 1988; Shewry et at., 1992), whereas on the short arm are located the *Glu-3* loci (*Glu-A3* and *Glu-B3*) as well as the *Glu-B2* loci that encode for the LMW-GS (Ruiz and Carrillo, 1993; Liu, 1995).

Studies about the relationship between the allelic composition at HMW-GS and gluten quality of durum wheat offered contrasting results. While some authors reported a positive association between certain *Glu-B1* allelic variants and gluten strength (Boggini and Pogna, 1989), others found poor relationships (DuCros, 1987). However, a positive association has been found between HMW-GS composition, particularly among landraces, and the breadmaking quality of both bread and durum wheat (Tarekegne and Labuschagne, 2005).

Early studies identified two combinations for LMW-GS in durum wheat, named model LMW-1 and model LMW-2. These models are associated with γ-42 and γ-45 gliadin, respectively, although links with gluten strength are likely to be caused by LMW glutenin subunits linked to γ-gliadin 45 (Payne *et al.*, 1984). Subsequent works found additional models, such as LMW-1^{-}, LMW-2^{-} and LMW-2* (Carrillo *et al.*, 1990). LMW-2 and LMW-2^{-} have been related with high gluten strength, while LMW-1 and LMW-1^{-} result in poor quality (Carrillo *et al.* 1990; Pogna *et al.*, 1990). Several authors have demonstrated that the quality of durum wheat gluten depends mainly on LMW-GS because they regulate gluten viscoelasticity (Ruiz and Carrillo, 1995; Vázquez *et al.*, 1996; Carrillo *et al.*, 2006). However, the association between gluten quality and LMW-models seem to be imprecise due to the existing interaction between HMW-GS and LMW-GS in the co-formation of the gluten complex. Allelic variation at the *Glu-B3* locus seems to affect much more gluten quality than the allelic differences at *Glu-B1* or *Glu-A3* (Vázquez *et al.*, 1996). Nieto-Taladriz *et al.* (1997) proposed a nomenclature for the commonly used LMW-models taking into account the specific LMW-GS encoded at the *Glu-A3, Glu-B3* and *Glu-B2* loci.

The present study was conducted with a set of 155 durum wheat landraces and old varieties from 21 Mediterranean countries and 18 representative modern varieties with the following aims: I) to study the allelic/banding pattern combinations for HMW-GS and LMW-GS present in the collection and their frequency, II) to identify the combinations having significant effect on gluten strength and the genotypes carrying them, and IIII) to assess whether a geographic distribution exists in the population according to the LMW-models detected.

Material and methods

Experimental setup

Plant material consisted on a collection of 155 durum wheat landraces and old varieties derived from them, from 21 Mediterranean countries including the major durum producers/users (Table 1) and 18 representative modern cultivars. Landraces were selected ─from a larger collection of 231 accessions─ on the basis of their genetic variability determined by 33 SSR-markers, in order to represent the genetic diversity of ancient local durum populations from the Mediterranean Basin (Nazco *et al.*, 2012). Seeds provided by public gene banks (Centro de Recursos Fitogenéticos INIA-Spain, ICARDA Germplasm Bank and USDA Germplasm Bank) were increased in bulk and purified by eliminating off types. The modern set included Spanish, Italian, and French cultivars, as well as the U.S. desert durum cultivar Ocotillo. Increase plots were planted in the same field in years previous to each experiment to ensure a common origin for seeds of all lines.

The collection was grown during 2007, 2008 and 2009 crop seasons in Gimenells (41°40 'N, 0°20' E, and 200 m a.s.l.) in Lleida province (north-eastern Spain). Experiments consisted on non-replicated plots of 6 m^2 (comprising eight 5-m rows, spaced 0.15 m apart), arranged according to a modified augmented design with three replicated checks (cultivars Claudio, Simeto and Vitron). Sowing dates were 21 Nov in 2006 and 20 Nov in 2007 and 2008. Sowing density was adjusted to 250 viable seeds m^{-2}. Water input was 208 mm, 308 mm and 237 mm in 2007, 2008 and 2009, respectively. Plots were mechanically harvested at ripening (first half of July), and the weeds and diseases were controlled according to standard cultural practices.

Gluten strength

A sample of about 250 g was randomly drawn from each plot, cleaned and 1 g of whole grain flour sample was used to determine gluten strength by the SDS- (sodium dodecyl sulfate) sedimentation-test, following the method of Axford *et al.* (1978), further modified by CIMMYT (Peña *et al.*, 1990), using stoppered 25 ml graduate cylinders.

Glutenin composition

Electrophoretic analysis (1D SDS-PAGE) of high and low molecular weight glutenin subunit composition at 5 loci (*Glu-A1*, *Glu-B1*, *Glu-A3*, *Glu-B3*, and *Glu-B2*) was performed according to Peña *et al.* (2004), following the nomenclature of Nieto-Taladriz *et al.* (1997) and Martinez *et al.* (2004). The combinations with a frequency below 0.05 were classified as rare (Russell *et al.*, 2000).

Table 1. Country of origin of the 155 landraces included in the study and number of allelic/banding pattern combinations detected by SDS-PAGE at the HMW- and LMW-glutenin subunits loci.

Country of origin	Number of entries
Algeria	6
Bulgaria	2
Croatia	5
Cyprus	3
Egypt	11
France	6
Greece	3
Israel	6
Italy	13
Jordan	8
Lebanon	7
Lybia	1
Macedonia	6
Montenegro	5
Morocco	11
Portugal	11
Serbia	3
Spain	28
Syria	4
Tunisia	5
Turkey	11

Combination	Landraces	Modern
HMW glutenin	32	4
LMW glutenin	98	5
LMW model	2	1
HMW and LMW glutenin	126	9

Statistical analysis

Raw data were fitted to a linear mixed model with the check cultivars as fixed effects, and the row number, column number and variety as random effects (Little *et al.*, 1996). Restricted Maximum Likelihood (REML) was used to estimate the variance components and to produce the best linear unbiased predictors (BLUPs) for the gluten strength data of each cultivar each year, achieved using the MIXED procedure of the SAS-STAT statistical package (SAS Institute Inc. 2009), which was used for all the analyses.

The SDS-sedimentation test value of each accession across experiments was used to include them in one of the following groups of gluten strength: outstanding ($SDS \geq 11$), very high ($10 < SDS < 11$), high ($9 \leq SDS \leq 10$), medium ($7 \leq SDS < 9$) and low ($SDS < 7$). A standard ANOVA, in which the genotype effect was partitioned according to this classification, was conducted with the BLUPs of gluten strength data. Means were compared by the Student-Newman-Keuls (SNK) test at $P=0.05$. Genetic diversity was calculated with the D index (Weir, 1996), according to the following expression:

$$D_j = 1-\Sigma\, p^2_{ij}$$

where p is the frequency of the i^{th} allele/banding pattern combination at j^{th} country. The effect of each combination on gluten strength was calculated as the difference between the mean SDS-sedimentation test values of the accessions carrying it and the remainder accessions. The frequency of the combination of LMW models by country was used to perform hierarchical cluster analysis by the Ward method of the JMP V.8 software (SAS Institute Inc. 2009).

Results

A total number of 114 banding patterns, potentially *Glu-1*/*Glu-3/Glu-2* allele-specific, were identified in the collection, with the following distribution: 5 and 20 in the *Glu-A1* and *Glu-B1* loci respectively, at HMW-GS, and 15, 72 and 2 in the *Glu-A3, Glu-B3* and *Glu-B2* loci, respectively at LMW-GS (Nazco *et al.*, submitted). Thirty two and 4 banding pattern combinations were detected at the HMW-GS loci in landraces and modern varieties respectively, whilst at the LMW-GS loci, 98 and 5 combinations, putatively allele-specific for *Glu-2*/*Glu-3* loci, were identified in the same germplasm sets (Table 1). The interaction between gluten subunit loci resulted in a total number of 126 combinations in landraces and 9 in modern varieties, from which 5 were specific of the modern genotypes (Table 1). A total number of 131 different allelic/banding pattern combinations were recorded in the whole germplasm collection. The large number of combinations identified in the landraces resulted in a very low frequency of many of them. The most frequent combination in modern varieties was number 1 (null allele at *Glu-A1* and band 7+8 at *Glu-B1* for HMW-GS loci, and bands 6, 2+4+15+19 and 12 at *Glu-A3*, *Glu-B3* and *Glu-B2,* respectively, at LMW-GS loci), which appeared in 7 (38.89%) entries (Table 3). On the other hand, combination 21, whose only difference with combination 1 was the banding pattern at *Glu-B1* locus, was the most common in the landraces, but it only was recorded in 13 (8.39%) of them. All the allelic/banding patterns identified in modern varieties were also present in the landraces (Tables 2 and 3).

Two LMW models, LMW-2 and LMW-2^-, were identified in the collection, the first one with two different combinations (Table 2). Model LMW-2 was the most frequent, both in landraces and old varieties (14.19%) and in modern cultivars 77.78 %. LMW-2 (aaa) model was part of the combinations identified as numbers 1, 21, 38, 73 and 83 in Table 2. It was the only model present in Algerian, Lebanese, Jordanian and Portuguese landraces, with frequencies of 83.3%, 28.6%, 12.5% and 9.1%, respectively. LMW-2 (dab) was present in combinations numbers 15, 36 and 54 (Table 2), and it was exclusive of two Spanish landraces (Table 3). LMW-2^- corresponded to combinations 26, 42 and 88 (Table 2) and was recorded in landraces from Morocco (36.4%), Italy (23.1%), Tunisia (20.0%), Turkey (9.1%), and Spain (3.6%). The only country having the three LMW model combination represented was Spain. Eleven, among the 18 modern varieties (61%), had LMW-2 (aaa) (Tables 2 and 3), while LMW-2(dab) was detected in 17% of them, the varieties 'Amilcar', 'Svevo' and 'Vitronero'. The most frequent alleles at *Glu1* loci associated with LMW models were the null allele at *Glu-A1* (96%) and *Glu-B1* GS 20 (54%), 7+8 (22%) and 6+8 (22%).

The overall genetic diversity index (D), calculated for the allelic/banding pattern combinations, was higher for the landraces than for modern varieties (Table 4). Mean D values by country ranged from 0.50 to 0.96 and were very high for landraces from Spain, Portugal, Egypt, Jordan and Lebanon, and low for landraces from Bulgaria, Algeria, Serbia, Greece and Cyprus (Table 4).

The results of the ANOVA for gluten strength (data not shown) indicated that both year and genotype effects were significant (*P*<0.0001), explaining respectively 9% and 73% of total variation. The partitioning of the genotype effect into its components allowed quantifying the percentage of the genotype variance accounted by differences between the five groups of gluten strength, and differences within each of them. The results revealed that differences between the groups of accessions formed according to SDS-sedimentation test values accounted for 91.5% of the genotype effect, while variability within each sedimentation group was not significant for any of them.

The effect of combinations and models on gluten strength, computed as the difference between the SDS-value of the accessions carrying it and all the remainder, was calculated for landraces and modern varieties separately (Table 5). None of the combinations or models had a significant effect on the gluten strength of modern varieties which, on average, had an SDS-sedimentation test value of 10.3 ml. However, two combinations and the two LMW models were significant among the landraces. The 13 landraces or old varieties (8.39%) that carried combination 21 (Table 2), had a SDS-value 1.30 ml higher than the remainder landraces. Similarly, the SDS-value of the 7 landraces carrying combination 26, absent in modern varieties and only differing from the previous one by the presence of the *Glu-A3* null allele, was 1.19 ml greater than that of the remainder landraces (Table 5). Combination aaa (bands 6, 2+4+15+19 and 12 at *Glu-A3*, *Glu-B3* and *Glu-B2* loci, respectively), included in combination 21, was the only significant and positively affecting gluten strength at the LMW-2 model, while model LMW-2^- ,included in combination 26, also significantly increased the gluten strength of landraces (Table 5). Only three among the rare combinations found in the landraces had a significant effect on gluten strength and the three drastically increased it. Combination 95, which in average increased the SDS-value in 2.33 ml, was found in four Turkish landraces (Table 3). Combinations 45 and 123, which showed very high SDS-sedimentation values, were only detected in one landrace each, the first in the French variety 'Trigo Glutinoso' and the last one in an Egyptian accession (PI-366109) (Table 3).

Table 2. Allelic/banding pattern combinations and LMW models identified and their frequency in the 155 landraces and 18 modern varieties. Known combinations are in bold type.

Combination number	High Molecular Weight		Low Molecular Weight			Frequency (%)	
	Glu-A1	*Glu-B1*	*Glu-A3*	*Glu-B3*	*Glu-B2*	Landraces	Modern
1	**null**	**7+8**	**6**	**2+4+15+19**	**12**	0.65	38.89
2	null	7+8	6	2+4+15+18	12	0.65	-
3	null	7+8	6	4+15+19	12	-	5.56
4	null	7+8	6	4+16+19	12	0.65	-
5	null	7+8	6	2+4+14+15+16	null	0.65	-
6	null	7+8	null	1+3+13+16	null	0.65	-
7	null	7+8	null	2+4+15+18	null	0.65	-
8	null	7+8	11	3+14+16	null	0.65	-
9	null	7+8	11	2+4+14+15+16	null	0.65	-
10	null	7+8	5	1+15+17	null	0.65	-
11	null	7+8	5	3+7+16+19	null	0.65	-
12	null	7+8	5	9+13+17+18	12	0.65	-
13	null	7+8	5+11	14+16	12	0.65	-
14	null	7+8	5+11	14+17	null	0.65	-
15	**null**	**7+8**	**6+11**	**2+4+15+19**	**null**	-	11.11
16	null	7+8	6+20	2+4+15+19	null	1.29	-
17	null	7+8	11+10	7+13+19	null	0.65	-
18	null	20	6	2+4+13+17	null	0.65	-
19	null	20	6	2+4+13+16+17	null	0.65	-
20	null	20	6	2+4+15+16	12	0.65	-
21	**null**	**20**	**6**	**2+4+15+19**	**12**	8.39	11.11
22	null	20	6	2+4+16+17	12	0.65	-
23	null	20	6	2+4+16+19	12	0.65	-
24	null	20	6	2+4+17	12	0.65	-
25	null	20	null	2+4+15+16	12	0.65	-
26	**null**	**20**	**null**	**2+4+15+19**	**12**	4.52	-
27	null	20	null	2+4+16+19	null	0.65	-
28	null	20	11	2+4+14+18	null	0.65	-
29	null	20	11	2+4+15+19	null	0.65	-
30	null	20	11	9+13+17	12	0.65	-
31	null	20	5	3+7+14+15+19	null	0.65	-
32	null	20	5+11	14+17	12	0.65	-
33	null	20	5+11	3+15+19	null	-	5.56
34	null	20	6+11	2+4+14+18	null	0.65	-
35	null	20	6+11	4+15+19	null	-	5.56
36	null	20	6+11	2+4+15+19	null	0.65	-
37	null	20	10	14+18	null	0.65	-
38	**null**	**6+8**	**6**	**2+4+15+19**	**12**	2.58	11.11
39	null	6+8	6	2+4+15+18	12	1.29	-
40	null	6+8	6	2+4+15+18	null	0.65	-
41	null	6+8	6	4+15+19	null	0.65	-
42	null	6+8	null	2+4+15+19	12	1.29	-
43	null	6+8	null	2+4+15+19	null	0.65	-
44	null	6+8	null	1+3+14+18	null	0.65	-
45	null	6+8	11	2+4+15+18+19	null	0.65	-
46	null	6+8	11	3+14+16	null	0.65	-
47	null	6+8	5	14+17	12	0.65	-
48	null	6+8	5	3+9+14+17+18	null	0.65	-
49	null	6+8	5+10+11	3+14+16+18	null	0.65	-
50	null	6+8	5+11	13+16	null	0.65	-
51	null	6+8	5+11	3+15+18+19	null	0.65	-
52	null	6+8	5+11	14+16+19	null	0.65	-
53	null	6+8	6+10	2+4+13+16+19	null	0.65	-
54	**null**	**6+8**	**6+11**	**2+4+15+19**	**null**	0.65	5.56
55	null	6+8	6+11	2+4+13+15+18	null	0.65	-
56	null	6+8	6+20	2+4+14+15+19	null	0.65	-
57	null	6+8	10	2+4+14+17+18	null	0.65	-
58	null	6+8	10+11	14+17+18	null	0.65	-
59	null	6+8	11+20	14+15+18+19	null	0.65	-
60	null	6+ + 17	5+11	14+17	null	0.65	-
61	null	6+18	5+11	14+17	null	0.65	-
62	null	6+18	6	2+4+15+16	12	0.65	-
63	null	6+18	11	3+13+15+19	null	0.65	-
64	null	6+22	6	2+4+15+19	null	0.65	-
65	null	7+8 -	5+11	14+16	12	0.65	-
66	null	7+17	6+11	4+15+19	null	-	5.56
67	null	7+22	6	2+4+16+17	null	0.65	-
68	null	13+16	6	3+15+19	null	0.65	-
69	null	13+16	11	1+3+14+18	null	0.65	-
70	null	13+16	null	2+4+15+18	null	0.65	-
71	null	13+16	null	2+4+15+19	null	0.65	-
72	null	14+ + 18	null	2+4+16+17	12	0.65	-
73	null	14+15	6	2+4+15+19	12	0.65	-
74	null	14+15	11	2+4+14+18+19	null	0.65	-
75	null	19+22	10	3+8+14+17	12	0.65	-
76	null	19+22	6+11	16+18	null	0.65	-
77	null	20+18	6+11	2+4+7+13+17+19	null	0.65	-

