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New insights to durum wheat breeding in the genomics era through the exploration of a diversity panel of Mediterranean landraces

Martina Roselló Hernández

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TESI DOCTORAL

New insights to durum wheat breeding in the genomics era through the exploration of a diversity panel of Mediterranean landraces

Martina Roselló Hernández

Memòria presentada per optar al grau de Doctor per la Universitat de Lleida
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Abstract

Durum wheat is a major crop in the Mediterranean Basin, which accounts for 60% of total durum production. The main objective of this PhD Thesis was to provide information for durum wheat breeding programmes helping to develop new varieties with enhanced productivity and the quality attributes meeting the standards of the food industry. Plant material consisted on a set of about 180 Mediterranean landraces from 21 countries and a collection of 205 world-wide modern cultivars. Phenotyping was conducted on field experiments during six harvesting seasons under rainfed Mediterranean conditions. Genotyping was done with DArT (1,149) and DArTseq (46,161) markers. Landraces collected on Eastern and Western Mediterranean countries, and belonging to different genetic subpopulations, were used to investigate their adaptation strategies to environmental conditions. They showed contrasting patterns of adaptation based on a different use of the water available before and after anthesis to generate yield. Eastern Mediterranean (EM) landraces showed early anthesis and used the water available before it to produce spikes and to accumulate water-soluble carbohydrates that were further remobilized to filling grains. For the seminal root system architecture (RSA), EM landraces showed a large root size and a wide seminal root angle. In contrast, Western Mediterranean (WM) landraces were efficient using post-anthesis water to increase the number and weight of grains. They had the highest number of seminal roots, and the narrowest angle. Two QTL meta-analysis were performed to identify consensus QTL regions for a number of agronomic and quality traits relevant for breeding purposes. Information was synthetized for 26 QTLs studies dealing with agronomic traits and 20 QTLs studies analysing quality traits in durum wheat. Two genome wide association-mapping studies (GWAS) with DArTseq markers detected differential genome regions for yield-related traits between Mediterranean landraces and world-wide modern cultivars. Twenty-four stable regions were identified in landraces for yield, grain number, grain weight and plant height, while in modern cultivars 31 stable regions were detected for grain weight and plant height. A GWAS identified 176 DArTseq markers in landraces associated to the seminal RSA, grouped in 82 genome regions, of which 64 had not been reported previously and 37 could present pleiotropic effect with yield-related traits. Another GWAS identified 70 DArT markers associated to quality traits. Four markers presented great stability and an increasing effect. These results proved that durum wheat Mediterranean landraces are a valuable source of genetic variability useful for widening the durum wheat genetic background and improving relevant traits in durum wheat breeding programmes.

Resum

El blat dur és un cultiu important a la Conca Mediterrània ja que aquesta representa el 60% de la producció mundial de blat dur. L'objectiu principal d'aquesta Tesi Doctoral va ser provenir d'informació els programes de millora genètica de blat dur per ajudar a desenvolupar noves varietats més productives i amb la qualitat requerida per la indústria alimentària. El material vegetal va consistir en un conjunt d'unes 180 varietats tradicionals originàries de 21 països de la conca Mediterrània i una col·lecció mundial de 205 cultivars moderns. El fenotipat es va realitzar durant sis campanyes en experiments al camp en condicions de secà. El genotipat es va dur a terme amb marcadors DArT (1.149) i DArTseq (46.161). Es van utilitzar varietats tradicionals dels països de l'est (EM) i l'oest (WM) del Mediterrani per investigar les seves estratègies d'adaptació a l'ambient i van mostrar diferents patrons adaptatius basats en l'ús diferencial de l'aigua disponible abans i després d'antesi per la formació del rendiment. Les varietats EM van ser més precoces a antesi i van utilitzar l'aigua disponible abans de l'antesi per produir espigues i acumular carbohidrats solubles en aigua que van ser mobilitzats al gra durant el seu ompliment. En l'arquitectura del sistema radicular primari, van mostrar arrels més llargues amb un major angle. Les varietats WM van ser eficients utilitzant l'aigua després de l'antesi per augmentar el nombre i pes dels grans i van presentar el nombre més elevat d'arrels primàries i l'angle més estret. Es van realitzar dos estudis de meta-anàlisi de QTLs per caràcters agronòmics i de qualitat per identificar regions de consens amb rellevància per la millora genètica. Aquestes anàlisis van utilitzar 26 estudis sobre caràcters agronòmics i 20 sobre caràcters de qualitat. Dos mapejos d'associació van detectar regions genòmiques diferents pels caràcters associats al rendiment entre les varietats tradicionals i els cultivars moderns. Per les varietats tradicionals, es van identificar 24 regions estables pel rendiment, nombre de grans, pes del gra i alçada de la planta, mentre que pels cultivars moderns es van identificar 31 regions estables pel pes del gra i l'alçada de la planta. Una anàlisi d'associació amb varietats tradicionals va identificar 176 marcadors DArTseq associats a l'arquitectura de les arrels primàries, agrupats en 82 regions genòmiques, de les quals 64 no havien estat identificades prèviament i 37 podrien presentar efectes pleiotròpics amb els caràcters relacionats amb el rendiment. Un altre anàlisi va identificar 70 marcadors DArT associats a caràcters de qualitat. Quatre marcadors van presentar una gran estabilitat i efecte positiu. Aquests resultats demostren que les varietats tradicionals mediterrànies de blat dur són una valuosa font de variabilitat genètica útil per ampliar el bagatge genètic del blat dur i millorar característiques importants en els programes de millora genètica.

Resumen

El trigo duro es un cultivo importante en la Cuenca Mediterránea ya que la misma representa el 60% de la producción global de este. El objetivo principal de esta Tesis Doctoral fue proporcionar información a los programas de mejora genética de trigo duro para el desarrollo de nuevas variedades más productivas y que cumplan con los estándares de calidad de la industria alimentaria. El material vegetal consistió en un conjunto de unas 180 variedades tradicionales originarias de 21 países de la cuenca Mediterránea y una colección mundial de 205 cultivares modernos. El fenotipado se realizó en campo durante seis campañas en condiciones de sequía. El genotipado se llevó a cabo mediante marcadores DArT (1.149) y DArTseq (46.161). Se utilizaron variedades tradicionales de los países del este (EM) y del oeste (WM) del Mediterráneo para estudiar las distintas estrategias de adaptación al ambiente, mostrando un gran contraste en su patrón adaptativo basado en el uso diferencial del agua antes y después de antesis en la formación del rendimiento. Las variedades EM mostraron una antesis temprana y utilizaron el agua disponible antes de la misma para producir espigas y acumular carbohidratos solubles en agua que se movilizaron al grano durante su llenado. El estudio de la arquitectura del sistema radicular primario, mostró que las variedades EM presentaron raíces más largas y con un mayor ángulo. En cambio, las variedades WM fueron eficientes utilizando el agua disponible después de la antesis para aumentar el número y el peso de los granos, presentando un mayor número de raíces primarias y un ángulo más estrecho. Se realizaron dos estudios de meta-análisis de QTLs para identificar regiones consenso para caracteres agronómicos y de calidad relevantes en mejora genética. Estos análisis comprendieron 26 estudios de caracteres agronómicos y 20 de caracteres de calidad. Dos análisis de asociación detectaron regiones genómicas diferentes para los caracteres de rendimiento entre variedades tradicionales y modernas. En las variedades tradicionales, se identificaron 24 regiones estables para el rendimiento, el número y peso de granos, y la altura de la planta, mientras que en las modernas se identificaron 31 regiones estables para el peso del grano y la altura de la planta. Un análisis de asociación con variedades tradicionales identificó 176 marcadores DArTseq asociados a la arquitectura radicular primaria, agrupados en 82 regiones genómicas, de las cuales 64 no habían sido identificadas previamente y 37 podrían presentar efectos pleiotrópicos con los caracteres relacionados con el rendimiento. Otro análisis identificó 70 marcadores DArT asociados a caracteres de calidad. Cuatro marcadores presentaron una gran estabilidad y un efecto positivo. Estos resultados demuestran que las variedades tradicionales mediterráneas de trigo duro son una valiosa fuente de variabilidad genética útil para ampliar el acervo genético del trigo duro y mejorar caracteres importantes en los programas de mejora genética.

Introduction

Introduction

1. Durum wheat origin and taxonomy

Wheat (*Triticum sp*) is a grass belonging to the *Poaceae* family, *Triticeae* tribe and *Triticum* genera. *Triticum* species are classified, based on the number of chromosomes, in diploid (2n=2x=14), tetraploid (2n=4x=28) and hexaploid (2n=6x=42), the basic number of chromosomes is 7 and their genomes AA, AABB and AABBDD respectively. Among the tetraploid wheats, durum wheat (*Triticum turgidum* L. var. *durum*) is one of the most important and oldest cereal species cultivated in the world.

Primitive parents of durum wheat, diploid grasses called einkorn, originated in the eastern Mediterranean coast regions, in the Fertile Crescent, by around 11,500 before present (BP). They were well adapted to the harsh and variable climate of the eastern Mediterranean regions and to a wide range of altitudes and landscapes due to their ontogenetic development that allow their adaptation to different environments and ecological conditions (Mac Key, 2005). There were three different einkorn types, i.e. *Aegilopoides*, *Thaoudar* and *Urartu* that did not have isolating barrier, thus genetic crosses may happen. **Tetraploidization** would develop from them in nature, one of those three species could be the first parent (donating the genome A) and the second parent (genome B) is thought to be *Aegilops speltoides*. The wild tetraploid wheat, wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides* [Körn. ex Asch. & Graebn.] Thell), was well adapted to extreme drought periods and presented a wide range of morphological ecotypes (Mac Key, 2005).

Around 10,000 BP due to both natural selection and human action, **domesticated emmer wheat** (*T. turgidum* ssp. *dicoccum* [Schrank ex Schübl.] Thell) arose and durum wheat evolved from it (Maccaferri et al., 2019). All cultivated forms may had originated in south-eastern Turkey, they were well adapted to hot and dry climates but also to hardier ones, characteristic that allowed them to expand northward (Mac Key, 2005).

Primitive durum wheat spread across the Mediterranean Basin and reached the Iberian Peninsula around 7,000 BP. It possibly followed two dispersion routes: through south Europe countries and through north Africa, the latter probably during the Arabic empire in the Middle Age (Moragues et al., 2006a, b). Due to the durum wheat's capacity to adapt to the different climatic conditions, during the domestication and subsequent dispersion new adaptive traits suitable for new environments and with interest for human activities and nourishment were stablished resulting in the development of local **landraces** (Peng et al., 2011). A landrace is defined as a traditional variety with a high capacity to tolerate biotic and abiotic

stresses, resulting in high yield stability and an intermediate yield level under a low input agricultural system (Zeven, 1998). They are generally considered endemic to a particular region to which they are well adapted. From these landraces, by both traditional and modern breeding techniques, durum wheat modern commercial varieties were developed.

2. Importance and distribution of the crop

Wheat is currently the most widespread crop and is a basic staple food of mankind, providing humans with 18% of their daily intake of calories and 20% of their protein (http://faostat.fao.org/)**.** It can be cultivated in almost all regions of the world with more than 220 million hectares planted annually and 763 million of tones harvested in 2018, representing the 36% of the total cereal production (International Grains Council, IGC, https://www.igc.int/en/default.aspx). The unique properties of the gluten protein fraction makes wheat grain an appropriate raw material for the production of a high amount of different sorts of food (Büchsenmann-Budzinski et al., 2017). Moreover, wheat grain can be stored for long periods of time as food reserves to be prepared for bad crop years and prevent extreme price increases, which is interesting since wheat is the most traded cereal crop in the international agricultural food market (Peña-Bautista et al., 2017).

Durum wheat represents about a 5-10% of the total wheat production (13 million hectares and 38 million tonnes on 2017), it is mainly cropped in three different regions: the Mediterranean Basin, the Northern Plains between United States of America and Canada, and within the desert areas of South West of United States and Northern Mexico (Ranieri, 2015). EU-28 is the first global durum wheat producer, accounting for about 25% of global durum wheat production (IGC 2015/2016 data, https://www.igc.int/en/markets/marketinfo-sd.aspx). The Mediterranean Basin countries are the main durum wheat producers (60% of total), being Italy the highest with 1.4 million hectares and 5 million tonnes, followed by France (0.4 million hectares and 1.6 million tonnes) and Spain (0.4 million hectares and 1 million tonnes) (Willems, 2017; http://www.italmopa.com/wp-content/uploads/2017/05/144_all_2.pdf). However, EU actually imports half of the total incomes from the second producer worldwide, Canada.

The major users of durum wheat are concentrated around the Mediterranean Basin, but it is also largely consumed in West Asia regions and India. Ninety five per cent of durum wheat production is used for **pasta** (a mixture of semolina and water) that is widely consumed in southern Europe, North Africa, North America and the former Soviet Union. Durum wheat **flat breads** such as chapatti, tortilla, baladi, tanoori, and pita are mainly consumed in North Africa, West Asia and India. **Couscous** is another durum wheat-based food, prepared by moistening semolina,

rubbing and kneading, mainly by hand, to agglomerate wet semolina particles and form granules that are steamed and then traditionally dried under the sun. It is highly consumed in North Africa mixed with vegetables and meat. Finally, **bulgur**, more common in West and South Asia, can be prepared by boiling the clean durum wheat grains and subsequently drying them to 10–12% moisture. Dried grains are then cracked in a stone mortar (Peña-Bautista et al., 2017).

3. Impact of climate change on Mediterranean durum wheat production

The United Nations Framework Convention on Climate Change (https://unfccc.int/) makes a clear distinction between **climate change** that refers to a human-induced changes in the climate system, and **climate variability** that is attributable to natural causes. According to the Intergovernmental Panel on Climate Change (IPCC, 2014), it is projected that mean global temperatures will rise to be 1.5-4.8°C higher by the year 2100 depending on the CO_2 concentration in the atmosphere. In Europe temperatures have been estimated to increase from 2 to 4°C, and reductions in precipitation are expected to be of −10 to −50% by the 2080s (Iglesias and Garrote, 2015). Heat waves will be longer and more frequent, and extreme cold temperatures in winter will be occasional. Precipitation changes will not be uniform, precipitation would decrease in subtropical and dry regions of middle latitudes while it would increase at high latitudes and tropical regions (IPCC, 2014).

In the Mediterranean Basin, durum wheat is mainly grown under rainfed conditions where drought stress and high temperature often occur together during the grain-filling period causing a reduction on yield potential of about 50% (Altenbach, 2012). Warmer and drier summers (precipitation decrease exceeding 25–30% and warming increase exceeding 4–5°C) are expected in the region for the next decades (Giorgi and Lionello, 2008). Moreover, climate change will cause more frequent and adverse conditions for durum wheat culture in every local region specially affecting its sensitive periods during the growing season, which will probably be accompanied by strong yield reductions. In this scenario, crop breeding and biotechnology are powerful tools for contributing to food security. New durum wheat varieties with wide diversity in their adaptation responses to the different crop culture regions should be released to reduce the impact of climate change. The development of resilient cultivars able to mitigate the effect of drought events and to reduce irrigation requirements may be robust adaptation strategies. Additionally, crop diversification has been recommended to react in front of the expected loss of diversity due to climate change (Iglesias and Garrote, 2015). A large number of scientific organizations are currently involved in the description of the climate dynamics and its effects of the life in the Earth (http://www.opr.ca.gov/facts/list-of-scientific-organizations.html).

4. From landraces to modern agricultural systems

At the beginning of the 20th century, the first breeding programmes were implemented in some Mediterranean countries. Nazareno Strampelli in Italy and Juan Bautista Camacho in Spain started to select those plants from landrace populations that presented the most suitable traits (in terms of vigour, phenological adaptation, spike length and yield) to produce superior lines. Actually, through this process, they achieved the identification and isolation of the best lines already existing within the original landrace (Royo et al., 2017).

After the Second World War, Norman Borlaug with the support of the Rockefeller Foundation started a wheat breeding programme in Mexico. Using the Japanese variety 'Norin 10' and crossing it with durum wheat landraces, he introduced the dwarfing gene *Rht-B1b* that confers insensitivity to the gibberellic acid. New **semi-dwarf varieties** presented tolerance to lodging and a reduction in plant height (without significant decreases in total plant dry weight and a larger allocation of resources in grains) that resulted in an increased yield and an improved harvest index (Royo et al., 2017). The breeding programme was shuttled in two different Mexican environments with contrasting photoperiod conditions, which resulted in the release of photoperiod-insensitive durum wheat varieties, a long-day species, which could be grown under short winter days, permitting the world-wide spread of Mexican semi-dwarf durum wheats. During all this process they were also selecting for rust resistance that was causing devastating epidemics at that time (Borlaug, 2007; Royo et al., 2009).

The adoption of these improved semi-dwarf varieties (supported by agricultural research, development and technology transfer initiatives), together with the intensification of management practices, the expansion of irrigation infrastructures and the supply of agrochemicals produced great yield increases leading to the so called **Green Revolution** (Reeves et al., 2016).

Durum wheat germplasm developed by CIMMYT (International Maize and Wheat Improvement Centre) in Mexico has been the most widely used by national programmes worldwide. However, breeding programmes in Italy were also very relevant due to its existence from the very beginning of the 20th century, and so Italian germplasm can be considered the most representative within the Mediterranean Basin (Royo et al., 2009). The main achievements of durum wheat breeding programmes have been: 1) the increase of grain yield (De Vita et al., 2007; Royo et al., 2008) and its stability in high-yielding environments (Subirà et al., 2015); 2) the phenological adjustment, translated in a shortening of the crop cycle with the introduction of photoperiod insensitivity and lower vernalisation requirement (Motzo and Giunta, 2007); 3) the harvest index increase associated to a higher

number of grains per unit area and to the reduction of plant height (Royo et al., 2007), and 4) a better efficiency for biomass production and grain filling (Álvaro et al., 2008). In relation to grain quality, protein yield per hectare increased very significantly as well as yellow colour index and gluten strength, but no significant changes were observed in test weight and vitreousness (Subirà et al., 2014).

On the 1960's and 1970's period, the global population started growing faster than it was previewed, and there were serious shortfalls in cereal production that led to widespread hunger and undernourished in the developing countries. To face this food crisis and the productivity's increase demand, many countries intensified the monocrop production with the new high-yielding crop varieties developed during the Green Revolution. They also increased the use of heavy farm machinery and external inputs as irrigation systems, fertilizers and pesticides. This modern monocrop cereal culture required uniform fields, which led to the planting of large areas with a single or a small number of varieties, managed under similar intensive agronomic practices. Almost all the available arable land was used and production was intensified. The negative consequences were land degradation and the soil erosion associated to the conventional tillage systems, as well as salinization of irrigated areas and over-extraction of groundwater (Collette et al., 2011).

The replacement of old cultivars by the modern more uniform and productive semi-dwarf varieties had also a huge effect on the natural habitat of wheat, as landraces and cultivars derived from them practically disappeared from cultivation (Royo et al., 2017). Landraces contain an extensive genetic diversity due to their different adaptation patterns according to the place of origin (Nazco et al., 2012), their resilience to abiotic stresses (Kyzeridis et al., 1995) and resistance to pests and diseases (Talas et al., 2011). The abandon of their use derived on a loss of genetic diversity, phenomenon known as "**genetic erosion**" (Hammer and Teklu, 2008). Currently the number of cultivars grown in the world is relatively small and most of them have a common number of ancestors, which has drastically reduced the genetic background underlying successful modern wheat varieties (Maccaferri et al., 2005; Soleimani et al., 2002). Narrowing the genetic background increases the vulnerability of the modern germplasm to diseases and pests, and decreases the abiotic stress tolerance, particularly to the drought and high temperatures that are typical of many growing regions of durum wheat (Hovmøller and Justesen, 2007; Royo et al., 2009; Shiferaw et al., 2013). Biodiversity decrease led also to a loss of soil fertility and an ecosystems services disruption till the point that sustainability of food production could be in danger (Tscharntke et al., 2012).

5. Landraces as sources of genetic variability

Genetic improvement of a crop is based in the recombination within genetic variability. Pre-breeding programmes try to identify and create new sources of genetic variability in order to find new and useful candidate alleles and genes for their introgression into commercial cultivars. Thus, genetic uniformity in crops can be reduced (Shimelis and Laing, 2012).

Hybridization is the main source for creation of variability, parents to be used in crosses should be chosen according their performance in the target traits and should carry the favourable alleles for them. Parents can proceed from different sorts of genetic resources including obsolete or primitive cultivars, wild or semi-wild species, landraces or modern cultivars. All members belonging to the tribe *Triticeae* were originated by alloploidization via hybrid speciation and, in consequence, the available genepool of *Triticum* spp is exceptionally wide (Zaharieva and Monneveux, 2006). Other ways to take in consideration as sources of variability are the creation of polyploids or the introgression of new traits through mutation breeding followed by backcrosses with good parents (Shimelis and Laing, 2012).

Landrace populations are of prime importance among the sources of **genetic diversity**. Unlike modern cultivars, landraces are dynamic populations with high intern competitiveness because they are formed by sets of plants with different genetic constitutions. Knowledge of this huge genetic diversity and population structure is essential for landrace conservation (Royo et al., 2017). The genetic variability of landraces can be exploited by breeding programmes, especially with respect to the field of adaptation to climate change and the quality of the end-products (Lopes et al., 2015).

Landraces, wild types and exotic germplasm are preserved in gene banks, such as those belonging to CIMMYT and ICARDA (International Center for Agricultural Research in the Dry Areas), the two international centres operating with durum wheat. These centres have largely helped to widen the genetic pool of current cultivars; shuttle breeding and germplasm exchange all around the world have been key factors in creating the current overall variation in durum wheat (Royo et al., 2009).

6. The IRTA Diversity Panel of Durum Wheat Mediterranean Landraces

The panel of durum wheat landraces used in the current PhD Thesis started to be assembled in 1999 in the framework of the IRTA's durum wheat breeding programme, with the objective of holding a collection of landraces gathering the genetic variability existing in the old Mediterranean germplasm, to be characterised and used for breeding purposes.

Initial studies, conducted with a lower number of entries than the used in the

current PhD Thesis, investigated the spreading of durum wheat through the Mediterranean Basin, the strategies of yield formation and glutenin subunit diversity (Moragues et al., 2006 a, b, c, 2007). The ability of the whole collection to improve grain quality of modern cultivars was analysed by Nazco et al. (2012). Further research conducted on allelic variants at *Glu-1/Glu-3* loci allowed the identification of landraces carrying new banding patterns positively affecting gluten strength (Nazco et al., 2014a). A geographic structure of landraces in East Mediterranean, West Mediterranean and North Balkan was established and the potential value of landraces in breeding to broaden the genetic basis of gluten quality improvement was demonstrated (Nazco et al., 2014 a, b).

From long-term climatic data of the main growing wheat areas in the countries of origin of the landraces, Royo et al. (2014) associated the physiology and yield formation strategies of Mediterranean landraces to the climate of the zones where they had been collected. Cycle length of landraces, their biomass, plant height and grain yield increased from the warmest and driest zone to the coldest and wettest one, while the number of grains per unit area and grain weight decreased.

The genetic structure of the panel was determined using 44 simple sequence repeat (SSR) markers that classified landraces in four Genetic Subpopulations (GSP). Most of the landraces assigned to any GSP followed a geographical pattern: Eastern Mediterranean (EM), Eastern Balkan and Turkey (EB+T), Western Balkan and Egypt (WB+E) and Western Mediterranean (WM) (Soriano et al. 2016). The genetic diversity increased from East to West of the Mediterranean Basin, accordingly to the pattern of dispersal of durum wheat. Genetic subpopulations also presented differences in their agronomic performance. Although grain yield was similar for all them, they present different yield formation strategies: EM landraces produced more number of spikes per unit area whereas WM presented the highest kernel weight. Moreover, landraces from the Balkan Peninsula lengthened their development probably as an adaptation to the cooler climate, while those from the eastern Mediterranean region reduced it as a mechanism to escape from terminal drought (Soriano et al., 2016).

On a further study Soriano et al. (2017) carried out association mapping, thus identifying 245 marker-trait associations for yield components (86), phenology (70) and biomass (89). This study also demonstrated that EM and WM landraces showed contrasting yield formation strategies. Subsequently, Soriano et al. (2018) demonstrated that markers associated to these traits presented different allele distribution and frequencies in both subpopulations.

7. Breeding challenges on a climate change scenario

The main challenge for durum wheat breeding is to release new **drought tolerant varieties** with improved **grain yield**, reaching the industry **quality** requirements and accomplishing with a guarantee of environmental sustainability. Moreover, varieties should present **durable resistance** to the main diseases. To achieve these goals the knowledge of the genotype by environment interaction should improve as it really constrains the phenotype-genotype association, and new biotechnological and bio-information tools should be incorporated into breeding programmes (Royo et al., 2009).

Durum wheat breeding will play a critical role in the needed sustainable intensification of its cropping systems and to accomplish with this main challenge, it is essential to better understand yield formation and the plant mechanisms involved in drought tolerance and their adaptation to the environment. Increasing the knowledge of these physiologic processes and their genetic regulation is critical to design strategies to increase genetic gains in productivity and yield stability.

7.1. Grain yield

According to the United Nations, by 2050 it is predicted to be more than 9 billion people, thus a significant improvement in wheat production will be required to feed human population (FAO, 2009). The global population's demand will require wheat production to increase until 2050 by 1.7% per year, which is more than the improvement reached by the Green Revolution (Leegood et al., 2010). This will require an increase of investment in research and development for sustainable productivity. Opportunities for important yield increases through breeding will depend on the precise knowledge of both genetic and environmental yield-limiting factors and how future breeding strategies are planned (Reynolds et al., 2011).

A large number of studies have investigated the genetic control of grain yield in wheat and several quantitative trait loci (QTLs) have been reported for durum wheat (Kidane et al., 2017; Maccaferri et al., 2008; Marcotuli et al., 2017; Mengistu et al., 2016; Soriano et al., 2017; Sukumaran et al., 2018).

Grain yield formation in durum wheat can be analysed in terms of yield components, i.e. number of spikes per unit area, number of grains per spike and grain weight. The product of the first two results on grain number per unit area. These components appear sequentially with later-developing components under control of earlier-developing ones (García del Moral et al., 1991; Hamid and Grafius, 1978), therefore interacting in compensatory patterns during plant development (Gibson and Paulsen, 1999; Simane et al., 1993a). Compensation of yield components occurs as a result of competition for limited resources (Miralles et al., 2000; Simane et al., 1993a). A negative correlation exists between grain number and grain weight

(Sadras, 2007), which limits the breeder's capacity to increase net yield through the improvement of both simultaneously. Grains per spike and spikes per square metre are the yield components most sensitive to drought while grain weight remains relatively stable due to high remobilization of the assimilates stored during pre-anthesis (Zhong-hu and Rajaram, 1994).

7.2. Phenology fitting

The development of the wheat plant undertakes several phases: germination, seedling establishment and leaf production, tillering and head differentiation, stem and head growth, head emergence and flowering, grain filling and maturity. Once the first leaf has reached the soil surface, wheat development starts with leaf initiation, which ends at the double ridge stage of the developing spike, giving way to the beginning of the reproductive phase. During the stem elongation phase floret primordia develop (Kirby and Appleyard, 1984).

Flowering time is a critical stage that delimits the duration of spike formation and marks the transition into the grain-filling period, when the number of grains per spike and its weight are defined. For this reason flowering time is considered a primary trait determining wheat adaptation to a particular set of growing conditions (Snape et al., 2001; Worland et al., 1998). During the last growing period, grain filling is supported by a high transient photosynthesis after anthesis and the remobilization of stored reserves accumulated in the stems and leaf sheaths prior to it (Blum, 1988; Royo et al., 2018). In Mediterranean environments grain filling is limited by several abiotic stresses, mainly rising temperatures and reducing water supply, which reduce photosynthesis rate after anthesis, increasing the contribution of remobilization of pre-anthesis assimilates, thus constraining yield potential (Álvaro et al., 2008; Simane et al., 1993b).

Phenological adjustment or the optimization of the duration of the different wheat developmental phases, has been one of the most useful strategy for adaptation to harsh or/and highly erratic environmental conditions (Loss and Siddique, 1994) and for maximizing performance under highly favorable environments. Controlling the time to reach heading and/or anthesis is a powerful approach for plant adaptation to the environmental conditions and allowing them to escape from terminal drought stress or to avoid an early flowering when temperatures are too cold (Habash et al., 2009). Time to flowering is one of the 'constitutive' traits that have proven to be very useful in escaping drought (Habash et al., 2009).

In wheat, genetics of flowering time is complicated due to a strong interaction between genotype and environment (Mastrangelo et al., 2005). The main genetic factors involved are vernalization requirement (*Vrn* genes), photoperiod sensitivity (*Ppd* genes), and intrinsic earliness (*Eps* genes). **Vernalization** requirement is

controlled by three major genes in wheat (Distelfeld et al., 2009a) called *VRN1*, *VRN2* and *VRN3*. *VRN1* includes three loci (*Vrn-A1*, *Vrn-B1* and *Vrn-D1*) mapped in co-linear regions of the chromosomes 5A, 5B and 5D (Yan et al., 2003). Low response to vernalization or absence results from the dominant allele presence. The *VRN2* region includes *Vrn-A2* and *Vrn-B2* loci in durum wheat (Distelfeld et al., 2009b). Spring growth habit (*vrn2*) is associated with mutations or deletions in both *ZCCT* genes simultaneously (Yan et al., 2004). *VRN3* is known to have an integrator role of the photoperiod and vernalization pathways (Trevaskis et al., 2007). Two major loci have been mapped for **photoperiod response** in durum wheat, *Ppd-A1* and *Ppd-B1* (Snape et al., 1996). Dominant alleles confer low sensitivity to photoperiod. A mutation conferring photoperiod insensitivity has been mapped on durum wheat *Ppd-A1* on chromosome 2A (Wilhelm et al., 2009). Two different deletions are known in durum wheat *Ppd-A1* gene upstream the coding region, denoted 'GS-100' and 'GS-105' alleles. It has been shown that the photoperiod insensitive mutations in *Ppd-A1* arose after domestication of durum wheat (Bentley et al., 2011). Minor genes have been located in chromosomes 1B, 4B, 5B, 6B, 7B, 1A, 5A and 7A (Hanocq et al., 2004; Shindo et al., 2003; Worland et al., 1998). Intrinsic earliness or **earliness *per se*** (*Eps*) consists in the genotypic difference for flowering date once vernalization and photoperiod requirements are accounted for (Boyd et al., 2003). Earliness *per se* confers subtle manipulation of life cycle within a region (Griffiths et al., 2009). Significant QTLs for earliness *per se* have been confirmed on chromosomes 1B, 2A, 3A, 3B, 4B, 5A, 5B, 6A, 7A, 7B additionally to 1D, 4D and 7D which are exclusive of bread wheat (Båga et al., 2009; Griffiths et al., 2009). Despite their effects being relatively modest these QTLs may cause variation in flowering date even in the presence of major genes as *Ppd* and/or *Vrn* (van Beem et al., 2005).

7.3. Adaptation to environmental conditions

Furthermore, yield depends on plants adaptation to the environmental conditions and it is highly determined by the **Genotype × Environment interaction** (GE) effect. GE is the differential phenotypic expression of cultivars across environments as cultivars perform in a different way when they are grown in diverse environmental conditions. This behaviour arises from the diverse response of genotypes to climatic variables (mainly temperatures and rainfall) and soil characteristics during plant growth and development (Blum and Pnuel, 1990). GE weakens the association between phenotype and genotype and complicates the identification of superior genotypes because a unique best genotype across all the target environments cannot be detected, thus reducing the genetic progress in breeding programmes. GE can be partitioned in subcomponents studying it as

genotype × site, genotype × year, and genotype × site × year interactions, or using the additive main effects and multiplicative interaction (AMMI) model defined by Gauch (1988), where the total sum of squares is divided in interaction principal component axis (IPCAs). Another useful methodology to analyse the GE interaction is partitioning it into those components that contribute to a change in rank of genotypes (cross-over interactions) and those that do not (non-cross-over interactions). In cross-over interactions, the lack of correlations among environments and genotypes is what complicates the selection of the superior ones (Basford and Cooper, 1998). The study of GE using advanced statistical models facilitates the development of appropriate breeding strategies and reduces the uncertainty associated to it (Romagosa et al., 2009). The concept of **stability** is also concerned with the consistency of genotype performance across environments. Yield stability can be analysed from two different points of view: static or dynamic. Static stability (or biological concept) is defined as the lack of response of a cultivar to any variation of the environment and it is usually related to low yields. On the contrary, the dynamic stability (or agronomic concept) refers to the fact that a cultivar will respond predictably to improved growing conditions (Becker and Léon, 1988).

Drought resistance involves a complex pool of mechanisms adopted by plants to mitigate the negative effects of water deficit (Reynolds et al., 2005). Among the traits related to drought adaptation, phenological adjustment, early vigour, water and radiation use efficiency, osmolytes accumulation and remobilization of water-soluble carbohydrates, carbon isotope discrimination and root system architecture are associated with yield culture under rainfed conditions (Tuberosa, 2012).

Roots are the main organs for water and nutrient absorption, so their characteristics play an important role in drought tolerance, nutrient and water uptake efficiency, lodging resistance and tolerance to mineral toxicity. In durum wheat there are two main types of roots: seminal roots arising from the embryonic seed part and nodal or crown roots arising from the basal part of the tiller when the fourth leaf emerges. Seminal roots include one primary root, two pairs of symmetric roots, and, sometimes, a sixth central root. They penetrate the soil earlier and more deeply than nodal roots and remain functional for the entire plant cycle. For this reason they are equally important than nodal roots for sustaining yield (Manschadi et al., 2013). Root system architecture (RSA) involves a wide number of traits such as seminal and crown roots number, seminal root angle, total root biomass, length and root system depth and total root surface area. They are considered to be complex traits with a quantitative nature and controlled by polygenes (Hamada et al., 2012). These traits present a wide range of variation and high phenotypic plasticity among cultivars, especially within landraces (Canè et al., 2014; Ruiz et al., 2018), depending on the environment where plants are grown, consequently it has a significant effect on grain

yield. Under drought conditions, when water and nutrient absorption may be reduced, a higher number of seminal roots, with a narrow angle and a deeper root system results in higher yields. Similarly, fine roots increase nutrient and water absorption through increased root surface area per unit mass (Wasson et al., 2012). Optimization of the root system could lead to varieties better adapted to drought tolerance but it is highly complicated due to the high variability of RSA (that also hinders the identification of genotypic variation) and the difficulties in root phenotyping under field conditions (Wasaya et al., 2018).

7.4. Grain quality

Durum wheat flour and semolina allow formulating different kinds of food as pasta, couscous, bulgur and flat breads that are obtained through different industrial processes or artisanal and home-made techniques (Duveiller et al., 2007). Semolina's durum wheat are coarse flour particles obtained from the grain's endosperm which is very hard and with high yellow pigment content. According to these end-products, there are some valuable traits related to flour quality that should be taken into account in durum wheat breeding: grain protein content, gluten strength, grain colour and yellow pigment content, vitreousness, starch characteristics, and grain weight (Carrillo et al., 2006). **Grain protein content** is highly depending on the composition of the storage proteins. Glutenins (polymeric proteins) and gliadins (monomeric proteins), are the main components of gluten and are responsible of the viscoelastic properties and dough extensibility, respectively, and so they define the **gluten strength**. Due to 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). Both of them are encoded by polymorphic genes: *Glu-A1* and *Glu-B1*, located on the long arm of group 1 homologous chromosomes (Shewry et al., 1992; Singh and Shepherd, 1988) code for HMW-GS, and *Glu-A3, Glu-B3* for LMW-GS (Ruiz and Carrillo, 1995; Vázquez et al., 1996). Each gene has multiple alleles and combinations between them determine the dough-forming properties of a durum wheat variety. *Glu-3* loci are genetically linked to *Gli-1* loci (those corresponding to gliadins) while *Gli-2* is placed to another chromosome (Gale, 2005; Ruiz et al., 2005).

In relation with the flour **colour**, it is convenient to obtain a bright yellow colour. It is determined by the yellow pigment content gene *Phytoene synthase* (*Psy*) and the carotenoid presence (Borrelli et al., 2008) primarily trans-lutein at the semolina level (Ramachandran et al., 2010), and from oxidative enzymes that degrade carotenoid compounds (Borrelli et al., 2008). Furthermore, a low

lipoxygenase activity is desirable because it degrades carotenoids during pasta processing. Semolina yellowness is a complex trait, with high heritability (Clarke et al., 2006; Geng et al., 2011), and a number of studies have reported QTLs associated to it along all wheat chromosomes (find a review in Schulthess and Schwember, 2013). A major QTL at chromosome 7AL has been found to account for up to 55% of yellow pigment in durum wheat (Patil et al., 2008), and a QTL at chromosome 7B has been reported to be responsible for 53% of the durum wheat grain yellow pigment content variation (El Ouafi et al., 2001). In addition, the homologous copies of *Psy-1* were mapped in durum wheat within these QTLs positioned on chromosomes 7A (Singh et al., 2009) and 7B (Pozniak et al., 2007; Zhang and Dubcovsky, 2008).

The genes **hardness**, *Ha*, and those that coded for the puroindolines, *pin-a* and *pin-b*, control one of the most important quality characteristic which affects milling, baking and end-use quality since according to the grain hardness, the flour obtained will have an increased starch damage with the subsequent increased water adsorption. Finally, the synthesis of amylose in stored **starch** in the endosperm is coded by the Waxy genes (*Wx*) or Granule-bound starch synthase1 (*GBSS1*), a lower amylose content increases starch viscosity and flour swelling volume (Gale, 2005).

The end-product assessment through milling and baking is highly costly and time-consuming, so some indirect test have been developed to achieved it, but they are also time-consuming (Goutam et al., 2013).

In the European Union, in order to evaluate durum wheat varieties according to their flour quality, there is the EU quality index (QI) calculated weighting four traits relative to check varieties, according to the following percentages: grain protein content (40%), gluten strength (30%), yellow index (20%) and grain weight (10%) (European Commission Regulation No 2237/2003, 23 Dec, 2003; Official Journal of 24.12.2003; Royo and Briceño-Félix, 2011).

8. From classical breeding to genomic selection

Despite of the large improvements in genetic gains for yield, to achieve the new challenges in the coming years to skip biotic and abiotic stresses, there is a need to find new tools to make breeding more efficient and faster. **Empirical or classical breeding**, based in the selection of the trait *per se* (Loss and Siddique, 1994), was the main approach used for breeders to increase crop productivity during the 20th century. It implies the selection of varieties carrying the desired characteristics for the target trait, usually morphological or visual characteristics such as yield, yield components or diseases resistance. Breeders chose the best varieties using them as progeny and back-crossed it to a recurrent parent to dilute the irrelevant or undesired trait (Hou et al., 2014). However this process takes several years to finally get a

commercial variety and has some limitations, especially when target traits are highly dependent on the environment and have low heritability (Jackson et al., 1996).

Analytical approaches complement and improve the results obtained by the empirical breeding. Their main objective is to identify in the earlier stages of development by indirect methods, the traits enhancing the agronomic performance. These features are usually physiological processes (phenology, photoperiod and vernalisation responses, root architecture system, osmolytes accumulation, biotic and abiotic stresses responses…) that involve several traits (Austin, 1993). However the use of physiological criteria in breeding programmes is expensive and time-consuming. Physiological features have been evaluated in controlled conditions during the process of their discovery, but carrying out field trials is not practical due to the thousands of entries comprising the segregating generations of breeding programmes (Monneveux et al., 2012).

With these approaches, durum wheat improvement was dependent on the selection of traits without knowing their molecular mechanisms of inheritance. Most of the important agronomic and quality traits are complex traits highly affected by the environmental conditions and regulated by several genes with small effect, they are quantitative trait loci (**QTLs**). Additionally, many of the traits proposed by plant physiologists are measured with complex, time-consuming and not applicable techniques in a breeding programme, so it became necessary to search for alternative tools that supplemented conventional durum wheat breeding. The development of molecular biology techniques brought a new type of markers based on the sequence or polymorphisms in the DNA, the **molecular markers**, and open the doors for new challenges in plant breeding. The advantage of these type of markers is that they can be widely distributed in the genome, they are not affected by the environment and they can be identified in any tissue and developmental stage. From their development, their use in agriculture increased through the construction of genetic maps in crop species, the association between molecular markers and important agronomic traits, the dissection of quantitative traits and the positional cloning of genes of interest. Besides the estimation of genetic distances and molecular cloning, molecular markers provide the most suitable tool for the evaluation of genetic diversity, allow the selection of the most suitable parental lines in the breeding programmes, the management of germplasm collections and varietal identification (Phougat et al., 2018).

In durum wheat pre-breeding, the screening and genotypic characterization of diverse germplasms is a requisite and genetic markers can be highly efficient in the identification of genotype–phenotype associations by biparental mapping or association mapping (Maccaferri et al. 2011; Laidò et al. 2014; Giraldo et al. 2016; Mengistu et al. 2016; Soriano et al. 2017). Once the association between a marker

and a gene is detected, it can be deployed into a breeding programme through **marker assisted selection** (MAS). The success of this technique relies on the identification of markers tightly linked with the genes or genomic region of a target trait. Thus, MAS may improve the efficiency of selection of both Mendelian traits and QTLs as it facilitates the introgression of single genes with the desired alleles into enhanced lines of candidate cultivars, it removes rapidly the undesirable genome of the donor parent in a back-crossing programme once the gene of interest is identified and, finally, it allows the identification and protection of commercial cultivars through fingerprinting (Monneveux et al., 2012; Shimelis and Laing, 2012).

Due to the great advantages of MAS, it is being integrated together with traditional breeding methods to enhance the efficiency of cultivar development, especially with Mendelian traits. However, since many of the agronomic traits present a multigenic and quantitative nature and the effect of the environment on them needs to be assessed, MAS will not replace traditional breeding methods, especially in later generation screening and cultivars evaluation, they will become complementary methods (Goutam et al., 2013). Additionally, in QTL biparental mapping, results depend absolutely on parental populations. These populations cannot cover the allelic distribution needed in a breeding programme, and marker effect can be overestimated. On its part, association mapping or genome-wide association study (GWAS) detect DNA markers with low heritability and few large-effect QTL. Finally, genomic selection is a form of MAS that attempts to eliminate the limitations of biparental and association mapping (Talukder and Saha, 2017).

In **genomic selection** (GS), all marker data in a training population are utilised as breeding value predictors. Genomic estimated breeding values (GEBVs) for all genotyped germplasm are obtained from markers, phenotyping and pedigree information of the training population (using a diverse germplasm), to predict their performance in the breeding programme and allowing the selection of superior individuals (Meuwissen et al., 2001). GS has emerged as a valuable tool for improving complex traits controlled by multiple QTLs with small effect. This, together with high-throughput phenotyping techniques have brought a revolution in breeding by enhancing the accuracy level of selection. Particularly, GS can be widely used in genetic resources to predict breeding values in pre-breeding studies (Rasheed and Xia, 2019). In durum wheat, GS is starting being used in complex traits such as yield and quality traits (Haile et al., 2018; Mérida-García et al., 2019).

9. Genomic tools for durum wheat breeding

9.1. Molecular markers

The firstly and mainly used molecular markers in plant breeding were the restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and microsatellites or SSR (Adhikari et al., 2017). These markers are based on PCR amplification or DNA cleavage with restriction enzymes, which makes their use time consuming and reporting low genome coverage. Among them, **microsatellites** or **SSR**, tandemly repeated nucleotide sequences flanked by conserved sequences, were widely used based on their characteristics. They present high level of polymorphisms and large variability, they have codominant inheritance and an abundant distribution through the genome (Rasheed and Xia, 2019). Another type of molecular markers widely distributed in the genome are the single nucleotide polymorphisms (**SNPs**). These markers can detect a difference in only one base in the DNA sequence. However, as they are based on sequencing resulted expensive.

In the last decade, the need of a wider genome coverage resulted in the development of a new type of molecular markers, the Diversity Arrays Technology (**DArT**) markers developed by Diversity Arrays Technology Pty Ltd (Canberra, Australia) with the microarray technology platform (https://www.diversityarrays.com/). Through hybridisation-based methods, Diversity Arrays detect single base pair changes (SNPs) (Jaccoud et al., 2001). DArT markers are becoming highly used since they provide an extensive genome coverage and they are obtained through a low-cost marker system. Maccaferri et al. (2014) developed a durum wheat consensus map combining mainly SSR and DArT markers.

Recently, the advances in next generation sequencing (NGS) technologies decreased the costs of DNA sequencing making feasible the genotyping based on sequence data. This fact allowed the development of two high-throughput SNPs platforms, one with 9,000 SNPs (Cavanagh et al., 2013) and the other with 90,000 SNPs (Wang et al., 2014). With these data Maccaferri et al. (2015a) constructed a second consensus map for durum wheat. In addition, Diversity Arrays Technology developed a new genotyping by sequencing platform, **DArTseq,** with the same principle than DArT markers by using the new NGS techniques to obtain short DNA sequences including the marker polymorphism. These new markers have wider genome coverage than conventional DArTs and provide an opportunity to select genome fractions corresponding predominantly to active genes. Restriction enzymes used in this method separate low copy sequences from the repetitive fraction of the genome, being these low copy sequences informative for marker discovery. Finally, representative fragments are sequenced on NGS platforms without the previous knowledge of the DNA sequence. DArTseq generates two types of data: SNPs and

presence/absence variants (PAVs) markers. Nowadays DArTseq and SNP arrays are the most widely used platforms in *Triticum* spp research and breeding (Rasheed and Xia, 2019).

Molecular markers have been widely used for the construction of linkage maps. A **linkage map** describes the position and relative genetic distance between markers and genes. The principle for their construction is the chromosomal recombination during meiosis, those markers close to each other will be transferred together more commonly than those separated by larger distance because there is no recombination between them. Molecular markers can be used as landmarks on genetic-linkage maps and enable the anchoring of markers to their true physical locations in the genome (Narain, 2010).

The first linkage map in durum wheat was developed by Blanco et al. (1998). This map was mainly constructed with restriction fragment length polymorphisms (RFLPs) markers. From this point other linkage maps including mainly SSR markers were constructed and both major genes and QTL were mapped (Mantovani et al., 2008; Nachit et al., 2001).

9.2. QTL mapping

QTL mapping has been performed classically using **biparental populations** derived from two parents with different phenotypic performance. The most used population types are F_2, recombinant inbred lines (RILs), doubled haploid (DH) and backcross populations (BC), they represent permanent resources that can be replicated over sites and years (Collard and Mackill, 2008). The success to detect a QTL depends on the marker density, the population size and the heritability of the trait. A large amount of QTL studies have been performed in wheat and durum wheat for disease resistance, yield performance, phenology, root system architecture or quality traits. A revision for different traits can be found in Soriano and Royo (2015), Soriano et al. (2017), Roselló et al. (2018) and Soriano and Álvaro, (2019).

The genetic resolution of QTL mapping often remains confined to a range of 5-10 cM due to the restricted number of recombination events produced in a biparental mapping population, which needs additional steps of generating more recombinant individuals to fine map a locus. In order to overcome this constraint, the association mapping was developed as a complementary approach.

