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Increasing genotypic productivity in post Green Revolution durum wheat: the case of Spain

Fadia Chairi

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Barcelona, September 2019

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Fadia Chairi

para optar al título de **Doctor** por la

Universidad de Barcelona.

Visto bueno de los directores de tesis

Director	Codirectora
Dr. José Luis Araus Ortega	Dra. M. Dolors Serret Molins

Barcelona, September 2019

“Se tourner vers ce dont on n’a pas à s’occuper et compter sur la partie obéissante de l’âme est une faiblesse. Se réjouir de la parure de sa propre essence en tant qu’elle appartient à celle-ci, bien que cette parure soit réelle, est une orgueilleuse erreur. Mais se diriger par l’universalité vers le vrai, c’est le salut.”

Avicenne Ibn Sina.

*Dear **God** thank you for everything...*

To my dear parents,
Noureddine** and **Rachida
Thank you for your unconditional love, your guiding hand, your support ...
Thank you for everything you have done for me
I cannot pay off your deeds but sure can make you now feel proud and happy

*To my husband, **Amine***
for your patience, love, friendship,
humor and willingness to eat out.

To my distracting little daughter
Elaa Yamina
who was no help in writing this thesis

To my brothers and Sister,
Alexandre, Ahmed** and **Mariem
Thank you for forcing me to write your beautiful names
in the dedications

To my family in law
(Douina family)
thank you is the least I can say to you

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Adrian, Armando, Fatima, Luisa, Shuan, Cristina, Noura, Safia, Rubèn, Susan how can I ever express my appreciation and gratitude to you? Thank you all for being both exemplary colleagues and faithful friends.

Abstract

It is of strategic importance for Mediterranean agriculture to develop new varieties of durum wheat with greater production potential, together with a better adaptation to adverse environmental conditions and better quality of grain. While durum wheat, which is the most strategic crop in the south Mediterranean agriculture, has benefited as other cereals of the Green Revolution, genetic progress in recent decades has been less evident, partly masked by climate change, and the underlying physiological mechanisms are not evident.

For this propose, the objective is to evaluate whether there have been agronomic and physiological changes associated with the genetic improvement of durum wheat grown in Spain after the Green Revolution and the environmental conditions where breeding gains have been higher. The study was based on a collection of 20 commercial varieties, grown in Spain between the beginning of the 70s of the last centuries and the present. The set was readapted during the last two cropping cycles and increased to a total of 23 cultivars, including more recent cultivars, released during the present decade. Cultivars were compared through consecutive years in wide range growing conditions secured by growing in three different sites differing in latitude and temperature, together with the implementation of support irrigation and different planting times. Changes in the genetic gain of the yield were evaluated between 2014 and 2016. Change in the agronomic and physiological parameters related to the genetic progress was evaluated between 2015-2016, and the changes caused by the improvement in the adaptation patterns through the study of the genotype by environment (GE) interaction was evaluated between 2017 and 2018.

It has been observed that the rate of genetic progress in the yield of durum wheat in Spain after the Green Revolution has been low it was estimated on 24 kg ha^{-1} y^{-1} (0.44% y^{-1} in term of relative gain) between 1980 and 2003 and has even stopped during the last decade until 2010. It was mainly due to increases in the number of kernels per spike (117 kernels m^{-2} y^{-1}), and spikes per unit area (0.24 kernels $spike^{-1}$ y^{-1}), while no clear trend in some grain quality traits (TKW and grain N concentration). Moreover, areal biomass at harvest

and grain nitrogen yield increased with the year of release of the cultivars for the entire period.

In addition, it has been observed that the more productive genotypes were characterized by a plant height of around 85 cm, small erect flag leaves, more open stomata, a better balance between N sources and N sinks and a higher capacity to re-fix CO_2 respired by the grain. Moreover, in general the non-laminar parts of the plants play a key role in providing assimilates during grain filling. Also, that the high heritability of most of the studied parameters allows their consideration as traits for phenotyping durum wheat better adapted to a wide range of Mediterranean conditions.

On the other hand, an improvement in genetic yield has been reported in warm environments and under optimal water conditions, environments similar to those of from where the germplasm provenance release or origin. The adaptation of semi-dwarf durum in Spain has shown a tendency to specific adaptation rather than large-scale adaptation. Two different patterns of selection have been reported due the G×E interaction and change in the ranking of genotypes: in the high yielding environments, plant favors more water uptake, with more transpiration and more open stomata (more negative value of $\delta^{13}C$, whereas, in low yielding environments, plant close stomata and favors more water use efficiency (positive value of $\delta^{13}C$).

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Introduction

1. Wheat importance and production in the world

Wheat is along with maize and rice one of the most important crops in the world and the first strategic staple for the majority of word's populations. The annual production of wheat is estimated to be around 771 million tones which makes it the second largest crop in the world behind maize (FAOSTAT, 2019). It is grown on about 219 million hectares all over the world (FAOSTAT, 2019) (Figure 1). Wheat suffices for 21% of the food calories and 20% of the protein requirement to more than 4.5 billion people in 94 developing countries (Braun et al., 2010). While main wheat crop is the so-called bread wheat (*Triticum aestivum* L.) the durum wheat (*Triticum turgidum* L. *subsp. Durum* (Desf) Husn) even if it occupies the ninth position as a crop in terms of cultivation Worldwide, it is a major crop around on the Mediterranean basin as well as a cultural pillar (FAOSTAT, 2019).

The worldwide demand for wheat is increasing and predictions suggest that grain production must increase annually by 2.4% to meet human needs in 2050 (Ray et al., 2013). However, current increases in worldwide wheat productivity are just 0.9% per year, which makes it imperative to found ways to raise the increase in wheat production (Ray et al., 2013).

The Introduction of the Thesis examines the evidences worldwide for recent genetic gains in wheat yield accounted after the Green Revolution and, in the cases genetic advanced has been recorded, what target environments, as well as agronomical and morphophysiological traits have been involved. Further the Thesis develops the specific case of durum wheat in Spain, through three chapters depicting research results (using scientific paper format) achieved using a post-Green Revolution panel grown in Spain through several years, sites and growing conditions. Finally, a general Discussion focuses on future prospects for identifying traits for raising yield with particular reference to durum wheat grown in Spain.

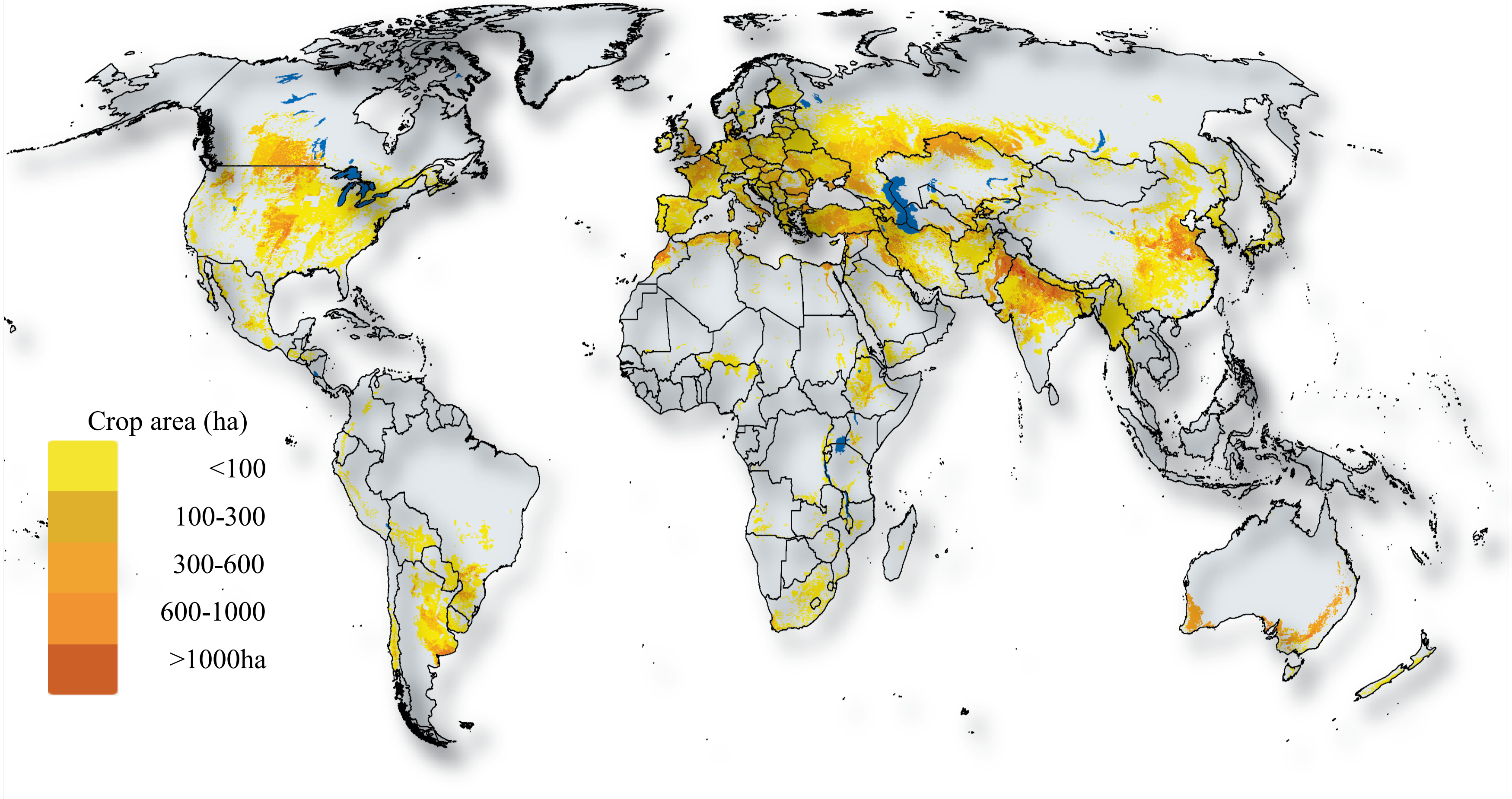

Figure 1: Global Wheat Cultivation. Darker colors indicate areas where more wheat is grown. Map based on You et al. 2014

2. Recent trends in post-Green Revolution genetic gain

The ways in which breeding has improved yield in the past can be properly assessed through retrospective studies. Cultivars released at different times in the past are cultivated simultaneously under certain conditions, thereby eliminating the effects on yield of improved management practices (Slafer et al., 1994).

During the Green Revolution of the 1960s and 1970s, the yield increases of the first semi-dwarf cultivars were associated with reduction in stature and a concomitant increase in harvest index, together with a reduction in lodging, and hence a greater responsiveness to high soil fertility and irrigation (Foulkes et al., 2011).While the jump in grain yield associated with the Green Revolution has accounted for more than doubling grain yields during the sixties and even seventies of the past century, the further trends in genetic gain, once the main benefits from new genotypes and associated crop management practices have been realized, remain less evident.

At the word level, the yield increases of wheat from 1961 to 1990 was 2.6% per year, but from 1990 to 2007 the average yield increase was only 0.52% per year (Alston et al., 2010). From 1961 to present, genetic gains reported in the high yielding environments (particularly irrigated areas or high rainfall areas together with adequate management practices) have been of the order of 1.2% to 0.5% (Fig. 2). Thus for the CIMMYT conditions in NE Mexico values range between 0.59% and 1% (Aisawi et al. 2015, Lopes et al. 2012, Manès et al. 2012) , while in the case of China these range between 0.48% and 1.05% (Zhou et al. 2007; Zheng et al. 2011; Xiao et al. 2012), and the United Kingdom (Shearman et al., 2005; Pennacchi et al. 2018) values ranged between 1.1 % and 1.2%. In Chile the genetic gain reached 63.4 kg $ha^{-1}y^{-1}$ (0.67% y^{-1}) (Pozo et al., 2019). However, there are also some cases in which the genetic progress appears to be showing a plateau, for example Brazil spring wheat between 1999 and 2009 (Beche et al., 2014). In these study, genetic gain has ranched 0.45%. In addition, Brisson et al. (2010) found genetic progress in yield potential in France had been counteracted from 1990 onward by climate change (particularly by heat stress) and in Spain Chairi et al. (2018) found an increase in grain yield with the year of release of cultivars at a rate of 0.44% y^{-1} from 1980 to 2003, with no clear additional improvements thereafter.

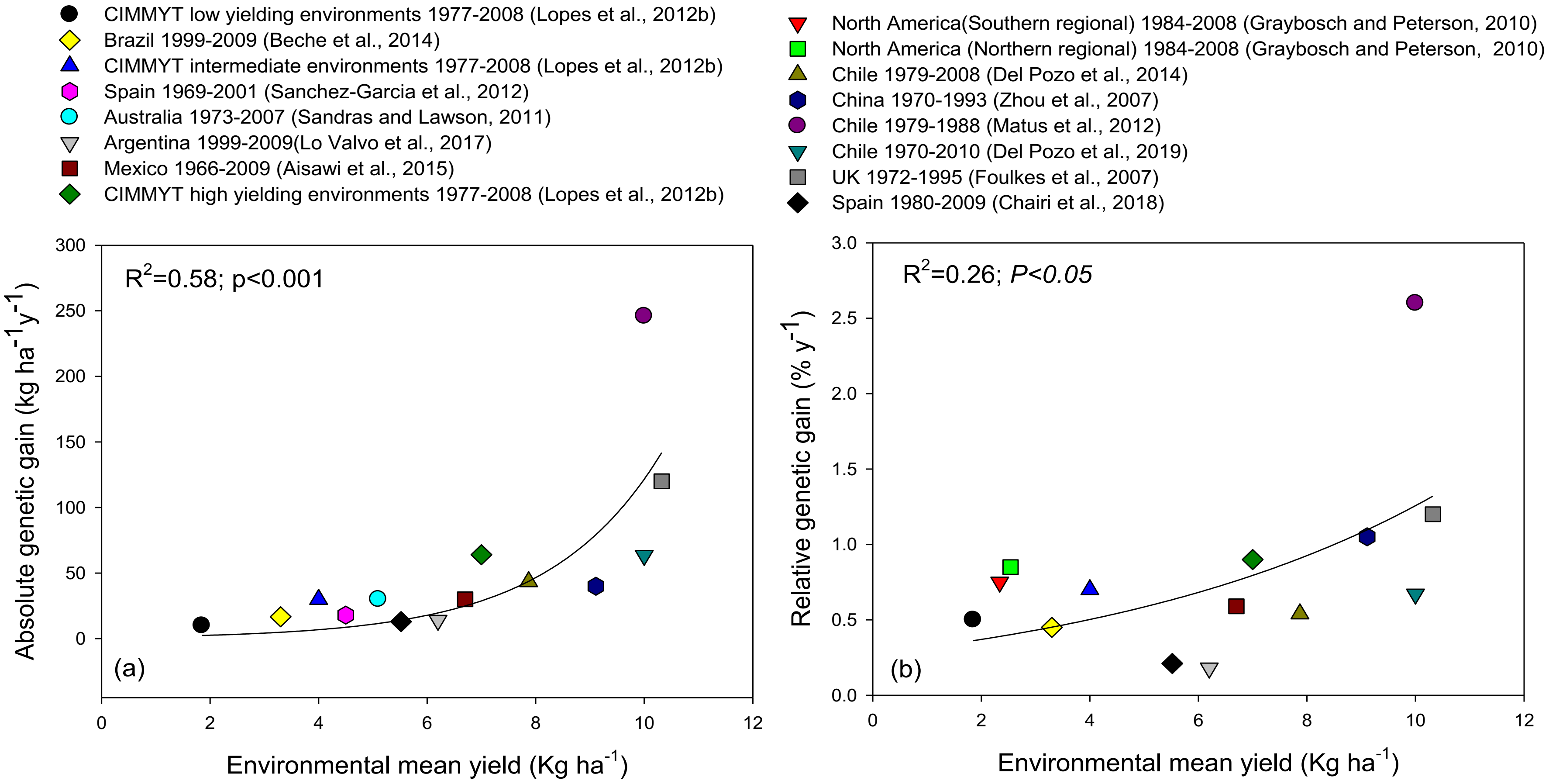

Figure 2: Relationship between the rate of post-Green Revolution yield improvement of wheat (a) absolute genetic gain and (b) relative genetic gain and the growing conditions, expressed as environmental mean yield

From the previous paragraph, it is evidenced that current wheat genetic advance, even in the best cases, is clearly below the annual rate of 2.4 % required to meet human needs in 2050 (Ray et al., 2013). Moreover in the less productive environments, where abiotic stress limit the actuals grain yield the evidences of genetic advance during the decades after the Green Revolution seems even less evident (Acreche et al., 2008; Alston et al., 2010) .For example, winter wheat in the great plains of North America between 1984 and 2008 ranged between 0.75% in the Southern Regional and 0.85% in Northern Regional Performance Nurseries (Graybosch and Peterson, 2010), in Argentina, the genetic progress in term of yield was 0.18% after 1999 (Lo Valvo et al., 2017) and In South Australia, under rain fed conditions, the annual rate of increase in grain yield (GY) was 30 kg ha^{-1} between 1973 and 2007 (Sadras and Lawson, 2011) (Fig. 2).

Knowledge of the changes in agronomical, physiological and morphological traits associated with genetic gains in yield potential is essential to improve understanding of yield-limiting factors and to inform future breeding strategies.

2.1.Agronomical traits

Crop yield is a quantitative trait of great complexity, controlled by many plant traits. Indeed, it is the result of complex interactions throughout the growing season with the direct or indirect expression from most of the genes (Slafer., 2003; Slafer and Rawson., 1994) (Fig. 3). Any attempt to increase it would be more likely based on a deep understanding of its generations. Commonly, yield can be decomposed into fractions that allows a better understanding of the yield components traits that are behind the increase of production. One of the most common ways to address performance improvement is the conceptual framework proposed by Donald and Hamblin (1976), which consists of considering it as the product of the plant biomass and harvest index:

Yield = Aerial biomass at maturity x Harvest index (HI: the proportion of the above-ground biomass allocated to grain)

Also, yield can be divided into its main agronomical components, i.e. spikes per unit ground area (or spike density), kernels spike^{-1}, and individual kernel weight. Thus, grain yield expressed on unit area basis it typically decomposed as.

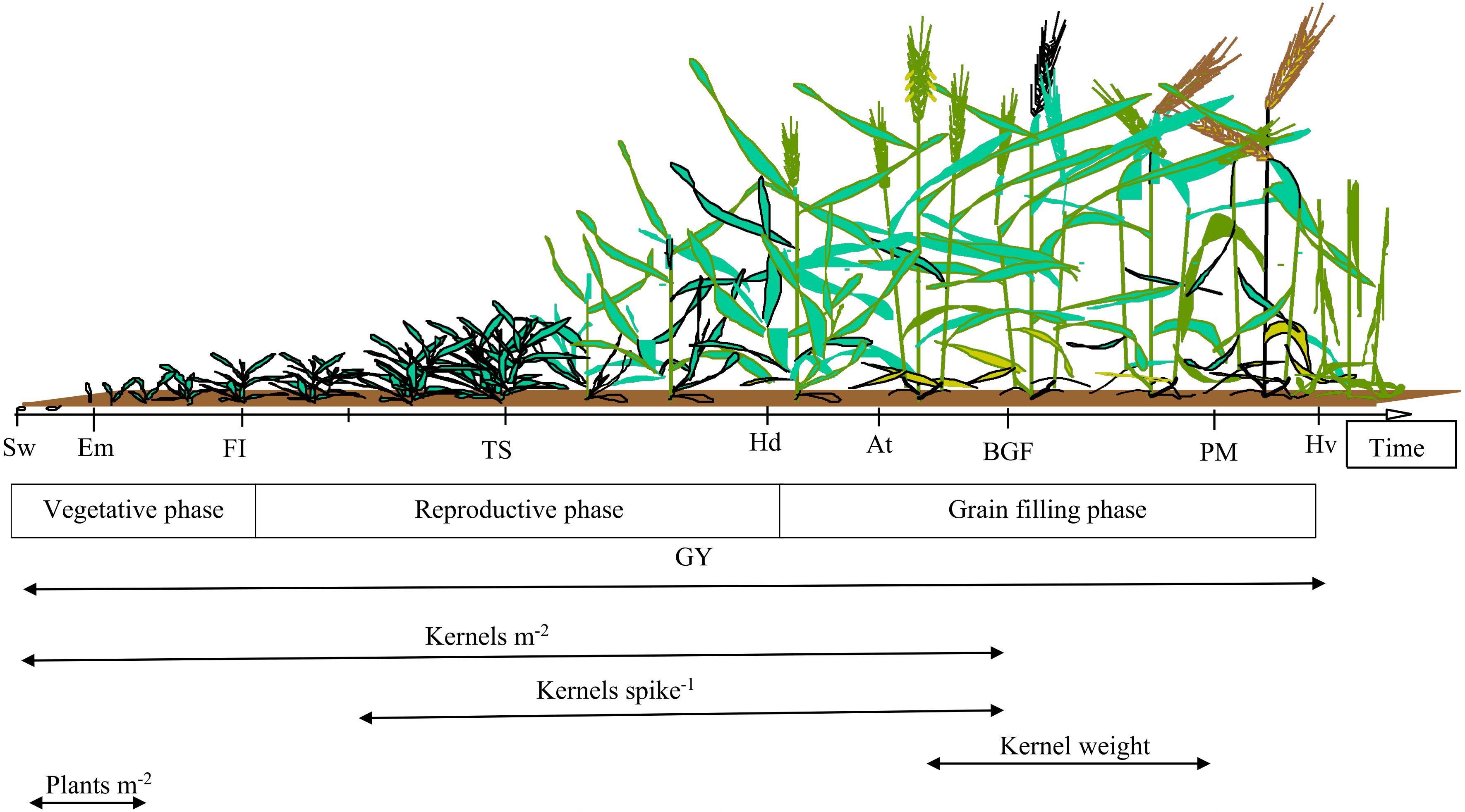

Figure 3:: Diagram of wheat growth and development showing the different stages of the crop: sowing (Sw), seedling emergence (Em), floral initiation (Fl), terminal spikelet initiation (TS), heading (Hd), anthesis (At), beginning of the grain-filling period (BGF), physiological maturity (PM), harvest (Hv). Boxes indicate the periods of differentiation or growth of some organs within the vegetative, reproductive, and grain-filling phases. Redrawn from Slafer and Rawson (1994)

Yield = Spike density (spikes per unit ground area) x kernels spike^{-1} (number of kernels per spike) x kernel weight (1000-kernels weight /1000 seeds)

As a consequence, of the Green Revolution, yield gains been reported to be associated with increases in HI, while has been little change in total aerial biomass. Alternatively it may be also shown that genetic gain associated with the Green Revolution was basically the consequence of an increase in kernels m^{-2} (Calderini et al., 1995; Reynolds et al., 1999) because an increase in the number of kernels spike^{-1}, (Fig. 4a) (Royo et al., 2007; Sayre et al., 1997). Further studies have found that semi dwarf wheat cultivars showed more fertile florets per ear as a consequence of increased assimilate partitioning to the ear during the pre-flowering period (Calderini et al., 1995; Miralles et al., 1998). Concerning spike density and individual kernel weight reports indicate minor changes (Fig. 4b.c) (Shearman et al., 2005; Sayre et al., 1997), even when for kernel weight there are contradictory results about the changes occurred after the Green Revolution. Thus where there are studies which conclude an increase with grain weight has occurred (Aisawi et al., 2015; Calderini et al., 1995a, Lopes et al., 2012), other reports conclude that a trend towards reduced grain weight has occurred for cultivars released before 1980 (Calderini et al., 1995a; M. Sanchez-Garcia et al., 2012).

The increase in the number of kernels spike^{-1} and therefore in HI caused by the Green Revolution was due to the introduction of dwarfing genes (Rht) which decreased the height of the plant without altering its final biomass. The the maximum achievable HI appear to be placed around 0.6 (Austin et al., 1980). However, recent studies have shown little or no improvement in the harvest index achieved during the last decades of the twentieth century, which seems to have stagnated at values close to 0.50-0.55 (Fig. 5) in the most optimal environments (Aisawi et al., 2015; Sadras and Lawson, 2011; Sayre et al., 1997). Despite the theoretical physiological limit, developing varieties that exceed a harvest index of 0.50 remains difficult in many environments (Fischer and Edmeades, 2010). These apparent limitations contribute to consider the increase of the biomass of the crop in maturity, without altering the harvest index, as one of the main strategies to be promoted in future improvement programs (Parry et al., 2011).

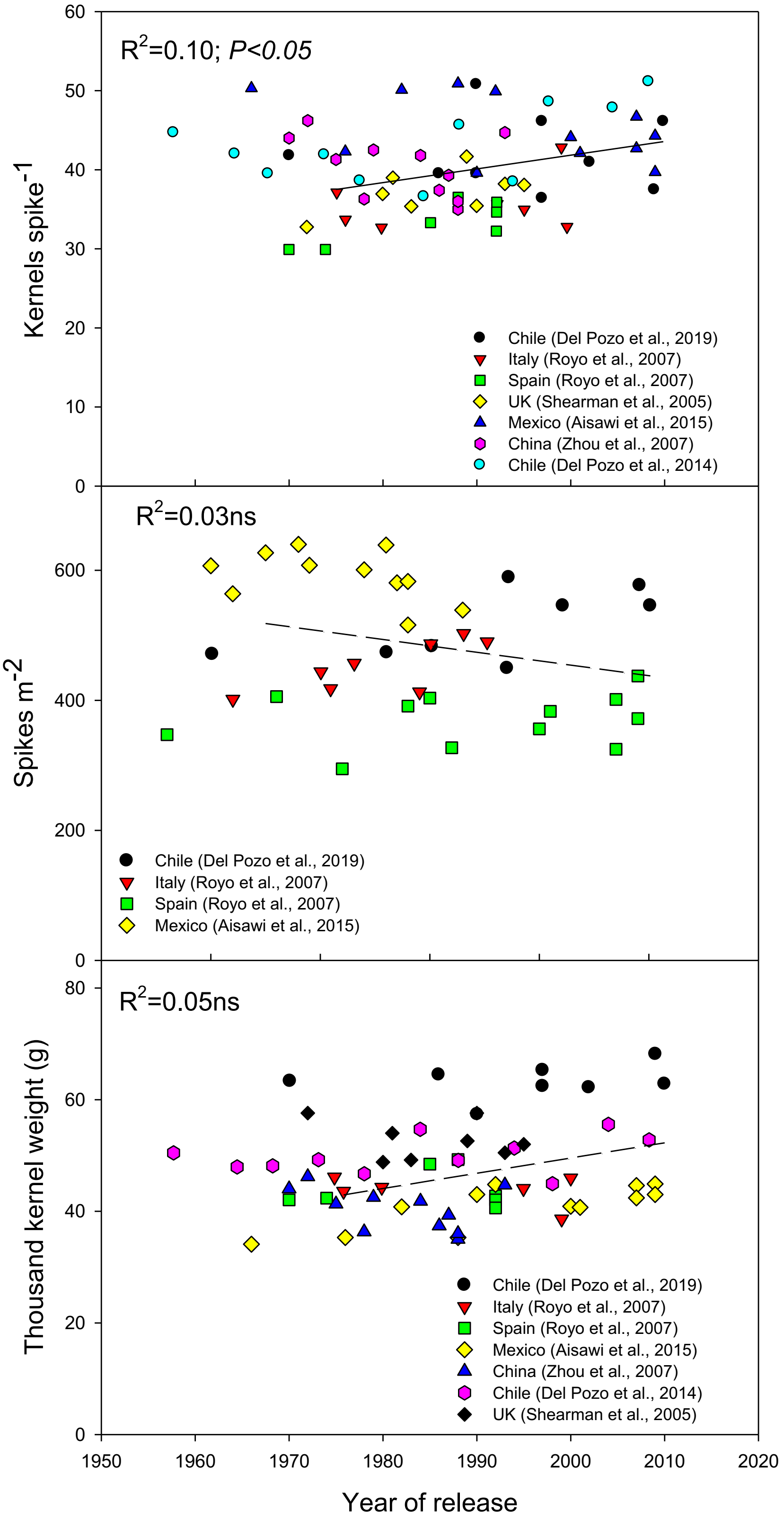

Figure 4:kernels spike^{-1} (a); Spikes m^{-2} (b) and Thousand kernels weight (c) of cultivars released in distinct years in some countries

Studies after the Green Revolution reports contradictory results about the change on biomass. For example, an increase was reported in Mexico between 1966 and 2009 (Aisawi et al., 2015), in Australia between 1980 and 2008 (Sadras and Lawson., 2011) and in UK between 1983 and 1995 (Shearman et al., 2005), while no changes were reported in Spain between 1940 and 1998 (Acreche et al., 2008; Royo et al., 2007), and Italy between 1930-1992 (Royo et al., 2007), and in the case of Argentina a decrease was reported between 1999 and 2011 (Lo Valvo et al., 2017).

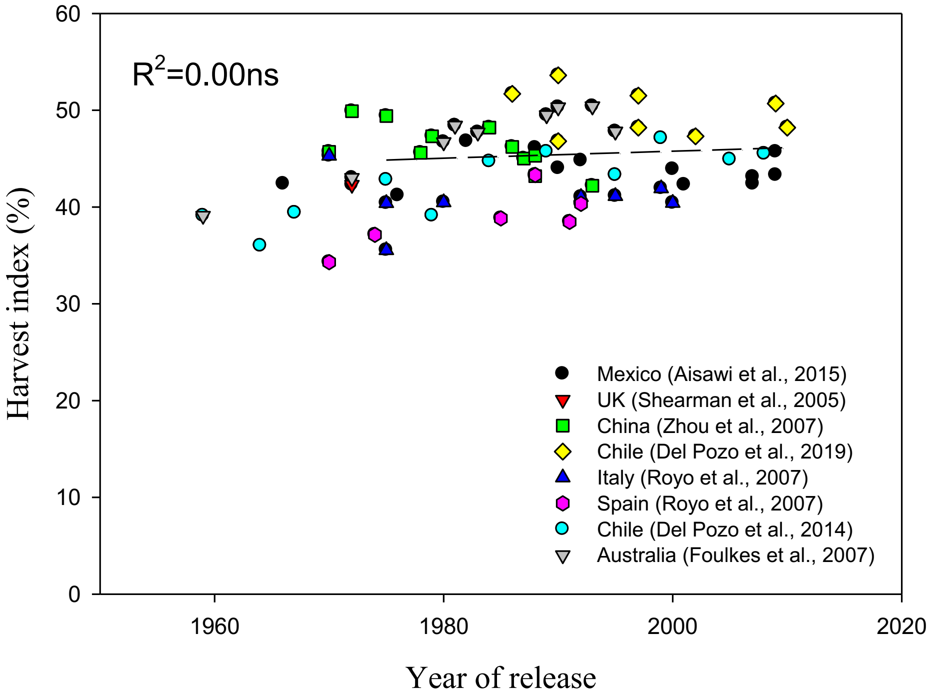

Figure 5:harvest index of cultivars released in distinct years in some countries

2.2.Morphophysiological traits

An increase in yield depends on a myriad number of morphophysiological traits contributing to a more efficient uptake and further use of the resources the plant needs (light, water, and nutrient). Thus for example, water and nutrient acquisition and a canopy's efficiency in capturing light, and photosynthesis efficiency, sink strength and translocation, are some of the factors which largely contribute to crop yield (Parry and Hawkesford, 2012). If we consider the light as the main resource, then biomass and yield may be formulated in terms of crop photosynthesis and will depend on (i) the ability of the canopy to intercept and capture light; (ii) the duration of light capture; and (iii) the photosynthetic capacity and efficiency of the canopy. For example, when water is the main resource limiting yield, crop productivity will depend on (i) the ability to extract water from the soil; (ii) the duration of water extraction and (iii) the efficiency with which the water is used.

Yield as a function of incoming radiation can be expressed as follows:

$$GY = IR \times AR \times PE \times HI$$

where IR is the total quantity of incident solar radiation received by the crop throughout the growing period; AR is absorbed radiation; PE is the overall photosynthetic efficiency of the crop: the total dry matter produced per unit of intercepted photo synthetically active radiation (PAR); and Harvest index.

In dry conditions, according to Passioura (1977) grain yield depends on (i) water use (i.e. the amount of water used by the crop), (ii) the water use efficiency (WUE: the efficiency for producing above-ground dry biomass per unit of water used), and (iii) the Harvest Index.

$$GY = WU \times WUE \times HI$$

2.2.1. Photosynthetic activity

The products of photosynthesis are the primary determinants of plant productivity, and increasing photosynthesis has been widely recognized as a key trait to increase future yields (Long et al., 2006; Zhu et al., 2010; Parry et al., 2011; Raines, 2011). While biomass is a function of the total photosynthesis of the canopy over time, the flag leaves have, been traditionally identified as the major contributors to grain yield (Thorne, 1973; Evans i Rawson, 1970; Araus i Tapia, 1987). However, recent studies are putting more and more emphasis in the photosynthetic contribution of non-laminar tissues to grain yield (Sanchez-Bragado et al., 2014b, 2014a). Thus Maydup et al. (2012) reports an increase in the photosynthetic contribution of the ear to grain filling of bread wheat during the breeding period 1920–2008 in Argentina.

Few studies have reported a genetic advance of the photosynthetic rate in semi-dwarf genotypes. Shearman et al., (2005) found that genetic progress in GY in UK winter wheat from 1972 to 1995 was based mainly on improvement in harvest biomass that was associated with a higher radiation use efficiency (RUE) during the stem elongation period, which suggest sink versus source interactions being also involved. Thus, longer stem elongation phase mean accumulated crop growth during stem elongation would be greater -as more radiation would be intercepted by the canopy Slafer et al., 2005). In Henan Province, in China an increase in grain yield in genotypes released between 1981 and 2008 were positively correlated with flag leaf net photosynthetic rate during grain filling with the most recent cultivars exhibiting higher photosynthesis together with higher stomatal conductance and transpiration rates (Zheng et al., 2011) An study for wheats released in the Shandong Province (China) suggested that genetic gains in grain yield were achieved through improving crop photosynthesis at and after heading, and the source for grain filling may have benefited from increased stem WSC in stems at anthesis (Xiao et al., 2012).

Stay-green - delay senescence

Senescence is a genetically programmed and environmentally influenced process resulting in the destruction of chlorophyll and the remobilization of nutrients to younger or reproductive parts of plants (Vijayalakshmi et al., 2010). Wheat plants exhibiting a functional stay-green phenotype are able to maintain photosynthesis for longer during the grain filling period (Thomas et al., 2000; Thomas and Smart, 1993), therefore increasing RUE of the crop and thus grain yield (Hawkesford et al., 2013) and contributing to yield stability under stress (Vijayalakshmi et al., 2010). Grain- yield progress of CIMMYT advanced lines released from 1977 to 2008 was associated with fewer days to heading, cooler canopy temperature at grain filling, increased stay green, and higher grain weight (Lopes et al., 2012b). For the high yielding conditions of UK, Pennacchi et al., (2018), reported that early vigor, accumulated green area, and stay green correlated positively with yield and that these traits determine the length of the period over which plants will intercept light and be able to convert it into biomass, thus, indirectly, these traits contribute to both efficiency of the plant to intercept and convert light into biomass by extending the period over which plants are photosynthetically active. Moreover, flag leaf longevity also appears to be more beneficial in terms of increasing yield potential than the photosynthetic rate (Carmo-Silva et al., 2017).

Morphological basis

Similar changes in flag leaf morphology with breeding progress (e.g., smaller, more erect flag leaves, higher N per unit area) were reported at CIMMYT (Fischer et al., 1998) and United Kingdom (Shearman et al., 2005) as associated to post Green Revolution breeding. Leaf posture and area considerably affects the interception of incident radiation and its distribution within the canopy. Light penetration into the canopy is improved in genotypes with erect leaves therefore improving the RUE at the single leaf and whole canopy levels. Consequently, upper leaves are less likely to be over-saturated by light and leaf sheaths may become significant sources of assimilates and contribute more to total crop biomass (Hay and Walker 1989). A slower senescence of lower leaves was also noted in genotypes with erect leaves (Austin et al. 1976). When hydraulic conditions are not optimum, plants in general, and crop plants in particular, will limit above-ground biomass essentially through leaf area reduction, in order to maintain hydraulic balance within leaves with a concomitant increase in density of veins (for water supply) and stomata (for water exit). (Roche et al., 2015).

Open stomata, canopy temperature and carbon isotope composition

Studies under irrigated conditions in Mexico suggest that improvements in photosynthesis per unit leaf area may already have occurred with an increase in stomatal conductance (e.g. Fischer et al., 1998; Reynolds et al., 1999), although apparently this has not been translated to a greater total biomass in field-. In fact, reports on wheat and other crops suggest increase in stomatal conductance as being one of the factors behind genetic advance in the modern cultivars (Roche 2015). The results on the stable carbon isotope signature also supports the contribution of higher stomatal conductance increasing photosynthesis and eventually yield. Interdependence between water and CO_2 is not limited to stomata and its subcavity, as it seems that these two molecules share diffusion pathways also through the mesophyll (Ferrio et al., 2012). Therefore, it is not surprising that rates of photosynthesis and stomatal conductance have been tightly correlated (Wong et al., 1979). Canopy temperature depression (Lopes and Reynolds 2010), together with discrimination against the heavier stable carbon isotope (^{13}C), are two physiological traits that help to estimate which genotypes extract more water than others Araus et al., 2003; Slafer et al., 2005). Indeed, genotypes having lower canopy temperature at midday have relatively better water status (Blum et al., 1982). Cool canopy temperatures have been associated with increased plant access to water, as a result of deeper roots (Li et al., 2019; Lopes and Reynolds, 2010), and therefore with better stomatal conductance. Higher stomatal conductance have been proposed as being associated with genetic advance in yield potential (Fischer et al., 1998) as well as under moderate to medium stress conditions (Roche, 2015). This is further supported by the positive phenotypic correlations usually found between yield and carbon isotope discrimination ($\Delta^{13}C$) or the negative correlations with carbon isotope composition ($\delta^{13}C$) within Mediterranean environments (Araus et al., 1998, 2003, 2013, 1998; Merah et al., 2001; Monneveux et al., 2006) meaning that genotypes that are able to maintain higher water use (even if it is at the expense of a lower WUE) are the most productive (Araus et al., 2013, 2008). In fact the effective use of water (EUW) and not WUE is the target of crop yield improvement even under drought stress (Blum, 2009). Several previous studies showed phenotypic correlations between CTD and genetic gains in grain yield among CIMMYT wheat releases (Aisawi et al., 2015; Fischer et al., 1998; Lopes et al., 2012b).