Profile	High Molecular Weight		Low Molecular Weight			Frequency (%)	
	Glu-A1	*Glu-B1*	*Glu-A3*	*Glu-B3*	*Glu-B2*	Landraces	Modern
78	1	7+8	6	2+4+15+18	12	0.65	-
79	1	7+8	5+11	3+9+15+18	null	0.65	-
80	1	7+8	10+11	3+14+17+19	null	0.65	-
81	1	7+8	10+11	15+18	null	0.65	-
82	1	20	6	2+4+15+18	12	0.65	
83	1	20	6	2+4+15+19	12	0.65	
84	1	20	6	2+4+15+19	null	0.65	
85	1	20	6	2+4+14+15+19	null	0.65	
86	1	20	null	3+15+17	12	0.65	-
87	1	20	null	2+4+15+18	null	0.65	-
88	1	20	null	2+4+15+19	12	0.65	-
89	1	20	null	2+4+15+19	null	0.65	-
90	1	20	null	2+4+15+17+19	null	0.65	-
91	1	20	11	2+4+16+17+18	null	0.65	-
92	1	20	5+10	9+15+17+19	null	0.65	-
93	1	20	5+10+11	15+16+18	null	0.65	-
94	1	20	5+11	16+17+19	12	0.65	-
95	1	20	6+20	2+4+15+19	12	2.58	-
96	1	20	10+11	15+18	null	0.65	-
97	1	20	20	2+4+15+19	12	0.65	-
98	1	20	20	2+4+15+19	12	0.65	-
99	1	6+8	null	2+4+15+19	null	0.65	-
100	1	6+8	11	2+4+13+17+18	null	0.65	-
101	1	6+8	5	1+15+17+18	12	0.65	-
102	1	6+8	5+10+11	16+19	null	1.29	-
103	1	7	null	2+4+15+19	null	0.65	-
104	1	13+19	6+11	2+4+15+18+19	null	0.65	-
105	1	13+18	5	14+15+17	12	0.65	-
106	1	14	5+11	1+14+17	null	0.65	-
107	1	19+22	10	15+16+17	12	0.65	-
108	1 '	13+16	5+11	13+19	null	0.65	-
109	1/2**	7+17	6+11	2+4+15+18	null	0.65	-
110	2*	7+8	5+11	9+15+18	null	0.65	-
111	2*	20	null	8+13+14+16	null	0.65	-
112	2*	6+8	10	2+4+14+17	null	0.65	-
113	2*	6+8	6	2+4+15+19	null	0.65	-
114	2*	6+8	null	2+4+15+19	null	0.65	-
115	2*	6+17	5+10+11	13+14+16	null	0.65	-
116	2*	6+17	5	9+15+17+18	null	0.65	-
117	2*	6+18	5+11	14+17	12	0.65	-
118	2*	7	6	2+4+15+19	null	0.65	-
119	2*	7	5	1+13+14+17	null	0.65	-
120	2*	7	5	3+13+18	null	0.65	-
121	2*	7	6+10	2+4+17	12	0.65	-
122	2*	7	null	2+4+15+18	null	0.65	-
123	2*	7+17	6	2+4+14+15+18	null	0.65	-
124	2*	7+17	5	1+3+14+15+18	null	0.65	-
125	2*	7+17	5	1+3+13+14+17	null	0.65	-
126	2*	7+17	6+11	14+17	null	0.65	-
127	2*	7+17	11	14+17	null	0.65	-
128	2*	7+17	null	1+7+15+16	null	0.65	-
129	2*	14+ + 18	null	2+4+9+13+17	null	0.65	-
130	2*	19+8	6	2+4+13+19	null	1.29	-
131	2*	19+8	11	1+3+14+15+17	null	0.65	-

Model	Combination	*Glu-A3*	*Glu-B3*	*Glu-B2*	Landraces	Modern
LMW-2	aaa	6	2+4+15+19	12	12.90	61.11
	dab	6+11	2+4+15+19	null	1.29	16.67
LMW-2-	haa	null	2+4+15+19	12	6.45	-

The frequency of known allelic/banding pattern combinations and models and that of the new combinations with significant effect on gluten strength was calculated for each of the five gluten strength groups considered in the ANOVA. All modern varieties had SDS values ≥9. Among them, 'Claudio', 'Meridiano' and 'Ocotillo' had the highest gluten strength and a common LMW-2 model (Table 6). The only difference between combinations 1 (present in 'Claudio' and 'Meridiano') and 38 (present in 'Ocotillo') was the banding pattern at *Glu-B1* locus, that is 7+8 at combination 1 and 6+8 at combination 38 (Table 2). Four among the modern varieties with very high gluten strength had combinations previously described (Table 6), while new combinations were found in the varieties 'Boabdil', 'Astigi 'and 'Ancalei'. The pattern of 'Svevo' at LMW-2 model differed from that of 'Simeto', 'Sula' and 'Bolo' in the banding pattern/alleles at *Glu-A3* and *Glu-B2* loci. The variety 'Arment' was, among the ones showing high gluten strength, the only one carrying a novel combination (number 33 in Table 2), while the most frequent in this group was combination 1. 'Amilcar' and 'Vitronero' showed a different LMW-2 pattern than the remainder varieties of this group (Table 6).

None of the combinations significantly increasing gluten strength were present in landraces with SDS values lower than 7 ml (Table 6). In addition, known combinations 21 and 26 were also missing in the five landraces with outstanding SDS values. Combination 21 was detected in 2 (14.3%) of the 14 landraces with very high gluten strength, in 9 (29%) of the 31 landraces with high gluten strength and only in 2 (2.8%) of the 71 accessions with medium gluten strength. One landrace (7.1%) of the 14 having very high SDS value carried combination number 26, which was also present in 4 (12.9%)and 2 (2.8%),respectively, of the landraces with high and medium gluten strength. Unknown combinations 45, 95 and 123were each identified in one (20%) of the 5 landraces with outstanding SDS-values. Combination 95 was also present in one (7.1%) and 2 (6.5%) of the landraces with very high and high gluten strength, respectively (Table 6). LMW models were missing in landraces with outstanding gluten strength. Model LMW-2 was the most frequent in landraces with high gluten strength. Model LMW-$2^{-}$was present in 2 (14.3%), 4 (12.9%) and 4 (5.6%) landraces with very high, high and medium gluten strength, respectively and it was not detected in any landrace with low gluten strength (Table 6). The genetic diversity indices calculated for each gluten strength group were greater for landraces than for modern varieties, showing the outstanding modern varieties the lowest overall genetic diversity (bottom part of Table 6).

Table 3. Landraces and modern varieties in which the allelic/banding pattern combinations were identified. See Table 2 for combinations description.

Combination number	Landraces*	Modern	Combination number	Landraces
1	JO: Salti na Zinia	Claudio, Gallareta, Jupare, Meridiano, Simeto, Sula, Vitron	67	IS: Etith
2	TUR: BGE-019263		68	SP: Arisnegro de Tenerife
3		Ancalei	69	GR: IG-96851
4	EG: PI-60726		70	AL: IG-92967
5	SY: IG-95841		71	SP: Farto cañifino
6	SY: IG-95931		72	SP: Pinet
7	IT: Cicirelo		73	AL: IG-92895
8	FR: Tounse		74	CY: Muri
9	EG: Sinai No.8		75	PO: Espanhol
10	IS: Juljulith		76	MO: Cobros
11	LE: PI-182666		77	MO: Maghoussa Amizmiz
12	SP: Candeal de Salamanca		78	CY: IG-82549
13	IT: Aziziah 17/45		79	SP: Blanco de Corella
14	LI: Tripshiro		80	MA: PI-405908
15		Amilcar, Svevo	81	GR: Rapsani
16	IS: PI-572901, PI-572903		82	CY: Vroulos
17	IS: Abu Fashit		83	TUR: Mindium
18	JO: Safra Maan		84	SP: Raspinegro de Alcalá Guadaira
19	JO: Zoghbiyeh Safra		85	EG: Reading
20	LE: PI-182667		86	SE: Belgrade 9
21	AL: Dur de Medeah, IG-93030, IG-93621 IT: Capeiti 8, Hymera, Razza 96, Senatore Capelli LE: IG-84856; MO: Mahmoudi C, Morocco TU: Biskri, Realforte; TUR: BGE018351	Bolo, Hispasano	87	IT: Balilla Falso
22	LE: Hourah		88	TUR: BGE019266
23	MO: Haj Mouline		89	PO: Tremes rijo
24	JO: PI-420946		90	MA: PI-345249
25	JO: Safra Jerash		91	SE: PI-378303
26	IT: Carlo jucci, Razza 208, Trinakria MO: Merzaga, Oned Zenati, Red Beard SP: Recio de Cañete		92	MA: PI-362629
27	LE: PI-182671		93	MA: PI-374658
28	SP: Claro de Balazote		94	MA: PI-345260
29	TUR: BGE-018354		95	TUR: BGE018353, BGE019262, BGE019264, BGE019265
30	SE: PI-585195		96	GR: Mavraani
31	MO: Maghoussa		97	TUR: BGE-018192
32	JO: Zugbieh Sutra		98	TUR: BGE-019270
33		Arment	99	PO: Dezassete
34	SP: Blancal		100	CR: PI-435057
35		Astigi	101	CR: PI-345441
36	SP: Recio de Almería		102	SP: Griego de Baleares, Gros de Cerdaña
37	BU: Lozen 76		103	MON: PI-435024
38	AL: IG-94009; LE: Reyati PO: Raspinegro; SP: Fartó	Ocotillo, Senadur	104	PO: Lobeiro de grao escuro
39	IT: Carlantino; SP: Enano de Andújar		105	MO: Zoco Yebel Hebil
40	SP: Ruso		106	SP: Rubio de Montijo
41	TU: Hamira		107	CR: PI-345442
42	MO: Ble Dur 250; TU: Louri AP 5		108	SP: Pisana cañihueca
43	TU: Souri		109	EG: Giza 2
44	IT: IG-83905		110	PO: Alentejo
45	FR: Trigo Glutinoso		111	BU: Tchirpan
46	FR: Lumillo		112	SP: Verdial
47	PO: Amarelo Barba Preta		113	SP: Basto Duro
48	SP: Colorado de Jerez		114	CR: Dalmatia 1
49	SP: Azulejo de Villa del Río		115	SY: IG-95812
50	EG: PI-60727		116	MA: PI-362638
51	PO: Raposinho		117	JO: Horani Howawi
52	FR: De Santa Marta		118	CR: Dalmatia 3
53	SP: Raspinegro Canario		119	SP: Blanquillón de Boñar
54	SP: Rubio de Miajadas	Vitronero	120	MON: PI-435043
55	SP: Alonso		121	SP: Heraldo del Rhin
56	PO: Marques		122	MON: PI-345357
57	SP: Entrelargo de Montijo		123	EG: PI-366109
58	FR: Rubio enlargado d'Atlemteje		124	EG: PI-576803
59	FR: Beladi Rouge		125	EG: Girgeh
60	IS: Hati		126	EG: PI-113397
61	JO: Harani Auttma		127	EG: PI-559973
62	SY: IG-95847		128	PO: Durazio Rijo Glabro
63	LE: PI-182669		129	SP: Blanquillo
64	PO: Anafil		130	MON: PI-435034, PI-435038
65	IT: IG-83920		131	EG: Mishriki
66		Boabdil		

* Country codes: AL: Algeria, BU: Bulgaria, CR: Croatia, CY: Cyprus, EG: Egypt, FR: France, GR: Greece, IS: Israel, IT: Italy, JO: Jordan, LE: Lebanon, LI: Libya, MA: Macedonia, MON: Montenegro, MO: Morocco, PO: Portugal, SE: Serbia, SP: Spain, SY: Syria, TU: Tunisia, TUR: Turkey. Accessions codes: BGE-numbers are codes from the Centro de Recursos Fitogenéticos (I.N.I.A., Madrid), IG-numbers are codes from ICARDA Germplasm Bank. PI-numbers are codes from USDA Germplasm Bank.

Table 4. Genetic diversity indices calculated for the allelic/banding pattern combinations identified in 155 landraces and 18 modern varieties, and per country of origin of the landraces.

	D
Modern	0.80
Landraces	0.98
Algeria	0.67
Bulgaria	0.50
Croatia	0.80
Cyprus	0.67
Egypt	0.91
France	0.83
Greece	0.67
Israel	0.78
Italy	0.82
Jordan	0.88
Lebanon	0.86
Libya	0.00
Macedonia	0.83
Montenegro	0.72
Morocco	0.84
Portugal	0.91
Serbia	0.67
Spain	0.96
Syria	0.75
Tunisia	0.72
Turkey	0.81

The cluster of the countries based on the frequencies of the LMW models identified in the collection (Figure 1) grouped them in three branches corresponding to high frequencies (branch A), low frequencies (branch B) and absence of the LMW models (branch C). The highest frequencies were recorded in Algerian (83.3%) landraces and modern varieties (77.8%), in both of which only model LMW-2 was present. Landraces from Italy, Tunisia and Morocco shared both models, with total frequencies ranging from 54% to 60%. Model LMW-2^- was also missing in three of the countries clustered in branch B (Jordan, Portugal and Lebanon), in which the frequencies of LMW-2 model ranged from 9% to 28%. Both models were present in Spanish and Turkish landraces, with total frequencies of 14% and 27%, respectively. The mean SDS-sedimentation values of the genotypes included on each cluster branch were 9.2, 8.2 and 8.0 for branches A, B and C, respectively.

Table 5. Known allelic/banding pattern combinations and models and unknown combinations with significant effect on SDS-sedimentation volume (ml) in the collection of 155 landraces and 18 modern cultivars and their frequencies on each population.

		Landraces				Modern			
	Combination number	Frequency (%)	Present (ml) (a)	Absent (ml) (b)	Main effect (ml) (a-b)	Frequency (%)	Present (a)	Absent (b)	Main effect (a-b) (ml)
Known combinations	1	0.65	8.11	8.27	-0.16	38.89	10.38	10.24	0.14
	15					11.11	10.04	10.32	-0.29
	21	8.39	9.45	8.16	1.30 **	11.11	10.38	10.28	0.10
	26	4.52	9.40	8.21	1.19 *				
	38	2.58	8.62	8.26	0.37	11.11	10.42	10.28	0.14
	54	0.65	8.59	8.26	0.33	5.56	9.63	10.33	-0.70
	LMW-2	14.19	9.07	8.13	0.94 **	77.78	10.28	10.33	-0.05
	aaa	12.90	9.04	8.15	0.89 *	61.11	10.39	10.15	0.24
	dab	1.29	9.41	8.25	1.16	16.67	9.90	10.37	-0.47
	LMW-2-	6.45	9.33	8.19	1.14 *				
Unknown combinations	45	0.65	11.56	8.24	3.31 *				
	95	2.58	10.53	8.20	2.33 **				
	123	0.65	11.68	8.24	3.44 *				

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

Discussion

Although the number of modern varieties included in this study was much lower than that of landraces, they were a representative set of the durum grown variability in the Mediterranean Basin. The results of a previous study (Royo *et al.*, 2010), conducted with a collection of 191 accessions representing the existing genetic diversity within the Mediterranean Basin, demonstrates that, according to their genetic structure assessed based on SSR markers, the modern varieties included in this research represent different genetic groups. Thus, varieties ‘Gallareta’, ‘Sula’ and ‘Astigi’ joined in the mentioned study a subpopulation related to the CIMMYT hallmark founder ‘Altar 84’, whose derivatives have been largely grown under different names in North Africa. On the other hand, ‘Vitron’ and ‘Meridiano’ belong to a different group genetically close to the CIMMYT founder ‘Yavaros 79’, released with different names in several Mediterranean countries. ‘Simeto’ was found to be part of an Italian genetic pool formed by genotypes derived from the founder variety ‘Valnova’, and ‘Boabdil’ was assigned within the Spanish genetic pool (Royo *et al.*, 2010). In the same study the varieties ‘Claudio’, ‘Svevo’, ‘Senadur’ and ‘Bolo’ could not be assigned to any structured subpopulation, thus suggesting large genetic diversity among them. Actually, the alleles or banding patterns that would be present in the overall modern Mediterranean germplasm are highly likely to be represented within the group of 18 modern varieties included in this research (Nazco *et al.*, submitted).

The genetic diversity found in landraces was much greater than that of modern varieties, but there were big differences between countries. Our results confirm the conclusions of previous studies reporting large genetic diversity in landraces from the Iberian Peninsula (Moragues *et al.*, 2006c), and low genetic diversity in Bulgarian and Algerian durum landraces (Moragues *et al.*, 2006c; Hamdi *et al.*, 2010). Low levels of genetic diversity may have resulted from a process of environmental adaptation or by farmers' selection. The 32 haplotypes found at HMW-GS loci were due to the combination of 5 and 20 allelic variants at *Glu-A1* and *Glu-B1* loci, respectively, while the 98 detected at LMW-GS loci were due to 15 allelic/banding patterns at *Glu-A3* locus, 72 at *Glu-B3* and 2 at *Glu-B2* (Nazco *et al.* submitted). This variability is much higher than the reported by previous studies conducted in durum wheat (Turchetta *et al.*, 1995; Cherdouch et.al, 2005; Moragues *et al.*, 2006c). The huge variability of glutenin combinations found in landraces enhances their value as a germplasm source to broaden the genetic background of durum wheat breeding programs.