Association mapping (**AM**) or **GWAS** allows the dissection of the genetic basis of complex traits providing broader allelic coverage and offering higher mapping resolution. It is based on linkage disequilibrium (LD), defined as the non-random association of alleles at different loci, and is used to detect the relationship between phenotypic variation and genetic polymorphisms (Flint-Garcia et al., 2003). It is important, however, to differentiate the LD due to physical linkage from LD due

to population structure that can be caused by selection, genetic drift and species-dependent characters such as the mating system. Germplasm collections characterised by medium to high LD levels are suitable for the identification of chromosome regions harbouring genes/QTL controlling agronomic traits (Breseghello and Sorrells, 2006). In wheat AM has been recently conducted for investigating the genetic basis of yield and yield components in different environments (Laidò et al., 2014; Maccaferri et al., 2011; Soriano et al., 2017), grain quality traits (Tadesse et al., 2015; Roselló et al., 2018), root traits (Canè et al., 2014; Roselló et al., 2019), resistance to diseases (Maccaferri et al., 2015b), and crop phenology (Soriano et al., 2017). A comparison between linkage mapping and association mapping is shown in Table 1.

Table 1. Linkage QTL mapping vs Association mapping.

Linkage QTL mapping	Association (LD) mapping
Two known ancestors (parents)	Multiple (unknown) ancestors
Short known recombination history	Long (unknown) recombination history
Population structure is simple	Complex population structure
LD caused by linkage	LD caused by different population genetic reasons
Requires construction of specific maps	Existing maps can be used
Contrasting genetic background	Diverse genetic background
Phenotyping is required for new populations	Phenotyping data might already be available

Since the first published QTLs, the number of studies reporting QTLs associated to different agronomic traits has notably grown. These studies identified hundreds of QTLs in different mapping populations using different types of markers. In order to identify consensus QTL regions in the genome, Goffinet and Gerber (2000) developed the QTL meta-analysis. This method allows the integration of results from independent QTL studies in a consensus map. The power of QTL meta-analysis lies in identifying regions of the genome that are most frequently involved in trait variation and narrowing down the QTL supporting intervals, thus facilitating the identification of candidate genes for positional cloning. QTL meta-analysis has been performed in the last few years in wheat for traits such as grain yield (Soriano et al., 2017; Zhang et al., 2010), crop phenology (Hanocq et al., 2007; Soriano et al., 2017), disease resistance (Goudemand et al., 2013; Löffler et al., 2009; Marone et al., 2013; Soriano and Royo, 2015), plant height (Griffiths et al., 2012), grain-related traits (Tyagi et al., 2015), quality traits (Roselló et al., 2018) and root related traits (Soriano and Álvaro, 2019).

9.3. Wheat genome sequence

In the last years, two achievements will speed up molecular biology in wheat, the release of both bread and durum wheat reference genomes (IWGSC, 2018; Maccaferri et al., 2019). These sequences represent an essential tool to study wheat domestication, evolution and breeding as well as to gain new insights into gene function and the genome-wide organization of QTLs for relevant agronomic traits.

10. Future prospects

Durum wheat based foods highly contribute in human's diet with the intake of calories, vitamins and minerals. Durum wheat production has to increase to achieve the growing population demand in a sustainable agricultural ecosystem. Although past yield improvements relied on the development of improved varieties that needed the intensification of agricultural practices to maximize yields, the new released varieties have to be able to produce equal or higher yields with the minimum environmental impact (Royo et al., 2017). In a framework of climate change, knowing the plants mechanisms for drought resistance and their adaptation to the environment to avoid yield losses, and matching the industry quality requirements becomes critical Increasing the knowledge of the genetic regulation of all these attributes with new biotechnology techniques will facilitate the development of strategies to accelerate the national and international breeding programmes work (Iglesias and Garrote, 2015).

New high-throughput genotyping and phenotyping technologies will facilitate the characterization and utilization of exotic germplasm (Wang et al., 2017). Exploring durum wheat Mediterranean landraces will increase the knowledge of genetic diversity in the Mediterranean Basin. As the genetic variation contained in landraces is usually unknown, identifying markers associated to the target traits and varieties carrying them, will provide new favourable potential genes to be incorporated into modern cultivars (Lopes et al., 2015). This information will facilitate the creation of new strategies and tools in the breeding programmes to release new durum wheat varieties with improved yield production and stability, drought resistance and keeping the industry quality requirements. The greater efficiency and efficacy of breeding programmes will reduce the costs to obtain new improved varieties that will benefit all the durum wheat chain, from farmers to consumers.

11. References

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Objectives

Objectives

The main objective of this PhD Thesis was to provide information useful for durum wheat breeding programmes to develop new varieties with enhanced productivity in Mediterranean environments on a context of climate change, and the grain quality attributes meeting the standards of the food industry. To achieve this general objective the specific aims were:

1. To conduct QTL meta-analysis to identify consensus QTL regions controlling durum wheat development, yield, yield related traits and grain quality (Chapters 1 and 5).
2. To explore the variability of the durum wheat yield QTLome among Mediterranean landraces and modern world-wide cultivars to detect differences in yield formation and identify new interesting alleles for breeding purposes (Chapter 2).
3. To analyse the pattern of adaptation to the environment and yield formation strategies of eastern and western Mediterranean landraces and the influence of meteorological variables on them (Chapter 3).
4. To study the architecture of the seminal root system in a structured panel of durum wheat Mediterranean genotypes, its relationship with yield and related traits and to identify molecular markers associated to its main characteristics (Chapter 4).
5. To identify stable genomic regions controlling pasta-making quality traits through association mapping and to ascertain whether a geographic structure exists for the identified QTLs (Chapter 5).

The plant material used to achieve these objectives was the IRTA Diversity Panel of Durum Wheat Mediterranean Landraces and a collection of 205 world-wide modern cultivars. This PhD Thesis is structured in five chapters written as scientific articles, so they can be read as individual entities. At the moment of elaborating the present manuscript Chapter 1 is part of the paper entitled: '*Dissecting the old Mediterranean durum wheat genetic architecture for phenology, biomass and yield formation by association mapping and QTL Meta-analysis'* published in PLoS ONE in 2017, Chapter 2 has been submitted to an international journal, Chapter 3 has been published in 2019 in the European Journal of Agronomy, Chapter 4 has been published in Agronomy in 2019 and Chapter 5 was published in Frontiers in Plant Science in 2018.

Chapter 1:
QTL meta-analysis for phenology, biomass and yield-related traits in durum wheat

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QTL meta-analysis for phenology, biomass and yield-related traits in durum wheat

Abstract
Durum wheat is a major crop in the Mediterranean Basin, which is the largest producer area worldwide. Unravelling the genetic mechanisms of yield formation and development under water limited conditions is one of the major challenges for durum wheat production. To synthesise the large amount of available information on QTLs for agronomic traits, a QTL meta-analysis was done to integrate the results of 26 independent QTL studies in wheat in a consensus map. A total of 477 QTLs, implying 25 different traits related to yield, yield components, phenology and biomass were condensed to 71 meta-QTLs (MQTL) and left 13 QTLs as singletons. The supporting interval of a meta-QTLs was reduced up to a 50% and the number of clustered QTLs ranged from 2 to 29. Candidate genes in the vicinity of a MQTL were identified.

Keywords: QTLs, yield formation, drought resistance

Abbreviations
CDW_{21}, crop dry weight at tillering
GD, grain diameter
GFD, grain-filling duration
GW, grain weight
GWS, grain weight per spike
GY, grain yield
HI, harvest index
LG, percentage of large grains
MG, percentage of medium grains
MQTL, meta-QTL
NDVI, normalized difference vegetation index at vegetative (NDVIv) and grain filling (NDVIg) stages
NFs, number of florets per spikelet
$NFTm^2$, number of fertile tillers per square metre
NGm^2, number of grains per square metre
NGS, number of grains per spike
NSm^2, number of spikes per square metre
NSP, number of spikes per plant
NsS, number of spikelets per spike

NTP, number of tillers per plant
PH, plant height
QTL, quantitative trait locus
SG, percentage of small grains
SL, spike length

1. Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is grown on around 17 million hectares worldwide. 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. Wheat was domesticated in the Fertile Crescent (10,000 before present, BP), and spread to the west of the Mediterranean Basin reaching the Iberian Peninsula around 7,000 years BP (Feldman, 2001). Natural and human selection occurring during this migration resulted in the establishment of local landraces specifically adapted to a diversity of agro-ecological zones, and is considered the largest source of biodiversity within the species (Nazco et al., 2012). The cultivation of local landraces was progressively abandoned from the early 1970s due to its replacement with the improved, more productive and genetically uniform semi-dwarf cultivars derived from the Green Revolution. However, evidence supports the hypothesis that landraces can provide new alleles for the improvement of commercially valuable traits. Mediterranean landraces represent an important group of genetic resources because of their genetic diversity, their documented resilience to abiotic stresses and their resistance to pests and diseases (Lopes et al., 2015).

In the Mediterranean Basin, wheat is mainly grown under rainfed conditions and yield is often constrained by water and heat stresses that are common during the grain filling period, due to the low and unpredictable seasonal rainfalls and high temperatures during the last stages of the crop season (Subirà et al., 2015). Moreover, according to the intergovernmental panel for climate change (http://www.ipcc.ch/), drought conditions are expected to worsen, with warmer temperatures and lower and more erratic water availability affecting the major wheat producing areas, including the Mediterranean Basin. In a context of climate change, improving the knowledge of yield and the most important traits underlying the adaptive response of durum wheat to Mediterranean environments is essential to enhance the development of varieties adapted to sub-optimal environments. Exploiting genetic diversity from local landraces in breeding programmes is important for adaptation to harsh environments and end-product quality, given the high level of polymorphism found between and within Mediterranean landraces for traits of commercial importance (Soriano et al., 2016). Thus, unravelling the genetic mechanisms underlying

development, growth and yield formation, under water limited conditions is one of the major challenges for wheat production worldwide.

To synthesise the large amount of information currently available on QTLs for relevant agronomic traits in wheat and its integration on a consensus map is essential in order to accelerate the breeding programmes. One way to synthesise and integrate all QTL information is the QTL meta-analysis approach developed by Goffinet and Gerber (2000). QTL meta-analyses have been performed in the last years mostly in bread wheat for grain traits (Tyagi et al., 2015), plant height (Griffiths et al., 2012), sprouting tolerance and dormancy (Tyagi and Gupta, 2012), dietary fibre content in grain (Quraishi et al., 2011), grain yield (Zhang et al., 2010), crop phenology (Hanocq et al., 2007), and resistance to septoria tritici blotch (Goudemand et al., 2013), powdery mildew (Marone et al., 2013), fusarium head blight (Liu et al., 2009; Löffler et al., 2009; Mao et al., 2010), leaf rust (Soriano and Royo, 2015) and Ug99 stem rust (Yu et al., 2014). However, studies in durum wheat are scarce. A QTL meta-analysis was conducted in the current study to narrow down the QTL intervals and to identify consensus QTL regions controlling the target traits in durum wheat.

2. Materials and methods

Four hundred and seventy-six QTLs were reported in the 26 published studies examined. A total number of 25 traits were studied: CDW_{21}, DSH, DSA, DSM, GD, GFD, GW, GWS, GY, HI, LG, MG, $NDVI_g$, $NDVI_v$, NFs, $NFTm^2$, NGm^2, NGS, NSP, NsS, NSm^2, NTP, PH, SG and SL.

For each study, the following information was collected: parents of the cross, type of cross, number of progenies, name of QTLs, trait, environment, LOD score, PVE (phenotypic variance explained) by each QTL, QTL position on the authors' linkage map, flanking markers and QTL supporting interval (SI).

To compare the QTLs detected in different populations, original QTL data were projected onto the consensus map of durum wheat developed by Maccaferri et al. (2014). QTLs were projected following the homothetic approach proposed by Chardon et al. (2004). The SI were defined as reported by Soriano and Royo (2015) and estimated at 95% on the consensus map using the empirical formula proposed by Darvasi and Soller (1997) and Guo et al. (2006):

SI=163/($N \times R^2$) for recombinant inbred line (RIL).

SI = 530/($N \times R^2$) for doubled haploid (DH), backcrosses (BC) and F_2 progenies.

where N is the size of the population and R^2 the proportion of variance explained by the QTL.

QTL meta-analysis was conducted following the approach of Goffinet and Gerber (2000) and Veyrieras et al. (2007) using BioMercator v4.2 (Arcade et al.,

2004; Sosnowski et al., 2012), available at http://moulon.inra.fr/. Additionally, MQTLs reported previously by Zhang et al. (2010) for yield components and Hanocq et al. (2007) for phenology were projected onto the consensus map for further comparisons. Graphical representation of the genetic position of MQTLs was carried out using MapChart 2.3 (Voorrips, 2002).

3. Results

This survey collected data from 24 studies reporting QTLs for wheat yield and yield components, biomass related traits and phenology, published from 2008 to 2015 (Table 1.1). The studies covered 23 different experimental crosses involving 44 parental lines and 4438 progenies. The traits were evaluated in a total of 117 environments and 477 QTLs were subjected for QTL projection onto the durum wheat consensus map developed by Maccaferri et al. (2014).

One hundred and ninety three QTLs (40%) were found in the A genome and 284 (60%) in the B genome. Chromosome 4B was the chromosome with the highest number of QTLs (103), whereas chromosome 1A was the one with the lowest (11) (Figure 1.1A). The number of QTLs per trait ranged from one for biomass, GFD and HI to 137 for GW. Grain weight traits (GWS, GW) included a total of 170 QTLs (36%) (Figure 1.1B). The distribution between the number of QTLs and size of the SI (Figure 1.1C) ranged from 1.9 to 51.9 cM with an average of 14.5 cM. Thirty-eight percent of the QTLs had a SI less than 10 cM and 82% of the QTLs less than 20 cM. The PVE explained by single QTL followed an L-shape distribution, with the majority of the QTLs (92%) showing a PVE < 0.2 (Figure 1.1D). PVE ranged from 0.03 to 0.68 with an average of 0.11.

The 477 QTLs projected onto the durum wheat consensus map were subjected to meta-analysis using the functions of Goffinet and Gerber (2000) when the number of QTLs in a chromosome was lower than ten and those of Veyrieras et al. (2007) when the number was 10 or more. Following an AIC criterion, 409 QTLs were grouped into 71 MQTLs (Supplementary material S3 File). Thirteen QTLs remained as single QTLs clearly defined as not overlapping with other MQTLs and finally, 55 QTL SIs overlapped with different MQTLs and were not included in any of them based on the membership coefficient given by the software and if the peak of the QTL was located out of the MQTL SI.

The number of clustered QTLs ranged from two in 16 MQTLs on different chromosomes to 29 in MQTL40 on chromosome 4B, involving eight traits. The SI reported for the MQTLs ranged from 0.37 to 22.67 cM with an average of 5.44 cM. This means a reduction of more than 50% from those observed in the initial QTLs. The number of traits involved in each MQTL ranged from one in 14 MQTLs to eight in MQTL40 (Supplementary material S3 File).

Table 1.1. Summary of QTL studies included in the meta-analysis for phenology, biomass and yield-related traits.

Cross	Progeny[a]	Size	Traits[b]	Environments	QTLs[c]	Reference
Louise × Penawawa	RIL	188	PH	5	7	Carter et al., (2011)
CDC Teal × CDC Go	RIL	187	DSA, DSH, DSM, FTm^2	4	15	Chen et al., (2015)
Wimai 8 × Jimai 20 (WJ)	RIL	175	NGS, GWS, NSP, GW	4	23	Cui et al., (2014)
Wimai 8 × Yannong 19 (WY)	RIL	172	NGS, GW	4	19	Cui et al., (2014)
Wimai 8 × Luohan 2 (WL)	RIL	179	NGS, NSP, GW	3	19	Cui et al., (2014)
05210 × Laizhou953	F2	166	NGS, NSP	1	2	Deng et al., (2011)
Kofa × Svevo	RIL	249	FTm^2, NGS, NDVI, NGm^2, GW	1	17	Graziani et al., (2014)
Nanda2419 × Wangshuibai	RIL	130	GW, NGS, PH, NSP, NTP	4	34	Jia et al., (2013)
AC Barrie × Cutler	RIL	177	DSA, DSM	5	9	Kamran et al., (2013)
Keumkang x Olgeuru	DH	122	GY, NGS, NSm^2, GW	1	6	Lee et al., (2014)
CNN × CNN (WI 3A)	RIL	223	DSA, GY, NGS, NSm^2, PH, GW	6	25	Liakat Ali et al., (2011)
Nanda 2419 × Wangshuibai	RIL	230	DSA	4	8	Lin et al., (2008)
ND3331 × Zang1871	RIL	217	NGS, GWS, NSP, PH, GW	9	66	Liu et al., (2014)
Lang × CSCR6	RIL	82	NGS, PH, GW	1	3	Ma et al., (2012a)
Kofa × Svevo	RIL	249	DSH	1	3	Maccaferri et al., (2008)
Halberd × Karl92	RIL	118	CDW_{21}, DSH, DSM, GFD, GY, NGS, NSm^2, GW	1	14	Mason et al., (2013)
Rye selection 111 × Chinese spring	RIL	92	GW	1	6	Mir et al., (2012)
HTRI 11712 × HTRI 105	F2:3	133	GW	2	10	Nezhad et al., (2012)
Kitanokaori × Ldn/KU-2097	F2	132	DSA, DSH, DSM	1	3	Nguyen et al., (2015)
PDW 233 × Bhalegaon 4	RIL	140	GY, NGS, GWS, GW	5	18	Patil et al., (2013)
Seri M82 × Babax	RIL	167	DSA, DSM, GY, NGm^2, $NDVI_g$, $NDVI_v$, PH, GW	1	11	Pinto et al., (2010)
Rye selection 111 × Chinese spring	RIL	185	GW	8	17	Ramya et al., (2010)

Cross	Progeny[a]	Size	Traits[b]	Environments	QTLs[c]	Reference
Chuanmai 42 × Chuannong 16 (F8)	RIL	127	GY, NGS, GWS, NGm^2, NSm^2, GW	7	35	Tang et al., (2011)
MN98550 × MN99394	RIL	139	DSH, GY, LG, MG, PH, GD, GW, SG	4	41	Tsilo et al., (2010)
Line 3228 × Jing 4839	F2:3	237	NFs, NGS, SL, NsS, GW	3	24	Wang et al., (2011)
Arche × Récital	DH	222	NGm^2, GW	31	42	Zheng et al., (2010)

[a]RIL: recombinant inbred line; DH: double haploid.

[b]For the extended name see list of acronyms.

[c]Number of QTLs subjected for projection onto the durum wheat consensus map.

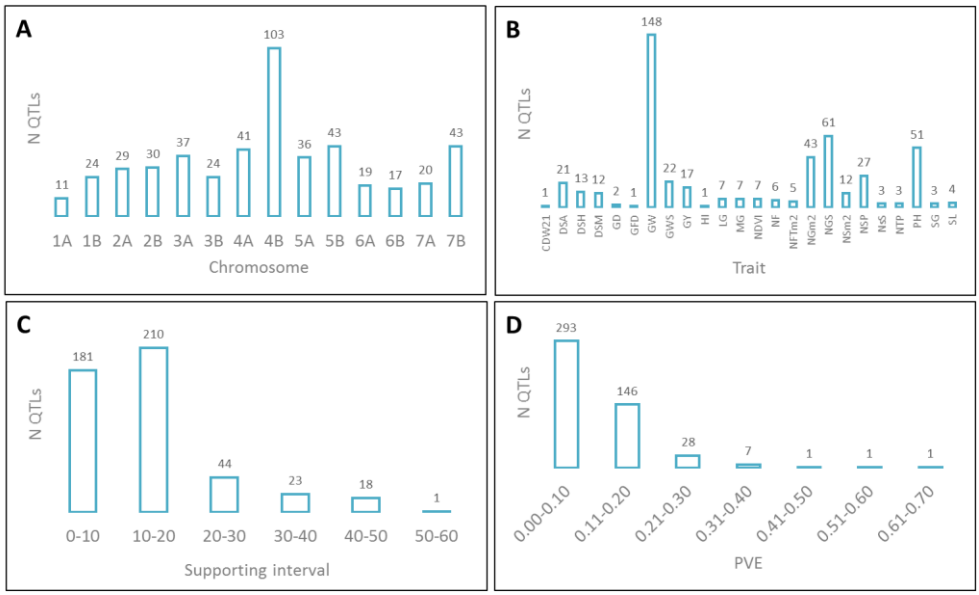

Figure 1.1. Parameters estimated in QTL studies collected for meta-analysis. Number of QTLs per A) chromosome, B) trait, C) supporting interval (cM) and D) phenotypic variance explained.

Meta-QTLs for phenology and yield and components reported in previous studies (Hanocq et al., 2007; Zhang et al., 2010) were also projected onto the consensus map developed by Maccaferri et al. (2014) in order to find common regions with our analysis. Only MQTLs flanked by common markers among the original maps and Maccaferri et al. (2014) were projected (Supplementary material S4 File). Based on overlapping MQTL SIs, three MQTLs were found in common locations with those reported by Hanocq et al. (2007) on chromosomes 2B, 5B and 7B, and 12 were in common with those reported by Zhang et al. (2010) located on chromosomes 1A, 2A, 3A, 4A, 4B, 5A, 6A and 7A (Figure 1.2, Supplementary Material S3 and S3 Files).

4. Discussion

From a breeding perspective, QTL meta-analysis could gain power and precision if raw genotypic and phenotypic data from published QTL experiments are available (Salvi and Tuberosa, 2015). As described by Salvi and Tuberosa (2007) this approach will help breeders and scientists to prioritize the selection of loci for breeding programmes and for QTL cloning.

Several studies have identified QTLs controlling traits related to wheat yield and phenology development (for references see: Hanocq et al. (2007), Zhang et al. (2010) and studies analysed in this study). QTL meta-analysis helps to integrate the QTL information available in order to determine regions of the genome that are

frequently involved in trait variation and to narrow down the SI of QTLs. The results of QTL meta-analysis are strongly dependent on the precision of the initial QTL mapping, SI and projection quality (Goffinet and Gerber, 2000). In the current study, we only used QTLs fulfilling the requirements for QTL projection following the homothetic approach of Chardon et al. (2004) and the BioMercator v4.2 software. QTL data from 26 independent studies were collected. Then, a QTL meta-analysis approach was carried out to detect genomic regions involved in yield, phenology and biomass traits previously identified.

QTL meta-analysis performed in this study revealed the presence of 84 (71 MQTL + 13 singletons) genomic regions involved in yield, yield components, and phenological development. Meta-analysis allowed for a remarkable simplification of the QTL regions, since the number was 6-fold fewer than the initial number of QTLs. MQTL positions reported in this study have some congruency with other results recently published. Three MQTLs were shared with Hanocq et al. (2007) and 12 with Zhang et al. (2010) after projection of MQTL of those authors on the consensus map (Maccaferri et al., 2014). QTL data compilation showed that all wheat homologous groups of chromosomes are involved in the genetic control of yield, its related traits and biomass, whereas group 6 chromosomes lacked phenology QTLs.

Chromosomes 1B, 2B, 3A, 3B, 4A, 5A, 5B, 7A and 7B reported MQTLs for phenology traits. No MQTLs were detected on chromosome 2A where the photoperiod sensitivity gene *Ppd-A1* is located. On 2B two MQTLs were found, but their position did not correspond with *Ppd-B1*. These observations would suggest that there is little or no variation for *Ppd* genes in this collection of genotypes. Three MQTLs were detected on chromosome 5A from 34 to 104cM, from them MQTL44 and 45 could be related to a photoperiod sensitivity QTL reported by Hanocq et al. (2004) and the vernalisation gene *Vrn-A1*, respectively. Chromosome 5B presented three MQTLs in a position from 54 to 100cM. In this case MQTL50 could be related to *Vrn-B1*, whereas MQTL48 could be associated with intrinsic earliness or earliness *per se* (*Eps*) QTL detected by Hanocq et al. (2004). Chromosomes 4A and 7B reported four and five MQTLs, respectively in the locations 60cM (4B) and 70cM (7B) suggesting important new regions controlling earliness. In addition, several QTLs were identified on chromosomes 1B, 3A, 3B, and 7A suggesting other regions with minor effects on earliness.

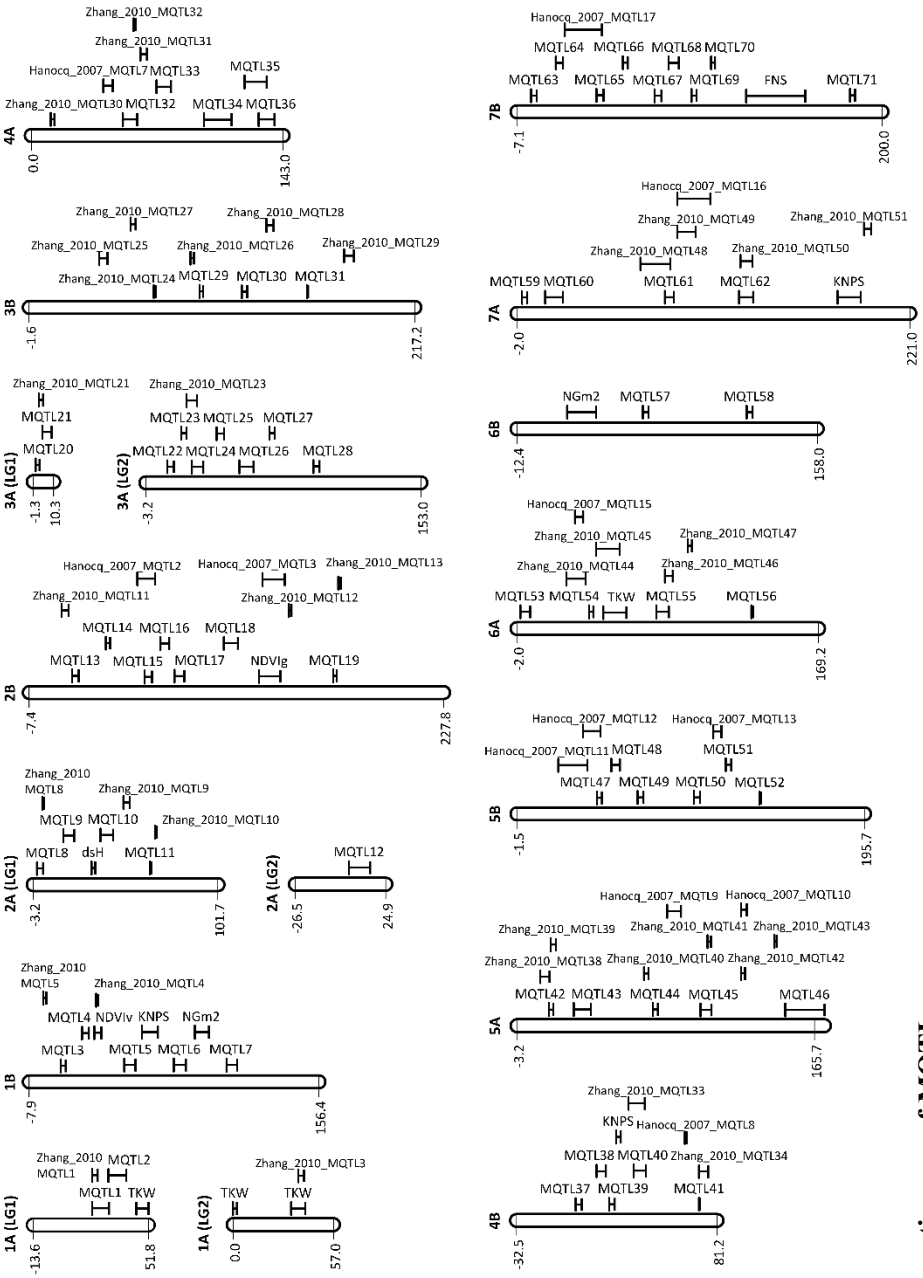

Figure 1.2. Genetic map of MQTLs.
Genetic position in the durum wheat consensus map from (Maccaferri et al., 2014) of MQTLs reported in this study and by other authors (Hanocq et al., 2007; Zhang et al., 2010). When a MQTL involved only one QTL, it is reported as the trait for that QTL. For the extended name of the traits, see the list of acronyms.

Grain yield can be partitioned into three major components: number of spikes per unit area, number of grains per spike and grain weight. These yield components are sequentially formed and are affected by other traits such as plant height, crop phenology and biomass. Together these traits make up grain yield, a complex trait controlled by multiple loci. However, some of these components are more stably inherited, such as grain weight (Gupta et al., 2006; Subirà et al., 2015). Candidate genes for grain weight have been identified in bread wheat in recent years. The sucrose synthase gene *TaSus2*, an orthologue of the maize gene *SuSy* (Carlson et al., 2002), was isolated and mapped in wheat by Jiang et al. (2011). The gene was mapped on chromosome 2B in a region where two MQTLs (MQTL15 and 17) were found in our study including QTLs for grain weight. The cell wall invertase (CWI) is a critical enzyme for sink tissue development and carbon partition, and has a high association with grain weight Ma et al. (2012b). These authors characterised the full-length genomic DNA sequence of a *Cwi* gene located on wheat chromosome 2A, designated *TaCwi-A1*. MQTL11, including QTLs for TKW, is located in the vicinity of this gene. Rustgi et al. (2013), from a search of candidate genes in rice, determined the location of two orthologous sequences underlying yield QTLs on chromosome 3A of wheat, *CKX2* and *GID2-like*. The location of these two genes may correspond with MQTL22, 23 and 24 but only the last two were associated with QTL involved in grain yield, grain weight and spikes per square metre. In rice, *OsGW2* encodes a RING-type E3 ubiquitin ligase and functions as a negative regulator of grain width and weight (Song et al., 2007). More recently, Simmonds et al. (2014) positioned the wheat orthologous *TaGW2* on chromosome 6A. Opposite results found by other authors, i.e. a positive regulation of grain size (Bednarek et al., 2012) and a negative regulation (Yang et al., 2012), did not allow to conclude the exact effect of the gene on grain size and weight. In the present study MQTL54 and 55 were located within the QTL region reported by Simmonds et al. (2014). In rice, the *TGW6* gene determines grain weight and encodes a protein with indole-3-acetic acid (IAA)-glucose hydrolase activity (Ishimaru et al., 2013). Its orthologue in wheat, *TaTGW6*, is considered as a candidate gene related to grain development (Hu et al., 2016). The gene was located on chromosome 4A, but no MQTLs for grain weight were found at that location in this study indicating that there is little or no variation for that gene in this collection of genotypes.

The meta-QTL analysis allowed us the identification of previously detected genomic regions harbouring QTLs for yield, phenology and biomass in durum wheat. Future studies using marker sequence and the recently updated wheat genome sequence assembly will be useful for searching and identifying putative candidate genes controlling the analysed traits.

Supplementary material

Supplementary material is available on the online version of the article '*Dissecting the old Mediterranean durum wheat genetic architecture for phenology, biomass and yield formation by association mapping and QTL Meta-analysis*', doi: 10.1371/journal.pone.0178290

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Chapter 2:
Exploring the genetic architecture for yield and related traits under rainfed Mediterranean climate in pre- and post-green revolution durum wheat collections

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Exploring the genetic architecture for yield and related traits under rainfed Mediterranean climate in pre- and post-green revolution durum wheat collections

Abstract

Differences in the genetic architecture for yield formation and plant height between a collection of 183 Mediterranean durum wheat landraces and 205 modern cultivars from the main growing regions in the world were analysed by a genome-wide association study. The whole panel was genotyped with a total of 46,161 DArTseq markers and phenotyped under rainfed conditions for three years in north-east Spain. Results of analysis of variance revealed differences in the source of variation among the landraces and the modern cultivars for number of spikes and grains, and grain weight. A total of 741 and 548 marker-trait associations (MTAs) were identified for landraces and modern cultivars, respectively, and simplified to 120 and 77 quantitative trait loci (MTA-QTLs). Of these, landraces showed 24 stable MTA-QTLs for number of grains per unit area, plant height, grain weight and grain yield and modern cultivars showed 31 for grain weight and plant height. Common associations with other studies were identified according to the genetic position of MTA-QTLs, and the involvement of known genes for yield-related traits is discussed. Additionally, gene annotation within the MTA peak for stable associations was carried out using the wheat genome sequence.

Keywords: Durum wheat, landraces, modern cultivars, marker-trait association, yield, yield components, plant height

1. Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) originated in the Fertile Crescent (10,000 before present) and spread across the Mediterranean Basin to the Iberian Peninsula following two routes, southern Europe and northern Africa (Mac Key, 2005; Moragues et al., 2006). During this migration, natural and human selection occurred and new adaptive traits suitable for the new environments were selected, resulting in the development of local landraces (Peng et al., 2011). Landraces were widely cultivated until the middle of the 20th century, when they were replaced by the improved semi-dwarf cultivars as a consequence of the Green Revolution. However, due to their wide genetic diversity, landraces are considered key for avoiding genetic erosion (Hammer and Teklu, 2008) and are useful for crop breeding, providing new alleles for the improvement of commercially valuable traits.

Mediterranean landraces are an important group of genetic resources because of their adaptation to their regions of origin, their huge genetic diversity (Nazco et al., 2012, 2014), their documented resilience to abiotic stresses (Kyzeridis et al., 1995) and their resistance to pests and diseases (Du Toit, 1989; Talas et al., 2011; Valdez et al., 2012). The Mediterranean Basin represents around 60% of the world's growing area for durum wheat, which is mainly cultivated there under rainfed conditions. The climate is characterised by low and variable annual rainfall and high temperatures during the grain-filling period, which constrain yield (Subirà et al., 2015), so improving yield under a water-limited scenario is a major challenge for wheat production in this area.

Genome-wide association studies (GWAS) have become an important approach to identifying genotype-phenotype associations as a complementary tool for linkage mapping. The power of GWAS resides in the broad allelic coverage and high mapping resolution in comparison with linkage mapping, thanks to the use of high genetic diversity and the history of allele recombination within the association mapping panels. GWAS is based on linkage disequilibrium (LD), defined as the non-random association of alleles at different loci (Flint-Garcia et al., 2003). Thus, it is important to differentiate LD due to physical linkage from LD due to population structure caused by selection, genetic drift and characters depending on the species, such as the mating system (Flint-Garcia et al., 2003).

In the last few years, high-throughput genotyping technologies such as single nucleotide polymorphisms (SNP) arrays and genotyping by sequencing platforms such as DArTseq have been widely used in wheat to identify marker-trait associations (MTAs). Some of these studies (Mangini et al., 2018; Mwadzingeni et al., 2017; Sukumaran et al., 2018; Wang et al., 2017) have identified MTAs for grain yield and related traits in different collections of cultivars.

The main objectives of the current study were a) to identify stable quantitative trait loci (QTLs) affecting yield formation in a GWAS panel including Mediterranean durum wheat landraces and modern cultivars from the main durum wheat–growing countries in the world, and b) to identify differences in the genetic architecture of yield formation between landraces and modern cultivars under rainfed Mediterranean conditions.

2. Material and methods

2.1. Plant material

The association mapping panel comprised a collection of 388 durum wheat genotypes, including 183 landraces from 24 Mediterranean and East Europe countries and a set of commercial varieties from the main durum wheat–growing

countries in the world (205 genotypes) (Supplementary materials Table S1, Annex 1). The landrace populations were provided by public gene banks (the Centro de Recursos Fitogenéticos CRF-INIA, Spain, the ICARDA Germplasm Bank and the USDA Germplasm Bank) and were increased in bulk and purified to select the dominant type (usually with a frequency above 80% of the bulk). Elite cultivars were provided by the IRTA durum wheat collection, international centres (CIMMYT and ICARDA) and breeding companies.

2.2. Phenotyping

Field experiments were carried out in the 2013, 2014 and 2015 harvesting seasons in Gimenells, Lleida, north-east Spain (41°38'N and 0°22'E, 260 m a.s.l) under rainfed conditions. The experiments followed a non-replicated augmented design with two replicated checks (the cultivars 'Avispa' and 'Euroduro') and plots of 3.6 m^2 (8 rows, 3 m long with 0.15 m spacing). Sowing density was adjusted to 250 germinable seeds per m^2. Weeds and diseases were controlled following standard practices at the site. Meteorological data were recorded by a weather station placed in the experimental field (Table 2.1). Zadoks growth stages (Zadoks et al., 1974) 10 (emergence), 55 (heading), 65 (anthesis), and 87 (physiological maturity) were determined in each plot.

Grain yield (t/ha), yield components (number of spikes per square metre, NSm^2; number of grains per square metre, NGm^2; and thousand kernel weight, TKW, g) and plant height (PH, cm) were calculated as described by Soriano et al. (2016).

2.3. Genotyping

DNA isolation was performed from leaf samples following the method reported by Doyle and Doyle (1987). High-throughput genotyping was performed at Diversity Arrays Technology Pty Ltd (Canberra, Australia) (http://www.diversityarrays.com) with the DArTseq genotyping by sequencing platform (Sansaloni et al., 2011). A total of 46,161 markers were used to genotype the association mapping panel, including 35,837 presence/absence variants (PAVs) and 10,324 SNPs.

Markers were ordered according to the consensus map of wheat v4 available at Diversity Arrays Technology Pty Ltd (Canberra, Australia), https://www.diversityarrays.com/technology-and-resources/genetic-maps/.

2.4. Data analysis

Phenotypic data were fitted to a linear mixed model with the check cultivars as fixed effects and the row number, column number and cultivar as random effects (Little et al., 1997). Restricted maximum likelihood was used to estimate the variance components and to produce the best linear unbiased predictors (BLUPs) for

the traits of each cultivar and year with the SAS-STAT statistical package (SAS Institute, 2014). Analyses of variance (ANOVAs) were performed for each phenotypic trait across experiments through the GLM procedure of the SAS-STAT (SAS Institute, 2014), considering genotype and year as the sources of variation and the year × cultivar interaction as the error term. Means were compared using the Tukey test (Tukey, 1949) with the JMP v12Pro statistical package (SAS Institute, 2009).

Table 2.1. Details of the experimental fields during the three years of experiments.

Year	**2013**	**2014**	**2015**
Soil texture	Sandy-loamy-clay	Sandy-clay	Sandy-loamy-clay
Sowing date	4-Dec-12	27-Nov-13	21-Nov-14
Harvest date	5-Jul-13	11-Jul-14	6-Jul-15
Environmental conditions from sowing to anthesis			
Tmin (°C)	3.4	2.8	2.9
Tmax (°C)	13.7	13.8	13.9
WI (rainfall, mm)	186	95	163
Environmental conditions from anthesis to maturity			
Tmin (°C)	9.7	8.9	10.1
Tmax (°C)	22.4	23.9	25.2
WI (rainfall, mm)	52	8.6	6.4
Phenology. Days from:			
Sowing to anthesis	155	147	156
Anthesis to maturity	39	30	29

2.5. Genome-Wide Association Study

A GWAS was performed independently for the landraces and modern cultivars using the BLUPs of the measured traits for each year and across years using a mixed linear model with TASSEL software version 5.0 (Bradbury et al., 2007). The model used a principal components matrix as the fixed effect and a kinship (K) matrix as the random effect (PCA + K) at the optimum compression level. A false discovery rate (FDR) threshold (Benjamini and Hochberg, 1995) was established at $P < 0.05$, taking into account the LD decay reported by Roselló et al. (2019) in the same set of markers, for considering an MTA significant and the results were expressed with the associated *P*-values on a $-\log_{10}$ scale. For landraces also a second threshold at $-\log_{10}P=3$ was considered as reported by other authors (Mangini et al., 2018; Mwadzingeni et al., 2017; Sukumaran et al., 2018; Wang et al., 2017).

Graphical representation of the genetic position of MTAs was carried out

using MapChart 2.3 (Voorrips, 2002).

2.6. Gene Annotation

Gene models for the stable traits within MTA-QTLs were identified using the gene models for high-confidence genes reported for the wheat genome reference sequence (IWGSC, 2018), https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies and the durum wheat reference sequence (Maccaferri et al., 2019), https://wheat.pw.usda.gov/GG3/jbrowse_Durum_Svevo. Due to the high number of genes within QTL intervals, only the closest gene to the MTA within stable MTA-QTLS was identified.

3. Results

3.1. Phenotypic Analyses

The climatic and soil conditions of the experimental fields are shown in Table 2.1. ANOVAs of phenotypic data (Table 2.2) revealed that for yield and number of spikes per unit area the year effect was more important than the cultivar effect in the phenotypic expression of the trait in both landraces and modern cultivars, whereas PH was mostly explained by the cultivar effect. For the number of grains per unit area, although the interaction of year and cultivar explained most percentage of the variance, for landraces was more important the year effect whereas for modern cultivars the cultivar effect. For grain weight, the year and cultivar effects showed a similar importance for the landraces, but for the modern cultivars the year effect has more contribution to the variation.

Table 2.2. Percentage of the sum of squares of the ANOVA for the five phenotypic traits of durum wheat genotypes grown during three years in Lleida, Spain.

Source of Variation	df	Yield	NSm^2	NGm^2	TKW	PH
		Landraces				
Year	2	68^{***}	40^{***}	34^{***}	30^{***}	7^{***}
Cultivar	182	15^{***}	30^{***}	26^{*}	31^{***}	75^{***}
Year × Cultivar	364	17^{NT}	30^{NT}	40^{NT}	39^{NT}	18^{NT}
		Modern				
Year	2	64^{**}	55^{**}	10^{**}	46^{**}	3^{**}
Cultivar	204	17^{**}	15	37^{*}	20	78^{**}
Year × Cultivar	408	19^{NT}	30^{NT}	53^{NT}	34^{NT}	19^{NT}

NSm^2, number of spikes per m^2; NGm^2, number of grains per m^2; TKW, thousand kernel weight; PH, plant height. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ^{NT} Not testable.

Yearly mean values of phenotypic traits for landraces and modern cultivars are shown in Table 2.3. The year with the lowest yield and grain weight was 2014, which, as shown in Table 2.1, received the lowest rainfall during the crop cycle. By contrast, in 2013, the year with the highest rainfall, landraces and modern cultivars reached the highest yields, and modern cultivars showed the highest grain weight. For landraces, although the grain weight was the highest in 2015, the difference between values of 2013 and 2015 was not statistically significant at $P < 0.05$. Additionally, in 2014, with the lowest rainfall before anthesis, both the landraces and the modern cultivars showed the highest number of spikes per unit area.

Table 2.3. Means comparison

	Year	Yield	NSm^2	NGm^2	TKW	PH
Landraces	2013	4889 ^{a}	321 ^{b}	11330 ^{a}	46 ^{a}	113 ^{a}
	2014	3139 ^{c}	366 ^{a}	10932 ^{b}	37 ^{b}	102 ^{b}
	2015	3843 ^{b}	325 ^{b}	8736 ^{c}	47 ^{a}	109 ^{a}
Modern	2013	5347 ^{a}	320 ^{b}	11386 ^{b}	52 ^{a}	75 ^{b}
	2014	3772 ^{c}	371 ^{a}	12024 ^{a}	36 ^{c}	78 ^{a}
	2015	4766 ^{b}	325 ^{b}	10531 ^{c}	46 ^{b}	80 ^{a}

NSm^2, number of spikes per m^2; NGm^2, number of grains per m^2; TKW, thousand kernel weight; PH, plant height. Means within columns and germplasm set with the same letter are not significantly different at $P < 0.05$ according to a Tukey test.

3.2. Genotyping

A total of 46,161 DArTseq markers were used to genotype the set of 388 durum wheat genotypes, of which 183 corresponded to Mediterranean and East Europe landraces and 205 to modern cultivars. In order to reduce the risk of false positives, markers and accessions were analysed for the presence of duplicated patterns and missing values.

Out of 35,837 PAVs, 24,188 had a known map position in the wheat v4 consensus map (Diversity Arrays Pty Ltd, Canberra, Australia). Of these, 4,745 markers with a minor allele frequency lower than 5% were removed from the analysis, leaving a total of 19,443 PAVs. Out of 10,324 SNPs, 6957 were mapped onto the wheat v4 consensus map. Of these, 1260 markers with more than 30% of missing data and 1,011 markers with minor allele frequency lower than 5% were removed from the analysis, leaving a total of 4,686 SNPs. Additionally, 413 markers were duplicated between PAVs and SNPs, so the corresponding PAVs were eliminated, leaving a total of 23,716 markers for the subsequent analyses. Forty-one percent of the markers corresponded to genome A and 59% to genome B. Total length of the map was 2,129.2 cM, with a mean coverage of 11 markers/cM.

3.3. Marker-Trait Associations

GWAS was performed using 23,716 markers in the five traits for three years and the mean across years. Results are reported in Supplementary materials Table S2. Based on the previous results of the LD decay for a maximum distance of 1 cM (Roselló et al., 2019), an FDR threshold at $P<0.05$ using 2135 markers was established for a $-\log_{10} P=4.6$.

A total of 22 and 548 MTAs were identified for the landraces and modern cultivars, respectively, above the FDR threshold. Thus a common threshold at a $-\log_{10} P=3$ (Mangini et al., 2018; Mwadzingeni et al., 2017; Sukumaran et al., 2018; Wang et al., 2017) was established for landraces identifying a total of 741 MTAs. The number of MTAs per trait in the landraces ranged from 77 for grain yield to 313 for PH, whereas in the modern cultivars it ranged from 1 for NSm^2 to 410 for PH (Supplementary materials Table S2, Figure 2.1A). The number of MTAs per chromosome in the landraces ranged from 20 in chromosome 1A to 95 in chromosome 7B. Seventy-six of the 95 MTAs in 7B corresponded to PH, whereas 55% of the MTAs on 2A corresponded with number of grains per unit area. In the modern cultivars the number of MTAs per chromosome ranged from 9 in 4A to 102 in 5B, 94% of the MTAs being related for PH in this chromosome. Only three chromosomes harboured almost 50% of the MTAs (Figure 2.1B). The mean percentage of variance explained (PVE) per MTA was higher in modern cultivars for number of grains and spikes per unit area and grain weight, whereas in landraces PH and grain yield showed higher PVE (Figure 2.1C). The percentage of MTAs with PVE lower than 0.1 was 91% and 86% for landraces and modern cultivars respectively (Figure 2.1D).

In order to simplify the MTA information, firstly, the LD decay at 1cM reported by Roselló et al. (2019) was used to identify QTLs based on linkage disequilibrium blocks. However, due to the low level of simplification, as most of the MTAs remained as unique QTLs, and in agreement with other authors (Laidò et al., 2014; Roselló et al., 2018; Soriano et al., 2017), those MTAs located within a region of 5–10 cM were considered as belonging to the same QTL. Thus, the 741 and 548 MTAs were restricted to 120 and 77 QTLs (named MTA-QTLs) for the landraces and modern cultivars, respectively (Tables 2.4 and 2.5, Figure 2.2).

For the landraces, 34 MTA-QTLs were reported in only one environment, 37 in two environments, 28 in three environments (three years or two years and mean across years) and 21 were reported during the three years and across years. Among those MTA-QTLs reported in 3 or 4 environments stable associations within an MTA-QTL, i.e. when they were reported in at least three environments, were found for 24 MTAs. From them 6 corresponded to NGm^2, 11 to PH, 4 to TKW and 3 to

grain yield. The MTA-QTLs lr_mtaq-2B.5 included stable traits for NGm^2 and TKW, lr_mtaq-5A.3 for NGm^2 and PH, and lr_mtaq-7B.8 for PH and TKW. Modern cultivars showed 30 MTA-QTLs in one environment, 11 in two environments, 29 in three environments and 7 in all four environments. In this case 31 MTA-QTLs included stable traits across environments, 29 corresponded to PH and 4 to TKW. MTA-QTLs mod_mtaq-1B.4 and mod_mtaq-5A.4 showed stable traits for both PH and TKW.