2.2.2. Root characteristics

Root systems play an important role in crop performance. Roots are important for anchorage and water and nutrient uptake from the soil solution (Foulkes et al., 2011). For example, deeper rooting has been demonstrated to be an important trait to capture water stored at depth. This is especially important during later growth stages when water is used for grain filling as it directly impacts on grain yield (Lopes and Reynolds, 2012). It has been reported that under moderate drought, access to additional water from the subsoil during grain filling can contribute to an increase in wheat yield as high as 0.62 Mg ha^{-1} (Kirkegaard et al., 2007). In dry areas, Aziz et al. (2017) have reported that reductions in root biomass with wheat cultivars released over time resulted in higher grain yields and that the root:shoot ratio—which depend on the partitioning of photosynthate carbon—and the proportion of total biomass allocated to the roots were higher in varieties released early (between 1958 and 1989) than those released between 1994 and 2007.

2.3. Adaptation to the environment

Understanding the mechanisms involved in adapting varieties to the environment, as well as identifying the main climatic variables that determine the sensitivity of the varieties to the environment, is crucial for the development of new varieties, more adapted and with a higher and more stable performance.

Most studies published to date around the world on the genetic improvement of wheat have focused their attention on yield and eventually its agronomical and/or morphopysiological components. However, there are few works that address the study of the changes produced by the improvement on the germplasm adaptation patterns. The breeders have followed several strategies to favor the adaptation of the varieties to the environment. In the CIMMYT the selection is carried out in environments of high potential with the aim of obtaining varieties with wide adaptation, leading to significant improvements in yield in a wide range of agroecological conditions (Braun et al., 1997). Recent studies have shown that in limiting environments, the improvement in yield has been due to the selection of varieties with low interaction with the environment and high potential yield (Cattivelli et al., 2008), while in high potential environments, the most successful varieties of cereals have shown a tendency towards a more specific adaptation (Royo et al., 2008; Sánchez-Garcia, 2012).

The adaption to the environment may be behind the contradictory reports about the existence or lack of genetic advance after the Green Revolution. In general, breeding for specific adaptation tends to imply greater genetic gains for these particular environmental conditions but in exchange of increased costs in terms of breeding relative to those for a wide adaptation strategy. The relatively high costs may be due to increased field testing rather than to duplication of breeding stations, because crossing and hybridization operations can be centralized in a single national station providing each subregion with novel germplasm for local selection (and, possibly, genetic resources for local testing to identify parent material of specific interest) (Annicchiarico, 2002).

The fact that durum wheat is comparatively the small brother of bread wheat, together with the perception that the genetic variability available for durum wheat is lesser than for the hexaploid bread wheat, makes the absence of studies on durum wheat a matter of fact, particularly those focused on the decades after the jump in grain yield due to the Green Revolution. This is important in the case of Spain which, after Italy is one of the main producers on durum wheat at World level.

Objectives

Objectives

The general objective of this Thesis is to evaluate whether there have been agronomic and physiological changes associated with the genetic improvement of durum wheat grown in Spain after the Green Revolution and the environmental conditions where breeding gains have been higher. The study was based on a collection of 20 commercial varieties, grown in Spain between the beginning of the 70s of the last century and the present. The set was readapted during the last two cropping cycles and increased to a total of 23 cultivars, including more recent cultivars, released during the present decade. Cultivars were compared through consecutive years in wide range growing conditions secured by growing in three different sites differing in latitude and temperature, together with the implementation of support irrigation and different planting times. Together with grain yield, a set of relevant agronomical and physiological characteristics related with crop productivity and adaptation were studied. The ultimate goal of the Thesis is to generate information that may help to speed the efficiency of wheat genetic improvement programs. This general objective is specified in the following specific objectives:

1- Quantify the genetic gain of the yield of durum wheat in Spain after the Green Revolution.

2- Dissect the agronomic and physiological parameters related to the genetic progress of durum wheat in Spain and propose a high-performance ideotype and better adapted to the Spanish Mediterranean environment.

3- To study the changes caused by the improvement in the adaptation patterns of wheat to the main growing areas of the country, through the study of the genotype by environment (GxE) interaction.

This Doctoral Thesis Report is made up of three chapters prepared as independent information entities, in order to be published as scientific articles in impact journals. At the time of writing this Doctoral Thesis Report, Chapter 1 has been published in Field Crops Research, Chapter 2 has been published in Plant Science and Chapter 3 is under evaluation at Agronomy.

Director’s Report

Integrative Crop Ecophysiology Group

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Director’s Report

Dr. José Luis Araus Ortega and Dra. Maria Dolors Serret Molins as Directors of the PhD Thesis entitled: Increasing genotypic productivity in post-Green Revolution durum wheat: the case of Spain carried out by the doctorate Fadia Chairi,

They inform about the index of impact and the participation of the doctorate in each one of the articles included in the memory of the Thesis. In all articles the PhD student is the first author of the work.

Chapter 1. Paper: “Post-green revolution genetic advance in durum wheat: The case of Spain” published in the journal Field Crops Research, Impact of 3.868 in 2018, year of publication, which places this journal in the top ten percent in the field of Agronomy and Crop Science. This paper is the first study that evaluates the genetic gain of durum wheat released in Spain with only Green Revolution cultivars with respect to yield, agronomical and physiological traits. It has been observed that the rate of genetic progress in the yield of durum wheat in Spain after the Green Revolution has been low and has even stopped during the last decade, while no clear trends in some grain quality traits (TKW and grain N concentration) are evidenced. In addition, kernels m^{-2} and kernels per spike were the main agronomical components related to genetic gain and genetic gain was positively related with the mean and maximum daily temperatures of the testing sites.

The doctorate participates in the sampling, has carried out the sample analysis, statistical processing and results elaboration. In addition, she participated in the discussion of the results and drafted the article.

Chapter 2. Paper: "Agronomic and physiological traits related to the genetic advance of semi-dwarf durum wheat: The case of Spain" published in the journal Plant Science, Impact of 3.785 in 2018, which places this journal in the top ten percent in the field of Plant Science. This study investigates the agronomic and physiological traits associated with genetic gains in yield and provides a combination of characters that define an ideotype of durum wheat for Spanish Mediterranean conditions. Thus, a set of semi-dwarf (post-green revolution) durum wheat cultivars were grown in a wide range of environments in Spain during two successive years. Grain yield and agronomic yield components were evaluated along with phenology, plant height, leaf morphology, staygreen, and different traits that informed about the water and nitrogen status of the crop. While no interaction between genotype and growing conditions existed for grain yield, this study shows that the more productive genotypes were characterized by a plant height around 85 cm, relatively small erect flag leaves, more open stomata, a better balance between N sources and N sinks, and a greater capacity to re-fix CO_2 respired by the grain.

The doctorate participates in the sampling, has carried out the sample analysis, statistical processing and results elaboration. In addition, she participated in the discussion of the results and drafted of the article.

Chapter 3. Paper: "Breeding effects on the genotype x environment interaction for yield of durum wheat grown after the Green Revolution: the case of Spain" Ready to send to the journal Agronomy, Impact of 2.259 in 2018, which places this journal in the first quartile in the field of Agronomy and Crop Science. This study evaluates the changes caused by breeding in the genotype x environment (G×E) interaction of the durum wheat varieties most widely cultivated in Spain after the Green Revolution. It has been observed an improvement in genetic yield in warm environments and under optimal water conditions, environments similar to those where the advanced lines, from which cultivar released in Spain comes, were selected Results also suggest a tendency of specific

adaptation of semi-dwarf durum in Spain rather than large-scale adaptation. In addition, two different patterns of selection have been reported due the G×E interaction and change in the ranking of genotypes: in the high yielding environments, plant favors more water uptake, with more transpiration and more open stomata (more negative value of carbon isotope composition), whereas, in low yielding environments plant close stomata and favors more water use efficiency (positive value of carbon isotope composition).
The doctorate has carried out the statistical processing and results elaboration. In addition, she participated in the discussion of the results and the drafted the article.

Other articles where the doctoral student participated as a co-author:

- Environmental and Experimental Botany. Impact factor of 3.712 in 2018. Cited 5 times, to date.

Vicente, R., Vergara-Díaz, O., Medina, S., Chairi, F., Kefauver, S. C., Bort, J., Serret, M.D., Aparicio, N. & Araus, J. L. (2018). Durum wheat ears perform better than the flag leaves under water stress: Gene expression and physiological evidence. Environmental and Experimental Botany, 153, 271-285.

- Frontiers in Plant Science. Impact factor of 4.106 in 2018. Cited 2 times, to date

Medina, S., Vicente, R., Nieto-Taladriz, M. T., Aparicio, N., Chairi, F., Vergara-Diaz, O., & Araus, J. L. (2018). The plant-transpiration response to vapor pressure deficit (VPD) in Durum Wheat is associated with differential yield performance and specific expression of genes involved in primary metabolism and water transport. Frontiers in plant science, 9.

- Journal of Experimental Botany impact factor of 5.36 in 2018,

Vergara-Díaz, O., Chairi, F., Vicente, R., Fernandez-Gallego, J.A, Nieto-Taladriz, M.T., Aparicio, N., Kefauver, S.C., Araus, J.L., 2018. Leaf dorsoventrality as a paramount factor determining spectral performance in field-grown wheat under contrasting water regimes Journal Experimental Botany, 69, 3081-3094.

To certify this for corresponding purposes,

Dr. Jose Luis Araus Ortega Dra.M.Dolors Serret Molines

Barcelona, 12th September 2019

Results

Chapter 1

Post-green revolution genetic advance in durum wheat: The case of Spain

Fadia Chairi, Omar Vergara-Diaz, Thomas Vatter, Nieves Aparicio, Maria Teresa Nieto-Taladriz, Shawn C. Kefauver, Jordi Bort, María Dolores Serret, José Luis Araus

Highlights

- Genetic gain of durum wheat in Spain slowed after the green revolution until reach a plateau in the last decade.
- However, genetic gain was positively related with the mean and maximum daily temperatures of the testing sites.
- The genetic advance was related to improvement in kernels m^{-2} and kernels $spike^{-1}$.

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Post-green revolution genetic advance in durum wheat: The case of Spain

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ABSTRACT

This paper addresses the question of whether there has been any genetic gain in yield for durum wheat released in Spain after the Green Revolution and assesses the agronomical and physiological traits associated with evolution of the crop during this time. Field experiments were carried out with a wide range of durum wheat cultivars (released in Spain from 1980 to 2009) and were conducted in different sites embracing a wide range of growing temperatures and water regimes at Aranjuez and Zamadueñas during three consecutive growing seasons (2013/14, 2014/15, 2015/16) under rainfed and supplemental irrigation and at Coria for two consecutive seasons (2014/15 and 2015/16) under rainfed conditions alone.

Grain yield increased with the year of release of cultivars at a rate of 24 kg ha^{-1} y^{-1} (0.44% y^{-1}) from 1980 to 2003, with no clear additional improvements thereafter. The moderate grain yield improvement from 1980 and 2003 was associated with kernels m^{-2} and kernels $spike^{-1}$, with an increase of 117 kernels m^{-2} y^{-1} and 0.24 kernels $spike^{-1}$ y^{-1}, respectively. Moreover, aerial biomass at harvest and grain nitrogen yield increased with the year of release of cultivars for the entire period. However, no differences were found for thousand kernel weight, number of spikes m^{-2}, days to heading, plant height, harvest index, canopy temperature depression, carbon isotope discrimination or grain nitrogen concentration. Overall, these results indicated that the rate of genetic progress in the yield of durum wheat in Spain after the Green Revolution has been low and has even stopped during the last decade, while no clear trend in some grain quality traits (TKW and grain N concentration) was recorded. However, the absolute and relative genetic gains estimated for yield were positively associated with the average mean and maximum daily temperatures from sowing to harvest of the testing site, which suggest that breeding has been performed under high-temperature environments.

1. Introduction

Spain is one of the largest producers of durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf) Husn.) in the Mediterranean basin (Royo, 2005). More than 400,000 ha of durum wheat are grown in Spain and over the last three years the harvest production was estimated to range between 713,699 Mg and 1,124,402 Mg (Cooperativas Agro-alimentarias, 2016). Crop breeding along with more efficient crop management, are mechanisms by which food supply can meet demand without significant expansion of global cropland area.

The history of durum wheat breeding in Spain dates back to the beginning of 20th century, with the production of varieties derived from bulk selection within landraces and others from crosses between the Italian variety Senatore Capelli and Spanish landraces (Royo and Briceño-Félix, 2013). As for bread wheat (*Triticum aestivum* L.), the huge improvement in durum wheat yield in Spain was realized during the second half of the last century. It was primarily a consequence of the introduction of semi-dwarfing germplasm by CIMMYT (International Center for Improvement of Maize and Wheat), which involved the replacement of traditional tall cultivars by semi dwarf and fertilizer-responsive varieties (Garcıa del Moral et al., 2005). However, in the case of bread wheat, there is strong evidence that the rate of genetic advance

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Abbreviations: CTD, canopy temperature depression; $\delta^{13}C$, carbon isotope composition; $\Delta^{13}C$, carbon isotope discrimination; GY, grain yield; GNY, grain nitrogen yield; NDVI, normalized difference vegetation index; HI, harvest index; grain N, grain nitrogen concentration; TKW, thousand kernel weight; leaf chl, leaf chlorophyll content

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Table 1
Experimental conditions of the field trial.

	Zamadueñas experimental station - ITACyL	Colmenar de Oreja experimental station - INIA	Coría del Rio experimental station - INIA
Soil texture	Loam	Clay-loam	Loam
Soil pH	8,44	8,1	
Sowing date	25.11.2013 /24.11.2014/30.11.2015	22.11.2013/21.11.2014/27.11.2015	1.12.2014/10.12.2015
Harvest date	22.07.2014/22.07.2015/20.07.2016	09.07.2014/20.07.2015/07.07.2016	10.07.2015/10.06.2016
Sowing density (seeds m^{-2})	250	250	250
Plot surface (m^2)	10.5 (7 × 1.5)	10.5 (7 × 1.5)	10.5 (7 × 1.5)
Irrigation provided[a] (mm)	125	180	–
Fertilization			
1st application	300 kg ha^{-1} NPK 8:15:15	400 kg ha^{-1} NPK 15:15:15	400 kg ha^{-1} NPK 15:15:15
2nd application	300 kg ha^{-1} CAN 27%N	150 kg ha^{-1} Urea 46%	150 kg ha^{-1} Urea 46%
Max. mean T[b] (ºC)	17/17/17	20/21/20	23/21
Min. mean T[b] (ºC)	5/5/4	5/5/5	9/9
Precipitation[b] (mm)	212/258/362	203/206/277	168/368

[a] in the irrigated treatments.
[b] during growing season.

in yield that started in the Green Revolution seems to have declined or even ceased in some regions of the world (Aisawi et al., 2015; Fischer and Edmeades, 2010; Ray et al., 2012; Sanchez-Garcia et al., 2012). Thus, at a global level, the yield increase of bread wheat from 1961 to 1990 was 2.6% per year, but from 1990 to 2007 the average yield increase was only 0.52% per year (Alston et al., 2010).

Genetic improvement of a crop pivots on the strength of genetic diversity within the crop species (Ogunniyan and Olakojo, 2014). Some studies have reported that genetic diversity in bread wheat has been reduced due to breeding (Huang et al., 2007; Hysing et al., 2008; Reif et al., 2005; Warburton et al., 2006). For durum wheat, it also seems that in some regions genetic variability has decreased as a result of modern breeding (Henkrar et al., 2016; Martynov et al., 2005; Van de Wouw et al., 2010). Thus, in the case of Spain after the Green Revolution, there is a lack of continuity with local breeding programs conducted in the first half of the twentieth century, and this coincided with the massive introduction of foreign durum wheat varieties. In fact, Spanish durum germplasm grown before 1945 constitutes a completely different gene pool to that of the varieties released more recently (Martos et al., 2005).

In this context, it is critical to deepen our understanding of yield changes and its related agronomical and physiological traits in order to design future breeding strategies In the case of bread wheat (Austin et al., 1980; Reynolds et al., 1999), studies of the physiological basis of genetic gains in grain yield (GY) that compared historical (i.e. pre Green Revolution) genotypes (landraces and old varieties) with post Green Revolution cultivars have generally indicated that the number of kernels m^{-2} (usually related to the number of grains per spike) and Harvest Index (HI) are positively associated with GY progress However, retrospective studies in bread wheat varieties produced by CIMMYT, including recently released varieties, have revealed that GY progress was associated with increased grain weight and aboveground dry matter at harvest (Aisawi et al., 2015).

Information on the genetic advance of durum wheat is scarce, given the comparatively smaller acreage and regionally localized cultivation of the crop compared to bread wheat. Indeed, durum wheat has received far less attention than bread wheat, and only a few reports on yield increases can be found in the literature (Álvaro et al., 2008; Royo et al., 2007; Pfeiffer et al., 1996). Moreover, to the best of our knowledge, there are no reports dedicated to evaluating the agronomical and physiological changes associated with post Green Revolution breeding advances in durum wheat. In fact, studies investigating genetic advance in wheat yield have included landraces and/or old (i.e. tall) varieties, which are clearly pre-Green Revolution. Even those studies that focus on post Green Revolution genetic advance (which are only available for bread wheat) frequently include varieties released at the beginning of the Green Revolution, and this may introduce a bias into the results due to the transitional values for some traits, such as greater plant height and lower HI (Aisawi et al., 2015; Reynolds et al., 1999)

This paper reports on the genetic advance in GY realized in durum wheat in Spain after the Green Revolution. In addition, the eventual changes in agronomic yield components, biomass, and other traits informing on growth, and nitrogen and water use efficiency have also been investigated. To that end a set of 20 fully semi-dwarf cultivars (i.e. non-transitional) released in Spain from 1980 until the end of the first decade of the 21 st century have been compared. This panel of cultivars has been grown in three successive seasons (2013–2016) under a wide range of water conditions. Finally, we aimed to identify the existence or absence of genetic advance in durum wheat in Spain and to assess which agronomical and physiological traits were involved, or at least affected.

2. Materials and methods

2.1. Experimental design and varieties

Experiments were carried out under field conditions in three experimental stations embracing a wide range of latitudes and elevations (providing a wide range of growing temperatures) in Spain for several consecutive growing seasons. Experiments were conducted at the experimental stations of the Spanish "Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria" (INIA) of Coria del Rio (Cor), Seville (37°14´N, 06°03´W, 5 masl) during 2014/15 and 2015/16, at Colmenar de Oreja – Aranjuez (Aran), Madrid (40°04´N, 3°31´W, 590 masl) during 2013/14, 2014/15, and 2015/16, as well as at the "Instituto Tecnólogico Agrario de Castilla y León" (ITACyL) in Zamadueñas (Zam), Valladolid (41°41´N, 04°42´W, 700 masl) during 2013/14, 2014/15, and 2015/16. For details see Table 1.

Twenty durum wheat (*Triticum turgidum* L. subsp. *Durum* (Desf) Husn.) cultivars were selected to represent the germplasm grown in Spain after the Green Revolution. These included cultivars widely grown in Spain since the introduction of the first semi-dwarf cultivars (from 1 to 18, Table 2), together with two cultivars (19 and 20) that have been chosen for their high yield in a national trial of new varieties (GENVCE). Plots were sown in a randomized block design with three replicates. Rainfed (R-) and supplemental irrigation (R+) conditions were imposed at Aran and Zam. Due to the presence of a shallow water table, caused by proximity to the Guadalquivir River, genotypes could only be evaluated under rainfed conditions at the Cor experimental site. Considering location (Aran, Zam, and Cor), water regime (R- and R+), and crop seasons, a total of fourteen growing conditions were evaluated. Each plot consisted of six rows 7 m long and 0.2 m apart, with a planting density of 250 seeds m^{-2}.

Table 2
Set of modern (semi dwarf) durum wheat cultivars tested in this study. Year of release, country of registration and pedigree or origin are presented with the period during which each variety was considered among the main cultivated varieties in Spain and the maximum and minimum percentage of area occupied during this period.

	Variety	Year of release	Country	Pedigree/cross name or origin	Period[a]	Max[a]	Min[a]
1	Mexa	1980	Spain	GERARDO-VZ-469/3/JORI(SIB)//ND-61-130/LEEDS	1993–2004	14.9	1.5
2	Vitron	1983	Spain	TURCHIA-77/3/JORI-69(SIB)/(SIB)ANHINGA//(SIB)FLAMINGO	1993–2011	34.0	3.1
3	Regallo	1988	Italy	Diputación General de Aragón CIMMYT	1995–2012	8.2	2.6
4	Simeto	1990	Spain	RUFF/FLAMINGO//MEXICALI-75/3/SHEARWATER	1993–2010	15.3	1.2
5	Gallareta	1994	Spain	CIMMYT	1993–2012	10.5	3.3
6	Bolo	1994	Spain	CARCOMUN/AUK	1998–2005	4.2	0.8
7	Don Pedro	1994	Spain	SHEARWATER(SIB)/(SIB)REDNECK//(SIB)YAVAROS	2001–2010	4.4	0.6
8	Sula	1994	Spain	CIMMYT	1993–2010	4.3	0.5
9	Bólido	1996	Italy	STN'S'//HUI'S'/SOMO'S'	2007–2010	2.3	1.0
10	Dorondon	1998	Spain	Genética y Gestión,S.C.	2004–2010	4.5	0.6
11	Iride	1998	Italy	Altar 84 × Ares sib	2001–2012	7.3	0.4
12	Burgos	1999	Spain	SUDDEUTSCHE SAATZ	2003–2012	5.3	1.0
13	Claudio	1999	Spain	(Sel. Cimmyt × Durango) × (IS193B × Grazia)	2008–2012	3.6	0.5
14	Amilcar	2001	Italy	ZEGZAG-1/LUNDE-5//GREENSHANK-32	2005–2012	10.6	2.0
15	Pelayo	2002	Spain	CAPEITI-8/VALNOVA	2005–2012	16.9	0.4
16	Avispa	2003	Spain	Limagrain-CIMMYT	2009–2010	1.2	0.5
17	Don Sebastián	2004	Spain	Agrovegetal-CIMMYT	2005–2010	5.6	3.6
18	D Ricardo	2008	Spain	Agrovegetal-CIMMYT	2008–2012	5.0	0.5
19	Kiko Nick[b]	2009	Spain	SEL.CIMMYT-35/DURANGO//ISEA-1938/GRAZIA			
20	Ramirez[b]	2009	Spain	ALTAR-84/IONIO			

[a] data from Asociación Española de Técnicos Cerealistas (AETC) 1992/93 to 2011/12.
[b] high yielding cultivars in the Spanish national trials (GENVCE).

2.2. Measurements and determinations

Days from emergence to heading (DH) were determined through periodic (twice per week) observations, when approximately half of the spikes in the plot had already emerged. At maturity and for each plot the plant height (PH), which was the distance from ground to the ear tip (including awns), was measured and the number of kernels per m^2 (kernels m^{-2}) and thousand kernel weight (TKW) were determined after harvest. For each plot the number of spikes per m^2 (spike m^{-2}) was also determined at maturity by sampling and counting the spikes contained in two 0.5m-length portions of one of the central-rowsat Zam and one 0.5 m length of a central-row at Cor and Aran. Further, kernels spike^{-1} was determined using a subset of ten representative stems per plot. GY was assessed by harvesting the whole plot.

Leaf chlorophyll content (Leaf Chl) was measured with a portable meter (SPAD-502 Chlorophyll Meter, Minolta Co. Ltd., Osaka, Japan), in late flowering-early grain filling stage. The measurements were taken on the flag leaves of five randomly selected plants within each plot. For each plant, the average of three readings around the midpoints of the flag leaves was taken.

The normalized difference vegetation index (NDVI) was calculated using readings from a portable spectroradiometer (Greenseeker Hand Held™ optical sensor unit, model 505; NTech Industries, Inc., Ukiah, CA, USA) made during the late flowering-early grain filling stage. Measurements were taken around midday on sunny days by passing the sensor over the subplots at a height of approximately 0.40–0.60 m above the canopy.

A portable thermal infrared camera (MIDAS 320 L, DIAS Infrared Systems, Dresden, Germany) was used to measure canopy temperature during the late flowering-early grain filling stage. Canopy images of whole plots were taken at midday on sunny days, at a distance of approximately one meter away from the plot edge, with an angle of about 45° with regard to the upper surface of the plot and having the sun behind the photographer. These pictures were processed using PYROSOFT Professional software (DIAS Infrared Systems, Dresden, Germany) selecting a representative area of each plot. Air temperature was recorded with a thermo-hygrometer (Testo AG, Lenzkirch, Germany) at the same time as each thermal picture. Canopy temperature depression (CTD) was then calculated as:

$$CTD = T_a - T_c$$

Where T_a depicts the air temperature and T_c the temperature of the canopy.

The total N content of mature grains was analyzed using an Elemental Analyzer (Flash 1112 EA; ThermoFinnigan, Bremen, Germany) for each individual plot within the entire set of trials. The same EA coupled with an Isotope Ratio Mass Spectrometer (Delta C IRMS, ThermoFinnigan, Bremen, Germany), operating in continuous flow mode, was used to determine the stable carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios of the same mature grains. Samples of ~1 mg and reference materials were weighed into tin capsules, sealed, and then loaded into an automatic sampler (ThermoFinnigan) before EA-IRMS analysis. Nitrogen was expressed as a concentration (g N per g DW) and atropine was used as a system check in the elemental analyses of nitrogen. The ^{13}C:^{12}C ratios of plant material were expressed in δ notation (Coplen, 2008).

$$\delta^{13}C = \frac{(^{13}C\!:\,^{12}C)\ sample}{(^{13}C\!:\,^{12}C)\ standard} - 1$$

Where *sample* refers to plant material and *standard* to international secondary standards of known ^{13}C:^{12}C ratios (IAEA CH7 polyethylene foil, IAEA CH6 sucrose, and USGS 40 L- glutamic acid) calibrated against Vienna Pee Dee Belemnite calcium carbonate (VPDB) with an analytical precision (s.d.) of 0.10‰.

^{15}N:^{14}N ratios were also expressed in δ notation ($\delta^{15}N$) using international secondary standards of known ^{15}N:^{14}N ratios (IAEA N_1 and IAEA N_2 ammonium sulfate and IAEA NO_3 potassium nitrate) referred to N_2 in air, with an analytical precision of 0.18‰.

Measurements were conducted at the Scientific Facilities of the University of Barcelona.

Carbon isotope discrimination was further calculated as:

$$\Delta^{13}C = \frac{\delta_a - \delta_s}{1 + \delta_s}$$

Where δ_a and δ_s refer to the $\delta^{13}C$ of the air and plant, respectively (Farquhar et al., 1989). On the PDB scale, free atmospheric CO_2 has a current value, δ_a, of approximately –8‰ (Farquhar et al., 1989)

2.3. Data analyses

The response of the studied genotype, environment, and environment by genotype interaction was tested with ANOVA. Absolute and relative genetic gains (AGG and RGG), which are the changes due exclusively to plant breeding, were calculated as the slope of the linear regression between either the absolute or relative value of the trait and the year of cultivar release, respectively. Relative values were computed for each genotype as a percentage with regard to the average value of all genotypes.

Heritability for these genotypes of diverse background was calculated, although this was not to determine the inheritance of the traits, but to see the degree to which a genotype determined a phenotype (Falconer and Mackay, 1996) and moreover, as a proof of concept of the quality of the traits. Broad sense heritability across environments for GY and the different traits included in this study was calculated as:

$$h^2 = \frac{V_G}{V_G + \frac{V_{GE}}{e} + \frac{V_R}{er}}$$

Where genotypic variance is coded by V_G, genotype x environment variance is coded by V_{GE}, and residual variance is coded by V_R. The terms e and r indicate the number of environments and replicates, respectively.

To estimate variance components for use in the calculation of broad sense heritability, all model parameters were set as random.

3. Results

3.1. Grain yield

Average yield across the set of 20 cultivars and 14 environments was 5.59 Mg ha^{-1}. GY was affected by environment (*$P < 0.001$*), genotype (*$P < 0.001$*) and for the interaction environment by genotype (*$P < 0.001$*) (Table 4). Across varieties, yield ranged from 5.09 Mg ha^{-1} to 6.13 Mg ha^{-1}.

Given the statistical significance of the environment x genotype interaction, genetic gains in yield were calculated for each location. The results indicated great variability between locations, with AGG for yield ranging from -4.43 kg ha^{-1} y^{-1} in Zam14R + to 34.86 kg ha^{-1} y^{-1} in Aran15R-, and RGG ranging from ≈ 0 in Zam14R + to 0.79%/yr in Aran16R- (Table 3). When the whole set of environments was considered together, yield increased linearly with year of cultivar release throughout the first 23 years of the breeding period analyzed (1980 to 2003), with an absolute genetic grain (AGG) of 24 kg ha^{-1} y^{-1}, which represents a relative genetic gain (RGG) of 0.44% y^{-1}, with no clear additional improvements thereafter (Fig. 1a). Considering the whole period studied, the annual rate of increase in GY was below 13 kg ha^{-1} y^{-1}, which represents only an RGG of 0.21% y^{-1}. A similar trend in the relationship between the grain yield and the year of release was found when analyzed in the high-yielding environments (with grain yield above 5600 kg ha^{-1}) and the low-yielding environments (grain yield below 5600 kg ha^{-1}), indicating a lineal increase in grain yield with the year of release of the cultivars throughout the 1980–2003 period, and with an absolute genetic gain of 23 kg ha^{-1} y^{-1} and 28 kg ha^{-1} y^{-1}, respectively (Fig. 1a). While no clear increase occurred thereafter (2003–2009). The rate of yield increase was not related to the environment mean yield (Fig. 1b). However, AGG was positively correlated with the average mean ($r^2 = 0.53$, $P < 0.01$) and maximum daily temperature ($r^2 = 0.58$; $P < 0.01$) across the growing season when considering all 14 growing environments (Fig. 2a,b). When the Aranjuez and Valladolid rainfed trials were excluded, the correlation between AGG and the mean ($r^2 = 0.85$, $P < 0.001$) and the maximum daily temperature ($r^2 = 0.86$, $P < 0.001$) increased even more (Fig. 2c,d). However, the best fit between the daily mean temperature and AGG was quadratic (Fig. 2a,c). Further, we evaluated the AGG for GY within the subset of environments exhibiting the highest ($T_{max} > 20$ °C) and the lowest ($T_{max} < = 20$ °C) average daily maximum temperatures during the complete growth cycle. A positive linear relationship ($P < 0.05$) was observed between the year of release (1980–2009) and GY with AGG = 26 Kg ha^{-1} y^{-1} in the environments with the highest daily max temperature, while no relationship was recorded in the environments with the lowest daily maximum temperatures (Fig. 3).

Table 3

Mean values, AGG and RGG of grain yield of twenty durum wheat in each environment.

Experiment Code[a]	Mean GY (Mg ha^{-1})	AGG (Kg $ha^{-1}y^{-1}$)	RGG (% y^{-1})	r^2	P
Cor15	6.49	27.38	0.42	0.09	ns
Aran15R +	5.08	21.72	0.43	0.109	ns
Aran15R-	4.66	34.86	0.75	0.259	0.02
Zam15R +	7.19	7.95	0.12	0.02	ns
Zam15R-	3.79	1.52	0.04	0.001	ns
Aran14R +	6.70	15.89	0.24	0.08	ns
Aran14R-	5.53	−3.05	−0.055	0.06	ns
Zam14R +	6.55	−4.43	−0.006	0.003	ns
Zam14R-	3.00	3.28	0.11	0.005	ns
Zam16R-	6.71	5.55	0.08	0.01	ns
Zam16R +	9.41	−0.67	−0.01	–	ns
Cor16	4.89	23.62	0.48	0.098	ns
Aran16R-	4.44	35	0.79	–	ns
Aran16R +	3.83	6.99	0.18	0.018	ns

[a] Experiment code: Aran, Aranjuez; Cor,Coría; Zam, Zamadueñas; 14, 2014; 15, 2015; 16, 2016; R-, Rainfed and R + , supplemental irrigation.

3.2. Phenological development

Significant differences existed in heading time among genotypes (*$P < 0.001$*) and environments (*$P < 0.001$*), whereas the genotype by environment interaction was also significant (*$P < 0.001$*) (Table 4). However, the slope of the regression between heading time and the year of release (Fig. 4) was statistically indistinguishable from zero (*$P \geq 0.12$*).

3.3. Yield components

All agronomical yield components were affected by environmental conditions (*$P < 0.001$*), whereas the genotype effect was significant for all the traits (*$P < 0.001$*), except for the spikes m^{-2}. The interaction between genotype and environment was significant (*$P < 0.001$*) for all the traits (Table 4). Spikes m^{-2} and TKW ranged among varieties from 303 to 431 m^{-2} and 37.2 to 40.1 g, respectively (Table 4). Likewise, no associations with the year of release were observed for either of the agronomical yield components (Fig. 5b,d). Kernels m^{-2} and number of kernels per spike (kernels $spike^{-1}$) ranged among varieties from 11,789 to 16,947 kernels m^{-2} and 27.7 to 40.6 kernels $spike^{-1}$, respectively, (Table 4). There was a trend of linear increase with year of variety release observed for kernels m^{-2} and kernels $spike^{-1}$ (P = 0.07 and 0.06, respectively) throughout the first 23 years of the breeding period analyzed, but there were no clear increases thereafter (Fig. 5a,c). The AGG from 1980 to 2003 was 117 kernels m^{-2} y^{-1} and 0.24 kernels $spike^{-1}$ y^{-1}, which represents an RGG of 0.81% y^{-1} and 0.60% y^{-1}, respectively. Overall, the HI ranged among varieties from 0.30 to 0.40 (Table 4), and there was no association with the year of release (Fig. 6c). When analyzing in high-yielding and low-yielding environments separately, we observed an increase in kernels $spike^{-1}$ in high-yielding environments ($r^2 = 0.324$, $P < 0.05$; AGG = 0.50 kernels $spike^{-1}$ y^{-1}) from 1980 to 2003 and in low-yielding environments ($r^2 = 0.414$, $P < 0.01$; AGG = 0.22 kernels $spike^{-1}$ y^{-1}) during the whole period studied. However, no changes in other agronomical yield components were found (Table 7).

Table 4

Mean values of grain yield (GY), plant height, agronomical yield components and number of days from planting to heading for 20 durum wheat cultivars released in Spain after the Green Revolution. Data presented are the mean values for the whole set of 14 growing environments (combinations of site, year and water regime) tested. Means followed by different letters were significantly different by Tukey's b test.