The 131 different allelic/banding pattern combinations recorded in the whole germplasm collection, representing 76% of the 173 theoretical possible haplotype combinations, reveals large genetic variability for glutenin composition. The greatest number of combinations detected in landraces can not only be attributed to their quantity in comparison with the lower number of modern cultivars, given that 93% of the HMW/LMW-GS combinations identified in landraces were present in a single genotype, while this percentage decreased until 55% in modern varieties.

Combinations of LMW-GS corresponded to 2 of the five LMW models described by Nieto-Taladriz *et al.* (1997). The number of LMW models and the percentage of landraces having them in this study (20.6%) were lower than those found in previous studies conducted with Mediterranean landraces (Moragues *et al.*, 2006c; Carrillo et al 1990; Carrillo *et al.*, 1995). This could be due to the fact that our study included landraces from Croatia, France, Israel, Jordan, Libya, Macedonia, Montenegro and Serbia, not represented in the mentioned studies, in which LMW models were absent. Model LMW-2 (aaa) was frequent in landraces from Lebanon, Turkey and Jordan, thus suggesting that the origin of this combination could be in one of these countries. On the other hand, model LMW-2 (dab) was merely detected in two Spanish landraces (Recio de Almería and Rubio de Miajadas), likely resulting from a mutation at *Glu-A3* locus followed by local selection or specific adaptation. The presence of LMW-2^{-} in a Turkish landrace supports the hypothesis of this combination having its origin in Turkey.

Table 6. Frequency (%) of known allelic/banding pattern combinations and models and unknown combinations with significant effect on gluten strength in genotypes with outstanding ($SDS \geq 11$), very high ($10 < SDS < 11$), high ($9 \leq SDS \leq 10$), medium ($7 \leq SDS < 9$) and low ($SDS < 7$) SDS-sedimentation test values (ml) and genetic diversity indices for the same groups. The number of genotypes in each strength group is shown between parenthesis and the name of the modern varieties corresponding to each combination and model are also indicated.

	Combination number	Landraces					Modern							
		Outstanding (5)	Very high (14)	High (31)	Medium (71)	Low (34)	Outstanding (3)		Very high (7)		High (8)		Medium (0)	Low (0)
Known combinations and models	1	0.0	0.0	0.0	1.4	0.0	66.7	Claudio, Meridiano	28.6	Simeto, Sula	37.5	Jupare, Gallareta, Vitron	-	-
	15	-	-	-	-	-	0.0		14.3	Svevo	12.5	Amilcar	-	-
	21	0.0	14.3	29.0	2.8	0.0	0.0		14.3	Bolo	12.5	Hispasano	-	-
	26	0.0	7.1	12.9	2.8	0.0	-		-		-		-	-
	38	0.0	0.0	3.2	4.2	0.0	33.3	Ocotillo	0.0		12.5	Senadur	-	-
	54	0.0	0.0	0.0	1.4	0.0	0.0		0.0		12.5	Vitronero	-	-
	LMW-2	0.0	21.4	32.3	11.3	2.9	100		57.1		87.5		-	-
	aaa	0.0	66.7	100.0	87.5	100.0	100	Claudio, Meridiano, Ocotillo	75.0	Simeto, Sula, Bolo	71.4	Jupare, Hispasano, Gallareta, Senadur, Vitron	-	-
	dab	0.0	33.3	0.0	12.5	0.0	0.0		25.0	Svevo	28.6	Amilcar, Vitronero	-	-
	LMW-2$^-$	0.0	14.3	12.9	5.6	0.0	-		-		-		-	-
Unknown combinations	45	20.0	0.0	0.0	0.0	0.0	-		-		-		-	-
	95	20.0	7.1	6.5	0.0	0.0	-		-		-		-	-
	123	20.0	0.0	0.0	0.0	0.0	-		-		-		-	-
Genetic diversity indices (D)		0.80	0.92	0.88	0.98	0.97	0.44		0.82		0.78		-	-

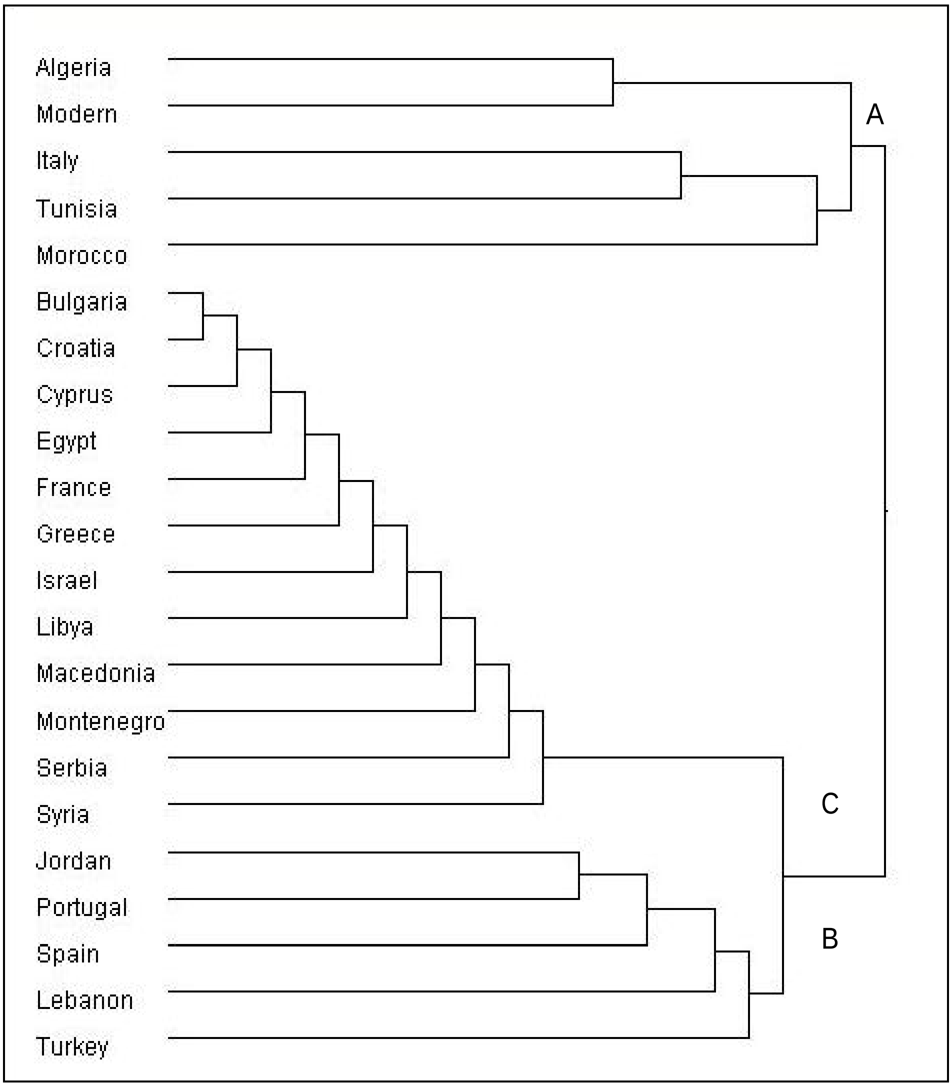

Figure 1. Cluster analysis based on LMW models frequency in modern varieties and by country for landraces.

In agreement with previous studies (Liu and Shepherd, 1996; Cherdouh *et al.*, 2005; Zarkti *et al.* 2010), cluster analysis grouped modern varieties with genotypes from Italy and the three Maghreb countries due to the high frequency of LMW models type 2. A second group clustered countries in which LMW-2 models were found from medium to low frequencies (Turkey, Jordan, Lebanon, Portugal and Spain), while a third branch joined 12 countries in which LMW-2 models were absent. The presence of LMW models in landraces from eastern (Jordan, Lebanon and Turkey) and western (Tunisia, Algeria, Morocco, Italy, Spain and Portugal) Mediterranean countries, and their absence in central ones (all Balkan Peninsula countries plus Libya and Egypt) matches up with the hypothesis of durum wheat being introduced in western Mediterranean countries from germplasm arrived by a maritime route from Turkey to Italy, and from there spread to North Africa and the Iberian Peninsula (Feldman, 2001; Mackey, 2005; Moragues *et al.*, 2006c).

Our results associated the presence of LMW-2 models to greater gluten strength in durum wheat, in agreement with previous studies showing the favorable effects of LMW-2 on durum wheat quality (Pogna *et al.*, 1990; Masci *et al.*, 2000; Bechere *et al.*, 2002; Raciti *et al.*, 2003). Liu and Shepherd (1996) related a high frequency of LMW-2 models with a deliberate selection for better quality. The high frequency of LMW models type 2 found in this study in genotypes from Italy and the Maghreb countries may be a consequence of local preferences in areas of great consumption of durum wheat products, such as pasta in Italy and couscous in North African countries.

The *Glu-A1* null allele was the most frequently associated with LMW models. For *Glu-B1* locus bands 7+8, 20 and 6+8 were the most frequently related with both combinations of the LMW-2 model. In this study the combination LMW-2 (aaa) with the null allele at *Glu-A1* locus and the 7+8 at *Glu-B1* (number 1) was the most frequent in modern varieties and was identified in about 39% of them. Although the effect of this combination on gluten strength was not statistically significant, all modern varieties carrying it had high to outstanding gluten, thus supporting the results of previous studies identifying it as suitable for high gluten strength (Peña *et al.*, 1995; Boggini *et al.*, 1997; Bechere *et al.*, 2002; Raciti *et al.*, 2003; Sissons *et al.*, 2005). Our results disagreed with previous studies (Carrillo *et al.*, 1990; Brites and Carrillo, 2001; Raciti *et al.*, 2003; Sissons *et al.*, 2005) as they showed that the presence of band 20 at *Glu-B1* locus was not detrimental for gluten strength. Actually, three of the combinations resulting in a significant enhancement of gluten strength (numbers 21, 26 and 95) had band 20 at *Glu-B1*. Nevertheless, in two of them band 20 was accompanied by LMW-2 models, and this could be the reason of this band not having a negative effect on gluten strength, as reported by Raciti *et al.* (2003). It can be hypothesized that in the case of combination 95 the putative negative effect of band 20 was overcame by the positive effect of banding pattern 2+4+15+19 at *Glu-B3* as reported by Nieto-Taladriz *et al.* (1997) and Carrillo *et al.* (2000). These results point out the interaction existing between HMW-GS and LMW-GS in the co-formation of the gluten complex (Peña and Pfeiffer, 2005; Ruiz and Carrillo, 1995), and the dominant effect of LMW-GS over the effect of HMW-GS, reported by previous studies (Boggini *et al.*, 1995; Edwards *et al.*, 2003). The discrepancies between studies addressing the effect of alleles at *Glu-B1* locus on grain quality led some authors to discourage the use of alleles at this locus to select for durum wheat quality (Sissons, 2008). Moreover, the effect of the alleles at *Glu-B1* locus seems also to depend on the allelic pattern at *Glu-B3* (Martinez *et al.*, 2005).

The ANOVA for gluten strength showed that the component of variation due to the genetic effect was much higher than the component due to the environment. This result confirms the large genetic control of this trait in durum wheat as reported by

Ames *et al.* (1999) and Taghouti *et al.* (2010). Total variance was mostly explained (67%) by differences in SDS-sedimentation test values between groups, which in turn, were homogeneous regarding the gluten strength of the genotypes included on them. These results indicate that the grouping of the accessions in five gluten strength clusters was appropriate.

The lack of significance of any of the combinations identified in modern varieties on their gluten strength was probably consequence of all them having SDS-sedimentation test values >9, which resulted in small differences between the SDS-values of the genotypes carrying or not a given combination. Among the landraces and old varieties there were only two known combinations, both involving LMW models, that significantly improved the gluten strength of the genotypes carrying them. Their presence increased the SDS-sedimentation test values by about 14.6% (from around 8.2 to about 9.4). The first of these combinations (number 21: null allele at *Glu-A1* locus, band 20 at *Glu-B1* locus and bands 6, 2+4+15+19 and 12 at *Glu-A3*, *Glu-B3* and *Glu-B2* loci, respectively), which included the LMW-2 (aaa) model, was present in 11.1% of modern varieties and about 8.4% of landraces and old Italian varieties. The second combination (number 26 only differing from the previous one by the presence of the null allele at *Glu-A3* locus), which included the LMW-2^- (haa) model, was absent in modern varieties and was found in 7 landraces and old Italian varieties, representing the 4.5% of them. Despite LMW-2 (dab) increased the SDS-sedimentation value by about 14%, its effect was not statistically significant, probably due to the fact that it was only detected in two Spanish landraces.

This study identified LMW-2 (aaa) in three modern Italian varieties (‘Simeto’, ‘Meridiano’ and ‘Claudio’), and LMW-2 (dab) in ‘Svevo’, another modern Italian variety. The presence of LMW-2 (aaa) in ‘Simeto’ and ‘Meridiano’ was probably consequence of their parental lines carrying it. The original source was likely the Algerian landrace ‘Jean Retifah’, one of whose lines was selected and released in Italy in 1915 as ‘Senatore Capelli’ which, according to our results, has LMW-2 (aaa). This variety, also known as ‘Capelli’, was the parental line most used in crosses in Italy during the first half of the twentieth century (De Cillis, 1942), thus being an ancestor of many Italian varieties, some of them included in this study and identified as having LMW-2 (aaa), such as ‘Capeiti 8’ (derived from the cross Senatore Capelli/Eiti), and the modern varieties ‘Simeto’ (Capeiti 8/Valnova) and ‘Meridiano’ (Simeto/WB881// Duilio/F21). Accordingly, LMW-2^- (haa) in ‘Trinakria’ (derived from the cross B14/Capeiti 8) could not come from Capeiti 8, but probably from the parent line B14. LMW-2 (aaa) in ‘Claudio’ (CIMMYT selection/ Durango//IS139b/Grazia) and LMW-2 (dab) in ‘Svevo’ (CIMMYT selection/Zenit) probably came from the CIMMYT lines used as parents, which probably incorporated LMW-2 (aaa) from the Italian germplasm used in its crossing block as parents for good quality and LMW-2 (dab) from other sources.

Our results showed that 78% of the modern varieties included in this study and the only three with outstanding gluten strength (‘Ocotillo’, ‘Claudio’ and ‘Meridiano’) had LMW-2 model, thus revealing the extensive use by breeding programs in different countries of the allele combinations with proved positive effect on gluten strength. This finding reflects the efforts made by most durum breeding programs to adapt the grain quality of the new varieties to the requirements of the pasta industry. LMW-2 was only absent in four modern varieties, one with high gluten strength (the French variety Arment), and three with very high gluten strength (the Spanish varieties ‘Ancalei’, ‘Astigi’ and ‘Boabdil’), all three containing CIMMYT germplasm on their pedigree.

This study identified three novel combinations that significantly increased the gluten strength of the landraces carrying them. Combination number 45 (null allele and band 6+8 at *Glu-A1* and *Glu-B1* loci, respectively, and bands 11, 2+4+15+18+19 and the null allele at *Glu-A3*, *Glu-B3* and *Glu-B2* loci, respectively) was identified in the French landrace ‘Trigo Glutinoso’ (PI-174699), which was reported in a previous study as having high EU quality index and a high sedimentation index calculated as the quotient between gluten strength and protein content (Nazco *et al.*, 2012). A second combination (number 123), formed by bands 2*and 7+17 at *Glu-A1* and *Glu-B1* loci, respectively and bands 6, 2+4+14+15+18 and the null allele at *Glu-A3, Glu-B3* and *Glu-B2* loci, respectively, was present in the Egyptian landrace identified in the USDA gene bank as PI-366109, which reached the highest SDS-sedimentation test value of all the genotypes included in this study (11.7 ml). This landrace had been previously reported to have very high EU quality index, protein content and sedimentation index (Nazco *et al.*, 2012). Despite they are promising these outcomes should be taken with caution since they were based on results of individual genotypes. The conclusion about the positive effect of combination 95 (bands 1 and 20 at *Glu-A1* and *Glu-B1* loci, respectively, and bands 6+20, 2+4+15+19 and 12 at *Glu-A3*, *Glu-B3* and *Glu-B2* loci, respectively) was based on the results obtained in four Turkish landraces. Among them genotypes BGE-019265 and BGE-019264 seem to be the most interesting for breeding purposes due to their outstanding gluten strength and overall quality reported in a previous study (Nazco *et al.*, 2012).