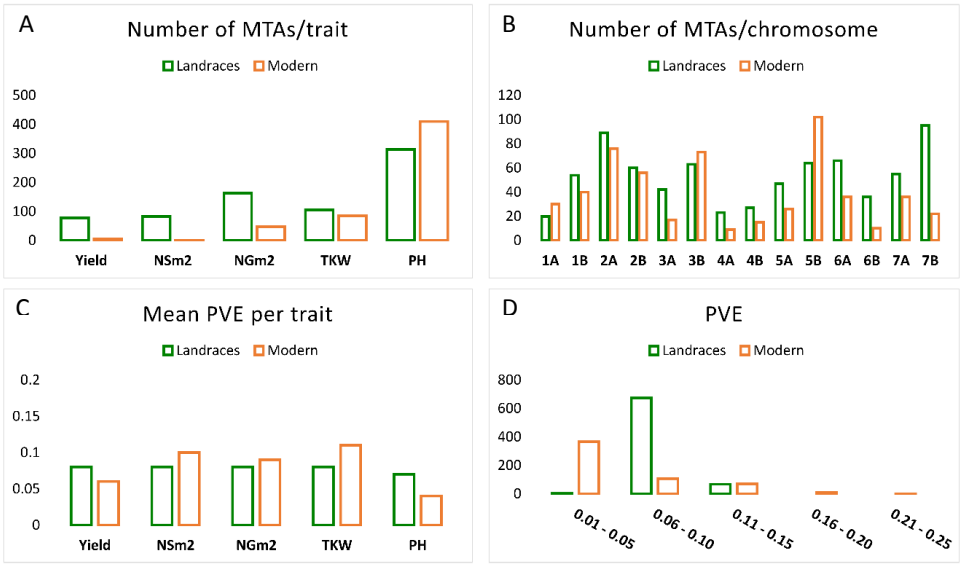

Figure 2.1. Marker Trait Associations (MTAs) summary.
(A) Number of MTAs per trait. (B) Number of MTAs per chromosome. (C) Mean percentage of variance explained (PVE) per trait. (D) PVE. NSm^2, number of spikes per m^2; NGm^2, number of grains per m^2; TKW, thousand kernel weight; PH, plant height.

Comparison of MTA-QTLs between landraces and modern cultivars (Figure 2.2, Tables 2.4 and 2.5) showed that 8 MTA-QTLs harbouring different traits shared the same position, whereas 38 overlapping MTA-QTLs between landraces and modern cultivars shared at least one trait.

3.4. Gene annotation

Gene models were successfully identified for wheat and durum wheat using the Gbrowse tools available at https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies (IWGSC, 2018) and https://wheat.pw.usda.gov/GG3/jbrowse_Durum_Svevo (Maccaferri et al., 2019) for 26 and 17 of the 55 stable associations within the MTA-QTLs respectively for landraces and modern cultivars (Table 2.6). Ten MTA-QTLs were in common in both genomes and the gene prediction was the same for the two genomes (Table 2.6).

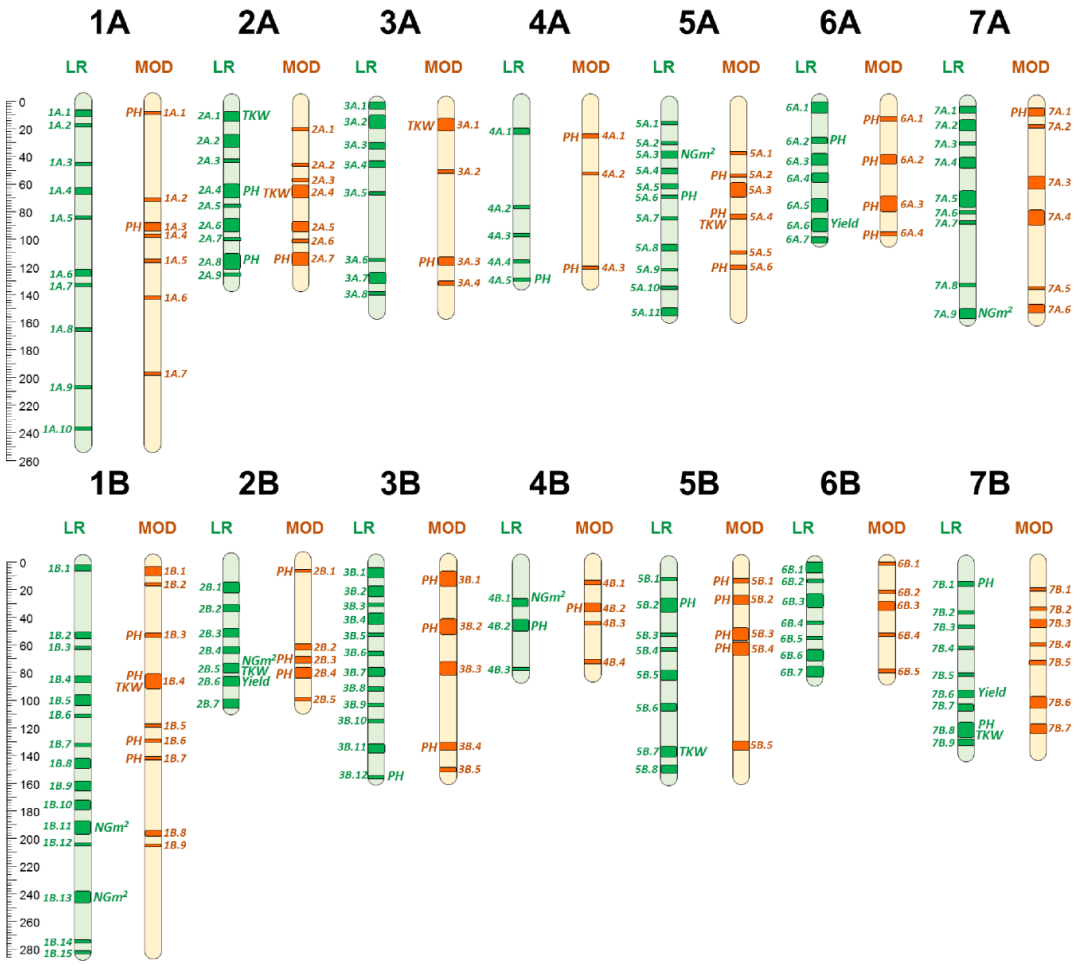

Figure 2.2. MTA-QTL map. Landraces (green) and modern cultivars (orange). The stable MTA-QTLs are indicated on the right of the green bars and on the left of the orange bars. The rule on the left indicates the genetic distance in cM. NGm^2, number of grains per m^2; TKW, thousand kernel weight; PH, plant height.

Table 2.4. MTA-QTLs in landraces.

MTA-QTL	Chr	Position (cM)	N env	Traits
lr_mtaq-1A.1	1A	3.4 - 8.9	3	NGm^2, NSm^2, Yield
lr_mtaq-1A.2	1A	17.6	1	PH
lr_mtaq-1A.3	1A	48.0	1	TKW
lr_mtaq-1A.4	1A	61.6 – 67.0	1	NSm^2
lr_mtaq-1A.5	1A	83.2	1	NSm^2
lr_mtaq-1A.6	1A	121.0 - 126.3	2	PH, TKW
lr_mtaq-1A.7	1A	133.6	1	PH
lr_mtaq-1A.8	1A	167.1	1	PH
lr_mtaq-1A.9	1A	208.9	1	PH
lr_mtaq-1A.10	1A	238.0 - 238.6	2	PH

MTA-QTL	Chr	Position (cM)	N env	Traits
lr_mtaq-1B.1	1B	4.2 - 9.3	1	PH
lr_mtaq-1B.2	1B	51.3 - 54.0	2	NSm^2, PH, Yield
lr_mtaq-1B.3	1B	63.9	1	PH
lr_mtaq-1B.4	1B	82.2 - 86.4	2	NGm^2, NSm^2, PH, TKW
lr_mtaq-1B.5	1B	94.4 - 103.3	2	PH
lr_mtaq-1B.6	1B	112.6	2	NGm^2, NSm^2
lr_mtaq-1B.7	1B	131.7 - 133.3	1	NGm^2, PH
lr_mtaq-1B.8	1B	141.4 - 148.4	2	PH, Yield
lr_mtaq-1B.9	1B	160.0 - 168.9	3	NGm^2, PH, TKW, Yield
lr_mtaq-1B.10	1B	172.2 - 179.8	2	NGm^2, PH, TKW
lr_mtaq-1B.11	1B	188.8 – 197.0	4	NGm^{2*}, NSm^2, PH, TKW
lr_mtaq-1B.12	1B	204.0	2	NGm^2, NSm^2
lr_mtaq-1B.13	1B	239.6 - 247.3	3	NGm^{2*}
lr_mtaq-1B.14	1B	273.9	1	TKW
lr_mtaq-1B.15	1B	285.9	2	NGm^2
lr_mtaq-2A.1	2A	8.3 - 16.2	4	NGm^2, NSm^2, PH, TKW*
lr_mtaq-2A.2	2A	24.6 - 33.8	3	PH, TKW, Yield
lr_mtaq-2A.3	2A	44.7 - 45.6	2	TKW
lr_mtaq-2A.4	2A	59.82 - 69.5	4	NGm^2, NSm^2, PH*, TKW, Yield
lr_mtaq-2A.5	2A	73.1 - 75.6	2	PH, Yield
lr_mtaq-2A.6	2A	87.9 - 95.6	2	PH, Yield
lr_mtaq-2A.7	2A	100.0	1	PH
lr_mtaq-2A.8	2A	111.0 - 121.1	4	NGm^2, NSm^2, PH*, TKW
lr_mtaq-2A.9	2A	122.2 - 123.7	4	NSm^2, PH, Yield
lr_mtaq-2B.1	2B	17.8 - 23.1	2	PH, TKW
lr_mtaq-2B.2	2B	36.3 - 39.9	1	PH
lr_mtaq-2B.3	2B	49.3 - 54.3	3	NGm^2, PH, Yield
lr_mtaq-2B.4	2B	62.1 - 67.7	2	NGm^2, NSm^2, TKW
lr_mtaq-2B.5	2B	73.2 - 81.7	4	NGm^{2*}, NSm^2, PH, TKW*
lr_mtaq-2B.6	2B	83.7 - 90.6	4	NGm^2, NSm^2, PH, TKW, Yield*
lr_mtaq-2B.7	2B	99.5 - 107.0	4	NGm^2, NSm^2, PH, Yield
lr_mtaq-3A.1	3A	0.6 - 4.7	4	NGm^2, PH
lr_mtaq-3A.2	3A	12.0 - 20.3	2	NGm^2, PH, Yield
lr_mtaq-3A.3	3A	30.0 - 34.1	3	NGm^2, PH, TKW
lr_mtaq-3A.4	3A	43.6 - 48.9	2	NGm^2, PH, TKW
lr_mtaq-3A.5	3A	65.6	1	Yield
lr_mtaq-3A.6	3A	114.9	1	Yield
lr_mtaq-3A.7	3A	125.2 - 132.8	3	PH, TKW
lr_mtaq-3A.8	3A	138.1	1	NGm^2

MTA-QTL	Chr	Position (cM)	N env	Traits
lr_mtaq-3B.1	3B	4.7 - 13.4	4	NGm^2, NSm^2, PH, TKW, Yield
lr_mtaq-3B.2	3B	17.4 - 27.4	2	NSm^2, PH, TKW, Yield
lr_mtaq-3B.3	3B	28.8 - 30.1	1	NSm^2
lr_mtaq-3B.4	3B	37.4 - 45.5	3	NGm^2, TKW
lr_mtaq-3B.5	3B	53.3	1	TKW
lr_mtaq-3B.6	3B	65.6 - 68.8	2	NGm^2, NSm^2, TKW
lr_mtaq-3B.7	3B	76.1 - 83.4	2	TKW
lr_mtaq-3B.8	3B	93.6 - 96.5	3	NGm^2, Yield
lr_mtaq-3B.9	3B	102.7 - 103.4	1	NGm^2
lr_mtaq-3B.10	3B	116.9 - 117.0	2	NGm^2, NSm^2, TKW
lr_mtaq-3B.11	3B	133.9 - 138.1	2	PH
lr_mtaq-3B.12	3B	155.2 - 157.1	4	NGm^2, NSm^2, PH^*
lr_mtaq-4A.1	4A	20.1 - 24.7	2	NSm^2, PH, TKW
lr_mtaq-4A.2	4A	76.2	1	TKW
lr_mtaq-4A.3	4A	96.1 - 98.4	3	NGm^2, PH, TKW, Yield
lr_mtaq-4A.4	4A	115.9 - 118.2	3	NGm^2, NSm^2, TKW
lr_mtaq-4A.5	4A	124.1 - 131.5	4	NSm^2, PH^*, TKW, Yield
lr_mtaq-4B.1	4B	28.9 - 33.0	4	NGm^{2*}, NSm^2, PH, TKW, Yield
lr_mtaq-4B.2	4B	46.7 - 51.6	3	PH^*
lr_mtaq-4B.3	4B	77.9	1	PH
lr_mtaq-5A.1	5A	13.7	1	PH
lr_mtaq-5A.2	5A	27.3	1	NSm^2
lr_mtaq-5A.3	5A	35.2 - 40.4	4	NGm^{2*}, NSm^2, PH^*
lr_mtaq-5A.4	5A	48.6 - 51.7	3	NGm^2, NSm^2, PH, Yield
lr_mtaq-5A.5	5A	58.4 - 61.7	2	PH, TKW
lr_mtaq-5A.6	5A	68.5	3	PH^*
lr_mtaq-5A.7	5A	84.4 - 86.7	2	NGm^2, NSm^2, TKW
lr_mtaq-5A.8	5A	106.3 - 109.1	2	NGm^2, PH
lr_mtaq-5A.9	5A	121.3	1	TKW
lr_mtaq-5A.10	5A	134.3	1	Yield
lr_mtaq-5A.11	5A	148.8 - 153.7	1	NGm^2
lr_mtaq-5B.1	5B	12.1 - 13.5	1	PH
lr_mtaq-5B.2	5B	25.1 - 36.1	3	PH^*, TKW
lr_mtaq-5B.3	5B	52.9 - 53.7	3	NGm^2, NSm^2, PH, TKW
lr_mtaq-5B.4	5B	65.4 – 66.0	2	NGm^2, Yield
lr_mtaq-5B.5	5B	79.5 - 86.0	2	NGm^2, NSm^2
lr_mtaq-5B.6	5B	102.2 - 108.6	1	NGm^2, NSm^2, TKW
lr_mtaq-5B.7	5B	132.8 - 140.3	3	TKW^*, Yield
lr_mtaq-5B.8	5B	148.7 - 151.3	3	PH, Yield
lr_mtaq-6A.1	6A	0.0 - 7.7	3	PH, TKW, Yield

MTA-QTL	Chr	Position (cM)	N env	Traits
lr_mtaq-6A.2	6A	23.8 - 29.3	4	NGm^2, NSm^2, PH*, TKW
lr_mtaq-6A.3	6A	36.2 - 45.1	3	NSm^2, PH, TKW
lr_mtaq-6A.4	6A	50.1 -58.1	2	PH, TKW
lr_mtaq-6A.5	6A	69.5 - 79.1	3	NSm^2, PH, TKW
lr_mtaq-6A.6	6A	86.5 - 96.7	3	NSm^2, PH, Yield*
lr_mtaq-6A.7	6A	98.2 -102.9	2	NSm^2, PH
lr_mtaq-6B.1	6B	1.6 - 8.2	3	NSm^2, PH, TKW, Yield
lr_mtaq-6B.2	6B	12.3 - 14.5	3	PH, TKW, Yield
lr_mtaq-6B.3	6B	23.2 - 32.7	3	NGm^2, NSm^2, PH, Yield
lr_mtaq-6B.4	6B	42.4 - 44.1	2	PH
lr_mtaq-6B.5	6B	54.6	1	TKW
lr_mtaq-6B.6	6B	63.5 - 71.8	3	NSm^2, Yield
lr_mtaq-6B.7	6B	77.5 - 83.5	2	NGm^2, PH
lr_mtaq-7A.1	7A	5.3 - 8.2	1	PH, Yield
lr_mtaq-7A.2	7A	15.0 - 23.8	4	NGm^2, NSm^2, PH, Yield
lr_mtaq-7A.3	7A	28.1 - 30.4	1	PH
lr_mtaq-7A.4	7A	40.6 - 46.6	1	PH
lr_mtaq-7A.5	7A	65.1 - 75.6	4	NGm^2, NSm^2, PH, TKW
lr_mtaq-7A.6	7A	77.9 - 78.5	2	PH
lr_mtaq-7A.7	7A	89.7 - 90.3	1	NGm^2, PH
lr_mtaq-7A.8	7A	130.9	1	NSm^2
lr_mtaq-7A.9	7A	149.6 - 157.4	4	NGm^{2*}, NSm^2, TKW, Yield
lr_mtaq-7B.1	7B	14.1 - 17.5	3	PH*
lr_mtaq-7B.2	7B	36.1 - 36.8	1	PH
lr_mtaq-7B.3	7B	47.6 - 48.3	2	PH
lr_mtaq-7B.4	7B	62.4	2	PH
lr_mtaq-7B.5	7B	82.0	1	NGm^2
lr_mtaq-7B.6	7B	93.0 - 97.4	3	PH, TKW, Yield*
lr_mtaq-7B.7	7B	104.5 - 109.2	4	NGm^2, NSm^2, PH, Yield
lr_mtaq-7B.8	7B	118.5 - 128.4	4	NGm^2, NSm^2, PH*, TKW*
lr_mtaq-7B.9	7B	128.6 - 134.7	4	NGm^2,PH, Yield

*Stable traits within the MTA-QTLs (present in at least three environments).

Chr, chromosome; N env, number of environments (three years and means across years); NSm^2, number of spikes per m^2; NGm^2, number of grains per m^2; TKW, thousand kernel weight; PH, plant height.

Table 2.5. MTA-QTLs in modern cultivars.

MTA-QTL	Chr	Position (cM)	N env	Traits
mod_mtaq-1A.1	1A	7.2–7.6	3	PH*
mod_mtaq-1A.2	1A	79.4	1	PH
mod_mtaq-1A.3	1A	89.3–95.0	3	PH*
mod_mtaq-1A.4	1A	102.0	1	PH
mod_mtaq-1A.5	1A	117.5	2	PH, TKW
mod_mtaq-1A.6	1A	143.9	1	PH
mod_mtaq-1A.7	1A	201.7	1	TKW
mod_mtaq-1B.1	1B	4.2–9.6	2	PH
mod_mtaq-1B.2	1B	17.8	1	PH
mod_mtaq-1B.3	1B	51.3–53.3	3	PH*
mod_mtaq-1B.4	1B	82.2–91.9	4	PH*, TKW*
mod_mtaq-1B.5	1B	120.4	1	PH
mod_mtaq-1B.6	1B	130.7	3	PH*
mod_mtaq-1B.7	1B	142.2	3	PH*
mod_mtaq-1B.8	1B	194.1–197.2	3	PH, TKW
mod_mtaq-1B.9	1B	204.9	2	PH
mod_mtaq-2A.1	2A	25.6	1	TKW
mod_mtaq-2A.2	2A	45.6	1	TKW
mod_mtaq-2A.3	2A	58.9	1	PH
mod_mtaq-2A.4	2A	62.4–71.3	3	NGm^2, TKW*
mod_mtaq-2A.5	2A	90.2–97.8	1	PH
mod_mtaq-2A.6	2A	103.8	1	PH
mod_mtaq-2A.7	2A	112.7 -120.7	3	PH*, TKW
mod_mtaq-2B.1	2B	12.9	3	PH*
mod_mtaq-2B.2	2B	64.1–67.4	1	PH
mod_mtaq-2B.3	2B	73.1–76.6	3	PH*
mod_mtaq-2B.4	2B	81.2–88.4	4	PH*, TKW
mod_mtaq-2B.5	2B	105.8	1	PH
mod_mtaq-3A.1	3A	13.1–21.2	3	PH, TKW*
mod_mtaq-3A.2	3A	50.6	1	NGm^2
mod_mtaq-3A.3	3A	116.0–118.1	3	PH*
mod_mtaq-3A.4	3A	130.1–132.8	1	PH
mod_mtaq-3B.1	3B	9.1–20.2	3	PH*
mod_mtaq-3B.2	3B	43.8–54.3	3	PH*, TKW
mod_mtaq-3B.3	3B	74.8–84.1	1	PH
mod_mtaq-3B.4	3B	132.0–138.7	3	PH*
mod_mtaq-3B.5	3B	153.7–155.3	2	PH, TKW
mod_mtaq-4A.1	4A	26.1–26.7	4	NGm^2, TKW, PH*
mod_mtaq-4A.2	4A	53.4	1	TKW

MTA-QTL	Chr	Position (cM)	N env	Traits
mod_mtaq-4A.3	4A	121.7	3	PH*
mod_mtaq-4B.1	4B	17.4–18.6	1	PH
mod_mtaq-4B.2	4B	31.9–37.9	3	PH*
mod_mtaq-4B.3	4B	47.5	1	PH
mod_mtaq-4B.4	4B	75.3–76.6	1	TKW
mod_mtaq-5A.1	5A	39.3	1	PH
mod_mtaq-5A.2	5A	55.1	3	PH*
mod_mtaq-5A.3	5A	59.5–69.8	2	PH, TKW
mod_mtaq-5A.4	5A	82.1–86.7	4	PH*, TKW*, Yield
mod_mtaq-5A.5	5A	109.6	2	TKW
mod_mtaq-5A.6	5A	121.2–122.5	3	PH*
mod_mtaq-5B.1	5B	15.8–16.4	3	PH*
mod_mtaq-5B.2	5B	25.5–32.6	3	PH*, TKW
mod_mtaq-5B.3	5B	49.3–58.2	4	NSm^2, PH*, TKW, Yield
mod_mtaq-5B.4	5B	59.1–68.8	4	PH*
mod_mtaq-5B.5	5B	132.5–137.4	2	PH
mod_mtaq-6A.1	6A	15.8–17.6	4	NGm^2, PH*, TKW
mod_mtaq-6A.2	6A	42.6–48.1	3	PH*
mod_mtaq-6A.3	6A	73.2–83.2	3	NGm^2, PH*
mod_mtaq-6A.4	6A	98.2–100.2	3	PH*, TKW
mod_mtaq-6B.1	6B	3.5	1	PH
mod_mtaq-6B.2	6B	23.7	1	TKW
mod_mtaq-6B.3	6B	31.5–37.8	2	PH, TKW
mod_mtaq-6B.4	6B	54.1	1	PH
mod_mtaq-6B.5	6B	81.5–82.8	1	PH
mod_mtaq-7A.1	7A	7.8–11.8	3	PH*, TKW
mod_mtaq-7A.2	7A	19.9	1	PH
mod_mtaq-7A.3	7A	57.8–64.9	2	PH, TKW
mod_mtaq-7A.4	7A	79.3–90.2	1	TKW
mod_mtaq-7A.5	7A	137.7	1	TKW
mod_mtaq-7A.6	7A	149.6–155.1	3	PH, TKW, Yield
mod_mtaq-7B.1	7B	22.4	2	TKW, Yield
mod_mtaq-7B.2	7B	36.8	3	PH
mod_mtaq-7B.3	7B	45.8–49.3	2	PH
mod_mtaq-7B.4	7B	62.1	3	PH, TKW, Yield
mod_mtaq-7B.5	7B	74.4–76.0	1	PH, TKW
mod_mtaq-7B.6	7B	100.0–108.8	1	PH
mod_mtaq-7B.7	7B	118.3–125.4	3	NGm^2, TKW

*Stable traits within the MTA-QTLs (present in at least three environments).

Chr, chromosome; N env, number of environments (three years and means across years);

NSm^2, number of spikes per m^2; NGm^2, number of grains per m^2; TKW, thousand kernel weight; PH, plant height.

4. Discussion

4.1. Phenotypic variation

As has been previously reported (García del Moral et al., 2003; Moragues et al., 2006; Royo et al., 2006, 2014), the contribution of yield components to yield formation in durum wheat is mainly affected by the temperature and water availability during the crop cycle. The environmental conditions during the three years of field experiments included in the current study were typical of a Mediterranean climate, showing a pattern of increasing temperatures during the spring and irregular rainfall distribution (Royo et al., 2014).

The ANOVA showed the different effect of environmental conditions on the phenotypic expression of agronomic traits for the landraces and modern cultivars. Whereas for the landraces the environmental effect accounted for most of the variation only for yield, in the modern cultivars the environmental effect accounted for a larger variation than the genotype effect also for number of spikes per unit area and grain weight. Most of the phenotypic variability for PH was explained by the cultivar effect, in accordance with the high heritability of the trait (Collaku, 1994). Similar results were reported previously by Royo et al. (2010) and Soriano et al. (2016).

Mean values of phenotypic traits across years showed that the year with the lowest yield and grain weight was 2014, with the lowest water in the soil during the grain-filling period (GFP). By contrast, 2013, the year with the highest rainfall both before and after anthesis, showed the highest yields for both the landraces and the modern cultivars, and also showed heavier grains. Additionally, in 2014, with the lowest rainfall before anthesis, both sets of cultivars showed the highest number of spikes per unit area. The potential number of spikes per unit area is determined during the vegetative phase and is strongly affected by water availability (García del Moral et al., 1991; Simane et al., 1993). The fact that during the year with lowest rainfall the cultivars had the largest numbers of spikes per unit area may be interpreted by the soil fertility (3.18% of organic matter) and the superficial sub-soil water layer at the site of the trials (Moragues et al., 2006). The large genetic control of the NSm^2 in the landraces revealed by the ANOVA suggests that, in terms of this yield component, they would be better adapted to water stress conditions than modern cultivars.

Table 2.6. Gene models within stable MTA-QTLs in the wheat Chinese Spring reference genome (IWGSC, 2018) and durum wheat Svevo reference genome (Maccaferri et al., 2019)

Only MTAs with markers mapped against the genome sequence are included. Grey shaded rows correspond to MTA-QTLs found in both genomes with the peak reporting the same prediction of the gene model.

	Wheat					Durum wheat				
Chr	**MTA-QTL**	**Trait**	**Pos.**	**Gene model**	**Prediction**	**MTA-QTL**	**Trait**	**Pos**	**Gene Model**	**Prediction**
1A	mod_mtaq-1A.1	PH	7.31	TraesCS 1A01G013200	Ankyrin repeat protein	-	-	-	-	-
	mod_mtaq-1A.3	PH	460.93	TraesCS 1A01G265300	Wound-induced protease inhibitor	mod_mtaq-1A.3	PH	452.83	TRITD 1Av1G167530	Wound-induced protease inhibitor
1B	mod_mtaq-1B.3	PH	9.55	TraesCS 1B01G020400	NBS-LRR disease resistance	-	-	-	-	-
	-	-	-	-	-	mod_mtaq-1B.4	TKW	114.32	TRITD 1Bv1G041340	Carboxypeptidase
	lr_mtaq-1B.11	NGm^2	633.09	TraesCS 1B01G403300	NBS-LRR disease resistance	-	-	-	-	-
2A	mod_mtaq-2A.4	TKW	153.45	TraesCS 2A01G188500	Homeobox leucine zipper	mod_mtaq-2A.4	TKW	151.47	TRITD 2Av1G066050	Homeobox leucine zipper
	lr_mtaq-2A.8	PH	733.02	TraesCS 2A01G503700	Protein kinase	lr_mtaq-2A.8	PH	742.56	TRITD 2Av1G279070	Protein kinase
2B	mod_mtaq-2B.3	PH	442.08	TraesCS 2B01G308900	Cytochrome P450	mod_mtaq-2B.3	PH	398.30	TRITD 2Bv1G135010	Carboxypeptidase
	lr_mtaq-2B.5	NGm^2	696.28	TraesCS 2B01G501200	Aldehyde dehydrogenase	-	-	-	-	-
	mod_mtaq-2B.4	PH	757.05	TraesCS 2B01G565600	NBS-LRR disease resistance	mod_mtaq-2B.4	PH	743.03	TRITD 2Bv1G247400	NBS-LRR disease resistance
	lr_mtaq-2B.6	NGm^2	758.14	TraesCS 2B01G567200	Flowering Locus T-like	lr_mtaq-2B.6	Yield	743.25	TRITD 2Bv1G247420	NBS-LRR disease resistance
3A	-	-	-	-	-	mod_mtaq-3A.3	PH	700.43	TRITD 3Av1G263160	2-oxoglutarate and Fe(II)-dependent oxygenase
3B	mod_mtaq-3B.4	PH	788.51	TraesCS 3B01G554800	Aspartic proteinase nepenthesin-1	mod_mtaq-3B.4	PH	796.87	TRITD 3Bv1G266540	Aspartic proteinase nepenthesin-1
4A	mod_mtaq-4A.1	PH	395.02	TraesCS 4A01G165600	3-oxoacyl-synthase	-	-	-	-	-
	mod_mtaq-4A.3	PH	703.53	TraesCS 4A01G432800	Anthocyanin 5-acyltransferase	-	-	-	-	-

	Wheat					Durum wheat				
Chr	**MTA-QTL**	**Trait**	**Pos.**	**Gene model**	**Prediction**	**MTA-QTL**	**Trait**	**Pos**	**Gene Model**	**Prediction**
	lr_mtaq-4A.5	PH	718.75	TraesCS 4A01G454600	F-box protein	-	-	-	-	-
4B	lr_mtaq-4B.2	PH	633.76	TraesCS 4B01G340600	Zinc finger CONSTANS	lr_mtaq-4B.2	PH	637.87	TRITD 4Bv1G191870	Zinc finger CONSTANS
5A	lr_mtaq-5A.6	PH	559.01	TraesCS 5A01G356700	Carboxyl-terminal-processing protease	lr_mtaq-5A.6	PH	523.05	TRITD 5Av1G193730	Anaphase-promoting complex
	mod_mtaq-5A.4	PH, TKW	575.32	TraesCS 5A01G377600	Auxin canalization protein	-	-	-	-	-
	mod_mtaq-5A.6	PH	661.22	TraesCS 5A01G492400	transcription repressor	-	-	-	-	-
5B	mod_mtaq-5B.1	PH	26.00	TraesCS 5B01G026900	F-box protein	mod_mtaq-5B.1	PH	24.48	TRITD 5Bv1G009290	F-box protein
	lr_mtaq-5B.2	PH	75.74	TraesCS 5B01G067600	Acetyl-CoA carboxylase	lr_mtaq-5B.2	PH	76.29	TRITD 5Bv1G028500	Acetyl-CoA carboxylase
	mod_mtaq-5B.2	PH	304.28	TraesCS 5B01G164300	DNA-directed RNA polymerase	-	-	-	-	-
	mod_mtaq-5B.3	PH	521.14	TraesCS 5B01G337600	Ankyrin repeat family protein	mod_mtaq-5B.3	PH	528.96	TRITD 5Bv1G183040	Ankyrin repeat protein family
6A	mod_mtaq-6A.2	PH	101.31	TraesCS 6A01G129200	F-box	mod_mtaq-6A.2	PH	99.61	TRITD 6Av1G043020	F-box
	lr_mtaq-6A.2	PH	392.19	TraesCS 6A01G214400	Flowering Locus T	-	-	-	-	-
7A	mod_mtaq-7A.1	PH	14.18	TraesCS 7A01G033100	Cellulose synthase	mod_mtaq-7A.1	PH	8.25	TRITD 7Av1G004930	F-box
	lr_mtaq-7A.9	NGm^2	732.91	TraesCS 7A01G561800	Elongation factor P	-	-	-	-	-
7B	lr_mtaq-7B.8	PH	740.89	TraesCS 7B01G482700	MADS-box transcription factor	lr_mtaq-7B.8	TKW	715.51	TRITD 7Bv1G231530	Chromodomain-helicase-DNA-binding family

Chr, chromosome; Pos, position in Mb; NSm^2, number of spikes per m^2; NGm^2, number of grains per m^2; TKW, thousand kernel weight; PH, plant height.

4.2. Marker-Trait Associations

In the course of domestication and breeding, crops gradually lose their genetic variability. A useful approach for recovering and broadening allelic variation of traits of interest is the use of landraces in breeding programmes (Lopes et al., 2015), which may be of particular interest for suboptimal environments such as the Mediterranean Basin. Mediterranean durum wheat landraces are an important group of genetic resources because of their huge genetic diversity and specific adaptation to local environments (Nazco et al., 2012).

The current study attempts to dissect the underlying genetics controlling yield-related traits in a collection of landraces from Mediterranean countries and a set of commercial varieties from the main durum wheat–growing countries in the world by association analysis under the dry and warm conditions typical of the Mediterranean Basin (Royo et al., 2014). In order to reduce the number of spurious associations, a mixed linear model accounting for the genetic relatedness between cultivars (random effect) and their population structure (fixed effect) (K+PCA model) was used. Additionally, an FDR threshold was established according to the distance of the linkage disequilibrium decay.

Yield is a genetically complex trait in wheat, being controlled by a large number of small-effect QTLs, and only 5 MTAs for the modern cultivars exceeded the restrictive FDR threshold used in this study. Thus, the dissection of the trait into components is a valuable strategy for identifying genomic regions controlling yield formation.

A total of 1289 significant associations were identified for the five yield-related traits during three years and across years in eastern Spain, of which 741 corresponded to landraces and 548 to modern cultivars. In order to simplify the MTA information and to integrate tightly linked associations in a single MTA-QTL, those MTAs located within short map intervals were considered as belonging to the same QTL, as reported previously (Laidò et al., 2014; Roselló et al., 2018; Soriano et al., 2017). Using this approach, the number of MTA-QTLs was reduced significantly to 120 and 77 for the landraces and modern cultivars, respectively. Of the MTA-QTLs in the landraces and modern cultivars, 38% and 67%, respectively, were MTA-QTLs for only one trait. The other 62% and 33% were pleiotropic MTA-QTLs including different traits, revealing the complex relationships among yield-related traits, as reported by Wang et al. (2017).

MTA-QTLs also showed stable associations across years, with differences between the landraces and modern cultivars. For the landraces, stable MTA-QTLs have been reported for NGm^2, PH, TKW and grain yield in all of the chromosomes except 1A, 3A and 6B, whereas the modern cultivars showed stable MTA-QTLs only

for PH and TKW in all chromosomes except 6B and 7B. These differences could indicate different adaptive mechanisms between landraces and modern cultivars, as reported by Soriano et al. (2018) with a set of durum wheat landraces from eastern and western Mediterranean countries. In that study, the authors pointed out that eastern landraces showed a higher frequency of alleles conferring a higher number of spikes and grains in landraces than those from western Mediterranean countries to compensate for the negative effect of water scarcity during the formation of spikes. Other authors (Motzo et al., 1996; Royo et al., 2014) have also reported a higher grain-filling rate in landraces from cold and wet areas, associated with increased grain weight in durum wheat. Grain weight in wheat is much more constrained under terminal drought conditions than in cold and wet environments (Royo et al., 2014). Additionally, the selection for larger grains during the spread of durum wheat across the Mediterranean Basin may also have contributed (Peng et al., 2011).

According to the genetic position of MTA-QTLs, comparisons with previous studies based on DArTseq or SNP arrays pointed out common genomic regions among the different studies. For the landrace collection, the position of lr_mtaq-3B.6 and lr_mtaq-4B.1 for NSm^2 could correspond to the associations found by Wang et al. (2017) for ESN (effective number of spikes per square metre), lr_mtaq-5A.7 and lr_mtaq-7A.9 harbouring MTAs for TKW co-localized with the regions on 5A and 7A found by Wang et al. (2017) for grain weight, whereas on 7B lr_mtaq-7B.9 for PH. Common positions for PH in lr_mtaq-1B.3, lr_mtaq-2B.1 and lr_mtaq-6B.7 and for TKW in lr_mtaq-6B.3 and lr_mtaq-7A.5 were shared with the results of Mwadzingeni et al. (2017). When compared with the results of Sukumaran et al. (2018), 19 common regions were found with our study. QTLs for yield were located in similar positions than lr_mtaq-3B.2 and lr_mtaq-4A.5, for grain number lr_mtaq-2A.4, lr_mtaq-2B.5 and lr_mtaq-7A.5 for grain weight lr_mtaq-2A.3, lr_mtaq-2A.4, and lr_mtaq-2B.5. Finally, eleven MTA-QTLs for PH reported common positions with Sukumaran et al. (2018). When compared with the study of Mangini et al. (2018) including wild and cultivated genotypes of tetraploid wheats, four regions were shared with the current study. On chromosome 2A a QTL for kernel number per spike and TKW was located in a similar region of lr_mtaq-2A.8 including MTAs for NGm^2 and TKW and on chromosome 6A a QTL for kernel number per spike and grain yield per spike was located in a similar region of lr_mtaq-6A.1. Finally other two MTA-QTLs reporting associations for grain number were in common with Mangini et al. (2018): lr_mtaq-5B.5 and lr_mtaq-7B.7. When the modern cultivar collection from our work was compared with previous studies, common positions with the study reported by Mwadzingeni et al. (2017) referred to PH: mod_mtaq-2A.6, mod_mtaq-2B.1, mod_mtaq-2B.5, mod_mtaq-3A.4, mod_mtaq-3B.1, mod_mtaq-6A.2 and mod_mtaq-7B.6. Four MTA-QTLs shared common regions

with the position of MTAs identified by Wang et al. (2017) for PH and TKW. On chromosome 5A, mod_mtaq-5A.4 including a stable association for TKW could correspond to the highly significant SNP Tdurum_contig71499_211 from which the authors developed a CAPS marker to be applied in breeding. The MTA-QTL mod_mtaq-7A.6 could also correspond to a TKW MTA identified by Wang et al. (2017). Regarding PH, two MTA-QTLs were found in similar positions to those reported by these authors, mod_mtaq-5B.5 and mod_mtaq-7B.5. Five MTA-QTLs reporting associations with TKW shared genetic positions with QTLs identified by Mangini et al. (2018): mod_mtaq-2A.7, mod_mtaq-5A.3, mod_mtaq-5A.5, mod_mtaq-6B.3 and mod_mtaq-7B.4, the last one also including an MTA for yield, as reported by Mangini et al. (2018), whereas mod_mtaq-6A.1 showed an association with grain number. Finally, 15 MTA-QTLs were in common with the associations reported by Sukumaran et al. (2018) under several conditions (yield potential, drought stress and heat stress). Although most of the common associations referred to PH, three of them included TKW (mod_mtaq-2A.2, mod_mtaq-2A.4, mod_mtaq-2B.4) and NGm^2 (mod_mtaq-2A.4). Common MTAs for PH were located on chromosomes 1A (mod_mtaq-1A.5), 1B (mod_mtaq-1B.3, mod_mtaq-1B.7), 2B (mod_mtaq-2B.2, mod_mtaq-2B.3, mod_mtaq-2B.4 and mod_mtaq-2B.5), 4B (mod_mtaq-4B.2), 5B (mod_mtaq-5B.4), 6A (mod_mtaq-6A.1), 6B (mod_mtaq-6B.3) and 7B (mod_mtaq-7B.3).

Dwarfing genes can be assigned to several of the MTA-QTLs identified in the modern cultivars on the basis of their genetic position, although the use of different types and numbers of markers in the mapping populations can lead to misestimating these positions. The locus mod_mtaq-4B.3 on chromosome 4B may correspond to *Rht-B1* (Fick and Qualset, 1973), whereas *Rht12* (Ellis et al., 2004) can be included within mod_mtaq-5A.4 on chromosome 5A, *Rht25* (Mo et al., 2018) within mod_mtaq-6A.1, *Rht18* (Grant et al., 2018) in mod_mtaq-6A.2, and *Rht13* (Rebetzke et al., 2011) within mod_mtaq-7B.6.

Among the yield-related genes reported in the review by Nadolska-Orczyk et al. (2017), three co-localized with MTA-QTLs reported in our work. The transcript elongation factor *TaTEF-7A* (Zheng et al., 2014) regulates tillering and increases grain number per spike, thus enhancing grain yield. This gene is located within the region of lr_mtaq-7A.5 where MTAs for NGm^2 and NSm^2 were identified. The gene *TaGW2* is the orthologous in wheat of the rice gene *OsGW2* involved in rice grain development (Su et al., 2011). *TaGW2* was significantly associated with wider grains and TKW by Su et al. (2011). In our study, the region covered by lr_mtaq-6A.3 including MTAs for TKW corresponded to the genetic position estimated by Su et al. (2011) for *TaGW2*. The locus *TaSus2-2A* (Jiang et al., 2011) involved in the

conversion of sucrose to starch produces differences among haplotypes correlated with grain weight differences. This locus is located on chromosome 2A in the region of lr_mtaq-2A.4 harbouring MTAs for TKW. Another gene associated with grain weight is the cell wall invertase (*CWI*) (Ma et al., 2012), which is involved in sink tissue development and carbon partition. In wheat, the gene was designated as *TaCwi-A1* and located on chromosome 2A, in a region where the MTA-QTL mod_mtaq-2A.2 involved in TKW was mapped.

Additionally, a search for candidate genes within stable MTA-QTLs was performed using the high-confidence gene annotation from the wheat genome sequence (IWGSC, 2018) and the durum wheat reference genome (Maccaferri et al., 2019). Because of the high number of gene models located within MTA-QTLs, only the closest one to the MTA marker was reported. On chromosome 1A and 5B, ankyrin repeat motives were reported for TraesCS1A01G013200 in mod_mtaq-1A.1 and TraesCS5B01G337600 and TRITD5Bv1G183040 in mod_mtaq-5B.3. This type of motif plays a role during plant growth and development (Sharma and Pandey, 2016). On chromosomes 1B and 2B, carboxypeptidases were reported for the durum wheat gene models TRITD1Bv1G041340, on mod_mtaq-1B.4, and TRITD2Bv1G135010, on mod_mtaq-2B.3. Carboxypeptidases were related with grain size control in rice by the regulation of grain width, filling and weight (Li et al., 2011a). These authors found that the expression of GS5 was correlated with larger grains in rice. On chromosome 2A an homeobox leucine zipper protein was found within mod_mtaq-2A.4 (TraesCS2A01G188500 and TRITD2Av1G066050). These kind of proteins regulate the plant development, and Khaled et al. (2005) found that the maize gene *ZmOCL1* had a role regulating kernel development. On chromosome 2B, cytochrome P450 coded by TraesCS2B01G308900 was located within mod_mtaq-2B.3. As reported in rice by Paul et al. (2012), OsTEF1 regulates tillering by inducing the expression of cytochrome P450. On chromosome 4A, the mod_mtaq-4A.1 included the TraesCS4A01G165600 coding for a 3-oxoacyl-(ACP) synthase. In barley, *Kas12*, belonging to this type of protein, was identified in roots, germinating embryos, developing kernels and leaves (Kauppinen, 1992). F-box proteins were found for lr_mtaq-4A.5 (TraesCS4A01G454600), mod_mtaq-5B.1 (TraesCS5B01G026900 and TRITD5Bv1G009290), mod_mtaq-6A.2 (TraesCS6A01G129200 and TRITD6Av1G043020) and mod_mtaq-7A.1 (TRITD7Av1G004930). Among the different functions of these genes, Li et al. (2011b) demonstrated that the F-box gene *LARGER PANICLE* improves the panicle architecture of rice, thus enhancing grain yield. In wheat, Hong et al. (2012) reported that members of the F-box *E3* ubiquitin ligases regulate spike development. A cellulose synthase was identified for TraesCS7A01G033100 within mod_mtaq-7A.1. According to Hyles et al. (2017), a wheat cellulose synthase-like gene is associated with the regulation of the tiller

inhibition gene (*tin*), which reduces tillering in wheat and is also associated with larger spikes and increased grain weight. Other gene models reported for the stable MTA-QTLs are involved in drought tolerance as the aldehyde dehydrogenase (Chen et al., 2015) in lr_mtaq-2B.5, or pest and disease resistance as reported on chromosomes 1A, 1B, 2B and 6A. These genes enhance yield by modulating the response to abiotic and biotic stresses. Other genes affecting yield are those related to phenology as the Flowering Locus T-like proteins detected in MTA-QTLs mod_mtaq-2B.6 and lr_mtaq-6A.2. This gene plays a central role in the regulation of the transition from vegetative to reproductive growth and can affect the spikelet formation (Dixon et al., 2018).

5. Conclusions

The use of local landraces in breeding programmes is considered a valuable approach to broadening the genetic variability of crops lost during the breeding process and improving traits of commercial importance (Lopes et al., 2015; Soriano et al., 2016). The results reported in the present study evidenced the differences in yield-related trait associations between landraces and modern cultivars. Whereas in the landraces stable MTAs across environments were found for grain number and weight, plant height and grain yield, in the modern cultivars the predominant stable traits were plant height and grain weight. Selecting the appropriate genotypes carrying favourable alleles for the differential traits will be useful for designing new crosses in durum wheat breeding programmes.

Supplementary material

Supplementary material will be available on the online version of the scientific article.

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Conflicts of interest

The authors declare no conflicts of interest

Data Availability Statement

The datasets analysed for this study can be found in https://www.diversityarrays.com/technology-and-resources/genetic-maps/

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Chapter 3:
Unravelling the relationship between adaptation pattern and yield formation strategies in Mediterranean durum wheat landraces

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Unravelling the relationship between adaptation pattern and yield formation strategies in Mediterranean durum wheat landraces

Abstract

Understanding the environmental and genetic factors behind the adaptation of landraces to different environments may help design breeding strategies and to promote yield improvement. Based on previous results that showed a differential frequency of alleles associated with important agronomic traits in landraces that originated in the east (EM) and the west (WM) of the Mediterranean Basin, this study analysed their patterns of adaptation and the influence this adaptation has on yield formation strategies. Thirteen and thirty-one genotypes selected according to their membership coefficient (q>0.900) from the EM and the WM genetic subpopulations, respectively, were tested during six crop seasons under rainfed Mediterranean conditions. Yearly yields ranged from 3173 to 4917 kg/ha. EM landraces showed more spikes per unit area, while WM ones showed consistently taller plants, larger cycle length to anthesis, a shorter grain filling period, a higher grain filling rate and heavier grains. The contrasting pattern of adaptation of the two subpopulations was based on a differential ability to use the water available before and after anthesis. The yield of EM landraces, originated in the warmest and driest area of the Mediterranean Basin, relied mostly on water input before anthesis, which was beneficial for spike production and for the accumulation of water-soluble carbohydrates in the stems prior to anthesis, to be remobilized to grains during grain filling. WM landraces performed better in environments with high water input during grain filling, which was efficiently used to increase grain setting and produce heavy grains. EM landraces could be used in breeding to improve the adaptation of modern cultivars to terminal drought.

Keywords: Drought; GE interaction; Water use; Water soluble carbohydrates; Yield components; Grain filling

Abbreviations

EM, East Mediterranean
GE, genotype × environment interaction
WI, water input
WM, West Mediterranean
WSC, water soluble carbohydrates

1. Introduction

Wheat is grown on about 219 million hectares worldwide and provides humans with about 20% of their intake of calories (FAOSTAT, 2016). Durum wheat (*Triticum turgidum* L. var. *durum*) represents about 10% of the global wheat production (Kantety et al., 2005). The Mediterranean Basin is the largest durum-producing region worldwide, as it comprises around 60% of the total growing area. In the region, durum wheat is mainly grown under rainfed conditions and yield is generally constrained by water scarcity, particularly during grain filling, when it is accompanied by high temperatures. Also, the unpredictable seasonal rainfalls cause large yield fluctuations between years (Anderson, 2010; Royo et al., 2010). The expected advent of more adverse weather conditions predicted by future climate change scenarios in the Mediterranean Basin (IPCC, 2014) will require the release of new cultivars adapted to the changing environments. Understanding the environmental and genetic factors behind plant adaptation to drought is critical in order to provide improved varieties with greater and more stable yields under stress environments.