Variety	Year of release	GY ($Mg\ ha^{-1}$)	PH (cm)	Kernels m^{-2}	Spikes m^{-2}	Kernels $spike^{-1}$	HI (%)	TKW (g)	DH
Mexa	1980	5.19^{def}	93.49^{bc}	12664^{hij}	402.5^{a}	$31,00^{fg}$	33.54^{ghi}	45.63^{de}	144^{l}
Vitron	1983	5.75^{ab}	88.68^{cde}	13927^{fgh}	423.1^{a}	$27,70^{efg}$	37.51^{abc}	47.32^{bcd}	147^{ijk}
Regallo	1988	5.67^{bcdef}	82.16^{gh}	14854^{efgh}	394.3^{a}	$28,01^{abcdef}$	34.65^{defgh}	43.82^{f}	148^{ghi}
Simeto	1990	5.19^{def}	83.08^{h}	11789^{j}	344.1^{a}	$30,13^{efg}$	35.01^{efgh}	50.12^{a}	148^{gh}
Gallareta	1994	5.49^{cdef}	89.31^{de}	14863^{defg}	380.5^{a}	$34,20^{abcde}$	35.57^{cdefg}	40.83^{gh}	148^{efg}
Bolo	1994	5.22^{ef}	92.8^{b}	12577^{j}	353.0^{a}	$40,65^{cdef}$	32.08^{hij}	45.72^{de}	154^{a}
Don Pedro	1994	5.21^{def}	88.73^{cde}	13805^{ghi}	404.7^{a}	$35,95^{cdef}$	33.82^{efgh}	42.63fg	148^{fg}
Sula	1994	5.82^{ab}	84.56^{gh}	16268^{abc}	420.9^{a}	$33,56^{abcde}$	37.58^{abc}	41.4^{gh}	148^{gh}
Bólido	1996	5.64^{abcde}	83.24^{gh}	17803^{a}	428.2^{a}	$39,11^{ab}$	36.44^{bcde}	37.2^{j}	149^{cd}
Dorondon	1998	5.58^{abcd}	90.41^{bcd}	14835^{cdef}	405.7^{a}	$40,47^{abcd}$	37.5^{abc}	40.74^{h}	147^{k}
Iride	1998	5.89^{ab}	84.77^{fgh}	15820^{bcde}	385.9^{a}	$34,32^{abc}$	40.01^{a}	42.17^{fg}	147^{hij}
Burgos	1999	5.42^{bcdef}	89.7^{de}	12446^{ij}	422.0^{a}	$28,34^{efg}$	31.12^{ij}	48.45^{bc}	150^{c}
Claudio	1999	5.65^{abcd}	$91.64bc^{d}$	13772^{fghi}	405.0^{a}	$34,92^{def}$	33.83^{fghi}	44.87^{e}	149^{de}
Amilcar	2001	5.94^{abc}	83.39^{gh}	16175^{bcde}	399.0^{a}	$34,95^{a}$	38.57^{ab}	41.9^{fg}	146^{jk}
Pelayo	2002	6.13^{a}	84.44^{fg}	14963^{cdef}	422.3^{a}	$31,00^{cdef}$	37.28^{abcd}	45.98^{e}	147^{gh}
Avispa	2003	5.94^{a}	84.48^{ab}	15456^{abcd}	403.8^{a}	$27,70^{abcd}$	38.23^{ab}	42.58^{f}	146^{ijk}
Don Sebastián	2004	5.09^{f}	97.71^{a}	11984^{j}	404.9^{a}	$28,01^{g}$	30.08^{j}	48.59^{b}	149^{def}
D Ricardo	2008	5.54^{abc}	91.03^{bcd}	13771^{efgh}	389.7^{a}	$30,13^{bcdef}$	35.79^{bcdefg}	46.48^{cde}	149^{cde}
Kiko Nick	2009	5.66^{ab}	88.09^{ef}	13570^{efgh}	431.0^{a}	$34,20^{fg}$	36.01^{bcdef}	46.95^{cde}	147^{ghi}
Ramirez	2009	5.8^{abcd}	89.67^{cde}	16947^{ab}	412.9^{a}	$40,65^{ab}$	$33.85g^{hij}$	39.18^{i}	152^{b}
G		< 0.001	< 0.001	< 0.001	ns	< 0.001	< 0.001	< 0.001	< 0.001
E		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
G*E		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
h^2		0.79	0.96	0.90	0.35	0.87	0.90	0.97	0.97

Parameters studied were: grain yield (GY), plant height (PH), number of grains m^{-2} (kernels m^{-2}), number of spikes m^{-2} (Spikes m^{-2}), number of kernels per spike (Kernels $spike^{-1}$),harvest index (HI), thousand kernel weight (TKW) and days to heading (DH).

3.4. Biomass, plant height, leaf chlorophyll content, nitrogen grain yield, and grain nitrogen concentration

Significant genotype effects were observed for biomass ($P < 0.05$), NDVI ($P < 0.001$), PH ($P < 0.001$), leaf chl ($P < 0.001$), total grain nitrogen yield (GNY) and grain nitrogen concentration (grain N) ($P < 0.001$), but not for the stable nitrogen isotope composition ($\delta^{15}N$) (Tables 4 and 5). Environment had a significant effect on biomass, PH, NDVI ($P < 0.001$), leaf chl, and all the nitrogen traits. The environment by genotype interaction was significant for biomass, leaf chl, NDVI ($P < 0.01$), PH, and nitrogen traits ($P < 0.001$), except for $\delta^{15}N$ (Tables 4 and 5).

Biomass at harvest ranged from 14.11 to 16.49 Mg ha^{-1} (Table 5) among varieties across the 14 environments and increased linearly with year of variety release throughout the studied period ($P < 0.05$). The AGG between 1980 and 2009 was 42 kg ha^{-1} y^{-1} (Fig. 6b), which represents an RGG of 0.27% y^{-1}.

Across varieties, GNY ranged from 120.28 to 140.37 kg ha^{-1}. GNY increased linearly with year of variety release ($P < 0.05$) (Tables 5 and 6). For the whole period, the AGG for GNY was 0.39 kg ha^{-1} y^{-1}, which represents an RGG of 0.29%y^{-1} (Table 6), whereas no significant correlations were observed for the $\delta^{15}N$ of mature kernels, the NDVI, or leaf Chl with the year of cultivar release (Table 6).

PH ranged between 0.83 m and 0.99 m (Table 4) among varieties across the 14 environments. PH was stable for varieties released after the Green Revolution. In fact, no significant correlation was observed between PH and the year of release ($P \geq 0.12$) (data not shown). However, as stressed above, when analyzing in high-yielding and low-yielding environments separately, no change was found in any of these traits (Table 7).

3.5. Water status

Carbon isotope discrimination ($\Delta^{13}C$) in mature kernels was analyzed as a surrogate of water use efficiency (WUE) and as an indicator of the overall plant water status. Canopy temperature depression (CTD) during grain filling was also measured as an indicator of plant water status. While environment had a significant effect ($P < 0.001$) on $\boldsymbol{\Delta}^{13}C$ and CTD, the genotype effect on these water status traits was not significant, and the interaction between environment and variety was only significant for CTD ($P < 0.01$) (Table 5). Consequently, there was no correlation of either $\boldsymbol{\Delta}^{13}C$ or CTD with the year of cultivar release (Table 6). Evaluating this trait under high-yielding and low-yielding environments, we only observed an increase in $\boldsymbol{\Delta}^{13}C$ under high-yielding environments ($r^2 = 0.292$, $P < 0.05$) from 1980 to 2003 with an AGG = 0.012‰ y^{-1} (Table 7).

3.6. Trait correlations

GY was positively correlated with kernels $spike^{-1}$, kernels m^{-2} (Fig. 5f,g) and HI (Fig. 6c) ($r^2 = 0.533$, $P < 0.001$; $r^2 = 0.252$, $P < 0.05$; $r^2 = 0.571$, $P < 0.001$, respectively) and negatively correlated with PH and grain N ($r^2 = 0.369$, $P < 0.01$; $r^2 = 0.202$, $P < 0.05$, respectively) (Table 6). However, no significant correlation was found between GY and TKW (Fig. 5h) GY and biomass (Fig. 6d) or any of the other parameters studied (Table 6). GNY was correlated positively with GY ($r^2 = 0.416$; $P < 0.01$) (Table 6) and biomass ($r^2 = 0.252$; $P < 0.05$) and negatively with PH ($r^2 = 0.229$; $P < 0.01$) (data not shown).

3.7. Broad sense heritability

High values of broad sense heritability (h^2) across environments were observed in GY ($h^2 = 0.79$), kernels m^{-2} ($h^2 = 0.90$), kernels $spike^{-1}$ ($h^2 = 0.87$), TKW ($h^2 = 0.97$), HI ($h^2 = 0.90$), NDVI ($h^2 = 0.87$), PH ($h^2 = 0.96$), DH ($h^2 = 0.97$), grain N concentration ($h^2 = 0.77$) and leaf chl ($h^2 = 0.87$). Moderate heritability values were observed in biomass ($h^2 = 0.47$), GNY ($h^2 = 0.57$), and CTD ($h^2 = 0.55$), and low heritability values were observed in spikes m^{-2} ($h^2 = 0.35$) and $\Delta^{13}C$ ($h^2 = 0.26$), (Tables 3 and 4).

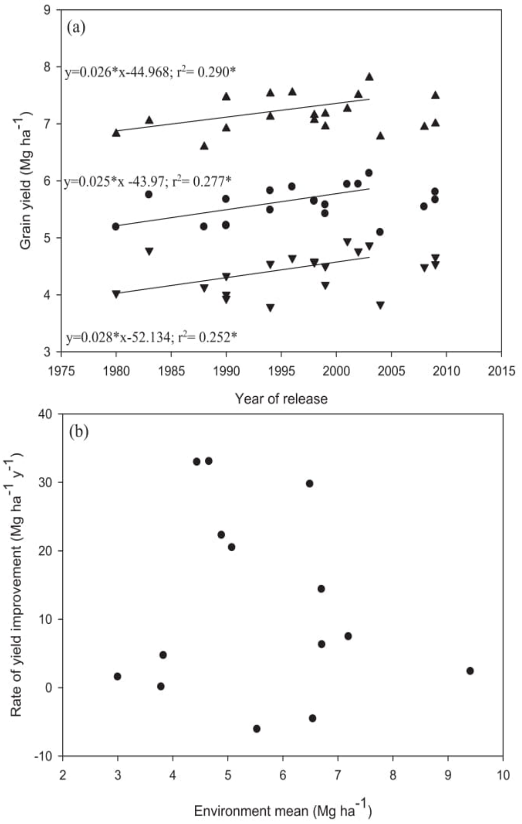

Fig. 1. Relationship between grain yield and the year of cultivar release for the average of all of the environments (circles), in high-yielding environments (i.e. above 5600 Mg ha^{-1}) (triangle-up) and low-yielding environments (below5600 Mg ha^{-1}) (triangle-down); solid lines refer to the relationships between 1980 and 2003. (b) Lack of a relationship between the rate of yield improvement and the environmental mean of the yield. Rates are the slope of the regression between the trait and year of cultivar release in each of the fourteen environments. The environmental mean is the mean grain yield across varieties for each environment (varieties between 1980 and 2003).

4. Discussion

The attainment of genetic gain in a breeding program requires genetic variability (Bered et al., 2002). Some studies have reported that losses in genetic diversity have been observed in recent times that are attributable to breeding in durum wheat (Altintaş et al., 2008; Henkrar et al., 2016) and bread wheat (Huang et al., 2007; Hysing et al., 2008; Reif et al., 2005; Warburton et al., 2006). The limited genetic variability may partly explain the low rate of genetic change in the case of durum wheat in Spain. However, the genetic variability of GY and many of the traits studied was significant, which suggested the absence of a clear breeding strategy. The extensive field trials, conducted in a wide range of growing conditions, allowed for an in-depth evaluation of the degree of genetic advance in durum wheat in Spain and for the identification of the agronomical and physiological traits involved. Moreover, the high to medium broad-sense heritability for GY and other traits in our study were possibly due to the large diversity in the genotypes studied, and this highlights the important role of genotype in portraying the phenotype. Moreover, the high heritability also implies that the overall quality of the trials is high.

The observed lack of a clear yield improvement after the Green Revolution agrees with earlier studies of spring wheat in the high-yielding conditions of irrigated agriculture in NW Mexico, which reported that the rate of yield improvement has been decreasing over the last 30 years and has generally not exceeded 1% per year (Fischer and Edmeades, 2010). Aisawi et al., (2015) have also reported a decrease in genetic gain in the last 25 years compared with the previous period for bread wheat in NW Mexico. Even though genetic gain in grain yield is still evidenced in the current CIMMYT breeding programs for bread wheat (Crespo-Herrera et al., 2017; Lopes et al., 2012; Manès et al., 2012), this progress has slowed in comparison with previous studies (Sayre et al., 1997; Waddington et al., 1986). In the case of Brazilian bread wheat, Beche et al. (2014) reported a non-significant increase in genetic gain for GY between 1999 and 2009. In Spain, the genetic gain in bread wheat has been low or null. Acreche et al. (2008) showed an absence of genetic advance in GY since the 1970s in high-yielding irrigated environment and Sanchez-Garcia et al. (2012) showed a low rate of genetic advance of 18 kg ha^{-1} y^{-1} between 1969 and 2001, when analyzed in different environmental conditions. Therefore, this represents an AGG that is even smaller than reported in our study for durum wheat in Spain over a similar time period, and clearly below the values reported for bread wheat at CIMMYT in NW Mexico.

The lack of genetic advance in the cultivars studied could be explained by the development of the varietal structure in Spain. Traditional tall varieties were preferred through to 1976 when new CIMMYT varieties were released, and during the 1980's one cultivar, Mexa, reached 80% of the crop area and has dominated the market for decades (Juan-Aracil and Michelena, 1995). In fact, most, if not all modern (bread and durum) cultivars grown across the country during the last 30 years have been developed using foreign germplasm (from CIMMYT, France and Italy) or are even cultivars adopted directly from elsewhere, rather than being bred locally (Acreche et al., 2008). Cultivars analyzed in the present study were selected from among those widely grown in Spain since the introduction of the first semi-dwarf cultivars. It should be noted that the rate of variety replacement in Spain is very low and almost half of them have been among the most widely gown for more than 10 years (Table 2). Moreover, Vitrón (1983), Simeto (1990), Don Pedro (1994), Gallareta (1994) and Claudio (1999) were the check cultivars at the National New Varieties Trials (GENVCE) until 2009–2010 (coinciding with the newest varieties included in our study), and in 2010–11 only two of them, Vitrón and Don Pedro, were substituted by Avispa (2003) and Amilcar (2001).

Other causes that might explain the low genetic gain in Spain, such as susceptibility of the new cultivars to major diseases, together with the emergence of new more virulent strains, or predisposition to lodging (particularly under high-yielding irrigated conditions) appear to be less evident. In fact during the past decades the breeding effort for durum wheat in Spain has been focused on improving lodging tolerance and restricting the number of non-productive tillers characteristic of the old varieties, while enhancing flour quality (Royo and Briceño-Félix, 2013). End-use quality may also be a factor to consider in further studies because a tradeoff between yield and grain quality is not unexpected. However, our results show no clear improving trend in some grain quality traits like TKW and grain N concentration. In the case of bread wheat from CIMMYT, end-use quality traits have been improved in the last 50 years. However, in parallel with our results, TKW and protein content (among other quality traits) have remained stable despite grain yield increases over the last 50 years (Guzmán et al., 2017).

When genetic gain for yield was calculated for each of the fourteen environments, the range of AGG was greater than 39 kg ha^{-1} y^{-1} (with the extreme values of -4.53 and 34.86 ha^{-1} y^{-1}), suggesting a very high dependence of the estimated genetic gain on the environment. In fact, variations in AGG across environments were positively correlated with the average mean and maximum daily temperatures of the testing trials from sowing to harvest. Interestingly, the correlations even increased when rainfed trials were removed. The lowest AGGs were recorded in environments with the lowest maximum temperatures and lowest mean temperature independent of the mean yield of the

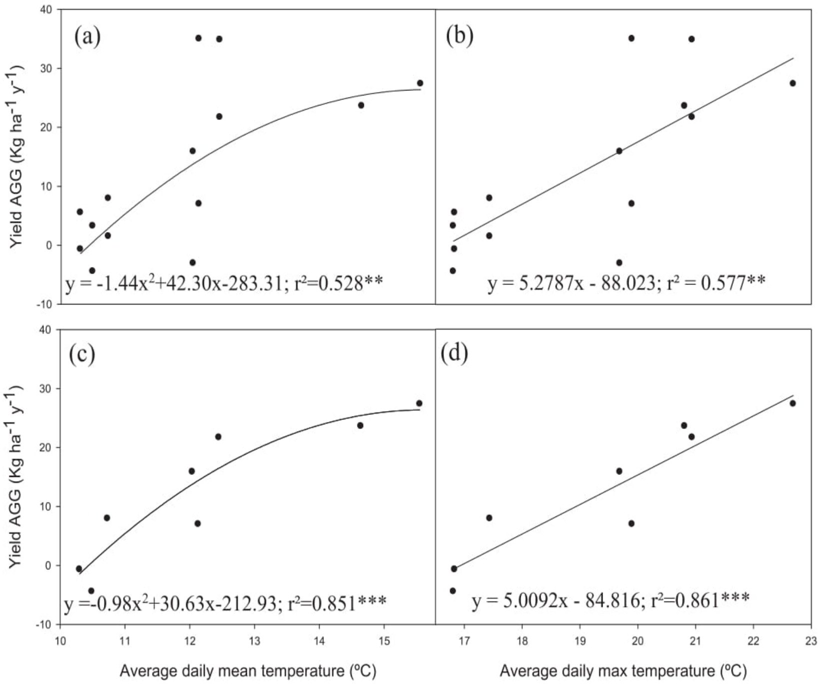

Fig. 2. Relationships between absolute genetic gain (AGG) for yield and the average daily mean and maximum temperatures from sowing to harvest. Each point represents the value for each growing environment including all environments in (a) and (b) and excluding the rainfed ones (from Aranjuez and Valladolid) in (c) and (d). The solid fitted lines refer to the relationships for the whole set of genotypes for the period between 1980 and 2010.

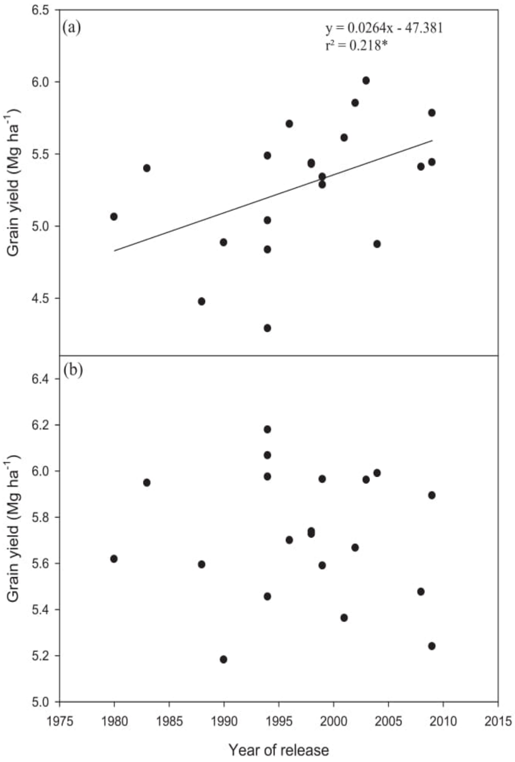

Fig. 3. Relationship between Grain yield and year of release in (a) environment with daily maximum temperature > 20 °C and (b) environment with daily max temperature < = 20 °C. Each point represents the average of GY across the three different year for each growing environment.

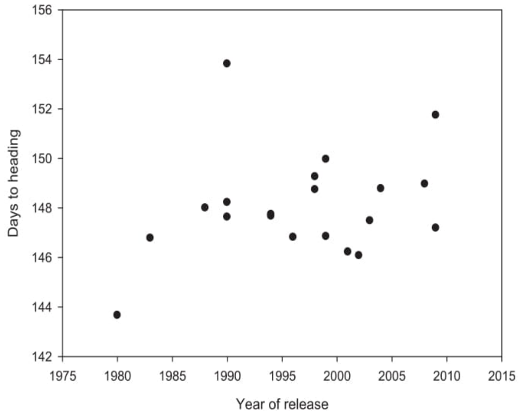

Fig. 4. Lack of a relationship between the number of days from planting to heading of the wheat varieties released between 1980 and 2010 and the year of release. Each point represents the value for a given cultivar and growing environment (combination of site, year and water regime).

environment, indicating that the yield differences between oldest and improved cultivars increased as the maximum and mean daily temperatures of the growing site increased.

In the case of Spanish bread wheat, Sanchez-Garcia et al. (2012) showed that variations in yield RGG across environments were largely explained by the average minimum daily temperatures from sowing to heading.

The positive correlation between the genetic advance and the temperature of the trial and the positive relationship between the GY and year of release in the environment with high daily maximum temperature may be interpreted in adaptive terms. If breeding has been conducted under warm conditions, adaptation to high temperature, including tolerance to heat shocks may appear as an implicit result of

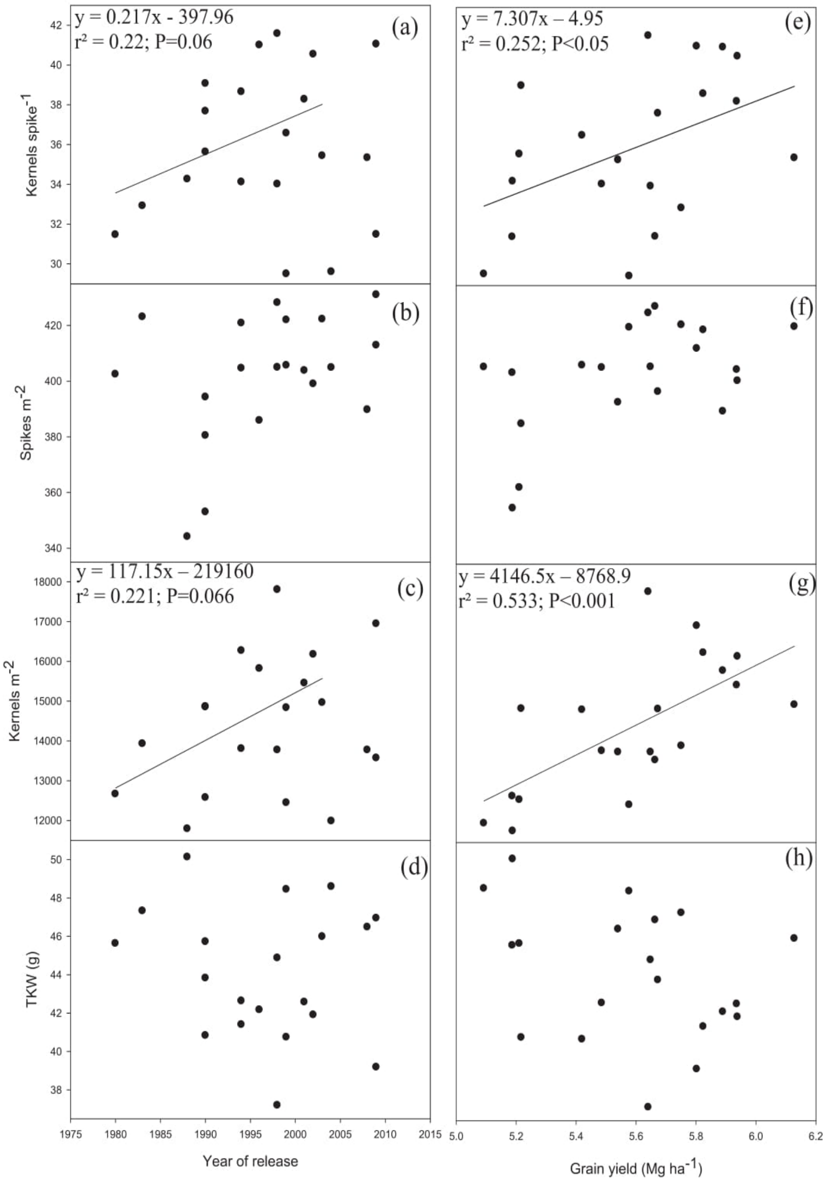

Fig. 5. Relationships between year of release and (a) the number of kernels per spike, (b) the number of spikes per m ^{2}(spikes m^{-2}), (c) the thousand kernel weight (TKW) and (d) the number of kernels per m^{2} (kernels m^{-2}), for the set of 20 durum wheat cultivars released between 1980 and 2009 in Spain. The relationship between grain yield and (e) the number of kernels per spike (kernels spike^{-1}), (f) the number of spikes per m^{2} (spike m^{-2}), (g) the number of kernels per m^{2} (kernels m^{-2}) and (h) the thousand kernel weight (TKW) for the same set of genotypes. The solid fitted lines refer to the relationship for the whole set of genotypes for those cases where significant relationships exist for the period between 1980 and 2003 (Fig. 4a and c) and for the whole period studied between 1980–2010 (Fig. 4e and g).

the breeding. In fact, our study showed a clearer relationship of genetic advance in AGG with maximum rather than mean temperatures. In fact, most of the durum wheat varieties grown in Spain after the Green Revolution have strong CIMMYT-derived genetic backgrounds. The main breeding site for CIMMYT, and the place where green revolution was developed, is in Cd. Obregon (NW Mexico) where high temperatures during growing season are common. This still makes sense at the Spanish level if we consider that 80% of the total production of durum wheat in the country is concentrated in Andalusia, which is the warmest region in Spain. However, genetic advance for cooler, more continental conditions, is not evident.

In our study the role of plant water status was apparently not important, as inferred in the lack of significant differences in Δ^{13}C and CTD across genotypes when all the environments were considered. However, in high-yielding environments Δ^{13}C increased significantly with the year of release until 2003 (AGG = 0.012‰ y^{-1}), This result suggests that in high-yielding environments the water use has increased (Araus et al., 2013) with plant breeding. Our results are in line with those achieved for bread wheat under the high-yielding conditions of the UK where Foulkes et al. (2016) showed increases in grain Δ^{13}C of 0.026 and 0.030‰ y^{-1} under irrigated and rain-fed conditions, respectively, when comparing seventeen wheat cultivars (fourteen of the cultivars were semi-dwarfs, and three were tall cultivars). However, there was no change in the oxygen isotope composition (δ^{18}O) of the leaf, which is an indicator of transpiration (Barbour et al., 2000) and thus stomatal conductance and water status. Del Pozo et al. (2014) reported no clear trend for Δ^{13}C when comparing 20 old and 75 modern cultivars of bread wheat (released before and after 1960, respectively) growing under high-yielding Mediterranean conditions in Chile. This was despite the observation of a clear decreasing trend for δ^{18}O in

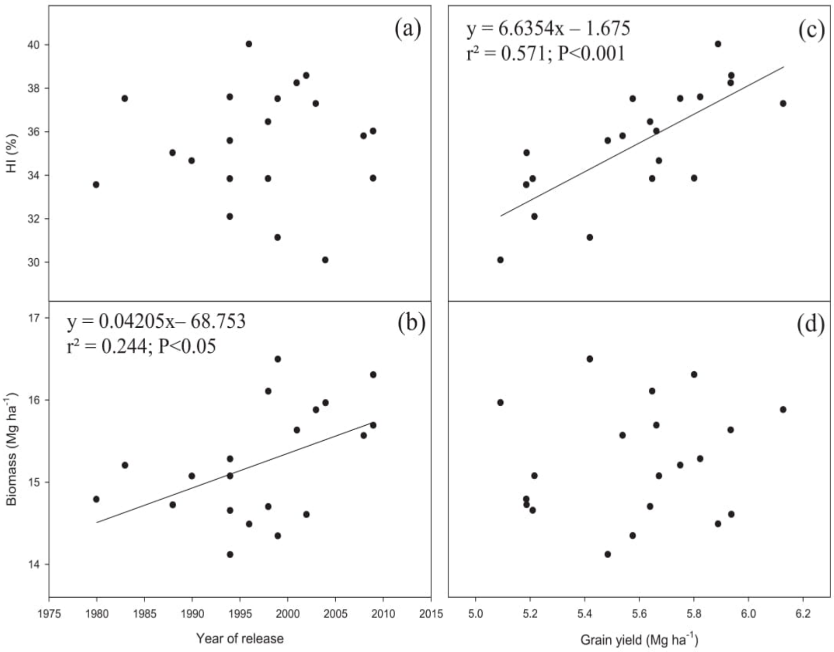

Fig. 6. Relationship between year of release and (a) the harvest index and (b) the biomass for the set of 20 durum wheat cultivars released between 1980 and 2009 in Spain. Relationship between grain yield and (c) the harvest index and (d) the biomass and grain yield for the same set of genotypes. Each point represents the mean value of one genotype across all experiments. The solid fitted lines refer to the relationship for the whole set of genotypes for the period between 1980 and 2010.

kernels, suggesting that higher transpiration and water use may exist in the more recent cultivars (Cabrera-Bosquet et al., 2009, 2011). Studies of bread wheat cultivars released between 1962 and 1988 under the high-yielding conditions of NW Mexico (CIMMYT) have reported a higher **Δ**^{13}C and lower δ^{18}O in the more modern wheats (Barbour et al., 2000; Fischer et al., 1998). When comparing modern varieties with ones released before the 1960s, the **Δ**^{13}C increased in the modern durum wheat cultivars under Mediterranean conditions (Araus et al., 2013; Royo et al., 2008), whereas no clear differences were found for δ^{18}O (Araus et al., 2013). The results of our study do not support any

Table 5
Mean values of crop growth, water and nitrogen status parameters for the set of 20 durum wheat cultivars released after the Green Revolution in Spain, measured in all environments. Data presented are the mean values for the whole set of 14 growing environments (combinations of site, year and water regime) tested. Means followed by different letters were significantly different by Tukey's b test.

Variety	Year of release	Biomass (Mg ha^{-1})	GNY (kg ha^{-1})	NDVI	N (%)	Leaf Chl	δ^{15}N (‰)	CTD (ºC)	Δ^{13}C (‰)
Mexa	1980	14.79^{abc}	119.02^{de}	0.66^{cde}	2.32^{ab}	54.02^{bcde}	4.26^{a}	4.27^{a}	18.35^{a}
Vitron	1983	15.2^{abc}	135.88^{abc}	0.69^{abc}	2.39^{a}	53.1^{cde}	4.56^{a}	4.99^{a}	18.33^{a}
Regallo	1988	15.07^{abc}	136.85^{abcd}	0.66^{de}	2.46^{a}	54.53^{cde}	4.87^{a}	4.14^{a}	17.77^{a}
Simeto	1990	14.72^{abc}	130.98^{abcde}	0.68^{abcde}	2.51^{a}	57.81^{a}	4.66^{a}	4.35^{a}	18.76^{a}
Gallareta	1994	14.11^{c}	126.04^{cde}	0.68^{abcde}	2.31^{ab}	55.34^{abcd}	4.3^{a}	3.77^{a}	18.45^{a}
Bolo	1994	15.07^{abc}	120.28^{e}	0.7^{abcd}	2.33^{ab}	55.49^{abcd}	4.37^{a}	4.09^{a}	18.30^{a}
Don Pedro	1994	14.65^{abc}	128.26^{bcde}	0.68^{bcde}	2.49^{a}	54.22^{cde}	4.54^{a}	3.88^{a}	18.56^{a}
Sula	1994	15.28^{abc}	135.11^{abcd}	0.66^{cde}	2.37^{a}	56.86^{abcd}	4.44^{a}	4.28^{a}	18.58^{a}
Bólido	1996	14.7^{abc}	131.97^{abcde}	0.67^{cde}	2.38^{a}	51.55^{e}	4.34^{a}	4.08^{a}	17.90^{a}
Dorondon	1998	14.34^{bc}	126.97^{abcde}	0.67^{bcde}	2.32^{ab}	55.75^{bcde}	4.15^{a}	4.03^{a}	18.50^{a}
Iride	1998	14.48^{abc}	125.95^{bcde}	0.68^{abcde}	2.16^{b}	56.08^{abcd}	4.18^{a}	3.68^{a}	18.37^{a}
Burgos	1999	16.49^{ab}	133.72^{abcde}	0.71^{a}	2.45^{ab}	52.43^{bcde}	4.27^{a}	4.53^{a}	18.03^{a}
Claudio	1999	16.1^{abc}	132.3^{abcde}	0.71^{ab}	2.35^{ab}	56.35^{abcd}	4.37^{a}	4.23^{a}	18.27^{a}
Amilcar	2001	14.6^{abc}	135.73^{abcd}	0.67^{cde}	2.33^{a}	56.88^{abc}	4.39^{a}	4.6^{a}	18.38^{a}
Pelayo	2002	15.88^{abc}	141.78^{ab}	0.68^{abcd}	2.33^{ab}	55.86^{abcd}	4.47^{a}	3.94^{a}	18.47^{a}
Avispa	2003	15.63^{abc}	140.37^{a}	0.67^{bcde}	2.41^{a}	57.45^{abcd}	4.36^{a}	4.06^{a}	18.46^{a}
Don Sebastián	2004	15.96^{abc}	130.62^{de}	0.68^{abcde}	2.57^{a}	55.22^{abcd}	4.41^{a}	3.91^{a}	18.26^{a}
D Ricardo	2008	15.56^{abc}	132.32^{abc}	0.66^{cde}	2.42^{a}	54.44^{bcde}	4.40^{a}	4.15^{a}	18.30^{a}
Kiko Nick	2009	15.69^{abc}	143.48^{a}	0.67^{e}	2.53^{a}	57.12^{ab}	4.47^{a}	3.78^{a}	18.36^{a}
Ramirez	2009	16.3^{a}	134.99^{abcd}	0.69^{abcde}	2.37^{ab}	52.54^{de}	4.73^{a}	4.42^{a}	17.65^{a}
G		< 0.05	< 0.001	< 0.001	< 0.001	< 0.001	ns	ns	ns
E		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
G*E		< 0.01	< 0.001	< 0.01	< 0.001	< 0.01	ns	< 0.01	ns
h^2		0.47	0.57	0.87	0.77	0.87	–	0.55	0.26

The parameters studied for crop growth: total biomass at maturity, the normalized difference vegetation index (NDVI) at around early grain filling; for nitrogen status, chlorophyll content of the flag leaf blade around early grain filling measured with a portable meter (Leaf Chl), nitrogen content (N) and nitrogen isotope composition (δ^{15}N) of mature kernels and, grain nitrogen yield (GNY); for water status: canopy temperature depression (CTD) around early grain filling and carbon isotope discrimination (Δ^{13}C) of mature kernels.

Table 6

Absolute and relative genetic changes in water status and nitrogen status traits for 20 durum wheat cultivars released after the Green Revolution in Spain, measured in all 14 environments and their relationships with GY.

Trait	r^2	Absolute changes(y^{-1})	Relative changes(y^{-1})	r^{2a}
GNY (Kg ha^{-1})	0.24*	0.3963	0.2998	0.416**
NDVI	ns	0.0001	0.0207	ns
N (%)	ns	0.0020	0.0844	0.202*
Leaf Chl	ns	0.0200	0.0363	ns
δ^{15}N (‰)	ns	−0.0009	−0.0213	ns
CTD (ºC)	ns	−0.0127	−0.3048	ns
Δ^{13}C (‰)	ns	−0.0106	−0.0512	ns

[a] Relationship with GY.

* $P < 0.05$.

** $P < 0.01$.

improvement in the water status of the more recent (from 2003 to 2009) durum wheat cultivars released in Spain and included in our study. This contrasts with the overall trend reported above of a higher **Δ^{13}**C in the scenarios where AGG has been more evident. In the same sense, the review of Roche (2015) also concluded that genetic advance in GY of cereals (including bread and durum wheat) has been associated with a higher water use (assessed either through a higher stomatal conductance of Δ^{13}C) even when many of the studies included in the review compare pre and post Green Revolution cultivars, which may bias the comparison due to different factors such as difference in phenology. In this sense, we observed that the least productive genotype (Don Sebastian), has the lowest Δ^{13}C of mature kernels among the whole set of 20 cultivars (data not shown), along with the lowest harvest index (Fig. 6a). The comparatively low Δ^{13}C from mature kernels suggests that this variety was the most water stressed during the reproductive stage, probably associated with low water use (Araus et al., 2003).

In agreement with the finding of a certain increase in Δ^{13}C with time, at least in high-yielding environments, which may indicate a higher water use, our results suggest that nitrogen uptake also does seem to have improved. Indeed, we found a linear increase in GNY over the whole period studied (1980–2010) which showed that the more recent cultivars did take up more nitrogen into the grain. However, we did not find any differences in the N concentration of kernels across varieties. This contrasts with the findings of Araus et al. (2013) in durum wheat and Del Pozo et al. (2014) in bread wheat after the adoption of Green Revolution cultivars. In our study, the increase in GNY was due to a parallel increase in GY, as shown by the positive relationship between both traits, while grain N did not play any role, at least until 2003.

Among the consequences of the introduction of semi-dwarfing genes, PH was reduced, which has been reported by many studies in different environmental conditions when comparing modern cultivars with old ones (released before the Green Revolution) in both durum wheat (Royo et al., 2008, 2007) and bread wheat (Del Pozo et al., 2014; Sanchez-Garcia et al., 2012). While the reduction in pH as a result of the Green Revolution subsequently increased the HI and kernels m^{-2}, other authors have also concluded that the moderate genetic advance reported after the Green Revolution was related to improvements in some agronomical yield components, including HI and kernels m^{-2} (Calderini and Slafer, 1998; Reynolds et al., 1999). However, in our study, which was based on fully semi-dwarf cultivars, the correlation between the year of release and PH was not significant. Likewise, HI and spikes per square meter did not change across years. This is similar to reports of spring bread wheat under the high-yielding conditions of NW Mexico (Aisawi et al., 2015), and in this sense no clear trend in HI has been evidenced since 1966 for the bread wheat varieties released by CIMMYT. The results reported by Shearman et al. (2005) for a set of bread wheat genotypes released in the UK between 1972 and 1995 have also indicated no changes in HI since 1980. As indicated above, whereas the increase in the HI during past breeding seems to have been the consequence of a reduction in plant height, no further reduction is expected. Miralles and Slafer (1995) and Richards (1992) reported that, in low stress environments, the modern cultivars have a stature close to the lower limit of optimum height. Therefore, any future efforts to increase the harvest index, should be independent of plant stature. In fact, 0.65 has been indicated as the theoretical maximum for the HI (Austin, 1982), which still gives some room for improvement in this trait.

Table 7

Absolute genetic changes in plant height, agronomical yield components, crop growth and water and nitrogen status traits for 20 durum wheat cultivars released after the Green Revolution in Spain. Evaluations were performed in high-yielding and low-yielding environments from 1980 to 2009 and from 1980 to 2003.