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CAPÍTULO 4

The climate of the zone of origin of Mediterranean durum wheat landraces affects their agronomic performance

Abstract

The genetic diversity of durum wheat is held by the landraces, generally considered as endemic to a particular region in which they are very well adapted. With the aim of evaluating the effect of the climate in the countries of origin on their agronomic performance, a collection of 172 durum wheat landraces from 21 Mediterranean countries was assembled and grown during three years under rainfed conditions in northeastern Spain. Average long-term climatic data of the main wheat growing areas at each country allowed identifying four climatic zones in the Mediterranean Basin, steadily varying from warm and dry to cool and wet: Z1 formed by southeastern countries, Z2 including southwestern countries plus Crete, Cyprus and Lebanon, Z3 grouping Turkey, Greece, Italy and Spain, and Z4 clustering north Balkan Peninsula countries, France, and Portugal. The climatic zone accounted for 32.8%, 28.3% and 14.5% of variation on the number of days from sowing to anthesis, plant height and grain filling rate, respectively. Landraces from countries with high solar radiation, evapotranspiration and temperature had the shortest cycle length, lowest grain filling rates and lightest grains, while abundant rainfalls during the spring in the zone of origin caused the opposite effects. Landraces from Z1 were earlier, with less biomass, more grains per unit area, longer grain filling period, lower chlorophyll content on the flag leaf one week after anthesis, lower grain filling rate, lighter grains and lower yields than the originated in other zones characterized by colder and wetter climatic conditions.

1. Introduction

Wheat is one of the founder crops of old world agriculture (Zohary and Hopf 2000), and currently the prime world crop plant. It is cultivated on 216.8 million ha worldwide in the temperate, Mediterranean-type and subtropical parts of both hemispheres (FAOSTAT 2011). Durum wheat (*Triticum turgidum* L. var. *durum*) represents about 8% of the wheat produced globally and is a major cereal crop in some regions, especially around the Mediterranean Basin. The world's durum wheat production is currently concentrated in latitudes ranging from 55°N (Canada) to 40°S (Argentina) (Palamarchuk 2005), corresponding mostly to the Mediterranean Basin, the North American Great Plains, India and the former USSR (International Wheat Council 2001).

Cultivated polyploid wheats derive from the wild tetraploid progenitor *T. dicoccoides*. Wheat domestication occurred in the Fertile Crescent between c. 12,000 and c. 10,000 years before present (Tanno and Willcox 2006). Archaeobotanical evidences suggest that wild emmer was possibly taken into cultivation independently in the southern and the northern Levant (Peng *et al.* 2011), spreading west through southern Europe and North Africa (MacKey 2005), which has been suggested to be one of the ways of introduction of durum wheat in the Iberian Peninsula (Moragues *et al.*, 2007). The genetic diversity of the species is currently located in traditional varieties, commonly referred to as landraces, which were originated with agriculture and have probably been grown for several millennia (Zeven 1998). Landraces are usually considered as endemic to a particular region in which they are very well adapted. The cultivation of local landraces was progressively abandoned from the advent, from the first decades of the 20th century, of new, improved and genetically uniform modern varieties derived from breeding programs.

It is generally accepted that during the domestication process and the spread of domesticated wheat from the Fertile Crescent, novel adaptive traits suited for the new environments were selected (Charmet 2011, Peng *et al.* 2011). Traits that facilitated harvesting and enabled colonization of new environments were probably primary targets. Among them larger seeded non-shattering plants (Fuller 2007), or fitting flowering time to the regional prevailing environmental conditions were probably critical traits in the post domestication spread of temperate cereals (Cockram *et al.* 2009). It has been suggested that many other traits, such as plant height, number of spikes and grains per plant, weight of spikes per plant, number of grains per spike and spikelet, and number of spikelets per spike, were also co-selected by ancient farmers (Peng *et al.* 2011).

The Mediterranean Basin comprises countries between about 27° to 47°N and 10°W to 37°E shoring on three continents, and a coastline of 46,000 km (http://www.fao.org/sd/ climagrimed/c_2_02.html). In this region, wheat yield is generally constrained by low and unpredictable seasonal rainfall, as well as higher temperatures towards the end of the crop cycle. In the region most rain falls during the spring, resulting in moderate stress for rainfed wheat around anthesis, which increases in severity throughout grain filling (Edmeades *et al.* 1989). According to the Koeppen's climate classification (Leemans and Cramer 1991; http://www.fao.org/sd/EIdirect/climate/EIsp0002.htm), the north and the south of the Mediterranean basin possess different climates. The predominant climates in the north part are types D (cold) and C (temperate), with average temperatures ranging between -4.5°C and 22°C, and a year rainfall between 300 and 1100 mm. In contrast, in the south of the Mediterranean Basin climate class B (dry) is the prevalent, except in some northern areas of Morocco and Tunisia that have climate type C (temperate). The south of the Mediterranean Basin is characterized by an annual evapotranspiration exceeding rainfall, and the presence of a dry season during the spring and summer. Mean temperatures are higher than in the north, and range between 10.5°C and 30.5°C, and annual precipitation ranges between 35 and 725 mm.

In this study a collection of 172 durum wheat landraces from 21 Mediterranean countries was used to study the effect of the prevalent climate in the regions in which they were collected on their agronomic performance when grown in a random Mediterranean-type environment.

2. Material and methods

2.1. Experimental setup

A collection of 172 durum wheat landraces and old varieties from 21 Mediterranean countries (Fig. 1) and 20 modern varieties, used as reference, were grown during three years in Gimenells (41°40 'N, 0°20' E, and 200 m a.s.l.) at Lleida province (north-eastern Spain). Landraces were selected ─from a larger collection comprising 231 accessions of different origin─ on the basis of their genetic variability determined by 33 SSR-markers, in order to represent the genetic diversity of ancient local durums from the Mediterranean Basin (Nazco *et al.* 2012). Seeds provided by public gene banks (Centro de Recursos Fitogenéticos INIA-Spain, ICARDA Germplasm Bank and USDA Germplasm Bank) were increased in bulks and purified. Increasing plots were planted in the same field the year previous to each experiment to ensure a common origin for the seeds of all genotypes. The modern set included Spanish, Italian, and French varieties, as well as the U.S. desert durum cultivar Ocotillo.

Experiments consisted of non-replicated plots of 6 m^2 (comprising eight 5-m rows, spaced 0.15 m apart), arranged according to a modified augmented design with three checks (cultivars Claudio, Simeto and Vitron). Sowing density was adjusted to 250 germinable seeds m^{-2}. Experiments were conducted under rainfed conditions, but the lack of rains after sowing in 2007 made necessary an irrigation to allow the germination of seeds. Climatic data were recorded by a weather station placed in the same field. Soil moisture was monitored from the seedling stage by means of soil probes (ECH$_2$O Dielectric Aquameter, Decagon Devices, Inc) located at 3 depths (0-10 cm, 10-25 cm and 25-40 cm). Experimental details are shown in Table 1. Plots were mechanically harvested at ripening. Weeds and diseases were controlled according to standard cultural practices.

2.2. Data recorded

Zadoks (Zadoks *et al.* 1974) growth stages 31 (beginning of jointing), 33 (mid jointing), 45 (booting), 55 (heading), 65 (anthesis) and 87 (physiological maturity) were determined in each plot. Samples consisting of the plants contained in a 0.5-m long row were pulled up in a central row of each plot at growth stages 21 (tillering), 31, 33 and 65, and 1-m long row was taken at GS87 (physiological maturity). 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 row had intact plant populations. In the laboratory, the number of plants, tillers and spikes contained in each sample were counted and the aerial portion was weight after being oven-dried at 70°C for 48 h. Then, crop dry weight (CDW, g m^{-2}) was calculated as the product of average dry weight per plant and the number of plants m^{-2} per sample. Fertile tillering was calculated as the quotient between number of spikes and stems per m^2. Plots were harvested mechanically at ripening, and grain yield (kg ha^{-1}) was determined for each plot and adjusted at 12 % of grain moisture. Thousand kernel weight (TKW, g) was calculated as mean weight of three sets of 100 g per plot. Grain filling rate (GFR, mg GDD^{-1}) was obtained as the quotient between average dry weight per grain and thermal-time (GDD, growing degree-days) from anthesis to maturity. Thermal-time was calculated by summing the daily values of mean temperatures minus the base temperature, considering 37°C and 0°C as upper and lower limits of temperature (Angus *et al.* 1981). Harvest index (HI) was calculated as the ratio between grain and plant weight on a whole sample basis. Plant height (cm) was measured at anthesis in ten main stems per plot, from the tillering node to the top of the spike excluding the awns. Chlorophyll content was measured on five flag leaf blades per plot one week after anthesis using a portable chlorophyll meter (SPAD-502, Soil-Plant Analysis Development Section, Minolta Camera Co., Osaka, Japan).

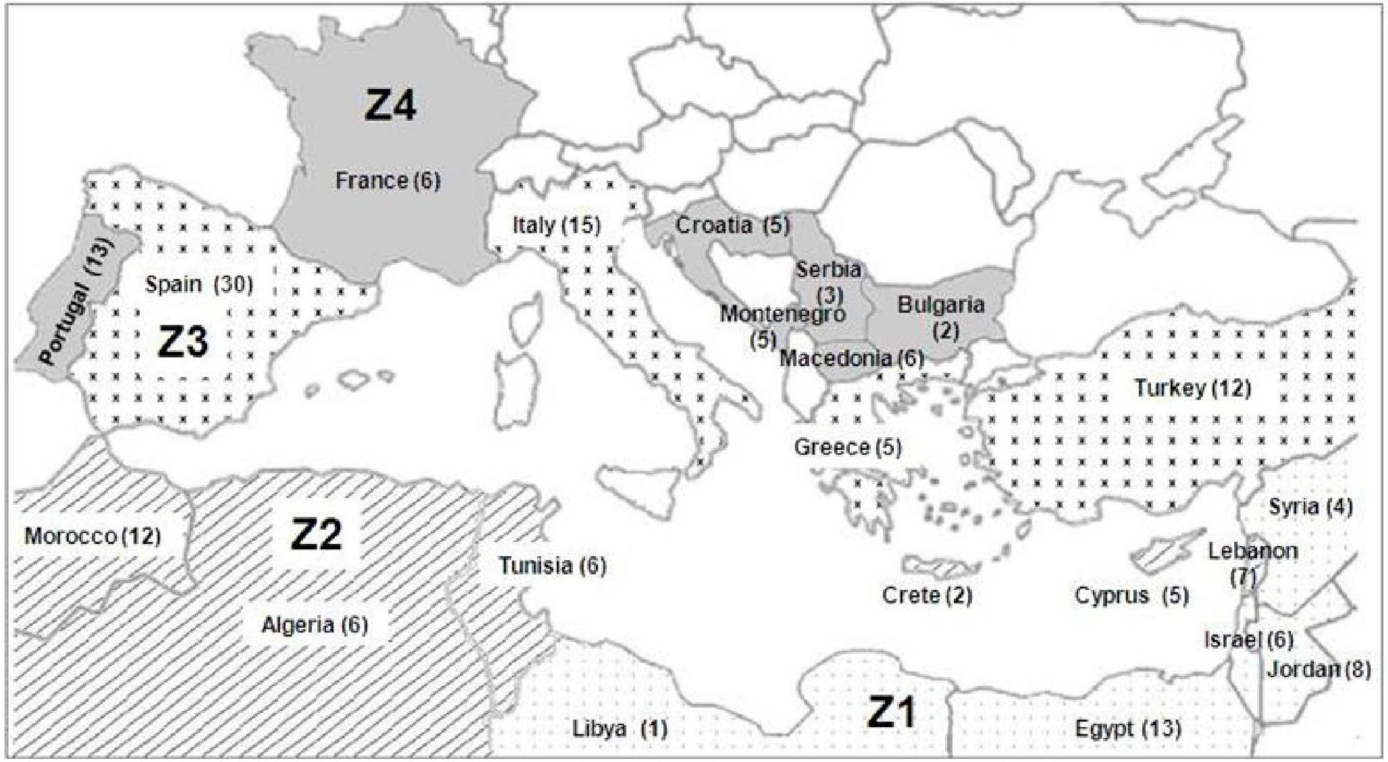

Figure 1. Countries of origin (Crete was considered as a different origin than Greece) of the 172 landraces included in the study. The number of landraces from each territory is between parentheses. The four climatic zones arisen from the PCA are also indicated by different drawings.

Table 1. Experimental details.

	2007		2008		2009	
Sowing date	21-Nov-06		20-Nov-07		20-Nov-08	
Enviromental	S-A[1]	A-M	S-A	A-M	S-A	A-M
Tmin (°C)	3	12	3	12	3	12
Tmax (°C)	14	26	14	23	13	27
Tmean (°C)	8	18	8	17	8	19
Radiation (MJ m^{-2} d^{-1})	11	25	12	21	12	24
HR (%)	79	59	74	71	76	58
ET_0 mean (mm)	252	131	277	162	266	119
Rainfall (mm)	155	37	98	141	183	7
Irrigation (mm)	0	0	50	0	0	0
Soil						
Texture	Clay-loamy		Loamy		Sandy-clay-loamy	
pH	8.1		8.3		8	
P (mg kg^{-1})	25		27		117	
K (mg kg^{-1})	163		118		590	
Organic matter (%)	2.18		2.2		3.11	
CE at 25°C (dS m^{-1})	0.42		0.29		0.34	
Fertilization (kg ha^{-1})						
N (top dressing)	32		30		20	
P_2O_5	110		128		68	
K_2O	183		213		113	
Harvest date	2-Jul-07		2-Jul-08		15-Jul-09	

[1] S-A and A-M correspond to the periods elapsed from sowing to anthesis and from anthesis to maturity, respectively, calculated from the mean experimental data across genotypes and years.

2.3. Climatic data of the territories origin of the landraces

Long-term climatic information −including a minimum of 15 years of data− of the 21 countries origin of the landraces were taken from the CLIMWAT 2.0 FAO database in combination with software CROPWAT (www.fao.org). Given its geographic location, Crete was handled as a different origin than Greece, and further in this paper it will be referred to as a different country. Daily climatic data from 3 to 7 climatic stations per country, located in the main wheat growing areas, were averaged. The data examined were: average daily minimum and maximum temperatures (Tmin and Tmax, ºC), average daily relative humidity (Rh, %), average daily solar radiation (Rad, MJ m^{-2} day^{-1}), average daily rainfall (Rain, mm), and reference evapotranspiration (ET_0, mm), calculated by the Penman-Monteith method. For each country climatic data were averaged considering two periods: November 20th - March 31st, and April 1st - June 30th, assuming that in the Mediterranean Basin the durum wheat growing periods from sowing to anthesis (S-A), and from anthesis to physiological maturity (A-M), nearly occur within them.

2.4. Statistical analysis

Field raw data were fitted to a linear mixed model with the check cultivars as fixed effects, and the row number, column number and accession as random effects (Little *et al.* 1996). Restricted Maximum Likelihood (REML) was used to estimate the variance components and to produce the Best Linear Unbiased Predictors (BLUPs) for the agronomic data of each accession each year, achieved using the MIXED procedure of the SAS-STAT statistical package (SAS Institute Inc. 2009) that was used for all the analyses.

Principal component analysis (PCA) was performed on the correlation matrix calculated with the average long-term climatic data of the 22 countries origin of the landraces, for the periods sowing-anthesis and anthesis-maturity, as indicated above. Analyses of variance were used to assess the variation of phenotypic traits measured in north-eastern Spain, representing a neutral Mediterranean environment, induced by the climate of the zone origin of the landraces arisen from the PCA. A mixed model was used with year and zone as fixed effects and the genotype within zone as random effect. Means were compared by the Duncan multiple range test at P=0.05. The relationship between long-term climatic data of the countries origin of landraces and their agronomic performance in north-eastern Spain was studied through correlation and linear regression analyses.

3. Results

Multivariate analysis of long-term climatic data

The first two axes of the PCA shown in Fig. 2, accounted for 80.7% of the total variance (axis 1, 69.4%; axis 2, 11.3 %). The eigenvectors of the various components, represented in Fig.2a, reflect the extent to which each variable weights the two components. Principal component 1 was positively related with minimum and maximum temperatures, radiation and ET_0 along the growing season, and negatively associated with the rainfall during grain filling and relative humidity, mainly after anthesis. Increases on principal component 2 were mostly related to rainfall and relative humidity from sowing to anthesis in the positive and negative directions, respectively. The cosine of the angles between vectors, which is inversely proportional to the correlation between them, indicated that strong positive correlations existed between radiation, temperatures and ET_0. On the other hand, the angles between vectors representing rainfall or relative humidity and average maximum temperature during grain filling, both close to 180º indicate negative relationships.

The points representing the countries origin of landraces, shown in Fig. 2b were grouped in four clusters along PC1. South-eastern Mediterranean countries were located in the bottom right part of the figure, thus indicating that their climate is characterized by high maximum temperatures, radiation and ET_0, and water scarcity during grain filling. On the opposite side of the figure, and with opposed climatic characteristics were placed the north Balkan countries, France and Portugal. The intermediate position between these two groups was occupied by other two clusters, one formed by the Maghreb countries plus Crete, Cyprus and Lebanon and another including Turkey, Greece, Italy and Spain. The location of Lebanon and Montenegro in the upper part of their corresponding clusters indicates that winters are particularly rainy in these countries. The average values of the climatic variables of the climatic zones represented by the clusters confirm the decreasing temperatures, radiation and ET_0, but increasing rainfall, mostly during the spring, when moving from Z1 to Z4 (Table 2).

Field testing environment

The meteorological data recorded at the experimental fields during the three years of field testing are shown in Table 1 and Fig. 3. The pattern of increasing temperatures during the spring and the uneven distribution of rainfalls properly represent a typical Mediterranean environment. The lowest temperatures were reached in December and January, and they drastically increased during the spring arriving to maximum close to 30°C in June, coinciding with the last phases of the grain filling period. Water input were 192 mm, 289 mm and 190 mm in 2007, 2008 and 2009 cropping seasons, respectively, and in 2009 soil moisture during the spring reached the lowest level of the three years (Table 1).