The genotype × environment (GE) interaction complicates selection for broad adaptation as the cultivars perform differently according to climatic variables and soil characteristics during plant growth and development (Blum and Pnuel, 1990; Cooper and Byth, 1996). GE interaction is a challenge for plant breeders as it weakens association between phenotype and genotype and restricts the identification of superior genotypes, hindering genetic improvement in breeding programmes.

Grain growth in wheat is supported by two major sources of carbon: transient photosynthesis during the grain filling period, and the remobilization of water-soluble carbohydrates (WSC) stored in the stem and leaf sheath up to anthesis (Blum, 1998; Gebbing et al., 1999; Xue et al., 2008). As the hot and dry conditions that generally occur during grain filling in Mediterranean environments limit photosynthesis (Palta et al., 1994; Papakosta and Gagianas, 1991), yield depends greatly on the translocation to the grain of WSC accumulated during pre-anthesis (Blum, 1998; Dreccer et al., 2014). The capacity to synthesise and store WSC in the stems before anthesis is one of the mechanisms used by the plant for drought resistance (Michiels et al., 2004). It has been reported that variation in WSC content is largely genetically determined (Xue et al., 2008). Stem carbohydrate reserves have been estimated to contribute 10% to 20% of the final grain yield under relatively non-stressed conditions but more than 40% under severe stress conditions during the grain filling period (Blum, 1998; Ehdaie et al., 2008; Gebbing et al., 1999; Rebetzke et al., 2008). $^{13}C/^{12}C$ carbon isotope discrimination (Δ) measured in mature grains may be used as an indirect indicator of the plant water status and the importance of

translocation processes during grain filling (Araus et al., 2013; Condon et al., 1992).

Archaeological evidence dates the earliest domesticated wheats from the Fertile Crescent to approximately 10,000 years before present (BP). They subsequently spread across the Mediterranean Basin, reaching the Iberian Peninsula around 7,000 years BP (Feldman, 2001; Mac Key, 2005). After arriving in a given territory, they adapted progressively to the varying conditions of the new area and gradually established new strategies for phenology fitting and yield formation, which likely conferred adaptive advantages under the new environmental conditions (Moragues et al., 2006). The evolution of wheat during this migration and the role of human selection after the advent of agriculture resulted in the establishment of local landraces that are generally considered to be endemic to a particular region to which they are well adapted. Landraces possess a useful source of stress-adaptive traits and a wide genetic diversity for adaptation to different conditions according to their place of origin (Lopes et al., 2015).

A previous study conducted with a collection of 172 durum wheat landraces and modern cultivars from 21 Mediterranean countries revealed that landraces collected in the warmest and driest zone of the Mediterranean Basin had a shorter cycle length to anthesis, more spikes and grains m^{-2}, lighter grains, and lower yields than those that originated in colder and wetter zones (Royo et al. 2014). A subsequent study using the same set of germplasm, clustered landraces from the east and the west of the Mediterranean Basin (hereafter EM and WM landraces, respectively) into different genetic subpopulations (Soriano et al. 2016). A more recent study demonstrated that the contrasting agronomic performance of EM and WM landraces was due to a differential frequency of alleles associated with important agronomic traits (Soriano et al., 2018). East Mediterranean landraces had higher frequencies of alleles associated with increased grain filling duration, spikes and grains per unit area, and others reducing cycle length and kernel weight (Soriano et al., 2018). Based on these previous results that suggest a different pattern of adaptation of EM and WM landraces, the current study aimed to: i) analyse and compare the yield formation strategies of eastern and western Mediterranean landraces, ii) evaluate the GE interaction for yield and the influence of meteorological variables before and after anthesis on the pattern of adaptation, and iii) identify putative main drivers for the evolutionary divergence of the two subpopulations in terms of adaptation.

2. Material and methods

2.1. Plant material

The current study was conducted with 44 genotypes selected from a panel of 172 durum wheat landraces and modern cultivars from 21 Mediterranean countries

developed by Royo et al. (2014) and structured into five genetic subpopulations (SP) by Soriano et al. (2016). Considering the differential frequency of alleles affecting agronomic performance in landraces from the east (EM) and the west (WM) regions of the Mediterranean Basin (Soriano et al., 2018), we used a membership coefficient of $q > 0.900$ to select 13 and 31 genotypes from the EM and WM landrace-subpopulations, respectively (supplementary Table 1, Annex 1).

2.2. Field experiments

The experiments were carried out during six crop seasons (harvesting years 2007, 2008, 2009, 2013, 2014 and 2015) in Lleida, north-east Spain (Table 3.1). The experiments were arranged following a non-replicated modified augmented design with replicated checks (cultivars 'Claudio', 'Simeto' and 'Vitron') and plots of 6 m^2 for the first three years and checks 'Avispa' and 'Euroduro' and plots of 3.6 m^2 for the last three. Sowing density was adjusted to 250 viable seeds/m^2 on experimental plots, with eight rows spaced 0.15 m apart. Average minimum and maximum daily temperatures (Tmin and Tmax, °C) and water input (WI, mm) were recorded from a weather station placed in the same field, and means were calculated for the periods from sowing to anthesis and from anthesis to physiological maturity. Soil moisture was monitored in one of the repeated checks from the seedling stage by means of soil probes (model EC-20, ECH2O Dielectric Aquameter, Decagon Devices, Inc.) located at three depths (0–10, 10–25 and 25–40 cm). Weeds and diseases were controlled following standard practices.

Zadoks et al. (1974) growth stages (GS) 65 (anthesis) and 87 (physiological maturity) were determined on each plot. At ripening, samples of the plants in a 0.5-m-long row were pulled up in a central row of each plot to determine the number of spikes per m^2 and the number of grains per spike. Thousand kernel weight (TKW, g) was assessed in three samples of 100 g of the mechanically harvested grain per plot. Plant height (cm) was measured at GS87 in three main stems per plot from the tillering node to the top of the spike, excluding the awns. Grain yield (kg/ha) was expressed on a 12% moisture basis after combine harvesting.

In the experiments conducted in 2007, 2008 and 2009 the main stems (including leaf sheaths and blades) of 10 plants per plot chosen at random were taken in 20 cultivars (5 EM and 15WM), at GS65 and GS87 to determine water-soluble carbohydrates (WSC). The samples were oven-dried at 70°C for 48 h, weighed, ground to pass through a 1-mm sieve, and scanned by the Scientific-Technical Services of the University of Lleida using a near-infrared reflectance spectroscopy (NIR) unit previously calibrated using the anthrone method (Ruuska et al., 2006; Yemm and Willis, 1954). Water-soluble carbohydrates concentration was expressed as a percentage. Dry matter translocation (DMT) was calculated per main stem as

the difference in WSC (g/stem) at anthesis and maturity. Dry matter translocation efficiency (DMTe, %) was computed as 100 x DMT/dry weight per stem at anthesis. In the experiments conducted in 2007 and 2008, carbon isotope discrimination (Δ) was determined on a sample of about 2 g of mature grains from each plot. The $^{13}C/^{12}C$ ratio was determined by mass spectrometry at the Stable Isotope Laboratory (COIL) of Cornell University following the methodology described in Farquhar et al. (1989) and Royo et al. (2008).

2.3. Statistical analyses

Raw 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 (Little et al., 1997). 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 in each environment using the SAS statistical package (SAS Institute, 2014). Combined analyses of variance were performed for all variables with the SAS statistical package with the Kenward-Roger correction due to the unbalanced number of genotypes within subpopulations. The sum of squares of the cultivar and the cultivar × year interaction were partitioned into differences between genetic subpopulations and differences within them. Means were compared using the Tukey test (Tukey, 1949) with the JMP v13 statistical package (SAS Institute, 2009).

The GE interaction was partitioned for yield and yield components according to the AMMI model (Gauch and Zobel, 1997) and the percentage of the sum of squares explained by each interaction principal component axis (IPCA) was calculated. Factorial regression analysis was performed in order to identify the meteorological covariates (Tmin, Tmax and WI from sowing to anthesis and from anthesis to physiological maturity) that best explained the GE interaction for yield and yield components. Following a sequential analysis of variance to establish their relative importance, covariates were introduced progressively in the factorial regression model, and finally those showing mean square values higher than the deviations were selected (Voltas et al., 2005). As the half-normal plot of the residuals of the GE interaction showed no obvious patterns, the deviations mean squares of the factorial regression were chosen as an estimate error for this analysis, which was carried out using GenStat v18 (VSN International, 2013) software. As WI from anthesis to maturity was the only covariate entered in the model that best explained the GE interaction for yield, vectors representing WI from sowing to anthesis and from anthesis to physiological maturity were depicted in the AMMI biplots for yield and yield components.

3. Results

3.1. Environmental

The experimental site has a typical Mediterranean climate characterised by low temperatures in winter that increase rapidly during the spring, accompanied by an irregular pattern of yearly rainfall distribution (Figure 3.1). The highest WI during the growth cycle was recorded in 2008 (285 mm) and 2013 (232 mm), and in the latter the maximum average yield (4917 kg/ha) was achieved (Table 3.1). The minimum yield (3173 kg/ha) was recorded in 2014, when the crop received only 104 mm of rainfall during the growth cycle and suffered severe water scarcity during grain filling. Water stress also occurred during grain filling in 2009 and 2015 (Table 3.1 and Figure 3.1).

3.2. Yield formation

The results of the ANOVA showed that the year effect was significant for all the analysed traits (Table 3.2). It accounted for most of the total variation for phenology, yield and yield components (except number of spikes per m^2), DMT and Δ, but had a lower effect on plant height. Differences between the subpopulations were significant for all traits except number of grains per spike, WSC at maturity and DMT, and explained 1.23% (for Δ) to 48.5% (for plant height) of the total variation of the model. Differences within subpopulations were significant for yield and yield components, and accounted for a larger percentage of the sum of squares of the model than differences between them. Within-subpopulations differences were still significant for plant height and length of developmental periods, but they accounted for a lower percentage of variation than the between-subpopulations differences. Variability within subpopulations was not significant for WSC accumulation at anthesis and maturity, DMT, DMT_e and Δ. The year × between genetic subpopulation interaction was significant for all traits (Table 3.2).

Table 3.1. Details of the six field experiments conducted at Gimenells (Lleida, north-east Spain, 41°40' N, 0°20' E; 200 m a.s.l.).

Year	2007	2008	2009	2013	2014	2015
Soil texture	Clay-loamy	Loamy	Sandy-clay-loamy	Sandy-loamy-clay	Sandy-clay	Sandy-loamy-clay
Sowing date	21-Nov-06	20-Nov-07	20-Nov-08	4-Dec-12	27-Nov-13	21-Nov-14
Harvest date	2-Jul-07	2-Jul-08	15-Jul-09	5-Jul-13	11-Jul-14	6-Jul-15
Environmental conditions from sowing to anthesis						
Tmin (°C)	3.50	3.10	2.50	3.05	2.88	2.98
Tmax (°C)	13.7	14.2	13.2	14.3	13.9	14.0
WI (rainfall + irrigation, mm)	165+0	102+50	183+0	186+0	95+0	166+0
Environmental conditions from anthesis to maturity						
Tmin (°C)	11.4	11.6	12.4	9.02	8.97	10.1
Tmax (°C)	25.7	23.2	27.2	22.6	23.9	25.4
WI (rainfall, mm)	24.3	133	8.60	46.0	8.60	3.60
Average agronomic data						
Days from sowing to anthesis	163a	163a	164a	155b	147c	157b
Days from anthesis to maturity	29.3c	44.2a	26.3e	38.0b	29.8c	27.9d
Yield (kg/ha)	3254c	3818b	3332c	4917a	3173c	4008b
Spikes/m^2	348bc	313d	386a	313d	363ab	324cd
Grains/Spike	20.6d	21.5d	22.6d	35.6a	29.8b	26.7cd
TKW (g)	49.4bc	58.7a	40d	45.1c	37.8d	49.8b

Tmin and Tmax, average minimum and maximum daily temperatures; WI, water input; TKW, thousand kernel weight. Means within rows with different letters are significantly different at P=0.05, following Tukey test.

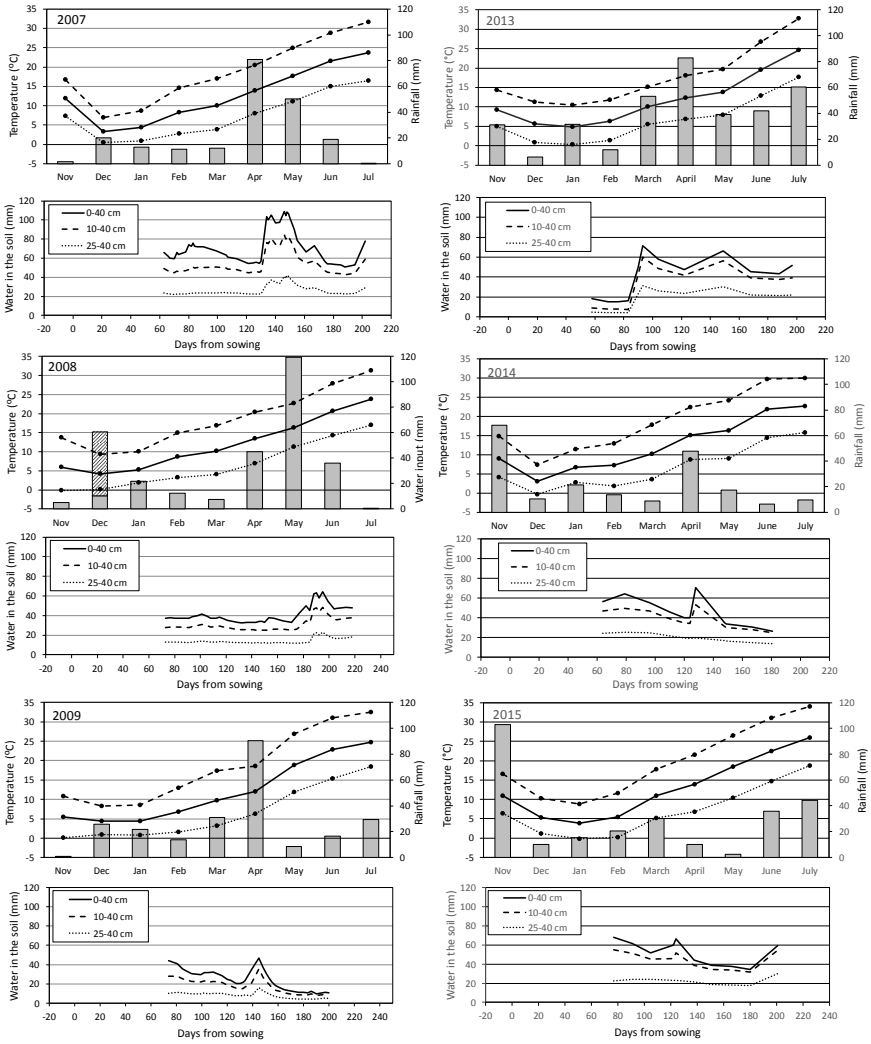

Figure 3.1. Monthly water input and maximum (dashed line), mean (solid line) and minimum (dotted line) temperatures during the growth cycle of each crop season.
The lowest figures indicate the water soil content at three depths (0-40, 10-40 and 25-40 cm) for each year

The comparison of mean values of the two subpopulations showed that the WM landraces outyielded the EM ones on average across years and in all experiments except the ones conducted in 2009 and 2015, when the differences were not statistically significant (Table 3.3). The number of spikes per m^2 was higher in EM landraces in all the experiments. The WM subpopulation showed consistently heavier grains, taller plants, a longer cycle length to anthesis and a shorter grain filling period. The number of grains per spike was significantly higher in the EM subpopulation in all experiments except in 2015, when the result was the opposite.

This contrasting behaviour resulted in a similar number of grains per spike on average across years (Table 3.3).

The results of the experiments conducted in 2007, 2008 and 2009 showed that the percentage of WSC accumulated at anthesis on main stems was significantly higher in EM landraces, but similar values were obtained for the WSC accumulated at physiological maturity (Table 3.3). On average, DMT per stem was similar in the two subpopulations, although in 2007 and 2008 it was higher in the WM subpopulation (Table 3.3). For DMT_e, differences between subpopulations were statistically significant only in 2009 and across years, but the tendency for EM landraces to have higher values was the same in all three experiments. Finally, results of the Δ data recorded in 2007 and 2008 showed significant differences between subpopulations in 2007 and across years, with EM landraces reaching the highest values for this variable (Table 3.3).

3.3. GE interaction for yield

The first two IPCAs of the AMMI model explained 77.4% of the GE interaction for yield (Table 3.4a). The biplot of the first two IPCAs of the AMMI model showed that IPCA1, which accounted for 45.6% of the GE variation, separated the genotypes into two partially overlapping clusters corresponding to EM and WM subpopulations (Figure 3.2). Variability among WM landraces appeared to be greater than that among EM ones. Points located on Figure 3.2 close to and far from the origin of the axes can be seen for both subpopulations, suggesting the presence of landraces with small and large yield variations between years, respectively.

The position of the environments within the biplot of the AMMI analysis (Figure 3.2) showed that the years 2008 and 2015 were separated from the remaining ones, as they were the only ones located on the negative side of the IPCA2 axis. However, a large distance separated them for IPCA1, as 2008 was located in the negative direction of this axis and 2015 in the positive direction.

The most explanatory model obtained by factorial regression analysis accounted for 38.1% of the SS of the GE interaction, with 20% of its degrees of freedom, and only included as a meteorological covariate the WI from anthesis to maturity (Table 3.4a). The vector of this covariable, depicted in the AMMI biplot shown in Figure 3.2, is located on the side of the WM landraces and close to the year 2008, suggesting that WM landraces performed better than EM ones in terms of yield in environments with high WI during grain filling, which was the case in 2008. On the other hand, the vector representing WI from sowing to anthesis was located on the opposite side of the biplot and closer to the points representing EM landraces, indicating their better performance in environments with high WI before anthesis.

Table 3.2. Percentage of the sum of squares of the ANOVA model for yield, yield components, remobilization of water-soluble carbohydrates and carbon isotope discrimination measured on a collection of 44 durum wheat landraces structured on East Mediterranean and West Mediterranean genetic subpopulations (SP).

Source of variation	df	Yield (kg/ha)		Spikes/ m^2		Grains/ spike		TKW		Plant height		$Days_{SA}$		$Days_{AM}$		df	WSC_A		WSC_M		DMT		DMTe		df	Δ	
Year	5	65.6	***	23.6	***	64.6	***	61.9	***	15.2	***	77.6	***	94.1	***	2	44.2	***	29.0	***	48.2	***	45.7	***	1	90.3	***
Between SP	1	2.57	***	9.08	***	0.54		8.63	***	48.5	***	14.9	***	1.79	***	1	14.7	***	0.01		0.01		12.5	***	1	1.23	***
Within SP	42	8.42	***	17.5	***	8.55	**	11.3	***	18.1	***	4.27	***	1.05	***	18	9.8		24.3		11.0		7.70		42	3.04	
Year × Between SP	5	5.05	***	8.70	***	3.55	***	2.56	***	1.77	***	0.70	***	0.65	***	2	13.4	***	10.8	*	20.8	***	18.4	***	1	1.66	***
Year × Within SP	210	18.3	NT	41.1	NT	22.7	NT	15.6	NT	16.4	NT	2.55	NT	2.39	NT	35	17.9	NT	35.9	NT	20.0	NT	15.7	NT	41	3.37	NT
Total	263															58									86		

TKW, thousand kernel weight; $Days_{SA}$ and $Days_{AM,}$ average number of days from sowing to anthesis and from anthesis to maturity, respectively; WSC_A and $WSC_{M,}$ water-soluble carbohydrates at anthesis and physiological maturity, respectively; DMT, dry matter translocation; DMTe, DMT efficiency; Δ, carbon isotope discrimination in mature kernels. *P<0.05; **P<0.01; ***P<0.001. NT: not testable.

Table 3.3. Mean values of yield, yield components, remobilization of water-soluble carbohydrates and carbon isotope discrimination for each year and across years of durum wheat landraces structured on East Mediterranean (EM) and West Mediterranean (WM) genetic subpopulations.

Year	SP	Yield (kg/ha)	Spikes/m^2	Grains/spike	TKW (g)	Plant height (cm)	Days$_{SA}$	Days$_{AM}$	WSC$_A$ (%)	WSC$_M$ (%)	DMT (g/stem)	DMTe (%)	Δ (^{o}/oo)
2007	EM	3205 ^{b}	371 ^{a}	19.6 ^{b}	42.7 ^{b}	91.0 ^{b}	157 ^{b}	30.7 ^{a}	28.0 ^{a}	7.59 ^{a}	0.25 ^{b}	19.7 ^{a}	16.4 ^{a}
	WM	3274 ^{a}	338 ^{b}	21.0 ^{a}	52.2 ^{a}	115 ^{a}	165 ^{a}	28.8 ^{b}	27.7 ^{a}	7.56 ^{a}	0.39 ^{a}	19.6 ^{a}	16.0 ^{b}
2008	EM	3133 ^{b}	324 ^{a}	19.1 ^{b}	51.5 ^{b}	88.6 ^{b}	159 ^{b}	45.8 ^{a}	40.9 ^{a}	10.1 ^{a}	0.54 ^{b}	31.4 ^{a}	17.6 ^{a}
	WM	4106 ^{a}	309 ^{a}	22.4 ^{a}	61.7 ^{a}	110 ^{a}	165 ^{a}	43.5 ^{b}	36.0 ^{b}	7.49 ^{b}	0.72 ^{a}	29.5 ^{a}	17.6 ^{a}
2009	EM	3398 ^{a}	467 ^{a}	19.7 ^{b}	37.8 ^{b}	107 ^{b}	162 ^{b}	26.7 ^{a}	36.6 ^{a}	8.93 ^{a}	0.58 ^{a}	30.2 ^{a}	
	WM	3304 ^{a}	351 ^{b}	23.8 ^{a}	40.9 ^{a}	125 ^{a}	165 ^{a}	26.1 ^{b}	19.5 ^{b}	11.5 ^{a}	0.25 ^{b}	10.6 ^{b}	
2013	EM	4783 ^{b}	336 ^{a}	34.6 ^{b}	42.9 ^{a}	88.1 ^{b}	151 ^{b}	40.4 ^{a}					
	WM	4974 ^{a}	291 ^{b}	35.9 ^{a}	46.0 ^{a}	117 ^{a}	157 ^{a}	37.1 ^{b}					
2014	EM	2840 ^{b}	375 ^{a}	29.3 ^{b}	35.7 ^{b}	91.1 ^{b}	143 ^{b}	30.0 ^{a}					
	WM	3312 ^{a}	358 ^{b}	30.0 ^{a}	38.6 ^{a}	108 ^{a}	148 ^{a}	29.8 ^{a}					
2015	EM	4035 ^{a}	340 ^{a}	29.8 ^{a}	46.2 ^{b}	95.2 ^{b}	153 ^{b}	30.2 ^{a}					
	WM	3996 ^{a}	317 ^{b}	25.4 ^{b}	51.4 ^{a}	115 ^{a}	158 ^{a}	27.0 ^{b}					
Average EM		3566 ^{b}	367 ^{a}	25.4 ^{a}	42.8 ^{b}	93.5 ^{b}	154 ^{b}	34.0 ^{a}	35.2 ^{a}	8.88 ^{a}	0.46 ^{a}	27.1 ^{a}	17.0 ^{a}
Average WM		3828 ^{a}	330 ^{b}	26.4 ^{a}	48.5 ^{a}	115 ^{a}	160 ^{a}	32.0 ^{b}	27.6 ^{b}	8.89 ^{a}	0.45 ^{a}	19.6 ^{b}	16.8 ^{b}

TKW, thousand kernel weight; Days$_{SA}$ and Days$_{AM,}$ average number of days from sowing to anthesis and from anthesis to maturity, respectively; WSC$_A$ and WSC$_{M,}$ water-soluble carbohydrates at anthesis and physiological maturity, respectively; DMT, dry matter translocation; DMTe, DMT efficiency; Δ, carbon isotope discrimination in mature kernels. EM and WM means within each year and across years with different letters are significantly different at P=0.05, following a Tukey test.

Table 3.4. Percentage of the sum of squares (SS) in the AMMI and factorial regression models for the partitioning of the GE interaction for yield and yield components of 44 durum wheat landraces representative of Mediterranean eastern and western genetic subpopulations and tested in six field experiments.

Source of variation	df	SS (%)	*p*-Value
a) Yield			
AMMI			
Year × Cultivar	215	23.4	
IPCA 1	47	45.6	< 0.001
IPCA 2	45	31.8	< 0.001
Residuals	123	22.6	
Factorial Regression			
Year × Cultivar	215	23.4	
WI_AM × Cultivar	43	38.1	< 0.001
Deviations	172	61.9	
b) Number of spikes/m^2			
AMMI			
Year × Cultivar	215	49.8	
IPCA 1	47	50.6	< 0.001
IPCA 2	45	34.3	< 0.001
Residuals	123	15.1	
Factorial Regression			
Year × Cultivar	215	49.8	
Tmax_SA × Cultivar	43	37.9	< 0.001
WI_AM × Cultivar	43	35.3	< 0.001
Deviations	129	26.8	
c) Number of grains/spike			
AMMI			
Year × Cultivar	215	26.3	
IPCA 1	47	51.8	< 0.001
IPCA 2	45	23.4	< 0.001
Residuals	123	24.9	
Factorial Regression			
Year × Cultivar	215	26.3	
WI_AM × Cultivar	43	29.8	0.0095
Deviations	172	70.2	
d) Thousand kernel weight			
AMMI			
Year × Cultivar	215	18.1	
IPCA 1	47	58.2	< 0.001
IPCA 2	45	19.1	< 0.001
Residuals	123	22.7	
Factorial Regression			
Year × Cultivar	215	18.1	
Tmin_AM × Cultivar	43	30.9	0.0047
Deviations	172	69.1	

WI_AM, water input from anthesis to physiological maturity, mm; Tmax_SA, average maximum daily temperature from sowing to anthesis, °C; Tmin_AM, average minimum daily temperature from anthesis to physiological maturity, °C.

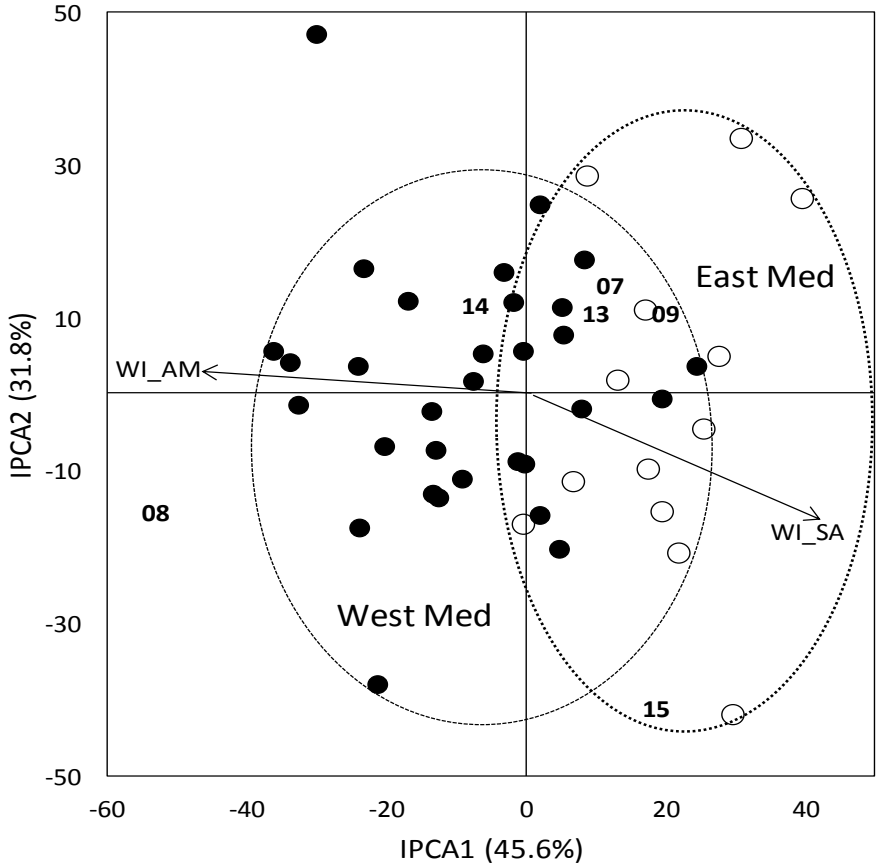

Figure 3.2. Biplot of the first two axes of the AMMI model summarizing the relationships between water input and yield.
Years are represented in bold with their last two digits. Cultivars are identified as follows for each genetic subpopulation: ○ East Mediterranean and ● West Mediterranean. WI_SA and WI_AM, water input from sowing to anthesis and from anthesis to physiological maturity, respectively.

3.4. GE interaction for yield components

The first two IPCAs of the AMMI model explained 84.9% of the SS of the GE interaction for number of spikes per m^2 (Figure 3.3a). The model obtained by factorial regression analysis accounted for 73.2% of the SS of the GE interaction with WI from anthesis to maturity explaining 35.3% of it (Table 3.4b). As in the case of yield, vectors representing WI before and after anthesis had opposite senses in the biplot. The years 2007 and 2013-2015 were close, and near to the vector representing WI before anthesis, suggesting that water availability during the early stages of crop development contributed considerably to the formation of spikes in these years. The years 2009 and 2008 were separated from the rest and situated on opposite sides of IPCA2. The points representing landraces were distributed along the two axes without a clear subpopulation structure for this trait. Nevertheless, most points

representing EM landraces were in the positive sense of IPCA2, some of them showing the greatest values for this axis, while the most negative ones were recorded in two WM landraces. The closeness of some points to particular years suggest that they were beneficial for the formation of spikes.

For number of grains per spike, the biplot of the first two axes of the AMMI model explained 75.2% of the SS of the GE interaction (Figure 3.3b). The factorial regression analysis included only WI from anthesis to maturity as covariate (Table 3.4c). The wide distribution of points representing WM landraces along the whole plot suggests that some WM landraces had a large GE interaction for number of grains per spike. The majority of points representing EM landraces were in the negative sense of IPCA1, on the opposite side of the vector representing WI from anthesis to maturity, suggesting that WI during grain filling had a low effect on the final number of grains per spike for the genotypes included in the EM subpopulation. On the other hand, the majority of points representing WM landraces were in the positive direction of IPCA1 and close to the same vector, indicating that WM landraces took advantage of the water available during grain filling to increase the number of grains per spike. The small length of the vector representing WI from sowing to anthesis suggests that the amount of water available before anthesis was irrelevant for grain setting. The experiments conducted in 2008 and 2015 were separated from the rest in the biplot (Figure 3.3b).

The biplot of the first two axes of the AMMI model for TKW explained 77.3% of the SS of the GE interaction (Figure 3.3c). The points corresponding to WM landraces were located in the upper right part of the figure, while the ones representing EM were in the lower left part, with very few overlapping between them. The only variable included in the factorial regression model was the minimum temperature from anthesis to maturity that accounted for 38.1% of the SS of the GE interaction (Table 3.4d). WI from anthesis to maturity was not included in the model though it accounted for 11.5% of its SS. The location of the points representing WM landraces close to the vector corresponding to WI after anthesis suggests that this subpopulation took advantage of it to increase its grain weight. By contrast, the points representing EM landraces were closer to the vector corresponding to WI before anthesis. IPCA2 was mostly associated with WI after anthesis and, accordingly, the years 2007, 2008 and 2013 were in the positive sense of this axis, while the remaining years were in the negative sense.

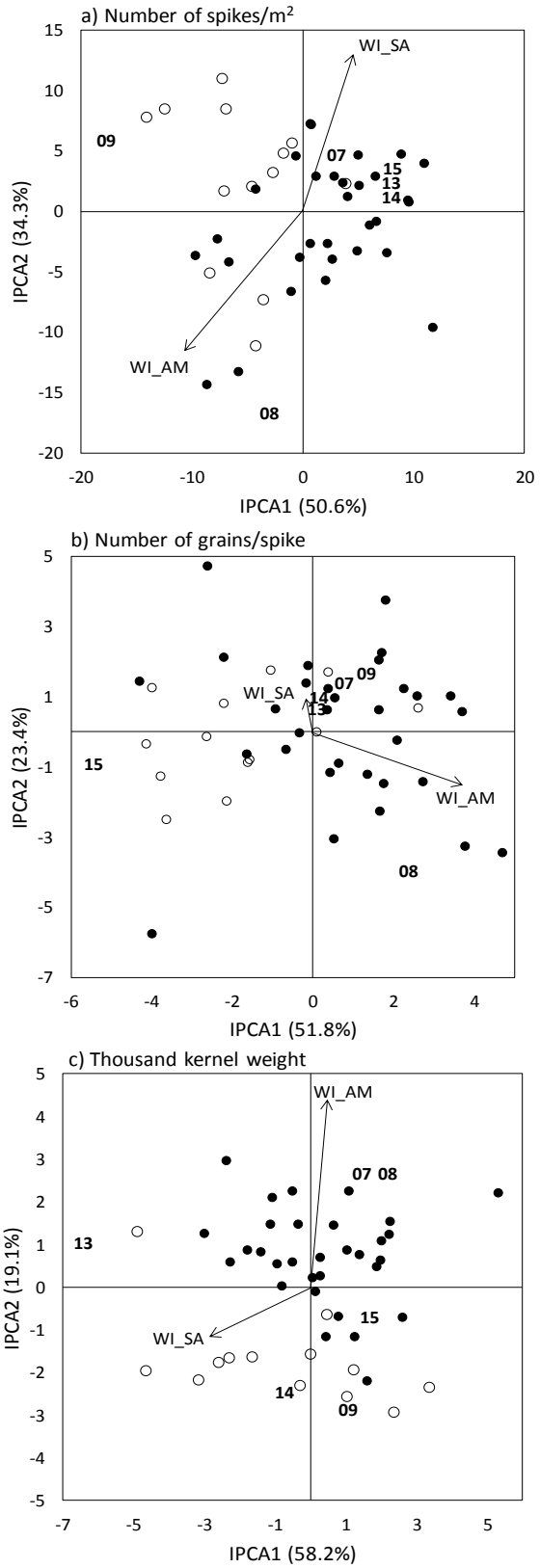

Figure 3.3. Biplot of the first two axes of the AMMI model summarizing the relationships between water input and: a) Number of spikes/m^2, b) Number of grains/spike and c) Thousand kernel weight (TKW).

Years are represented in bold with their last two digits. Cultivars are identified as follows for each genetic subpopulation: ○ East Mediterranean and ● West Mediterranean. WI_SA and WI_AM, water input from sowing to anthesis and from anthesis to physiological maturity, respectively.

4. Discussion

The current study explains, in terms of adaptation, previous results showing differences in yield and yield components between landraces collected in the east and the west of the Mediterranean Basin that conform two different genetic subpopulations (Soriano et al., 2016, 2018). Experiments were conducted during six years on a site located in the west of the Mediterranean Basin that showed the meteorological variability characteristic of the climate in the region.

Variation in weather conditions resulted in yields ranging from 3173 kg/ha in 2014, the year with the lowest rainfall, to 4917 kg/ha in 2013, a year in which rainfall was not the highest but was the most evenly distributed. The high yield recorded in 2014 considering the low water input that year could be attributed to the high soil fertility (about 3% of organic matter) and the superficial sub-soil water layer at this site (Moragues et al., 2006). The year effect explained 66% of yield variability and 24% to 65% of the variance observed for yield components, values slightly lower than those reported in previous studies conducted on durum wheat (Subirà et al., 2015). However, the year effect only accounted for a small proportion of the variability for plant height, which was consistently higher in WM landraces.

On average, WM landraces outyielded EM ones by 7.3%. This result was expected, given that the experimental site is located in the west of the Mediterranean Basin. Yield differences between the two subpopulations were statistically significant in four of the six experiments. In the biplot of the first two axis of the AMMI model that analysed the GE interaction for yield, years 2009 and 2015, when both subpopulations achieved a similar yield, were located close to the vector representing WI from sowing to anthesis. The points corresponding to the years 2007 and 2013, in which yield differences between subpopulations were significant but very low (2% and 4%, respectively), were also located on the positive side of the first IPCA axis. These results suggest that EM landraces had the best yield performance in environments with high water input before anthesis. On the other hand, the largest yield divergence between subpopulations was observed in 2008, when the WM subpopulation outyielded the EM one by 31%. The greatest rainfall received in 2008 after anthesis is in accordance with the location in the biplot of the point corresponding to this year close to the vector symbolizing water input during grain filling. The positioning of most points representing WM landraces in the same direction of this vector suggests that they had the best yield performance in the environment with the highest water input after anthesis. The WM subpopulation was more variable than the EM one with regard to the effect of the distribution of the water available to explain the GE interaction for yield, likely partially due to the larger number of genotypes included in this subpopulation. The points close to the

origin of the first axis denoted yield stability across years in terms of the distribution of the water available during crop cycle.

These results suggest that EM landraces showed good adaptation to environments with high water input before anthesis, but low water input during grain filling, denoting a high efficiency in the use of the water available for the crop before anthesis to generate yield. In contrast, the positioning of most points of the WM subpopulation on the left part of axis 2 reflects a better efficiency than the EM subpopulation in the use of the water available during grain filling to increase yield. To understand these results, the influence of the distribution of the water available in the two growing periods was analysed for each yield component.

In all experiments EM landraces produced a significantly higher number of spikes per unit area than the WM ones, on average 11.2% more. Hütsch et al. (2019) observed that under heat stress, wheat plants tend to increase the number of ear-bearing tillers as an adaptation strategy. The greater frequency of alleles increasing the number of spikes per unit area in EM than in WM landraces found previously in the collection used in the current study (Soriano et al., 2018) was likely a result of the adaptation of EM landraces to warm environments. However, differences between subpopulations ranged from 4.7% (in 2014) to 33% (in 2009). In the biplot, vectors representing water input before and after anthesis were in opposite sense, and only 2008 was located in the direction of WI after anthesis, in agreement with the fact that rainfall was highest during grain filling in this year. The position in the biplot of the points corresponding to 2008 and 2009 on opposite sides of IPCA2 concords with the lowest number of spikes per m^2 recorded in 2008 and the highest recorded in 2009. Furthermore, the position of the vector representing water input before anthesis is on the same side of IPCA2 as the points of all years except 2008, in agreement with the well-documented positive effect of water during early growth stages on the production and survival of tillers (Begg and Turner, 1976; Turner and Begg, 1978). The distance in the biplot between the point corresponding to 2009 and the vector symbolizing water input before anthesis denotes that other factors may have contributed to the large spike number that year. The low temperatures recorded before anthesis in 2009 could have also stimulated the production of tillers and the subsequent spikes, as low temperatures in the early stages of growth promote tiller formation in wheat and other cereals (Chaturvedi et al., 1981). The dispersion of points representing genotypes along both sides of the two axes denotes high variability within subpopulations regarding the strategy of water use for spike formation. However, a small number of points representing landraces from the two subpopulations were located in the biplot close to the vector representing water input after anthesis, which may indicate that in these genotypes late rainfall could benefit the survival of spikes or the emergence of new spikes in late-formed tillers.

Though the average number of grains per spike across years was similar for both subpopulations, WM landraces produced significantly more grains per spike than EM ones in five of the six experiments, and only in 2015 was the opposite observed. The biplot of the first two axes of the AMMI model helped to interpret this result. The position and length of the vectors in the biplot clearly showed that water input before anthesis had a negligible effect on the number of grains per spike when compared with the main effect of water input during grain filling. The only year positioned in the negative sense of IPCA1, on the opposite side of the vector representing water input after anthesis, was 2015, in agreement with the fact that the rainfall was lowest during grain filling in this year. The location of the remaining years and the majority of points representing the WM subpopulation in the same direction of this vector is in line with the largest number of grains per spike recorded in WM landraces in all years except in 2015. The placement of points representing EM landraces close to this year and in the opposite direction of the vector symbolizing water input after anthesis indicates a good adaptation of this subpopulation to water scarcity during grain filling. This finding can be at least partially explained by early anthesis in the EM landraces to escape terminal drought and exposure to slightly lower temperatures during grain filling, in agreement with results obtained by Lopes et al. (2018) in Turkey and Iran, where severe terminal drought and heat causes this type of response. The relative position of points of both subpopulations in the biplot also suggests that the WI after anthesis had a much lower effect on grain setting in EM landraces than in WM ones. Our results also indicate that water stress after anthesis in 2015 was more detrimental for grain setting in WM landraces than in EM ones. In consequence, the larger number of grains per spike of EM landraces in that year resulted in a similar yield in both subpopulations, though the grains were heavier in the WM landraces. Although the genotype effect only explained 9% of the total variation for number of grains per spike, high variability was found within the WM subpopulation for this trait, as revealed by the dispersion in the biplot of the points representing genotypes. The larger number of grains per unit area reported previously for EM landraces (Soriano et al., 2018) was due to a consistently higher number of spikes per unit area, as the number of grains per spike tended to be higher in the WM subpopulation.

Kernel weight of WM landraces was consistently higher than that of EM ones. The difference was 13.3% across years, but this percentage ranged from 8% to 22% depending on the year. The first and second axis of the AMMI biplot that analysed the GE interaction for kernel weight were related to water input before and after anthesis, respectively. Accordingly, the points corresponding to the years 2007, 2008 and 2013 were located on the positive part of IPCA2 close to the vector representing

water input after anthesis, and the remaining years were placed on the negative part of the same axis.

The position in the TKW biplot of the majority of points representing WM landraces in the positive sense of IPCA2 indicates that they made the most of the water input after anthesis to fill their grains. In contrast, the location of points corresponding to EM landraces on the negative part of IPCA2 and closer to the vector symbolizing water input before anthesis suggests that they relied mostly on the water available before anthesis to fill their grains. The results obtained when comparing the concentration of WSC on main stems at anthesis support this conclusion, as EM landraces had consistently higher average values than WM ones in the three years in which this analysis was carried out. These results suggest that the EM landraces used the water available for the crop before anthesis efficiently to accumulate carbohydrates on their stems, probably as a mechanism of adaptation to drought, as it has been reported that WSC content is enhanced in drought-resistant wheats (Hou et al., 2018).

The WM landraces flowered on average 6 days later than the EM ones (3 to 8 days depending on the year), suggesting that they filled their grains under hotter and drier conditions, which was probably the reason for their shorter grain-filling period. Thus, the heavier grains of WM landraces were a consequence of a higher grain filling rate (1.52 mg/day in WM and 1.25 mg/day in EM, derived from Table 3.3), in agreement with their greater ability to use water after anthesis to the benefit of kernel weight.

The similar WSC concentration on main stems at physiological maturity, in view of the fact that it was much higher in the EM landraces at anthesis, denotes a greater relative contribution of WSC to grain filling in this subpopulation than in the WM one. This indicates that the plant canopy of WM landraces had a greater capacity to photosynthesise after anthesis, as transient photosynthesis and translocation of stored reserves accumulated prior to anthesis are the two sources of carbon for grain growth (Blum, 1998; Royo et al., 2018). This is in agreement with the higher yields of WM landraces. However, in absolute values (g/stem), the remobilization to the filling grains of WSC accumulated prior to anthesis in main stems was higher in the WM landraces in 2007 and 2008, which concords with their taller plants. The only year in which DMT was greater in the EM subpopulation was 2009, coinciding with the crop season that received the lowest precipitation after anthesis. Drought during grain filling in this year likely promoted the remobilization of WSC to the filling grains of the EM landraces, which resulted in a DMT efficiency 19.6% superior in them than in the WM ones. Our results indicate that drought stress after anthesis decreased the deposition of dry matter on the filling grains of the WM landraces, as shown by the low differences in grain weight between the two subpopulations in

2009 (< 8%), which finally resulted in similar yields.

The large environmental effect on WSC reported in the literature (Blum, 1998; Ehdaie et al., 2006; Shearman et al., 2005) was confirmed by the current study, because in 2008 (the year of the three analysed that received the lowest water input before anthesis, particularly in April just before flowering) the concentration of WSC at anthesis and DMT in grams per stem were the highest for the two subpopulations. This may reflect the positive effect of water deficit before flowering in the accumulation of WSC in stems, in agreement with Ruuska et al. (2006) and Yang et al. (2000, 2001).

Results of carbon isotope discrimination measured in mature grains in 2007 and 2008 showed similar values in 2008 in both subpopulations, probably as a consequence of the relatively high water input after anthesis in this year. It has been reported that Δ is reduced by drought (Sayre et al., 1995), in agreement with the lower Δ values recorded in 2007, which received less water after anthesis than 2008. In addition, the higher values of EM landraces in 2007 are in agreement with the greater importance of translocation processes during grain filling in this subpopulation, and could indicate that water stress developed more rapidly and deeply during grain filling in WM landraces.

5. Conclusions

The results of the current study demonstrate that the dispersal of durum wheat landraces from the east to the west of the Mediterranean Basin caused important changes in their pattern of adaptation and yield formation strategies. When durum wheat migrated from the eastern zone, whose climate is characterised by high temperatures and low rainfall, particularly after anthesis (Royo et al., 2014), to the western one, the populations established new strategies for yield formation, which conferred adaptive advantages according to the new environmental conditions. The results of the current study showed that landraces from the eastern Mediterranean Basin had the best yield performance in environments with high water input before anthesis, which they used efficiently to produce spikes and to accumulate water soluble carbohydrates in the main stem prior to anthesis to be remobilized to the grains to support drought stress during grain filling. In contrast, landraces collected in the western Mediterranean countries, characterised by lower temperatures and more rain either before or after anthesis (Royo et al., 2014), were better adapted to environments with more water availability during grain filling, which they used to the benefit of a large grain setting and the production of heavier grains. Our results suggest that eastern Mediterranean landraces are more adapted to terminal drought than western ones. In addition to this general pattern suggesting a differential efficiency for water use before and after anthesis in the two subpopulations, the

dispersion of points representing landraces in all biplots indicated that variability within each subpopulation was high in terms of adaptation to Mediterranean environments.

Supplementary material

Supplementary material is available on the online version of the article, doi: 10.1016/j.eja.2019.04.003

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Chapter 4:
Genetic dissection of the seminal root system architecture in Mediterranean durum wheat landraces by genome-wide association study

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Abstract
Roots are crucial for adaptation to drought stress. However, phenotyping root systems is a difficult and time-consuming task due to the special feature of the traits to be analysed. Correlations between root system architecture (RSA) at the early stages of development and in adult plants have been reported. In this study the seminal RSA was analysed on a collection of 160 durum wheat landraces from 21 Mediterranean countries and 18 modern cultivars. The landraces showed large variability in RSA, and differences in root traits were found between previously identified genetic subpopulations. Landraces from the eastern Mediterranean region, the driest and warmest within the Mediterranean Basin, showed the largest seminal root size in terms of root length, surface and volume and the widest root angle, whereas landraces from eastern Balkan countries showed the lowest values. Correlations were found between RSA and yield-related traits in a very dry environment. The identification of molecular markers linked to the traits of interest detected 233 marker-trait associations for 10 RSA traits and grouped them in 82 genome regions named marker-train association quantitative trait loci (MTA-QTLs). Our results support the use of ancient local germplasm to widen the genetic background for root traits in breeding programmes.