		High-yielding environments				Low-yielding environments			
		Absolute changes (y^{-1})		r^2		Absolute changes (y^{-1})		r^2	
		1980–2003	1980–2009	1980–2003	1980–2009	1980–2003	1980–2009	1980–2003	1980–2009
Plant height and yield components	PH (cm)	−0.21	0.013	ns	ns	−0,122	0.0006	ns	ns
	Kernels m^{-2}	114.21	45.02	ns	ns	120.66	71.39	ns	ns
	Spikes m^{-2}	−0.025	0.798	ns	ns	1.82	0.86	ns	ns
	Kernels spike^{-1}	0.50	0.20	0.324*	ns	0.335	0.22	0.414**	0.260*
	HI (%)	0.082	−0.26	ns	ns	0.120	0.031	ns	ns
	TKW (g)	−0.025	0.79	ns	ns	−0.177	−0.061	ns	ns
Crop growth, water and nitrogen status	Biomass (Mg ha^{-1})	0.032	0.042	ns	ns	0.021	0.042	ns	ns
	GNY (Kg ha^{-1})	0.288	0.399	ns	ns	0.562	0.399	ns	ns
	NDVI	0.0004	0.0002	ns	ns	0.0004	–	ns	ns
	N (%)	−0.003	0.003	ns	ns	−0.001	0.0006	ns	ns
	Leaf Chl (SPAD units)	0.081	0.024	ns	ns	0.049	0.015	ns	ns
	δ^{15}N (‰)	−0.003	0.007	ns	ns	−0.014	−0.010	ns	ns
	CTD (ºC)	−0.022	−0.011	ns	ns	−0.010	−0.014	ns	ns
	Δ^{13}C(‰)	0.012	−0.011	0.292*	ns	−0.005	0.0004	ns	ns

The parameters studied were: plant height (PH), number of grains m^{-2} (kernels m^{-2}), number of spikes m^{-2} (Spikes m^{-2}), number of kernels per spike (Kernels spike^{-1}) harvest index (HI), thousand kernel weight (TKW), the normalized difference vegetation index (NDVI) at around early grain filling; for nitrogen status, chlorophyll content of the flag leaf blade around early grain filling measured with a portable meter (Leaf Chl), nitrogen content (N) and nitrogen isotope composition (δ^{15}N) of mature kernels and, grain nitrogen yield (GNY); for water status: canopy temperature depression (CTD) around early grain filling and carbon isotope discrimination (Δ^{13}C) of mature kernels.

* $P < 0.05$.

** $P < 0.01$.

Increasing the sink size through larger numbers of fertile florets and grains per spike may be an alternative strategy (Foulkes et al., 2010). In this context, we observed that the least productive genotype in our study (Don Sebastian), besides having the lowest Δ^{13}C of mature kernels and the lowest harvest index, was the tallest genotype among the set of 20 cultivars (Fig. 6a).

The increase in GY observed in our study, even if due to a rise in the kernels m^{-2} and the kernels spike^{-1}, was paralleled with an increase in biomass during the whole period studied. In the same sense, Shearman et al., (2005) found that genetic progress in GY in UK winter wheat from 1972 to 1995 was based mainly on improvement in harvest biomass that was associated with a higher radiation use efficiency (RUE) during the stem elongation period, driving increases in kernels m^{-2} and stem soluble carbohydrate reserves.

In addition, the GY progress of CIMMYT advanced lines (1977–2008) was associated with fewer days to heading, cooler canopy temperature at grain filling, increases in stay green and higher grain weight (Lopes et al., 2012). These trends in different physiological traits were not observed in the current study of durum wheat cultivated in Spain.

5. Conclusions

In summary, the genetic gain of wheat yield in Spain showed a weak AGG between 1980 and 2003, with no clear additional improvements thereafter. Yield improvement was associated with increased kernels m^{-2} and an increase in kernels spike^{-1} during the breeding period, 1980–2003. The low rate of genetic advance in yield could be partly due to the low magnitude of genetic variability, while the existence of genetic variability for GY and many of the traits studied also suggested the absence of a clear breeding strategy. The deficit in genetic gains in recent decades may be viewed as a clear indication of the need to increase the scale of and investment in wheat breeding programs in Spain to ensure future cultivar enhancement. The need for improvements in yield and stability will become even more relevant for farmers under decreasing subsidies and increasing climate change challenges. Nevertheless, the positive association of genetic advances with temperature during the growth period highlights the role played by adaptation mechanisms during past breeding achievements, and the need to continue improving crop adaptation to withstand future environmental challenges. However, our findings also illustrate the potential limitations linked to a (near) direct adoption of foreign germplasm. While this germplasm may be well suited to the warm conditions of southern Spain, its suitability to cooler areas of the country in terms of genetic advances remains under question.

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References

Acreche, M., Brice, G., Mart, J.A., Slafer, G.A., 2008. Physiological bases of genetic gains in Mediterranean bread wheat yield in Spain. Eur. J. Agron. 28 (3), 162–170. https://doi.org/10.1016/j.eja.2007.07.001.

Aisawi, K.A.B., Reynolds, M.P., Singh, R.P., Foulkes, M.J., 2015. The physiological basis of the genetic progress in yield potential of CIMMYT spring wheat cultivars from 1966 to 2009. Crop Sci. 55 (4), 1749–1764. https://doi.org/10.2135/cropsci2014.09.0601.

Alston, J.M., Beddow, J.M., Pardey, P.G., 2010. Global patterns of crop yields and other partial productivity measures and prices. The Shifting Patterns of Agricultural Production and Productivity Worldwide. pp. 39–61.

Altintaş, S., Toklu, F., Kafkas, S., Kilian, B., Brandolini, A., Özkan, H., 2008. Estimating genetic diversity in durum and bread wheat cultivars from Turkey using AFLP and SAMPL markers. Plant Breed. 127 (1), 9–14. https://doi.org/10.1111/j.1439-0523.2007.01424.x.

Álvaro, F., Isidro, J., Villegas, D., García del Moral, L.F., Royo, C., 2008. Old and modern durum wheat varieties from Italy and Spain differ in main spike components. Field Crops Res. 106 (1), 86–93. https://doi.org/10.1016/j.fcr.2007.11.003.

Araus, J.L., Villegas, D., Aparicio, N., García Del Moral, L.F., El Hani, S., Rharrabti, Y., Ferrio, J.P., Royo, C., 2003. Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. Crop Sci. 43 (1), 170–180.

Araus, J.L., Cabrera-Bosquet, L., Serret, M.D., Bort, J., Nieto-Taladriz, M.T., 2013. Comparative performance of δ^{13}C, δ^{18}O and δ^{1}5N for phenotyping durum wheat adaptation to a dryland environment. Funct. Plant Biol. 40 (6), 595–608. https://doi.org/10.1071/FP12254.

Austin, R.B., 1982. Crop characteristics and the potential yield of wheat. J. Agric. Sci. 98 (2), 447–453.

Austin, R.B., Bingham, J., Blackwell, R.D., Evans, L.T., Ford, M.A., Morgan, C.L., Taylor, M., 1980. Genetic improvements in winter wheat yields since 1900 and associated physiological changes. J. Agric. Sci. 94 (3), 675. https://doi.org/10.1017/S0021859600028665.

Barbour, M.M., Fischer, R.A., Sayre, K.D., Farquhar, G.D., 2000. Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and grain yield in irrigated wheat. Funct. Plant Biol. 27 (7), 625–637. https://doi.org/10.1071/PP99041.

Beche, E., Benin, G., da Silva, C.L., Munaro, L.B.B., Marchese, J.A., Lemes da Silva, C., et al., 2014. Genetic gain in yield and changes associated with physiological traits in Brazilian wheat during the 20th century. Eur. J. Agron. 61, 49–59. https://doi.org/10.1016/j.eja.2014.08.005.

Bered, F., Barbosa-Neto, J.F., de Carvalho, F.I.F., 2002. Genetic variability in common wheat germplasm based on coefficients of parentage. Genet. Mol. Biol. 25 (2), 211–215. https://doi.org/10.1590/S1415-47572002000200015.

Cabrera-Bosquet, L., Molero, G., Nogués, S., Araus, J.L., 2009. Water and nitrogen conditions affect the relationships of Δ^{13}C and Δ^{18}O to gas exchange and growth in durum wheat. J. Exp. Bot. 60 (6), 1633–1644. https://doi.org/10.1093/jxb/erp028.

Cabrera-Bosquet, L., Albrizio, R., Nogués, S., Araus, J.L., 2011. Dual Δ^{13}C/δ^{18}O response to water and nitrogen availability and its relationship with yield in field-grown durum wheat. Plant Cell Environ. 34 (3), 418–433. https://doi.org/10.1111/j.1365-3040.2010.02252.x.

Calderini, D.F., Slafer, G.A., 1998. Changes in yield and yield stability in wheat during the 20th century. Field Crops Res. 57 (3), 335–347. https://doi.org/10.1016/S0378-4290(98)00080-X.

Valoración del estado de la cosecha de cereales 2016 Cooperativas Agro-alimentarias. (2016). Cooperativas Agro-Alimentarias. Retrieved from http://www.agro-alimentarias.coop/ficheros/doc/04972.pdf.

T.B. Coplen, (2008). Explanatory glossary of terms used in expression of relative isotope ratios and gas ratios. IUPAC Recommendations 2008. (International Union of Pure and Applied Chemistry Inorganic Chemistry Division, Commission on Isotopic Abundances and Atomic Weights: Research Triangle Park, NC, USA). CanadaAustriaChina/Beijing); National Representatives: J. R. de Laeter (Australia. Retrieved from http://old.iupac.org/reports/provisional/abstract08/coplen_prs.pdf.

Crespo-Herrera, L.A., Crossa, J., Huerta-Espino, J., Autrique, E., Mondal, S., Velu, G., Singh, R.P., 2017. Genetic yield gains in CIMMYT'S international elite spring wheat yield trials by modeling the genotype × environment interaction. Crop Sci. 57 (2), 789–801. https://doi.org/10.2135/cropsci2016.06.0553.

Del Pozo, A., Matus, I., Serret, M.D., Araus, J.L., Pozo, A., Matus, I., Araus, J.L., 2014. Agronomic and physiological traits associated with breeding advances of wheat under high-productive Mediterranean conditions. The case of Chile. Environ. Exp. Bot. 103, 180–189. https://doi.org/10.1016/j.envexpbot.2013.09.016.

Falconer, D.S., Mackay, T.F.C., 1996. Introduction to Quantitative Genetics, Ed 4. Longmans Green, Harlow, Essex, UK.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40 (1), 503–537. https://doi.org/10.1146/annurev.pp.40.060189.002443.

Fischer, R.A., Edmeades, G.O., 2010. Breeding and cereal yield progress. Crop Sci. 50 (Supplement 1), S-85–S-98. https://doi.org/10.2135/cropsci2009.10.0564. Crop Science Society of America.

Fischer, R.A., Rees, D., Sayre, K.D., Lu, Z.M., Condon, A.G., Larque Saavedra, A., 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Sci. 38 (6), 1467–1475. https://doi.org/10.2135/cropsci1998.0011183X003800060011x.

Foulkes, M.J., Slafer, G.A., Davies, W.J., Berry, P.M., Sylvester-Bradley, R., Martre, P., Calderini, D.F., Griffiths, S., Reynolds, M.P., 2010. Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. J. Exp. Bot. 62 (2), 469–486. https://doi.org/10.1093/jxb/erq300.

Foulkes, M.J., DeSilva, J., Gaju, O., Carvalho, P., 2016. Relationships between δ^{13}C, δ^{18}O and grain yield in bread wheat genotypes under favourable irrigated and rain-fed conditions. Field Crops Res. 196, 237–250. https://doi.org/10.1016/j.fcr.2016.07.006.

Garcıa del Moral, L.F., Royo, C., Slafer, G.A., 2005. Genetic Improvement Effects on Durum Wheat Yield Physiology. Durum Wheat Breeding: Current Approaches and Future Strategies. Food Products Press, New York, pp. 379–396.

Guzmán, C., Autrique, E., Mondal, S., Huerta-Espino, J., Singh, R.P., Vargas, M., Crossa, J., Amaya, A., Peña, R.J., 2017. Genetic improvement of grain quality traits for CIMMYT semi-dwarf spring bread wheat varieties developed during 1965–2015: 50 years of breeding. Field Crops Res. 210, 192–196. https://doi.org/10.1016/j.fcr.2017.06.002.

Henkrar, F., El-Haddoury, J., Ouabbou, H., Nsarellah, N., Iraqi, D., Bendaou, N., et al., 2016. Genetic diversity reduction in improved durum wheat cultivars of Morocco as revealed by microsatellite markers. Sci. Agric. 73 (2), 134–141. https://doi.org/10.1590/0103-9016-2015-0054.

Huang, X.Q., Wolf, M., Ganal, M.W., Orford, S., Koebner, R.M.D., Röder, M.S., 2007. Did modern plant breeding lead to genetic erosion in European winter wheat varieties? Crop Sci. 47 (1), 343–349. https://doi.org/10.2135/cropsci2006.04.0261.

Hysing, S.-C., Säll, T., Nybom, H., Liljeroth, E., Merker, A., Orford, S., Koebner, R.M.D., 2008. Temporal diversity changes among 198 Nordic bread wheat landraces and cultivars detected by retrotransposon-based S-SAP analysis. Plant Genet. Resour. Charact. Util. 6 (2), 113–125. https://doi.org/10.1017/S1479262108983544.

Juan-Aracil, J., Michelena, A., 1995. Durum wheat in Spain. Options Méditerranéennes Série A 22, 117–121. http://omciheam.org/article.php?IDPDF = 95605360.

Lopes, M.S., Reynolds, M.P., Manes, Y., Singh, R.P., Crossa, J., Braun, H.J., 2012. Genetic yield gains and changes in associated traits of CIMMYT spring bread wheat in a “Historic” set representing 30 years of breeding. Crop Sci. 52 (3), 1123–1131. https://doi.org/10.2135/cropsci2011.09.0467.

Manès, Y., Gomez, H.F., Puhl, L., Reynolds, M., Braun, H.J., Trethowan, R., 2012. Genetic yield gains of the CIMMYT International semi-arid wheat yield trials from 1994 to 2010. Crop Sci. 52 (4), 1543–1552. https://doi.org/10.2135/cropsci2011.10.0574.

Martos, V., Royo, C., Rharrabti, Y., Garcia Del Moral, L.F., 2005. Using AFLPs to determine phylogenetic relationships and genetic erosion in durum wheat cultivars released in Italy and Spain throughout the 20th century. Field Crops Res. 91 (1), 107–116. https://doi.org/10.1016/j.fcr.2004.06.003.

Martynov, S.P., Dobrotvorskaya, T.V., Pukhalskiy, V.A., 2005. Analysis of genetic diversity of spring durum wheat (*Triticum durum* desf) cultivars released in Russia in 1929–2004. Russ. J. Genet. 41 (10), 1113–1122.

Miralles, D.J., Slafer, G.A., 1995. Yield, biomass and yield components in dwarf, semi-dwarf and tall isogenic lines of spring wheat under recommended and late sowing dates. Plant Breed. 114 (5), 392–396. https://doi.org/10.1111/j.1439-0523.1995.tb00818.x.

Ogunniyan, D.J., Olakojo, S.A., 2014. Genetic variation, heritability, genetic advance and agronomic character association of yellow elite inbred lines of maize (Zea mays L.). Niger. J. Genet. 28 (2), 24–28. https://doi.org/10.1016/j.nigjg.2015.06.005.

Pfeiffer, W.H., Sayre, K.D., Mergoum, M., 1996. Enhancing genetic grain yield potential in durum wheat and triticale. Increasing yield potential in wheat: breaking the barriers. Proceedings of a Workshop Held in Ciudad Obregón 208–213 CIMMYT.

Ray, D.K., Ramankutty, N., Mueller, N.D., West, P.C., Foley, J.A., 2012. Recent patterns of crop yield growth and stagnation. Nat. Commun. 3 (1), 1293. https://doi.org/10.1038/ncomms2296.

Reif, J.C., Zhang, P., Dreisigacker, S., Warburton, M.L., Van Ginkel, M., Hoisington, D., Bohn, M., Melchinger, A.E., 2005. Wheat genetic diversity trends during domestication and breeding. Theor. Appl. Genet. 110 (5), 859–864. https://doi.org/10.1007/s00122-004-1881-8.

Reynolds, M.P., Rajaram, S., Sayre, K.D.D., 1999. Physiological and genetic changes of irrigated wheat in the post-green revolution period and approaches for meeting projected global demand. Crop Sci. 39 (6), 1611–1621. https://doi.org/10.2135/cropsci1999.3961611x.

Richards, R.A., 1992. The effect of dwarfing genes in spring wheat in dry environments. I. Agronomic characteristics. Aust. J. Agric. Res. 43 (3), 517–527. https://doi.org/10.1071/AR9920517.

Roche, D., 2015. Stomatal conductance is essential for higher yield potential of C 3 crops. Crit. Rev. Plant Sci. 34 (4), 429–453. https://doi.org/10.1080/07352689.2015.1023677.

Royo, C., 2005. Durum wheat improvement in Spain. In: Royo, C., Nachit, M.N., Di Fonzo, N., Araus, J.L., Pfeiffer, W.H., Slafer, G.A. (Eds.), Durum Wheat Breeding: Current Approaches and Future Strategies. Food Products Press, New York, pp. 883–906.

Royo, C., Briceño-Félix, G.A., 2013. Spanish wheat pool. J. Chem. Inf. Model. 53, 1689–1699. https://doi.org/10.1017/CBO9781107415324.004.

Royo, C., Álvaro, F., Martos, V., Ramdani, A., Isidro, J., Villegas, D., García del Moral, L.F., 2007. Genetic changes in durum wheat yield components and associated traits in Italian and Spanish varieties during the 20th century. Euphytica 155 (1–2), 259–270. https://doi.org/10.1007/s10681-006-9327-9.

Royo, C., Martos, V., Ramdani, Villegas, D., Rharrabti, Y., García Del Moral, L.F., 2008. Changes in yield and carbon isotope discrimination of Italian and Spanish durum wheat during the 20th century. Agron. J. 100, 352–360. https://doi.org/10.2134/agronj2007.0060.

Sanchez-Garcia, M., Royo, C., Aparicio, N., Martín-Sánchez, J.A., Álvaro, F., 2012. Genetic improvement of bread wheat yield and associated traits in Spain during the 20th century. J. Agric. Sci. 151 (1), 105–118. https://doi.org/10.1017/S0021859612000330.

Sayre, K.D., Rajaram, S., Fischer, R.A., 1997. Yield potential progress in short bread wheats in northwest Mexico. Crop Sci. 37 (1), 36–42. https://doi.org/10.2135/cropsci1997.0011183X003700010006x.

Shearman, V.J., Scott, R.K., Foulkes, M.J., 2005. Crop Physiology and Metabolism. Physiological Processes Associated with Wheat Yield Progress in the UK. Crop Sci. 45 (1), 175–185. https://doi.org/10.2135/cropsci2005.0175.

Van de Wouw, M., Kik, C., van Hintum, T., van Treuren, R., Visser, B., 2010. Genetic erosion in crops: concept, research results and challenges. Plant Genet. Resour. 8 (1), 1–15. https://doi.org/10.1017/S1479262109990062.

Waddington, S.R., Ransom, J.K., Osmanzai, M., Saunders, D.A., Saunders, D.A., 1986. Improvement in the yield potential of bread wheat adapted to northwest Mexico improvement in the yield potential of bread wheat adapted to northwest Mexico1. Crop Sci. 26 (4), 698–703. Retrieved from. https://www.researchgate.net/profile/Stephen_Waddington/publication/250113933_Improvement_in_the_Yield_Potential_of_Bread_Wheat_Adapted_to_Northwest_Mexico/links/5570c86708ae2f213c2238ec.pdf.

Warburton, M.L., Crossa, J., Franco, J., Kazi, M., Trethowan, R., Rajaram, S., et al., 2006. Bringing wild relatives back into the family: recovering genetic diversity in CIMMYT improved wheat germplasm. Euphytica 149 (3), 289–301. https://doi.org/10.1007/s10681-005-9077-0.

Chapter 2

Agronomic and physiological traits related to the genetic advance of semi-dwarf durum wheat: The case of Spain

Fadia Chairi, Rut Sanchez-Bragado, Maria Dolores Serret, Nieves Aparicio, Maria Teresa Nieto-Taladriz and José Luis Araus

Highlights

- Traits associated with genetic gains in yield may inform future breeding strategies
- Genotypic differences exist in modern durum wheat cultivars released in Spain
- Kernels per spike is the main agronomic component determining yield improvement
- Delayed senescence is a negative trait related to limits on sink accumulation of N
- Higher yielding genotypes exhibit better water status across Mediterranean conditions

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Agronomic and physiological traits related to the genetic advance of semi-dwarf durum wheat: The case of Spain

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ABSTRACT

Knowledge of the agronomic and physiological traits associated with genetic gains in yield is essential to improve understanding of yield-limiting factors and to inform future breeding strategies. The aim of this paper is to dissect the agronomic and physiological traits related to genetic gain and to propose an ideotype with high yield that is best adapted to Spanish Mediterranean environments. Six semi-dwarf (i.e. modern) durum wheat genotypes were grown in a wide range of growing conditions in Spain during two successive years. Diverse agronomic, physiological and leaf morphological traits were evaluated. Kernels spike^{-1} was the yield component most affected by the genetic gain. While no interaction between genotype and growing conditions existed for grain yield, the more productive genotypes were characterized by a plant height of around 85 cm, small erect flag leaves, more open stomata, a better balance between N sources and N sinks and a higher capacity to re-fix CO_2 respired by the grain. Moreover, in general the non-laminar parts of the plants play a key role in providing assimilates during grain filling. The high heritability of most of the studied parameters allows their consideration as traits for phenotyping durum wheat better adapted to a wide range of Mediterranean conditions.

1. Introduction

Durum wheat is the 10th most important crop worldwide owing to its annual production of 37 million tons [1,2]. Moreover, it is one of the most widely cultivated herbaceous crops in the Mediterranean basin, where terminal abiotic stresses are the main yield constraints. Italy and Spain are the largest producers of durum wheat in the Mediterranean basin [3]. In Spain, durum wheat represents about 5.7% (1.32 million tons) of the national cereal production, and about 65% of the area under durum wheat cultivation is located in the Southern part of the country (Andalucía) [4].

Increasing grain yield in cereal crops in general and durum wheat in particular has been a major goal of most breeding programs, and the effects of genetic improvement on yield potential have been reported in several studies [5–7]. The grain yield of durum wheat has increased significantly worldwide from the early 1960s, coinciding with the adoption of the green revolution. Spain has followed the same trend, with the average yield of 2.8 Mg ha^{-1} for pre-green revolution genotypes rising beyond 5 Mg ha^{-1} for varieties released in the 1980s [7,8]. However, in the last three decades yield improvement for both durum and bread wheat has slowed, indicating that genetic gain is potentially leveling off [7,9,10]. Nevertheless, despite no clear advances in recent times, genotypic differences do exist in grain yield across the post green revolution durum wheat varieties released in Spain [7,11]. Dissecting the agronomic and physiological components behind the genotypic differences in grain yield may contribute towards genetic advances in future breeding.

The increase in yield during the green revolution, associated with the introduction of semi-dwarf (i.e. modern) cultivars, was mainly achieved by a decrease in plant height, thus reducing lodging, while increasing the harvest index, due to a reduced competition from the growing stem, resulting in larger spikes with more grains per spike [12]. However, further decreases in plant height do not seem feasible and in fact may penalize yield potential and even adaptation to

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Abbreviations: CT, canopy temperature; $\delta^{13}C$, carbon isotope composition; KWSP, kernel weight per spike; KNSP, total kernel nitrogen per spike; PH, plant height

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unfavorable conditions [13,14]. On the other hand, the green revolution was not associated with an increase in TKW, with the opposite occurring instead [7,11], nor was there an increase in the number of spikes per square meter [7]. Moreover, the increase in grain yield associated with the green revolution does not seem to have been related to more biomass but rather its redistribution [11,15], despite the fact that some reports indicate higher biomass in more recent cultivars [16]. Genetic gain in grain yield may also be achieved through targeting additional traits closely associated with improved plant adaptation to stress [17]. Under water limiting conditions, various physiological processes and traits have been associated with GY [18–20]. Among them are traits related to delays in senescence (i.e. staygreen) during grain filling assessed via changes in leaf greenness [21,22]. Moreover, staygreen also appears to be beneficial in terms of increasing yield potential [22]. Senescence is a genetically programmed and environmentally influenced process resulting in the destruction of chlorophyll and the remobilization of nutrients to younger or reproductive parts of plants, and this is the case for growing grains in cereals [23]. It has been widely assumed that extending the green canopy duration by delaying senescence will extend the grain filling period, increase grain yield [24] and contribute to yield stability under stress [23]. Other traits like higher stomatal conductance have also been proposed as being associated with genetic advance in yield potential [25], as well as under moderate to medium stress conditions [26]. This is further supported by the positive phenotypic correlations usually found between yield and carbon isotope discrimination (Δ^{13}C) or the negative correlations with carbon isotope composition (δ^{13}C) within Mediterranean environments [19,27–31]. In wheat, Δ^{13}C can provide an indirect determination of the effective water use of the crop [19,32]. Although a negative association between Δ^{13}C and yield has been found under very dry Mediterranean rainfed conditions, a positive association is more common because genotypes capable of sustaining greater stomatal conductance and water consumption are more productive [27,33]. The carbon isotope signature has also been used to evaluate the relative contribution of different photosynthetic organs during grain filling [34–36]

In this study we dissected the agronomic and physiological traits related to genetic gain in durum wheat during recent decades. We have selected a subset of six representative cultivars from a set of 20 post green revolution cultivars studied by Chairi et al. [7]. In this previous study, and except for the number of kernels per spike, for the three decades studied there was no significant temporal trend in the genetic advance of yield or other agronomic (grain yield, grain weight and spikes per square meter) and phenological (e.g. days to heading) components, or physiological (egg. δ^{13}C) traits [7]. Also, we investigated the role of different plant parts as photosynthetic contributors during grain filling.

2. Materials and methods

2.1. Plant material and growing conditions

Experiments were carried out under field conditions in three experimental stations embracing a wide range of latitudes and elevations, therefore providing a wide range of growing temperatures in Spain. Experiments were conducted at the experimental stations of the Spanish “Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria” (INIA) at Coria del Rio (Cor), Seville (37°14´N. 06°03´W. 5 masl) and Colmenar de Oreja-Aranjuez (Aran), Madrid (40°04´N. 3°31´W. 590 masl), as well as at the “Instituto Tecnológico Agrario de Castilla y León” (ITACyL) in Zamadueñas (Zam), Valladolid (41°41´N. 04°42´W. 700 masl). Trials were conducted during two successive crop seasons (2013-14, and 2014–15), except for Coria where only one trial was conducted during the second crop season (Table1).

Six durum wheat (*Triticum turgidum* L. subsp. *Durum* (Desf) Husn.) cultivars were selected among a set of 20 fully semi-dwarf (i.e. modern) cultivars, evaluated in a previous study and released in Spain during the three decades (1980–2009) after the green revolution [7]. Specific cultivars were chosen to be representative of variability in yield performance, including some extreme genotypes (with contrasting yield within trials), while maintaining similar phenology [7,37]: Sula (1994), Iride (1998), Pelayo (2002), Don Sebastian (2004), Don Ricardo (2008) and Kiko Nick (2009).

Plots were sown in a randomized block design with three replicates. Each plot consisted of six rows 7 m long and 0.2 m apart, with a planting density of 250 seeds m^{-2}. Rainfed (RF) and supplemental irrigation (IR) conditions were imposed at Aran and Zam. Due to the presence of a shallow water table caused by proximity to the Guadalquivir River, genotypes could only be evaluated under rainfed conditions at the Cor experimental site. A different irrigation regime was applied at each site, with the timing and amount of water applied designed to ensure good crop growth during the critical period from stem elongation to mid grain filling. To that end, at each site specific agronomic practices were followed. For both years at Aranjuez (A1IR and A2IR), irrigation was applied to compensate for evapotranspiration during the period between stem elongation and the milk-dough development stage. For both years at Zamadueñas (Z1IR and Z2IR), 25 mm of irrigation was applied approximately every week from heading to ripening.

Considering location (Aran, Zam and Cor), water regime (RF and IR) and seasons (1 and 2), a total of nine growing conditions were evaluated, four in the first year and five in the second. Phenology was recorded throughout the crops life cycle using the Zadoks scale for growth stages (GS) [38]. Days to heading (days from emergence until 50% of the spikes are half emerged, GS55) were recorded in all environments. Plant height (PH) was measured after anthesis as the distance from ground to the ear tip, excluding awns.

In the second year, together with PH, leaf length, leaf width and spike length were measured. Flag leaf blade, ear and peduncle samples were taken at early grain filling (GS 71) and were saved at -80 °C for carbon isotope composition and elemental carbon and nitrogen analyses.

2.2. Agronomic traits

For each plot the number of spikes per m^2 (spike m^{-2}) was determined at maturity by sampling and counting the spikes contained in two 0.5 m-length portions of one of the central-rows at Zam and one 0.5 m length of a central-row at Cor and Aran. Further, kernels spike^{-1} was determined using a subset of ten representative stems per plot. GY was assessed by harvesting the whole plot. Then the thousand kernel weight (TKW) was measured and subsequently the number of kernels per m^2 (kernels m^{-2}) was determined, together with the total kernel weight (KWPS) and the total kernel nitrogen (KNSP) per spike.

2.3. Remote sensing indices

In the first-year remote sensing measurements were undertaken once, around early grain filling (GS71). In the second year, measurements were carried at around booting (GS 45), early grain filling (GS 71), medium grain filling (GS 75) and late grain filling (GS77). The normalized difference vegetation index (NDVI) was determined with a portable spectroradiometer with an active sensor (GreenSeeker handheld crop sensor, Trimble, USA) scanning with the sensor held perpendicularly to the canopy and 0.5–0.6 m above the top canopy. NDVI was calculated using the equation: NDVI = (NIR − R) / (NIR + R), where R is the reflectance in the red band (660 nm) and NIR is the reflectance in the near-infrared band (760 nm).

One digital RGB picture was taken per plot, holding the camera at 0.8–1.0 m above the plant canopy, in a zenithal plane and focusing near the center of each plot. Photographs were taken with a Nikon D40 camera. The camera had a set focal length of 18 mm, shutter speed of 1/

Table 1
Experimental trial conditions of the nine trials.

Crop season	Station	Soil texture (organic matter)	Soil classification	Water regime	Trial code	Sowing	Harvest	Irrigation[a]	Rain[b]	ET_0	Fertilization			Average daily Temp (Min-Max)b
								mm	mm	mm	NPK (kg ha^{-1})	CAN 27%N (kg ha^{-1})	Urea 46% (kg ha^{-1})	(ºC)
2013-2014	Zamadueñas	Slimy sandy loam (0.8%)	Xerofluvent	RF	Z1RF	25-11-2013	22-07-2014	–						
									212	690				
				IR	Z1IR			125			300	300	–	(2 - 26)
	Colmenar de Oreja	Silty clay loam (0.5%)	Calcixerept	RF	A1RF	22-11-2013	09-07-2014	–						
									203	634				
				IR	A1IR			180			400	–	150	(0 - 25)
2014-2015	Zamadueñas	Slimy sandy loam (0.8%)	Xerofluvent	RF	Z2RF	24-11-2014	22-07-2015	–						
									258	690				
				IR	Z2IR			125			300	300	–	(4 - 17)
	Colmenar de Oreja	Silty clay loam (0.5%)	Calcixerept	RF	A2RF	21-11-2014	20-07-2011	–						
									206	727				
				IR	A2IR			180			400	–	150	(5 - 21)
	Coría del Rio	Loam (0.9%)		RF	C2RF	01-12-2014	10-07-2015	–	168	780	400	–	150	(5 - 20)

[a] in the irrigated treatments.
[b] during growing season.
IR: Supplemental irrigation; RF: Rainfed; ET_0: Evapotranspiration.

125 without flash, the aperture set to automatic and the images were saved in JPEG format with a size of 1920 × 1280 pixels. Pictures were subsequently analyzed with the open source Breedpix 0.2 software designed for digital photograph processing of different color properties [39]. This software enabled the determination of the RGB vegetation indices green area (GA) and greener area (GAA). Both are formulated based on the number of green pixels in the image, but differ due to GAA excluding yellowish-green tones and therefore more accurately describing the amount of photosynthetically active biomass and leaf senescence.

The leaf chlorophyll content (Leaf Chl) was measured using a portable chlorophyll meter (SPAD-502 Chlorophyll Meter, Minolta Co. Ltd., Osaka, Japan). Five flag leaves were measured for each plot. Measurements were taken from the middle portion of the lamina.

2.4. Total N content and C and N isotope analyses

For the first year, only mature kernels were analyzed. For the second year, together with mature kernels, the dry matter and water-soluble fraction in the flag leaf, peduncle and different ear parts (awns, glumes, lemma and palea) were analyzed. All the measurements were performed for each individual plot, within the entire set of trials.

Stable carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios, together with the total nitrogen content, were determined. Measurements of carbon and nitrogen isotopes were conducted at the Scientific Facilities of the University of Barcelona, using an elemental analyzer (Flash 1112 EA; Thermo Finnigan, Berman Germany) coupled with an isotope ratio mass spectrometer (Delta C IRMS, Thermo Finnigan), operating in continuous flow mode. Isotopic results were expressed in standard δ –notation [40].

$$X = \left(\frac{Rsample}{Rstandard} - 1\right) \times 100$$

where X is the $\delta^{13}C$ or $\delta^{15}N$ value, and R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios, respectively. The $\delta^{13}C$ values were reported relative to the Vienna PeeDee Belemnite standard, whereas the $\delta^{15}N$ values were reported relative to the standard N_2 in air [41].

2.5. Water soluble fraction

The protein-free water-soluble fraction (WSF) of the flag leaves and ears was extracted from the same dry samples tested for carbon isotope composition, as described previously [42–44]. Leaf and ear powder were suspended with 1 ml of Milli-Q water in an Eppendorf tube (Eppendorf Scientific, Hamburg. Germany). After centrifugation, the supernatant containing the WSF was collected. Soluble proteins in the supernatant were heat-denatured and precipitated. After centrifugation, an aliquot of 60 µl of the supernatants containing the protein-free WSF was transferred to tin capsules and dried at 60 °C for isotope analyses as indicated above.

2.6. Statistical analysis

The hypothesis of zero difference between means was tested with analysis of variance (ANOVA) performed using the general linear model procedure to calculate first the effects of year, environment (combination of specific site x water regime), genotype and environment by genotype interactions on the measured and calculated parameters. Then, because the year effect was not significant for GY we combined the two-year data and analyzed the environment (combination of year x specific site x water regime) and genotype effects and their interaction. Mean separation between genotypes for the different parameters was performed with Duncan's multiple range test ($P < 0.05$). Broad sense heritability (h2) was estimated for each trait over the different environments as:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{e} + \frac{\sigma^2}{re}}$$

where r = number of repetitions, e = number of environments, r2 = error variance, r2g = genotypic variance and r2ge = G x E variance. Calculations were done using either the subset of six genotypes or the original set of 20 genotypes [7].

A bivariate correlation procedure was constructed to analyze the relationships between the studied traits. Stepwise regression analyses were conducted with grain yield as the dependent variable and yield components as the independent ones. Data were analyzed using the SPSS 21.0 statistical package (SPSS Inc., Chicago, IL, USA). Figures were created using the Sigma-Plot 11.0 program for Windows (Systat Software Inc., Point Richmond, CA, USA). A principal component analysis (PCA) of yield components and physiological parameters of wheat were carried out in Rstudio version 3.4.3. In addition, yield stability of genotypes was assessed using the genotype main effects and genotype × environment interaction effect GGE biplot methodology, by an average environment coordinate (AEC) method [45] using GEA-R free software analysis [46] including the 20 genotypes evaluated in a previous study [7]. In this method, the average principal components were used for each of the nine growing conditions. A line was then drawn through this average environment and the biplot origin; this line is called the average environment axis and serves as the abscissa of the AEC. Unlike the AEC abscissa, this has one direction, with the arrow pointing to a greater genotype main effect; the AEC ordinate and either direction away from the biplot origin indicates a greater genotype x environment interaction (GEI) effect and reduced stability.

3. Results

3.1. Grain yield and agronomic yield components

GY across genotypes ranged between 5.96 Mg ha^{-1} (Pelayo) and 4.69 Mg ha^{-1} (Don Sebastian), considering the two crop seasons (nine growing conditions), with no significant year effect but significant genotypic variation (Table S1). When combining the two-year data, significant differences between both genotypes and growing conditions were found (Table 2). The same pattern of differences was found in the second year on its own (Table S2). In fact, Pelayo and Don Sebastian were the two extreme genotypes, when either the nine growing conditions or only the five growing conditions of the second season were considered. In addition, there were no differences across genotypes in days to heading (DTH).

Concerning the agronomic components, a significant year effect was observed for all traits, while the genotypic variation was also significant for all traits except biomass (BM) at maturity (Table S1). When combining the two-year data, all of the agronomic components exhibited genotypic variation except again BM. Thousand kernel weight (TKW) ranged from 49.95 g (Don Sebastian) to 41.60 g (Sula), kernels per spike (kernels $spike^{-1}$) ranged from 41.37 (Iride) to 25.11 (Don Sebastian), spikes m^{-2} ranged from 404.3 (Kiko Nick) to 333.5 (Iride), kernels m^{-2} ranged from 10,392 (Don Sebastian) to 14,498 (Iride), KWPS ranged from 1.25 g (Don Sebastian) to 1.76 g (Iride) and HI ranged from 32.51 (Don Sebastian) to 41.93 (Iride). The two extreme genotypes in terms of grain yield exhibited different response patterns in their agronomic yield components (Table 2 and Table S2). Among the six genotypes studied, Don Sebastian was characterized by the lowest HI and the highest TKW, together with a relatively high number of spikes m^{-2} but a relatively low number of kernels $spike^{-1}$, while Pelayo exhibited a relatively high HI, spike m^{-2} and TKW and intermediate numbers of kernels $spike^{-1}$. GY and all the agronomic parameters studied were significantly affected by the agronomic conditions (Table 2 and Table S2). The environment by genotype interaction was not significant for GY, BM, spikes m^{-2} or kernels m^{-2}, while significant interactions were

Table 2

Mean values for grain yield (GY), agronomic components, plant height, carbon isotope composition and N status parameters measured in the six selected genotypes in two crop seasons (2014–2015) Each value is the mean of the nine environments (combining specific site, year and growing conditions). Grain yield (GY), thousand kernel weight (TKW), number of kernels per spike (kernels spike^{-1}), number of spikes per square meter (spikes m^{-2}), number of kernels per square meter (kernels m^{-2}), kernel weight per spike (KWPS), biomass at harvest (BM), harvest index (HI), days to heading (DTH), plant height (PH), the normalized difference vegetation index (NDVI), green area (GA), greener area (GGA) and chlorophyll content (SPAD).