Phenology

The ANOVA for phenological data showed that the year, climatic zone and genotype significantly affected the phenologic development of the studied landraces (Table 3). The environmental conditions of the year accounted for most of the variability on total cycle length and grain filling duration. The percentage of the sum of squares explained by the climatic zone origin of the landraces was low for the time elapsed from sowing to early growth stages, but increased until anthesis when it accounted by about 33% of total variance. The mean values of the duration of the

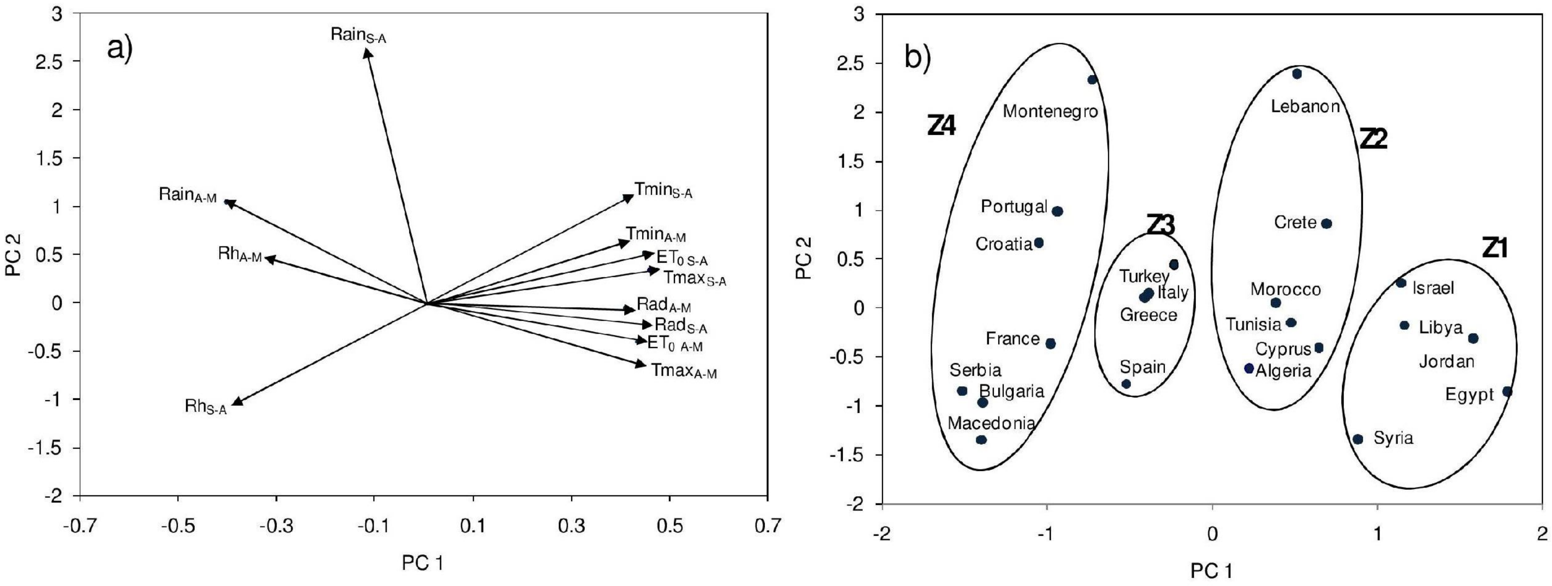

Fig. 2. Biplot of the first two axes of the principal component analysis summarizing the relationships between long-term climatic variables of the countries origin of the landraces. Fig. 2(a). Eigenvalues of the correlation matrix symbolized as vectors representing climatic variables of the period sowing-anthesis (November 20th – March 31st), and anthesis-physiological maturity (April 1st - June 30th): Tmin (average minimum daily temperature), Tmax (average maximum daily temperature), Rain (average daily rainfall), ET$_0$ (reference evapotranspiration), Rad (average daily solar radiation), Rh (average daily relative humidity). Fig. 2(b). Plot of the points corresponding to the 21 countries and Crete.

Table 2. Average long-term climatic variables of the four Mediterranean climatic zones arisen from the PCA. Tmin (average minimum daily temperature), Tmax (average maximum daily temperature), Tmean (average mean daily temperature), Rad (average daily solar radiation), Rh (average daily relative humidity), ET_0 (reference evapotranspiration), Rain (average daily rainfall). Sowing-anthesis and anthesis-maturity considered to be the periods elapsed from November 20th to March 31st, and from April 1st to June 30th, respectively.

Zone	Tmin (°C)	Tmax (°C)	Tmean (°C)	Rad (MJ $m^{-2}d^{-1}$)	Rh (%)	ET_0 (mm)	Rain (mm)
				Sowing-anthesis			
Z1	7.8 a	19.0 a	13.4 a	12.7 a	67.1 c	299 a	188 a
Z2	8.2 a	17.6 a	12.9 a	10.8 b	70.5 bc	255 a	318 a
Z3	5.3 a	13.6 b	9.5 b	8.2 c	74.2 ab	178 b	305 a
Z4	2.0 b	9.8 c	5.9 c	6.9 c	77.2 a	134 b	390 a
				Anthesis-maturity			
Z1	15.1 a	29.3 a	22.2 a	23.7 a	51.0 b	531 a	20 c
Z2	14.0 ab	25.3 b	19.7 b	22.4 a	63.0 a	401 b	60 bc
Z3	12.6 b	23.5 c	18.0 c	19.5 b	63.2 a	341 c	109 b
Z4	10.7 c	21.6 d	16.1 d	18.8 b	65.0 a	309 c	210 a

Means within columns and periods with different letters are significantly different at $P = 0.05$

different periods for the four climatic zones revealed that the number of days from sowing to the main growth stages consistently increased from Z1 to Z4, the opposite being true for the duration of the grain filling period (Table 4). The phenology of modern varieties was closer to that of landraces from Z1 than to the recorded in landraces from the remainder climatic zones.

Biomass and associated traits

The percentage of variability for CDW at different growth stages from tillering to maturity explained by the climatic zone origin of the landraces was much lower than the obtained for phenological development; it was greatest at maturity, but even so, it only accounted for about 8% of total variation (Table 3). The comparison of CDW values of the landraces from the different climatic zones indicated that, except at jointing when differences were not statistically significant, genotypes from Z1 (south-eastern Mediterranean countries) had less biomass than those of the remainder zones (Table 4). Despite CDW at tillering, jointing and anthesis did not differ significantly between Z2, Z3 and Z4, the common tendency at all growth stages was to a biomass increase when moving from Z1 to Z4. The lowest differences between zones were recorded at jointing. In spite of modern varieties having lower CDW values at the four studied growth stages than the landraces, their values were closer to those of landraces from Z1 than to the recorded in landraces from other climatic zones.

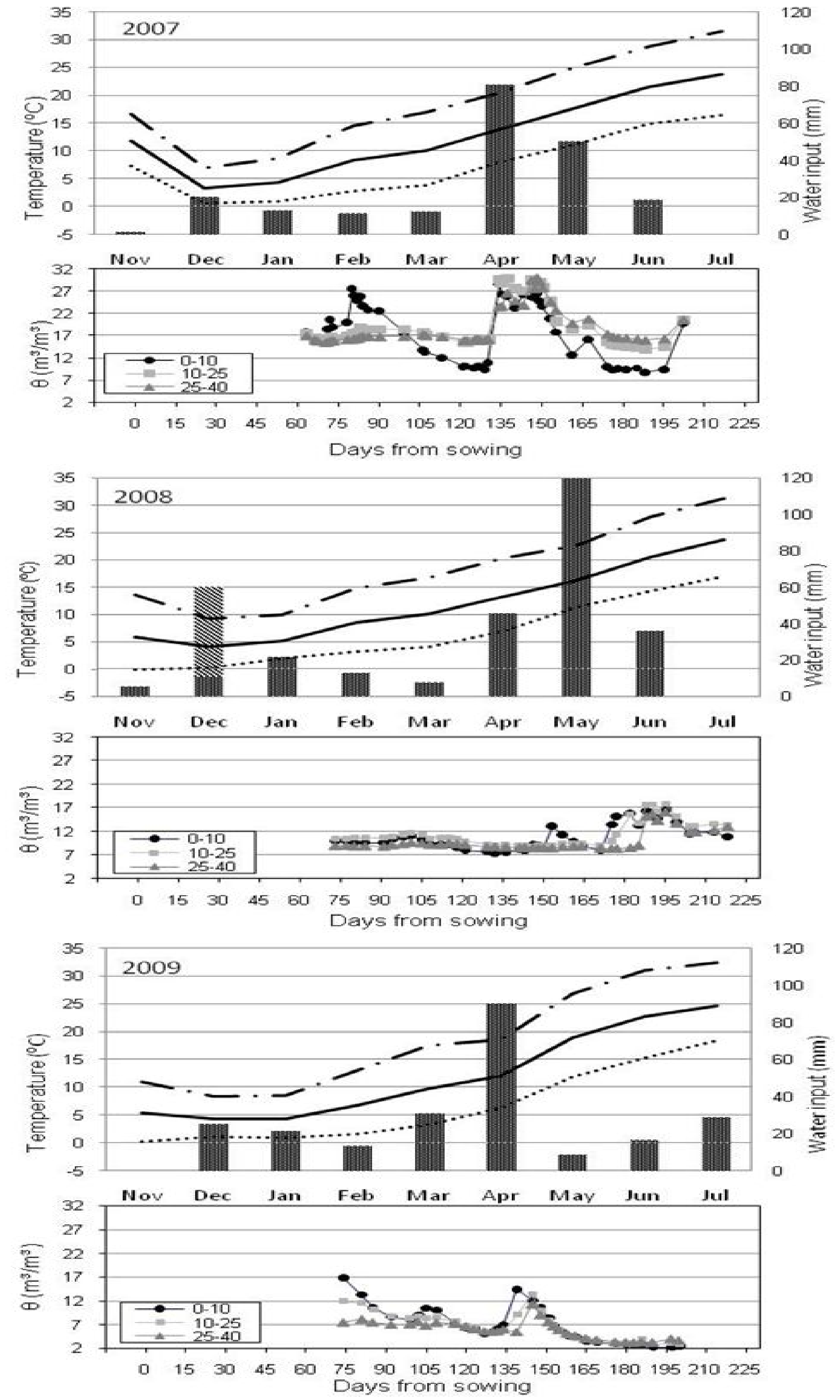

Fig. 3. Monthly water input and maximum (dashed line), mean (solid line) and minimum (dotted line) temperatures during the growth cycle each crop season. The lowest figures indicate for each year the volumetric water soil content (VWC) at three depths (0-10 cm, 10-25 cm and 25-40 cm).

Table 3. Percentage of the sum of squares of the ANOVA for phenology, biomass, yield and associated traits for 172 durum wheat landraces from four Mediterranean climatic zones and evaluated during 3 years in north-eastern Spain.

Source of variation	Year	Zone	Genotype (Zone)
		Phenology	
Days from sowing to:			
Beginning of jointing	16.2***	9.2***	44.7***
Mid jointing	58.8***	6.2***	19.6***
Booting	10.7***	26.4***	52.9***
Heading	2.5***	30.9***	60.0***
Anthesis	1.1***	32.8***	54.4***
Maturity	87.0***	3.1***	6.5***
Days anthesis-maturity	90.9***	1.3***	4.8***
		Biomass and associated traits	
Crop dry weight at:			
Tillering	30.4***	1.8**	23.0
Jointing	93.6***	0.02	2.2
Anthesis	29.9***	7.7***	26.3**
Maturity	5.3***	7.9***	38.1***
Plant height	4.4***	28.3***	53.4***
Harvest index	77.7***	0.5	12.5***
SPAD one week after anthesis	42.1***	2.9**	28.6***
		Yield and yield components	
Number of tillers per m^2	19.4***	3.2*	47.0***
Fertile tillering	77.0***	1.0***	7.3
Number of spikes per m^2	37.1***	3.7***	35.6***
Number of grains per spike	7.7***	0.5	46.1***
Number of grains per m^2	35.3***	6.1***	39.3***
Grain filling rate	15.9***	14.5***	45.5***
Thousand kernel weight	65.6***	5.7***	20.8***
Yield	65.6***	1.0**	13.3*

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

Table 4. Mean values of phenology, biomass, yield and associated traits of 172 durum wheat landraces from four Mediterranean climatic zones and evaluated during 3 years in north-eastern Spain. Mean values of modern varieties are included for reference.

	Z1	Z2	Z3	Z4	Modern varieties
Phenology					
Days from sowing to:					
Beginning of jointing	123 b	124 b	126 a	126 a	124
Mid jointing	139 d	140 c	141 b	142 a	138
Booting	147 d	149 c	152 b	153 a	145
Heading	154 d	157 c	159 b	161 a	151
Anthesis	161 d	163 c	165 b	167 a	159
Maturity	195 c	197 b	198 a	199 a	195
Days anthesis-maturity	34.1 a	34.0 a	32.4 b	31.6 c	36
Biomass and associated traits					
Crop dry weight (g m^{-2}) at:					
Tillering	68.6 b	75.7a	72.8 a	73.6 a	63.2
Jointing	406 a	406 a	408 a	411 a	399
Anthesis	838 b	1001 a	1039 a	1054 a	805
Maturity	1100 c	1185 b	1210 b	1274 a	1076
Plant height (cm)	94 c	108 b	114 a	118 a	75
Harvest index	0.38 a	0.37 a	0.36 a	0.36 a	0.42
SPAD one week after anthesis	50.2 b	51.7 a	51.5 a	51.3 a	53.9
Yield and yield components					
Number of tillers per m^2	369 a	332 b	336 b	337 b	341
Fertile tillering (%)	0.76 a	0.75 ab	0.73 c	0.74 bc	0.77
Number of spikes per m^2	406 a	370 b	365 b	369 b	389
Number of grains per spike	20.8 a	20.5 a	20.8 a	20.1 a	22
Number of grains per m^2	8157 a	7433 b	7386 b	7285 b	8570
Grain filling rate (mg GDD^{-1})	0.065 b	0.074 a	0.075 a	0.077 a	0.065
Thousand kernel weight (g)	42.8 b	48.9 a	48.9 a	49.4 a	46.4
Yield (kg ha^{-1})	3411 b	3525 a	3514 a	3509 a	3800

Means within rows with different letters are significantly different at $P = 0.05$

The climatic zone explained 28.3% of the variability observed for plant height (Table 3). Landraces from Z1 were 14 cm shorter than those from Z2, which in turn were 6 cm shorter that those of Z3. Despite landraces from Z4 were 4 cm taller than those of Z3, these differences were not statistically significant (Table 4). As expected, modern varieties were shorter than the landraces. Variability for harvest index was mostly explained by year variations and, to a lower extent, by genotypic differences, but the climatic zone did not have any effect on it. Chlorophyll content determined one week after anthesis in SPAD units, differed between landraces from different climatic zones (Table 3), and this difference was caused by the lowest SPAD values of the landraces from Z1 (Table 4). Modern varieties had the largest harvest index and chlorophyll content.

Yield and yield components

The year effect accounted for most of the variation in fertile tillering, yield and thousand kernel weight. The climatic zone origin of the landraces significantly affected yield and all the yield components studied, with the exception of the number of grains per spike (Table 3). Despite the effect of the zone was significant, the percentage of variability accounted for was in general very low, except for the mean rate of grain filling. The significance of the zone effect for yield and associated traits was mostly due to the differences existing between landraces from Z1 and those of the remainder zones (Table 4). Yield and kernel weight were lowest in genotypes from the south-eastern countries of the Mediterranean Basin (Z1) when compared with landraces from the remainder zones, but the number of tillers, spikes and grains per unit area were greatest in landraces from this zone. Fertile tillering was lower in genotypes from Z3 and Z4 than in those of Z1. When compared with the landraces, modern varieties had intermediate values for the number of tillers and spikes per unit area, more grains per spike and per unit area, fertile tillering and yield, and a similar mean grain filling rate than the landraces from Z1 (Table 4).

Relationships between the climate of the zone origin of the landraces and their agronomic performance

The relationship between long-term climatic data of the 22 countries origin of the landraces and the 3 years average agronomic data at Lleida are shown in Table 5. The duration of the cycle from sowing to anthesis and to maturity was significantly and negatively associated with the maximum and minimum temperatures, solar radiation and $ET_{0,}$ both before and after anthesis, of the countries in which the landraces were collected, and positively associated with the rainfall during grain filling (Table 5). The relationships between the climatic variables and the duration of the period anthesis-maturity had the opposite sign.

The climatic variables in the countries origin of the landraces did not have any significant relationship with their CDW at early growth stages, but some correlation coefficients were already significant at anthesis and increased at maturity, when above ground biomass was negatively correlated with the temperature, radiation and ET_0. A positive effect was observed of relative humidity and, particularly rainfall during grain filling, on biomass at maturity and plant height (Table 5). No consistent relationships were observed between climatic variables and harvest index or SPAD values one week after anthesis.

Table 5. Pearson correlation coefficients between the long-term climatic variables of the countries origin of 172 durum wheat Mediterranean landraces and their agronomic performance in north-eastern Spain. Tmin (average minimum daily temperature), Tmax (average maximum daily temperature), Rad (average daily solar radiation), Rh (average daily relative humidity), ET_0 (reference evapotranspiration), Rain (average daily rainfall). N = 22 (21 countries + Crete).