Keywords: Durum wheat, landraces, marker-trait association, root system architecture

Abbreviations
BP, Before Present
DArTseq, Diversity Arrays Technology sequencing
EB+T, Eastern Balkans and Turkey
EM, Eastern Mediterranean
FDR, False Discovery Rate
GWAS, Genome Wide Association Study
GY, Grain Yield
LRD, Lateral Roots Diameter
LRL, Lateral Roots Length
LRS, Lateral Roots Surface

LRV, Lateral Roots Volume
MTA, Marker-Trait Association
NGm^2, Number of Grains per square metre
NSm^2, Number of Spikes per square metre
PAV, Presence/Absence Variants
PRD, Primary Root Diameter
PRL, Primary Root Length
PRS, Primary Root Surface
PRV, Primary Root Volume
PVE, Phenotypic Variance Explained
QTL, Quantitative Trait Loci
RSA, Root System Architecture
SNP, Single Nucleotide Polymorphism
SP, Subpopulation
SRA, Seminal Root Angle
TKW, Thousand Kernel Weight
TRN, Total Root Number
WB+E, Western Balkans and Egypt
WM, Western Mediterranean

1. Introduction

Wheat is estimated to have been first cultivated around 10,000 years BP in the Fertile Crescent region. It spread to the west of the Mediterranean Basin and reached the Iberian Peninsula around 7,000 years BP (Feldman, 2001). During this migration, both natural and human selection resulted in the development of local landraces considered to be very well adapted to the regions where they were grown and containing the largest genetic diversity within the species (Nazco et al., 2012). From the middle of the 20^{th} century, as a consequence of the Green Revolution, the cultivation of local landraces was progressively abandoned and replaced by the improved, more productive and genetically uniform semi-dwarf cultivars. However, scientists are convinced that local landraces may provide new alleles to improve commercially valuable traits (Lopes et al., 2015). Introgression of these alleles into modern cultivars can be very useful, especially in breeding for suboptimal environments.

Drought is the most important environmental factor limiting wheat productivity in many parts of the world, so improving yield under water-limited conditions is one of the major challenges for wheat production worldwide. Breeding for adaptation to drought is extremely challenging due to the complexity of the target environments and the stress-adaptive mechanisms adopted by plants to withstand

and mitigate the negative effects of water deficit (Reynolds et al., 2005). These mechanisms allow the plant to escape (e.g. early flowering date), avoid (e.g. root system) and/or tolerate (e.g. osmolyte accumulation) the negative effects of drought, thus playing a role in determining final crop performance (Maccaferri et al., 2011). The crop traits to be considered as selection targets under drought conditions must be genetically correlated with yield and should have a greater heritability than yield itself (Royo et al., 2005; Royo and Villegas, 2011). Among these traits, early vigour, leaf area duration, crop water status, radiation use efficiency and root architecture have been identified to be associated with yield under rainfed conditions (reviewed by Tuberosa (2012)).

Root system architecture (RSA) is crucial for wheat adaptation to drought stress. Roots exhibit a high level of morphological plasticity in response to soil conditions, allowing plants to adapt better, particularly under drought conditions. However, evaluating root architecture in the field is very difficult, expensive and time-consuming, especially when a large number of plants need to be phenotyped. Several studies have reported a correlation of RSA in the early stages of development with RSA in adult plants (Løes and Gahoonia, 2004), Manschadi et al. (2008) reported that adult root geometry is strongly related to seminal root angle (SRA), and Wasson et al. (2012) described a relationship of root vigour between plants grown in the field and controlled conditions. Several systems have been adopted to enable early screening of the RSA in wheat (Canè et al., 2014).

Identifying quantitative trait loci (QTLs) and using marker-assisted selection is an efficient way to increase selection efficiency and boost genetic gains in breeding programmes. However, while numerous studies have reported QTLs for RSA in bi-parental crosses (Soriano and Álvaro, 2019), very few of them were based on association mapping (Alahmad et al., 2019; Ayalew et al., 2018; Beyer et al., 2019; Canè et al., 2014; Li et al., 2019; Sanguineti et al., 2007). Association mapping is a complementary approach to bi-parental linkage analysis and provides broader allelic coverage with higher mapping resolution. Association mapping is based on linkage disequilibrium, defined as the non-random association of alleles at different loci, and is used to detect the relationship between phenotypic variation and genetic polymorphism.

The main objectives of the present study were a) to identify differences in RSA among genetic subpopulations of durum wheat Mediterranean landraces, b) to find correlations of RSA with yield-related traits in different rainfed Mediterranean environments, and c) to identify molecular markers linked to RSA in the old Mediterranean germplasm through genome-wide association study.

2. Material and methods

2.1. Plant material

The germplasm used in the current study consisted of a set of 160 durum wheat landraces from 21 Mediterranean countries and 18 modern cultivars from a previously structured collection (Nazco et al., 2012; Soriano et al., 2016). The landraces were classified into four genetic subpopulations (SPs) that matched their geographical origin as follows: the eastern Mediterranean (19 genotypes), the eastern Balkans and Turkey (20 genotypes), the western Balkans and Egypt (31 genotypes), the western Mediterranean (71 genotypes), and 19 genotypes that remained as admixed (Supplementary Materials Table S1, Annex 1).

2.2. Phenotyping

Eight uniform seeds per genotype were cultured following the paper roll method (Rahnama et al., 2011; Watt et al., 2013) in two replicates of four seeds. The seeds were placed at the top of a filter paper (420×520 mm) with the embryo facing down and sprayed with a 0.4% sodium hypochlorite solution. Subsequently, the papers were folded in half to obtain a 210×520 mm rectangle with the seeds fixed at the top. The papers were misted with deionized water and rolled by hand. The rolls were placed in plastic pots with deionized water at the bottom that was regularly checked to ensure it did not evaporate. The experiment was conducted in a growth chamber at 25°C and darkness conditions. One week after sowing, the seeds were transferred to a black surface to take digital images that were processed by SmartRoot software (Lobet et al., 2011) (Figure 4.1). Nine traits for seminal root system architecture (RSA) were measured: total root number (TRN), primary root length (PRL, cm), total lateral root length (LRL, cm), primary root surface (PRS, cm^2), total lateral root surface (LRS, cm^2), primary root volume (PRV, mm^3), total lateral root volume (LRV, mm^3), primary root diameter (PRD, cm) and mean lateral root diameter (LRD, cm).

Additionally, the seminal root angle (SRA, °) was measured at the facilities of the International Center for Agricultural Research in the Dry Areas (ICARDA) in Rabat (Morocco) using the clear pot method described by Richard et al., (2015) (Figure 4.1). Using a randomized complete block design, eight seeds per genotype were grown in 4 L clear pots filled with peat. The seeds were placed with the embryo facing down and close to the pot wall to facilitate root growth along the transparent wall. The pots were then watered, placed inside 4 L black pots and kept at 20°C and darkness conditions in a growth chamber. Five days after sowing, digital images were taken and processed with ImageJ software (Rasband, 2012).

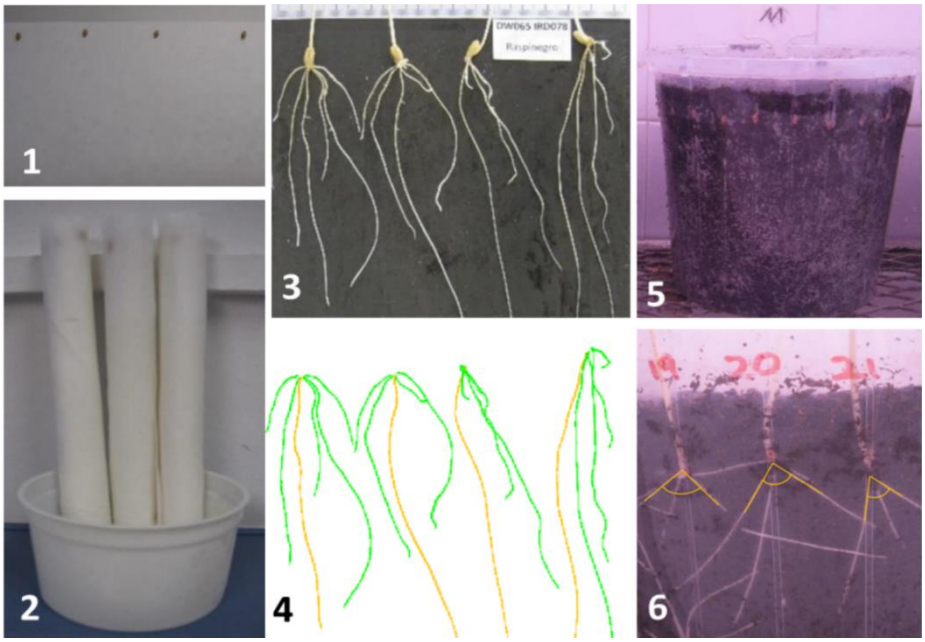

Figure 4.1. Experimental setup for root system architecture analysis.
Firstly seeds were placed on humid filter paper (1) and rolled. Paper rolls were placed in plastic pots with deionized water at the bottom for root growth (2). One week after sowing, the seeds were transferred to a black surface for digital imaging (3) that were processed by SmartRoot software (Lobet et al., 2011) (4). Seminal root angle was measured using the clear pots (5, 6).

Data from field experiments conducted under rainfed conditions during two years of contrasting water input from sowing to physiological maturity (285 mm in 2008 and 104 mm in 2014) in Lleida, north-eastern Spain (Roselló et al., 2019a) were used to assess the relationships between RSA traits and yield-related traits.

The experiments were carried out in a non-replicated modified augmented design with three replicated checks (the cultivars 'Claudio', 'Simeto' and 'Vitron') and plots of 6 m^2 (8 rows, 5 m long with a 0.15 m spacing). Sowing density was adjusted to 250 viable seeds m^{-2} and the plots were maintained free of weeds and diseases.

2.3. Statistical analyses

Combined analyses of variance (ANOVA) were performed for the RSA traits of the structured accessions (141 landraces and 18 modern cultivars), considering the accessions and the replicate as random effects. The sum of squares of the cultivar effect was partitioned into differences between SPs and differences within them. The Kenward-Roger correction was used due to the unbalanced number of genotypes within the SPs. Since the experiment was divided into six sets with one check, least squared means were calculated using Simeto as a check and compared using the Tukey test (Tukey, 1949) at $P < 0.01$.

Raw field 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 (Little et al., 1997). Restricted maximum likelihood was used to estimate the variance components and to produce the best linear unbiased predictors (BLUPs) for yield and yield components. The relationships between RSA traits and yield-related traits were assessed through correlation analyses. All calculations were carried out using the SAS statistical package (SAS Institute, 2014).

2.4. Genotyping

DNA isolation was performed from leaf samples following the method reported by Doyle and Doyle (Doyle and Doyle, 1987). High throughput genotyping was performed at Diversity Arrays Technology Pty Ltd (Canberra, Australia) (http://www.diversityarrays.com) with the genotyping by sequencing (GBS) DArTseq platform (Sansaloni et al., 2011). A total of 46161 markers were used to genotype the association mapping panel, including 35837 presence/absence variants (PAVs) and 10324 single nucleotide polymorphisms (SNPs). Markers were ordered according to the consensus map of wheat v4 available at https://www.diversityarrays.com/.

2.5. Linkage Disequilibrium

Linkage disequilibrium (LD) among markers was calculated for the A and B genomes using markers with a map position on the wheat v4 consensus map, and a minor allele frequency greater than 5%, using TASSEL 5.0 (Bradbury et al., 2007). Pair-wise LD was measured using the squared allele frequency correlations r^2 and the values for genomes A and B were plotted against the genetic distance to determine how fast the LD decays. A LOESS curve was fitted to the plot using the JMP v12Pro statistical package (SAS Institute, 2009).

2.6. Genome-Wide Association Study

A genome-wide association study (GWAS) was performed with 160 landraces for the mean of measured traits with TASSEL 5.0 software (Bradbury et al., 2007). A mixed linear model was conducted using the population structure determined by Soriano et al. (2016) as the fixed effect and a kinship (K) matrix as the random effect (Q + K) at the optimum compression level. A false discovery rate threshold (Benjamini and Hochberg, 1995) was established at $-\log_{10}P>4.6$ ($P<0.05$), using 2135 markers according to the results of the LD decay, to consider a marker-trait association (MTA) significant. Moreover, a second, less restrictive threshold was established at $-\log_{10}P>3$. To simplify the MTA information, those associations located within LD blocks were considered to belong to the same QTL and were

named marker-trait association quantitative trait loci (MTA-QTLs). Graphical representation of the genetic position of MTA-QTLs was carried out using MapChart 2.3 (Voorrips, 2002).

2.7. Gene Annotation

Gene annotation for the target region of significant MTAs was performed using the gene models for high-confidence genes reported for the wheat genome sequence (IWGSC, 2018) available at https://wheat-urgi.versailles.inra.fr/Seq-Repository/.

3. Results

3.1. Phenotypic Analyses

The ANOVA showed that for all traits the phenotypic variability was mainly explained by the cultivar effect, as it accounted for 63.41% (PRD) to 90.57% (LRD) of the total sum of squares (Table 4.1). A summary of the genetic variation of the RSA traits is shown in Supplementary Materials Table S2. The partitioning of the sum of squares of the cultivar effect into differences between and within SPs revealed that the variability induced by the genotype was mainly explained by differences within SPs on a range from 70.1% for TRN to 91.5 for PRV (Table 4.1). Differences between SPs were statistically significant for all traits, accounting for 8.5% (PRV) to 30.5% (TRN) of the sum of squares of the genotype effect (Table 4.1). Western Mediterranean landraces showed the highest number of seminal roots and the narrowest root angle, whereas the eastern Balkans and Turkey SP showed the widest angle (Table 4.2). The highest values for root size–related traits (length, surface and volume) in both primary and lateral roots were recorded in the eastern Mediterranean landraces. The western Balkans and Egypt subpopulation showed the largest root diameter (Table 4.2). The comparison of mean values of eastern Balkans and Turkish landraces revealed that the Turkish ones had high values for all traits except TRN, LRL and root diameter (Supplementary Materials Table S3). The modern cultivars showed intermediate values for all RSA traits (Table 4.2).

Correlation coefficients between RSA traits and yield-related traits were calculated for two field experiments with contrasting water input (285 and 104 mm of rainfall from sowing to physiological maturity). Whereas for the rainiest environment only the relationship between SRA and number of spikes per square meter (NSm^2) was statistically significant (P=0.043, r^2=0.16), for the driest environment 14 correlations involving all the yield-related traits and RSA traits except root diameter were statistically significant (Figure 4.2) (r^2 between 0.17 for NSm^2 and PRL and PRS to 0.30 for TKW and TRN). Most of the significant correla-

Table 4.1. Percentage of the sum of squares of the ANOVA model for the seminal root system architecture traits in a set of 159 Mediterranean durum wheat genotypes structured into five genetic subpopulations by Soriano et al. (2016).

Source of variation	df	TRN	SRA	PRL	LRL	PRS	LRS	PRV	LRV	PRD	LRD
Genotype	158	84.1***	69.4***	87.0***	86.8***	85.3***	86.1***	82.9***	86.8***	63.41***	90.57***
Between subpopulations	4	30.5***	16.6***	15.4***	22.7***	11.6***	18.5***	8.5**	12.7***	10.44**	17.96***
Within subpopulations	154	70.1***	83.4***	85.0***	77.8***	88.6***	82.0***	91.5***	87.6***	89.33**	81.87***
Replicate	1	0.001	1.83**	0.00	0.43*	0.36*	0.36*	0.86**	0.33**	1.35*	0.12
Error	157	15.9	28.8	13.0	12.9	14.3	13.6	16.1	12.9	35.25	9.33
Total	316										

TRN, total root number; SRA, seminal root angle; PRL, primary root length; LRL, total lateral root length; PRS, primary root surface; LRS, total lateral root surface; PRV, primary root volume; LRV, total lateral root volume; PRD, primary root diameter; LRD, mean lateral root diameter. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

Table 4.2. Means comparison of seminal root system architecture traits measured in a set of 159 Mediterranean durum wheat genotypes structured into five genetic subpopulations (Soriano et al., 2016).

	TRN	SRA	PRL	LRL	PRS	LRS	PRV	LRV	PRD	LRD
EM	4.8 ^{b}	94.7 ^{ab}	13.8 ^{a}	25.1 ^{a}	2.5 ^{a}	4.6 ^{a}	38.3 ^{a}	67.2 ^{a}	0.57 ^{b}	0.57 ^{b}
EB+T	4.8 ^{b}	98.2 ^{a}	10.3 ^{c}	17.2 ^{b}	1.9 ^{c}	3.2 ^{b}	28.5 ^{b}	47.6 ^{b}	0.57 ^{b}	0.58 ^{b}
WB+E	4.3 ^{c}	87.6 ^{bc}	10.4 ^{c}	16.5 ^{b}	2.1 ^{bc}	3.2 ^{b}	33.6 ^{ab}	52.3 ^{b}	0.61 ^{a}	0.62 ^{a}
WM	5.2 ^{a}	84.5 ^{c}	11.8 ^{ab}	23.5 ^{a}	2.2 ^{bc}	4.3 ^{a}	33.0 ^{ab}	63.9 ^{a}	0.58 ^{b}	0.58 ^{b}
Modern	4.5 ^{bc}	93.9 ^{ab}	12.8 ^{ab}	20.8 ^{ab}	2.4 ^{ab}	3.7 ^{ab}	35.2 ^{ab}	54.5 ^{ab}	0.56 ^{b}	0.57 ^{b}

Means within columns with different letters are significantly different at $P<0.01$ following a Tukey test. TRN, total root number; SRA, seminal root angle (°); PRL, primary root length (cm); LRL, total lateral root length (cm); PRS, primary root surface (cm^2); LRS, total lateral root surface (cm^2); PRV, primary root volume (mm^3); LRV, total lateral root volume (mm^3); PRD, primary root diameter (cm); LRD, mean lateral root diameter (cm). EM, eastern Mediterranean; EB+T, eastern Balkans and Turkey; WB+E, western Balkans and Egypt; WM, western Mediterranean.

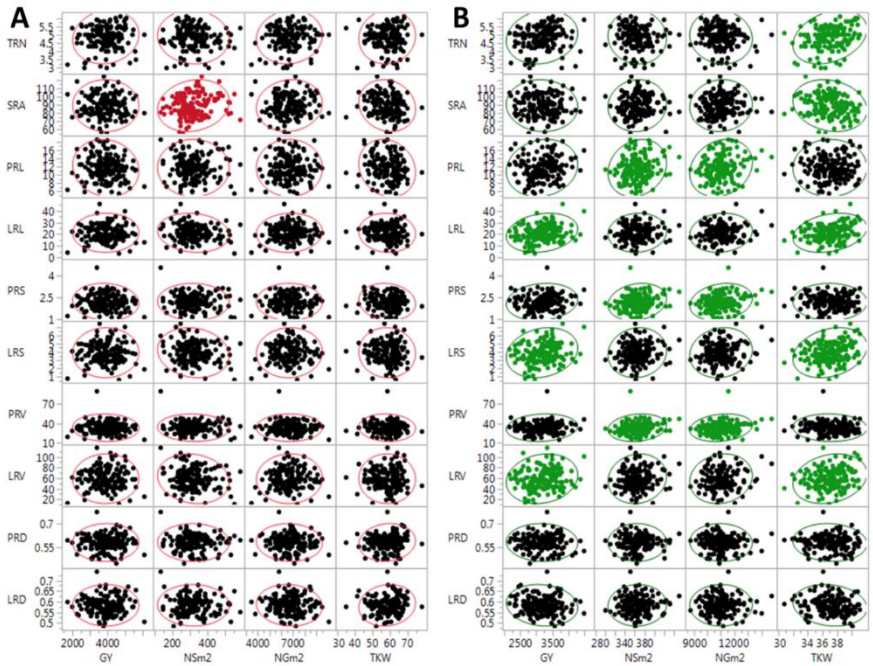

Figure 4.2. Correlations between seminal root system architecture traits and yield-related traits determined in field experiments receiving high (density ellipse in red, A) and low (density ellipse in green, B) water input from sowing to physiological maturity. Significant correlation coefficients ($P<0.05$) are indicated with red and green points. TRN, total root number; SRA, seminal root angle (°); PRL, primary root length (cm); LRL, total lateral root length (cm); PRS, primary root surface (cm^2); LRS, total lateral root surface (cm^2); PRV, primary root volume (mm^3); LRV, total lateral root volume (mm^3); PRD, primary root diameter (cm); LRD, mean lateral root diameter (cm); GY, grain yield (kg/ha); NSm^2, number of spikes per square metre; NGm^2, number of grains per square metre; TKW, thousand kernel weight (g).

tions were positive; only the relationship between SRA and thousand kernel weight (TKW) was negative

3.2. Marker-Trait Associations

A total of 46,161 DArTseq markers, including PAVs and SNPs, were used to genotype the set of 160 durum wheat landraces. To reduce the risk of false positives, markers and accessions were analysed for the presence of duplicated patterns and missing values. Of 35,837 PAVs, 24,188 were placed on the wheat v4 consensus map. Of these, those with more than 30% of missing data and those with a minor allele frequency lower than 5% were removed from the analysis, leaving 19,443 PAVs. A total of 6,957 SNPs were mapped, leaving a total of 4,686 SNPs after marker filtering as before. Additionally, 413 markers were duplicated between PAVs and SNPs, so the corresponding PAVs were eliminated. A total of 23,716 markers remained for the subsequent analysis.

Linkage disequilibrium was estimated for locus pairs in genomes A and B using a sliding window of 50 cM. A total of 471,319 and 681,389 possible pair-wise loci were observed for genomes A and B, respectively. Of these locus pairs, 52% and 43% showed significant linkage disequilibrium at $P<0.01$ and $P<0.001$, respectively. Mean r^2 was 0.12 for genome A and 0.11 for genome B. These means were used as a threshold for estimating the intercept of the LOESS curve to determine the distance at which LD decays in each genome. Markers were in LD in a range from less than 1 cM in genome B to 1 cM in genome A (Supplementary Materials Figure S1).

Results of the GWAS are reported in Figure 4.3 and in Supplementary Materials Table S4. Using a restrictive threshold based on false discovery rate at $P<0.05$ ($-\log_{10}P>4.6$) and the LD decay, only 12 MTAs corresponding to 7 markers were significant. Using a common threshold of $-\log_{10}P>3$, as previously reported by other authors (Mangini et al., 2018; Mwadzingeni et al., 2017; Sukumaran et al., 2018; Wang et al., 2017), a total of 233 MTAs involving 176 markers were identified. MTAs were equally distributed in both genomes (50.2% in the A genome and 49.8% in the B genome). Chromosomes 2B and 7A harboured the highest number of MTAs (39 and 32 respectively), carrying 30% of the total number of MTAs, whereas chromosomes 4B and 7B harboured the lowest number of MTAs, 8 and 6 respectively (Figure 4.3A). Root volume was the trait showing the highest number of MTAs (77), followed by root surface (46), root diameter (37), root length and number (26), and finally SRA (21) (Figure 4.3B). The mean percentage of phenotypic variance explained (PVE) per MTA was similar for all traits, ranging from 0.09 to 0.11 (Figure 4.3C). Most of the MTAs showed low PVE, in agreement with the quantitative nature of the analysed traits. The percentage of MTAs with a

PVE lower than 0.1 was 71%, whereas that of MTAs with a PVE lower than 0.15 was 98% (Figure 4.3D).

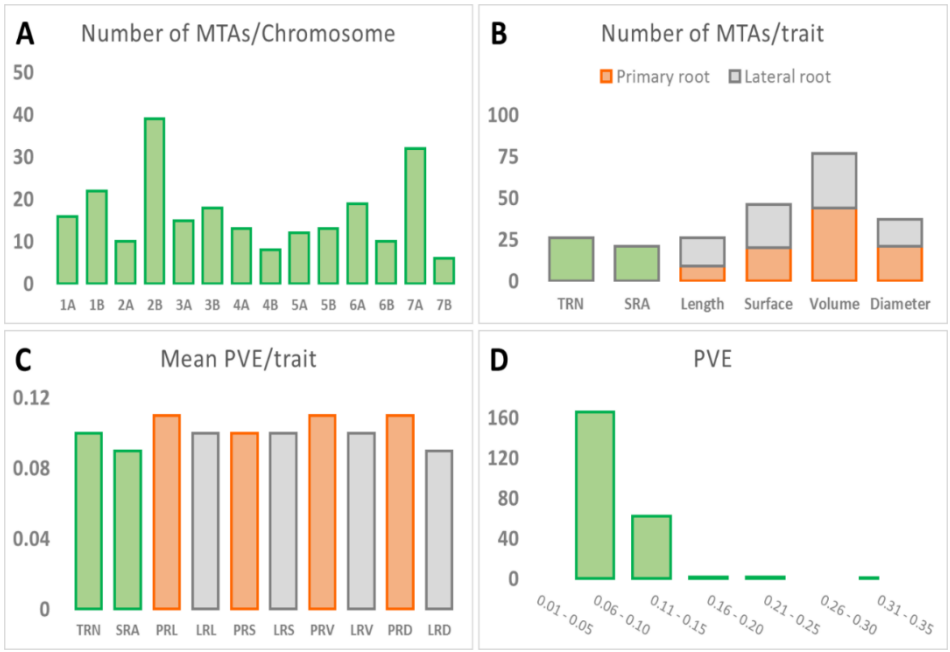

Figure 4.3. Summary of marker trait associations (MTA).
(A) Number of MTAs per chromosome. (B) Number of MTAs per trait. (C) Mean PVE per trait. (D) PVE. TRN, total root number; SRA, seminal root angle; PRL, primary root length; LRL, total lateral root length; PRS, primary root surface; LRS, total lateral root surface; PRV, primary root volume; LRV, total lateral root volume; PRD, primary root diameter; LRD, mean lateral root diameter

To simplify the MTA information, those MTAs located within a region of 1 cM as reported by the LD decay were considered part of the same QTL. Thus, the 233 associations were restricted to 82 MTA-QTLs (Figure 4.4 and Table 4.3). Of the 82 MTA-QTLs, 33 had only one MTA, whereas for the remaining 49 the number of MTAs per MTA-QTL ranged from 2 in 19 MTA-QTLs to 15 in mtaq-7A.1. When several consecutive pairs of MTAs were separated for a distance of 1 cM the whole block was considered as the same MTA-QTL. The genomic distribution of MTA-QTLs showed that chromosome 1A, 4A and 5B harboured 8 MTA-QTLs, chromosomes 1B, 3A , 3B and 5A 7 MTA-QTLs, 6A 6 MTA-QTLs, 7A 5 MTA-QTLs, 2A, 2B, 4B and 6B 4 MTA-QTLs and finally chromosome 7B harboured 3 MTA-QTLs. For the 48 MTA-QTLs with more than one MTA, 10 were related to one trait. Of these, mtaq-1A.5, mtaq-3A.1, mtaq-4A.4 and mtaq-4A.5 carried associations related to root volume, mtaq-2B.1 and mtaq-3B.6 to root diameter, mtaq-3B.1 and mtaq-7A.5 to root number, and finally mtaq-4A.3 and mtaq-6A.5 to root angle.

Among all significant MTAs, markers with different alleles between extreme genotypes for each trait (i.e. the upper and lower 10th percentile) were identified except for PRL (Table 4.4, Figure 4.5). Frequency of the most common allele among genotypes from the upper 10th percentile ranged from 67% for LRD to 90% for PRV, whereas for the lower 10th percentile they ranged from 74% for TRN to 93% for LRD (Figure 4.5).

3.3. Gene Annotation

Of the 176 markers showing significant associations, 31 were identified in the reference sequence of the wheat genome (IWGSC, 2018) (Table 4.5). Eight of them were positioned within gene models, whereas for the rest the closest gene model to the corresponding marker was taken into consideration. The gene models described in Table 4.5 included molecules related to abiotic stress resistance, seed formation, carbohydrate remobilization, disease resistance proteins and other genes involved in different cellular metabolic pathways.

4. Discussion

Roots exhibit a high level of morphological plasticity in response to soil conditions, allowing plants to better adapt, particularly under drought conditions. Several authors have reported the role of RSA traits in response to drought stress (Christopher et al., 2013; Paez-García et al., 2015). Wasson et al. (2012) suggested that a deep root system with the appropriate density along the soil profile would confer advantage on wheat grown in rainfed agricultural systems. Therefore, identifying new alleles for improving root architecture under drought conditions and introgressing them into adapted phenotypes is a desirable approach for breeding purposes. The current study analysed a collection of durum wheat landraces representative of the variability existing within the Mediterranean Basin in an attempt to broaden the genetic background present in commercial cultivars.

Evaluating root architecture in the field is a difficult, expensive and time-consuming assignment, especially when a large number of plants need to be phenotyped. It has been reported that the root geometry of adult plants is strongly related to SRA, with deeply rooted wheat genotypes showing a narrower SRA (Manschadi et al., 2008). Different systems have been adopted to enable early screening of the root system architecture in wheat, assuming that genotypes that differ in root architecture at an early developmental stage would also differ in the field at stages when nutrient and/or water capture become critical for grain yield (Canè et al., 2014).

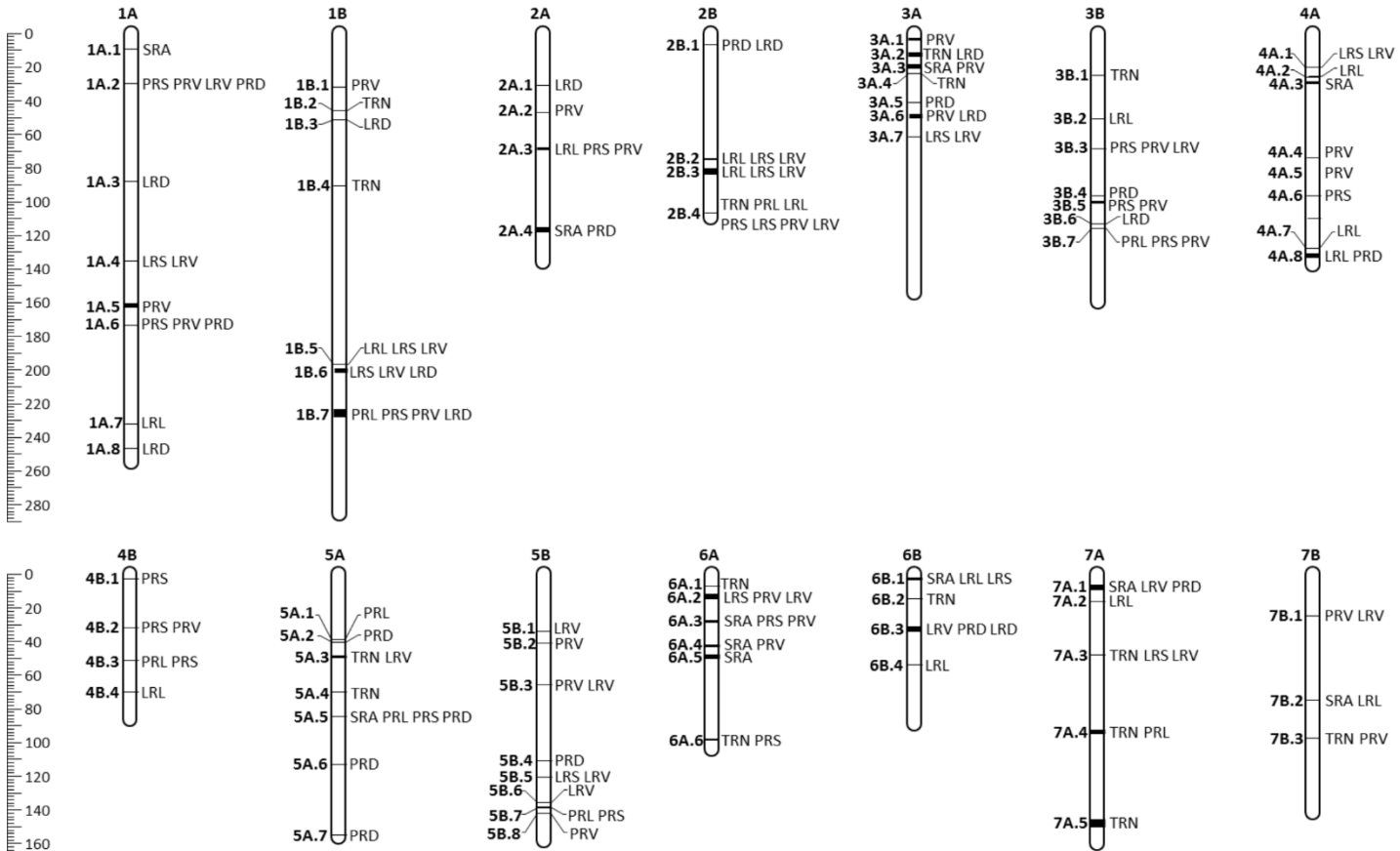

Figure 4.4. MTA-QTL map.

MTA-QTLs are indicated in bold on the left side of the chromosome and traits involved in each MTA-QTL are on the right side. The rule on the left indicates genetic distance in cM. TRN, total root number; SRA, seminal root angle; PRL, primary root length; LRL, total lateral root length; PRS, primary root surface; LRS, total lateral root surface; PRV, primary root volume; LRV, total lateral root volume; PRD, primary root diameter; LRD, mean lateral root diameter.

Table 4.3. MTA-QTLS.

MTA-QTLs	Chromosome	Position (cM)	MTAs	Trait
mtaq-1A.1	1A	9.24	1	SRA
mtaq-1A.2	1A	29.71	4	PRS PRV LRV PRD
mtaq-1A.3	1A	88.15	1	LRD
mtaq-1A.4	1A	135.37	2	LRS LRV
mtaq-1A.5	1A	160.75-163.11	3	PRV
mtaq-1A.6	1A	173.41	3	PRS PRV PRD
mtaq-1A.7	1A	231.76	1	LRL
mtaq-1A.8	1A	246.3	1	LRD
mtaq-1B.1	1B	31.69	1	PRV
mtaq-1B.2	1B	45.68	1	TRN
mtaq-1B.3	1B	51.29	1	LRD
mtaq-1B.4	1B	90.37	1	TRN
mtaq-1B.5	1B	196.56	3	LRL LRS LRV
mtaq-1B.6	1B	199.9-201.49	3	LRS LRV LRD
mtaq-1B.7	1B	223.51-227.36	12	PRL PRS PRV LRD
mtaq-2A.1	2A	31.13	1	LRD
mtaq-2A.2	2A	46.78	1	PRV
mtaq-2A.3	2A	68.39-68.96	4	LRL PRS PRV
mtaq-2A.4	2A	115.8-118.32	4	SRA PRD
mtaq-2B.1	2B	6.7	2	PRD LRD
mtaq-2B.2	2B	75.09-75.13	13	LRL LRS LRV
mtaq-2B.3	2B	80.79-83.84	16	LRL LRS LRV
mtaq-2B.4	2B	106.98-107.03	8	TRN PRL LRL PRS LRS PRV LRV
mtaq-3A.1	3A	3.32-3.58	3	PRV
mtaq-3A.2	3A	11.88-12.93	2	TRN LRD
mtaq-3A.3	3A	18.37-20.39	3	SRA PRV
mtaq-3A.4	3A	23.99	1	TRN
mtaq-3A.5	3A	40.97	1	PRD
mtaq-3A.6	3A	48.06-49.67	3	PRV LRD
mtaq-3A.7	3A	61.57	2	LRS LRV
mtaq-3B.1	3B	24.98-25	2	TRN
mtaq-3B.2	3B	50.7	1	LRL
mtaq-3B.3	3B	68.36	4	PRS PRV LRV
mtaq-3B.4	3B	96.48	1	PRD
mtaq-3B.5	3B	100.07-101.44	3	PRS PRV
mtaq-3B.6	3B	112.86	4	LRD

MTA-QTLs	Chromosome	Position (cM)	MTAs	Trait
mtaq-3B.7	3B	115.61	3	PRL PRS PRV
mtaq-4A.1	4A	20.42-26.03	2	LRS LRV
mtaq-4A.2	4A	26.03	1	LRL
mtaq-4A.3	4A	28.85-28.87	2	SRA
mtaq-4A.4	4A	74.09	2	PRV
mtaq-4A.5	4A	96.08	2	PRV
mtaq-4A.6	4A	109.72	1	PRS
mtaq-4A.7	4A	127.56	1	LRL
mtaq-4A.8	4A	131.42-132.72	2	LRL PRD
mtaq-4B.1	4B	2.79	1	PRS
mtaq-4B.2	4B	31.93	4	PRS PRV
mtaq-4B.3	4B	51.22	3	PRL PRS
mtaq-4B.4	4B	70.04	1	LRL
mtaq-5A.1	5A	38.83	1	PRL
mtaq-5A.2	5A	40.51	1	PRD
mtaq-5A.3	5A	48.57-48.65	2	TRN LRV
mtaq-5A.4	5A	69.82	1	TRN
mtaq-5A.5	5A	84.51	5	SRA PRL PRS PRD
mtaq-5A.6	5A	112.96	1	PRD
mtaq-5A.7	5A	155.41	1	PRD
mtaq-5B.1	5B	33.99	1	LRV
mtaq-5B.2	5B	40.83	1	PRV
mtaq-5B.3	5B	65.51	2	PRV LRV
mtaq-5B.4	5B	111.15	1	PRD
mtaq-5B.5	5B	120.34	2	LRS LRV
mtaq-5B.6	5B	135.45	1	LRV
mtaq-5B.7	5B	138.69	4	PRL PRS
mtaq-5B.8	5B	142.12	1	PRV
mtaq-6A.1	6A	7.11	1	TRN
mtaq-6A.2	6A	11.95-14.24	8	LRS PRV LRV
mtaq-6A.3	6A	27.82-28.69	3	SRA PRS PRV
mtaq-6A.4	6A	42.36	3	SRA PRV
mtaq-6A.5	6A	48.39-50.08	2	SRA
mtaq-6A.6	6A	98.51-98.82	2	TRN PRS
mtaq-6B.1	6B	2.41-3.31	5	SRA LRL LRS
mtaq-6B.2	6B	14.26	1	TRN
mtaq-6B.3	6B	31.49-33.46	3	LRV PRD LRD
mtaq-6B.4	6B	53.66	1	LRL

MTA-QTLs	Chromosome	Position (cM)	MTAs	Trait
mtaq-7A.1	7A	5.7-9.43	15	SRA LRV PRD
mtaq-7A.2	7A	16.28	1	LRL
mtaq-7A.3	7A	47.85	3	TRN LRS LRV
mtaq-7A.4	7A	92.69-94.34	2	TRN PRL
mtaq-7A.5	7A	145.94-150.31	11	TRN
mtaq-7B.1	7B	24.48	2	PRV LRV
mtaq-7B.2	7B	74.86-75.24	2	SRA LRL
mtaq-7B.3	7B	97.45	2	TRN PRV

TRN, total root number; SRA, seminal root angle; PRL, primary root length; LRL, total lateral root length; PRS, primary root surface; LRS, total lateral root surface; PRV, primary root volume; LRV, total lateral root volume; PRD, primary root diameter; LRD, mean lateral root diameter.

4.1. Phenotypic variation

The germplasm analysed in the present study, including mostly durum wheat landraces from the Mediterranean Basin, showed wide variability in RSA traits. The variability found was higher than that observed in other studies using elite accessions (Canè et al., 2014; Sanguineti et al., 2007) or even landraces, as reported by Ruiz et al. (2018) analysing a collection of Spanish durum wheat landraces. These results, and the intermediate values obtained for all traits in modern cultivars, support the use of ancient local germplasm to widening the genetic background in breeding programmes.

Means comparison of phenotypic traits revealed large differences among SPs associated with their geographical origin. Eastern Mediterranean landraces, collected in the area closest to the origin of tetraploid wheat, showed the largest root size in terms of length, surface and volume, and the widest root angle. The wheat-growing areas of this region, which comprises Syria, Jordan, Israel and Egypt, are the warmest and driest within the Mediterranean Basin (Royo et al., 2014). In addition, when RSA traits were analysed separately for the two components of the eastern Balkans and Turkey subpopulation, large differences appeared between them, with Turkish landraces being much more similar to the eastern Mediterranean ones than to the eastern Balkan ones, as the latter showed the lowest values for root length, surface and volume. Turkish landraces also showed a wide root angle, as did the eastern Mediterranean ones. The differences found in SRA between the eastern Balkans and Turkish landraces are sustained by two lines of evidence. One is the contrasting environmental conditions of the wheat-growing areas of northern Balkan countries and Turkey, since the analysis of long-term climate data demonstrated less rainfall and higher temperatures and solar radiation in the latter (Royo et al., 2014). The other

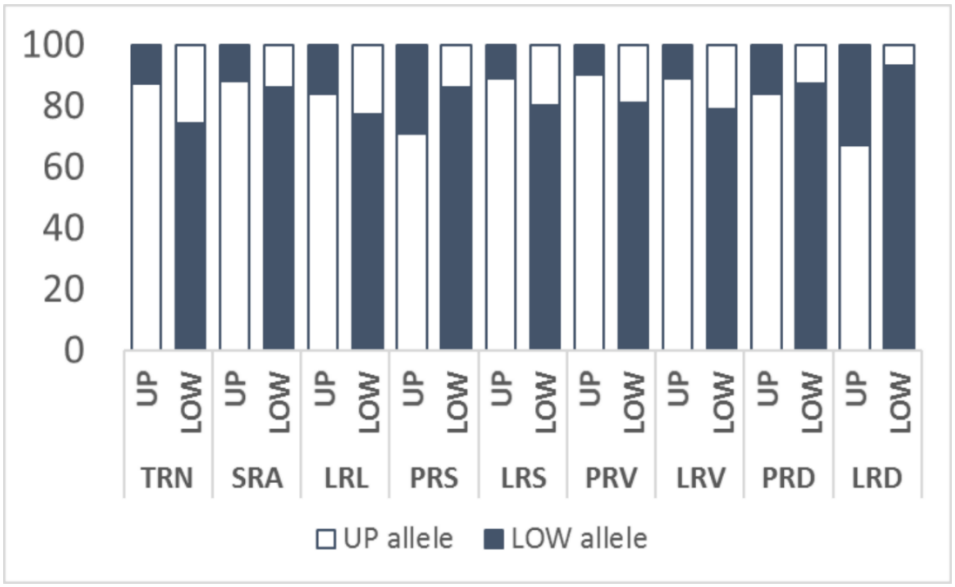

Figure 4.5. Figure 4.5. Marker allele frequency means from landraces within the upper and lower 10th percentile for the analysed traits.
All significant markers shown in Table 4.4 are included. TRN, total root number; SRA, seminal root angle; PRL, primary root length; LRL, total lateral root length; PRS, primary root surface; LRS, total lateral root surface; PRV, primary root volume; LRV, total lateral root volume; PRD, primary root diameter; LRD, mean lateral root diameter.

is that the northern Balkan landraces probably originated in the steppes of southern Russia and the Volga region (Dedkova et al., 2009; Nazco et al., 2012), also suggesting contrasting environmental conditions in the zones of origin of the eastern Balkan and Turkish landraces. The phenotypic analysis carried out in the current study revealed that landraces from regions where drought stress is prevalent have a larger root size and a wider root angle. This architecture should allow a larger proportion of the soil to be covered for more efficient water capture, and this hypothesis is supported by correlations between RSA and yield traits. Although low, probably due to the very early stage when the root traits were measured, differences in the number of significant correlations were observed between the two environments with highest and lowest water input reported by Roselló et al. (2019a). Root size–related traits were positively correlated with number of grains and spikes per unit area (primary roots) and with grain yield and grain weight (lateral roots) in the driest environment. SRA was negatively correlated with TKW, as reported previously by Canè et al. (2014), who concluded that it was due to the influence of root angle on the distribution of roots in the soil layers, which affects the water uptake from deeper layers. In our study, the genotypes with the narrowest angle corresponded to those from the western Mediterranean countries, which Royo et al. (2014) and Soriano et al. (Soriano et al., 2016), reported to have heavier grains.

4.2. Marker-Trait Associations

The current study attempts to dissect the genetic architecture controlling the seminal root system in a collection of landraces from the Mediterranean Basin by association analysis. A mixed linear model accounting for the genetic relatedness between cultivars (random effect) and their population structure (fixed effect) (K+Q model) was used in order to reduce the number of spurious associations.

A total of 233 significant associations were identified for the 10 RSA traits underlying the complex genetic control of RSA. However, in order to simplify this information and to integrate closely linked MTAs in the same QTL, those MTAs located within LD blocks were considered as belonging to the same MTA-QTL. As a result, the number of genome regions involved in RSA was reduced to 82. The relationships between RSA and yield-related traits was also suggested by the presence of pleiotropic MTA-QTLs. The comparison of the genome regions identified in the current study with those related to yield and yield components by Roselló et al. (2019b) showed that 45% of the RSA MTA-QTLs were located with yield-related trait MTA-QTLs. These results are in agreement with the findings of Canè et al. (2014), who found that 30% of the RSA-QTLs affected agronomic traits, providing evidence of the implications of RSA in field performance of durum wheat at early growth stages.

In the last few years GWAS for RSA have been limited in comparison with QTL mapping for root traits based on bi-parental populations (see Soriano and Álvaro (2019) for a review). Comparison with previous studies reporting MTAs for RSA resulted in few common regions with the current study. Four common regions were found with the study of Canè et al. (2014), but different traits were included for MTAs in those QTLs (mtaq-3A.3; mtaq-3A.5; mtaq-3A.6; mtaq-6B.2). Two MTAs were in common with those reported by Ayalew et al. (2018), who identified five significant associations with root length under stress (2) and non-stress (3) conditions. The MTA reported under stress conditions in chromosome 2B may correspond with mtaq-2B.2, which also shows an association with LRL. However, the association on chromosome 3B, although in a common region with mtaq-3B.4, differed in RSA. When MTA-QTLs were compared with QTLs from bi-parental populations, twelve genomic regions were located within the meta-QTL positions defined by Soriano and Álvaro (2019) after the compilation of 754 QTLs from 30 studies.

Candidate genes at the MTA peak were sought using the high-confidence gene annotation from the wheat genome sequence (IWGSC, 2018). Among these

Table 4.4. Selected significant markers from the GWAS with different allele composition for the upper (UP) and lower (LOW) 10th percentile of genotypes.

Different letters on the UP and LOW 10th phenotype indicate that means are significantly different at $P<0.01$ following a Tukey test.