	Year of release	GY (Mg ha^{-1})	TKW (g)	Kernels Spike^{-1}	spikes m^{-2}	kernels m^{-2}	KWPS (g)	BM (Mg ha^{-1})	HI	DTH	PH (cm)	NDVI	GA	GGA	SPAD
Pelayo	2002	5.96^{a}	46.08^{c}	34.05^{c}	390.26^{ab}	13959^{a}	1.57^{c}	16.57^{a}	38.46^{b}	145.7^{a}	85.37^{d}	0.685^{a}	0.737^{b}	0.550^{b}	56.7^{a}
Sula	1994	5.83^{a}	41.60^{d}	38.22^{b}	370.00^{bc}	14277^{a}	1.61^{bc}	15.87^{a}	38.79^{b}	145.4^{a}	84.66^{cd}	0.679^{a}	0.807^{a}	0.576^{b}	56.7^{a}
Iride	1998	5.80^{a}	42.20^{d}	41.37^{a}	333.45^{d}	14,498^{a}	1.76^{a}	14.98^{a}	41.93^{a}	145.9^{a}	85.21^{cd}	0.682^{a}	0.823^{a}	0.619^{ab}	57.4^{a}
Kiko Nick	2009	5.70^{ab}	48.56^{ab}	29.35^{d}	404.32^{a}	12438^{b}	1.43^{d}	15.87^{a}	38.77^{b}	145.2^{a}	86.74^{c}	0.693^{a}	0.796^{ab}	0.617^{ab}	57.2^{a}
D.Ricardo	2008	5.49^{b}	47.45^{bc}	35.59^{c}	346.62^{bc}	12532^{b}	1.69^{db}	15.84^{a}	37.19^{b}	147.1^{a}	91.69^{b}	0.675^{a}	0.820^{a}	0.620^{ab}	54.9^{b}
D.Sebastian	2004	4.69^{c}	49.95^{a}	25.11^{e}	396.76^{ab}	10,392^{c}	1.25^{e}	16.30^{a}	32.51^{c}	147.5^{a}	96.81^{a}	0.691^{a}	0.868^{a}	0.661^{a}	55.7^{ab}
G		0.000	0.000	0.000	0.000	0.000	0.000	0.248	0.000	0.432	0.000	0.530	0.000	0.000	0.002
Env		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G x E		0.130	0.019	0.001	0.115	0.263	0.011	0.140	0.000	0.231	0.000	0.913	0.393	0.682	0.383
$h^2_{(20\ \text{genotypes})}$		0.74	0.96	0.95	0.79	0.91	0.89	0.67	0.85	0.94	0.94	0.82	0.63	0.75	0.88
$h^2_{(6\ \text{genotypes})}$		0.95	0.95	0.97	0.86	0.97	0.96	–	0.96	0.96	0.96	0.60	0.79	085	0.65

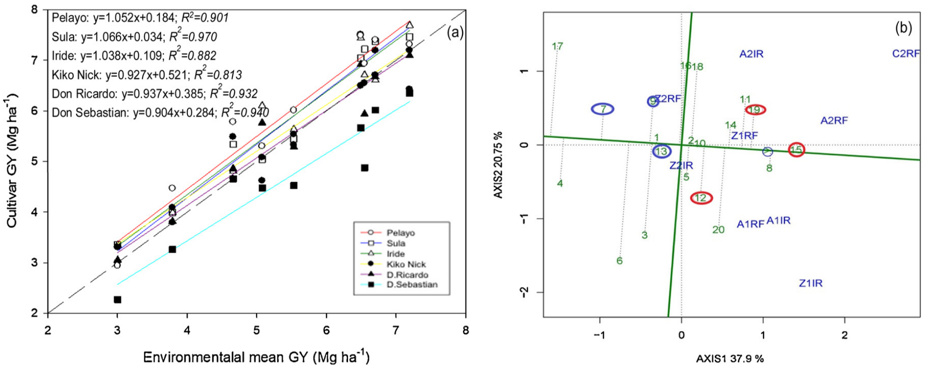

Fig. 1. (a) Regression lines showing the relationship between the individual mean grain yields (GY) of six cultivars of durum wheat in different environments and the mean yield of each environment. The environmental mean GY is the mean of a set of 20 semi-dwarf durum wheat varieties grown during the same trials. The broken line represents the 1:1 slope. All genotypes followed a similar pattern, with a regression coefficient around 1. (b) Average environment coordination (AEC) views of the GGE-biplot based on environment-focused scaling for the mean performance and stability of genotypes. Green numbers represent genotypes. (1) Amílcar, (2) Avispa, (3) Bólido, (4) Bolo, (5) Burgos, (6) Claudio, (7) Don Ricardo, (8) Don Pedro, (9) Don Sebastian, (10) Dorondon, (11) Gallareta, (12) Iride, (13) Kiko Nick, (14) Mexa, (15) Pelayo, (16) Ramirez, (17) Regallo, (18) Simeto, (19) Sula and (20) Vitron. Blue symbols represent environments: a combination of locations (A, Aranjuez; C, Coria; Z, Zamadueñas), years (1, 2014; 22,015) and treatments (RF, rainfed and IR, supplemental irrigation). The six selected genotypes have been designed by a red or blue circle, which refer to high productivity and low productivity genotypes, respectively. AXIS 1 and AXIS 2 refer to PC1 and PC2 (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

observed for HI, TKW, kernels spike^{-1} and KWPS. In the case of GY, Fig. 1a shows graphically a non-crossover pattern across growing conditions for the six varieties with the ranking of genotypes remaining constant across environments. In addition, the AEC ordinate separates genotypes with below-average means from those with above-average means (Fig. 1 b). The genotypes with above-average means were Pelayo, Iride, and Sula and those with below average means were Don Sebastian, Don Ricardo, and Kiko Nick. Both Pelayo and Don Sebastian seemed stable even though they had different yields. Conversely, Iride, Sula, Kiko Nick, and Don Ricardo were more variable.

3.2. Plant height, vegetation indices and morphological traits

Plant height (PH) and all vegetation indices measured in early grain filling exhibited a significant year effect and genotypic variation, except for NDVI (Table S1). When combining the two-year data, significant differences between genotypes existed for pH and all vegetation indices except NDVI (Table 2). Across varieties pH ranged between 96.8 cm (Don Sebastian) and 84.7 cm (Sula), with Pelayo being among the shortest of the genotypes (85.4 cm). The GA and GGA indices ranged from 0.868 and 0.661 (Don Sebastian) to 0.737 and 0.550 (Pelayo), respectively. No differences in leaf chlorophyll content were recorded between the two extreme genotypes (Pelayo and Don Sebastian). pH and all vegetation indices were significantly affected by the environment (Table 2), and the environment by genotype interaction was significant for pH but not for any of the vegetation indices. Considering only the second year (Table S3), the same pattern of differences was recorded between the canopy vegetation indices measured at early grain filling for the extreme genotypes, but they only reached significance for SPAD ($P < 0.05$) and marginally ($P = 0.09$) for GA. However, for the second year, significant genotype variation existed for GA ($P < 0.05$) measured at booting and mid grain filling, while the significance was marginal at early ($P = 0.09$) and late ($P = 0.07$) grain filling (Fig. 2). Genotype variation also existed for NDVI ($P < 0.01$) at mid grain filling, and for Leaf Chl at early grain filling. Throughout the productive period Don Sebastian tended to have the highest values for GA and NDVI and Pelayo the lowest (Fig. 2), and the same occurred for GGA (data not shown).

Significant genotype and environment effects were observed for leaf length ($P < 0.001$), leaf width ($P < 0.01$) and spike length ($P < 0.05$), which were measured in the second year (Table S3). The environment by genotype interaction was not significant for any of the traits except leaf length. Leaf length ranged between 18.2 cm (Iride) and 21.96 cm (Don Ricardo). Spike length ranged between 6.48 cm

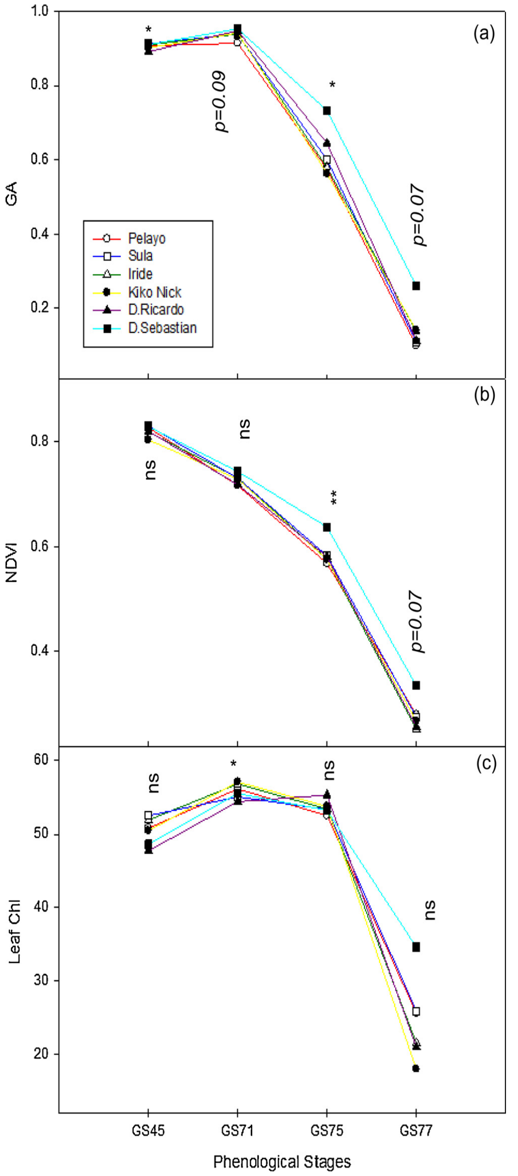

Fig. 2. Mean values of GA(a), the NDVI (b) and leaf chlorophyll content (c) at different phenological stages. Each point is the mean of five environments with three replicates in each environment. Each line represents a genotype. Probabilities (ns, not significant; *, $P < 0.05$; **, $P < 0.01$) are shown.

(Kiko Nick) and 7.19 (Iride).

3.3. Water and N status parameters

Water and N status parameters exhibited a significant year effect and genotypic variation for all traits except for canopy temperature depression (CTD) (Table S4). Considering the nine environments, both the growing condition and genotype had a significant effect on the kernel carbon isotope composition ($\delta^{13}C_{Kernel}$), CTD, kernel N content and KNSP (Table 3) and no genotypic effect on nitrogen isotope composition ($\delta^{15}N_{Kernel}$). Don Sebastian exhibited the highest $\delta^{13}C_{Kernel}$ (-25.25‰) and the lowest CTD (4.28 °C), while Pelayo had the lowest $\delta^{13}C_{Kernel}$ (-25.74‰), and a high CTD (5.15 °C). In addition, Don Sebastian exhibited the highest N_{kernel} (2.61%) but the lowest KNPS (31.7 mg), while Pelayo had the lowest N_{kernel} (2.35%) and the highest KNPS (36.2 mg). A genotype by environment interaction was identified for $\delta^{15}N_{Kernel}$, and also marginally for CTD and N_{kernel}, but not for $\delta^{13}C_{kernel}$. Even so, a trend for a crossover in $\delta^{13}C_{Kernel}$ was observed in the less productive environments (Fig. 3).

3.4. Carbon isotope composition in different tissues

Significant genotype variation was exhibited in $\delta^{13}C$ for all tissues except $\delta^{13}C_{glume.DM}$ and $\delta^{13}C_{peduncle.DM}$ and $\delta^{13}C_{awns.WSF}$ and $\delta^{13}C_{peduncle.WSF}$ (Table S5). The $\delta^{13}C$ of different organs was significantly affected by environmental conditions, except for $\delta^{13}C_{peduncle.DM}$, and only marginally for $\delta^{13}C_{lemma.WSF}$. The environment by genotype interaction was highly significant for all tissues except for $\delta^{13}C_{glume.DM}$, $\delta^{13}C_{peduncle.DM}$, and $\delta^{13}C_{awns.WSF}$, where it was not significant, and for $\delta^{13}C_{peduncleWSF}$, $\delta^{13}C_{leaf.WSF}$ and $\delta^{13}C_{leaf.WSF}$, where it was just marginally significant. While no differences in $\delta^{13}C$ existed between DM and WSF for awns, leaves and peduncles, the values were higher (less negative) in WSF compared to the DM of the other three organs of the spike. Significant differences in $\delta^{13}C$ values were observed across plant organs, with both the DM and WSF of the peduncle and flag leaf blades having the highest and the lowest $\delta^{13}C$ values, respectively (Fig. 4). The values of $\delta^{13}C_{Kernel}$ were significantly different to the $\delta^{13}C$ of the other plant parts with the exception of $\delta^{13}C_{lemma.DM}$ and $\delta^{13}C_{glume.WSF}$. When specifically considering the $\delta^{13}C$ of the WSF, organ values of the peduncles, glumes, lemmas and paleas were higher and those of the awns and the leaves were lower than the $\delta^{13}C_{Kernel.}$ With regard to the DM, the $\delta^{13}C$ organ values of the paleas and peduncles were higher while those of glumes, lemmas, awns and leaves were lower than the $\delta^{13}C_{Kernel}$ (Fig. 4).

3.5. Relationship between $\delta^{13}C$, GY and yield components across growing conditions

The correlations of $\delta^{13}C$ with GY and the agronomic yield components were examined (Fig. 5). The relationship of the $\delta^{13}C$ of kernels, flag leaves, peduncles and different parts of the ears (glumes, lemma, palea and awns) against GY and yield components were plotted using the whole set of individual measurements for each of the five growing conditions of this study (the second growing season). The $\delta^{13}C_{kernels}$ correlated negatively with GY in four of the five growing conditions. The $\delta^{13}C$ of the different plant parts other than kernels correlate in few cases with GY;indeed, the $\delta^{13}C$ of some ear parts when measured in WSF for a couple of growing conditions and the $\delta^{13}C$ of the flag leaf DM in one growing condition. The relationships between $\delta^{13}C_{kernels}$ and TKW were positive but only achieved statistical significance for two growing conditions. No correlation was found between $\delta^{13}C_{kernels}$ and the other agronomic components. $\delta^{13}C_{leaf.DM}$ correlated negatively with TKW, achieving significance in three of the five growing conditions and also correlated positively with kernels spike^{-1}, achieving significance in four of the five growing conditions. By contrast, when the $\delta^{13}C$ of the WSF was considered, the values of some ear parts correlated significantly with the two yield components determined during the last part of the crop cycle: against kernels spike^{-1} in three of the five environments and against TKW in four of the five environments.

3.6. Relationships of leaf length with $\delta^{13}C$ and GY

The relationships between GY and leaf length were negative but only achieved statistical significance under rainfed conditions in Zam and Aran (Fig. 6) using the six genotypes and three replicates of this study (18 values) as well as the whole set of 20 genotypes reported in Chairi et al. [7] (60 values). The leaf length correlated positively with $\delta^{13}C_{kernels}$ within the rainfed conditions of Zam and Aran.

Table 3
Mean values for water status and N status parameters measured in the six selected genotypes in two crop seasons (2014–2015). Each value is the mean of the nine environments (combining specific site, year and growing condition). Kernel carbon isotope composition ($\delta^{13}C_{Kernel}$), canopy temperature depression (CTD), kernel nitrogen isotope composition ($\delta^{15}N_{Kernel}$), kernel nitrogen content (N_{Kernel}) and total kernel nitrogen per spike (KNPS).

	$\delta^{13}C_{kernel}$ (‰)	CTD (°C)	$\delta^{15}N_{Kernel}$ (‰)	N_{Kernel} (%)	KNPS (mg)
Pelayo	-25.74^{b}	5.15^{ab}	4.19^{a}	2.35^{bc}	36.2^{b}
Sula	-25.70^{b}	5.35^{ab}	4.31^{a}	2.32^{cd}	35.5^{bc}
Iride	-25.65^{b}	4.73^{ab}	4.27^{a}	2.20^{d}	38.1^{ab}
Kiko Nick	-25.56^{b}	4.45^{b}	4.50^{a}	2.60^{a}	37.1^{ab}
D.Ricardo	-25.59^{b}	5.75^{a}	4.53^{a}	2.48^{ab}	41.0^{a}
D.Sebastian	-25.25^{a}	4.28^{b}	4.57^{a}	2.61^{a}	31.7^{c}
G	0.003	0.037	0.145	0.000	0.000
Env	0.000	0.000	0.000	0.000	0.000
G x E	0.250	0.046	0.018	0.050	0.231
$h^2_{(20\ genotypes)}$	0.38	–	–	0.69	0.79
$h^2_{(6\ genotypes)}$	0.48	–	–	0.86	0.86

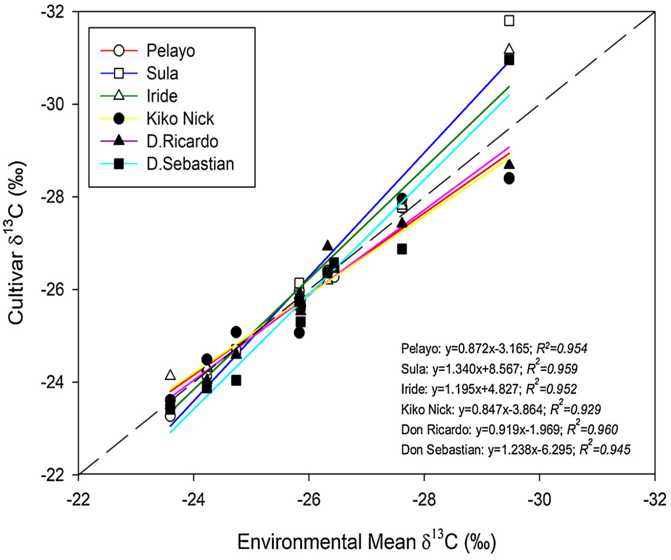

Fig. 3. Regression lines showing the relationship between the individual mean carbon isotope composition ($\delta^{13}C$) of kernels of six of durum wheat genotypes at different locations and the mean $\delta^{13}C$ of each environment. The environmental mean is the mean of a set of 20 durum wheat semi-dwarf varieties grown during the same trials. The broken line represents the 1:1 slope.

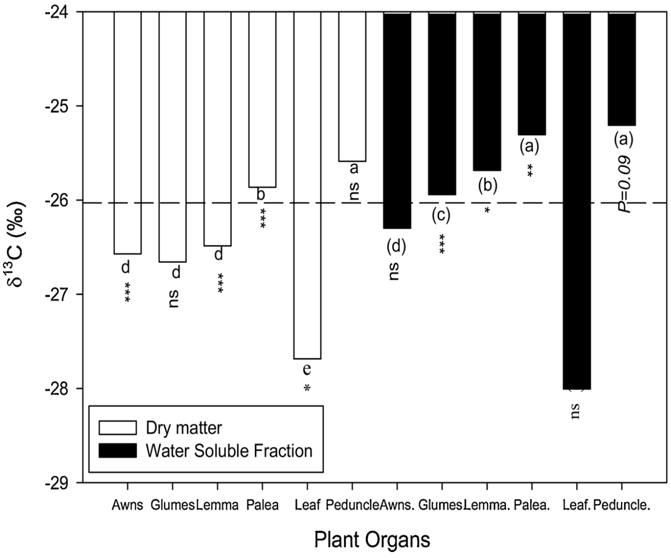

Fig. 4. Carbon isotope composition ($\delta^{13}C$) of the different organs at early-grain filling in dry matter and the water-soluble fraction (filled and open bars) compared to the $\delta^{13}C$ of the kernels (dashed horizontal line). Each bar represents the mean $\delta^{13}C$ of the six genotypes in all environments. Mean values with different superscript letters are significantly different between different organs according to Duncan's test ($P < 0.05$). Probabilities of the genotype by environment interaction (ns, not significant; *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$) are shown.

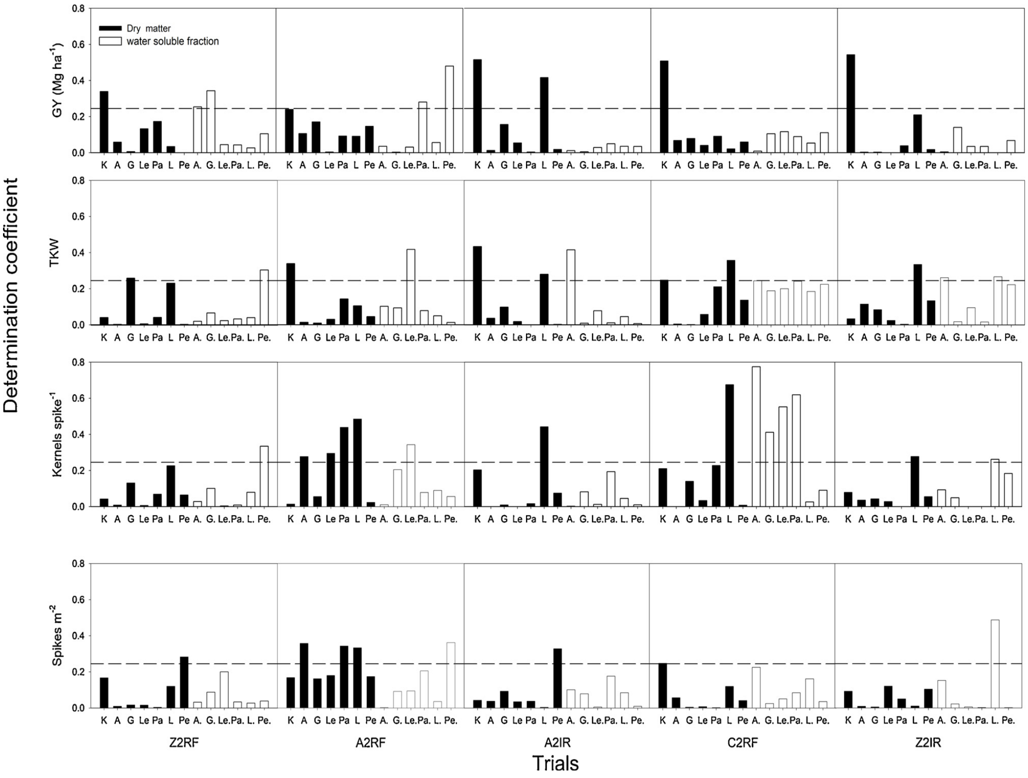

Fig. 5. Relationship between grain yield, TKW, kernels spike^{-1}, spikes m^{-2}, and carbon isotope composition of mature kernels (K), flag leaves (L), different parts of the ear (awns, A; glume, G; lemma, Le; palea; P) and the peduncle (Pe) in dry matter and the water-soluble fraction in each environment (see Trial codes in Table 1). The horizontal broken line refers to the significance level ($P < 0.05$) of the relationship of each isotopic signature against the grain yield.

3.7. Broad sense heritability

The broad sense heritability (h^2) of the different parameters studied in this work were in general high (Tables 2,3) using the six selected genotypes, as well as the whole set of 20 genotypes reported in the previous study [7]. Even so, h^2 values were in general slightly higher when calculated for the subset of six genotypes exhibiting contrasting grain yield than for the complete panel of 20 genotypes. Moreover, yield components had higher h^2 (TKW, 0.96; kernels spike^{-1}, 0.95; spikes m^{-2}, 0.79; kernels m^{-2}, 0.91; HI, 0.85; KWPS, 0.89) than yield (GY, 0.74) and biomass (BM, 0.67) when using the whole set of 20 durum wheat genotypes (Table 2, S2), while differences were not so evident when h^2 was calculated only using the six genotypes. The vegetation indices had a higher h^2 (NDVI, 0.82; GGA, 0.75; SPAD, 0.88) than grain yield except for GA (GA, 0.63) and $\delta^{13}C_{kernel}$ ($\delta^{13}C_{kernel,}$ 0.38) when h^2 was calculated for the whole set of 20 genotypes, while differences were not so evident when it was calculated for the six contrasting genotypes alone. N_{Kernel} and KNPS had comparable h^2 values (N_{Kernel}, 0.69 and KNPS, 0.79) than GY (Table 3). Considering only the second year, the h^2 of the DTH (0.97) and the morphological parameters (LL, 0.87; SL, 0.77, PH, 0.89) also had higher h^2 values than GY (0.63), while LW (LW, 0.55) was the exception (Table S2, S3). For the $\delta^{13}C$ of the different plant tissues measured for the six selected genotypes in the second growing season alone, the h^2 values observed were in general moderate to high but lower than the other parameters (Table S5). High values of h^2 were observed for $\delta^{13}C_{kernel}$ (0.87), $\delta^{13}C_{leaf.DM}$ (0.97), $\delta^{13}C_{awns.WSF}$ (0.62) and $\delta^{13}C_{peduncle.WSF}$ (0.82). Moderate values were observed for $\delta^{13}C_{lemme.DM}$ (0.47); $\delta^{13}C_{palea.DM}$ (0.42) and low values for $\delta^{13}C_{awns.DM}$ (0.32) and $\delta^{13}C_{glume.WSF}$ (0.31).

3.8. Stepwise analysis and PCA

The environments were separated into two subsets, high yielding (HY, $GY > 5.8$ Mg ha^{-1}, comprising A1RF, A1IR, Z1IR, C2RF and Z2IR growing conditions) and low yielding (LY, $GY < 5.8$ Mg ha^{-1}, including Z1RF, A2RF, A2IR and Z2RF growing conditions) and the stepwise regression analysis between GY, as the dependent variable, and the agronomic yield components (spikes m^{-2}, kernels spike^{-1} and TKW) as independent variables were calculated. Under high yielding conditions kernels spike^{-1} was the first variable chosen by the model and under low yielding conditions the spike m^{-2} was the first variable in the model (Table 4). Stepwise analysis was also performed within each agronomic condition in the second year. In four of the five environments, kernels spike^{-1} was the first component chosen followed by spikes m^{-2} (Table 4). Also, a stepwise analysis was performed under the two growing conditions, the HY and LY environments, using GY and yield components as dependent variables, and water status parameters ($\delta^{13}C_{kernels}$ and CTD) and remote sensing indices as independent variables (NDVI, SPAD, GA and GGA). Under both the HY and LY environments (Table 5) $\delta^{13}C_{kernels}$ was the first variable chosen followed by GGA. For TKW, $\delta^{13}C_{kernels}$ was the first variable chosen in the HY environment and SPAD in the LY environment. For kernels spike^{-1}, N_{kernel} was the first variable chosen in both the HY and LY environments. However, for spikes m^{-2}, NDVI was the first variable chosen in both the HY and LY environments, followed by CTD in the LY environment and $\delta^{13}C_{kernels}$ in HY environment. Together with the stepwise analysis a PCA was performed for each environment (Fig. 7) using first the GY and agronomic GY components and second the GY and physiological traits. In the four cases, the two first principal components (PC) explained > 60% of the observed variability under the HY

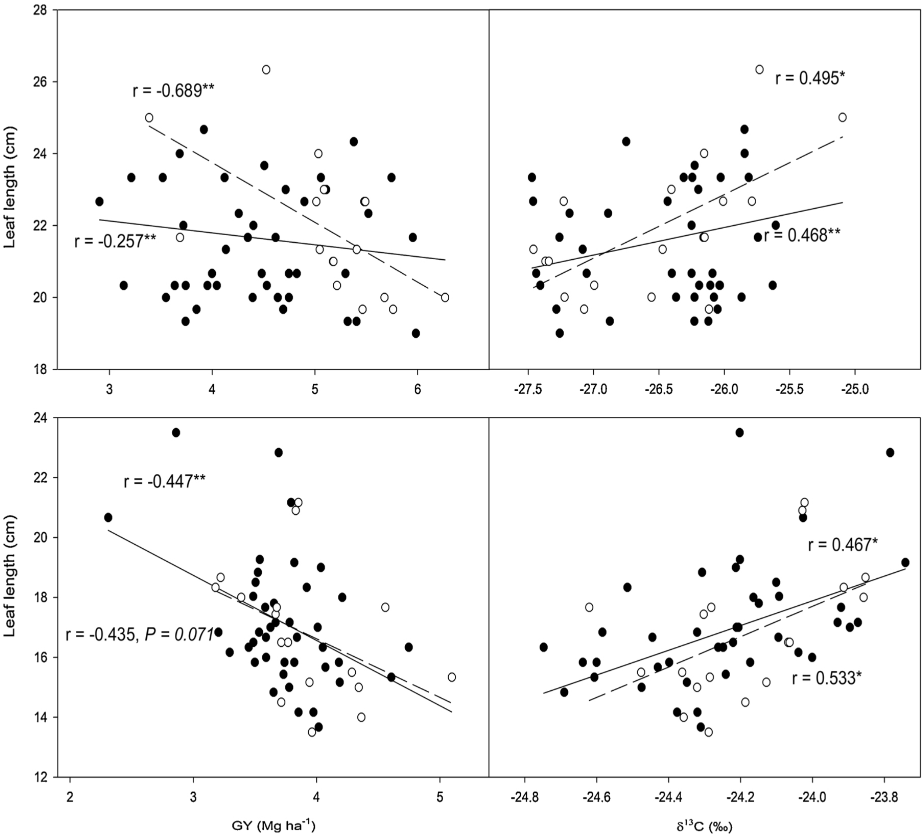

Fig. 6. Relationships between leaf length and grain yield (GY) and carbon isotope composition ($\delta^{13}C_{kernel}$) measured in twenty durum wheat (solid lines and filled circles) and six durum wheat genotypes (broken lines and open circles) grown under rainfed conditions in two locations: Aranjuez (a,b) and Zamadueñas (c,d). Probabilities (*, $P < 0.05$; **, $P < 0.01$) are shown.

Table 4

Stepwise regression analysis between grain yield (GY) as dependent variables and agronomic yield components (TKW, spikes m^{-2}, and kernels spike^{-1}) as independent variables of six genotypes of durum wheat grown under different environments. Top: under the two growing seasons (2014 and 2015) separated into high yielding (HY) and low yielding (LY) environments. LY included the Z1RF, A2RF, A2IR and Z2RF trials, while HY included the remaining A1RF, A1IR, Z1IR, C2RF and Z2IR trials. Bottom: under each environment in the second season (2015).

Environment	Variable/s chosen	Adjusted R^2	*P*
LY	Spikes m-2	0.241	0.000
	Spikes m-2, Kernels Spike-1	0.887	0.000
	Spikes m-2, Kernels Spike-1,TKW	0.982	0.000
HY	Kernels Spike-1	0.131	0.004
	Kernels Spike-1, Spikes m-2	0.567	0.000
	Kernels Spikes-1, Spikes m-2,TKW	0.675	0.000
Z2RF	Kernels Spike-1	0.226	0.027
	Kernels Spikes-1, Spikes m-2	0.549	0.001
A2RF	Spikes m-2	0.652	0.000
	Spikes m-2, Kernels Spike-1	0.868	0.000
	Spikes m-2, Kernels Spike-1,TKW	0.954	0.000
A2IR	Kernels Spike-1	0.328	0.024
	Kernels Spike-1, Spikes m-2	0.840	0.000
	Kernels Spikes-1, Spikes m-2,TKW	0.954	0.000
C2RF	Kernels Spike-1	0.288	0.013
	Kernels Spike-1, Spikes m-2	0.699	0.000
Z2IR	Kernels Spike^{-1}	0.399	0.003

and LY environments (Fig. 7). Kernels spike^{-1} and spikes m^{-2} were the two agronomic components most closely related to GY under the HY and LY environments (Fig. 7a, b). TKW was negatively related to TKW under low yielding conditions. Among the physiological traits, $\delta^{13}C$ and nitrogen kernel presented a negative association with GY in both environments while the vegetation indices were positively associated (Fig. 7). The CTD was closely associated with GY in the LY environment and to a lesser extent in the HY environment (Fig. 7c). The SPAD index was closely associated with GY under the HY environment (Fig. 7d). The two categories of genotypes (Pelayo, Sula and Iride on the one hand and Kiko Nick, Don Ricardo and Don Sebastian on the other) were clearly separated.

4. Discussion

The six semi-dwarf varieties used in this study were released over a period of just 15 years. In spite of the significant genotypic differences among grain yields, these varieties did not follow a pattern of increasing productivity over time. This fact reinforces the conclusion of a lack of a clear strategy in breeding durum wheat for Spanish conditions, at least in terms of grain yield as a target [7]. Moreover, the lack of a genotype by environment interaction for grain yield indicates that the varieties tested here do not seem to be suited to any particular environment, and hence this does not support the view that breeding has led to genetic advances for the warmer conditions of South Spain [7]. In the high yielding conditions of the UK [47] and NW Mexico [48], genetic advance in wheat grain yield has been reported. Moreover, and contrasting with our results, the study of Pennachi et al. [47] supports

Table 5

Stepwise analysis with grain yield (GY), thousand kernel weight (TKW), kernels per spike (kernels spike-1) and spikes per square meter (spikes m-2) as dependent variables for six durum wheat genotypes under two growing seasons (2014 and 2015) separated into high yielding (HY) and low yielding (LY) environments. The independent variables were chlorophyll content at grain filling (SPAD), carbon isotope composition ($\delta^{13}C_{kernel}$), vegetation indices at grain filling (GA, GGA and NDVI), canopy temperature depression (CTD), and N content (N_{Kernel}).

Environment	Predicted variable	Variable/s chosen	Adjusted R^2	Pvalue
LY	GY	$\delta^{13}C_{kernel}$(-)	0.676	0.000
		$\delta^{13}C_{kernel}$ (-); GGA (+)	0.726	0.000
		$\delta^{13}C_{kernel}$ (-); GGA (+); SPAD (+)	0.749	0.000
	TKW	SPAD(+)	0.056	0.034
		SPAD(+); NDVI(-)	0.179	0.001
		SPAD(+);NDVI(-); GGA(+)	0.292	0.000
		SPAD(+);NDVI(-); GGA(+); CTD(+)	0.340	0.000
	Kernels spike^{-1}	N_{kernel}(-)	0.007	0.007
		N_{kernel}(-);NDVI (-)	0.002	0.002
		N_{kernel}(-); NDVI(-); GGA(+)	0.000	0.000
	Spikes m^{-2}	NDVI (+)	0.433	0.000
		NDVI (+); CTD (+)	0.467	0.000
HY	GY	$\delta^{13}C_{kernel}$(-)	0.136	0.001
		$\delta^{13}C_{kernel}$ (-); GGA (+)	0.217	0.000
		$\delta^{13}C_{kernel}$ (-); GGA (+); N_{kernel} (+)	0.267	0.000
	TKW	$\delta^{13}C_{kernel}$ (-)	0.340	0.000
		$\delta^{13}C_{kernel}$ (-);GA	0.398	0.000
	Kernels spike^{-1}	N_{kernel}(-)	0.269	0.000
		N_{kernel}(-); GA(-)	0.527	0.000
		N_{kernel}(-); GA(-); NDVI(-)	0.577	0.000
	Spikes m^{-2}	NDVI(+)	0.453	0.000
		NDVI(+);$\delta^{13}C_{kernel}$ (-)	0.514	0.000
		NDVI(+);$\delta^{13}C_{kernel}$ (-);N_{kernel}(+)	0.539	0.000
		NDVI(+);$\delta^{13}C_{kernel}$ (-);N_{kernel}(+); GA(+)	0.570	0.000

the contention that breeding for yield potential does not select for climate resilience and yield stability in wheat. However, a recent study [49] using a large set of cultivars released over 50 years in Germany reported that breeding for high performance not only enhanced cultivar performance under optimal production conditions but also increased performance in production systems with reduced agrochemical inputs. This study concluded that new cultivars incrementally accumulated genetic variants conferring favorable effects on key yield parameters and disease resistance. In the present work, Don Sebastian exhibited the poorest yield in all environments and consistently produced below-average yields, proving that it is poorly adapted to all environments. The other varieties maintained higher yields in all environments, which indicates that they have general adaptability. This included Pelayo, which exhibited a linear fit to the Finlay and Wilkinson model [50] above the other genotypes and without any crossover in the lines. Also, the average environment coordination (AEC) method [45] revealed that Pelayo had the highest mean yield as well as the highest stability.

Subsequently we dissected the agronomic, physiological and morphological characters related to the consistent differences in yield across this set of six varieties. In this study, the number of days from sowing to heading (DTH) did not differ statistically among genotypes, which excludes differences in phenology as one of the causes of differences in $\delta^{13}C$ and grain yield [51,52].