	Sowing-Anthesis						Anthesis-Maturity					
	Tmin	Tmax	Rad	Rh	ET_0	Rain	Tmin	Tmax	Rad	Rh	ET_0	Rain
Days sowing-anthesis	-0.67 ***	-0.76 ***	-0.80 ***	0.57 **	-0.78 ***	0.21	-0.60 **	-0.69 ***	-0.81 ***	0.47 *	-0.82 ***	0.75 ***
Days sowing-maturity	-0.57 **	-0.67 ***	-0.73 ***	0.46 *	-0.68 ***	0.14	-0.47 *	-0.62 **	-0.76 ***	0.44 *	-0.74 ***	0.66 ***
Days anthesis-maturity	0.66 ***	0.72 ***	0.71 ***	-0.61 **	0.74 ***	-0.26	0.64 **	0.64 **	0.68 ***	-0.42	0.75 ***	-0.70 ***
CDW at tillering	0.13	0.01	-0.12	-0.16	0.02	0.32	0.05	-0.20	-0.05	0.07	-0.17	0.32
CDW at jointing	-0.31	-0.34	-0.39	0.17	-0.36	0.08	-0.17	-0.13	-0.23	-0.21	-0.10	0.39
CDW at anthesis	-0.36	-0.48 *	-0.65 **	0.37	-0.54 *	0.27	-0.42	-0.54 **	-0.59 **	0.32	-0.62 **	0.55 **
CDW at maturity	-0.53 *	-0.73 ***	-0.80 ***	0.52 *	-0.62 **	0.19	-0.50 *	-0.75 ***	-0.68 ***	0.54 **	-0.73 ***	0.72 ***
Plant height	-0.42	-0.47 *	-0.62 **	0.35	-0.57 **	0.28	-0.49 *	-0.50 *	-0.66 ***	0.26	-0.64 **	0.62 **
Harvest index	0.11	0.14	0.18	-0.13	0.10	0.12	0.18	0.29	0.36	-0.43 *	0.38	-0.14
SPAD one week after anthesis	-0.43 *	-0.38	-0.21	0.41	-0.41	0.00	-0.31	-0.29	-0.10	0.39	-0.32	0.24
Number of tillers per m^2	0.25	0.18	0.19	-0.21	0.34	-0.14	0.45 *	0.28	0.14	-0.13	0.36	-0.36
Fertile tillering	0.28	0.28	0.36	-0.16	0.35	0.00	0.32	0.29	0.40	-0.10	0.38	-0.22
Number of spikes per m^2	0.32	0.25	0.27	-0.25	0.41	-0.12	0.52 *	0.35	0.25	-0.17	0.44 *	-0.39
Number of grains per spike	0.01	0.14	0.18	0.03	-0.01	-0.24	-0.10	0.13	0.17	-0.18	0.06	-0.12
Number of grains per m^2	0.29	0.39 *	0.48 *	-0.21	0.40 *	-0.49 *	0.44 *	0.51 *	0.37 *	-0.31	0.48 *	-0.55 **
Grain filling rate	-0.58 **	-0.64 **	-0.68 ***	0.45 *	-0.68 ***	0.35	-0.61 **	-0.61 **	-0.60 **	0.40 *	-0.71 ***	0.70 ***
Thousand kernel weight	-0.44 *	-0.50 *	-0.58 **	0.32	-0.55 **	0.32	-0.48 *	-0.54 **	-0.50 *	0.38	-0.62 **	0.60 **
Yield	-0.46 *	-0.43 *	-0.43 *	0.28	-0.48 *	-0.14	-0.25	-0.23	-0.45 *	0.20	-0.43 *	0.31

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

Nor the number of tillers and spikes, fertile tillering or the number of grains per spike had any relationship with the long-term climatic variables before anthesis of the countries of origin, but the number of tillers and spikes per unit area were positively and significantly associated with the minimum temperature during grain filling, and the number of spikes was also positively associated with the ET_0 (Table 5). On the other hand, the number of grains per unit area was significantly correlated with most climatic traits, but the correlation coefficients were in general low (Table 5). Both the grain filling rate and grain weight were negatively associated with maximum and minimum temperatures, solar radiation and ET_0 before and after anthesis, while rainfall during grain filling was positively associated with both traits. The relationships between climatic variables before anthesis and yield followed the same trend than the recorded for thousand kernel weight, but after anthesis only radiation and ET_0 were negative and significantly associated with yield (Table 5).

In order ascertain whether the significant relationships shown in Table 5 were consistent with the climatic variations between zones, some of them are plotted in Fig. 4. Fig. 4a shows that the higher the radiation before anthesis in the climatic zone in which the landraces were collected, the shorter was their cycle length until anthesis when grown in northeastern Spain and lower their biomass at maturity (Fig. 4b). On the other hand, abundant rainfalls during grain filling in the climatic zone of origin were related with shorter grain filling periods (Fig. 4c), few number of grains per unit area (Fig. 4d), but heavy grains (Fig. 4e), and greater aboveground biomass at maturity (Fig. 4f) in northeastern Spain. The consistency of the arrangement of the climatic zones in all these figures confirms the connection between the agronomic performance of the landraces and the climatic characteristics of the zones in which they were collected and to which they are supposed to be well adapted.

4. Discussion

The multivariate analysis of the long-term climatic data of the countries origin of the landraces showed that most of the information contained in the climatic data could be summarized by projecting the points in the plane determined by the first two axes. The first axis, which explained about 70% of total variation, allowed the identification of four climatic zones based on two opposite groups of climatic traits. The right direction of PC1 located climatic variables related with high temperatures, radiation and evapotranspiration, while in the negative direction were positioned the eigenvectors representing rainfall −mostly during grain filling− and relative humidity. The two clusters located in the positive side of PC1 grouped southern Mediterranean countries, characterized by high temperatures, radiation and evapotranspiration, and very dry climates. The most extreme among them corresponded to the south-eastern countries, while the Maghreb region, jointly with the eastern islands and Lebanon −this last country joining this group due to the abundant rainfalls before anthesis− have similar, but more moderate characteristics. Northern Mediterranean countries were located in the negative side of PC1, signifying cooler and wetter climates. In this side, the Balkan countries jointly with France and Portugal –the last probably due to the Atlantic influence− had the most extreme conditions, while a strip joining north-side sea countries (Turkey, Italy, Greece and Spain) were located close to the origin of the axis. The results of the PCA classified the main wheat growing areas within the Mediterranean Basin in four climatic zones that followed a consistent trend, from warm and dry to cool and wet environments when moving from the south (Z1) to the north (Z4). Based on long-term climatic data, the two sides of the Mediterranean Basin were clearly separated, in close agreement with the Koeppen's climatic classification (Leemans and Cramer 1991).

The meteorological conditions of the three years of field experiments conducted in north-eastern Spain reflected the typical variations of Mediterranean climates between and within cropping seasons. The pattern of temperature was more similar the three years than the distribution of rainfalls. As a result, broad differences between years appeared in soil water content during the whole cycle length, which likely contributed to the wide year effect on some agronomic traits such as fertile tillering, harvest index, kernel weight and yield. Actually, the erratic distribution of rainfall has explained as much as 75% of the variation in wheat yield in the Mediterranean region (Blum and Pnuel 1990).

The 172 landraces used in this study showed great variability on their agronomic performance. Previous studies conducted with the same collection showed that it properly represents the diversity existing for durum wheat landraces in the Mediterranean Basin for yield, quality attributes (Nazco *et al.*, 2012), and glutenin alleles (Nazco *et al.*, in press).

Our results revealed a significant effect of the climatic zone on the number of days needed to reach the main growth stages. The varieties originated in Z4 elapsed from 3 to 7 more days from sowing to the different growth stages that those originated in Z1. The period elapsed from sowing to anthesis increased by 2 days when moving from one zone to the other in the direction Z1 to Z4, and this cycle enlargement when moving from Z1-Z2-Z3-Z4 was consistent at all growth stages. The climatic traits of the zones of origin mostly affecting cycle length of landraces were radiation, evapotranspiration and temperatures during the whole crop cycle, all them shortening cycle length. The percentage of variation in cycle length until anthesis explained by individual climatic variables ranged from 32% to 64%, and from sowing to maturity it ranged from 22% to 58%. The observed cycle enlargement of the landraces from cool areas in comparison with those of warm areas is in agreement with the reported enhanced crop growth period induced in wheat by lower mean temperatures during growing period (Chakrabarti *et al.* 2011). Moreover, rainfall during grain filling and relative humidity during the whole cycle in the zones of origin enlarged the cycle length, having an opposite effect to that of traits associated to warm environments. The earliness at anthesis of landraces from southern zones (Z1 and Z2) probably caused that

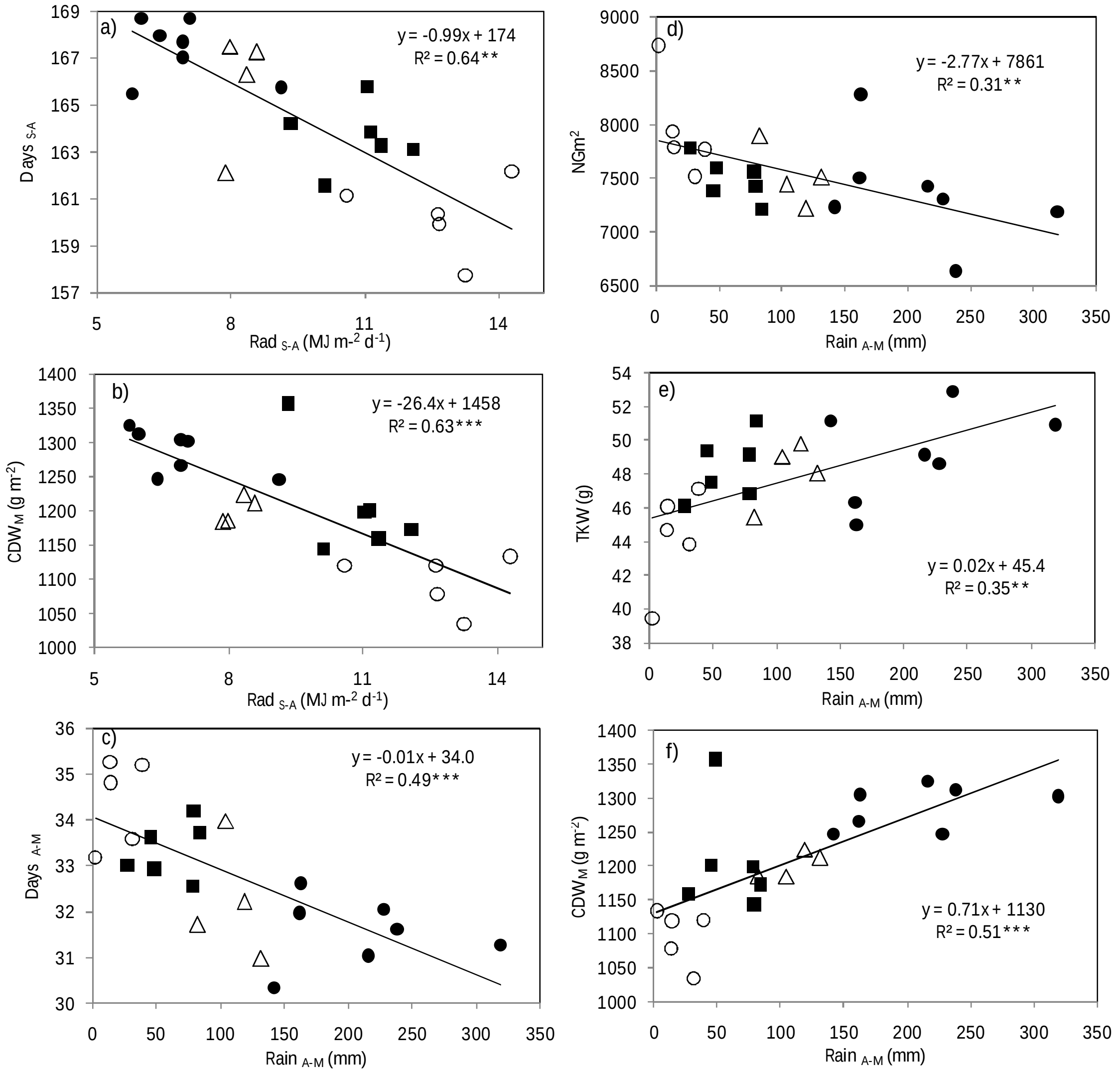

Fig. 4. Relationships between long-term average daily solar radiation (Rad) and rainfall (Rain) of the countries origin of the 172 durum wheat Mediterranean landraces and their agronomic performance in north-eastern Spain. CDW: crop dry weight, NGm^2: number of grains per m^2, TKW: thousand kernel weight, S: sowing, A: anthesis, M: physiological maturity. Climatic zones, Z1, Z2, Z3 and Z4, identified in the PCA are represented by open circles, solid squares, open triangles and solid circles, respectively.

their grain filling duration was more than 2 days longer than that of landraces from northern zones (Z3 and Z4). This is in agreement with the reported longer post-anthesis duration of early-anthesis wheat cultivars (Tewolde *et al.* 2006). Minimum and maximum temperatures, radiation and evapotranspiration in the countries origin of the landraces were positively related with the duration of their grain filling, explaining from

41% to 56% of variation in this period. On the contrary, landraces from areas with abundant rains after anthesis had shorter grain filling duration, with spring rains explaining 49% of variations on the number of days from anthesis to maturity. The large effect of the environmental conditions on grain filling duration observed in this study and reported by previous works (Santiveri *et al.* 2002; Wiegand and Cuellar, 1981), particularly the cessation of dry matter accumulation caused by excessive high temperatures at the end of the cycle (Nicolas *et al.* 1984; Egli 1998) could explain why the 6 days of delay on anthesis date of landraces from Z4 when compared with those of Z1, were reduced to 4 days of differences in the number of days at maturity.

As expected, the percentage of variability explained by the climatic zone origin of the landraces was lower for biomass, yield and associated traits than for phenologic development. However, except for biomass at jointing and harvest index, which largely depended on the year, and the number of grains per spike, all the remainder studied traits were significantly affected by the climatic zone origin of the landraces. Despite of the climatic zone of origin accounted for 1.8% of variation for CDW at tillering, any of the climatic variables studied could justify this effect, suggesting that other features, possibly associated with soil characteristics in the countries of origin, affected the early growth of landraces. Biomass at anthesis of landraces from warm areas with few rains during grain filling was lower than that of the landraces from cold regions with abundant spring rains. The unexpected effect of climatic variables during grain filling on traits determined at anthesis, as in the case of biomass, could be consequence of the close association existing, on a given environment, between climate characteristics before and after anthesis. Moreover, the estimation of the developmental periods in this study, which was an approximation, could also be in the origin of these results. The lack of significance of the correlation coefficients between rainfall before anthesis and biomass at any growth stage, but the positive and significant relationships between rainfall during the spring and biomass at anthesis and maturity demonstrate that landraces from areas with humid springs developed a greater capacity for biomass accumulation at the latest growth stages. Actually, long-term average rainfall during grain filling in the country of origin of the landraces explained 52% of biomass variations at maturity. The lowest biomass of landraces from Z1 is in agreement with the reported negative effects of water scarcity on durum wheat growth (Villegas *et al.* 2001; Royo *et al.* 2004). However, contrarily to the positive effect of rains and moisture in the countries origin of the landraces on their biomass accumulation, the negative consequences of high radiation, temperature and evapotranspiration during the whole cycle on biomass at anthesis and maturity was evident. These results point out again the opposite effects of warm and dry origin environments and the cold and wet ones on the agronomic performance of the landraces adapted to them when tested on a common environment. The largest effect of a climatic trait in the country origin of the landraces on their biomass accumulation at maturity was that of solar radiation before anthesis, which explained 64% of biomass variability at maturity.

The significant effect of long-term climatic variables on the flag leaf chlorophyll content one week after anthesis was due to the low SPAD values of landraces from climatic Z1, which had the highest solar radiation. Low leaf chlorophyll content is a photo-protection mechanism in cereals, characteristic of landraces adapted to semi-arid environments (Havaux and Tardy 1999), which is in accordance with the results obtained in this study. The climatic zone origin of landraces explained 28.3% of variations on their plant height. The climatic variables of the countries of origin and the correlation coefficients between them and plant height were similar to the calculated for biomass at anthesis, in agreement with the reported relationship between plant height and biomass of Mediterranean landraces (Annicchiarico and Pecetti 2003). The tendency of plant height increases when moving from climatic Z1 to Z4 could be attributed to two possible causes: i) the inhibition of plant height generally caused by a limited water supply (Xianshan *et al.* 2010), which would agree with landraces from drier areas being shorter than those from wet areas, or ii) a selection for taller plants by farmers during wheat expansion, as it has been suggested that ancient farmers selected the tall mutants because of their higher biomass and yield potential (Peng *et al.* 2011).