Trait	Phenotype			Marker	Chr	Pos (cM)	R^2	Most frequent allele			
	Mean	UP 10th	LOW 10th					UP 10th	Frequency	LOW 10th	Frequency
TRN (N)	4.9	5.8[a]	3.7[b]	2260740_SNP	7A	148.38	0.09	T	0.80	C	0.81
				1252655_PAV	7B	97.45	0.11	1	0.94	0	0.67
SRA (º)	88.5	111.0[a]	67.1[b]	1125557_PAV	2A	115.80	0.09	0	1.00	1	1.00
				1117775_PAV	2A	118.32	0.10	1	0.75	0	0.71
LRL (cm)	21.8	36.5[a]	9.3[b]	4408432_PAV	6B	3.31	0.09	1	0.88	0	0.73
				4408958_PAV	6B	3.31	0.09	1	0.88	0	0.73
				1098568_PAV	6B	53.66	0.08	1	0.77	0	0.86
PRS (cm^2)	2.2	3.2[a]	1.3[b]	4406631_PAV	4B	31.93	0.09	0	0.71	0	0.86
				4406980_PAV	4B	31.93	0.09	1	0.71	1	0.86
LRS (cm^2)	4.0	6.5[a]	1.8[b]	1201756_PAV	2B	107.03	0.15	1	1.00	0	0.73
				987263_PAV	3A	61.57	0.10	0	0.92	1	0.88
				4408432_PAV	6B	3.31	0.09	1	0.81	0	0.79
				4408958_PAV	6B	3.31	0.09	1	0.81	0	0.79
PRV (mm^3)	33.7	49.8[a]	20.2[b]	997799_SNP	1B	31.69	0.12	A	0.86	G	0.77
				1201756_PAV	2B	107.03	0.11	1	0.87	0	0.71
				4406631_PAV	4B	31.93	0.09	0	0.93	1	0.87
				4406980_PAV	4B	31.93	0.09	0	0.93	1	0.87
LRV (mm^3)	60.5	99[a]	27.7[b]	1201756_PAV	2B	107.03	0.15	1	0.94	0	0.73
				987263_PAV	3A	61.57	0.10	0	0.93	1	0.81
				1126050_SNP	5B	33.99	0.07	A	0.81	M	0.81
				1149356_PAV	7B	24.48	0.08	0	0.87	1	0.81
PRD (mm)	0.58	0.66[a]	0.48[b]	1113225_SNP	5A	84.51	0.09	G	0.87	C	0.92
				1864057_SNP	6B	33.46	0.07	C	0.81	M	0.81

Trait	Phenotype			Marker	Chr	Pos (cM)	R^2	Most frequent allele			
	Mean	UP 10th	LOW 10th					UP 10th	Frequency	LOW 10th	Frequency
LRD (mm)	0.58	0.66[a]	0.51[b]	4005012_PAV	1B	51.29	0.10	0	0.67	1	0.93

Chr, chromosome; Pos, Position; TRN, total root number; SRA, seminal root angle; PRL, primary root length; LRL, total lateral root length; PRS, primary root surface; LRS, total lateral root surface; PRV, primary root volume; LRV, total lateral root volume; PRD, primary root diameter; LRD, mean lateral root diameter.

Table 4.5. Gene models within MTA-QTL positions.
Only MTAs with markers mapped against the genome sequence are included. Markers in bold were located within gene models. Genome position of the gene model is indicated in Mb.

Marker	MTA-QTL	Gene Model	Position	Description
1109244_SNP	mtaq-1A.5	TraesCS1A01G363600	540.1	Jacalin lectin family protein
1210090_SNP	mtaq-1A.7	TraesCS1A01G424800	579.8	Cellulose synthase
997799_SNP	mtaq-1B.1	TraesCS1B01G022500	10.1	Protein trichome birefringence
1003552_SNP	mtaq-1B.7	TraesCS1B01G430400	654.8	F-box domain protein
1085277_SNP	mtaq-2A.3	TraesCS2A01G250600	378.4	9-cis-epoxycarotenoid dioxygenase
1083104_SNP	mtaq-2A.3	TraesCS2A01G281000	469.4	Dynamin-like family protein
1117775_PAV	mtaq-2A.4	TraesCS2A01G541700	752.9	LEA hydroxyproline-rich glycoprotein family
1075469_SNP	mtaq-2B.1	TraesCS2B01G004500	2.4	Cytochrome P450 family protein
1256467_PAV	mtaq-3A.1	TraesCS3A01G018600	11.5	F-box domain protein
1082068_PAV	mtaq-3A.2	TraesCS3A01G034100	19.3	Receptor-like kinase
1130621_PAV	mtaq-3A.5	TraesCS3A01G132300	108.9	Blue copper protein
987263_PAV	mtaq-3A.7	TraesCS3A01G393600	641.6	Pectin lyase-like superfamily protein
1101009_SNP	mtaq-3B.4	TraesCS3B01G516800	759.9	Ribosomal protein S4
3034109_PAV	mtaq-4A.6	TraesCS4A01G419000	688.9	Histone acetyltransferase of the CBP family 5
1250077_PAV	mtaq-4B.3	TraesCS4B01G345800	639.4	Basic helix-loop-helix DNA-binding protein
1240561_PAV	mtaq-6A.3	TraesCS6A01G041500	21.7	Transmembrane protein 97
1047867_PAV	mtaq-6A.3	TraesCS6A01G415600	615.3	Cobyric acid synthase
1105573_PAV	mtaq-6A.5	TraesCS6A01G242300	453.9	50S ribosomal protein L19
989287_PAV	mtaq-6A.6	TraesCS6A01G417400	615.8	F-box domain protein
1129380_PAV	mtaq-6B.1	TraesCS6B01G000200	0.1	NBS-LRR resistance-like protein
1864057_SNP	mtaq-6B.3	TraesCS6B01G335600	590.9	Hexosyltransferase
1098568_PAV	mtaq-6B.4	TraesCS6B01G399700	675.2	bZIP transcription factor family protein
1130796_PAV	mtaq-7A.1	TraesCS7A01G015100	0.0	Mitochondrial pyruvate carrier
2253648_PAV	mtaq-7A.1	TraesCS7A01G016700	7.3	Transmembrane protein DUF594
1139027_PAV	mtaq-7A.1	TraesCS7A01G015400	6.7	Signal peptidase complex catalytic subunit SEC11
1076865_PAV	mtaq-7A.1	TraesCS7A01G024800	9.7	WAT1-related protein
1059554_SNP	mtaq-7A.3	TraesCS7A01G100600	61.8	GDSL esterase/lipase
1665955_PAV	mtaq-7A.4	TraesCS7A01G442400	636.7	BTB/POZ domain
1149356_PAV	mtaq-7B.1	TraesCS7B01G058300	60.6	Glutamate receptor
1075278_SNP	mtaq-7B.2	TraesCS7B01G378200	642.6	Receptor-like kinase
1252655_PAV	mtaq-7B.3	TraesCS7B01G421300	690.2	NBS-LRR resistance-like protein

genes, those involved in plant growth and development and tolerance to abiotic stresses may be of special interest. On chromosome 1A, the marker 1210090_SNP in mtaq-1A.7 is located close to a cellulose synthase gene. This type of gene is involved in plant cell growth and structure (Lei et al., 2012). A trichome birefringence (TB) protein was identified in mtaq-1B.1. According to Zhu et al. (2014), the TB-like27 protein mutants in *Arabidopsis* increased aluminium accumulation in cell walls, inhibiting root elongation through structural and functional damage. Three peaks corresponded with F-box domains located in mtaq-1B.7, mtaq-3A.1 and mtaq-6A.6. According to Hua and Vierstra (2011), this is the protein subunit of E3 ubiquitin ligases involved in the response to abiotic stresses. Li et al. (2018) overexpressed the F-box *TaFBA1* in transgenic tobacco to improve heat tolerance, and one of the results was increased root length in the transgenic plants. 9-cis-epoxycarotenoid dioxygenase (NCED) is a key enzyme in the biosynthesis of ABA in higher plants, which regulates the response to various environmental stresses (Zhang et al., 2014). This enzyme is located within mtaq-2A.3. In mtaq-2A.4, the marker 1117775_PAV corresponded with a late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein. These proteins play a role in the response to abiotic stresses. They are mainly accumulated in seeds, but have been found in roots during the whole developmental cycle (Gao and Lan, 2016). The marker 1098568_PAV, in mtaq-6B.4, is located within a gene coding a bZIP transcription factor family protein. This type of transcription factor is involved in abiotic stresses (Sornaraj et al., 2016). Zhang et al. (2017) observed that the root growth of transgenic plants overexpressing the gene *TabZIP14-B* was hindered more severely than that of the control plants. Another gene involved in abiotic stress tolerance is the mitochondrial pyruvate carrier (*MPC*) located in mtaq-7A.1 (He et al., 2019). This gene is involved in cadmium tolerance in *Arabidopsis*, preventing its accumulation. Roots are the predominant plant tissue for cadmium absorption or exclusion. He et al. (2019) found that the root length of mutant plants of *Arabidopsis* for *MPC* genes was substantially shorter than the wild-type plants. A protein related to WAT1 (WALLS ARE THIN1) involved in secondary cell wall thickness (Ranocha et al., 2013) is located in the peak of mtaq-7A.1.

5. Conclusions

Including local landraces in breeding programmes is a useful approach to broadening genetic variability of crops (Lopes et al., 2015). The variability for root system architecture traits found in Mediterranean landraces and the high number of genome regions controlling them - most of them not reported previously - makes this germplasm a valuable source for root architecture improvement. The identification of extreme genotypes for root architecture traits can help to identify parents for the

development of new mapping populations to tackle a map-based cloning approach to the genes of interest. In the present study, we identified the molecular markers linked to these genotypes with different allele composition that will facilitate the introgression of the corresponding traits through marker-assisted breeding.

Supplementary material

Supplementary material is available on the online version of the article, doi: 10.3390/agronomy9070364

Author Contributions

Conceptualization, M.R. and J.M.S.; Methodology, M.R., M.S.-G., and J.M.S.; Formal Analysis, M.R.; Investigation, M.R., M.S.-G., J.M.S.; Resources, C.R.; Data Curation, M.R., C.R.; Writing—Original Draft Preparation, M.R., J.M.S.; Writing—Review & Editing, C.R., M.S.-G., and J.M.S.; Visualization, J.M.S.; Supervision, C.R., M.S.-G., and J.M.S.; Project Administration, C.R. and J.M.S.; Funding Acquisition, C.R. and J.M.S.

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Conflicts of interest

The authors declare no conflicts of interest

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Chapter 5:
Pasta-making quality QTLome from Mediterranean durum wheat landraces

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Pasta-making quality QTLome from Mediterranean durum wheat landraces

Abstract

In order to identify genome regions related to pasta-making quality traits, association mapping (AM) was performed in a set of 165 durum wheat landraces from 21 Mediterranean countries. The collection was genotyped using 1149 DArT markers and 872 of them with a known genetic position were used for AM. The collection was grown in north-east Spain during three years. Results of ANOVA showed that trait variation for quality traits, except for grain protein content (GPC), was mainly explained by genetic effects. Landraces showed higher GPC than modern cultivars but lower gluten strength (GS). Modern and eastern landraces showed the highest yellow colour index (YI). Balkan landraces showed the lowest test weight (TW). A total of 92 marker-trait associations were detected, 20 corresponding to GS, 21 to GPC, 21 to YI and 30 to TW. With the aim of detecting new genomic regions involved in grain quality, the position of the associations was compared with previously mapped QTL by a meta-QTL analysis. A total of 249 QTLs were projected onto the same map used for AM, identifying 45 meta-QTL (MQTL) regions and the remaining 15 QTLs as singletons. The position of known genes involved in grain quality was also included, and gene annotation within the most significant regions detected by AM was carried out using the wheat genome sequence.

Keywords: Association mapping, grain quality, protein content, gluten strength, test weight, yellow colour, sedimentation index.

1. Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is a staple food for 40% of the world's population (Peng et al., 2011a), and the Mediterranean Basin is the largest production area in the world (Nazco et al., 2012). Flour and semolina are used to make many traditional Mediterranean foods, such as pasta, couscous, bulgur and flat bread, and pasta is the most common durum end product consumed in Europe, America and West Asia. For pasta making, durum wheat grains must possess specific traits related to flour quality. The most challenging objective of durum wheat breeding programmes should not be restricted to yield increases, as generally occurred during the twentieth century (Duveiller et al., 2007), but grain quality traits appropriate for pasta making should also be considered to meet social demands (Groos et al., 2007).

Grain protein quantity and quality are highly depending on the amount and type of glutenins and gliadins, proteins that are the main components of gluten and are responsible for the viscoelastic properties and extensibility of dough, respectively. Other important traits for pasta making are flour colour and yellow pigment content, kernel weight, test weight and the starch characteristics related to grain hardness (Ruiz et al., 2005). Genetic improvement of durum wheat quality is subject to many constraints (Goutam et al., 2013). First, the quality traits are of a quantitative nature, and besides the known major genes determining gluten composition (glutenins and gliadins, reviewed in Ruiz et al., 2005), protein content (*Gpc-B1*, Olmos et al., 2003) and colour (*Psy-A1*, Patil et al., 2008; *Psy-B1*, El Ouafi et al., 2001), many other genes control their expression. Second, the quality traits require assessment of an end product, but direct estimation through milling and baking is costly and time-consuming, and requires a large grain sample. Indirect tests, such as the sodium dodecyl sulphate (SDS) sedimentation test, the alveograph and the mixograph, were developed to solve these problems but are still time-consuming. Alternative tools need to be sought, and the use of molecular markers can help identify new sources of desirable genes through association mapping (AM) and accelerated breeding programmes using marker-assisted selection (MAS). Association mapping is a complementary approach to traditional bi-parental linkage mapping. It uses linkage disequilibrium to identify genotype-phenotype associations, providing broader allelic variation and higher mapping resolution (Breseghello and Sorrells, 2006; Flint-Garcia et al., 2003). Because of the quantitative nature of grain quality traits, MAS will not replace traditional wheat quality testing procedures for the screening of advanced generations and cultivar evaluation. However, it will be an extremely valuable tool that allows breeders to identify new lines of interest for a more in-depth analysis at an earlier stage in the breeding programmes (Goutam et al., 2013).

Durum wheat originated in the Fertile Crescent approximately 10,000 before present and spread across the Mediterranean Basin (Feldman, 2001). During the migration, natural and human selection resulted in the establishment of local landraces adapted to the prevalent climatic conditions (Peng et al., 2011b). Due to their wide genetic diversity, landraces are considered useful for breeding as a source of new alleles (Lopes et al., 2015).

Grain protein content and composition are the main determinants of the end-use value of durum wheats. For that reason, in wheat breeding programmes it is important to develop wheat cultivars with well-balanced grain protein compositions. Advances in grain quality resulting from breeding activities conducted during the twentieth century resulted in a loss of genetic variability that may constrain breeding for quality in the future (Nazco et al., 2014a; Subirà et al., 2014). This is the case of

LMW glutenin subunits, which are restricted to a few alleles in modern cultivars. The large allelic variability found in Mediterranean landraces in previous studies (Moragues et al., 2006; Nazco et al., 2014a) provides tools for enhancing and diversifying gluten characteristics. Landraces may also provide new allelic sources for improving grain yellowness (Campos et al., 2016), and for improving grain weight due to the large genetic component of the trait (Nazco et al., 2012; Soriano et al., 2016). However, past breeding activities reduced grain protein content (Nazco et al., 2012; Subirà et al., 2014), possibly as a consequence of the negative relationship between this trait and yield. Therefore, exploring genetic diversity within pre-breeding materials is of interest to identify sources that allow protein content to be increased without a yield penalty.

The main objective of this study was to identify genome regions related to pasta-making quality traits through association mapping using a set of 165 Mediterranean durum wheat landraces representative of the genetic background existing in the species within the Mediterranean Basin. Additional goals were *i*) to conduct a meta-analysis to restrict the QTL intervals and discover consensus QTL regions affecting the target traits, and *ii*) to ascertain whether a geographic structure exists for the identified QTLs.

2. Material and methods

2.1. Plant material

The germplasm used consisted of a collection of 172 durum wheat landraces and old cultivars from 21 Mediterranean countries and 20 modern cultivars (Supplementary Material 1, Annex 1).

Seeds were provided by public gene banks (CRF-INIA, Spain, ICARDA germplasm bank and USDA germplasm bank) and were purified and increased in bulk, as described by Nazco et al. (2012). The term ‘old cultivars’ was used to designate a limited number of entries (6) corresponding to some of the first varieties obtained from selections within landraces (i.e. Andalucia 344 and Aziziah 17/45) or by crosses with landraces (i.e. Carlo jucci, Trinakria, Hymera and Lozen 76). Given that old cultivars did not cluster apart from landraces according to the structure results of Soriano et al. (2016) both landraces and old cultivars were designated as landraces onwards. The collection was divided into five genetic subpopulations (SPs), one of the modern cultivars and four of landraces corresponding to their geographical origin: the eastern Mediterranean (EM, 19 cultivars), the eastern Balkans and Turkey (EB+T, 21 cultivars), the western Balkans and Egypt (WB+E, 33 cultivars) and the western Mediterranean (WM, 73 cultivars). Finally, 19 cultivars were classified as admixed. Due to missing genetic and phenotypic data, only 165

landraces and 18 modern cultivars were analysed.

2.2. Phenotyping

Field experiments were carried out during three harvesting seasons (2007, 2008 and 2009) in Lleida, north-east Spain, under rain-fed conditions following a non-replicated modified augmented design with three replicated checks (the cultivars 'Claudio', 'Simeto' and 'Vitron') with plots of 6 m^2 (8 rows, 5 m long with 0.15-m spacing). Sowing density was adjusted to 250 viable seeds m^{-2}.

The plots were mechanically harvested at commercial maturity and a sample of 250 g of harvested grain from each plot was cleaned and used for quality tests. The analysed traits were grain protein content (GPC), gluten strength (GS), yellow colour index (YI) and test weight (TW). Additionally, sedimentation index (SI) was calculated as the quotient between GS and GPC and was expressed as mL/protein unit. GPC (%) was determined by a near-infrared spectroscope (NIT, Infratec® 1241 grain analyser, Foss Tecator AB, Sweden) calibrated against the standard Kjeldahl method (Kjeldahl, 1883). Whole-grain flour samples were obtained with a whole-meal grinder; fine particle size was ensured by attaching a 0.5-mm screen to the grinder. GS (mL) was determined on 1 g of flour samples by the SDS sedimentation test using the method of Axford et al. (1978), as modified by Peña et al. (1990). The YI (b, CIE L*a*b colour system) was estimated on whole-grain flour by a portable reflectance colorimeter (CR-400, Konica-Minolta Sensing, Inc., Tokyo) equipped with a filter tri-stimulate system. Yellow pigment content (YPC) was measured following the AACC method, as described in Santra et al. (2003). TW (kg/hL) was determined by the GAC2100 analyser (Dickey-John Co., Auburn, IL, USA). Subsequently, the EU quality index (QI) for durum (European Commission Regulation No 2237/2003, 23 Dec, 2003; Official Journal of 24.12.2003; Royo and Briceño-Félix, 2011) was calculated from these four quality traits using the cultivars 'Simeto', 'Gallareta' and 'Vitron' as reference checks and weighting each trait according to the following percentages: GPC (40%), GS (30%), YI (20%) and TW (10%).

2.3. Statistical analyses

Phenotypic data were fitted to a linear mixed model with the check cultivars as fixed effects and the row number, column number and cultivar as random effects (Little et al., 1997). Restricted maximum likelihood was used to estimate the variance components and to produce the best linear unbiased predictors (BLUPs) for the quality traits of each cultivar and year with the SAS-STAT statistical package (SAS Institute, 2014). Combined ANOVAs were performed across experiments through the GLM procedure of the SAS-STAT statistical package (SAS Institute,

2014), considering the cultivar and the year as random effects, and the year × cultivar interaction as the error term. The sum of squares of the cultivar effect was partitioned into differences between SPs and differences within them. Means were compared using the Tukey test (Tukey, 1949) with the JMP v12Pro statistical package (SAS Institute, 2009). ANOVAs and mean comparisons were carried out using only the structured cultivars (146 landraces and 18 modern cultivars).

2.4. Genotyping

DNA isolation was performed following the method of Doyle and Doyle (1987) from young leaf samples and sent to Diversity Arrays Technology Pty Ltd (Canberra, Australia) (https://www.diversityarrays.com/). Genotyping was carried out using the durum wheat PstI/TaqI array v2.0. A total of 1149 DArT markers were used to genotype the whole collection and were ordered according with the consensus map of durum wheat developed by Maccaferri et al. (2014). Markers with duplicated patterns, with more than 20% of values missing and with minor allele frequency lower than 5% were excluded from the analysis.

2.5. Association Mapping

Association mapping was performed for the BLUPs of the measured traits in all the landraces for each year and across years using a mixed linear model (MLM) at the optimum compression level, accounting for the genetic relatedness and population structure (K+Q model) determined in Soriano et al. (2017). The mapping was performed using TASSEL software version 5.0 (Bradbury et al., 2007). The threshold *p*-value for considering a marker-trait association (MTA) significant was defined for each year and trait based on the Q-Q plot pattern at the point at which the observed *F*-test statistics deviated from the expected *F*-test statistics (Supplementary Material 2), as described in Sukumaran et al. (2012).

2.6. QTL Meta-Analysis

Twenty published studies were examined and reported a total of 345 QTLs related to GPC, GS, YI, YPC and TW (Annex 2). For all of the studies, the following information was collected: parents of the cross, type of cross, number of progenies, name of QTLs, trait, environment, LOD score, phenotypic variance explained (PVE) by each QTL, QTL position on the author's linkage map, flanking markers and QTL confidence interval (CI). To compare the QTLs detected in different populations, original QTL data were projected onto the consensus map of durum wheat developed by Maccaferri et al. (2014). QTLs were projected following the homothetic approach proposed by Chardon et al. (2004). CIs were defined as reported by Soriano and Royo (2015) and estimated at 95% on the consensus map using the empirical formula proposed by Darvasi and Soller (1997) and Guo et al. (2006):

CI = 163/($N \times R^2$) for recombinant inbred line (RIL)

CI = 530/($N \times R^2$) for doubled haploid (DH), backcrosses (BC) and F_2 progenies

where N is the size of the population and R^2 the proportion of variance explained by the QTL.

QTL meta-analysis was conducted using BioMercator v4.2 (Sosnowski et al., 2012) following the approach of Goffinet and Gerber (2000) when the number of QTLs in a chromosome was lower than 10 and that of Veyrieras et al. (2007) when the number of QTLs was 10 or more.

Additionally, genes involved in grain quality previously mapped or reviewed (El Ouafi et al., 2001; Maccaferri et al., 2014; Olmos et al., 2003; Patil et al., 2008, 2009; Pozniak et al., 2007; Ruiz et al., 2005; Zhang and Dubcovsky, 2008a) were also projected onto the consensus map (Maccaferri et al., 2014) for further comparisons.

Graphical representation of the genetic position of the significant MTAs, MQTLs and quality trait genes was carried out using MapChart 2.3 (Voorrips, 2002).

2.7. Gene Annotation

Gene annotation for the target region of the most significant MTAs was performed using the gene models for high-confidence genes reported for the wheat genome sequence (IWGSC, 2018), available at https://wheat-urgi.versailles.inra.fr/Seq-Repository/. Intervals were defined by a genetic distance of 1cM above and below the corresponding marker or the linkage disequilibrium block (identified by TASSEL version 5.0 software) when present. Correspondence between genetic and physical distances was performed individually for each marker using the position of common markers in the consensus map (Maccaferri et al., 2014) and the wheat genome sequence.

3. Results

3.1. Phenotypic Data

The results of the ANOVA showed that variation in GPC was mostly explained by the environmental conditions of the year, whereas the remaining traits were influenced by large genetic effects mainly due to variations within SP (Figure 5.1).

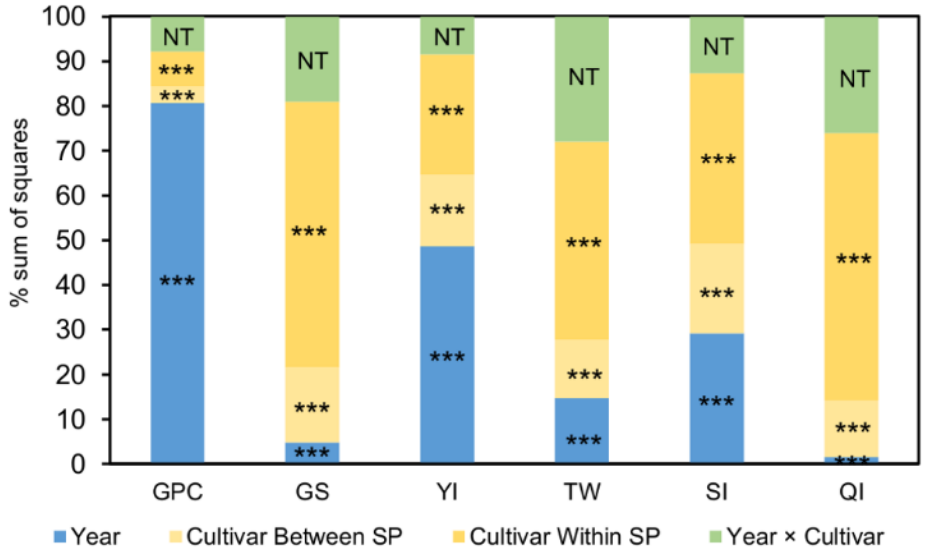

Figure 5.1. Percentage of the sum of squares (SS) of the ANOVA model for the pasta-making quality traits measured in three years on a collection of 183 durum wheat cultivars clustered in 5 genetic subpopulations (SPs).
The SS of the genotype effect is partitioned into cultivar differences between SPs and cultivar differences within SPs. GPC, grain protein content; GS, gluten strength; YI, yellow colour index; TW, test weight; SI, sedimentation index; QI, quality index. *** $P < 0.001$; NT, not testable.

Mean comparisons between SPs showed significant differences between modern cultivars and landraces in terms of GPC and GS (Figure 5.2). Regardless of their geographical origin, landraces had a higher GPC but a lower GS and SI than modern cultivars. For YI, cultivars from the WB+E had the lowest value (13.9), whereas cultivars from the EM showed the highest (16.2), but showed no statistically significant differences in comparison with modern cultivars (15.7) and the SPs from the EB+T (15.6). Balkan SPs showed lower TW values than landraces from the east and west of the Mediterranean Basin and modern cultivars. Finally, comparison of means for QI indicated that modern cultivars had the highest overall quality (102%), though it was similar to that of the SPs from the EM and the EB+T, while landraces from the WB+E showed the lowest quality (94%). This SP, however, showed the greatest variability for most traits, with some individuals showing the largest overall quality: e.g. the cultivar 'D-2' from Egypt had the highest QI (112%) and GS (11.7 mL) and the cultivar 'Heraldo del Rhin' had the highest TW (82.1 kg/hL).

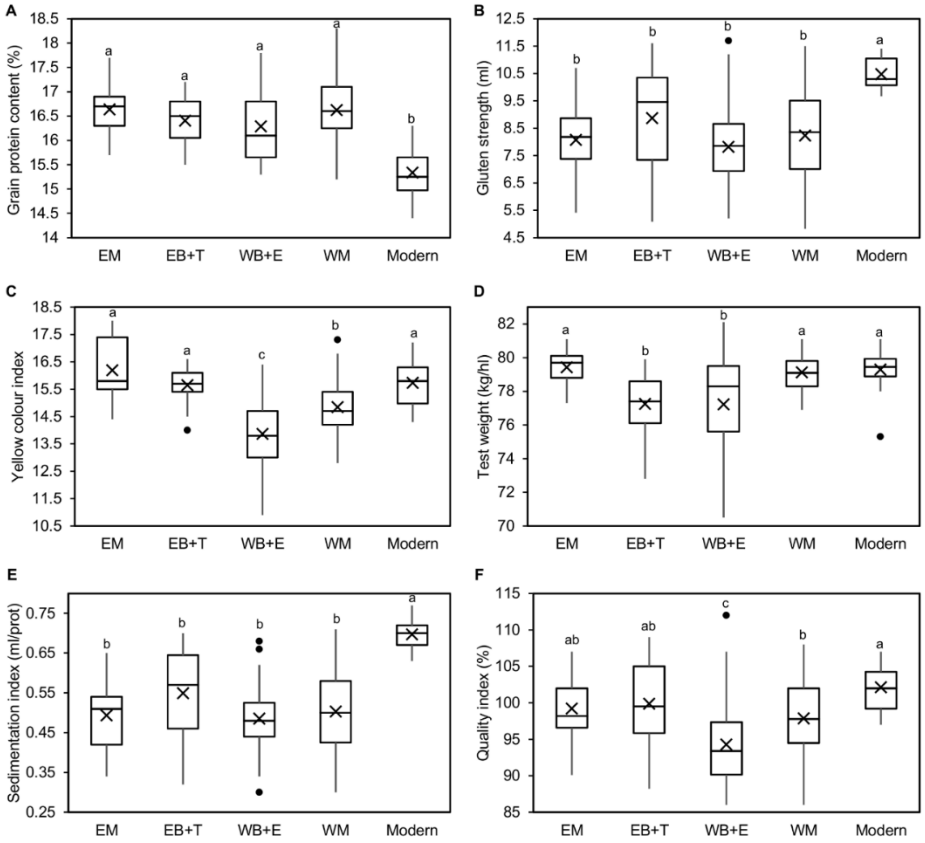

Figure 5.2. Boxplots for mean comparison of pasta-making quality traits [(A) grain protein content, (B) gluten strength, (C) yellow colour index, (D) test weight, (E) sedimentation index and (F) quality index] in a collection of 183 durum wheat cultivars divided into five genetic SPs and grown for three years.
EM, eastern Mediterranean; EB+T, eastern Balkans and Turkey; WB+E, western Balkans and Egypt; WM, western Mediterranean. Means within boxplots with different letters are significantly different at $P < 0.05$ following a Tukey test. × represents the mean value and • represents outlier values.

3.2. Association mapping

The landrace collection was genotyped using 1149 DArT markers. In order to reduce the risk of false positive MTAs, markers and cultivars were analysed for the presence of duplicated patterns and missing values. Markers and cultivars were excluded as follows: 46 markers with duplicated patterns, 5 markers with more than 20% of values missing and 24 markers with allele frequency lower than 5%. A total of 1074 markers remained. DArT markers were ordered according with the consensus map for durum wheat developed by Maccaferri et al. (2014), and only 872 with a known map position were used for mapping purposes. According to Soriano et al. (2017), using the same set of markers, linkage disequilibrium decay was

estimated up to 8 cM.

The results of the association mapping are reported in Table 5.1 and Figure 5.3. A total of 92 MTAs involving 70 markers were identified. A significance threshold for each trait and year was established considering the deviation of the observed from the expected test statistics in the Q-Q plots (Sukumaran et al., 2012), in all cases $-\log(P) > 2.0$ (Supplementary Material 2). The MTAs were located in 13 chromosomes, with 36 and 64% of the MTAs located on genomes A and B respectively. The highest number of MTAs (12) were identified on chromosomes 2B and 7A, whereas on chromosome 5A only one was reported. Twenty MTAs corresponded to GS (including 18 markers), 21 to GPC and YI (15 and 18 markers, respectively) and 30 to TW (24 markers) (Table 5.1). Eighteen MTAs were found significant across years, four of them, wPt-2737 (GPC) and wPt-1140, wPt-1441 and wPt-6204 (TW), overcoming a $P < 0.001$ threshold (Supplementary Material 3). Twelve markers were involved in two or more MTAs (5 for GPC, 2 for GS, 1 for YI and 4 for TW). The marker wPt-2737 on chromosome 7B was detected for GPC with a $-\log(P) > 3.0$ in two years and across years, explaining the highest percentage of PVE for the trait in this last association. Three other markers located in the same position on chromosome 7A (wPt-3883, wPt-7734 and wPt-9796) showed associations in 2008 and across the three years, and finally the marker wPt-2698 (3B) was detected in 2007 and across years. For GS, rPt-6127 and wPt-7653 were associated in at least one year and across years. For YI the marker wPt-1437 showed associations for 2007 and 2009 (showing the highest PVE for the trait in this year) and wPt-3729 showed three associations (2007, 2008 and across years). For TW, the marker wPt-6204 showed associations for the three years and across years, with a $-\log(P) > 3$, wPt-1140 and wPt-1441 each showed two associations with TW (2007 and across years), and the association between wPt-1140 and 2007 was the MTA with the highest PVE for TW. Finally, the marker wPt-8892 was detected in 2008 and across years, and the marker wPt-1140 was also associated with GPC and GS in 2007.

According to the results of Soriano et al. (2017) reporting a LD decay up to 8 cM depending on the chromosome and the approach used by Laidò et al. (2014), MTAs located within a region of 5-10 cM are considered as belonging to the same QTL. Thus, the 92 MTAs were restricted to 37 QTLs (named as MTA-QTLs) (Table 5.2). The number of MTAs per QTL ranged from 1 in 14 MTA-QTLs to 8 in 1 MTA-QTL. Taking into account the number of traits involved in each MTA-QTL, 65% of the MTA-QTLs involved only 1 trait, 27% involved 2 traits and the remaining 8% 3 traits. Twenty-three out of the 37 MTA-QTLs had 2 or more MTAs. Of those, 5 MTA-QTLs were detected in one environment, 10 in 2 environments, 7 in 3 environments, and finally 1 in four environments.

Table 5.1. Significant markers associated with pasta-making quality traits obtained in 165 Mediterranean durum wheat landraces.

Trait	Marker	Year	Chromosome	Position (cM)	-log(*P*)	Marker R^2
GPC (%)	wPt-1140	2007	2B	133.4	3.63	0.09
	wPt-8693	2009	2B	146.3	2.73	0.07
	wPt-4223	2009	2B	169.5	2.59	0.06
	wPt-2698	2007	3B	162.9	2.32	0.05
	wPt-2698	Across years	3B	162.9	2.39	0.05
	wPt-7355	2007	4A	59.8	3.00	0.08
	wPt-6123	2008	4B	16.2	2.56	0.06
	wPt-5497	2008	4B	16.3	2.56	0.06
	tPt-5342	2008	4B	16.5	3.22	0.08
	wPt-7400	2007	5B	172.4	2.34	0.05
	wPt-6959	2007	7A	6.1	2.74	0.06
	wPt-3883	2008	7A	63.2	2.61	0.06
	wPt-3883	Across years	7A	63.2	2.76	0.06
	wPt-7734	2008	7A	63.2	2.64	0.06
	wPt-7734	Across years	7A	63.2	2.84	0.07
	wPt-9796	2008	7A	63.2	2.64	0.06
	wPt-9796	Across years	7A	63.2	2.77	0.06
	wPt-4220	2008	7A	220.4	2.19	0.05
	wPt-2737	2007	7B	68.9	3.12	0.08
	wPt-2737	2008	7B	68.9	3.34	0.08
	wPt-2737	Across years	7B	68.9	4.30	0.11
GS (ml)	wPt-6280	2009	1A.1	2.7	3.48	0.08
	wPt-1310	2008	1A.2	30.4	2.88	0.07
	wPt-6853	2008	1A.2	30.4	2.84	0.07
	wPt-1011	2008	1A.2	30.5	2.87	0.07
	wPt-5274	2008	1A.2	34.2	2.53	0.07
	wPt-6754	2008	1A.2	34.2	2.00	0.05
	wPt-1140	2007	2B	133.4	2.94	0.07
	wPt-6894	2008	2B	227.1	2.50	0.06
	wPt-6854	2008	3A.1	6.2	2.11	0.05
	wPt-11691	2009	3B	172	2.95	0.07
	tPt-0353	2008	5A	83.6	2.03	0.04
	rPt-6127	2008	5B	10.6	2.56	0.06
	rPt-6127	Across years	5B	10.6	2.61	0.06

Trait	Marker	Year	Chromosome	Position (cM)	-log(*P*)	Marker R^2
	wPt-6880	2008	5B	145.4	2.49	0.06
	wPt-7954	2008	6B	23.6	2.06	0.05
	wPt-2056	2008	7A	8.2	2.11	0.05
	wPt-1853	2008	7B	18	3.07	0.08
	wPt-7653	2007	7B	37.8	2.73	0.06
	wPt-7653	Across years	7B	37.8	2.79	0.06
	wPt-4258	2008	7B	143	2.89	0.08
	wPt-2694	2007	1B	27.2	2.48	0.06
	wPt-2724	2007	2B	220.8	2.18	0.04
	wPt-8140	2007	3B	47.8	2.42	0.05
	wPt-1349	2007	3B	47.9	2.92	0.07
	wPt-8686	2007	3B	47.9	3.04	0.07
	wPt-2416	2009	3B	216.3	2.42	0.05
	wPt-0162	2007	4A	69.7	2.25	0.05
	wPt-3729	2007	4A	136.7	2.20	0.05
	wPt-3729	2008	4A	136.7	2.70	0.06
	wPt-3729	Across years	4A	136.7	2.61	0.06
YI	wPt-8443	2009	6A	0	2.39	0.05
	wPt-3247	2007	6A	137.1	2.32	0.05
	wPt-3774	2009	6B	4.4	2.60	0.06
	wPt-0245	2007	6B	5.1	2.56	0.06
	wPt-7662	2009	6B	6.2	2.76	0.06
	wPt-5673	2007	6B	14.7	2.45	0.05
	wPt-1437	2007	6B	24.7	2.52	0.06
	wPt-1437	2009	6B	24.7	3.22	0.08
	wPt-1429	2009	7A	216.1	2.85	0.06
	wPt-5228	2007	7B	184.8	2.24	0.05
	wPt-5138	Across years	7B	189	2.68	0.06
	wPt-2654	2008	1B	-0.9	2.84	0.06
	wPt-5562	Across years	1B	20.6	2.19	0.04
	wPt-1634	2007	2B	2.7	2.38	0.04
	wPt-7158	2008	2B	42.2	2.66	0.06
TW (Kg/hl)	wPt-1140	2007	2B	133.4	4.51	0.10
	wPt-1140	Across years	2B	133.4	3.60	0.08
	wPt-8569	Across years	2B	142.9	2.40	0.06
	wPt-7360	Across years	2B	220.5	2.17	0.04
	wPt-6204	2007	3A.1	3.4	3.45	0.07

Trait	Marker	Year	Chromosome	Position (cM)	-log(*P*)	Marker R^2
	wPt-6204	2008	3A.1	3.4	3.41	0.08
	wPt-6204	2009	3A.1	3.4	3.14	0.07
	wPt-6204	Across years	3A.1	3.4	4.01	0.09
	wPt-8480	2007	3B	157.3	2.13	0.04
	wPt-2491	2008	3B	181.6	2.35	0.05
	wPt-8892	2007	4B	15.7	3.51	0.07
	wPt-8892	Across years	4B	15.7	2.53	0.05
	wPt-6123	2007	4B	16.2	2.26	0.04
	wPt-5497	2007	4B	16.3	2.26	0.04
	tPt-5342	2007	4B	16.5	2.16	0.04
	wPt-6022	2007	5B	88.8	2.43	0.05
	wPt-2707	2007	5B	126.9	3.68	0.08
	wPt-6191	2007	5B	126.9	3.60	0.07
	wPt-4577	2007	5B	131.7	3.70	0.08
	tPt-3714	2008	5B	185	2.55	0.06
	wPt-9000	2007	6A	137.1	2.39	0.05
	wPt-6995	2007	6A	158.9	2.20	0.04
	wPt-1441	2007	7A	8.1	3.01	0.06
	wPt-1441	Across years	7A	8.1	3.38	0.07
	wPt-5343	Across years	7B	152.2	2.54	0.05
	wPt-8615	Across years	7B	152.2	2.36	0.05

GPC, grain protein content; GS, gluten strength; YI, yellow colour index; TW, test weight.

The analysis of the distribution among SPs of the significant markers for GPC across years showed that wPt-2737 was mainly present in the EM SPs, in contrast with the EB+T and modern SPs, which lacked this allele with a positive effect in most cultivars (Table 5.3). Protein content increased significantly on average by 3.6% in cultivars holding wPt-2737 (Table 5.3). Markers wPt-3883, wPt-7734 and wPt-9796 on chromosome 7A were present in most of the cultivars of each SP (from 70% in modern and WB+E cultivars to 100% in WM and EB+T cultivars), increasing GPC on average by 5.0%. Finally, wPt-2698 was mainly present in landraces (from 80% in WM cultivars to 100% in eastern cultivars) but showed a negative effect, decreasing protein content by 1%. The marker was present in 60% of the modern cultivars. The frequency of the markers showing a positive effect in the upper 10th percentile ranged from 0.8 (wPt-2737) to 1 (wPt-3883, wPt-7734 and wPt-9796), while in the lower 10th percentile the frequency of the marker wPt-2737 dropped to

0.055. Considering the extreme landraces, the five with the highest GPC (‘Morocco’, ‘D-2’, ‘Sinai no.8’, ‘Dezassete’ and ‘IG-95841’) carried the four markers, whereas in the ones with the lowest GPC (‘Haj Mouline’, ‘Ruso’, ‘VII/18-X24’, ‘37’ and ‘1P1’), only marker wPt-2737 was missing.

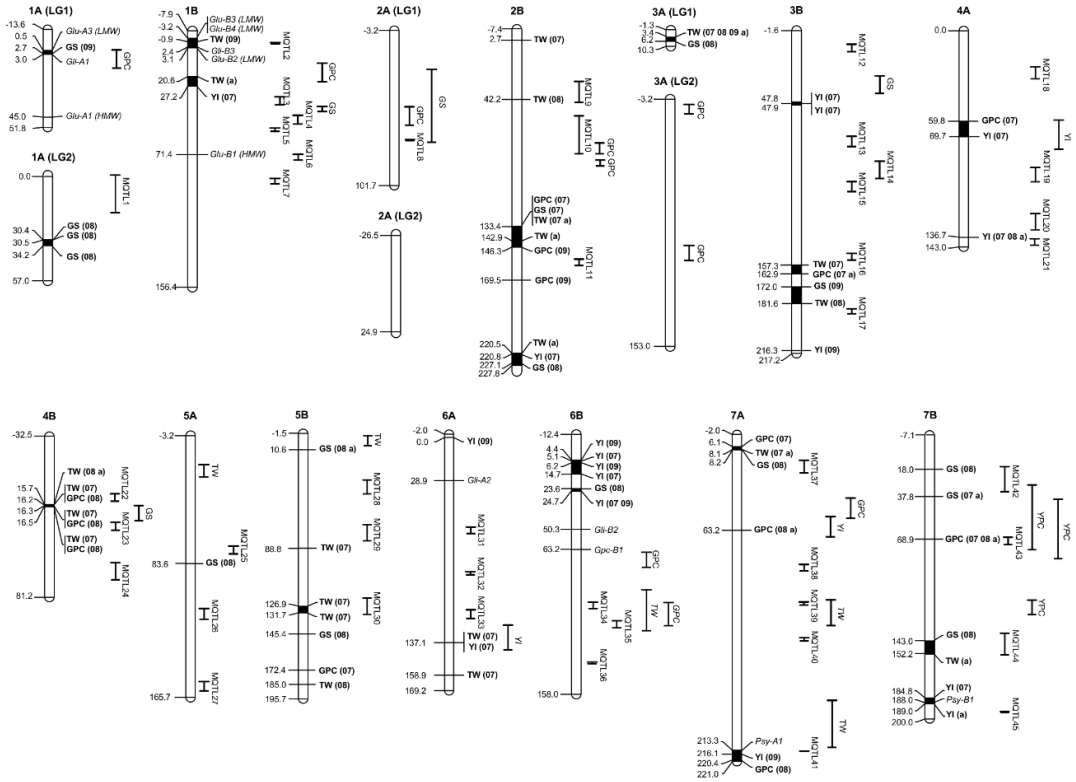

Figure 5.3. Genetic position of significant MTAs in the durum wheat consensus map developed by Maccaferri et al. (2014), together with the position of MQTLs reported in this study and known genes affecting grain quality.

MTAs and their traits are indicated in bold. Numbers in parentheses after a trait represent the number of environments with significant MTAs. Single QTLs are reported as the trait for that QTL. 07, MTA significant in 2007; 08, MTA significant in 2008; 09, MTA significant in 2009; a, MTA significant across years. GPC, grain protein content; GS, gluten strength; YI, yellow colour index; YPC, yellow pigment content; TW, test weight.

Table 5.2. MTA-QTLs.

MTA-QTL	Traits involved	MTAs	Environments*	Chromosome	Region (cM)
mtaq1A.1	GS	1	1	1A	2.7
mtaq1A.2	GS	5	1	1A	30.4-34.2
mtaq1B.1	TW	1	1	1B	0.0
mtaq1B.2	TW, YI	2	2	1B	20.6-27.2
mtaq2B.1	TW	1	1	2B	2.7
mtaq2B.2	TW	1	1	2B	42.2
mtaq2B.3	GPC, GS,TW	6	3	2B	133.4-146.3
mtaq2B.4	GPC	1	1	2B	169.5
mtaq2B.5	GS, TW, YI	3	3	2B	220.5-227.1
mtaq3A.1	GS, TW	5	4	3A	3.4-6.2
mtaq3B.1	YI	3	1	3B	47.8-47.9
mtaq3B.2	GPC, TW	3	2	3B	157.3-162.9
mtaq3B.3	GS, TW	2	2	3B	172-181.6
mtaq3B.4	YI	1	1	3B	216.3
mtaq4A.1	GPC, YI	2	1	4A	59.8-69.7
mtaq4A.2	YI	3	3	4A	136.7
mtaq4B.1	GPC, TW	8	3	4B	15.7-16.5
mtaq5A.1	GS	1	1	5A	83.6
mtaq5B.1	GS	2	2	5B	10.6
mtaq5B.2	TW	1	1	5B	88.8
mtaq5B.3	TW	3	1	5B	126.9-131.7
mtaq5B.4	GS	1	1	5B	145.4
mtaq5B.5	GPC	1	1	5B	172.4
mtaq5B.6	TW	1	1	5B	185.0
mtaq6A.1	YI	1	1	6A	0.0
mtaq6A.2	TW, YI	2	1	6A	137.1
mtaq6A.3	TW	1	1	6A	158.9
mtaq6B.1	YI	4	2	6B	4.4-14.7
mtaq6B.2	GS, YI	3	3	6B	23.6-24.7
mtaq7A.1	GPC, GS, TW	4	3	7A	6.1-8.2
mtaq7A.2	GPC	6	2	7A	63.2
mtaq7A.3	GPC, YI	2	2	7A	216.1-220.4
mtaq7B.1	GS	1	1	7B	18.0
mtaq7B.2	GS	2	2	7B	37.8
mtaq7B.3	GPC	3	3	7B	68.9
mtaq7B.4	GS, TW	3	2	7B	143-152.2
mtaq7B.5	YI	2	2	7B	184.8-189.0

GPC, grain protein content; GS, gluten strength; YI, yellow colour index; TW, test weight.
* Number of environments represent the number of years and/or across years where an MTA was significant

Marker wPt-7653, associated with GS, was present in 40% of the WB+E landraces and had a similar frequency (20-30%) to the remaining SPs. On the other hand, marker rPt-6127, whose presence significantly increased GS by 16.4% on average, was widely distributed across the Mediterranean Basin, being present in all modern cultivars. This marker was identified in all landraces of the upper 10th percentile and only in 44% of genotypes in the lowest 10th percentile. Taking into account only landraces, the five genotypes with the highest GS ('D-2', 'BGE019265', 'Trigo Glutinoso', '5P4' and 'Vroulos') carried the marker, whereas the five with the lowest GS ('Razza 181', 'Akathiotico Naurotheri', 'VII/13-X11', 'IG-96851' and 'Blanco de Corella') lacked it.