Retrospective studies on wheat, combining pre- and post-green revolution cultivars, indicate that the improvement in yield has more often been associated with augmented partitioning of biomass to the grain, and associated in turn with a decrease in stature, than with enhanced overall biomass [53–55]. Thus plant height is usually negatively correlated with the HI [11]. The few studies embracing only post green revolution genotypes frequently include transitional genotypes from the 1960s and 1970s, with relatively tall plants [5,56]. Otherwise no clear effect on differences in plant height or increased biomass have been reported [57], even though some studies report an increase in biomass associated with recent genetic advance in wheat cultivated under good agronomic conditions [54,58]. Indeed, in our study, the most productive genotypes (Pelayo, Sula and Iride) exhibited higher HI together with lower plant height than the less productive ones (Don Sebastian, Don Ricardo and Kiko Nick), with no differences in biomass. As reported in studies comparing pre- and post-green revolution genotypes [6,11,54], a higher HI seems related to greater numbers of kernels per spike, whereas the TKW did not change. As a consequence, a higher HI indirectly translates to greater numbers of kernels m^{-2}. Most studies agree that kernels m^{-2} best explains yield [59–61]. In our study, genotypes with higher GY were associated with more kernels m^{-2}, while the contribution of TKW was minor. The increase in kernels m^{-2} was mostly due to an increase in kernels spike^{-1} [7,11,62]. The stepwise and PCA analyses indicated that kernels spike^{-1} was better associated with GY in high and low yielding environments than spikes m^{-2}, while TKW was not associated at all. Other studies have also shown that kernels spike^{-1} was associated with GY under a high-yielding environment [63] and also under water stress conditions [64]. A positive relationship between the number of kernels spike- [7,11] and grain yield seems to have been derived from the fact that grain yield in wheat is frequently sink limited [65], and for this reason, kernels spike^{-1} has been reported as a promising trait for increasing wheat grain yield [64,65].

Regarding durum wheat in Spain, the above results suggest that a certain point was reached where new cultivars represented a step backwards in genetic improvement for grain yield. In fact, the least productive genotype (Don Sebastian) from the past three decades [7] was released in 2004, and regardless of growing conditions, in the current study it exhibited taller stature and lower HI than the most productive genotypes. Interestingly, the three least productive genotypes exhibited higher TKW and nitrogen concentration in kernels than the three most productive genotypes. This suggests that for some varieties grain quality has been a key breeding objective, even if this has been at the cost of lower yield. In fact, high TKW and N concentration in kernels are the two main quality traits in durum wheat [66].

A delay in leaf senescence (i.e. staygreen) would increase the amount of fixed carbon available for grain filling [22,67,68]. The positive effects of staygreen on yield have also been reported in other crops like sorghum [69] and maize [70]. However, in our study, the least productive cultivar, Don Sebastian, maintained a higher canopy green biomass during grain filling, assessed through different vegetation indices, as well as greener flag leaves during the last part of the grain filling period relative to the more productive cultivars. In the same sense, a negative relationship between a staygreen attitude and GY has been reported in rice [71]. In fact, because of the size of its flag leaf and low harvest index, Don Sebastian has a higher source of N for remobilization (large leaves; including the flag leaf) and a limited sink for N accumulation (low number of kernels per spike) compared to other cultivars (see also Sanchez-Bragado et al. [72]), which may contribute to delaying senescence [73–75].

In this study, $\delta^{13}C_{kernel}$ was negatively correlated with GY, not only across growing conditions, but also within each environment and across genotypes (Fig. 5). Negative correlations between $\delta^{13}C_{kernel}$ and grain yield across genotypes have been previously reported for durum wheat growing under a wide range of Mediterranean conditions, including different water, salinity or nitrogen fertilization conditions [28,76–79]. This negative association suggests that genotypes that can maintain higher water use and a greater stomatal conductance are the most

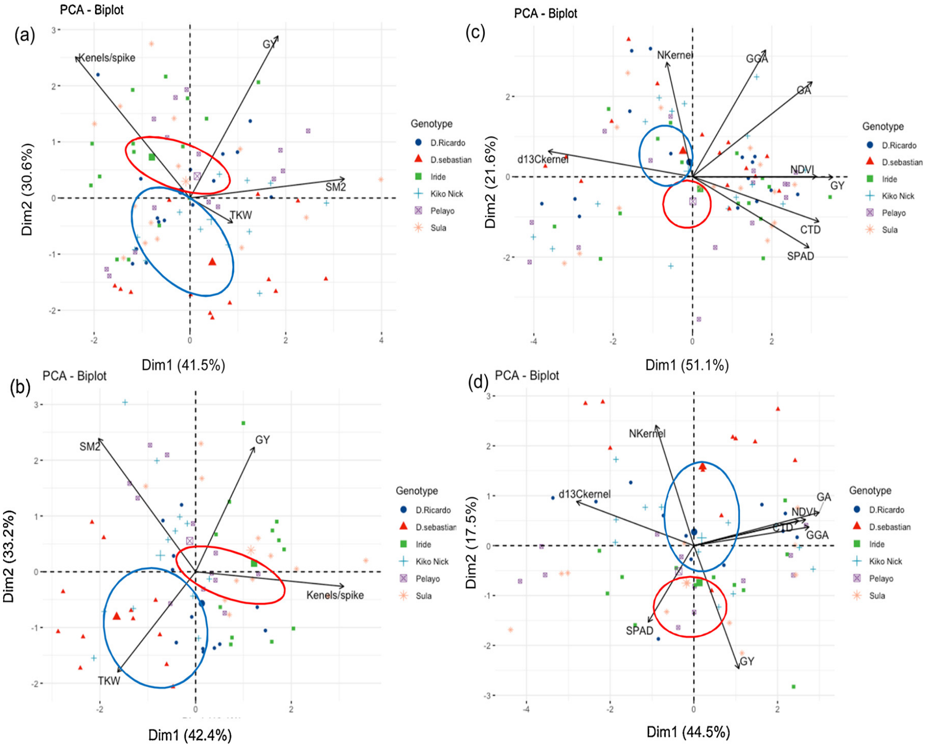

Fig. 7. The PCA of agronomic (a,b) and physiological (c,d) traits measured in six genotypes of wheat grown under low yielding (a,c) and high yielding (b,d) conditions in two growing seasons (2014 and 2015). Traits included in the PCA are: (a and b) grain yield (GY), kernels per spike (kernels spike^{-1}), spikes m^{-2} (SM2) and thousand kernel weight (TKW); (c and d) grain yield (GY), chlorophyll content at grain filling (SPAD), carbon isotope composition (δ^{13}Ckernel), vegetation indices at grain filling (GA, GGA and NDVI), canopy temperature depression (CTD) and N content (N$_{\text{Kernel}}$).

productive [28,32]. Indeed, in this study Pelayo was the most productive genotype and exhibited the most negative $\delta^{13}C_{\text{Kernels}}$ value alongside a cooler canopy compared with Don Sebastian. Despite the fact that no significant genotype by environment interaction was found for $\delta^{13}C_{\text{Kernels}}$, there was a trend towards a crossover in the graphic model of Finlay and Wilkinson [50] that placed this crossover in the most stressful growing conditions, was found. A negative interaction has been reported before [27,31,33,37] where a positive association between $\delta^{13}C_{\text{Kernels}}$ and yield has been found under very dry Mediterranean rainfed conditions. Even so, a negative association is more common because genotypes capable of sustaining greater stomatal conductance and water consumption are more productive and better adapted. Differences in plant architecture may contribute, at least in part, to the differences in $\delta^{13}C_{\text{Kernels}}$ across genotypes. Thus a large, prostrated, flag leaf, as in the case of Don Sebastian, Don Ricardo and Kiko Nick, may suffer water stress and then close its stomata earlier than genotypes with smaller and more erect flag leaves (Pelayo, Iride and Sula). In fact, we found a positive correlation between leaf length and $\delta^{13}C_{\text{Kernels}}$ across genotypes. Moreover, visual observations suggested that flag leaves (and the rest of leaves in general) tended to be more erect in the most productive compared with the least productive genotypes. See the examples of Pelayo and Don Sebastian, the two extreme genotypes (Fig. 8). Leaf posture and area can affect the δ^{13}C, and indeed, Don Sebastian with its droopy leaves, together with its high δ^{13}C and high canopy temperature suggests that it has a lower stomatal conductance [80] than Pelayo (Fig. 8).

To further understand the differences between the extreme genotypes in terms of grain yield and $\delta^{13}C_{\text{Kernels}}$, we analyzed δ^{13}C from the dry matter and the water-soluble fractions of different photosynthetic tissues (including non-laminar parts) potentially contributing to filling the grains. While the genotype by environment interaction was not significant for $\delta^{13}C_{\text{kernel}}$, and only marginally significant for $\delta^{13}C_{\text{leaf}}$, it achieved significance for the δ^{13}C of different ear tissues. The presence of a genotype by environment interaction suggests genetic variance for plasticity for these traits [81]. The flag leaf exhibited much lower (i.e. negative) δ^{13}C values than the mature kernels, the peduncle or other parts of the ear. Previous studies in durum and bread wheat [35,77,82,83] and triticale [84] have found similar patterns of lower δ^{13}C in the DM and the WSF of the flag leaves relative to different parts of the ears, while the mature kernels exhibited values between them but closer to the ear parts. Considering that no major fractionation occurs in the δ^{13}C of the assimilates moving to the growing grains [34,35], which may otherwise affect the isotopic signature, these results suggest that the ear and plant parts other than the flag leaf have a key role in contributing to kernel growth [34,35,77,83]. The higher δ^{13}C of the ear parts compared to the δ^{13}C of the flag leaf is a constitutive fact (i.e. evidenced by the absence of stress) and can be attributed to the lower stomatal conductance of the ear tissues [83] together with their positioning in the upper part of the canopy, exposed to direct sunlight conditions. In fact, regardless of the growing conditions, ears exhibit a higher temperature than the leaves, even though the ear as a photosynthetic organ is more resistant to water stress than the flag leaf [85]. We tried to elucidate the relative importance of each organ as a photosynthetic contributor to grain filling through the relationship of their δ^{13}C values with grain yield. The genotypic correlation of δ^{13}C from the different plant parts against GY within each growing conditions was simply absent or much lower than the correlation between $\delta^{13}C_{\text{kernels}}$ and GY. We gave more emphasis to the δ^{13}C values of the WSF because this fraction represents the photoassimilates recently produced by the organs, while the δ^{13}C signature analyzed in dry matter reflects the

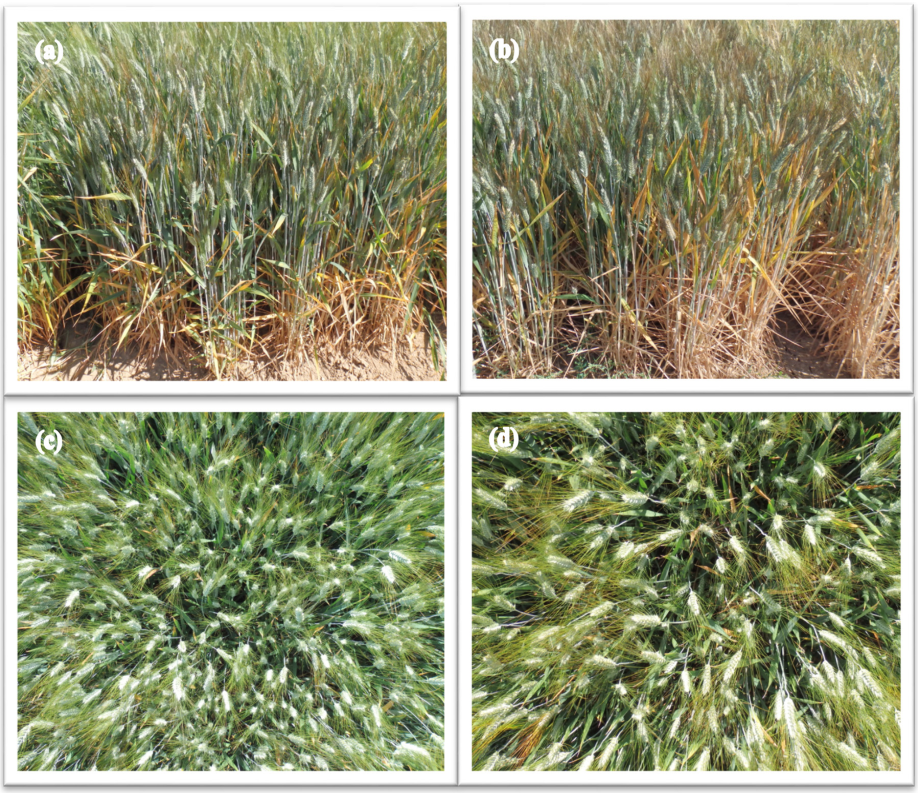

Fig. 8. Front and zenithal images of the durum wheat cultivars Don Sebastian (a,c) and Pelayo (b,d) grown under irrigated conditions at Aranjuez Station 2015.

signature of the assimilates used during the growth of each organ [44]. In terms of WSF and for a couple of environments, some non-laminar organs correlated with GY. These results suggest that there is no specific plant part that accounts for the majority of the assimilates moving to the grains, but rather there are several contributors, which is also the case for the nitrogen [72]. Nonetheless, the $\delta^{13}C$ in the WSF of the flag leaf did not correlate with any of the five growing conditions tested against grain yield or any of the two agronomic yield components defined during grain filling (TKW and kernels spike^{-1}), which suggests that the role of the flag leaf as a photosynthetic organ during grain filling is minor. By contrast, the photosynthetic contribution of the non-laminar parts to grain filling appears more important, considering the number of cases where a correlation with grain yield was found. The relationship between carbon isotope composition and agronomic yield components is poorly documented in durum wheat under Mediterranean conditions and usually is only addressed using the $\delta^{13}C$ of kernels or leaves [28,86]. Some studies using different approaches have concluded that the major source of carbon assimilates for grain filling in cereals was the flag leaf [87]. However, more recent studies have revealed that, under post-anthesis water stress, ear photosynthesis plays a major role in grain filling [34,35,85]. In the present work, significant differences existed for the $\delta^{13}C$ of different plant parts between the two extreme genotypes, but the photosynthetic tissue that exhibited the clearest difference between the two extreme genotypes was the palea for both DM and WSF, with the values in Pelayo being more negative than in Don Sebastian. Interestingly, and due to its position in the ear, the $\delta^{13}C$ of the palea seems to originate from the refixation of CO_2 respired by the grain rather than from assimilating atmospheric CO_2 [84,88]. Thus, the $\delta^{13}C$ of the palea exhibited the highest (least negative values), for both dry matter and the water-soluble fraction, compared to all the other plant parts. In fact, respired CO_2 derived from the growing grains is re-fixed close to its site of evolution, and the palea is the closest ear tissue to the grains and the furthest from the atmospheric air. The hypothesis is that the highly productive genotype Pelayo takes advantage of the respired CO_2 by re-fixing more of it than the least productive genotype Don Sebastian. Thus in Pelayo, the $\delta^{13}C_{palea}$ in the water-soluble fraction was less negative than $\delta^{13}C_{kernel}$ (2.75% less negative), however, in Don Sebastian $\delta^{13}C_{palea}$ presented a similar value to $\delta^{13}C_{kernel}$ (0.8% more negative).

5. Conclusion

In summary, the results of this study show that kernels spike^{-1} is the agronomic component most affected by the yield improvement of durum wheat cultivars released in Spain after the green revolution. This study has also identified a combination of characters that define an ideotype of durum wheat for the Mediterranean conditions of Spain. These genotypes should be characterized by a plant height of around 85 cm with a relatively small erect flag leaf, higher water use combined with a better water status (more negative $\delta^{13}C_{kernel}$ and higher CTD), a better balance between the N source and N sink during grain filling (even if this does not translate to a staygreen attitude), and a higher capacity to re-fix CO_2 respired by the grain. Also, it can be concluded that while the kernel is the most effective plant part for $\delta^{13}C$ assessment in durum wheat under Mediterranean conditions, the non-laminar parts of the plants play a key role in providing assimilates during grain filling.

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References

[1] R. Ranieri, Geography of the Durum Wheat Crop, Pastaria Int., 2015 (Accessed September 8, 2017), https://scholar.google.com/scholar?q=Geography+of+the+durum+wheat+crop+ranieri&btnG=&hl=en&as_sdt=0%252C5.

[2] R. Taylor D, W.W. Koo, Outlook of the U.S. and World Wheat Industries, 2015-2024, 2015, (2015) https://ideas.repec.org/p/ags/nddaae/201310.html.

[3] C. Royo, Durum wheat improvement in Spain, in: C. Royo, M.M. Nachit, N. Di Fonzo, J.L. Araus, W.H. Pfeiffer, G.A. Slafer (Eds.), Durum Wheat Breeding: Current Approaches and Future Strategies, Food Products Press, New York, 2005, pp. 883–906, , https://doi.org/10.1007/s10535-006-0137-7.

[4] J. Juan-Aracil, A. Michelena, Durum wheat in Spain, in: N. Di Fonzo, F. Kaan, M. Nachit (Eds.), Durum wheat quality in the Mediterranean region, Zaragoza, CIHEAM,durum wheat q, 2019http://ressources.ciheam.org/om/pdf/a22/95605360.pdf (Accessed April 28, 2019).

[5] K.A.B. Aisawi, M.P. Reynolds, R.P. Singh, M.J. Foulkes, The physiological basis of the genetic progress in yield potential of CIMMYT spring wheat cultivars from 1966 to 2009, Crop Sci. 55 (2015) 1749–1764, https://doi.org/10.2135/cropsci2014.09.0601.

[6] P. De Vita, O.L.D. Nicosia, F. Nigro, C. Platani, C. Riefolo, N. Di Fonzo, L. Cattivelli, Breeding progress in morpho-physiological, agronomical and qualitative traits of durum wheat cultivars released in Italy during the 20th century, Eur. J. Agron. 26 (2007) 39–53, https://doi.org/10.1016/j.eja.2006.08.009.

[7] F. Chairi, O. Vergara-Diaz, T. Vatter, N. Aparicio, M.T. Nieto-Taladriz, S.C. Kefauver, J. Bort, M.D. Serret, J.L. Araus, Post-green revolution genetic advance in durum wheat: the case of Spain, Field Crop. Res. 228 (2018) 158–169, https://doi.org/10.1016/j.fcr.2018.09.003.

[8] C. Royo, V. Martos, A. Ramdani, D. Villegas, Y. Rharrabti, L.F. García Del Moral, Changes in yield and carbon isotope discrimination of Italian and Spanish durum wheat during the 20th century, Agron. J. 100 (2008) 352–360, https://doi.org/10.2134/agronj2007.0060.

[9] M. Sanchez-Garcia, C. Royo, N. Aparicio, J.A. Martín-Sánchez, F. Álvaro, Genetic improvement of bread wheat yield and associated traits in Spain during the 20th century, J. Agric. Sci. 151 (2012) 105–118, https://doi.org/10.1017/S0021859612000330.

[10] M. Acreche, G. Brice, J.A. Mart, G.A. Slafer, Physiological bases of genetic gains in Mediterranean bread wheat yield in Spain, Eur. J. Agron. 28 (2008) 162–170, https://doi.org/10.1016/j.eja.2007.07.001.

[11] C. Royo, F. Álvaro, V. Martos, A. Ramdani, J. Isidro, D. Villegas, L.F. García del Moral, Genetic changes in durum wheat yield components and associated traits in Italian and Spanish varieties during the 20th century, Euphytica 155 (2007) 259–270, https://doi.org/10.1007/s10681-006-9327-9.

[12] R. Fischer, Y. Stockman, Increased kernel number in Norin 10-derived dwarf wheat: Evaluation of the c Kernel Number in Norin 10-Derived Dwarf Wheat: Evaluation of the Cause, Aust. J. Plant Physiol. 13 (1986) 767, https://doi.org/10.1071/PP9860767.

[13] D.J. Miralles, G.A. Slafer, Yield, biomass and yield components in dwarf, semi--dwarf and tall isogenic lines of spring wheat under recommended and late sowing dates, Plant Breed. 114 (1995) 392–396, https://doi.org/10.1111/j.1439-0523.1995.tb00818.x.

[14] R.A. Richards, The effect of dwarfing genes in spring wheat in dry environments. I. Agronomic characteristics, Aust. J. Agric. Res. 43 (1992) 517–527, https://doi.org/10.1071/AR9920517.

[15] F. Giunta, R. Motzo, G. Pruneddu, Trends since 1900 in the yield potential of Italian-bred durum wheat cultivars, Eur. J. Agron. 27 (2007) 12–24, https://doi.org/10.1016/j.eja.2007.01.009.

[16] M.J. Foulkes, J.W. Snape, V.J. Sherman, M.P. Reynolds, O. Gaju, R. Sylvester-Bradely, Genetic progress in yield potential in wheat: recent advances and future prospects, J. Agric. Sci. 145 (2007) 17–29, https://doi.org/10.1017/s0021859607006740.

[17] G.J. Rebetzke, T.C. Scientific, R. Richards, T.C. Scientific, G.D. Farquhar, Selection for Reduced Carbon Isotope Discrimination Increases Aerial Biomass and Grain Yield of Rainfed Bread Wheat G. (2002), pp. 739–745, https://doi.org/10.2135/cropsci2002.0739.

[18] M. Reynolds, F. Dreccer, R. Trethowan, Drought-adaptive traits derived from wheat wild relatives and landraces, J. Exp. Bot. 58 (2006) 177–186, https://doi.org/10.1093/jxb/erl250.

[19] J.L. Araus, G.A. Slafer, C. Royo, M.D. Serret, Breeding for yield potential and stress adaptation in cereals, Crit. Rev. Plant Sci. 27 (2008) 377–412, https://doi.org/10.1080/07352680802467736.

[20] E.A. Tambussi, J. Bort, J.L. Araus, Water use efficiency in C3 cereals under Mediterranean conditions: a review of physiological aspects, Ann. Appl. Biol. 150 (2007) 307–321, https://doi.org/10.1111/j.1744-7348.2007.00143.x.

[21] M.S. Lopes, M.P. Reynolds, Stay-green in spring wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology, J. Exp. Bot. 63 (2012) 3789–3798, https://doi.org/10.1093/jxb/ers071.

[22] E. Carmo-Silva, P.J. Andralojc, J.C. Scales, S.M. Driever, A. Mead, T. Lawson, C.A. Raines, M.A.J. Parry, Phenotyping of field-grown wheat in the UK highlights contribution of light response of photosynthesis and flag leaf longevity to grain yield, J. Exp. Bot. 68 (2017) 3473–3486, https://doi.org/10.1093/jxb/erx169.

[23] K. Vijayalakshmi, A.K. Fritz, G.M. Paulsen, G. Bai, S. Pandravada, B.S. Gill, Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature, Mol. Breed. 26 (2010) 163–175, https://doi.org/10.1007/s11032-009-9366-8.

[24] M.J. Hawkesford, J.L. Araus, R. Park, D. Calderini, D. Miralles, T. Shen, J. Zhang, M.A.J. Parry, Prospects of doubling global wheat yields, Food Energy Secur. 2 (2013) 34–48, https://doi.org/10.1002/fes3.15.

[25] R.A. Fischer, D. Rees, K.D. Sayre, Z.M. Lu, A.G. Condon, A. Larque Saavedra, Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies, Crop Sci. 38 (1998) 1467–1475, https://doi.org/10.2135/cropsci1998.0011183X003800060011x.

[26] D. Roche, Stomatal conductance is essential for higher yield potential of C 3 crops, Crit. Rev. Plant Sci. 34 (2015) 429–453, https://doi.org/10.1080/07352689.2015.1023677.

[27] J.L. Araus, L. Cabrera-Bosquet, M.D. Serret, J. Bort, M.T. Nieto-Taladriz, Comparative performance of δ13C, δ18O and δ^{15}N for phenotyping durum wheat adaptation to a dryland environment, Funct. Plant Biol. 40 (2013) 595–608, https://doi.org/10.1071/FP12254.

[28] J.L. Araus, D. Villegas, N. Aparicio, L.F.G. del Moral, S. El Hani, Y. Rharrabti, J.P.P. Ferrio, C. Royo, L.F. Garcıa del Moral, S. El Hani, Y. Rharrabti, J.P.P. Ferrio, C. Royo, Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions, Crop Sci. 43 (2003) 170–180, https://doi.org/10.2135/cropsci2003.1700.

[29] M. Hafsi, P. Monneveux, O. Merah, A. Djekoune, C. Royo, M.M. Nachit, N. Di-Fonzo, J.L. Araus, Carbon isotope discrimination and yield in durum wheat grown in the high plains of Setif (Algeria). Contribution of different organs to grain filling, Durum Wheat Improv. Mediterr. Reg. New Challenges. 40 (32676BC) Spain, 12-Spain, (2019), p. 14 (Accessed October 12, 2017), http://om.ciheam.org/article.php?IDPDF=600020http://www.ciheam.org/.

[30] R.A. Richards, G.J. Rebetzke, A.G. Condon, A.F. Van Herwaarden, Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals, Crop Sci. 42 (2002) 111–121, https://doi.org/10.2135/cropsci2002.0111.

[31] A.G. Condon, R.A. Richards, G.D. Farquhar, Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown Wheat1, Crop Sci. 27 (1987) 996, https://doi.org/10.2135/cropsci1987.0011183X002700050035x.

[32] A. Blum, Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress, Field Crop. Res. 112 (2009) 119–123, https://doi.org/10.1016/j.fcr.2009.03.009.

[33] A. Elazab, J. Bort, B. Zhou, M.D. Serret, M.T. Nieto-Taladriz, J.L. Araus, The combined use of vegetation indices and stable isotopes to predict durum wheat grain yield under contrasting water conditions, Agric. Water Manag. 158 (2015) 196–208, https://doi.org/10.1016/j.agwat.2015.05.003.

[34] R. Sanchez-Bragado, A. Elazab, B. Zhou, M.D. Serret, J. Bort, M.T. Nieto-Taladriz, J.L. Araus, Contribution of the ear and the flag leaf to grain filling in durum wheat inferred from the carbon isotope signature: genotypic and growing conditions effects, J. Integr. Plant Biol. 56 (2014) 444–454, https://doi.org/10.1111/jipb.12106.

[35] R. Sanchez-Bragado, G. Molero, M.P. Reynolds, J.L. Araus, Photosynthetic contribution of the ear to grain filling in wheat: a comparison of different methodologies for evaluation, J. Exp. Bot. 67 (2016) 2787–2798, https://doi.org/10.1093/jxb/erw116.

[36] O. Merah, P. Monneveux, Contribution of different organs to grain filling in durum wheat under mediterranean conditions I. Contribution of post-anthesis photosynthesis and remobilization, J. Agron. Crop Sci. 201 (2015) 344–352, https://doi.org/10.1111/jac.12109.

[37] S. Medina, R. Vicente, M.T. Nieto-Taladriz, N. Aparicio, F. Chairi, O. Vergara-Diaz, J.L. Araus, The plant-transpiration response to vapor pressure deficit (VPD) in durum wheat is associated with differential yield performance and specific expression of genes involved in primary metabolism and water transport, Front. Plant Sci. 9 (2019) 1–19, https://doi.org/10.3389/fpls.2018.01994.

[38] J.C. Zadoks, T.T. Chang, C.F. Konzak, A decimal code for the growth stages of cereals, Weed Res. 14 (1974) 415–421, https://doi.org/10.1111/j.1365-3180.1974.tb01084.x.

[39] J. Casadesús, Y. Kaya, J. Bort, M.M. Nachit, J.L. Araus, S. Amor, G. Ferrazzano, F. Maalouf, M. Maccaferri, V. Martos, H. Ouabbou, D. Villegas, M. Maccaferri, J. Bort, S. Amor, Using vegetation indices derived from conventional digital cameras as selection criteria for wheat breeding in water-limited environments, Ann. Appl. Biol. 150 (2007) 227–236, https://doi.org/10.1111/j.1744-7348.2007.00116.x.

[40] T.B. Coplen, Explanatory Glossary of Terms Used in Expression of Relative Isotope Ratios and Gas Ratios. IUPAC Recommendations 2008. (International Union of Pure and Applied Chemistry Inorganic Chemistry Division, Commission on Isotopic Abundances and Atomic Weights: Research Triangle Park, NC, USA), Canada Austria China/Beijing); Natl. Represent. J. R. Laeter (Australia, (2008) (Accessed November 28, 2017), http://old.iupac.org/reports/provisional/abstract08/coplen_prs.pdf.

[41] G.D. Farquhar, J.R. Ehleringer, K.T. Hubick, Carbon isotope discrimination and photosynthesis, Annu. Rev. Plant Physiol. Plant Mol. Biol. 40 (1989) 503–537, https://doi.org/10.1146/annurev.pp.40.060189.002443.

[42] L. Cabrera-Bosquet, R. Albrizio, S. Nogués, J.L. Araus, Dual Δ^{13}C/δ^{18}O response to water and nitrogen availability and its relationship with yield in field-grown durum wheat, Plant Cell Environ. 34 (2011) 418–433, https://doi.org/10.1111/j.1365-3040.2010.02252.x.

[43] R. Sanchez-Bragado, G. Molero, M.P. Reynolds, J.L. Araus, Relative contribution of shoot and ear photosynthesis to grain filling in wheat under good agronomical conditions assessed by differential organ δ^{13}C, J. Exp. Bot. 65 (2014) 5401–5413, https://doi.org/10.1093/jxb/eru298.

[44] S. Yousfi, M.D. Serret, J.L. Araus, Comparative response of δ13C, δ18O and δ^{15}N in durum wheat exposed to salinity at the vegetative and reproductive stages, Plant, Cell Environ. 36 (2013) 1214–1227, https://doi.org/10.1111/pce.12055.

[45] W. Yan, M.S. Kang, GGE Biplot Analysis, CRC Press, 2002, https://doi.org/10.1201/9781420040371.

[46] A. Pacheco, M. Vargas, G. Alvarado, F. Rodríguez, GEA-R (Genotype Environment Analysis With R for Windows) Version 4.0. CIMMYT, (2015) (Accessed May 22, 2019), https://scholar.google.es/scholar?cluster=8720594121169897844&hl=es&as_sdt=2005&sciodt=0,5&scioq=GEA-R+analysis+genotype+by+environment.

[47] J.P. Pennacchi, E. Carmo-Silva, P.J. Andralojc, T. Lawson, A.M. Allen, C.A. Raines, M.A.J. Parry, Stability of wheat grain yields over three field seasons in the UK, Food Energy Secur. (2018), https://doi.org/10.1002/fes3.147.

[48] R.A. Fischer, G.O. Edmeades, Breeding and cereal yield progress, Crop Sci. (2010), https://doi.org/10.2135/cropsci2009.10.0564.

[49] K.P. Voss-Fels, A. Stahl, B. Wittkop, C. Lichthardt, S. Nagler, T. Rose, T.-W. Chen, H. Zetzsche, S. Seddig, M. Majid Baig, A. Ballvora, M. Frisch, E. Ross, B.J. Hayes, M.J. Hayden, F. Ordon, J. Leon, H. Kage, W. Friedt, H. Stützel, R.J. Snowdon, Breeding improves wheat productivity under contrasting agrochemical input levels, Nat. Plants (2019), https://doi.org/10.1038/s41477-019-0445-5.

[50] K.W. Finlay, G.N. Wilkinson, The analysis of adaptation in a plant-breeding programme, Aust. J. Agric. Res. 14 (1963) 742–754, https://doi.org/10.1071/AR9630742.

[51] E. Acevedo, Potential of carbon isotope discrimination as a selection criterion in Barley Breeding, stable isot, Plant Carbon-Water Relations (1993) 399–417, https://doi.org/10.1016/B978-0-08-091801-3.50035-0.

[52] P. Annicchiarico, L. Pecetti, Yield vs. Morphophysiological trait-based criteria for selection of durum wheat in a semi-arid Mediterranean region (northern Syria), F. Crop. Res. 59 (1998) 163–173, https://doi.org/10.1016/S0378-4290(98)00116-6.

[53] R.B. Austin, J. Bingham, R.D. Blackwell, L.T. Evans, M.A. Ford, C.L. Morgan, M. Taylor, Genetic improvements in winter wheat yields since 1900 and associated physiological changes, J. Agric. Sci. 94 (1980) 675, https://doi.org/10.1017/S0021859600028665.

[54] K.D. Sayre, S. Rajaram, R.A. Fischer, Yield potential progress in short bread wheats in northwest Mexico, Crop Sci. 37 (1997) 36–42, https://doi.org/10.2135/cropsci1997.0011183X003700010006x.

[55] D.F. Calderini, M.F. Dreccer, G.A. Slafer, Genetic improvement in wheat yield and associated traits. A re-examination of previous results and the latest trends, Plant Breed. 114 (1995) 108–112, https://doi.org/10.1111/j.1439-0523.1995.tb00772.x.

[56] M.J. Foulkes, G.A. Slafer, W.J. Davies, P.M. Berry, R. Sylvester-Bradley, P. Martre, D.F. Calderini, S. Griffiths, M.P. Reynolds, Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance, J. Exp. Bot. 62 (2011) 469–486, https://doi.org/10.1093/jxb/erq300.

[57] A. Del Pozo, I. Matus, M.D. Serret, J.L. Araus, Agronomic and physiological traits associated with breeding advances of wheat under high-productive Mediterranean conditions. The case of Chile, Environ. Exp. Bot. 103 (2014) 180–189, https://doi.org/10.1016/j.envexpbot.2013.09.016.

[58] V.J. Shearman, R.K. Scott, M.J. Foulkes, Crop Physiology and Metabolism. Physiological Processes Associated with Wheat Yield Progress in the UK, Crop Sci. 185 (2005) 175–185, https://doi.org/10.2135/cropsci2005.0175.

[59] G.A. Slafer, D.F. Calderini, D.J. Miralles, Yield Components and Compensation in Wheat: Opportunities for Further Increasing Yield Potential, (1996) (Accessed April 23, 2019), http://agris.fao.org/agris-search/search.do?recordID=QY9700152.

[60] G.A. Slafer, R. Savin, V.O. Sadras, Coarse and fine regulation of wheat yield components in response to genotype and environment, Field Crop. Res. 157 (2014) 71–83, https://doi.org/10.1016/J.FCR.2013.12.004.

[61] M.J. Foulkes, M.P. Reynolds, R. Sylvester-Bradley, Genetic Improvement of Grain Crops, Crop Physiol., 2009, pp. 355–385, https://doi.org/10.1016/b978-0-12-374431-9.00015-3.

[62] G.A. Slafer, F.H. Andrade, Genetic improvement in bread wheat (Triticum aestivum) yield in Argentina, F. Crop. Res. Elsevier Sci. Publ. B.V. 21 (1989) 289–296, https://doi.org/10.1016/0378-4290(89)90010-5.

[63] G.A. García, A.K. Hasan, L.E. Puhl, M.P. Reynolds, D.F. Calderini, D.J. Miralles, Grain yield potential strategies in an elite wheat double-haploid population grown in contrasting environments, Crop Sci. 53 (2013) 2577, https://doi.org/10.2135/cropsci2012.11.0669.

[64] S. Denčić, R. Kastori, B. Kobiljski, B. Duggan, Evaluation of grain yield and its components in wheat cultivars and landraces under near optimal and drought conditions, Euphytica 113 (2000) 43–52, https://doi.org/10.1023/A:1003997700865.

[65] G.A. Slafer, J.L. Araus, C. Royo, L.F. García, D. Moral, Promising Eco-physiological Traits for Genetic Improvement of Cereal Yields in Mediterranean Environments, (2005) https://www.ugr.es/˜lfgm/images/Slafer et al. 2005.pdf (Accessed December 19, 2018).

[66] C. Royo, G.A. Briceño-Félix, Spanish wheat pool, J. Chem. Inf. Model. (2013) 1689–1699, https://doi.org/10.1017/CBO9781107415324.004.

[67] A. del Pozo, A. Yáñez, I.A. Matus, G. Tapia, D. Castillo, L. Sanchez-Jardón, J.L. Araus, Physiological traits associated with wheat yield potential and performance under water-stress in a mediterranean environment, Front. Plant Sci. 7 (2016), https://doi.org/10.3389/fpls.2016.00987.

[68] M. Bogard, M. Jourdan, V. Allard, P. Martre, M.R. Perretant, C. Ravel, E. Heumez, S. Orford, J. Snape, S. Griffiths, O. Gaju, J. Foulkes, J. Le Gouis, Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield, and grain protein concentration in a winter wheat population segregating for flowering time QTLs, J. Exp. Bot. 62 (2011) 3621–3636, https://doi.org/10.1093/jxb/err061.

[69] A.K. Borrell, G.L. Hammer, A.C.L. Douglas, Does maintaining green leaf area in sorghum improve yield under drought? I. Leaf growth and senescence, Crop Sci. 40 (2000) 1026–1037, https://doi.org/10.2135/cropsci2000.4041026x.

[70] E.A. Lee, M. Tollenaar, Physiological basis of successful breeding strategies for maize grain yield, Crop Sci. 47 (2007), https://doi.org/10.2135/cropsci2007.04.0010IPBS.

[71] G.H. Jiang, Y.Q. He, C.G. Xu, X.H. Li, Q. Zhang, The genetic basis of stay-green in rice analyzed in a population of doubled haploid lines derived from an indica by japonica cross, Theor. Appl. Genet. 108 (2004) 688–698, https://doi.org/10.1007/s00122-003-1465-z.

[72] R. Sanchez-Bragado, M.D. Serret, J.L. Araus, The nitrogen contribution of different plant parts to wheat grains: exploring genotype, water, and nitrogen effects, Front. Plant Sci. 7 (2017), https://doi.org/10.3389/fpls.2016.01986.