The effect of the climatic zone on yield and yield components was much lower than the year effect. The great number of tillers and spikes per unit area of landraces from the warmest and driest climatic zone (Z1), when compared with those of the other zones, did not compensate for their lighter grains, thus resulting in lower average yields. The greater tiller and spike number and lighter grains of landraces from Z1 when compared with the other three could be related with the strategy followed by durum wheat for its yield formation under warm Mediterranean environments. Previous studies have demonstrated that durum wheat yield under cooler Mediterranean conditions is mostly determined by kernel weight, whereas the number of spikes per unit area predominantly influences grain production in the warmer environments (Royo *et al.* 2006; García del Moral *et al.* 2003; Moragues *et al.* 2006). The significant and negative relationships obtained in this study between temperatures, radiation and evapotranspiration in the countries origin of the landraces and kernel weight, as well as the positive relationship between the minimum temperatures and evapotranspiration during grain filling on the number of spikes per unit area is also in agreement with this statement. The lower percentage of fertile tillering recorded in landraces from northern zones (Z3 an Z4) when compared with the southern ones (Z1 and Z2) is in agreement with previous results (Moragues *et al.* 2006), despite we did not found any significant relationship between climatic traits in the area of origin of the landraces and their fertile tillering.

Differences in grain weight and grain filling rate were only significant between Z1 and the remainder zones, but both traits tended to increase from climatic Z1 to Z4. Similar correlation coefficients were obtained between climatic variables and both, grain weight and filling rate, in agreement with the reported close relationship between these two components in durum wheat (Motzo *et al.* 1996), and the reduction caused by drought environments in the rate of grain filling (Nicolas *et al.* 1985) and grain weight

(Egli 1998; Royo *et al.* 2000). The shorter grain filling duration of the landraces from northern areas was compensated by a higher rate of grain filling, thus leading to heavier grains. This was probably consequence of the opportunity for obtaining heavier grains in wetter areas than in the dry ones due to the negative effect of drought on grain weight (Royo *et al.* 2000), and the selection for heavier grains during the dispersal of durum wheat along the Mediterranean basin that occurred in all domesticated cereals (Peng *et al.* 2011).

The data obtained in this study in the modern varieties used for comparison are in agreement with previous results of studies involving historical series of durum wheat cultivars. For many traits, such as cycle length, fertile tillering, number of spikes and grains per unit area and grain filling rate, the values recorded in modern varieties were closer to the ones of landraces from Z1 than to the obtained in landraces from other climatic zones, particularly the colder and wetter ones. These results suggest that breeding programs in the Mediterranean Basin resulted in the introgression of traits that during centuries ensured the good adaptation of durum wheat landraces to the warmer and drier areas within the region. However, the greater harvest index of the modern varieties and their superior number of grains per spike, a pleiotropic effect of major dwarfing genes (Álvaro *et al.* 2008), justify the greater yield achieved by them, even under the low water input conditions of our experiments.

5. Conclusions

The countries origin of the landraces widely represented the climatic conditions of the main durum wheat growing areas within the Mediterranean Basin. The four different climatic zones, identified through the multivariate analysis of long-term climatic data of the main wheat growing areas at each country, differed on their environmental characteristics, but the greatest differences appeared between southern (Z1 and Z2) and northern (Z3 and Z4) environments. High temperatures, solar radiation and evapotranspiration, all them typical of warm environments, were associated with low rainfall and relative humidity, while cold environments were associated with more rainfalls, especially during the spring.

Our results demonstrate that the climatic conditions of the zone in which the landraces were developed and to which they are very well adapted, significantly affected their agronomic performance when cultivated on a random Mediterranean type-environment. The climatic features on the countries of origin had large effects on cycle length, particularly concerning the cycle duration until last growth stages, plant height and, to a lesser extent, grain filling rate. While the effects of climatic traits on cycle length were steady when moving in the direction Z1 to Z4, in agreement with the consistent environmental differences between the four climatic zones identified, differences on agronomic traits tended to be only significant between landraces originated in south-eastern Mediterranean countries (Z1), and the three remainder.

Landraces originated in the warmest and driest climatic zone, corresponding to the south-eastern Mediterranean countries (Israel, Libya, Jordan, Egypt and Syria) were earlier, with less biomass, more grains per unit area, longer grain filling period, lower grain filling rate, lighter grains and lower yields than the originated in other zones characterized by colder and wetter climatic conditions.

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DISCUSIÓN GENERAL

DISCUSIÓN GENERAL

Germoplasma utilizado

El material vegetal utilizado para la realización de esta Tesis Doctoral consistió en una colección de 172 variedades tradicionales y antiguas de trigo duro (*Triticum turgidum* L. var. *durum*) de 21 países de la cuenca mediterránea (Argelia, Bulgaria, Croacia, Chipre, Egipto, Francia, Grecia, Israel, Italia, Jordania, Líbano, Libia, Macedonia, Montenegro, Marruecos, Portugal, Serbia, España, Siria, Túnez y Turquía), y 20 cultivares modernos (Ancalei, Almilcar, Astigi, Bolido, Boabdil, Bolo, Gallareta, Hispasano, Senadur, Sula, Svevo, Vitron, Vitronero, Arment, Claudio, Simeto, Kronos, Meridiano, Ocotillo y Jupare) de distintas procedencias. Al efecto de reunir una colección representativa de la variabilidad existente en variedades tradicionales de la especie en la cuenca mediterránea, inicialmente se reunió una colección de 231 accesiones procedentes de bancos de germoplasma públicos, como el Centro de Recursos Fitogenéticos del INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria) en España, el Banco de Germoplasma del ICARDA (*International Center for Agricultural Research in the Dry Areas*) en Siria y el Banco de Germoplasma del USDA (*United States Department of Agriculture*), entre otros. Los resultados de análisis moleculares llevados a cabo mediante 33 SSR aleatoriamente distribuidos entre los 7 grupos de ligamiento y 1149 marcadores DArT, permitieron seleccionar un subconjunto de accesiones representativas de la variabilidad existente en el ámbito geográfico objetivo. Los cultivares modernos utilizados como referencia incluyen algunas variedades ampliamente cultivadas en la cuenca mediterránea y pertenecientes a distintas subpoblaciones de acuerdo a su estructura genética (Royo *et al.*, 2010), así como otras con características especiales de interés para los objetivos de esta investigación.

Las poblaciones de variedades tradicionales recibidas de los Bancos de Germoplasma se cultivaron en parcelas de multiplicación en Gimenells (Lleida) durante la campaña 2005-2006. En ellas se identificaron y describieron someramente las características morfológicas diferenciales de los distintos fenotipos distinguibles visualmente. Además se cuantificó de manera aproximada el porcentaje de cada uno de los tipos en la población original, seleccionando una espiga del tipo dominante en cada población. Las parcelas se cosecharon masalmente y la semilla obtenida se utilizó la siguiente campaña para la siembra de los ensayos de campo y el establecimiento de nuevas parcelas de multiplicación y depuración. En las mismas se eliminaron las plantas distintas al tipo dominante y, tras la cosecha, la semilla se utilizó para la siembra del ensayo de campo 2007-2008 y nuevas parcelas de multiplicación y depuración esa misma campaña, en la que se procedió de la misma forma que el año anterior.

Las espigas identificadas en 2006 en cada población como pertenecientes al tipo dominante se sembraron la siguiente campaña en surcos individuales en los que se embolsaron algunas de ellas para evitar posibles cruzamientos. En cada surco se tomaron 10 granos de las espigas embolsadas, que se utilizaron para los análisis electroforéticos de los capítulos 2 y 3 de esta Memoria. Los resultados obtenidos detectaron mezclas en 17 variedades tradicionales y 2 variedades modernas, por lo que no se incluyeron en los capítulos 1, 2 y 3 de la presente Memoria. Además, en el capítulo 1 no se incluyó la única accesión procedente de Libia al considerar que no representaba suficientemente al país en el marco del estudio de variabilidad geográfica que en el mismo se planteó.

Estructuras geográficas

En todos los capítulos de esta Memoria se refleja la existencia de estructuras geográficas en la población utilizada, que varían según las variables utilizadas en cada caso. Así, en el capítulo 1 en el que se estudió el rendimiento y la calidad global de las entradas de la colección, aparecieron tres zonas geográficas correspondientes al norte de la península de los Balcanes y las regiones este y oeste del Mediterráneo. En el capítulo 2, la frecuencia del alelo nulo en el locus *Glu-A1* permitió establecer similitudes entre accesiones de diversos países. En el tercer capítulo la agrupación de países en base a la frecuencia de los modelos de combinaciones alélicas para gluteninas de bajo peso molecular proporcionó información para fortalecer una de las hipótesis sobre la dispersión del trigo duro en la cuenca mediterránea. Finalmente, en el capítulo 4 la estructura geográfica se asoció a diferencias climáticas entre zonas dentro de la región.

Considerando la calidad global, en base a las variables consideradas en el índice europeo de calidad del trigo duro y su influencia sobre el mismo, las variedades tradicionales del este del Mediterráneo, donde se encuadra el origen de la domesticación del trigo (Feldman, 2001; Mac Key, 2005), demostraron tener mejor calidad que las originarias del resto de zonas. Sin embargo, se mantuvieron algunas similitudes cualitativas con variedades de áreas situadas más al oeste, como la Península Ibérica, sur de Europa y países del Magreb. La similitud entre características de calidad de ambas zonas puede sugerir un origen común, lo que se sustenta en los numerosos estudios que han mostrado diversas vías y etapas en la dispersión del trigo desde el Creciente Fértil hacia el oeste de Europa (Feldman, 2001; Mac Key, 2005; Moragues *et al.*, 2006).

La frecuencia del alelo nulo en el locus *Glu-A1* demostró las similitudes existentes entre variedades de la zona de origen (Israel, Líbano, Jordania, Siria) y las de países situados más al oeste, en concreto los de la zona del Magreb (Marruecos, Argelia, Túnez y Libia), todos ellos situados en la vertiente sur de la cuenca mediterránea, así como dos países de la vertiente norte, Italia y Francia. Por otro lado, la mayoría de los países de la vertiente norte se separaron de los anteriores debido a la baja frecuencia de dicho alelo, lo que estaría de acuerdo con resultados de estudios anteriores que identificaron una ruta de expansión del trigo a través del norte de África (Moragues *et al.*, 2006). La inclusión de Italia y Francia en el grupo de los países del sur podría explicarse por las estrechas relaciones mantenidas por ambos países con los del norte de África (Di Fonzo *et al.*, 2005).

Los resultados obtenidos también sugieren que en algunos de los países asociados a la ruta sur de dispersión del trigo a través de la cuenca mediterránea, la selección no solamente consideró aspectos relacionados con la adaptación y la productividad, sino también el tamaño del grano y su peso específico, tal como han demostrado diversos estudios (Peng *et al.*, 2011). La distribución geográfica de la fuerza del gluten, analizada en el capítulo 2 de esta Memoria, demostró que, salvo algunas excepciones, las variedades tradicionales procedentes de países cercanos al Creciente Fértil, poseen mayor fuerza del gluten que las procedentes del norte de África, la Península Ibérica, Francia e Italia. Estos resultados, unidos a la mayor diversidad de alelos y combinaciones de gluteninas encontrados en variedades recolectadas en la proximidad de la zona de origen del trigo, pueden ser un indicador de que durante la expansión del cultivo hacia el oeste de la cuenca mediterránea se fueron perdiendo alelos asociados a la fuerza del gluten en favor de otros relacionados con una mayor capacidad de llenado y peso de grano, tal como sugieren estudios previos (Peng *et al.*, 2011) y corroboran los resultados mostrados en los capítulos 1 y 4 de esta Memoria. A pesar de esta tendencia general, los resultados obtenidos sugieren que en la zona del Magreb hubo una cierta selección por parte de antiguos agricultores dirigida a la calidad, sobre todo asociada a la fuerza del gluten. Cabe destacar en este sentido el caso de Argelia como máximo exponente, ya que las 6 variedades tradicionales procedentes de dicho país mostraron una diversidad mínima y, en su conjunto, demostraron poseer una alta calidad global, fuerza del gluten en índice de sedimentación. La reducida diversidad genética se asocia generalmente a un efecto propio de la selección tradicional por parte de los agricultores debida a aspectos comerciales o agroecológicos (Ganeva *et al.*, 2010). La menor diversidad hallada en las subunidades de gluteninas de bajo peso molecular (LMW) de los trigos argelinos, asociadas a la fuerza del gluten en diversos estudios (Ruiz y Carrillo, 1995; Vázquez *et al.*, 1996, Carrillo *et al.*, 2006), sugiere que uno de los objetivos de la selección de poblaciones en ese país, fue la fuerza del gluten y las características beneficiosas que de ella derivan para la elaboración de productos alimenticios muy característicos en dicho país, como el couscous (Elias y Manthey, 2005).

La hipótesis de la selección temprana por calidad en países del Magreb explicaría también la alta frecuencia encontrada en los mismos de los modelos de combinación alélica de gluteninas LMW-2 y LMW-2^{-} asociados a alta calidad (Liu y Shepherd, 1996; Peña y Pfeiffer, 2005). La gran utilización de germoplasma procedente de esta región del norte de África para la mejora de variedades italianas (Di Fonzo *et al.*, 2005), y su introducción en la Península Ibérica (Moragues *et al.*, 2006; Royo y Briceño-Félix, 2011), justifican la alta frecuencia de estos dos modelos en las variedades Italianas, así como la detección de los mismos en accesiones de España y Portugal.

Los resultados obtenidos sugieren que la dispersión del trigo duro desde el Creciente Fértil a lo largo de la vertiente norte de la cuenca mediterránea estuvo menos asociada al mantenimiento de las propiedades del gluten que a un mayor peso del grano, lo que explicaría la inferior calidad de las variedades tradicionales procedentes del norte de la península de los Balcanes. La disociación entre características productivas y de calidad es probablemente consecuencia de que los genes asociados con caracteres de calidad no están ligados a los de adaptación al ambiente y productividad. De hecho, son conocidas las relaciones negativas existentes entre algunos caracteres productivos y cualitativos, como por ejemplo, entre contenido de proteína y rendimiento (Boggini *et al.*, 1997), sobre todo en ambientes con escaso potencial productivo (Rharrabti *et al.*, 2001b). Sin embargo, el mayor peso del grano de las variedades tradicionales procedentes de los países del norte de la península de los Balcanes podría también estar asociado a las condiciones ambientales existentes en la zona ya que, como se demuestra en el capítulo 4 de esta Memoria, el clima de los países de dicha región se caracteriza por menores temperatura, radiación, evapotranspiración y mayor pluviometría que las existentes en el norte de África o el Medio Oriente. Ello podría influir negativamente en la expresión de variables de calidad, tales como el contenido en pigmentos o la concentración de proteínas (Rharrabti *et al.*, 2003), favoreciendo además la obtención de un mayor peso del grano (Royo *et al.*, 2000). Por otro lado, las grandes diferencias observadas en caracteres de calidad entre las variedades del norte de la península de los Balcanes y las del resto de orígenes geográficos podrían también ser parcialmente atribuidas a su posible introducción desde el sur de Rusia y de la región del Volga, tal y como han sugerido algunos estudios previos (Dedkova *et al.*, 2009; Melnikova *et al.*, 2010).

Variabilidad fenotípica y genética para caracteres de calidad

En el capítulo 1 de esta Memoria se cuantificó la variabilidad fenotípica inducida por el genotipo y el ambiente para caracteres de calidad. Los resultados indicaron que, teniendo en cuenta la totalidad de las accesiones, tanto variedades tradicionales y antiguas como modernas, el genotipo explicó entre el 13% (para contenido de proteína) y el 73% (para fuerza del gluten e índice de calidad global) de la variabilidad observada en los ensayos de campo. Sin embargo, al considerar el grupo de variedades modernas dentro del análisis, podría interpretarse que la variabilidad fue debida fundamentalmente a las diferencias existentes entre variedades antiguas y modernas. Si bien ello es cierto en parte, como sugiere el análisis de componentes principales, el porcentaje de varianza debido al genotipo explicado por las diferencias entre accesiones dentro de cada zona geográfica, fue para la mayoría de variables (como fuerza del gluten, índice de calidad global, índice de amarillo, peso del grano, índice de sedimentación) superior al explicado por las diferencias entre grupos. Ello pone de manifiesto la variabilidad fenotípica existente para variables de calidad incluso dentro de las propias zonas geográficas en las que se detectaron tendencias comunes en calidad del grano. Sin embargo, la variabilidad existente dentro de estos grupos geográficos no fue similar para todos ellos, ya que en el conjunto de variedades del este y oeste de la cuenca mediterránea la variabilidad fenotípica para caracteres de calidad fue muy superior a la detectada en las variedades del norte de la península de los Balcanes y en las variedades modernas.

La ausencia de diferencias significativas dentro de las variedades modernas para el índice de calidad global, fuerza del gluten e índice de sedimentación, caracteres en los que los valores de las mismas fueron particularmente elevados, demuestra una gran uniformidad fenotípica para estos caracteres en este conjunto de variedades. Esta menor diversidad de los cultivares modernos en comparación con las variedades tradicionales ha sido detectada en estudios anteriores (Newton *et al.*, 2010), y fue la causa de que dentro del grupo de variedades modernas no aparecieran alelos o combinaciones con efecto estadísticamente significativo sobre la fuerza del gluten. Estos resultados demuestran el éxito de los programas de mejora de la calidad al haber conseguido acumular genes favorables para algunos caracteres de gran importancia para la industria de la sémola y la pasta, y que se han mantenido durante las últimas décadas.