For YI, wPt-3729 was mainly restricted to the eastern regions and most modern cultivars. The marker was present in 83% of the upper 10th percentile genotypes and only in 5% of the genotypes of the lower 10th percentile. Additionally it was present in the five landraces with highest YI values ('Harani Auttma', 'Safra Maan', '26', 'Hati' and 'Safra Jerash') and absent in the five landraces with the lowest values ('28', '441-IX/97', 'Heraldo del Rhin', '440-IX/96', and 'Dalmatia 3').

Markers associated with TW showed a different distribution among SPs. The marker wPt-6240, with the strongest effect on TW (3.6%), was present in the majority of cultivars of all SPs, but in the EB+T the frequency decreased to 50%. It was also present in almost 90% of modern cultivars. The marker was present in all landraces of the upper 10th percentile and in the five with the highest TW ('Heraldo del Rhin', 'Espanhol', 'Harani Auttma', 'Haiti' and 'Rubio de Montijo'), but it was also present in 61% of the genotypes included in the lower 10th percentile, and in three out of five landraces with the lowest TW ('5P4', '28 Giza', 'D-2' and 'BGE019265'). Markers wPt-1140 and wPt-1441 were associated with low TW. The first marker was present in all cultivars from the EM, EB+T and WM SPs, but its frequency was lower in the WB+E and modern cultivars. It was present in most genotypes of both 10th percentiles, 89% for the upper and 94% for the lower. Moreover, it was present in four and five landraces showing the highest and lowest TW, respectively. The second marker (wPt-1441) was present mainly in the WM and EM SPs, decreasing in landraces from the Balkans and in modern cultivars. This marker was present only in 50% of the genotypes from the upper 10th percentile and in 67% of those from the lower 10th percentile. The marker was present only in one out of the five landraces with the highest TW and in the five landraces with the lowest TW. Finally, marker wPt-8892, with a low positive effect on TW, was present in all eastern cultivars and in a high percentage of western cultivars, but its presence decreased to 60% in modern cultivars. It was present in a high percentage in both 10th percentiles (94% and 78% for the upper and lower ones, respectively) and in

four and three out of five landraces with the highest and lowest TW.

3.3. QTL meta-analysis

In order to compare the genomic regions involved in grain quality identified by association mapping with previous reported QTLs, a QTL meta-analysis was carried out. The analysis collected data from 345 QTLs in 20 studies published from 2003 to 2016 reporting QTLs for GPC (129), GS (79), YI (37), YPC (74) and TW (26) (Supplementary Material 4, Annex 2). The study covered 21 experimental crosses in 93 different environments. Of the 345 QTLs, 249 were subjected for projection onto the consensus map developed by Maccaferri et al. (2014). The remaining QTLs were not suitable for projection due to the lack of common markers between original and consensus maps.

Of the 249 projected QTLs, 43% were found in genome A and 57% in genome B. QTLs were detected in all chromosomes, ranging from 2 QTLs in chromosome 3A to 42 QTLs in chromosome 1B. The PVE by single QTLs followed an L-shaped distribution, with the majority of QTLs showing a low PVE (<0.20 for 82%) (Supplementary Material 5). Phenotypic variance explained a range from 0.01 to 0.542 with an average of 0.14. Size of the CIs ranged from 1.4 cM to 175.3 cM with an average of 18.0 cM. Approximately two-thirds of the QTLs (64%) had CIs < 20 cM (Supplementary Material 5).

The 249 QTLs projected onto the consensus map (Maccaferri et al., 2014) were subjected to meta-analysis. Following an Akaike information criterion, 204 QTLs were grouped into 45 meta-QTLs (MQTLs) (Table 5.4). The software did not include in the analysis 10 QTLs with low LOD scores and/or large CIs. Twenty QTLs were excluded as their CIs overlapped with different MQTLs, and it was not possible to determine the MQTL to which they belonged based on the membership coefficient given by the software. Fifteen QTLs remained as single QTLs not overlapping with other QTLs or MQTLs. Figure 5.3 shows the position of the MQTLs and the single QTLs. The number of QTLs clustered in a single MQTL ranged from 2 to 20 (Table 5.4). The CI for the MQTLs ranged from 0.1 to 24.3 cM, with an average of 6.4 cM, indicating a significant reduction of 64% in the CIs from the initial QTLs. Seventeen MQTLs were related to a single trait.

Table 5.3. A) Frequency of cultivars within each SP holding markers with significant associations with each trait at least in one year and across years and B) mean values for each pasta-making quality trait of cultivars holding or not holding each marker.

		Grain protein content (%)					Gluten strength (mL)		Yellow index	Test weight (kg/hL)			
	N	wPt-2737	wPt-3883	wPt-7734	wPt-9796	wPt-2698	rPt-6127	wPt-7653	wPt-3729	wPt-6204	wPt-1140	wPt-1441	wPt-8892
A)													
EM	19	0.7	0.9	0.8	1.0	1.0	0.9	0.3	0.8	1.0	1.0	0.8	1.0
EB+T	21	0.1	1.0	1.0	1.0	1.0	0.9	0.2	0.9	0.5	1.0	0.4	1.0
WB+E	33	0.4	0.7	0.7	0.7	0.9	0.6	0.4	0.2	0.9	0.5	0.3	0.8
WM	73	0.3	1.0	1.0	1.0	0.8	0.7	0.2	0.2	0.9	1.0	0.6	0.9
Modern	18	0.1	0.7	0.7	0.7	0.6	1.0	0.2	0.8	0.9	0.4	0.3	0.6
B)													
Absence		16.2 b	15.6 b	15.6 b	15.6 b	16.5 a	7.31 b	8.31 a	14.5 b	75.9 b	79.3 a	78.7 a	77.7 a
Presence		16.8 a	16.5 a	16.5 a	16.5 a	16.4 a	8.74 a	8.83 a	15.7 a	78.8 a	78.4 b	78.4 a	78.6 a

EM, eastern Mediterranean; EB+T, eastern Balkans and Turkey; WB+E, western Balkans and Egypt; WM, western Mediterranean. Means within columns with different letters are significantly different at $P < 0.05$.

In addition to the integration of reported QTLs, genes involved in grain quality previously mapped were also projected onto the consensus map (Maccaferri et al., 2014) (Table 5.5 Figure 5.3). Only one MTA for GS (wPt-6280 in 2009) was observed in the region of chromosome 1A, where the complex *Glu-A3/Gli-A1* has been mapped. However, no relation was observed between the presence of the marker and any of the 15 different alleles at this locus identified by Nazco et al. (2014a) in the same collection. No other MTA for GS of the 20 identified in this work mapped close to a locus with a known mapping position coding for HMW or LMW-GS. Three MTAs for YI were placed in the vicinity of the phytoene synthase genes *Psy-A1* (wPt-1429) and *Psy-1B* (wPt-5228 and wPt-5138).

Table 5.4. Summary of MQTL information for grain quality traits.

Chr	MQTL	Pos[a]	CI[b]	Left marker	Right marker	N QTL	Traits
1A LG2	1	0.5	24.3	cfa2129	wPt-11558	2	YPC
1B	2	0.0	1.10	wPt-2654	wPt-8627	8	GS
1B	3	33.0	5.06	gwm18	ksum28	9	TW, GPC, YPC
1B	4	49.0	5.57	barc302	wmc419	7	GS, YPC, YI
1B	5	55.5	2.09	wmc419	wPt-9937	5	GS
1B	6	73.0	3.55	kbo-0425	wPt-0506	4	GS
1B	7	88.4	3.41	wPt-5011	rPt-7906	4	GPC, GS
2A LG1	8	71.0	0.81	gwm95	gwm372	11	GPC, YPC, YI
2B	9	32.0	4.86	wmc112	wPt-7932	5	TW, GPC
2B	10	57.1	6.93	gwm429	wPt-6477	2	GPC
2B	11	157.0	2.72	wPt-7305	wmc332	4	GPC
3B	12	10.4	5.09	cfb6018	gpw7774	4	TW, GPC
3B	13	73.6	6.94	CA499601b	wPt-10530	3	GPC
3B	14	92.9	11.8	wPt-5390	wmc1	3	TW, GS
3B	15	104.0	6.81	wPt-0446	barc164	2	GPC, GS
3B	16	152.0	4.70	wPt-7145	wPt-0384	5	GPC, YPC
3B	17	187.0	3.25	wPt-4401	wPt-6956	2	YI
4A	18	27.8	8.01	gwm610	Lp-A3	5	GPC, YPC
4A	19	95.1	9.42	wPt-1584	wPt-1701	2	GPC, YI
4A	20	126.0	10.6	wPt-3596	wPt-7354	3	GS, YI
4A	21	140.0	4.32	wmc723	wPt-9103	6	TW, GPC, YPC, YI
4B	22	10.6	5.26	gwm857	gwm368	2	TW, GPC
4B	23	31.0	5.92	wPt-1491	gwm781	2	GPC, GS
4B	24	62.7	12.1	wPt-8092	wPt-9625	2	GPC
5A	25	70.6	4.94	barc197	gwm639	3	GPC
5A	26	112.0	6.97	wPt-730410	barc142	3	GS
5A	27	159.0	5.89	gwm126	gwm291	2	TW

Chr	MQTL	Pos[a]	CI[b]	Left marker	Right marker	N QTL	Traits
5B	28	38.4	10.3	wPt-1951	gwm213	2	GPC
5B	29	72.3	12.0	wmc415	kbo-0077	3	GPC, YPC, YI
5B	30	127.0	12.2	wPt-6014	barc140	2	GPC
6A	31	62.0	4.46	gwm132	gwm4675	4	YPC, YI
6A	32	90.7	2.03	gwm1150	wPt-2014	18	GS, YPC, YI
6A	33	118.0	5.59	gwm169	BE483091	2	YPC, YI
6B	34	100.0	4.22	wPt-6889	R18-370	2	TW, GPC
6B	35	112.0	4.55	gwm1682	850	2	GPC
6B	36	137.0	1.23	barc125a	wPt-5270	7	GS
7A	37	19.8	8.65	gwm1187	wmc168	3	GS
7A	38	88.7	4.38	wmc83	cfa2147	4	TW, GPC, YPC, YI
7A	39	113.0	2.11	gwm573	wPt-0321	2	GPC
7A	40	137.0	2.26	barc29	barc121	7	GS, YPC, YI
7A	41	214.0	0.11	BJ262177B	gwm1061	6	YPC, YI
7B	42	25.1	18.2	wmc323	wmc405	2	GPC, YI
7B	43	70.1	5.36	wPt-8273	gwm540a	6	TW, GPC, YPC, YI
7B	44	145.0	15.8	barc315	kbo-0372	2	GPC, YPC
7B	45	195.0	0.68	Psy-B1	wPt-7387	20	GPC, YPC, YI

Chr, chromosome; GPC, grain protein content; GS, gluten strength; YI, yellow colour index; YPC, yellow pigment content; TW, test weight.
[a] Most probable position on the consensus map.
[b] Length of the 95% confidence interval (CI) centred on the most probable position in cM

Table 5.5. List of genes involved in grain quality previously mapped and projected onto the consensus map.

Gene	Trait	Reference
Gpc-B1	Protein content	Olmos et al., (2003)
Gli-A1	Gluten strength	Patil et al., (2009)
Gli-A2	Gluten strength	Maccaferri et al., (2014); Ruiz et al., (2005)
Gli-B2	Gluten strength	Maccaferri et al., (2014)
Gli-B3	Gluten strength	Patil et al., (2009); Ruiz et al., (2005)
Glu-A3	Gluten strength	Patil et al., (2009); Ruiz et al., (2005)
Glu-B1	Gluten strength	Maccaferri et al., (2014); Patil et al., (2009)
Glu-B2	Gluten strength	Patil et al., (2009)
Glu-B3	Gluten strength	Patil et al., (2009)
Psy-A1	Yellow colour	Patil et al., (2008)
Psy-B1	Yellow colour	El Ouafi et al., (2001); Pozniak et al., (2007)

3.4. Gene annotation

Candidate genes within 1cM above and below the most significant markers identified by association mapping were detected using the high-confidence gene annotation from the wheat genome sequence (IWGSC RefSeq v1.0; IWGSC, 2018). Using the position of common markers in the consensus map (Maccaferri et al., 2014) and the wheat genome sequence, genetic distances were converted to physical distances. Annotated genes for the corresponding regions are shown in Supplementary Material 6.

For GPC, two loci were selected: the locus comprising markers wPt-3883, wPt-7734 and wPt-9796 located at the same position (63.2 cM) on chromosome 7A, and wPt-2737 located at 68.9 cM on chromosome 7B. For the locus at chromosome 7A the correspondence was 1:1.5 (genetic:physical). The evaluated region of approximately 3.5Mb showed 72 gene models. One gene located at 64.6 Mb was also included in the interval because of its homology with previously identified genes increasing GPC. For the locus on chromosome 7B the correspondence was 1:15. The physical region analysed covered a distance of 30 Mb with 119 gene models.

For TW, markers wPt-1140 (133.4 cM, 2B) and wPt-6204 (3.4 cM, 3A) were analysed. The region covered for the former was 4 Mb (ratio 1:2) and included 24 gene models. As marker wPt-6204 was not mapped on the wheat genome sequence and the sequence of the DArT clone was not available on the Diversity Arrays Technology website (www.diversityarrays.com) to perform BLAST, its position was defined by the SSR marker barc294, mapped by Maccaferri et al. (2014) in the same genetic position. The physical interval on the wheat genome sequence to this region corresponded to 4.6 Mb (ratio 1:2.3) and 94 gene models were found within it.

For YI only the marker wPt-3729 (136.7 cM, 4A) was selected, as the marker wPt-1437, which was also present in two MTAs, was not mapped on the wheat genome, and the sequence of the DArT clone was not available on the website to perform BLAST against the genome sequence. The marker was included in a linkage disequilibrium block with three other close markers, covering a genetic region of 0.1 cM. For this region, the ratio of genetic to physical distance was 1:2.5 and the region covering 5 Mb surrounding the marker had 101 gene models.

Finally, for GS, as occurred for YI, only one marker among others that were significant in at least 2 years or across years was identified in the genome sequence and was included in the analysis. The physical region for the marker wPt-7653 (38.7 cM, 7B) was identified through the linked marker wPt-7064 and the ratio of genetic to physical distance was defined as 1:2.3. Fifty-two gene models were included within the approximately 4.7 Mb flanking region.

4. Discussion

4.1. Quality traits

Results of ANOVA showed the variability existing in the phenotypic expression of pasta-making quality traits in durum wheat. Cultivar effect was partitioned into variation within and between the genetic SPs defined by Soriano et al. (2016). Grain protein content was largely explained by the environmental effect (harvesting year), whereas for GS, TW, SI and QI the cultivar accounted for a larger variation than environment, suggesting a higher genetic control for these traits. These results agree with those of previous studies reporting a large environmental influence on durum wheat GPC in Mediterranean environments (De Vita et al., 2007; Rharrabti et al., 2001, 2003; Taghouti et al., 2010). For YI, although the environment effect was higher, the genotypic effect was also large (48 and 43% of the explained variance, respectively). When the genotype effect was partitioned, most of the variation accounted for all studied traits within SPs, revealing an enormous intra-population variability. These results based on classification of genetic SPs (Soriano et al., 2016) are in agreement with those reported by Nazco et al. (2012) using a population structure based on the different climatic zones of the Mediterranean Basin.

When comparing mean differences among SPs, modern cultivars showed the highest values for overall quality and sedimentation indices, probably due to the higher GS of the cultivars belonging to this SP. In agreement with these results, a previous study that analysed the changes caused by breeding in quality traits of Italian and Spanish durum wheat reported a lack of progress in TW, a loss of GPC and a substantial improvement in GS and yellow colour (Subirà et al., 2014). The high level of GPC found in Mediterranean landraces in this and previous studies (Nazco et al., 2012) was associated in the current study with a high frequency of markers with a positive and significant effect on GPC, thus offering a potential tool for protein content improvement in breeding programmes.

A previous study that used the same germplasm as the present one demonstrated that the greater GS values found in modern cultivars were due to a very few allelic combinations of high (HMW-) and low (LMW-) molecular weight glutenin subunit loci (Nazco et al., 2014a), which could be a constraint for future quality improvement. However, allelic banding patterns drastically increasing GS were identified in landraces (Nazco et al., 2014b), showing their potential to broaden the genetic basis for gluten quality improvement.

Significant differences in YI appeared between two groups: the highests values were found in modern cultivars and landraces from EM countries, whereas the lowest ones were found in western SPs. These results suggest that yellow colour

of wheat grain decreased during the migration of wheat from the Fertile Crescent, the area of origin and domestication, to the west of the Mediterranean Basin, and was recently improved by breeding, as demonstrated with the use of historical series of genotypes (Subirà et al., 2014).

For TW we found a large genetic component accounting for the total variance explained (57%) and only 14% due to environment. These results disagree with those reported by Taghouti et al. (2010) and Subirà et al. (2014), who found a large environmental effect accounting for the variation of the trait. A possible explanation for these differences may be the lower number of genotypes used by those authors compared with the large collection used in the current study. The largest differences between SPs appeared between landraces from the Balkans peninsula and the remaining SPs, the latter showing heavier grains but much lower internal variability.

4.2. Genetic architecture

In addition to the studies conducted by Nazco et al. (2014 a, b) that used glutenin subunit composition to study the genetic bases of GS, this study is one of the first attempts to elucidate the molecular bases of pasta-making quality traits in Mediterranean durum wheat landraces. The collection of landraces was grown under the dry and warm conditions typical of the Mediterranean Basin (Royo et al., 2014). A genome-wide association study (GWAS) was performed following a mixed linear model method accounting for the genetic relatedness between cultivars and their population structure (K+Q model) in order to reduce the number of spurious associations.

A total of 92 associations involving 4 traits and 70 markers were detected in three years and across years in north-eastern Spain. As reported previously by Laidò et al. (2014), MTAs located within short map intervals (ca. 5-10 cM) should be considered as belonging to the same QTL. Thus, following this suggestion in the present study, 37 genomic regions (or quality MTA-QTLs) involving the 92 MTAs were identified. Eight of these regions were detected across 3 and 4 environments and were considered the most stable QTLs. MTAs were widely distributed across the genome, with all chromosomes except 2A showing significant associations.

In order to compare the MTA-QTLs identified in the present research with previously reported QTLs, a QTL meta-analysis was carried out, summarizing data from 249 QTLs for quality traits published from 2003 to 2016. These QTLs were projected onto the same consensus map (Maccaferri et al., 2014). The meta-analysis revealed the presence of 60 genomic regions (45 MQTLs + 15 singletons) controlling quality traits in the genomes A and B of durum wheat. The meta-analysis produced a simplification in the genome regions containing QTLs, as their number was reduced up to four times and the CI also diminished significantly by 64%. Eleven

out of the 37 MTA-QTLs reported in the present study were located within the CI of MQTLs and five of them had QTLs for the same traits. On chromosome 2B, the *mtaq2B.2* for TW was located within the interval of MQTL9, which had QTLs for TW and GPC. On chromosome 4A, *mtaq4A.1*, which had MTAs for GPC and YI, was located with a single QTL for YI reported by Roncallo et al. (2012). Additionally, in the same chromosome, the *mtaq4A.2* associated with YI was flanked closely by two MQTLs (20 and 21), both of them with QTLs for YPC and YI also described by Roncallo et al. (2012). The importance of this region on chromosome 4A for flour yellow colour lies in the stability across years found in the present study. Additionally, Roncallo et al. (2012) found epistatic effects of these QTLs on chromosome 4A, with others located on the bottom of chromosomes 6A and 7A, where MTAs for YI were also found in the present work: *mtaq6A.2*, which was located within the CI of a QTL for YI; and *mtaq7A.3*, also with MTAs for GPC, which mapped with MQTL41, which had QTLs for GPC, YI and YPC. Finally, the *mtaq7B.3* for GPC was identified in the region of MQTL43, which had six QTLs for GPC, TW, YI and YPC. The MTA for GPC in this region was also considered stable, as it was detected in two years and across years. Although other genomic regions containing MTAs were located within MQTL positions, they identified QTLs for different traits to those reported by association analysis. Thus, most MTA-QTLs identified by association analysis corresponded to new regions for quality traits present in durum wheat Mediterranean landraces.

Previous studies reported the association of molecular markers with grain quality traits. Laidò et al. (2014) performed a GWAS for different agronomical, morphological and grain quality traits using a collection of 230 inbred lines, 128 of them corresponding to durum wheat cultivars and 102 to wild and domesticated cultivars from six other subspecies. These authors found 39 MTAs for GPC in the whole collection, only 14 of them corresponding to the durum wheat cultivars. Only one MTA from the durum cultivars corresponded to an MTA-QTL located in the present study (*mtaq3B.3*) harbouring associations with GS and TW. When the whole collection was analysed, 3 MTAs were placed in a similar location to *mtaq4A.1*, *mtaq7A.1* and *mtaq7A.2*, all of them associated with GPC. More recently, Giraldo et al. (2016) reported an association analysis for agro-morphological and grain quality traits in a structured collection of Spanish durum wheat landraces including different subspecies (*T. durum*, *T. turgidum* and *T. diccocon*). The authors found 33 MTAs for quality traits (2 for GPC, 26 for GS, 3 for TW and 2 for YI) and 6 of them can be integrated within MTA-QTLs reported in the current study: wPt-8780 for GS in *mtaq1A.1* (GS), on chromosome 3B; wPt-3599 (TW) in *mtaq3B.2* (GPC, TW); wPt-0990 (GS) in *mtaq3B.3* (GS, TW); wPt-0665 (YI) in *mtaq3B.4* (YI); wPt-6916 (GS) in *mtaq6B.2* (GS, YI); and finally, wPt-6869 (YI) in *mtaq7B.5* (YI). Interestingly, all

common associations between the study of Giraldo et al. (2016) and ours corresponded to the same quality traits. The low number of common MTAs detected between the current study and those reported by Laidò et al. (2014) and Giraldo et al. (2016) could be explained by the different plant material used by the three groups: durum wheat inbred lines (Laidò et al., 2014) and Spanish durum wheat landraces (Giraldo et al., 2016), both authors including other durum subspecies; and durum wheat landraces from 21 Mediterranean countries in our work. The findings of the present study highlight the importance of the Mediterranean landraces as a source of new alleles to improve durum wheat quality traits, as reported previously by Nazco et al. (2012, 2014a, b). Another reason for the differences could be the different maps used for the GWAS. Laidò et al. (2014) and Giraldo et al. (2016) used the map reported by Marone et al. (2012) to locate the polymorphic markers, whereas the present study used the consensus map reported by Maccaferri et al. (2014).

Finally, to support the association of the MTA-QTLs with previously reported genes involved in pasta-making quality traits, known genes were also included in the map (Table 5.5). On chromosome 1A, the LMW glutenin locus *Glu-A3* (Ruiz et al., 2005) and the gliadin locus *Gli-A1* (Patil et al., 2009) were located in the vicinity of an MTA for GS, within *mtaq1A.1*. Additionally, a QTL for GPC was also located in this region (Suprayogi et al., 2009). On chromosome 2B the HMW and LMW glutenin loci *Glu-B2* and *Glu-B3* (Patil et al., 2009) and the gliadin *Gli-B3* (Patil et al., 2009; Ruiz et al., 2005) were located together with an MTA for TW in *mtaq1B.1* and with MQTL2, harbouring 8 QTLs for GS. In the same chromosome HMW-GS *Glu-B1* (Maccaferri et al., 2014; Patil et al., 2009) was located within MQTL6, harbouring 4 QTLs for GS. The *Gli-A2* locus identified by Ruiz et al. (2005) and Maccaferri et al. (2014) on chromosome 6A and *Gli-B2* (Maccaferri et al., 2014) on chromosome 6B were located in regions without MTAs or MQTLs. For protein content, the *GPC-1B* locus was located on chromosome 6B (Olmos et al., 2003) and was projected onto the consensus map close to a QTL for GPC (Prasad et al., 2003). Finally, for yellow colour the phytoene synthase genes *Psy-A1* (Patil et al., 2008) and *Psy-1B* (El Ouafi et al., 2001; Pozniak et al., 2007) were located within the YI MTA-QTLs *mtaq7A.3* and *mtaq7B.5*, respectively. In both cases, MQTLs for YI were also identified in the same regions (MQTL41 and MQTL45, respectively).

4.3. Gene annotation

Potential candidate genes for the studied traits were searched using the high-confidence gene annotation from the wheat genome sequence (IWGSC RefSeq v1.0; IWGSC, 2018). The position of common markers between the durum wheat consensus map (Maccaferri et al., 2014) and the wheat genome sequence at https://wheat-urgi.versailles.inra.fr/Seq-Repository/ was used to define CIs. The

limitation of using only DArT markers to find regions in the genome sequence reporting candidate genes resides in the uncovered regions for this type of markers in the sequence, as reported in this work for some of the MTAs. In this case closely linked markers or blasting the marker sequence (if available) resulted in useful approaches to identify gene models. If no closely linked markers or the marker sequence is not available, the identification of candidate genes becomes a difficult task and highly saturated maps are needed. The join analysis of GWAS together with QTL meta-analysis using reference maps also helps to identify genome regions uncovered by DArT markers.

Among the gene models within CIs of 1 cM above and below the selected marker, candidate genes previously described in the literature were found for GPC and TW.

For GPC two regions were subjected to analysis. The first region on chromosome 7A comprised the markers wPt-3883, wPt-7734 and wPt-9796 located at 61.8 Mb, and the second one on chromosome 7B the marker wPt-2737 at 199.3 Mb. In both cases, the gene models TraesCS7A01G106300.1 (7A) and TraesCS7B01G143900.1 (7B) encoded for an NAC domain containing protein transcription factor. This kind of domain was described by Uauy et al. (2006) for *Gpc-B1* and is associated with an increase in GPC, Zn and Fe content in wheat. This transcription factor accelerates senescence and increases the nutrient remobilization from leaves to develop grains.

For TW the analysed loci were wPt-1140 (601.9 Mb, 2B) and wPt-6204 (7.9 Mb, 3A). For wPt-1140, the gene model TraesCS2B01G419400.1 was found, encoding for an E3 ubiquitin protein ligase. This is the same kind of protein encoded by the gene *TaGW2-A1* (Simmonds et al., 2016), which has a role as a negative regulator of grain size and weight in hexaploid wheat (Yang et al., 2012). A mutant allele of this gene significantly increased grain weight, grain width and grain length in tetraploid and hexaploid wheat. For wPt-6204, several candidate genes within the defined CI were found. The gene model TraesCS3A01G007600.1 encodes for a transcription elongation factor as the gene *TaTEF-7A* (Zheng et al., 2014), which increases potential grain yield and yield-related traits and confers complex pleiotropic effects on growth, yield and quality. Two other gene models within the region of wPt-6204 were associated with expansin proteins (TraesCS3A01G011100.1 and TraesCS3A01G011200.1). According to Lizana et al. (2010), the expression of expansins in wheat is associated with grain size. The results of these authors support an association between the expression levels of expansins and fast growth of the wheat grain taking place at early developmental stages.

4.4. Breeding potential

As a consequence of domestication and breeding, the genetic variability of crops has been gradually reduced. Exploiting genetic diversity from local landraces in breeding programmes is a valuable approach to recovering and to broadening allelic variation of traits of interest (Lopes et al., 2015). Mediterranean durum wheat landraces are an important group of genetic resources because of their specific adaptation to local environments and their end-product quality (Nazco et al., 2012), in view of the enormous genetic diversity found among Mediterranean landraces in traits of commercial importance (Soriano et al., 2016).

The results reported in the present study can be exploited to improve wheat cultivars by selecting the most significant and stable MTAs across environments. Protein content has decreased as a consequence of past breeding activities, which concentrated on increasing yield potential (De Vita et al., 2007; Motzo et al., 2004; Royo et al., 2008; Subirà et al., 2014). Marker wPt-2737 located on chromosome 7B shows the greatest variance explained for this trait; it is linked to an increase in GPC and is present mainly in EM cultivars. Further studies are needed in order to develop new molecular markers from the sequence of wPt-2737 and validate them in progenies and breeding material. As reported previously by Subirà et al. (2014), the greatest improvements for GS were produced with the introduction and release of the first improved cultivars in Italy and Spain, and were due to the use of a limited number of HMW- and LMW-GS alleles associated with GS. Thus, breeding for GS should focus on increasing the genetic diversity of glutenin allelic combinations rather than increasing the trait itself. In the current study, mean values for GS were higher in modern cultivars than in landraces, and the frequency of the markers with higher R^2 and stability across environments did not differ between the two types of cultivar. The marker wPt-3729 for YI showed that eastern landraces and modern cultivars differed clearly from western landraces. Although the value of YI in modern cultivars is high, the use of eastern landraces to improve yellowness would help increase genetic diversity for the trait. Finally, for TW the maker wPt-6204 appeared to be the most stable across environments, as it was detected in all three years and across years. However, except for the EB+T SP, the marker was present in most cultivars belonging to the other landrace SPs and modern cultivars. Although markers wPt-1140 and wPt-1441 were mainly present in landraces, they produced a negative effect. The marker wPt-8892 would be the most suitable for increasing the trait in modern cultivars because it is present mainly in landraces.

Recently the genome sequence of the cultivar ‘Chinese Spring’ was published (IWGSC, 2018), becoming a useful tool for the wheat breeding community. The sequence will allow the identification of candidate genes through map based

approaches, the development of new molecular markers in order to saturate genome regions or identifying new ones in low recombination regions, cloning candidate genes in other cultivars to study mutations and differences in expression levels. Knowing the whole sequence of candidate genes will also help in speeding breeding programmes by the use of gene editing technologies.

Supplementary material

Supplementary material is available on the online version of the article, doi: 10.3389/fpls.2018.01512

Author contributions

CR and JMS obtained funding. CR, FA, DV and JMS designed the experiments. CR and DV assembled and purified the germplasm collection. DV and RN phenotyped the collection for quality traits. MR performed association mapping, meta-analysis and statistical analysis. FA and JMS conceived the manuscript. MR, FA and JMS wrote the manuscript. CR, FA and JMS edited and provided a critical review of the manuscript. MR, CR, FA, DV, RN and JMS read and approved the final manuscript.

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Discussion

Discussion

1. Introduction

Wheat breeding will play a critical role in the next decades as an essential instrument to face the current global challenges of increasing food production reducing, at the same time, the environmental impact. United Nations has predicted that world population will be up to 9 billion by 2050. To meet the food demand, cereal production will have to rise by about 40%, considering that more land for agricultural uses will not be available. For wheat, needs of yield increases have been estimated in 1.7% y^{-1} (Leegood et al., 2010), largely exceeding the gains obtained by the Green Revolution in the 1970's.

Considered globally, until now, wheat breeding programmes have been very successful, as around 50% of production rises from 1930 are generally attributed to improved genotypes, while the remainder 50% has been due to changes in agricultural practices. However, the general adoption of high-productive varieties, particularly during the second half of the 20th century, led to an enormous loss of biodiversity. Moreover, ecosystem alterations to give crops the most favourable environmental conditions to express their yield potential caused a strong soil degradation and an over-exploitation of natural resources. One of the consequences of the situation generated has been the promotion by FAO and other organisms of the '*sustainable intensification of crop production*' concept (http://www.fao.org/policy-support/policy-themes/sustainable-intensification-agriculture/ en/). This paradigm, which entails to '*produce more with less*' will be unavoidably linked to the development and cultivation of varieties with characteristics specifically designed to address it.

In contrast with the classical model of wheat production based on adjusting the environment to the crop needs, breeders are now focusing on delivering cultivars customised to the farming environment (Royo et al., 2017). On a climate change scenario, with a growing water scarcity and rising temperatures, resilience to climate change effects is already one of the main characteristics pursued by breeding programmes. One of the most important issues for environmental adaptation is phenology fitting, which allows the crop to develop each growing period under the best environmental conditions. For durum wheat grown under Mediterranean conditions, flowering time will have to be early enough to allow the crop to escape from terminal drought and heat stresses, but at the same time, late frosts during flowering have to be avoided. New cultivars will have to be also more efficient in the use of inputs, particularly water and fertilizers, principally nitrogen. In addition, they will have to incorporate genetic resistance to the traditional and upcoming

diseases and pests to limit the use of pesticides (Ceoloni et al., 2017), thus hampering the appearance of damaging epidemics that could provoke serious shrinkages in production (Araus et al., 2008). Finally, but also essential, the grain of the new cultivars will have to reach the quality standards demanded by the food industry.

This enormous challenge that wheat-breeding programmes are already facing, can only be effectively addressed through international cooperation between multidisciplinary teams, and with the incorporation of last-generation technologies into breeding programmes. The huge potential of recently developed technologies offers opportunities to generate information and develop tools for wheat improvement that were unimaginable few decades ago (Royo et al., 2017). A traditional breeding programme that grows a generation per year could delay around 15 years to release a commercial variety (Shimelis and Laing, 2012). Molecular tools applied to breeding, such as marker assisted selection (MAS) and genomic selection (GS), can accelerate the process by reducing the need of certain phenotype characterization and make selection more efficient due to the increase of the genotype-phenotype relationship (Charmet, 2011).

In this context, the research conducted by this PhD Thesis was conceived to generate information and contribute to the development of tools that could help breeders to address the current challenges. All traits analysed in this research are relevant for breeding purposes. In many cases, particularly for farmers, yield is the most important trait. However, its complex genetic nature and phenotypic expression as being affected by many other traits related to plant morphology and physiology, counsels dissecting it on different components and analyse them independently. This was the approach used in the current research, in which in addition to yield other traits largely influencing it such as yield components, root architecture, plant height or harvest index, were also investigated. Durum wheat was chosen as the target crop due to its relevance in the Mediterranean Basin, which represents 60% of the world's growing area for the species. For this reason, knowledge of the crop adaptation strategies to environmental conditions is essential for breeding programmes. Finally, the significance of durum wheat in the Mediterranean diet, as it is the raw material not only for producing pasta, but also flat breads, couscous and bulgur (Royo et al., 2017), justified devoting a chapter of this report to analyse the genetic control of the main quality traits.

The germplasm used in this research included sets of durum wheat landraces and modern cultivars. The old germplasm consisted on the 'IRTA Diversity Panel of Durum Wheat Mediterranean Landraces', formed by a collection of about 180 landraces from 21 countries. This panel started to be investigated in previous studies that showed its huge genetic and phenotypic diversity, and its genetic and geographic

structures (Nazco et al., 2012, 2014a, b; Royo et al., 2014; Soriano et al., 2016, 2017, 2018). All or part of the panel has been used to address all the objectives of this PhD Thesis. Modern cultivars were additionally used to address objectives 2, 4 and 5 in order to identify changes made by breeding on target traits. Phenotyping was carried out on field experiments conducted during six cropping seasons (2007 to 2009 and 2013 to 2015) under rainfed conditions in Mediterranean environments. Genotyping was done with 1,149 DArT markers and 46,161 DArTseq markers.

Given that the agronomic traits examined in this research have a quantitative nature, a **QTL meta-analysis** was carried out in the **first chapter** to synthesise the information contained in 26 QTLs studies published until the moment in wheat, for a number of **agronomic traits** related with phenology, biomass production and allocation, plant height, yield and yield components.

The **second chapter** entailed a genome-wide association mapping study (**GWAS**) addressed to identify loci that explained most of the variability for **yield related traits** on contrasting germplasm collections representing old-unimproved and modern-improved durum wheat cultivars.

The two subsequent chapters analyse mechanisms and traits related to durum wheat adaptation to environmental conditions. An attempt was made on **chapter 3** to assess the **pattern of adaptation** and its influence on yield formation strategies of landraces collected on Mediterranean countries with distinct rainfall and temperature, whose agronomic performance had been associated to different frequency of specific alleles.

Chapter 4 analysed the **seminal root system architecture** of modern cultivars and landraces structured in different subpopulations, its relationship with yield formation and identified molecular markers associated to root traits through GWAS.

Chapter 5 was devoted to **grain quality**. Association mapping was used to identify genome regions related to pasta-making quality traits, and a meta-analysis was conducted to discover consensus QTL regions affecting the EU quality index and the traits comprised on it.

2. Phenotypic traits related to durum wheat adaptation to Mediterranean environments

2.1. Adaptive traits of Eastern and Western Mediterranean landraces

Although the Mediterranean climate has distinctive and very well know characteristics, such as the low and unpredictable rainfalls that mostly fall in autumn and winter and the temperature and drought rises during the spring and summer, within the Mediterranean Basin marked climate heterogeneity exists between zones

(Nicault et al., 2008; Xoplaki et al., 2004). The region comprises countries between about 27°– 47°N and 10°W–37°E shoring on three continents and with a coastline of 46,000 km (http://www.fao.org/sd/ climagrimed/c_2_02.html), which entails a range of environmental conditions varying from favourable to dry-land areas.

A previous study conducted assembling long-term climatic data of the main wheat-growing regions of the 21 Mediterranean countries origin of the IRTA Diversity Panel of Durum Wheat Mediterranean Landraces, identified four climatic zones in the Mediterranean Basin, steadily varying from warm and dry to cold and wet (Royo et al., 2014). This climate variability explains the need of progressive adaptation of ancient wheats to the varying environmental conditions of the new growing areas during their dispersal from the Fertile Crescent to Western Europe (Royo et al., 2017). It has been suggested that the different climates prevalent in the new regions of adaptation induced gradual changes in crop phenology and in the strategies of yield formation that conferred adaptive advantages under the new environmental conditions (Moragues et al., 2006a). This process of migration and natural and human selection led to the establishment of a wide diversity of local landraces specifically adapted to different agro-ecological zones, which makes them as an excellent germplasm for adaptation studies, such as the conducted in this PhD Thesis.

Large number of studies have evaluated the field performance and quality characteristics of landrace collections from specific countries. Scientific papers have been published concerning Spain (Giraldo et al., 2016; Ruiz et al., 2012), Italy (Giunta et al., 2007, 2019), Bulgaria (Ganeva et al., 2010), Turkey (Karagöz and Zencirci, 2005), Syria (Talas et al., 2011), Jordan (Rawashdeh et al., 2007), Tunisia (Sourour et al., 2010), Morocco (Zarkti et al., 2012), Iran (Mohammadi et al., 2014) and Ethiopia (Mengistu et al., 2016). However, the use of landrace collections including accessions from a large number of countries is unusual. Landraces are likely sources of highly beneficial untapped diversity because they are potential providers of new favourable genes to be introgressed into adapted phenotypes. However, as the genetic variation contained in them is usually unknown, their effective use in breeding programmes makes necessary to evaluate the existing diversity in the gene pool and to characterize the available accessions (Lopes et al., 2015). Identifying variants of potential interest for breeding purposes in landraces may be particularly useful when breeding programmes are oriented to suboptimal environments. However, old germplasm is currently not sufficiently exploited for breeding purposes because there is a lack of efficient strategies to identify beneficial alleles and transferring them into elite germplasm. Therefore, evaluating germplasm pools to discover and identify valuable genotypes and favourable alleles will

facilitate breeder's work to incorporate them into their breeding programmes (Wang et al., 2017).

Previous studies conducted with the IRTA Diversity Panel of Durum Wheat Mediterranean Landraces used in this research showed that it may be considered a representative sample of the variability existing within the species in the region (Moragues et al., 2003, 2006a,b; Nazco et al., 2012, 2014a,b). Initial studies trying to link the agronomic performance of landraces and the environmental conditions of the area where they were collected demonstrated significant relationships for phenology, biomass production and allocation and yield components (Moragues et al., 2006a, b; Royo et al., 2014). However, none of these studies took into account the genetic structure of the population, which was further assessed by Soriano et al. (2016). A subsequent study revealed that the contrasting agronomic performance of subpopulations from the east and the west of the Mediterranean Basin was consistently based on a different genetic background (Soriano et al., 2018). These previous results suggested that differences in the agronomic performance of eastern (EM) and western (WM) Mediterranean landraces, collected in the zones with the largest climate disparities within the Mediterranean Basin, were likely consequence of the mechanism of adaptation of durum wheat during its migration from the east to the west of the Mediterranean Basin, and inspired the third objective of this PhD Thesis. The results of the study conducted to address it, shown in chapter 3, added consistent information that is essential to explain previous results and to understand the evolutionary adaptation of durum wheat resulting from its migration across the Mediterranean Basin. To give more reliability to the results of this study, only landraces with a high membership coefficient to each of the two subpopulations (q>0.900) were used, and phenotypic data from six years of field experiments was used to analyse the GE interaction. The results showed that landraces collected in the Eastern Mediterranean countries (Syria, Lebanon, Israel and Jordan), close to the area of wheat domestication and characterised by being the warmest and driest within the Mediterranean Basin (Royo et al., 2014), based their yield on the water available before anthesis. The EM subpopulation showed also the earliest anthesis time and the longest grain filling period, a combination that has been reported to be appropriate to escape from terminal drought stress (Annicchiarico et al., 2009).

The results of the current research demonstrated that the superior number of tillers and spikes reported for landraces from dry and warm regions, when compared with those from wet and cold (Royo et al., 2014), may be very likely associated to the water used by the crop before flowering. It is well known that the development of a high number of ear-bearing tillers is an adaptation mechanism to heat stress (Hütsch et al., 2019), which agrees with the adaptive response of EM landraces to the high temperatures recorded in the eastern Mediterranean countries. Moreover,

the accumulation of water-soluble carbohydrates in the stems and leaf-sheaths before anthesis and its remobilization to grains after flowering is a very typical drought-resistance feature genetically determined (Dreccer et al., 2014; Michiels et al., 2004; Xue et al., 2008). The results shown in chapter 3 demonstrated that EM landraces use this mechanism, and this evidence, jointly with the production of large number of spikes per unit area and the early flowering time that allow them to escape from terminal stress, point out their adaptive response to terminal drought.

On the other hand, a number of adaptive traits were identified in landraces collected on western Mediterranean countries (Spain, Italy, Portugal, Morocco, Algeria and Tunisia). In this region, temperatures are lower than in the east, and more water is available during crop cycle, particularly after anthesis (Royo et al., 2014). The adaptive traits identified in WM landraces were a delay of flowering time, a reduction of the grain-filling period, and a greater efficiency in the use of water after anthesis to produce large number of heavy grains due to an increased grain-filling rate. It has been demonstrated that in optimal environments the contribution to kernel weight to yield is enhanced, while in warmer environments the number of spikes becomes more relevant (García del Moral et al., 2003; Hütsch et al., 2019; Moragues et al., 2006a). The results of the current study support this assumption giving a substantiated explanation to it in terms of adaptation, and pointing out the suitability of landraces for this type of investigation.

2.2. Adaptive traits concerning root architecture of landraces and modern cultivars

One of the most important contributions of the Green Revolution to durum wheat breeding was the introgression of the dwarfing gene *Rht-B1*, which confers a reduced response to gibberellin (Peng et al., 1999) and has diverse pleiotropic effects on plants, as reported for durum wheat (Álvaro et al., 2008a, b, c). A previous study demonstrated that the incorporation of this dwarfing allele reduced root biomass at anthesis by 28.1% (Subirà et al., 2016). Chapter 4 of the current PhD Thesis analysed the seminal root architecture of a set of 160 landraces structured in four subpopulations with a clear geographic structure, and 18 semi-dwarf modern cultivars. The comparison of the mean values of the four landrace subpopulations with those of the modern cultivars shown on Table 4.2 indicates that the *Rht-B1* dwarfing gene did not affect the seminal root system as it did with the crown roots, as reported previously by Subirà et al. (2016). Actually, comparing the average values of the four subpopulations shown on Table 4.2 with those of the modern cultivars shown in the same table, it can be observed that differences are minimum, and in average, landraces only show superior values for root number and lateral root

surface and volume. Moreover, the comparison of the individual values of each subpopulation with those of the modern varieties revealed that modern cultivars had intermediate values for all the analysed traits, which is in agreement with the assumption of a negligible breeding effect on seminal root system architecture, probably due to the very early developmental stage when the roots were measured.

However, large differences appear when the comparison is made between landraces from the two most geographically distant subpopulations (eastern and western). Concerning the primary root, the WM landraces analysed in chapter 4 showed lower values for length (14.4%), surface (12%), and volume (13.8%) than the EM ones. The comparison of lateral roots showed a similar tendency, although the differences between both subpopulations were minor (6.4% for length, 6.5% for surface and 4.9% for volume). For root diameter values were very similar, but the root angle was significantly higher in the EM landraces. These results are in line with the good adaptation of EM landraces to the driest and warmer area within the region, as they showed the largest root size and the widest root angle, traits associated to the adaptation to drought environments (Christopher et al., 2013; Wasson et al., 2012).

Besides these differences between EM and WM landraces, the values of root traits recorded in the genetic subpopulations involving East Balkan + Turkey and West Balkan + Egypt did not allow inferring a geographic pattern associated to the countries origin of the landrace subpopulations, as intermediate values were observed for many of them. This is not an unexpected result as landraces from very diverse climatic environments were included on the same genetic subpopulations, as discussed in chapter 4.

3. Genomic approaches

3.1. Discovering consensus genomic regions by QTL meta-analysis

In the last years, numerous studies identifying QTLs controlling agronomic traits have been published in wheat, each of them using different traits, genetic backgrounds, mapping populations and/or environmental conditions. Goffinet and Gerber (2000) developed a way to synthesise QTL information, the QTL meta-analysis, in order to reduce redundancies and to find consensus genomic regions harbouring the most robust and reliable QTLs among the mapping populations. QTL meta-analysis can result of special interest narrowing down the supporting intervals (SI) of QTLs to tackle map-based cloning strategies more efficiently. However, QTL meta-analysis is highly dependent on the individual QTL mapping studies, their SI and projection quality. For this reason, we projected only QTLs that had all the information required for QTL projection following the homothetic approach defined by Chardon et al. (2004) and meta-analysis using the BioMercator v4.2 software,

such as LOD score, phenotypic variance explained (PVE), peak position, SI and flanking markers.

QTL meta-analysis became very popular and different QTL meta-analysis studies were performed in wheat for traits such as grain yield (Soriano et al., 2017; Zhang et al., 2010), crop phenology (Hanocq et al., 2007; Soriano et al., 2017), disease resistance (Goudemand et al., 2013; Löffler et al., 2009; Marone et al., 2013; Soriano and Royo, 2015), plant height (Griffiths et al., 2012), grain-related traits (Quraishi et al., 2011; Tyagi et al., 2015), sprouting tolerance and dormancy (Tyagi and Gupta, 2012), grain quality (Roselló et al., 2018) and root related traits (Soriano and Álvaro, 2019). Two QTL meta-analysis from this revision were performed in the framework of this PhD Thesis: chapter 1 collected information from 26 different QTLs studies collecting information for agronomic traits related with yield, yield components, phenology and biomass; and in chapter 5 it is shown the results defining consensus QTL regions for quality traits from 20 QTL studies.

From a breeding point of view, Löffler et al. (2009) defined the criteria for MQTL selection to be used to accelerate breeding programmes: 1) presenting a small SI, 2) clustering of a high number of initial QTLs, and 3) initial QTLs should present a high effect of the PVE. Based on these criteria we identified the most promising MQTL for breeding purposes (Table 1).

The sequence of the genomes of wheat (IWGSC, 2018) and durum wheat (Maccaferri et al., 2019) will be an excellent tool for research and for the breeding community allowing the identification of putative candidate genes within MQTLs that will accelerate the breeding process through finely directed research of specific gene models.