[73] L. Borrás, G.A. Slafer, M.E. Otegui, Seed dry weight response to source-sink manipulations in wheat, maize and soybean: a quantitative reappraisal, Field Crop. Res. 86 (2004) 131–146, https://doi.org/10.1016/j.fcr.2003.08.002.

[74] I. Rajcan, M. Tollenaar, Source: sink ratio and leaf senescence in maize: II. Nitrogen metabolism during grain filling, F. Crop. Res. 60 (1999) 255–265, https://doi.org/10.1016/S0378-4290(98)00143-9.

[75] I. Rajcan, M. Tollenaar, Source : sink ratio and leaf senescence in maize:: I. Dry matter accumulation and partitioning during grain filling, FieldCrop. Res. 60 (1999) 245–253, https://doi.org/10.1016/S0378-4290(98)00142-7.

[76] J. Bort, M. Belhaj, K. Latiri, Z. Kehel, J.L. Araus, Comparative performance of the stable isotope signatures of carbon, nitrogen and oxygen in assessing early vigour and grain yield in durum wheat, J. Agric. Sci. 152 (2014) 408–426, https://doi.org/10.1017/S0021859613000269.

[77] O. Merah, E. Deléens, B. Teulat, P. Monneveux, Association between yield and carbon isotope discrimination value in different organs of durum wheat under drought, J. Agron. Crop Sci. 188 (2002) 426–434, https://doi.org/10.1046/j.1439-037X.2002.00594.x.

[78] P. Monneveux, D. Rekika, E. Acevedo, O. Merah, Effect of drought on leaf gas exchange, carbon isotope discrimination, transpiration efficiency and productivity in field grown durum wheat genotypes, Plant Sci. 170 (2006) 867–872, https://doi.org/10.1016/j.plantsci.2005.12.008.

[79] Z. Chamekh, S. Ayadi, C. Karmous, Y. Trifa, H. Amara, K. Boudabbous, S. Yousfi, M. Dolors Serret, J.L. Araus, Comparative effect of salinity on growth, grain yield, water use efficiency, δ 13 C and δ^{15} N of landraces and improved durum wheat varieties, (2016), https://doi.org/10.1016/j.plantsci.2016.07.005.

[80] J.L. Araus, M.P. Reynolds, E. Acevedo, Leaf posture, grain yield, growth, leaf structure, and carbon isotope discrimination in wheat, Crop Sci. 33 (1993) 1273, https://doi.org/10.2135/cropsci1993.0011183X003300060032x.

[81] C.D. Schlichting, The evolution of phenotypic plasticity in plants, Annu. Rev. Ecol. Syst. 17 (1986) 667–693, https://doi.org/10.1146/annurev.es.17.110186.003315.

[82] B. Zhou, Á. Sanz-Sáez, A. Elazab, T. Shen, R. Sánchez-Bragado, J. Bort, M.D. Serret, J.L. Araus, Physiological traits contributed to the recent increase in yield potential of winter wheat from Henan Province, China, J. Integr. Plant Biol. 56 (2014) 492–504, https://doi.org/10.1111/jipb.12148.

[83] J.L. Araus, H.R. Brown, A. Febrero, J. Bort, M.D. Serret, Ear photosynthesis, carbon isotope discrimination and the contribution of respiratory CO_2 to differences in grain mass in durum wheat, Plant Cell Environ. 16 (1993) 383–392, https://doi.org/10.1111/j.1365-3040.1993.tb00884.x.

[84] J.L. Araus, P. Santiveri, D. Bosch-Serra, C. Royo, I. Romagosa, Carbon isotope ratios in ear parts of triticale : influence of grain filling, Plant Physiol. 100 (1992) 1033–1035 (Accessed April 27, 2019), http://www.ncbi.nlm.nih.gov/pubmed/16653012.

[85] R. Vicente, O. Vergara-Diaz, S. Medina, F. Chairi, S.C. Kefauver, J. Bort, M.D. Serret, N. Aparicio, J.L. Araus, Title : Differential Physiological and Gene Expression Responses of Durum Wheat Leaves and Ears to Contrasting Water Regimes, (2017), pp. 1–37.

[86] I.F. Wardlaw, The control of carbon partitioning in plants, New Phytol. 116 (1990) 341–381, https://doi.org/10.1108/08880451111169124.

[87] O. Merah, E. Deléens, P. Monneveux, Relationships between carbon isotope discrimination, dry matter production, and harvest index in durum wheat, J. Plant Physiol. 158 (2001) 723–729, https://doi.org/10.1078/0176-1617-00273.

[88] J. Bort, R.H. Brown, J.L. Araus, Refixation of respiratory CO_2 in the ears of C3 cereals, J. Exp. Bot. 47 (2007) 1567–1575, https://doi.org/10.1093/jxb/47.10.1567.

Supplementary Material

Table S1: Mean values for grain yield (GY), agronomic components, plant height, carbon isotope composition and N status parameters measured in the six selected genotypes in two crop seasons (2014-2015)

Each value is the mean of the nine environments (combining specific site, year and growing conditions). Grain yield (GY), thousand kernel weight (TKW), number of kernels per spike (kernels spike^{-1}), number of spikes per square meter (spikes m^{-2}), number of kernels per square meter (kernels m^{-2}), kernel weight per spike (KWPS), biomass at harvest (BM), harvest index (HI), days to heading (DTH), plant height (PH), the normalized difference vegetation index (NDVI), green area (GA), greener area (GGA) and chlorophyll content (SPAD). *G = genotype; Env refers to the specific growing conditions of the trials in a given year.*

	Year of release	GY (Mg ha^{-1})	TKW (g)	kernels Spike^{-1}	spikes m^{-2}	kernels m^{-2}	KWPS (g)	BM (Mg ha^{-1})	HI	DTH	PH (cm)	NDVI	GA	GGA	SPAD
Pelayo	2002	5.96^{a}	46.08^{c}	34.05^{c}	390.26^{ab}	13959^{a}	1.57^{c}	16.57^{a}	38.46^{b}	145.7	85.37^{d}	0.685^{a}	0.737^{b}	0.550^{b}	56.7^{a}
Sula	1994	5.83^{a}	41.60^{d}	38.22^{b}	370.00^{bc}	14277^{a}	1.61^{bc}	15.87^{a}	38.79^{b}	145.4	84.66^{cd}	0.679^{a}	0.807^{a}	0.576^{b}	56.7^{a}
Iride	1998	5.80^{a}	42.20^{d}	41.37^{a}	333.45^{d}	14498^{a}	1.76^{a}	14.98^{a}	41.93^{a}	145.9	85.21^{cd}	0.682^{a}	0.823^{a}	0.619^{ab}	57.4^{a}
Kiko Nick	2009	5.70^{ab}	48.56^{ab}	29.35^{d}	404.32^{a}	12438^{b}	1.43^{d}	15.87^{a}	38.77^{b}	145.2	86.74^{c}	0.693^{a}	0.796^{ab}	0.617^{ab}	57.2^{a}
D.Ricardo	2008	5.49^{b}	47.45^{bc}	35.59^{c}	346.62^{bc}	12532^{b}	1.69^{db}	15.84^{a}	37.19^{b}	147.1	91.69^{b}	0.675^{a}	0.820^{a}	0.620^{ab}	54.9^{b}
D.Sebastian	2004	4.69^{c}	49.95^{a}	25.11^{e}	396.76^{ab}	10392^{c}	1.25^{e}	16.30^{a}	32.51^{c}	147.5	96.81^{a}	0.691^{a}	0.868^{a}	0.661^{a}	55.7^{ab}
G		0.000	0.000	0.000	0.000	0.000	0.000	0.175	0.000	0.324	0.000	0.479	0.000	0.004	0.005
Year		0.424	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.176	0.000	0.000	0.000	0.000	0.019
Env		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006
G x Year		0.178	0.920	0.001	0.540	0.711	0.016	0.811	0.016	0.487	0.284	0.151	0.483	0.019	0.187
G x E		0.275	0.014	0.004	0.292	0.649	0.072	0.101	0.009	0.768	0.000	0.998	0.289	0.016	0.509
Year x E		0.000	0.087	0.000	0.031	0.005	0.000	0.077	0.314	0.060	0.000	0.000	0.000	0.000	0.000
G x Year x E		0.096	0.744	0.000	0.126	0.026	0.320	0.079	0.021	0.002	0.009	0.620	0.250	0.011	0.454

Table S 2: Mean values for grain yield (GY), agronomical components and phenology in the six selected genotypes during the second season (2015)
Each value is the mean of five locations. Grain yield (GY), Thousand Kernels weight (TKW), number of kernels per spike (kernels spike^{-1}), number of spikes per square meter (spikes m^{-2}), number of kernels per square meter (kernels m^{-2}), biomass at harvest (BM), harvest index (HI) and days to heading. *G = genotype; Env = environment (combining specific site and growing condition)*

	GY (Mg ha^{-1})	TKW(g)	kernels spike^{-1}	spikes m^{-2}	kernels m^{-2}	BM (Mg ha^{-1})	HI	DTH
Pelayo	6.07^{a}	45.20^{b}	32.40^{b}	446.35^{ab}	14266^{abc}	17.53^{a}	34.52^{a}	143.6^{a}
Iride	6.00^{a}	40.62^{c}	40.12^{a}	382.23^{b}	15268^{a}	16.66^{a}	35.89^{a}	141.6^{a}
Sula	5.78^{ab}	40.63^{c}	34.68^{b}	435.06^{ab}	14456^{ab}	17.21^{a}	33.40^{a}	144.2^{a}
Kiko Nick	5.78^{ab}	47.63^{ab}	27.65^{c}	462.30^{a}	12750^{c}	17.52^{a}	32.96^{ab}	139.9^{a}
D.Ricardo	5.69^{ab}	46.70^{ab}	33.82^{b}	384.00^{b}	12945^{bc}	17.20^{a}	32.85^{ab}	144.6^{a}
D.Sebastian	4.94^{c}	49.80^{a}	24.99^{c}	424.48^{ab}	10573^{d}	17.56^{a}	29.52^{c}	142.8^{a}
G	0.001	0.000	0.000	0.068	0.000	0.982	0.016	0.998
Env	0.000	0.000	0.000	0.653	0.000	0.000	0.000	0.000
G x E	0.848	0.929	0.105	0.793	0.844	0.859	0.914	0.999
$h^2_{(20\ genotypes)}$	0.63	0.93	0.90	0.61	0.82	0.39	0.77	0.97
$h^2_{(6\ genotypes)}$	0.81	0.93	0.93	0.75	0.91	-	0.84	0.95

Table S 3: Mean values for plant height, chlorophyll content, vegetation indices and morphological parameters in the six selected genotypes during the second season (2015).

Each value is the mean of five locations plant height (PH), normalized difference vegetation index (NDVI), green area (GA), greener area (GAA), leaf length (LL), leaf width (LW) amd spike length (SL). *G = genotype; Env = environment (combining specific site and growing condition)*

	PH (cm)	NDVI	GA	GGA	SPAD	LL (cm)	LW (cm)	SL (cm)
Pelayo	89.12^{c}	0.717	0.800	0.569	56.1^{abc}	18.98^{c}	1.57^{b}	6.98^{ab}
Iride	89.17^{c}	0.731	0.868	0.621	56.4^{ab}	18.20^{c}	1.55^{b}	7.19^{a}
Sula	89.44^{c}	0.731	0.876	0.582	56.1^{abc}	18.55^{c}	1.68^{c}	7.02^{a}
Kiko Nick	91.89^{c}	0.729	0.836	0.602	56.6^{a}	20.12^{b}	1.53^{b}	6.48^{b}
Don Ricardo	95.92^{b}	0.718	0.881	0.635	54.4^{c}	21.96^{a}	1.39^{c}	7.04^{a}
Don Sebastian	103.52^{a}	0.743	0.901	0.662	54.6^{bc}	20.56^{b}	1.48^{bc}	6.72^{ab}
G	0.000	0.142	0.151	0.200	0.049	0.000	0.001	0.025
Env	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G x E	0.000	0.432	0.689	0.805	0.926	0.009	0.305	0.465
$h^2_{(20\ genotypes)}$	0.89	0.74	0.60	0.69	0.79	0.87	0.55	0.77
$h^2_{(6\ genotypes)}$	0.95	0.35	-	-	0.73	0.22	0.82	0.54

Table S4: Mean values for water status and N status parameters measured in the six selected genotypes in two crop seasons (2014-2015)

Each value is the mean of the nine environments (combining specific site, year and growing condition). Kernel carbon isotope composition ($\delta^{13}C_{Kernel}$), canopy temperature depression (CTD), kernel nitrogen isotope composition ($\delta^{15}N_{Kernel}$), kernel nitrogen content (N_{Kernel}) and total kernel nitrogen per spike (KNPS). *G = genotype; Env refers to the specific growing conditions of the trials in a given year.*

	$\delta^{13}C_{kernel}$ (‰)	CTD (ºC)	$\delta^{15}N_{Kernel}$ (‰)	N_{Kernel} (%)	KNPS (mg)
Pelayo	-25.74^{b}	5.15^{ab}	4.19^{a}	2.35^{bc}	36.2^{b}
Sula	-25.70^{b}	5.35^{ab}	4.31^{a}	2.32^{cd}	35.5^{bc}
Iride	-25.65^{b}	4.73^{ab}	4.27^{a}	2.20^{d}	38.1^{ab}
Kiko Nick	-25.56^{b}	4.45^{b}	4.50^{a}	2.60^{a}	37.1^{ab}
D.Ricardo	-25.59^{b}	5.75^{a}	4.53^{a}	2.48^{ab}	41.0^{a}
D.Sebastian	-25.25^{a}	4.28^{b}	4.57^{a}	2.61^{a}	31.7^{c}
G	0.003	0.193	0.080	0.000	0.000
Year	0.000	0.000	0.000	0.039	0.000
Env	0.000	0.000	0.000	0.000	0.000
G x Year	0.539	0.047	0.454	0.298	0.068
G x Env	0.261	0.574	0.007	0.061	0.978
Year x Env	0.000	0.000	0.001	0.000	0.019
G x Year x Env	0.404	0.903	0.170	0.319	0.615

Table S 5: Mean values for carbon isotope composition of mature kernel, flag leaf, peduncle and ear (awns, glume, lemma and palea) in dry matter and water-soluble fraction in the six selected genotypes during the second season (2015). Each value is the mean of five locations. *G = genotype; Env = environment (combining specific site and growing condition)*

	Dry matter							Watter soluble fraction					
	$\delta^{13}C_{kernel}$	$\delta^{13}C_{awns}$	$\delta^{13}C_{glume}$	$\delta^{13}C_{lemme}$	$\delta^{13}C_{palea}$	$\delta^{13}C_{leaf}$	$\delta^{13}C_{peduncle}$	$\delta^{13}C_{awns}$	$\delta^{13}C_{glume}$	$\delta^{13}C_{lemme}$	$\delta^{13}C_{palea}$	$\delta^{13}C_{leaf}$	$\delta^{13}C_{peduncle}$
Pelayo	-26.16	-26.69	-26.68	-26.57	-26.01	-28.04	-25.44	-26.62	-25.86	-25.77	-25.44	-28.06	-25.25
Iride	-26.14	-26.24	-26.56	-26.33	-25.72	-26.05	-25.59	-25.93	-25.63	-25.36	-25.14	-28.00	-25.60
Sula	-26.11	-26.56	-26.66	-26.37	-25.73	-28.08	-25.26	-25.93	-25.71	-25.50	-24.99	-27.65	-25.15
Kiko Nick	-26.08	-26.74	-26.72	-26.68	-26.08	-28.33	-26.11	-26.33	-26.03	-25.62	-25.32	-28.05	-25.18
Don Ricardo	-25.95	-26.47	-26.55	-26.36	-25.91	-27.67	-25.42	-26.45	-26.25	-26.06	-25.69	-28.10	-25.09
Don Sebastian	-25.71	-26.66	-26.71	-26.56	-25.63	-28.13	-25.81	-26.30	-25.93	-25.49	-25.04	-28.27	-25.16
G	0.072	0.003	0.730	0.041	0.019	<0.001	0.826	0.577	0.057	0.024	0.017	<0.001	0.185
Env	<0.001	<0.001	0.013	<0.001	<0.001	<0.001	0.678	<0.001	0.002	0.054	<0.001	<0.001	<0.001
G x E	0.845	<0.001	0.182	<0.001	<0.001	0.050	0.714	0.920	<0.001	0.038	0.002	0.080	0.091
$h^2_{(6\ genotypes)}$	0.87	0.32	-	0.47	0.42	0.97	-	0.62	0.31	-	-	-	0.82

Chapter 3

Breeding effects on the genotype x environment interaction for yield of durum wheat grown after the Green Revolution: the case of Spain

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Highlights

- Genetic gain in durum wheat in Spain increased in warmer and optimal environments
- The G x E interaction was affected by mean and maximum temperature
- In high-yielding environments plants favored higher water uptake
- In low-yielding environments plants favored greater WUE

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Breeding effects on the genotype x environment interaction for yield of durum wheat grown after the Green Revolution: the case of Spain

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Abstract:

This study evaluates the changes caused by breeding in the genotype x environment (G×E) interaction of the durum wheat varieties most widely cultivated in Spain after the Green Revolution. A set of 12 cultivars was tested in 27 environments, which are understood as the combination of different sites, years, and treatments (water regime and planting dates), representative of the durum wheat growing conditions in Spain with average grain yields (GY) ranging between 2.8 to 9.1 Mg ha^{-1}. The most important environmental factors affecting the G×E interaction for yield were maximum and mean temperature during the entire crop cycle. An improvement in genetic yield was observed in warm environments and under optimal water conditions that resemble those where the germplasm originated before its release in Spain (essentially as advanced lines). Therefore, the adaptation of semi-dwarf durum in Spain has shown a tendency to specific adaptation rather than large-scale adaptation. Two different patterns of selection have been reported due the G×E interaction and changes in the ranking of genotypes: in the high yielding environments (GY > 5 Mg h^{-1}), plants favor increased water uptake, with higher levels of transpiration and more open stomata (more negative values of $\delta^{13}C$ and higher Canopy Temperature Depression, CTD), whereas, in low yielding environments (GY < 5 Mg ha^{-1}) plants close their stomata and favor greater water use efficiency (less negative $\delta^{13}C$ values and lower CTD values).

Keywords: Durum wheat; breeding; genotype × environment interaction (G×E); adaptability; carbon isotope composition; canopy temperature.

1. Introduction

Durum wheat (*Triticum turgidum L.* spp. durum) is a crop of global significance that is cultivated on about 30–35 million hectares, and has a particular economic and cultural relevance in the Mediterranean basin where it represents a staple crop [1]. Under Mediterranean conditions, drought and its frequent association with heat are the stresses that most limit cereal yield because they usually occur together during the reproductive stages of the crop [2]. Moreover, the variability of thermo-pluviometry patterns results in large spatial and temporal yield fluctuations [3,4]. Predictions of climate change will result in a more unfavorable environment for wheat production that will require unprecedented efforts to release new cultivars that are not only more productive but also offer better yield stability under various environmental conditions. This is particularly urgent for durum wheat because the Mediterranean basin is a hotspot for negative predictions of climate change in terms of decreased rainfall and increased temperatures [5].

Studies addressing genetic gains in wheat yield during the last century focused on the eventual changes through time in agronomical yield components and/or relevant physiological traits [6–10] within a wide range of environments. However, few studies have addressed the relationship between genetic gain and the pattern of adaptation in terms of examining the genotype by environment interaction (G×E) [11,12]. In addition, to the best of our knowledge, there are no studies that have focused exclusively on semi-dwarf (i.e. post Green Revolution) durum wheat genotypes. This is despite the fact that the G×E interaction, which is a critical question for cultivar evaluation, may lead to contrasting responses to a given environment [13]. Indeed, it is important to understand the adaptation mechanisms of wheat cultivation and the parameters responsible for the G×E interactions in grain yield caused by the large and unpredictable seasonal and geographical fluctuations in rainfall and temperature typical of the Mediterranean drylands [3,4].

The assessment of the relative contributions of the cultivar, the environment and the G×E interaction to cultivar performance is essential to determine the adaptation capacity, which is the cultivar’s ability to reach its full potential in a specific environment in spite of the constraints imposed on the crop [11]. Nevertheless, there are two breeding strategies: selecting for wheat under stress-free conditions (aimed at wide adaptation) [14] and selecting under limiting environments (specific adaptation)[15,16].

Durum wheat breeding activities in Spain started during the first half of 20th century, with local breeding programs targeting a bulk selection within landraces, as well as selecting from crosses between the Italian variety Senatore Capelli and Spanish landraces [17]. However, the huge rise in yield during the second half of the 20th century in Spain [17] was primarily a consequence of the introduction of CIMMYT semi-dwarf germplasm (the so called Green Revolution). As a consequence, most commercial cultivars grown in Spain until this day are (to a greater or lesser extent) of foreign origin, and they were primarily selected for the specific environmental conditions of these external locations [17]. However, the Spanish environments that have determined the pattern of adaptation of these semi-dwarf genotypes remain essentially unknown.

The objectives of this research were: (i) to assess the impact of breeding for yield over the the last few decades within the wide range of Spain environments and (ii) to investigate their adaptation patterns. To that end, a set of commercial semi-dwarf cultivars released and widely grown in Spain during the last few decades were tested in 27 environments (resulting from a combination of different locations, years water regimes and planting dates).

2. Materials and Methods

2.1. *Plant material, growing conditions and experimental design*

Twelve durum wheat (*Triticum turgidum L.* subsp. Durum (Desf) Husn.) cultivars were selected to represent the germplasm grown in Spain after the Green Revolution: Mexa, Vitron, Regallo, Simeto, Gallareta, Dorondon, Burgos, Claudio, Amilcar, Avispa, Don Ricardo and Kiko Nick (Table 1).

Table 1: Set of twelve modern (semi-dwarf) durum wheat cultivars tested in this study. Year of release, country of registration and pedigree or origin are presented.

Code	Variety	Year of release	Country	Pedigree/cross name or origin
1	Mexa	1980	Spain	GERARDO-VZ-469/3/JORI(SIB)//ND-61-130/LEEDS
2	Vitron	1983	Spain	TURCHIA-77/3/JORI-69(SIB)/(SIB)ANHINGA//(SIB)FLAMINGO
3	Regallo	1988	Italy	Diputación General de Aragón CIMMYT
4	Simeto	1990	Spain	RUFF/FLAMINGO//MEXICALI-75/3/SHEARWATER
5	Gallareta	1994	Spain	CIMMYT
10	Dorondon	1998	Spain	Genética y Gestión,S.C
12	Burgos	1999	Spain	SUDDEUTSCHE SAATZ
13	Claudio	1999	Spain	(Sel. Cimmyt×Durango) × (IS193B×Grazia)
14	Amilcar	2001	Italy	ZEGZAG-1/LUNDE-5//GREENSHANK-32
16	Avispa	2003	Spain	Limagrain-CIMMYT
18	D Ricardo	2008	Spain	Agrovegetal-CIMMYT
19	Kicko Nick	2009	Spain	SEL.CIMMYT-35/DURANGO//ISEA-1938/GRAZIA

Twenty-seven field experiments were conducted in Spain during five consecutive crop seasons, 2013-2014 until 2017-2018, at three locations at different latitudes, and exhibiting a wide range of growing temperatures, and these were representative of the main wheat growing areas in the country. Two of the three locations are experimental stations of the Spanish "Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria" (INIA): Coria del Rio (C), Seville (37°14′N, 06°03′W, 5 masl), and Colmenar de Oreja – Aranjuez (A), Madrid (40°04′N, 3°31′W, 590 masl). The third station belongs to the "Instituto Tecnológico Agrario de Castilla y León" (ITACyL) in Zamadueñas (Z), Valladolid (41°41′N, 04°42′W, 700 masl). For all of the twenty-seven trials, plots were sown in a randomized block design with three replicates (plots) per cultivar. Each plot consisted of six rows of 7 m length and 0.2 m apart, with a planting density of 250 seeds m^{-2}. Concerning normal planting, rainfed (RF) and supplemental irrigation (IR) conditions were imposed at Aranjuez and Zamadueñas. In the case of Coria del Rio and due to the presence of a shallow water table, caused by proximity to the Guadalquivir River, the genotypes did not experience water stress even if they grew under rainfed conditions. Late planting was also performed in Aranjuez and Zamadueñas during the last two growing seasons. Sowing took place between the end of November and the beginning of December for the normal planting at the three sites, and during the first week of February for the late plantings performed at Aranjuez and Zamadueñas (Table 2). In that last case, trials were irrigated to ensure that only temperature was the main environmental variable. During all experimental campaigns fertilizer was applied in two steps, a first basal application of 300 Kg ha^{-1} of 8-15-15 (N:P:K) in Zamadueñas and 400 Kg ha^{-1} in both Aranjuez and Coria del Rio, and then a second top dressing application of 300 kg ha^{-1} nitric acid (NAC) in Zamadueñas and 150 Kg ha^{-1} in both Aranjuez and Coria de Rio. All trials were controlled for weeds, insect pests, and diseases by recommended chemical doses. Plants were harvested mechanically at maturity and grain yield assessed.

During the first three years these twelve genotypes were evaluated within a set of 20 genotypes released in Spain between 1980 and 2009 (see [9]) while in the last two years these genotypes were cultivated side by side with another twelve modern cultivars released in Spain between 1980 and 2014.

2.2. *Environment characterization*

The growing environments were characterized on the basis of agro-climatic variables measured from sowing to physiological maturity (Zadoks stage 92) [18]. The following variables were measured: average daily minimum, mean and maximum temperature (T_{min}, T_{mean}, T_{max}; ºC), water input (WI, mm, including accumulated rainfall during the crop cycle, plus irrigation when appropriate), reference evapotranspiration (ET_0, mm) and the water deficit experienced by the crop, represented by the ratio of water input (WI) to ET0 (WI/ET0). Climate data was extracted through the SiAR network of agrometeorological stations (www.eportal.mapama.gob.es/websiar).

2.3. *Canopy temperature depression*

Canopy temperature depression (CTD) was measured in the early grain filling stage (Zadocks stage 71 [18]) as CTD = Ta – Tc, where Ta and Tc were the air temperature and canopy temperature for each plot, respectively. The canopy temperature of each plot was measured with an infrared thermometer (PhotoTempTM MX6TMTM, Raytek Corporation, Santa Cruz, USA). Ambient temperature was measured simultaneously above each plot using a thermo-hygrometer (Testo 177-H1 Logger, Germany). Measurements were taken around midday on sunny days.

2.4. *Stable carbon and nitrogen isotope signatures and N content*

The total N content of mature grains was analyzed using an Elemental Analyzer (Flash 1112 EA; ThermoFinnigan, Bremen, Germany) for each individual plot within the entire set of trials. The same EA coupled with an Isotope Ratio Mass Spectrometer (Delta C IRMS, ThermoFinnigan, Bremen, Germany), operating in continuous flow mode, was used to determine the stable carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios of the same mature grains. Finely ground samples of ~1 mg and reference materials were weighed into tin capsules, sealed, and then loaded into an automatic sampler (ThermoFinnigan) before EA-IRMS analysis. Nitrogen was expressed as a concentration (g N per g DW) and atropine was used as a system check in the elemental analyses of nitrogen. The ^{13}C:^{12}C ratios of plant material were expressed in δ notation [19].

$$\delta^{13}C\ (‰) = [(^{13}C{:}^{12}C)\ \textit{sample} / ((^{13}C{:}^{12}C)\ \textit{standard} - 1)\ ], \qquad (1)$$

Where sample refers to plant material and standard to international secondary standards of known ^{13}C:^{12}C ratios (IAEA CH7 polyethylene foil, IAEA CH6 sucrose, and USGS 40 L-glutamic acid) calibrated against Vienna Pee Dee Belemnite calcium carbonate (VPDB) with an analytical precision (SD) of 0.10‰.

The 15N:14N ratios were also expressed in δ notation ($\delta^{15}N$) using international secondary standards of known ^{15}N:^{14}N ratios (IAEA N_1 and IAEA N_2 ammonium sulfate and IAEA NO_3 potassium nitrate) referred to N_2 in air, with an analytical precision of 0.18‰.

$$\delta^{15}N\ (‰) = [(^{15}N{:}^{14}C)\ \mathit{sample}\ /\ ((^{15}C{:}^{14}C)\ \mathit{standard}\ -\ 1)], \qquad (2)$$

Measurements were conducted at the Scientific Facilities of the University of Barcelona. Grain nitrogen yield (GNY) was calculated as follows:

$$\text{GNY (Kg ha}^{-1}) = [(\mathit{Ncontent}\ (\%) \times \mathit{GY}\ (\text{Kg ha}^{-1}))\ /\ 100], \qquad (3)$$

2.5. *Statistical analysis*

The response of the studied genotype, environment, and environment by genotype interaction was tested with ANOVA. The GxE interaction was evaluated through the Additive Main effect and Multiplicative Interaction (AMMI) analysis [20] using GEA-R statistical software [21]. The AMMI results were graphically presented in the form of a biplot [22], where genotypes and environment scores of the first two bilinear terms are represented by vectors, with their starting points at the origin (0.0) and end points (markers) determined by their scores. Two kinds of biplots are reported in this paper. The AMMI1 biplot shows genotype and environment means and the grand mean on the abscissa and its IPCA1 scores for genotypes and environments on the ordinate. The AMMI2 biplot shows its IPCA1 on the abscissa and IPCA2 on the ordinate.

3. **Results**

3.1. *Environmental classification*

The experiments were carried out over a large range of climatic and growing conditions. There were large differences in growing conditions, as shown not only by the variability in environmental variables during the growing cycle (temperature, water input and evapotranspiration), but also by the range of variability in crop water status traits ($\delta^{13}C$ and CTD) and grain yield (GY and GNY) (Table 2).

Table 2. Description of the 27 environments (trials) included in this study.

Groups	Trials	T_{min} (ºC)	T_{max} (ºC)	T_{mean} (ºC)	WI (mm)	ET0 (mm)	WI/ET0	GY (Mg ha^{-1})	GNY (Kg ha^{-1})	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	CTD (ºC)
Coria	C2RF	6.8	19.9	12.9	163.4*	780	0.21	6.5	159.4	-27.6	13.4	5.7
Coria	C3RF	8.5	19.4	13.5	351.2*	673	0.52	5.0	116.0	-27.0	9.6	2.7
Coria	C4RF	9.7	22.6	12.5	204.0*	569	0.36	5.4	-	-	-	2.6
Coria	C5RF	6.8	18.0	12.1	297.8*	669	0.45	5.6	123.5	-27.5	4.9	4.8
IR	A1IR	3.4	17.7	10.2	557.7	634	0.88	6.7	121.1	-25.8	3.9	4.6
IR	A2IR	3.0	18.0	9.9	386.0	726	0.53	5.1	125.0	-25.8	3.9	6.9
IR	A3IR	3.3	17.3	9.9	477.1	632	0.75	4.5	135.5	-26.2	3.5	2.9
IR	A4IR	7.2	24.3	11.42	537.2	804	0.67	4.9	-	-	-	2.4
IR	A5IR	2.4	16.0	8.7	475.8	726	0.66	7.4	183.5	-26.2	3.1	-
IR	Z1IR	2.8	14.2	8.2	337.3	690	0.49	6.5	185.8	-25.8	3.0	1.2
IR	Z2IR	2.4	14.0	7.9	387.8	695	0.56	7.2	185.6	-25.8	3.1	4.9
IR	Z3IR	2.6	13.5	7.6	429.7	656	0.66	9.1	170.1	-25.7	3.0	5.7
IR	Z4IR	2.7	15.5	8.6	279.0	633	0.44	7.0	176.0	-24.9	2.6	3.4
IR	Z5IR	2.4	12.9	7.3	585.8	605	0.97	6.3	117.5	-26.5	2.0	
Late	A4L	8.9	22.0	15.5	559.6	559	1.00	3.7	-	-	-	12.1
Late	A5L	7.2	21.4	14.3	481.3	513	0.94	4.0	-	-	-	-
Late	Z4L	6.6	22.1	14.3	370.6	669	0.55	4.8	-	-	-	3.2
RF	A1RF	3.4	17.7	10.2	203.7	490	0.42	5.6	109.1	-25.7	3.7	5
RF	A2RF	3.0	18.0	9.9	206.0	726	0.28	4.6	115.2	-26.7	3.8	4.9
RF	A3RF	3.3	17.3	9.9	277.1	632	0.44	3.9	123.0	-27.7	4.2	2.6
RF	A4RF	6.9	23.9	11.42	230.5	804	0.29	2.9	-	-	-	1.5
RF	A5RF	2.4	16.0	8.7	325.8	727	0.45	3.5	99.6	-24.4	1.0	-
RF	Z1RF	2.8	14.2	8.2	212.3	690	0.31	3.1	105.5	-24.5	1.9	-0.6
RF	Z2RF	2.4	14.0	7.9	262.8	696	0.38	3.8	104.0	-24.5	2.4	0.84
RF	Z3RF	2.6	13.5	7.6	359.7	673	0.53	6.6	105.3	-24.5	2.4	1.8
RF	Z4RF	2.7	15.5	8.6	179.0	631	0.28	2.9	84.5	-23.7	0.9	1.3
RF	Z5RF	2.2	13.0	7.2	476.4	605	0.79	6.9	155.8	-26.5	3.3	-

T_{min}, T_{max} and T_{mean} are average minimum, maximum and mean daily temperatures, respectively. WI: water input (rainfall + irrigation); ET_0: potential evapotranspiration; $\delta^{13}C$ and $\delta^{15}N$ correspond to the stable carbon and nitrogen isotope compositions, respectively, of mature kernels. Site code; A, C and Z correspond to location (A: Aranjuez, C: Coria and Z: Zamadueñas); numbers (1-5) correspond to the respective growing season (e.g. 1: 2013-2014), and RF, IR and L correspond to rainfed, irrigation and late planting, respectively. The effect of a shallow water table in Coria is not reflected in the WI, but in the low $\delta^{13}C$ and high CTD values typical of good crop water availability [23] as well as the high $\delta^{15}N$, which suggested that the nitrogen source was not solely from chemical fertilizer (characterized by $\delta^{15}N$ values near 0 ‰) but strongly affected by animal and urban $\delta^{15}N$ sources ([24]).

According to the data in Table 2, we classified growing environments in four major groups. Group 1 "Coria" was characterized by a high mean and maximum temperature values and high availability of water thanks to the proximity of the water table. Group 2 "Late" was characterized by high mean and maximum temperature values like "Coria" and strong support irrigation but a late sowing date. Group 3 "RF" and groups 4 "IR" were constituted by the rainfed and irrigated environments of the normal planting trials, respectively.

3.2. *Genetic advance in grain yield within each group of environments*

The analysis showed a significant progress in grain yield (GY) in Coria and Late (Fig. 1A and B).

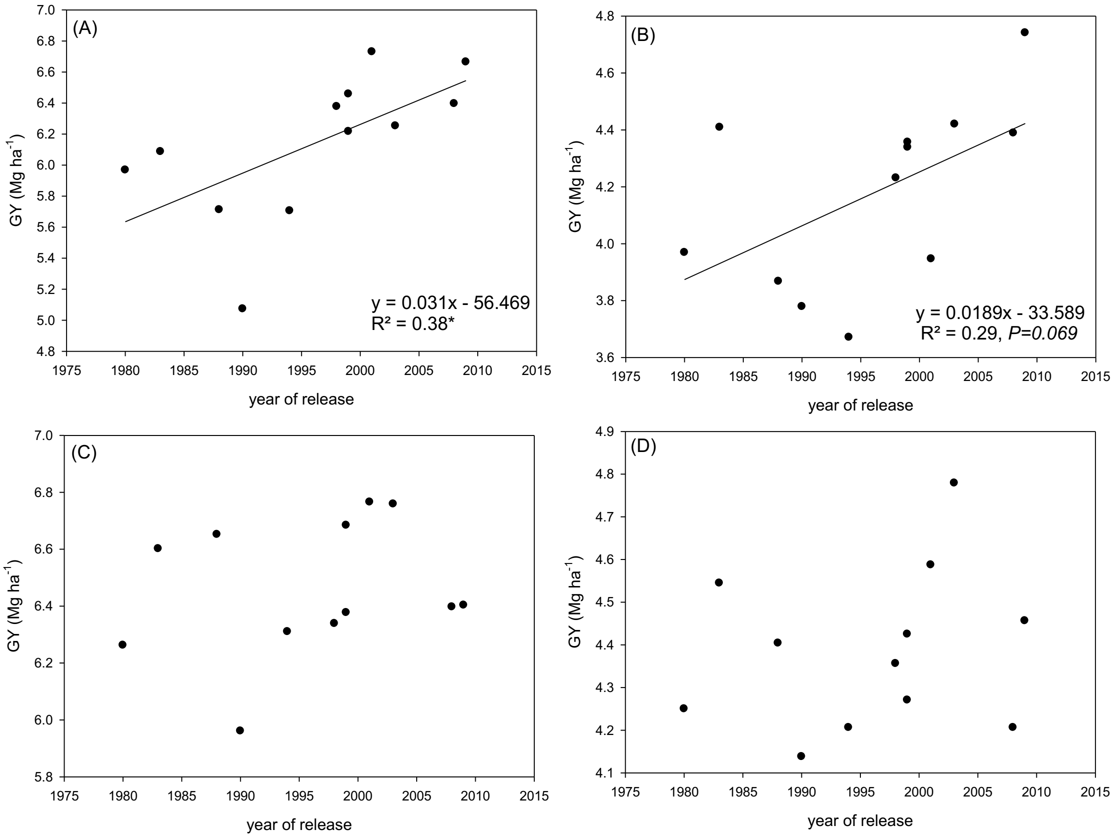

Figure 6: Relationship between grain yield and the year of cultivar release under each group of environments A: Coria, B: Late, C: IR and D: RF. For each group of environments, each point represents the average GY value of a given cultivar across the environments and replicates.