La gran diversidad de alelos de gluteninas detectada en la población de estudio de esta Tesis Doctoral, fundamentalmente para el locus *Glu-B3*, fue mucho mayor que la publicada en estudios previos (Moragues *et al.*, 2006), confirmando así la utilidad de la colección de germoplasma utilizada para dar respuesta a los objetivos inicialmente planteados. También fue muy superior a la variabilidad detectada en variedades modernas, ya que solamente 13 de los 114 alelos o patrones de bandas identificados en las variedades tradicionales estuvieron presentes en las obtenciones recientes y el número de combinaciones entre los loci asociados a gluteninas de alto y bajo peso molecular fue solamente de 9 para los cultivares modernos, frente a los 126 de las variedades tradicionales. Una gran cantidad de los alelos o bandas detectados en las variedades tradicionales se encontraron en frecuencias inferiores al 5% −por lo que se consideran alelos raros− y cerca del 85% de los mismos no habían sido descritos previamente. Los resultados que se muestran en el capítulo 2 confirman la mayor variabilidad existente en variedades tradicionales de trigo duro para alelos/bandas de gluteninas de bajo peso molecular (LMW) que en las de alto peso molecular (HMW), como han descrito previamente otros autores (Brites y Carrillo, 2001; Sissons *et al.*, 2005). En sintonía con los resultados del capítulo 2, el tercero de ellos demostró que el número de combinaciones fue también muy superior al detectado en estudios previos (Turchetta *et al.*, 1995; Cherdouch *et al.*, 2005; Moragues *et al.*, 2006).

Relación genotipo-fenotipo para la fuerza del gluten

Los análisis de varianza de la fuerza del gluten determinada mediante el test de sedimentación SDS de Axford *et al.* (1978), con las modificaciones introducidas posteriormente por el laboratorio de calidad de CIMMYT (Peña *et al.*, 1990), confirmaron el reducido efecto ambiental sobre dicho carácter. La clasificación de las accesiones en cinco categorías de fuerza del gluten (baja, media, alta, muy alta y extrema), en base a los valores del test de sedimentación, resultó ser apropiada para la evaluación de los efectos de los alelos/bandas y sus combinaciones sobre la fuerza del gluten.

El efecto de la composición de gluteninas presentes en la población sobre la fuerza del gluten, reveló que los efectos de la combinación de varios alelos/bandas ejercen un efecto muy superior al del de los alelos evaluados individualmente. Así, la diferencia entre los valores SDS de variedades portadoras de alelos que afectaron significativamente a la fuerza del gluten y aquellas en los que dichos alelos no estaban presentes osciló entre 0.73 y -1.29 ml, mientras que el rango de dichas diferencias varió entre 0.89 y 3.44 ml cuando se comparó el efecto de combinaciones de alelos y/o bandas. Ello explica que no se detectaran combinaciones alélicas que ejercieran un efecto significativamente negativo sobre la fuerza del gluten a pesar de ser portadoras de alelos que al ser estudiados de forma independiente, afectaron negativamente a los valores del test de sedimentación. Tal fue el caso del alelo *Glu-B1e* (banda 20), que habiendo demostrado su efecto perjudicial sobre la fuerza del gluten en múltiples estudios (Carrillo *et al.*, 1990; Brites y Carrillo, 2001; Raciti *et al.*, 2003; Sissons *et al.*, 2005), reveló un efecto positivo en esta investigación. Ello pudo ser debido a que el 70% de las combinaciones que contenían este alelo también incluían, al menos, uno de los alelos de gluteninas LMW que elevaban significativamente la fuerza del gluten, como el caso de *Glu-A3a* (banda 6), *Glu-A3d* (bandas 6+11), *Glu-B3a* (bandas 2+4+15+19) y *Glu-B2a* (banda 12). La interacción entre las subunidades de gluteninas de alto y bajo peso molecular en la co-formación del gluten, podría ser la causa de que el efecto de un determinado alelo varíe en distinto germoplasma, particularmente en trigo duro donde el efecto de las gluteninas LMW sobre la fuerza del gluten, especialmente la de los modelos LMW tipo 2, domina sobre el de las HMW, como han sugerido diversos autores (Boggini *et al.* 1995; Edwards *et al.* 2003; Peña y Pfeiffer, 2005). Las gluteninas LMW se han mostrado como las más importantes a la hora de seleccionar variedades tradicionales y antiguas capaces de proporcionar nuevos genes para mejorar la fuerza para el gluten en programas de mejora.

Es de destacar que los efectos favorables sobre la fuerza del gluten detectados en el capítulo 2 como consecuencia de la presencia de los patrones de bandas 2+4+14+15+18 y 2+4+15+18+19 en el locus *Glu-B3*, así como de las combinaciones números 45 [nulo (*Glu-A1*), 6+8 (*Glu-B1*), 11 (*Glu-A3*), 2+4+15+18+19 (*Glu-B3*) y nulo (*Glu-B2*)], 95 [1 (*Glu-A1*), 20 (*Glu-B1*), 6+20 (*Glu-A3*), 2+4+15+19 (*Glu-B3*) y 12 (*Glu-B2*)] y 123 [2* (*Glu-A1*), 7+17 (*Glu-B1*), 6 (*Glu-A3*), 2+4+14+15+18 (*Glu-B3*) y nulo (*Glu-B2*)] (capítulo 3) han de ser valorados como orientativos de la posible utilidad de las variedades que las contienen como parentales en programas de mejora, ya que la baja frecuencia a la que se encuentran en la población impide hacer afirmaciones rotundas sobre sus efectos.

Identificación de variedades de alta calidad

En general, los cultivares modernos mostraron mayores rendimientos, mejor coloración de grano y menor contenido en proteína que las variedades tradicionales y antiguas, aunque con mejor calidad de proteína como consecuencia de la mejora genética. Debido a ello el gluten de las variedades modernas fue de gran fuerza, tal y como se ha confirmado en estudios previos (Motzo *et al.*, 2004). Si bien la calidad global de las variedades modernas fue la más destacada, diversas variedades tradicionales sobresalieron en más de una variable de interés para la mejora de la calidad. La variedad egipcia número 18 (PI-366109) y la portuguesa ‘*Lobeiro de Grao Escuro’* mostraron altos índices de calidad, concentraciones de proteína y valores de fuerza de gluten debido a ser portadoras de los patrones de bandas identificados como beneficiosos (2+4+14+15+18 y 2+4+15+18+19, respectivamente). La primera de estas combinaciones de bandas fue detectada en la combinación nueva número 123 [2* (*Glu-A1*), 7+17 (*Glu-B1*), 6 (*Glu-A3*), 2+4+14+15+18 (*Glu-B3*) y nulo (*Glu-B2*)] (Tabla 2, en el capítulo 3), de efecto beneficioso para la calidad, mientras que la segunda también estuvo presente en la variedad francesa ‘Trigo Glutinoso’, formando parte de otra nueva y destacada combinación, la número 45 [nulo (*Glu-A1*), 6+8 (*Glu-B1*), 11 (*Glu-A3*), 2+4+15+18+19 (*Glu-B3*) y nulo (*Glu-B2*)]. La variedad española Raspinegro de Alcalá destacó por sus buenos índices de calidad, color de grano y fuerza de gluten, mientras que el mayor peso de grano fue alcanzado por otra variedad española, ‘Enano de Andújar’, debido a su altísima tasa de llenado de grano. Otras 4 variedades turcas también destacaron por contener la combinación nueva identificada con el número 95 [1 (*Glu-A1*), 20 (*Glu-B1*), 6+20 (*Glu-A3*), 2+4+15+19 (*Glu-B3*) y 12 (*Glu-B2*)] en la Tabla 2 del capítulo 3, que incrementó significativamente la fuerza del gluten de las variedades portadoras de la misma. El cultivar ‘Svevo’, de origen italiano, y el estadounidense ‘Ocotillo’ fueron los que en general mostraron mejor calidad dentro del grupo de los cultivares modernos.

Clima y comportamiento agronómico

La agrupación de los países de origen de las variedades de la colección, en base a series históricas de datos climáticos (capítulo 4), mostró una estructura geográfica que guardó cierta similitud con la obtenida en los capítulos anteriores, referidos a características fundamentalmente asociadas con la calidad del grano. La zona sureste del mar Mediterráneo, la más próxima a la zona de domesticación del trigo, se diferenció también del resto de la cuenca por sus características climatológicas. En las cuatro zonas climáticas identificadas se observa una tendencia sur-norte, desde las más cálidas y secas a las más frías y húmedas.

En lo que respecta al efecto que, sobre los caracteres agronómicos, confirieron los climas de las zonas de origen de las variedades analizadas, destacaron los mostrados sobre la fenología, la altura de la planta y la tasa media de llenado. Las variedades originarias de ambientes más cálidos y secos mostraron una mayor precocidad en todos los estadios de desarrollo analizados. Ello quedó especialmente puesto de manifiesto en el estadío de antesis, en el que la zona climática de origen de la variedad explicó el

32.8% de la varianza observada para el número de días transcurridos entre siembra y antesis, frente al 1.1% que explicó el año de ensayo. Estos resultados demuestran que la variabilidad inducida en la fecha de antesis se debió mucho más a las características de adaptación de la variedad a la zona donde se originó que a los efectos del ambiente en que se cultivaron. Ello está en sintonía con el elevado porcentaje de variabilidad explicada por el genotipo (54.4%), lo que indica que se trata de una carácter de alta heredabilidad y explica el éxito de las variedades precoces en ambientes con sequía terminal en los que el escape ha demostrado ser una herramienta muy eficaz de adaptación (Worland y Snape, 2001). Por el contrario, las variedades de zonas más frías y húmedas fueron más tardías, tal como se ha observado en otras especies (Kato *et al.* 1998; Goldringer *et al.*, 2006). El consiguiente acortamiento del periodo de llenado que se derivó de este hecho fue compensado por la mayor capacidad de estas variedades para llenar sus granos, dando lugar a los granos de mayor peso.

La altura de planta fue menor en las variedades procedentes de climas más cálidos, lo que justifica que la biomasa aérea de éstas también lo fuese, dada la relación existente entre las mismas (Annicchiarico y Pecetti, 2003). El menor porte de estas plantas se justifica por el efecto de las condiciones más áridas de sus lugares de origen, debido al efecto que sobre la elongación de los tallos tienen factores como la alta intensidad lumínica (Becerril, 2008) o el déficit hídrico (Xianshan *et al.*, 2010). Las condiciones opuestas, propias de los climas de zonas de mayor latitud, favorecieron la mayor altura de las plantas y la mayor biomasa, lo que ha sido relacionado con mejores rendimientos potenciales (Peng *et al.*, 2011). Los resultados de este estudio indican que los mayores rendimientos de variedades procedentes de zonas frías se deben a su mayor peso del grano, asociado a una mayor tasa de llenado, mientras que en las variedades de zonas más cálidas, la estrategia de formación del rendimiento se basa en el desarrollo de un número de granos mayor.

Los elevados rendimientos de las variedades originarias de climas fríos estuvieron asociados a peor calidad, al contrario de lo detectado en las variedades de zonas más cálidas. Esta relación inversa entre los parámetros de calidad y los rendimientos estuvo en concordancia con estudios anteriores (Rharrabti *et al.*, 2003) y está muy influenciada por el hecho de que a mayor peso del grano, menor es la concentración de proteínas en el mismo, dada la dilución de éstas por compuestos no nitrogenados, opuestamente a lo que sucede en ambientes áridos donde el estrés hídrico reduce el llenado del grano y la acumulación de almidón (Rharrabti *et al.*, 2001a). El aumento en la concentración de proteínas originado por las temperaturas más altas y el descenso de la misma a consecuencia de las mayores precipitaciones también ha sido demostrado en otros estudios (Correll *et al.*, 1994).

La relación entre la actividad fotosintética y el rendimiento de los cultivos es plenamente conocida. Los resultados que se presentan en el capítulo 4 muestran que las variedades procedentes de la zona más árida, el sureste del Mediterráneo, tuvieron los menores valores SPAD medidos en hoja bandera una semana después de la antesis. Ello puede interpretarse como un mecanismo de fotoprotección frente a la elevada radiación solar en la zona de origen, en concordancia con lo indicado por otros autores (Havaux y Tardy, 1999) y una consecuencia de lo innecesario de desarrollar un uso eficiente de la luz (Wu *et al.* 2009) en dicha zona. Los resultados de este estudio muestran que la relación inversa entre precipitación y temperaturas detectada en previos trabajos no sólo afecta significativamente a la calidad del grano, sino también a muchas de las variables agronómicas analizadas en este trabajo, como ya se ha comentado (capítulo 4).

La utilización de cultivares modernos en los distintos capítulos de esta Memoria sirvió como elemento comparativo para las variedades tradicionales. En todos ellos se observó un patrón común. Los cultivares mejorados mostraron características agronómicas y de calidad más próximas a las de las variedades originarias de zonas con condiciones ambientales más áridas, especialmente este o sureste del Mediterráneo y área del Magreb. Así, los mayores índices de calidad fueron registrados en los cultivares modernos y en las variedades tradicionales del este del Mediterráneo y la duración de sus ciclos y días entre los estadios de crecimiento también fueron similares. La composición de gluteninas también relacionó a estos países, especialmente los del Magreb, con variedades mejoradas (capítulos 2 y 3). A semejanza de los cultivares modernos, las variedades el sureste del Mediterráneo tuvieron menos biomasa, menores altura de planta y tasa media de llenado del grano, así como mayores índice de cosecha, fertilidad de hijuelos y número de granos por unidad de superficie, en comparación con el resto de variedades tradicionales. Si bien las diferencias entre variedades modernas y tradicionales no pueden interpretarse en términos de adaptación, al ser en gran medida consecuencia de la presencia de genes de enanismo en las variedades mejoradas (Álvaro *et al.*, 2008a, b), la existencia de una tendencia es innegable. Los resultados muestran que la mejora de variedades de trigo duro para los países de la cuenca mediterránea ha llevado a la obtención de genotipos con muchas características agronómicas similares a las de las variedades tradicionales procedentes de la zona más cálida y seca de la región, donde tuvo lugar la domesticación de la especie.

CONCLUSIONES

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1.- De acuerdo a su rendimiento y a las características de calidad incluidas en el índice de calidad de la UE para trigo duro, las variedades tradicionales y antiguas de la cuenca mediterránea se estructuran geográficamente en tres áreas: este del Mediterráneo, oeste del Mediterráneo y norte de la península de los Balcanes.

2.- Las variedades tradicionales originarias de la zona este del Mediterráneo poseen la mayor calidad global y una gran variabilidad. Las de la zona oeste tienen tasas de llenado superiores y granos de mayor peso, mientas que las procedentes del norte de la península de los Balcanes se caracterizan por su baja calidad global y reducida variabilidad.

3.- Las variedades modernas fueron el grupo más productivo, uniforme y con menor contenido de proteína en el grano, si bien poseen el mayor índice de calidad global, fuerza del gluten, índice de amarillo e índice de sedimentación.

4.- La variabilidad para características de calidad y la diversidad genética para gluteninas de alto (HMW) y bajo (LMW) peso molecular fue muy superior en las variedades tradicionales que en las modernas.

5. Los alelos *Glu-B1e*, *Glu-A3a*, *Glu-A3d*, *Glu-B3a* y *Glu-B2a*, así como los patrones de bandas 2+4+14+15+18 y 2+4+15+18+19, identificados en muy baja frecuencia, aumentaron la fuerza del gluten de las variedades portadoras, mientras que *Glu-A3b*, *Glu-B2b* y *Glu-B1a* la redujeron.

6.- Además de dos combinaciones conocidas, que incluyen los modelos LMW-2 y LMW-2^{-}, se detectaron tres combinaciones nuevas en muy baja frecuencia en la población, pero con efectos positivos sobre la fuerza del gluten.

7.- El efecto de determinados alelos de gluteninas en las subunidades de alto peso molecular (HMW) estuvo muy influenciado por la composición alélica en las subunidades de bajo peso molecular (LMW).

8.- Las variedades tradicionales identificadas como potenciales parentales en programas de mejora de la calidad fueron: la egipcia ‘PI-366109’, la portuguesa ‘Lobeiro de Grao Escuro’ (ambas por su alto índice de calidad, contenido de proteína y fuerza de gluten), la francesa ‘Trigo Glutinoso’ (por la fuerza de su gluten) y las españolas ‘Raspinegro de Alcalá’ (por sus elevados índices de calidad e índice de amarillo y la fuerza de su gluten) y ‘Enano de Andújar’ (por su alta tasa de llenado y peso del grano). Entre las variedades modernas destacaron por la fuerza del gluten ‘Ocotillo’, ‘Claudio’ y ‘Meridiano’.

9. La climatología de la zona de origen de las variedades tradicionales influyó en su fenología, crecimiento, rendimiento y componentes del rendimiento cuando se cultivaron en el noreste de España.

10. La zona de origen de las variedades tradicionales afectó principalmente al desarrollo fenológico, altura de la planta y tasa de llenado del grano, mientras que no tuvo ningún efecto sobre el índice de cosecha y el número de granos por espiga.

11. Altas temperaturas, radiación solar y evapotranspiración potencial en la zona de origen de las variedades tradicionales dan lugar a genotipos más precoces, con menores tasas de llenado de grano y granos ligeros, mientras que la presencia de lluvias en primavera en la zona de origen da lugar a genotipos de características opuestas.

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