3.2. Identification of genomic regions regulating relevant traits for durum wheat breeding by GWAS

This PhD Thesis addresses the previous studies of the research group related to yield formation, adaptation to Mediterranean environments and grain quality from a different point of view, this is, taking into account the genetic structure defined by Soriano et al. (2016) and using molecular approaches (GWAS) to find genome regions controlling these traits. GWAS accelerates the identification of genome regions involved in trait variation, allowing 1) the development of new molecular markers to carry out marker assisted selection (MAS) in the breeding programmes, and 2) the identification of potential candidate genes thanks to the release of the genome sequence of wheat (IWGSC, 2018) and durum wheat (Maccaferri et al., 2019). Comparison of the genetic architecture of landraces and modern cultivars as reported in chapter 2 allowed the identification of new alleles from the old

germplasm to be incorporated in the commercial varieties for their improvement.

Table 1. Breeding MQTLs inform the QTL meta-analysis of chapters 1 and 5 that followed the criteria of Löffler et al. (2009).

Chr	MQTL	Pos	SI_{95}	N QTLs	PVE_{max}	PVE_{mean}	N studies	Traits
2A	11	63.4	0.6	18	0.14	0.08	2	Yield
2A	8	71.0	0.8	11	0.35	0.16	3	Quality
3B	29	96.0	1.8	10	0.26	0.13	3	Yield
4A	32	56.0	8.5	17	0.21	0.09	6	Yield
4A	35	127.3	12.4	10	0.20	0.12	3	Yield, phenology
4B	39	21.6	3.1	26	0.28	0.13	6	Yield
4B	40	37.5	6.9	29	0.68	0.14	8	Yield
5A	42	16.3	2.1	11	0.26	0.14	3	Yield, phenology
5A	44	75.0	2.8	14	0.14	0.08	3	Yield, phenology
5B	47	45.3	2.6	15	0.21	0.13	3	Yield
6A	32	90.7	2.0	18	0.43	0.19	3	Quality
6B	57	60.5	3.7	13	0.13	0.08	1	Yield
7B	45	195.0	0.7	20	0.16	0.09	5	Quality

Chr, chromosome; MQTL, meta-QTL; Pos, position in cM; SI_{95}, length of the 95% supporting interval centred on the most probable position (in cM); PVE_{max}, maximum phenotypic variance explained of a QTL within a MQTL; PVE_{mean}, average phenotypic variance explained of all the QTLs of a MQTL; N studies, number of studies reported in the MQTL.

Biparental QTL mapping is the most common method to detect QTLs for complex traits. However, in the last decade, GWAS became very popular as a complementary tool for QTL mapping. The powerful of GWAS resides in a higher mapping resolution than biparental mapping due to the use of germplasm collections with higher number of recombination events. The analyses conducted during the current PhD Thesis allowed the detection of numerous QTLs with small-effect contributing to the genetic control of agronomic, architectural and quality traits. The genome regions associated with the analysed traits will be of special interest for the identification of particular alleles to be introgressed in the breeding programmes.

In order to reduce the number of spurious associations, a mixed linear model accounting for the genetic relatedness between cultivars (random effect) and their population structure (fixed effect) was used. Additionally, an FDR threshold (Benjamini and Hochberg, 1995) was established and calculated according to the distance of the LD decay. To simplify the number of associations, marker-trait associations (MTAs) were grouped in QTLs (named as MTA-QTLs) based on linkage disequilibrium blocks or setting a confidence interval as reported in Laidò et al. (2014).

In chapter 2 it is shown a comparative GWAS for yield, yield components

and plant height (PH), between worldwide modern cultivars and Mediterranean landraces cultivated in the Mediterranean Basin. Yield is the most important and genetically complex trait in wheat, being controlled by a large number of small effect QTLs. In fact only 5 MTAs in modern cultivars were above the FDR threshold. Thus, its dissection into components helps to identify genomic regions involved in yield formation. The complexity of the yield related traits is revealed by the presence of MTAs for yield and components in all chromosomes. Grain number and grain weight are the main components affecting grain yield, and are usually negatively correlated (Sadras, 2007). The results of the comparison between pre and post green revolution accessions pointed out different stable genomic regions for the yield components, i.e. in modern cultivars only stable MTA-QTLs for grain weight were found, whereas in landraces most of the stable MTA-QTLs corresponded to grain number. This result could point out different yield formation strategies among the origin of the accessions.

The main interest of using landraces as genetic resources is their excellent adaptation to the local environments and their resilience to biotic and abiotic stresses. Among the traits for adaptation to drought prone environments, roots play an important role maintaining plant productivity under water limiting conditions. Roots exhibit a large plasticity depending on the environmental conditions of their place of origin as reported above and in chapter 4. In this chapter the seminal root system architecture (RSA) of Mediterranean landraces was analysed by GWAS. Out of 82 MTA-QTLs related to RSA, 64 were new identifications not coincident with other QTLs already mapped and 37 were located in the same position than yield related traits MTA-QTLs identified in chapter 2, suggesting the presence of pleiotropic QTLs.

Yield is one of the most important trait and the ultimate goal for plant breeding, thus other traits as grain quality remained in the background in the breeding programmes. However, the obtained flour is of high importance to produce high quality end-use products by the manufacturer industry. Most of quality traits have already been improved and there is a large knowledge of their genetic control. Nevertheless, as quality traits are also of quantitative nature, Mediterranean landraces could provide new alleles to widen their genetic diversity especially for grain protein content and yellow colour index. GWAS of chapter 5 identified 92 MTAs related to quality traits grouped in 37 genomic regions, of which 11 were located within the SI of previously identified QTLs (reviewed in Roselló et al. (2018)).

In this PhD Thesis two types of markers were used for GWAS, DArT and DArTseq markers. Although they were developed by the same company using the

same approach to identify a polymorphism, i.e. the identification of a SNP within a restriction site, the considerable reduction of the sequencing price made possible the use of next generation sequencing (NGS) techniques in DArTseq. This change enabled to increase the number of genomic fragments analysed and developing thousands of markers. The polymorphic markers in the collections used in this PhD Thesis were mapped against two different maps depending on the type of the marker: the consensus map of Maccaferri et al. (2014) for DArT markers, and the DArT v4 map for the DArTseq, available at https://www.diversityarrays.com/. As there are no common markers between both maps, in order to compare the genomic positions of the stable associations from the three studies, the markers were mapped against the durum wheat genome sequence (Maccaferri et al., 2019) and were depicted in figure 1. For those markers without position in the genome, their sequences were blasted against the durum wheat genome sequence to obtain their physical position (https://wheat.pw.usda.gov/cgi-bin/seqserve/blast_wheat.cgi).

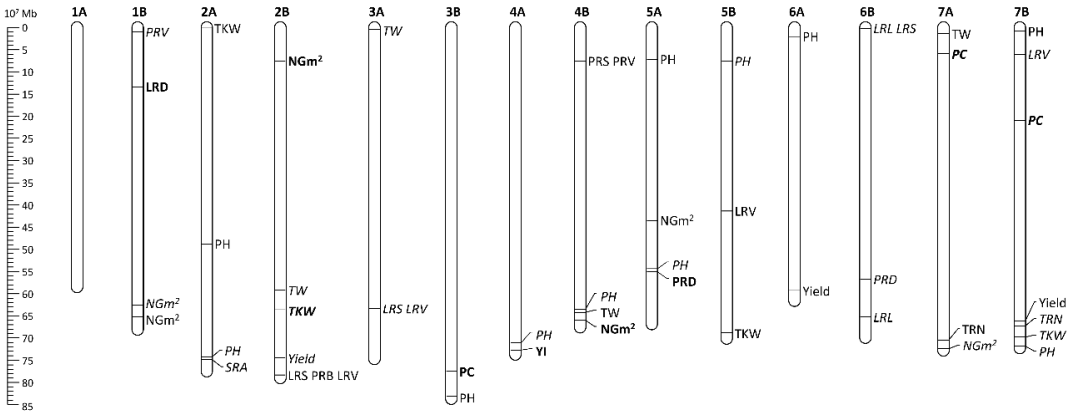

Figure 1. Marker trait associations with higher relevance for breeding identified from the GWAS for yield, RSA and quality related traits

Traits in bold were located within a MQTL or with a QTL previously mapped. Traits in italics were closely linked to a candidate gene model. The rule on the left indicates physical distance in Mb. NGm^2, number of grains per square metre. TKW, thousand kernel weight. PH, plant height. TRN, total root number. SRA, seminal root angle. LRL, total lateral root length. PRS, primary root surface. LRS, total lateral root surface. PRV, primary root volume. LRV, total lateral root volume. PRD, primary root diameter. LRD, mean lateral root diameter. PC, grain protein content. YI, yellow colour index. TW, test weight.

In total, 46 genome regions represented the most interesting associations for breeding. Eight chromosomes (2A, 2B, 3B, 4A, 4B, 5A, 7A and 7B) reported regions associated to yield related traits, seminal root traits and/or quality traits that were close to each other suggesting pleiotropic effect. Thirty-seven out of the 46 stable regions were reported for the first time in Mediterranean landraces and 9 corresponded with QTLs previously mapped. Additionally, 22 markers were located

in the vicinity of gene models showing homology with genes involved in the expression of the studied traits. These findings revealed the importance of the Mediterranean landraces searching new alleles to be introgressed in adapted phenotypes.

Although GWAS has been revealed as a powerful tool to identify molecular markers linked to traits of interest exploiting the diversity present in the durum wheat gene pool (Rasheed et al., 2018), translating the research to breeders is still a huge challenge as reviewed by Li et al. (2018). The first step for using the identified markers in breeding is to convert them into reliable PCR markers as SSR (when present in the sequence allele) or KASP (Kompetitive Allele-Specific PCR) assays.

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Conclusions

Conclusions

1. QTLs meta-analyses allowed the identification of consensus genomic regions (MQTL) related with agronomic and grain-quality traits, decreasing the confidence interval of the QTLs up to 50% in agronomic characteristics and up to 64% for quality traits.

2. QTLs meta-analyses grouped 477 QTLs in 71 MQTL for yield traits, phenology and biomass, and 249 QTLs for grain quality in 45 MQTLs distributed throughout the genome.

3. GWAS for yield and related traits revealed molecular differences matching with breeding intensity: whereas modern cultivars reported significant associations mainly for plant height and grain weight, Mediterranean landraces showed significant associations for spike and grain number.

4. Landraces originated on eastern and western Mediterranean countries showed a contrasting pattern of adaptation that was based on a different use of the water available before and after anthesis to generate yield.

5. Eastern Mediterranean landraces were well adapted to terminal drought due to their early anthesis and the efficient use of the water available in pre-anthesis to produce spikes and to accumulate water-soluble carbohydrates in the stems, which were lately remobilized to filling grains.

6. Western Mediterranean landraces had later anthesis time, shorter grain-filling period and higher grain-filling rate than Eastern Mediterranean landraces. They were efficient using post-anthesis water to increase the number and weight of grains.

7. Landraces from eastern Mediterranean countries had the largest seminal root size and the widest seminal root angle.

8. Correlations between seminal root architecture traits and yield related traits on landraces increased in the very low water-input environment.

9. GWAS identified 176 DArTseq markers for seminal root system architecture traits, which were grouped in 82 genomic regions. From them, 64 corresponded to *loci* non-previously reported and 37 were pleiotropic with yield related ones reported in this PhD Thesis.

10. For quality traits, GWAS identified 70 significant DArT markers that were grouped in 37 genomic regions. Four of these markers are of high interest due to their environmental stability and their positive phenotypic effect. Three out of them showed specificity for genetic subpopulations.

11. The information provided by the current PhD Thesis allowed the identification in the public wheat and durum wheat genome sequences of *hot spots* for putative candidate genes associated to relevant traits for wheat breeding.

12. The collection of durum wheat Mediterranean landraces used in the current PhD Thesis showed a large diversity for yield-related traits, seminal root system architecture and quality characteristics, and may be used to widen the genetic background managed in breeding programmes.

Conclusions

1. La meta-anàlisi de QTLs va permetre la identificació de regions genòmiques de consens (MQTLs) relacionades amb els caràcters agronòmics i de qualitat del gra, reduint l'interval de confiança dels QTLs fins a un 50% en els caràcters agronòmics i un 64% en els de qualitat.

2. La meta-anàlisi de QTLs va agrupar 477 QTLs en 71 MQTLs per a caràcters relacionats amb el rendiment, la fenologia i la biomassa i 249 QTLs dels caràcters de qualitat en 45 MQTLs distribuïts per tot el genoma.

3. El mapeig per associació pel rendiment i els caràcters associats va revelar diferències relacionades amb la intensitat de la millora genètica: mentre els cultivars moderns van mostrar associacions significatives, principalment, per l'alçada de la planta i el pes del gra, les varietats tradicionals mediterrànies van presentar associacions significatives pels nombres d'espigues i grans.

4. Les varietats tradicionals originàries dels països de l'est i l'oest del Mediterrani van mostrar diferents patrons d'adaptació basats en l'ús diferent de l'aigua disponible abans i després de l'antesi per la formació del rendiment.

5. Les varietats tradicionals de l'est del Mediterrani van mostrar una bona adaptació a la sequera terminal degut a la seva precocitat a antesi i a la utilització eficient de l'aigua disponible abans de la mateixa per produir espigues i acumular carbohidrats solubles en aigua que van ser mobilitzats al gra durant el seu ompliment.

6. Les varietats tradicionals de l'oest del Mediterrani van ser més tardanes a antesi, el seu període d'ompliment del gra va ser més curt i la taxa d'ompliment del gra va ser més elevada que en les varietats tradicionals de l'est. Van ser eficients utilitzant l'aigua després de l'antesi per augmentar el nombre i el pes dels grans.

7. Les varietats tradicionals dels països de l'est del Mediterrani van mostrar arrels seminals més llargues amb un angle més ample.

8. En les varietats tradicionals les correlacions entre les característiques que defineixen l'arquitectura del sistema radicular seminal i els caràcters relacionats amb el rendiment van augmentar en l'ambient on l'aportació hídrica va ser molt baixa.

9. El mapeig per associació va identificar 176 marcadors DArTseq pels caràcters relacionats amb l'arquitectura del sistema radicular seminal, que es van agrupar en 82 regions genòmiques. D'aquestes, 64 van correspondre a *loci* no identificats prèviament i 37 van ser pleiotròpiques amb regions genòmiques relacionades amb el rendiment descrites en aquesta Tesi Doctoral.

10. Pels caràcters de qualitat, el mapeig per asociació va identificar 70 marcadors DArT significatius que es van agrupar en 37 regions genòmiques. Quatre d'aquests marcadors tenen un gran interès degut a la seva estabilitat a través del ambients i el seu efecte fenotípic positiu. Tres d'ells van mostrar especificitat per les subpopblacions genètiques.

11. La informació proporcionada per aquesta Tesi Doctoral va permetre la identificació de *punts calents* a les seqüències públiques dels genomes del blat fariner i el blat dur per potencials gens candidats relacionats amb caràcters rellevants per la millora genètica del blat.

12. La col·lecció de varietats tradicionals mediterrànies de blat dur utilitzada en aquesta Tesi Doctoral va mostrar una àmplia diversitat per els caràcters relacionats amb el rendiment, l'arquitectura del sistema radicular seminal i la qualitat del gra, i podria ser utilitzada per ampliar la variabilitat genètica existent en els programes de millora genètica.

Conclusiones

1. El meta-análisis de QTLs permitió identificar regiones genómicas consenso (MQTLs) relacionadas con los caracteres agronómicos y de calidad del grano, reduciendo el intervalo de confianza de los QTLs hasta un 50% en los caracteres agronómicos y un 64% en los de calidad.

2. El meta-análisis de QTLs agrupó 477 QTLs en 71 MQTLs para caracteres relacionados con el rendimiento, la fenología y la biomasa y 249 QTLs de caracteres de calidad en 45 MQTLs distribuidos por todo el genoma.

3. El mapeo per asociación para el rendimiento y caracteres asociados reveló diferencias relacionadas con la intesisdad de la mejora genética: mientras que los cultivares modernos mostraron asociaciones significativas, principalmente, para la altura de la planta y el peso del grano, las variedades tradicionales mediterráneas presentaron asociaciones significativas para el número de espigas y de granos.

4. Las variedades tradicionales originarias de los países del este y del oeste del Mediterráneo mostraron patrones adaptativos contrastantes basados en el uso diferencial del agua disponible antes y después de la antesis para la formación del rendimiento.

5. Las variedades tradicionales del este del Mediterráneo mostraron una buena adaptación a la sequía terminal debido a su precocidad a antesis y al uso eficiente del agua disponible antes de la misma para producir espigas y acumular carbohidratos solubles en agua que se movilizaron al grano durante su llenado.

6. Las variedades tradicionales del oeste del Mediterráneo fueron más tardías a antesis, tuvieron un periodo de llenado de grano más corto y una tasa de llenado de grano más elevada que las variedades tradicionales del este. Fueron eficientes utilizando el agua después de antesis para aumentar el número y el peso del grano.

7. Las variedades tradicionales de los países del este del Mediterráneo mostraron unas raíces seminales más largas con un ángulo más ancho.

8. En las variedades tradicionales las correlaciones entre las características que definen la arquitectura del sistema radicular seminal y los caracteres relacionados con el rendimiento aumentaron en el ambiente donde el aporte hídrico fue muy bajo.

9. El mapeo por asociación identificó 176 marcadores DArTseq para los caracteres relacionados con la arquitectura del sistema radicular seminal, que se agruparon en 82 regiones genómicas. De las mismas, 64 correspondieron a *loci* no identificados previamente y 37 fueron pleiotrópicas con regiones genómicas relacionadas con el rendimiento descritas en esta Tesis Doctoral.

10. Para los caracteres de calidad, el mapeo por asociación identificó 70 marcadores DArT significativos que fueron agrupados en 37 regiones genómicas. Cuatro de estos marcadores tienen un gran interés debido a su estabilidad a través de los ambientes y su efecto fenotípico positivo. Tres de ellos mostraron especificidad para las subpoblaciones genéticas.

11. La información proporcionada en la presente Tesis Doctoral permitió la indentificación en las secuencias públicas de los genomas del trigo harinero y el trigo duro de *puntos calientes* para potenciales genes candidatos relacionados con caracteres relevantes para la mejora genética del trigo.

12. La colección de variedades tradicionales mediterráneas de trigo duro utilizada en la presente Tesis Doctoral mostró una amplia diversidad para los caracteres relacionados con el rendimiento, la arquitectura del sistema radicular seminal y la calidad del grano, y podría ser utilizada para ampliar el acervo genético de los programas de mejora genética.

Annexes

Annex 1

Table 1. Plant Material used in the PhD Thesis.

Cultivar[a]	Country of origin	Type	Genetic Subpopulation	q_i[b]	Chapter
Sinai No.8	Egypt	Landrace	East Mediterranean	0.548	2, 4, 5
Etith	Israel	Landrace	East Mediterranean	0.996	2, 3, 4, 5
Hati	Israel	Landrace	East Mediterranean	0.995	2, 3, 4, 5
IG-83920	Italy	Landrace	East Mediterranean	0.784	2, 4, 5
Hymera	Italy	Landrace	East Mediterranean	0.598	2, 4, 5
Aziziah 17/45[c]	Italy	Landrace	East Mediterranean	0.937	2, 3, 4, 5
Safra Jerash	Jordan	Landrace	East Mediterranean	0.859	2, 4, 5
Harani Auttma	Jordan	Landrace	East Mediterranean	0.997	2, 3, 4, 5
Horani Howawi	Jordan	Landrace	East Mediterranean	0.997	2, 3, 4, 5
Zugbieh Sutra	Jordan	Landrace	East Mediterranean	0.996	2, 3, 4, 5
Zoghbiyeh Safra	Jordan	Landrace	East Mediterranean	0.988	2, 3, 4, 5
Safra Maan	Jordan	Landrace	East Mediterranean	0.961	2, 3, 4, 5
PI-420946	Jordan	Landrace	East Mediterranean	0.766	2, 4, 5
PI-182667	Lebanon	Landrace	East Mediterranean	0.954	2, 3, 4, 5
PI-182669	Lebanon	Landrace	East Mediterranean	0.992	2, 3, 4, 5
PI-182671	Lebanon	Landrace	East Mediterranean	0.973	2, 3, 4, 5
Hourah	Lebanon	Landrace	East Mediterranean	0.977	2, 3, 4, 5
Tripshiro	Libya	Landrace	East Mediterranean	0.510	2, 4, 5
IG-95812	Syria	Landrace	East Mediterranean	0.994	2, 3, 4, 5
Vroulos	Cyprus	Landrace	East Balkan and Turkey	0.993	2, 4, 5
IG-82549	Cyprus	Landrace	East Balkan and Turkey	0.765	2, 4, 5
Mavraani	Greece	Landrace	East Balkan and Turkey	0.604	2, 4, 5
Capeiti	Italy	Landrace	East Balkan and Turkey	0.663	2
248-VII/7	Macedonia	Landrace	East Balkan and Turkey	0.995	2, 4, 5
259-VII/12	Macedonia	Landrace	East Balkan and Turkey	0.971	2, 4, 5
VII/13-X11	Macedonia	Landrace	East Balkan and Turkey	0.978	2, 4, 5
196/71	Macedonia	Landrace	East Balkan and Turkey	0.935	2, 4, 5
II/4	Macedonia	Landrace	East Balkan and Turkey	0.950	2, 4, 5
Belgrade 9	Serbia	Landrace	East Balkan and Turkey	0.996	2, 4, 5
1575	Serbia	Landrace	East Balkan and Turkey	0.991	2, 4, 5
IG-95847	Syria	Landrace	East Balkan and Turkey	0.994	2
BGE-018192	Turkey	Landrace	East Balkan and Turkey	0.992	2, 4, 5
BGE018350	Turkey	Landrace	East Balkan and Turkey	0.818	5
BGE018351	Turkey	Landrace	East Balkan and Turkey	0.621	2, 4, 5
BGE018353	Turkey	Landrace	East Balkan and Turkey	0.995	2, 4, 5
BGE-018354	Turkey	Landrace	East Balkan and Turkey	0.598	2, 4, 5
BGE019262	Turkey	Landrace	East Balkan and Turkey	0.994	2, 4, 5
BGE-019263	Turkey	Landrace	East Balkan and Turkey	0.914	2, 4, 5
BGE019264	Turkey	Landrace	East Balkan and Turkey	0.996	2, 4, 5
BGE019265	Turkey	Landrace	East Balkan and Turkey	0.996	2, 4, 5
BGE019266	Turkey	Landrace	East Balkan and Turkey	0.994	2, 4, 5
BGE-019270	Turkey	Landrace	East Balkan and Turkey	0.732	2, 4, 5

Cultivar[a]	Country of origin	Type	Genetic Subpopulation	q_i[b]	Chapter
Dalmatia 1	Croatia	Landrace	West Balkan and Egypt	0.632	2, 4, 5
Dalmatia 3	Croatia	Landrace	West Balkan and Egypt	0.796	2, 4, 5
440-IX/96	Croatia	Landrace	West Balkan and Egypt	0.844	2, 4, 5
441-IX/97	Croatia	Landrace	West Balkan and Egypt	0.678	2, 4, 5
PI-435057	Croatia	Landrace	West Balkan and Egypt	0.980	5
Milagro	Egypt	Landrace	West Balkan and Egypt	0.557	2, 4, 5
PI-366109	Egypt	Landrace	West Balkan and Egypt	0.967	2, 4, 5
PI-113395	Egypt	Landrace	West Balkan and Egypt	0.896	2, 4, 5
PI-113397	Egypt	Landrace	West Balkan and Egypt	0.990	2, 4, 5
Giza 2	Egypt	Landrace	West Balkan and Egypt	0.967	2, 4, 5
PI-559973	Egypt	Landrace	West Balkan and Egypt	0.986	2, 4, 5
MG 26429	Egypt	Landrace	West Balkan and Egypt	0.955	2, 4, 5
PI-60726	Egypt	Landrace	West Balkan and Egypt	0.965	2, 4, 5
PI-60727	Egypt	Landrace	West Balkan and Egypt	0.637	2, 4, 5
Mishriki	Egypt	Landrace	West Balkan and Egypt	0.682	2, 4, 5
Girgeh	Egypt	Landrace	West Balkan and Egypt	0.847	2, 4, 5
VII/18-X24	Macedonia	Landrace	West Balkan and Egypt	0.936	5
356-I/9	Montenegro	Landrace	West Balkan and Egypt	0.897	2, 4, 5
PI-435024	Montenegro	Landrace	West Balkan and Egypt	0.942	2, 4, 5
PI-435034	Montenegro	Landrace	West Balkan and Egypt	0.988	2, 4, 5
PI-435038	Montenegro	Landrace	West Balkan and Egypt	0.986	2, 4, 5
PI-435043	Montenegro	Landrace	West Balkan and Egypt	0.995	2, 4, 5
Zoco Yebel Hebil	Morocco	Landrace	West Balkan and Egypt	0.551	2, 4, 5
Dezassete	Portugal	Landrace	West Balkan and Egypt	0.495	2, 4, 5
Durazio Rijo Glabro	Portugal	Landrace	West Balkan and Egypt	0.892	2, 4, 5
Alentejo	Portugal	Landrace	West Balkan and Egypt	0.503	2, 4, 5
Caxudo de sete espigas	Portugal	Landrace	West Balkan and Egypt	0.630	2, 4, 5
Blanco de Corella	Spain	Landrace	West Balkan and Egypt	0.986	2, 4, 5
Blanquillo	Spain	Landrace	West Balkan and Egypt	0.993	2, 4, 5
Griego de Baleares	Spain	Landrace	West Balkan and Egypt	0.683	2
Gros de Cerdaña	Spain	Landrace	West Balkan and Egypt	0.994	2, 4, 5
Heraldo del Rhin	Spain	Landrace	West Balkan and Egypt	0.543	2, 4, 5
Pisana cañihueca	Spain	Landrace	West Balkan and Egypt	0.869	2, 4, 5
Blanquillón de Boñar	Spain	Landrace	West Balkan and Egypt	0.679	2, 4, 5
IG-92895	Algeria	Landrace	West Mediterranean	0.574	2, 4, 5
IG-92967	Algeria	Landrace	West Mediterranean	0.987	2, 3, 4, 5
IG-93030	Algeria	Landrace	West Mediterranean	0.994	2, 3, 4, 5
IG-93621	Algeria	Landrace	West Mediterranean	0.904	2, 3, 4, 5
IG-94009	Algeria	Landrace	West Mediterranean	0.964	2, 3, 4, 5
Dur de Medeah	Algeria	Landrace	West Mediterranean	0.981	2, 3, 4, 5
Tchirpan	Bulgaria	Landrace	West Mediterranean	0.685	2, 4, 5
Lozen 76	Bulgaria	Landrace	West Mediterranean	0.649	2, 4, 5
IG-96802	Crete	Landrace	West Mediterranean	0.644	2, 4, 5
IG-96851	Crete	Landrace	West Mediterranean	0.622	2, 4, 5

Cultivar[a]	Country of origin	Type	Genetic Subpopulation	q_i[b]	Chapter
Muri	Cyprus	Landrace	West Mediterranean	0.727	2, 4, 5
Reading	Egypt	Landrace	West Mediterranean	0.984	2, 3, 4, 5
Beladi Rouge	France	Landrace	West Mediterranean	0.658	2, 4, 5
Tounse	France	Landrace	West Mediterranean	0.485	2, 4, 5
Trigo Glutinoso	France	Landrace	West Mediterranean	0.697	2, 4, 5
Rubio enlargado d'Atlemteje	France	Landrace	West Mediterranean	0.711	2, 4, 5
Rapsani	Greece	Landrace	West Mediterranean	0.561	2, 4, 5
Carlantino	Italy	Landrace	West Mediterranean	0.825	2, 4, 5
Cicirelo	Italy	Landrace	West Mediterranean	0.690	2, 4, 5
IG-83905	Italy	Landrace	West Mediterranean	0.863	2, 4, 5
Carlo jucci	Italy	Landrace	West Mediterranean	0.673	2, 4, 5
Senatore Capelli	Italy	Landrace	West Mediterranean	0.899	2, 4, 5
Trinakria	Italy	Landrace	West Mediterranean	0.766	2, 4, 5
Razza 208	Italy	Landrace	West Mediterranean	0.867	2, 4, 5
Balilla Falso	Italy	Landrace	West Mediterranean	0.716	2, 4, 5
Milazzo	Italy	Landrace	West Mediterranean	0.810	2, 4, 5
Razza 181	Italy	Landrace	West Mediterranean	0.862	2, 4, 5
Razza 96	Italy	Landrace	West Mediterranean	0.911	2, 3, 4, 5
Reyati	Lebanon	Landrace	West Mediterranean	0.717	2, 4, 5
Maghoussa	Morocco	Landrace	West Mediterranean	0.936	2, 3, 4, 5
Merzaga	Morocco	Landrace	West Mediterranean	0.653	2, 4, 5
Red Beard	Morocco	Landrace	West Mediterranean	0.883	2, 4, 5
Morocco	Morocco	Landrace	West Mediterranean	0.986	2, 3, 4, 5
Saffi	Morocco	Landrace	West Mediterranean	0.994	2, 3, 4, 5
Ble Dur 250	Morocco	Landrace	West Mediterranean	0.993	2, 3, 4, 5
Oned Zenati	Morocco	Landrace	West Mediterranean	0.996	2, 3, 4, 5
Mahmoudi C	Morocco	Landrace	West Mediterranean	0.991	2, 3, 4, 5
Maghoussa Amizmiz	Morocco	Landrace	West Mediterranean	0.987	2, 3, 4, 5
Cobros	Morocco	Landrace	West Mediterranean	0.721	2, 4, 5
Raposinho	Portugal	Landrace	West Mediterranean	0.974	2, 3, 4, 5
Durazio Rijo	Portugal	Landrace	West Mediterranean	0.910	2, 3, 4, 5
Raspinegro	Portugal	Landrace	West Mediterranean	0.942	2, 3, 4, 5
Anafil	Portugal	Landrace	West Mediterranean	0.728	2, 4, 5
Espanhol	Portugal	Landrace	West Mediterranean	0.619	2, 4, 5
Amarelo Barba Preta	Portugal	Landrace	West Mediterranean	0.656	2, 4, 5
Tremes rijo	Portugal	Landrace	West Mediterranean	0.518	2, 4, 5
Arisnegro de Tenerife	Spain	Landrace	West Mediterranean	0.866	2, 4, 5
Basto Duro	Spain	Landrace	West Mediterranean	0.943	2, 3, 4, 5
Candeal de Salamanca	Spain	Landrace	West Mediterranean	0.644	2, 4, 5
Colorado de Jerez	Spain	Landrace	West Mediterranean	0.992	2, 3, 4, 5
Enano de Andújar	Spain	Landrace	West Mediterranean	0.985	2, 3, 4, 5
Fartó	Spain	Landrace	West Mediterranean	0.926	2, 3, 4, 5

Cultivar[a]	Country of origin	Type	Genetic Subpopulation	q_i[b]	Chapter
Pinet	Spain	Landrace	West Mediterranean	0.952	2, 3, 4, 5
Raspinegro Canario	Spain	Landrace	West Mediterranean	0.796	2, 4, 5
Raspinegro de Alcalá Guadaira	Spain	Landrace	West Mediterranean	0.994	2, 3, 4, 5
Recio de Almería	Spain	Landrace	West Mediterranean	0.953	2, 3, 4, 5
Verdial	Spain	Landrace	West Mediterranean	0.756	2, 4, 5
Alonso	Spain	Landrace	West Mediterranean	0.685	2, 4, 5
Andalucía 344	Spain	Landrace	West Mediterranean	0.627	2, 4, 5
Azulejo de Villa del Río	Spain	Landrace	West Mediterranean	0.949	2, 3, 4, 5
Blancal	Spain	Landrace	West Mediterranean	0.964	2, 3, 4, 5
Claro de Balazote	Spain	Landrace	West Mediterranean	0.959	2, 3, 4, 5
Entrelargo de Montijo	Spain	Landrace	West Mediterranean	0.974	2, 3, 4, 5
Farto cañifino	Spain	Landrace	West Mediterranean	0.613	2, 4, 5
Rubio de Miajadas	Spain	Landrace	West Mediterranean	0.706	5
Rubio de Montijo	Spain	Landrace	West Mediterranean	0.853	2, 4, 5
Ruso	Spain	Landrace	West Mediterranean	0.643	5
Semental	Spain	Landrace	West Mediterranean	0.689	2, 4, 5
Recio de Cañete	Spain	Landrace	West Mediterranean	0.912	2, 3, 4, 5
Souri	Tunisia	Landrace	West Mediterranean	0.736	2, 4, 5
Realforte	Tunisia	Landrace	West Mediterranean	0.984	2, 3, 4, 5
Biskri	Tunisia	Landrace	West Mediterranean	0.995	2, 3, 4, 5
Mindium	Turkey	Landrace	West Mediterranean	0.544	2, 4, 5
Akathiotico Naurotheri	Cyprus	Landrace	non classified	0.467	2, 4, 5
FAO 29.912	Cyprus	Landrace	non classified	0.401	2, 4, 5
De Santa Marta	France	Landrace	non classified	0.429	2, 4, 5
Iumillo	France	Landrace	non classified	0.417	2, 4, 5
Abu Fashit	Israel	Landrace	non classified	0.422	2, 4, 5
Juljulith	Israel	Landrace	non classified	0.399	2, 4, 5
JM-3987	Israel	Landrace	non classified	0.376	2, 4, 5
JM-3989	Israel	Landrace	non classified	0.414	2, 4, 5
Salti na Zinia	Jordan	Landrace	non classified	0.375	2, 4, 5
IG-84856	Lebanon	Landrace	non classified	0.470	2, 4, 5
PI-182666	Lebanon	Landrace	non classified	0.475	2, 4, 5
Haj Mouline	Morocco	Landrace	non classified	0.448	2, 4, 5
Marques	Portugal	Landrace	non classified	0.476	2, 4, 5
Lobeiro de grao escuro	Portugal	Landrace	non classified	0.332	2, 4, 5
18/71	Serbia	Landrace	non classified	0.447	2, 4, 5
IG-95841	Syria	Landrace	non classified	0.348	2, 4, 5
IG-95931	Syria	Landrace	non classified	0.407	2, 4, 5
Louri AP 5	Tunisia	Landrace	non classified	0.426	2, 4, 5
Hamira	Tunisia	Landrace	non classified	0.416	2, 4, 5
Zagorka	Bulgaria	Landrace			2

Cultivar[a]	Country of origin	Type	Genetic Subpopulation	q_i[b]	Chapter
Pansyiotico	Cyprus	Landrace			2
Kambourico Famaquita	Cyprus	Landrace			2
Kyperounda Yiallouriko	Cyprus	Landrace			2
Greece 14	Greece	Landrace			2
Greece 23	Greece	Landrace			2
Greece 24	Greece	Landrace			2
Iran 1	Iran	Landrace			2
Capeiti 8	Italy	Landrace			2
1640	Macedonia	Landrace			2
II/10	Macedonia	Landrace			2
Douro Boukowo	Morocco	Landrace			2
Du Maroc Battandier	Morocco	Landrace			2
D-1995	Russia	Landrace			2
IC 7640	Russia	Landrace			2
Rubio de Belalcázar	Spain	Landrace			2
Blanco Verdeal	Spain	Landrace			2
Clarofino	Spain	Landrace			2
Haurani 79-b	Syria	Landrace			2
Jennah Khetifa Rp4	Tunisia	Landrace			2
Gallareta	CIMMYT	Modern	Modern	0.996	2, 4, 5
Jupare	CIMMYT	Modern	Modern	0.996	2, 4, 5
Sula	CIMMYT	Modern	Modern	0.996	2, 4, 5
Vitron	CIMMYT	Modern	Modern	0.968	2, 4, 5
Arment	France	Modern	Modern	0.944	2, 4, 5
Claudio	Italy	Modern	Modern	0.994	2, 4, 5
Meridiano	Italy	Modern	Modern	0.993	2, 4, 5
Simeto	Italy	Modern	Modern	0.558	2, 4, 5
Svevo	Italy	Modern	Modern	0.979	2, 4, 5
Amilcar	Spain	Modern	Modern	0.987	2, 4, 5
Astigi	Spain	Modern	Modern	0.989	2, 4, 5
Boabdil	Spain	Modern	Modern	0.834	2, 4, 5
Bolido	Spain	Modern	Modern	0.995	2, 4, 5
Bolo	Spain	Modern	Modern	0.996	2, 4, 5
Senadur	Spain	Modern	Modern	0.993	2
Vitronero	Spain	Modern	Modern	0.992	2
Ancalei (PNTD/1)	Spain/CIMMYT	Modern	Modern	0.995	2, 4, 5
Hispasano (PNTD/3)	Spain/CIMMYT	Modern	Modern	0.978	2, 4, 5
Kronos	USA	Modern	Modern	0.995	2, 4, 5
Ocotillo	USA	Modern	Modern	0.886	2, 4, 5
Sahel 77	Algeria	Modern			2
Bonaerense valverde	Argentina	Modern			2
Buck candisur	Argentina	Modern			2
Buck cristal	Argentina	Modern			2

Cultivar[a]	Country of origin	Type	Genetic Subpopulation	q_i[b]	Chapter
Arivato	Australia	Modern			2
Bellaroi	Australia	Modern			2
Kalka	Australia	Modern			2
Saintly	Australia	Modern			2
Tamaroi	Australia	Modern			2
Macoun	Canada	Modern			2
Wakooma	Canada	Modern			2
Waskana	Canada	Modern			2
Ac Avonlea	Canada	Modern			2
Ac Morse	Canada	Modern			2
Ac Navigator	Canada	Modern			2
Ac Pathfinder	Canada	Modern			2
Commander	Canada	Modern			2
Strongfield	Canada	Modern			2
Chagual Inia	Chile	Modern			2
Chonta Inia	Chile	Modern			2
Guayacan Inia	Chile	Modern			2
Quilafen	Chile	Modern			2
Ucaro 1	Chile	Modern			2
Somat	CIMMYT	Modern			2
CIMMYT 67 - Plata 16	CIMMYT	Modern			2
CIMMYT 73 - Porto 5	CIMMYT	Modern			2
Aronas	Cyprus	Modern			2
Mesaoria	Cyprus	Modern			2
Arendeto	Ethiopia	Modern			2
Boohai	Ethiopia	Modern			2
Hora	Ethiopia	Modern			2
Marou	Ethiopia	Modern			2
Arcalis	France	Modern			2
Arcodur	France	Modern			2
Aronde	France	Modern			2
Artimon	France	Modern			2
Attila	France	Modern			2
Auroc	France	Modern			2
Epidur	France	Modern			2
Flodur	France	Modern			2
Bonitec	Germany	Modern			2
Burgos	Germany	Modern			2
Malavika	India	Modern			2
Malvaraj	India	Modern			2
Narbada 215	India	Modern			2
Raj 1555	India	Modern			2
Wh 896	India	Modern			2

Cultivar[a]	Country of origin	Type	Genetic Subpopulation	q_i[b]	Chapter
Oscar	Iran	Modern			2
Hazera	Israel	Modern			2
Creso	Italy	Modern			2
Flavio	Italy	Modern			2
Ambral	Italy	Modern			2
Anento	Italy	Modern			2
Ardente	Italy	Modern			2
Casiello	Italy	Modern			2
Fenice	Italy	Modern			2
Appulo	Italy	Modern			2
Fortore	Italy	Modern			2
Adamello	Italy	Modern			2
Cirillo	Italy	Modern			2
Zenit	Italy	Modern			2
Annouar	Morocco	Modern			2
Karim	Morocco	Modern			2
Massa	Morocco	Modern			2
Ouedezena	Morocco	Modern			2
Sarif	Morocco	Modern			2
Tassaout	Morocco	Modern			2
Yasmine	Morocco	Modern			2
1804	Morocco	Modern			2
1805	Morocco	Modern			2
1807	Morocco	Modern			2
1808	Morocco	Modern			2
1809	Morocco	Modern			2
Enduro	Netherland	Modern			2
Wadhanak 85	Pakistan	Modern			2
Alcamin	Portugal	Modern			2
Bakht	Russia	Modern			2
Selinogradskaja	Russia	Modern			2
Mexa	Spain	Modern			2
Camacho	Spain	Modern			2
Bidi 17	Spain	Modern			2
Abadia	Spain	Modern			2
Anibal	Spain	Modern			2
Donduro	Spain	Modern			2
Asdrúbal	Spain	Modern			2
Belladur	Spain	Modern			2
Bonzo	Spain	Modern			2
Boreal	Spain	Modern			2
Borgia	Spain	Modern			2
Carpio	Spain	Modern			2
Debano	Spain	Modern			2
Duradero	Spain	Modern			2

Cultivar[a]	Country of origin	Type	Genetic Subpopulation	q_i[b]	Chapter
Excalibur	Spain	Modern			2
Grecale	Spain	Modern			2
Imhotep	Spain	Modern			2
Jabato	Spain	Modern			2
Jaguar	Spain	Modern			2
Jiloca	Spain	Modern			2
Kidur	Spain	Modern			2
Lebrija	Spain	Modern			2
Mellaria	Spain	Modern			2
Mexidur	Spain	Modern			2
Paramo	Spain	Modern			2
Pingüino	Spain	Modern			2
Ponferrada	Spain	Modern			2
Prospero	Spain	Modern			2
Ramirez	Spain	Modern			2
Randur	Spain	Modern			2
Safari	Spain	Modern			2
Santadur	Spain	Modern			2
Semolero	Spain	Modern			2
Severo	Spain	Modern			2
Taranto	Spain	Modern			2
Tejón	Spain	Modern			2
Tetradur	Spain	Modern			2
Valgera	Spain	Modern			2
Valira	Spain	Modern			2
Esquilache	Spain	Modern			2
Ariesol	Spain	Modern			2
Euroduro	Spain	Modern			2
Awalbit-7	Syria	Modern			2
Brachoua	Syria	Modern			2
Chahba 88	Syria	Modern			2
Chanst	Syria	Modern			2
Fardes	Syria	Modern			2
Massara-1	Syria	Modern			2
Moosabil-1	Syria	Modern			2
Omrabi 5	Syria	Modern			2
Sabil 1	Syria	Modern			2
Stojocri-2	Syria	Modern			2
Stork	Syria	Modern			2
Aghrass-1	Syria	Modern			2
Ammar-1	Syria	Modern			2
Arislahn-5	Syria	Modern			2
Awali-1	Syria	Modern			2
Bicre	Syria	Modern			2
Chacan	Syria	Modern			2

Cultivar[a]	Country of origin	Type	Genetic Subpopulation	q_i[b]	Chapter
Cham-1	Syria	Modern			2
Derra	Syria	Modern			2
Guerou-1	Syria	Modern			2
Kabir1	Syria	Modern			2
Khabur-1	Syria	Modern			2
Lagonil-2	Syria	Modern			2
Lagost 3	Syria	Modern			2
Lahn	Syria	Modern			2
Loukos-1	Syria	Modern			2
Maamouri-1	Syria	Modern			2
Marsyr-1	Syria	Modern			2
Moulsabil 2	Syria	Modern			2
Murlagost-1	Syria	Modern			2
Omgenil-3	Syria	Modern			2
Omrabi 3	Syria	Modern			2
Omruf-2	Syria	Modern			2
ORT-1	Syria	Modern			2
Ouaserl-1	Syria	Modern			2
Ouasloukos-1	Syria	Modern			2
Quabrach-1	Syria	Modern			2
Sebah	Syria	Modern			2
Stojocri-3	Syria	Modern			2
Terbol97-3	Syria	Modern			2
Wadalmez-1	Syria	Modern			2
Zeina 1	Syria	Modern			2
Waha	Syria	Modern			2
Karim 80	Tunisia	Modern			2
Khiar 92	Tunisia	Modern			2
Mâali	Tunisia	Modern			2
Nasr 99	Tunisia	Modern			2
Razzak 87	Tunisia	Modern			2
Amanos 97.3.1	Turkey	Modern			2
Duraking	USA	Modern			2
Fjord	USA	Modern			2
Lakota	USA	Modern			2
Lloyd	USA	Modern			2
Matt	USA	Modern			2
Medora	USA	Modern			2
Modoc	USA	Modern			2
Monroe	USA	Modern			2
Orita	USA	Modern			2
Vic	USA	Modern			2
Ward	USA	Modern			2
West Bred Laker	USA	Modern			2
Colorado	USA	Modern			2

Cultivar[a]	Country of origin	Type	Genetic Subpopulation	q_i[b]	Chapter
Cortez	USA	Modern			2
Durex	USA	Modern			2
West Bred Turbo	USA	Modern			2

[a] IG-numbers are codes from ICARDA Germplasm Bank, PI-numbers are codes from USDA Germplasm Bank.

[b] q_i membership coefficient to a genetic subpopulation according to Soriano et al. (2016).

[c] Aziziah 17/45 was developed in Italy derived from an early maturing pure line selected from Syro-Palestinian landraces (Scarascia Mugnozza, 2005).

Annex 2

Table 1. Summary of the QTL studies for quality related traits reviewed in the meta-analysis for the quality-related traits. Number of QTLs for each trait in the studies are indicated.

Reference	Cross	Population type	Size	Environments (n)	Grain protein content	Gluten strength	Yellow colour index	Yellow pigment content	Test weight
Blanco et al., (2011)	Latino × Primadur	F2:F3	121	4	-	-	10	20	-
Conti et al., (2011)	UC1113 × Kofa	RIL	93	6	15	26	-	-	-
Groos et al., (2004)	Renan × Recital	RIL	194	3	1	1	-	-	-
Huang et al., (2006)	AC Karma × 87E03-S2B1	DH	414	4	1	-	-	-	3
Kunert et al., (2007)	Batis × Syn022	BC2F3:5	250	10	4	-	-	-	6
Kunert et al., (2007)	Zentos × Syn086	BC2F3:6	150		1	-	-	-	3
Li et al., (2009)	Neixiang 188 × Yanzhan 1	RIL	198	2	14	13	-	-	-
Ma et al., (2012)	Lang × CSCR6	RIL	82	4	2	1	-	-	-
McCartney et al., (2006)	RL4452 × AC Domain	DH	182	6	2	3	-	-	-
Patil et al., (2008)	PDW 233 (YAV'S'/TEN'S') × Bhalegaon 4	RIL F2:7	140	4	-	-	-	6	-
Patil et al., (2009)	PDW 233 × Bhalegaon 4	RIL	140	4	1	11	-	-	-
Prasad et al., (2003)	WL711 × PH132	RIL	100	5	8	-	-	-	-
Roncallo et al., (2012)	UC1113 × Kofa	RIL	93	6	-	-	27	24	-
Sun et al., (2008)	Chuan 35050 × Shannong 483	RIL	131	2	3	-	-	-	-
Sun et al., (2010)	Ning7840 × Clark	RIL	132	7	2	-	-	-	6
Sun et al., (2016)	Chuan 35050 × Shannong 483	RIL	131	6	7	-	-	-	-
Suprayogi et al., (2009)	DT695 × Strongfield	DH	185	6	19	-	-	-	-
Tsilo et al., (2010)	MN98550 × MN99394	RIL	139	3	4	-	-	-	-
Turner et al., (2004)	Avalon × Hobbit	RIL	200	3	6	-	-	-	-
Wang et al., (2012)	Weimai 8 × Luohan 2	RIL	302	3	28	-	-	-	-
Zhang et al., (2008)	UC1113 × Kofa	RIL	93	5	11	24	-	24	8
Total				93	129	79	37	74	26

RIL (recombinant inbred line); DH (double haploid); BC2 (backcross).