The genetic advance using the set of 12 genotypes was around 31 kg ha^{-1} y^{-1} in Coria and around 19 kg ha^{-1} y^{-1} in Late. A similar pattern was seen when using 23 genotypes and 13 environments from the last two seasons (Fig. S1). However, no pattern of increase was seen in the RF and IR groups when using either 12 (Fig1 C and D) or 23 genotypes (Fig. S1).

3.3. *Analysis of variance*

Mean environment yields ranged between 2.88 Mg ha^{-1} (A4RF) and 9.11 Mg ha^{-1} (Z3IR) (Table 1). The combined analysis of variance (ANOVA) revealed significant environment (E), genotype (G) and G×E interaction. The ANOVA analyzed in each group of environments separately revealed significant E effects for GY (Table 3). The G effect was highly significant in Coria, IR and RF and marginally significant in Late. The G×E interaction was highly significant for Coria and RF, marginally significant in Late, and not significant in IR.

Even when the analyses were performed within each of the four groups of environments, the ANOVA revealed that most of the total variation for GY within each group (calculated as the proportion of the total sum of square) was due to the effect of environments, with high values of 60.4%, 79.9%, and 72.1% in Coria, RF and IR, respectively, while in Late the values were lower (27%). The proportion of the total sum of squares due to differences among genotypes was much higher for Coria (13.7%) and Late (10.6 %) than for the other two groups (2.2%, IR and 1.3%, RF) and was significant in all groups except for Late (where it was marginally significant). Also, it was different for the G×E interaction (14.1%, Coria; 20.9%, Late; 6.1, RF and 10.4%, IR) (Table 3) and the G×E interaction was significant in all groups except IR.

Table 3: Analysis of variance for grain yield (GY) for twelve durum wheat genotypes grown in each of the four groups of environments as well as the combined set of environments

Source of variation in GY	df	SS	SS (%)	F	*p-value*
Combined					
Total	968	3099.810			
E	26	2516.154	81.2	198.0	*.000*
G	11	43.307	1.4	8.056	*.000*
G × E	286	219.686	7.1	1.572	*.000*
Error	645	315.225			
Coria					
Total	143	211.01			
E	3	127.52	60.4	163.8	*.000*
G	11	28.88	13.7	10.12	*.000*
G × E	33	29.69	14.1	3.468	*.000*
Error	96	24.91			
Late					
Total	107	96.817			
E	2	26.272	27.1	23.64	*.000*
G	11	10.294	10.6	1.684	*.094*
G × E	22	20.247	20.9	1.656	*.057*
Error	72	40.004			
IR					
Total	358	7831.8			
E	9	740.9	79.9	165.4	*.000*
G	11	11.6	1.3	2.123	*.019*
G × E	99	56.3	6.1	1.144	*.205*
Error	239	118.9			
RF					
Total	357	852.6			
E	9	614.8	72.1	124.8	*.000*
G	11	18.7	2.2	3.099	*.001*
G × E	99	88.6	10.4	124.8	*.001*
Error	238	130.3			

The mean environment grain nitrogen yield (GNY) ranged between 84.5 kg ha^{-1} (A4RF) and 185.8 kg ha^{-1} (Z1IR) (Table 1). The combined analysis of variance (ANOVA) revealed significant E and G effect and no G×E interaction was observed. The ANOVA analyzed separately for each of the four groups of environments revealed significant E effects for GNY (Table S1). The G effect was highly significant only in Coria and the G×E interaction was marginally significant in Coria and not significant in the other groups of environments (Table 3). Most of the total variation within each group was due to significant differences among environments with around 50% for Coria and IR and lower for RF (28%). The proportion of the total sum of squares due to differences among genotypes was much lower in RF (1.7%) than in IR (2.4%) and in Coria (13.7%), whereas the GxE interaction was quite similar (around 12%) for the three groups.

The mean environment carbon isotope composition ($\delta^{13}C$) ranged between -27.7 ‰ (A3RF) and -23.7 ‰ (Z4R) (Table 4). The combined ANOVA revealed a significant E effect but no effect for G and G×E interaction was observed. The ANOVA for the four groups of environments separately and revealed significant E effects for $\delta^{13}C$ (Table 4). The G effect was highly significant only in Coria and no G×E interaction was observed in any group of environments. Therefore, most of the total variation was due to significant differences among environments: 57% for Coria; 39.6% for RF and a lower value for IR (21.4%). The three groups showed similar G×E interaction values (around 12%).

Table 4: Analysis of variance for carbon isotope composition ($\delta^{13}C$) (‰) for twelve durum wheat genotypes grown in each group of environments as well in the combined set of environments.

Source of variation	$\delta^{13}C$ (‰)	df	SS	SS (%)	F	*p-Value*
	Combined					
Total		748	1853.5			
E		20	878.27	47.4	25.99	*.000*
G		11	15.403	0.83	0.829	*.611*
G × E		220	99.516	5.36	0.268	*1.000*
Error		497	839.56			
	Coria					
Total		107	18.937			
E		2	8.074	42.6	57.85	*.000*
G		11	4.056	21.4	5.284	*.000*
G × E		22	1.784	9.4	1.162	*.308*
Error		72	5.024			
	RF					
Total		318	1237.8			
E		8	489.5	39.6	19.60	*.000*
G		11	11.7	0.9	0.340	*.976*
G × E		88	57.6	4.7	0.210	*1.000*
Error		211	658.4			
	IR					
Total		321	269.4			
E		8	57.7	21.4	8.873	*.000*
G		11	6.4	2.4	0.720	*.719*
G × E		88	32.1	11.9	0.440	*1.000*
Error		214	173.8			

3.4. AMMI analysis

G, E and G x E interaction effects were also estimated by the AMMI model. The results showed that the first two principal-component axes of the interaction (IPCA) explained most of the G x E interaction effect for GY in all four groups of environments evaluated, ranging from 58% (IR) to 100% (Late). In Coria, 37% of the sum of squares corresponding to the interaction was captured by the IPCA1 axis and a further 36% was explained by the IPCA2 axis while IPCA3 explained an additional 26%. In Late, 77% of the interaction sum of squares was captured by the IPCA1 axis and a further 23% was explained by the IPCA2 axis. Values of 37% and 32% for the interaction sum of squares were captured by IPCA1 in IR and RF, respectively, and a further 21% and 30%, respectively, were explained by the IPCA2 axis. Therefore, most information could be graphically displayed in an AMMI1 biplot (Fig. 2), which allowed visualization of relationships between the eigenvalues for the first principal component axis (IPCA1) and the genotype and environment means.

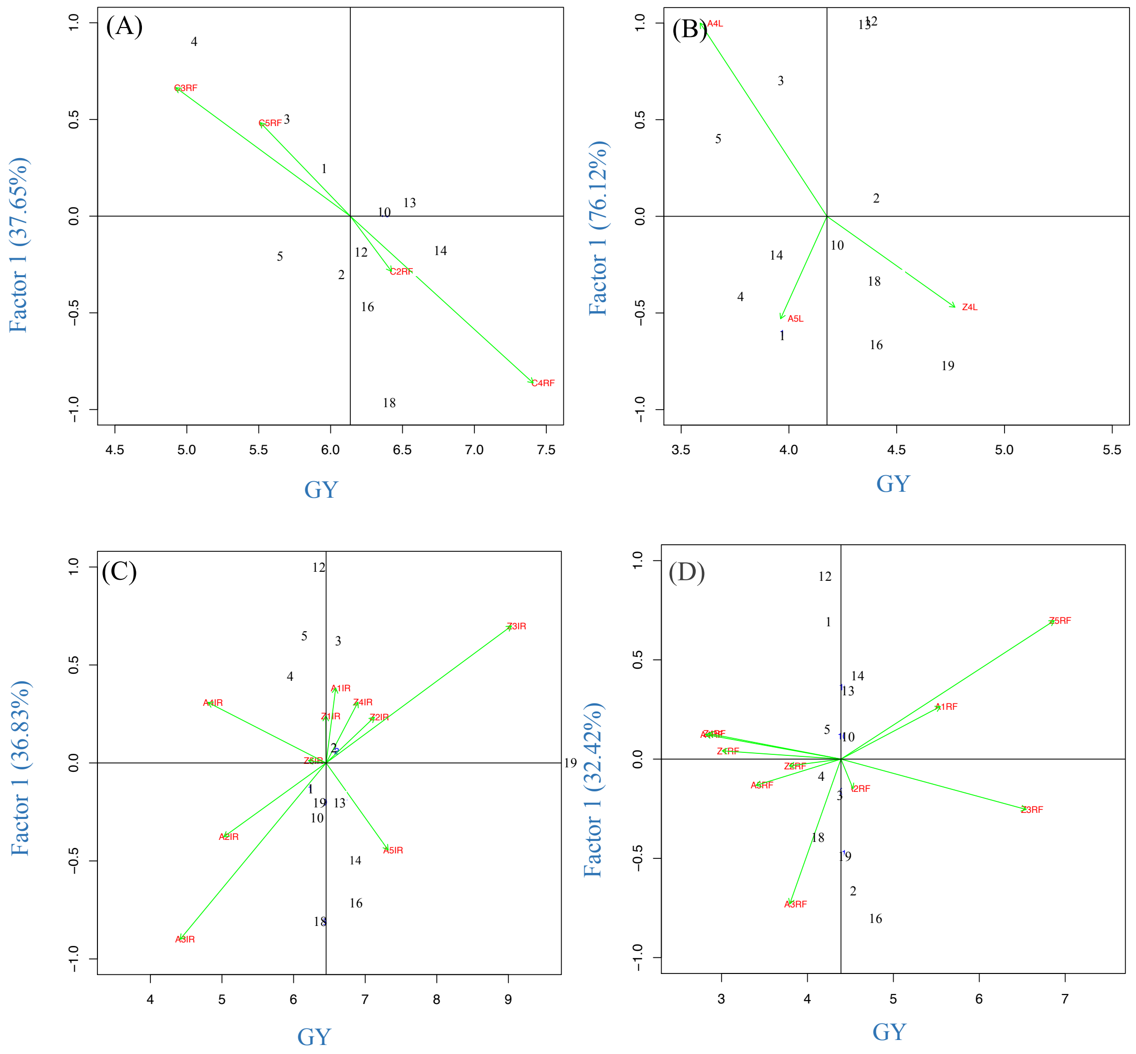

Figure 7: AMMI1 biplot for GY (Mg ha^{-1}) of the 12 semi-dwarf durum wheat genotypes evaluated in each group of environments (A, Coria; B, Late; C, IR and D, RF).

Large deviations in the genotypic and environmental scores on the ordinate indicated specific adaptation to the environments with the same IPCA1 sign, while the values on the abscissa reflected the agronomical potential for environments and the general improvement status for genotypes [25]. Values close to zero characterized genotypes and environments that had a low contribution to the interaction, being considered stable with a wide adaptation. Therefore, genotypes can be characterized based on their interactions with the environments. Genotypes with IPCA1 scores close to zero had small interactions and showed wide adaptation to the tested environments. These included: Dorondon (10), Claudio (13) and Amilcar (14) in Coria; Dorondon (10) and Vitron (2) in Late; Vitron (2) and Mexa (1) in IR; and Dorondon (10) and Regallo (3) in RF (Fig. 2). In contrast, large deviations from zero on the ordinate indicated specific adaptation to the environment with the same IPCA1 sign. In Coria, the more recently released Dorondon (10), Burgos (12), Claudio (13), Amilcar (14), Avispa (16), Don Ricardo (18) and Kiko Nick (19) had higher mean yields and were specifically adapted to the most productive environments (C2RF and C4RF). On the other hand, old genotypes (1-5: Mexa, Vitron, Regallo, Simeto and Gallareta) with low mean yields were specifically adapted to the poorer sites (C3RF and C5RF). A similar trend to Coria was seen in the Late group of environments.

The AMMI2 biplot shows its IPCA1 on the abscissa and IPCA2 on the ordinate (Figure 3). The relative position of the genotypes versus the environmental vectors is based on their interaction (not on main effects). The GE biplot illustrated the role of the IPCA on the performance of genotypes and environments through the distance of each point (genotype/environment) from the origin.

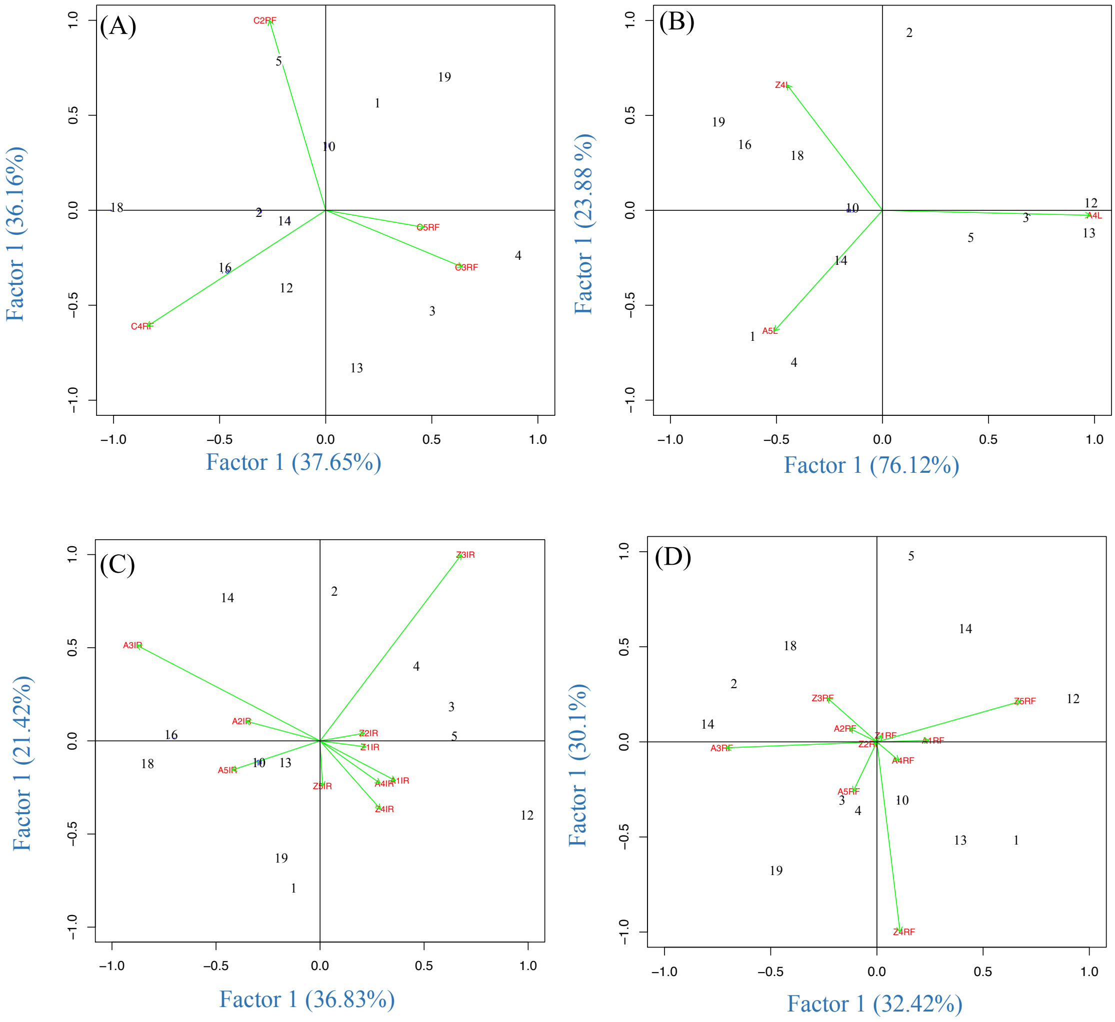

Figure 8: AMMI2 biplot for GY (Mg ha^{-1}) of the 12 semi-dwarf durum wheat genotypes evaluated in each group of environments (A, Coria; B, Late; C, IR and D, RF).

It was observed that most of the genotypes and environments were dispersed around the biplot. Genotypes further from the center of biplot showed specific adaptation. In order to estimate specific adaptation and study their stability, the biplot diagram was used. Busey et al. [26] in a study of G×E interaction in durum wheat revealed that genotypes that were far from the center of a biplot had high G×E interactions while those placed nearest to the center of a biplot, had high stability. In our study, while far more genotypes were positioned away from the origin than close to or at the origin, the few genotypes close to the origin were Amilcar (14) and Vitron (2) in Coria (Fig. 3A), Dorondon (10) and Amilcar (14) in Late (Fig. 3B), Dorondon (10) and Claudio (13) in IR (Fig 3C) and Regallo (3), Simeto (4) and Dorodon (10) in RF (Fig. 3D).

3.5. Climate conditions and genetic gain

To understand the relationship between genetic gain and agro-climatic parameters that form the basis of G x E interactions, correlations have been made between the coefficient of correlation of year of cultivar release vs GY (that is, the genetic gain for grain yield) and different climate parameters (T_{max}, T_{min} and T_{mean}). Positive relationships were found for the correlation coefficient of the genetic gain for GY with T_{max} and T_{mean}, while no relationships existed with T_{min} (Fig. 4A and B).

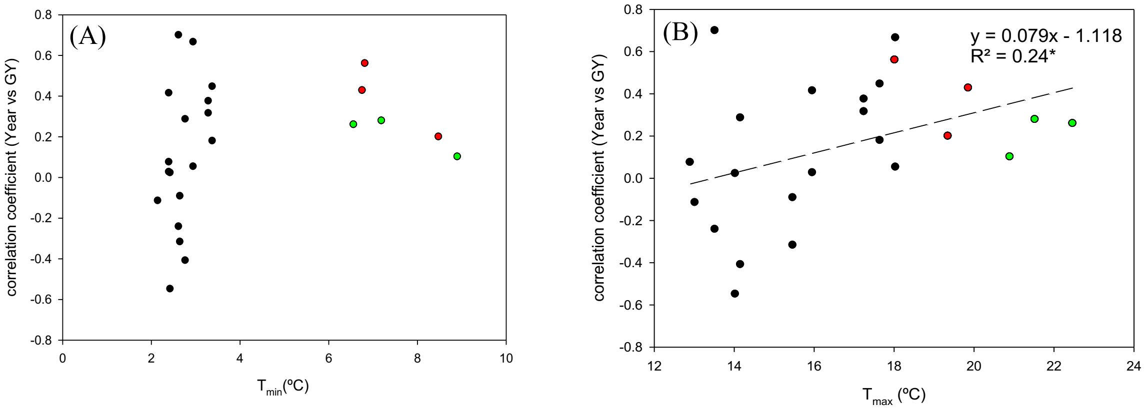

Figure 9: Relationship between correlation coefficient of GY vs year of cultivar release and (A) average minimum temperature (T_{min}) and (B) average maximum temperature (T_{max}). The red points correspond to Coria and the green ones to Late.

3.6. Relationship between GY, $\delta^{13}C$ and CTD

Across the set of 24 environments where the $\delta^{13}C$ of mature kernels was analyzed (Table 1) the GY was negatively correlated with the correlation coefficient between the year of cultivar release and the δ^{13}C of mature kernels across the same set of environments (R^2=0.25,P<0.05), (Fig. 5A) and marginally with the coefficient of correlation between GY and δ^{13}C (R^2=0.14, P=0.08), (Fig. 5B).

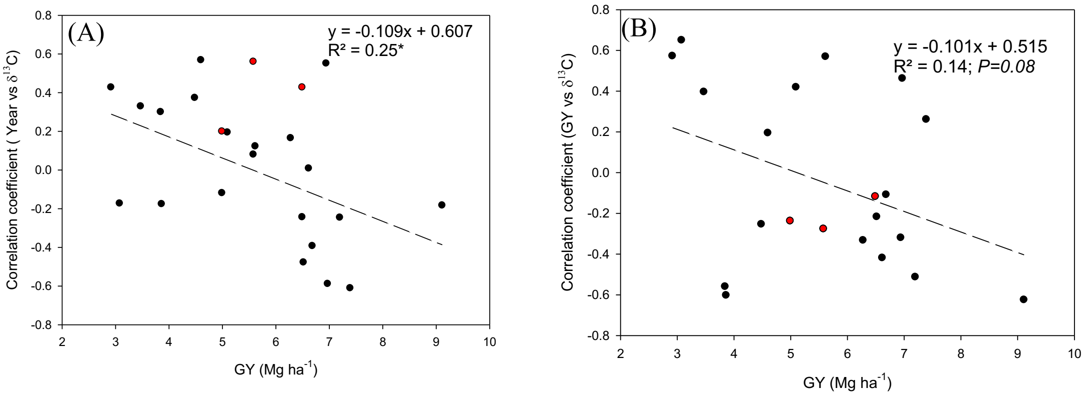

Figure 5: Relationship between the average GY within each environment and (A) correlation coefficient of the year of cultivar release vs δ^{13}C within each of the environments and (B) correlation coefficient of the GY vs δ^{13}C within each of the environments (B). Each point represents average GY value of twelve cultivars (X-axis) and the correlation coefficient across the twelve genotypes for each environment (combination between location, year and treatment). The red points correspond to Coria.

Across the set of 22 environments where CTD was measured (Table 1) the GY was positively correlated with the correlation coefficient between GY and CTD (R^2=0.20; P <0.05) (Figure 6).

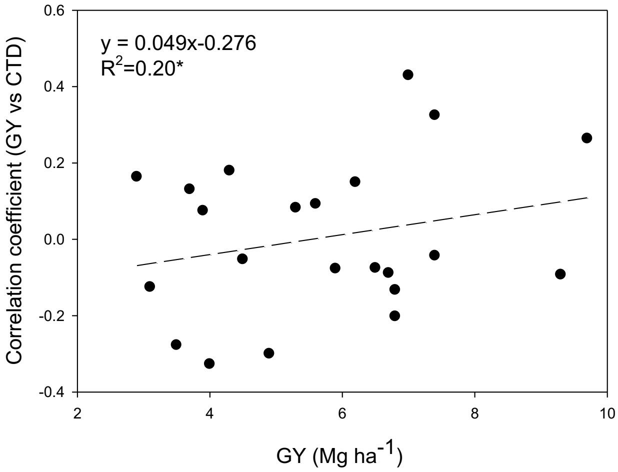

Figure 6: Relationship between the average GY within each environment and the correlation coefficient of GY vs CTD within each of the environments. Each point represents the average GY value of twelve cultivars (X-axis) and the correlation coefficient across the twelve genotypes for each environment (combination of location, year and treatment)

4. Discussion

The environmental parameters affecting the growth of durum wheat showed large variability across the 27 environments included in this study. This variability caused a difference of 6.2 Mg ha^{-1} between the most- and the least-yielding experiments, which represents a range of more than three times the yield obtained in the least-yielding environment. The environmental effect explained around 83% of the yield variability, which is in the range of that reported in previous studies in durum wheat (76.4% [11] and 98% [26]) and in bread wheat (57% [12]). Our results revealed the existence of different adaptation patterns among the 12 semi-dwarf genotypes included in this work, as 7.1% of the total yield variance was explained by the different sensitivities of the varieties to the environmental conditions, namely the G x E interaction. In our study, significant genetic gain for durum wheat in Spain was mostly observed in the warmer environments with optimum conditions in terms of water availability. This result and the AMMI analysis suggest that breeding efforts after the Green Revolution for yield increases in Spain focused on adaptation to specific environments. The earliest released cultivars were the lowest yielding varieties in the warmest (but well irrigated) growing conditions (Coria and Late) but not in the cooler environments (environments with low T_{max} and T_{mean} ; either rainfed or irrigated). Conversely, the most modern cultivars performed the best in the warmest sites there were no clear differences with regard to the oldest cultivars in the cooler environments.

Apparently, breeding in Spain has produced little progress in low yielding environments and the less warm environments (RF and IR) typical of the inland (i.e. cooler) areas of Spain [9]. However, the yield improvement is not a complete measure of progress achieved by breeding programs, because breeding for stress conditions, for example, has focused on stability rather than yield potential. Variability in water supply and temperatures, and the related G x E interactions are the main causes for the slow breeding progress in stressed environments [27]. As a result, many farmers in marginal environments have not benefited from major crop research successes [28]. Mediterranean environments are typically stressful due to drought and associated high temperatures, particularly during the later crop stages, [29]. In the same context, De Vita et al. [3] concluded that the breeding strategies adopted during recent decades have contributed to reducing G x E and selecting genotypes with better stability across a wide range of locations and years. As a consequence, modern genotypes outperform the old ones in all test environments with a strong adaptability to improved fertility.

The current study showed that the post-Green Revolution genotypes in Spain were better adapted to warmer environments (high T_{max} and high T_{mean}), which agrees with a study done previously in a set of 20 semi-dwarf durum wheat genotypes in Spain [9]. This highlights the influence that the selection environment has on adaptation. In fact, 80% of the total production of durum wheat in the country is concentrated in Andalusia, which is the warmest region of Spain and most of the durum wheat varieties grown in Spain after the Green Revolution have strong Italian and CIMMYT-derived genetic backgrounds. The main breeding site for CIMMYT, and the place where the Green Revolution was developed, is in Ciudad Obregon (NW Mexico) where high temperatures during the growing season are common and plants are grown under well-irrigated conditions. In the case of cultivars derived from Italian germplasm, the material has been selected in South Italy where climatic conditions are comparable to Andalusia.

The importance of the selection environment has led some breeders to follow the strategy of conducting selection in the target environments [30]. When the number of different target environments is large so as to select for suitable genes for each specific target environment, breeders eventually share early segregating populations (F2) with other breeders or even with farmers [31]. The establishment of separate programs is, no doubt, expensive, but it should yield greater genetic gains. The selection of widely adapted genotypes that generally perform well across a wide range of environmental conditions (i.e. representing what is known as a mega environment), is less expensive due to the economy-of-scale component, but this may also produce reduced genetic gain within a given area [32].

As observed in this study, Spain's target environments showed wide differences, which included contrasting conditions, as can be deduced from the broad yield range (a difference of more than 6 Mg ha^{-1} between the extreme environments). Where such contrasting environmental conditions exist, the response of genotypes (yield traits or other traits) is different. In fact, the change in the sign of the correlation coefficients across the set of the twelve cultivars, of the relationship between the average GY within each environment against the correlation coefficient of the relationship between release year vs $\delta^{13}C$, from negative in the high yielding environments (GY > 5 Mg ha^{-1}) to positive in the low yielding environments (GY < 5 Mg ha^{-1}), (Fig. 5A), confirms the existence of different responses of the genotypes. Likewise, the change in the sign of the correlation coefficient between GY vs $\delta^{13}C$ from the most- to the least- yielding environments (Fig. 5B) revealed two different response patterns. In the low yielding environments, the advantage is given to cultivars exhibiting a clearer trend towards a survival strategy, with a higher water use efficiency (WUE, understood as the ratio of net assimilation versus transpiration) as inferred by a higher $\delta^{13}C$ in mature kernels and a positive phenotypic relationship between GY vs $\delta^{13}C$ [33,34]. By contrast, under optimum conditions, cultivars exhibiting a drought avoidance response, with a higher water use (in spite of a lower WUE), as inferred by a lower $\delta^{13}C$ in mature kernels and a negative relationship between GY vs $\delta^{13}C$ [15,30,31] were the best performers. In fact, the effective use of water (EUW) and not the WUE is the target of crop yield improvement even under drought [34]. The first reaction of virtually most of the plants to severe drought is the closure of their stomata to prevent water loss via transpiration, which leads to a decrease in canopy temperature depression and an increase in the WUE [34]. However, for the high-yielding environments, the more productive cultivars are able to use more water than others and would have more open stomata and therefore higher canopy temperature depression and lower $\delta^{13}C$ in plant matter [16,33,34]. In fact, the genetic advances by CIMMYT for bread wheat, for example, seems related to lower $\delta^{13}C$ and higher CTD [35], which agrees with a higher EUW conferring better performance to cultivars selected under warm albeit well-watered conditions.

5. Conclusion

Breeding in Spain has made genetic progress in warmer environments with optimum conditions (optimum water input) -environments that resemble those from where the original germplasm (mostly advanced lines) was selected-. Nevertheless, current breeding is driving adaptation patterns more towards specific adaptation. Two different patterns of selection have been reported due the G×E interaction and changes in the ranking of genotypes: in the high-yielding environments (GY 5 Mg ha^{-1}), plants favor increased water uptake, with high levels of transpiration and more open stomata (negative value of $\delta^{13}C$ and higher CTD), whereas, in low yielding environments (GY 5 Mg ha^{-1}) plants close stomata and favor greater WUE (positive value of $\delta^{13}C$ and lower value of CTD).

Supplementary Materials: Supplement Figure S1: Relationship the year of cultivar release and grain yield across the set of twenty-three genotypes within each of the four groups of environments A: Coria, B: Late, C: IR and D: RF. Each point represents GY average value of any of the twenty-three genotypes for each group of environments and during two growing seasons (2016-2018). Supplemental Table S1: Analysis of variance for nitrogen grain yield (GNY) for twelve durum wheat genotypes grown in each group of environments

Author Contributions: N.A., M.T.N.T., M.D.S. and J.L.A. conceived and designed the experiment. N.A. managed and directed the wheat trials at the experimental station of Zamadueñas (Valladolid, Spain) and

M.T.N-T. the trials in Aranjuez and Coria. F.C. and M.D.S. run the stable isotope analyses. F.C. did the statistical analysis and wrote the draft paper under the supervision of J.L.A. and M.D.S.

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References

[1] C. Guzmán, J.E. Autrique, S. Mondal, R.P. Singh, V. Govindan, A. Morales-Dorantes, G. Posadas-Romano, J. Crossa, K. Ammar, R.J. Peña, Response to drought and heat stress on wheat quality, with special emphasis on bread-making quality, in durum wheat, F. Crop. Res. 186 (2016) 157–165. doi:10.1016/J.FCR.2015.12.002.

[2] J.L. Araus, T. Amaro, J. Voltas, H. Nakkoul, M.M.Nachit, Chlorophyll fluorescence as a selection criterion for grain yield in durum wheat under Mediterranean conditions, F. Crop. Res. 55 (1998) 209–223. doi:10.1016/S0378-4290(97)00079-8.

[3] P. De Vita, A.M. Mastrangelo, L. Matteu, E. Mazzucotelli, N. Virzì, M. Palumbo, M. Lo Storto, F. Rizza, L. Cattivelli, Genetic improvement effects on yield stability in durum wheat genotypes grown in Italy, F. Crop. Res. 119 (2010) 68–77. doi:10.1016/j.fcr.2010.06.016.

[4] L.F. García Del Moral, Y. Rharrabti, S. Elhani, V. Martos, C. Royo, Yield formation in Mediterranean durum wheats under two contrasting water regimes based on path-coefficient analysis, Euphytica. 146 (2005) 203–212. doi:10.1007/s10681-005-9006-2.

[5] D. Lobell, C. Field, D.B. Lobell, C.B. Field, Global scale climate–crop yield relationships and the impacts of recent warming, Environ. Res. Lett. 2 (2007) 7. doi:10.1088/1748-9326/2/1/014002.

[6] C. Royo, F. Álvaro, V. Martos, A. Ramdani, J. Isidro, D. Villegas, L.F. García del Moral, Genetic changes in durum wheat yield components and associated traits in Italian and Spanish varieties during the 20th century, Euphytica. 155 (2007) 259–270. doi:10.1007/s10681-006-9327-9.

[7] F. Álvaro, J. Isidro, D. Villegas, L.F. García del Moral, C. Royo, Old and modern durum wheat varieties from Italy and Spain differ in main spike components, F. Crop. Res. 106 (2008) 86–93. doi:10.1016/j.fcr.2007.11.003.

[8] K.A.B. Aisawi, M.P. Reynolds, R.P. Singh, M.J. Foulkes, The physiological basis of the genetic progress in yield potential of CIMMYT spring wheat cultivars from 1966 to 2009, Crop Sci. 55 (2015) 1749–1764. doi:10.2135/cropsci2014.09.0601.

[9] F. Chairi, O. Vergara-Diaz, T. Vatter, N. Aparicio, M.T. Nieto-Taladriz, S.C. Kefauver, J. Bort, M.D. Serret, J.L. Araus, Post-green revolution genetic advance in durum wheat: The case of Spain, F. Crop. Res. 228 (2018) 158–169. doi:10.1016/j.fcr.2018.09.003.

[10] A. Del Pozo, I. Matus, M.D. Serret, J.L. Araus, A. Pozo, I. Matus, M.D. Serret, J.L. Araus, A. Del Pozo, I. Matus, M.D. Serret, J.L. Araus, Agronomic and physiological traits associated with breeding advances of wheat under high-productive Mediterranean conditions. The case of Chile, Environ. Exp. Bot. 103 (2014) 180–189. doi:10.1016/j.envexpbot.2013.09.016.

[11] J. Subira, F. Álvaro, L.F. García del Moral, C. Royo, Breeding effects on the cultivar×environment interaction of durum wheat yield, Eur. J. Agron. 68 (2015) 78–88. doi:10.1016/j.eja.2015.04.009.

[12] M. Sanchez-Garcia, F. Álvaro, J.A. Martín-Sánchez, J.C. Sillero, J. Escribano, C. Royo, Breeding effects on the genotype × environment interaction for yield of bread wheat grown in Spain during the 20th century, F. Crop. Res. 126 (2012) 79–86.

doi:10.1016/J.FCR.2011.10.001.
[13] H. Becker, J.L.-P. breeding, undefined 1988, Stability analysis in plant breeding, Wiley Online Libr. (n.d.). https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1439-0523.1988.tb00261.x (accessed August 20, 2019).
[14] M.P. Reynolds, S. Rajaram, A. McNab, International Maize and Wheat Improvement Center., Increasing yield potential in wheat : breaking the barriers : proceedings of a workshop held in Ciudad Obregón, Sonora, Mexico, CIMMYT, 1996. https://books.google.es/books?hl=es&lr=&id=4naO7vUxl4wC&oi=fnd&pg=PA1&dq=Breaking+the+yield+barriers+in+wheat:+a+brief+summary+of+the+outcomes+of+an+international+consultation.+In:&ots=plXDBDi8F1&sig=zWJ5G0oaAms8ZQy0eCrMxCpjLC4#v=onepage&q=Breaking the yield barriers in wheat%3A a brief summary of the outcomes of an international consultation. In%3A&f=false (accessed August 20, 2019).
[15] S. Ceccarelli, S. Grando, Drought as a challenge for the plant breeder, Plant Growth Regul. 20 (1996). doi:10.1007/BF00024011.
[16] J.L. Araus, G.A. Slafer, M.P. Reynolds, C. Royo, Plant breeding and drought in C3 cereals: What should we breed for?, Ann. Bot. 89 (2002) 925–940. doi:10.1093/aob/mcf049.
[17] C. Royo, G.A. Briceño-Félix, Spanish wheat pool, in: J. Chem. Inf. Model., 2013: pp. 1689–1699. doi:10.1017/CBO9781107415324.004.
[18] J.C. Zadoks, T.T. Chang, C.F. Konzak, A decimal code for the growth stages of cereals, Weed Res. 14 (1974) 415–421. doi:10.1111/j.1365-3180.1974.tb01084.x.
[19] T.B. Coplen, Explanatory glossary of terms used in expression of relative isotope ratios and gas ratios. IUPAC Recommendations 2008. (International Union of Pure and Applied Chemistry Inorganic Chemistry Division, Commission on Isotopic Abundances and Atomic Weights: Research Triangle Park, NC, USA), CanadaAustriaChina/Beijing); Natl. Represent. J. R. Laeter (Australia. (2008). http://old.iupac.org/reports/provisional/abstract08/coplen_prs.pdf (accessed November 28, 2017).
[20] H.G.. J. Gauch, Statistical analysis of regional yield trials: AMMI analysis of factorial designs., Stat. Anal. Reg. Yield Trials AMMI Anal. Factorial Des. (1992). https://www.cabdirect.org/cabdirect/abstract/19931643324 (accessed August 21, 2019).
[21] A. Pacheco, M. Vargas, G. Alvarado, F. Rodríguez, GEA-R (genotype environment analysis with R for Windows) Version 4.0. CIMMYT, (2015).
[22] K.G.- Biometrika, undefined 1971, The biplot graphic display of matrices with application to principal component analysis, Academic.Oup.Com. (n.d.). https://academic.oup.com/biomet/article-abstract/58/3/453/233361 (accessed August 21, 2019).
[23] P. Monneveux, M.P. Reynolds, R. Trethowan, H. González-Santoyo, R.J. Peña, F. Zapata, Relationship between grain yield and carbon isotope discrimination in bread wheat under four water regimes, Eur. J. Agron. 22 (2005) 231–242. doi:10.1016/j.eja.2004.03.001.
[24] W.J. Choi, J.H. Kwak, S.S. Lim, H.J. Park, S.X. Chang, S.M. Lee, M.A. Arshad, S.I. Yun, H.Y. Kim, Synthetic fertilizer and livestock manure differently affect δ15N in the agricultural landscape: A review, Agric. Ecosyst. Environ. 237 (2017) 1–15. doi:10.1016/j.agee.2016.12.020.
[25] J. Crossa, P.N. Fox, W.H. Pfeiffer, S. Rajaram, H.G. Gauch, AMMI adjustment for statistical analysis of an international wheat yield trial, Theor. Appl. Genet. 81 (1991) 27–37. doi:10.1007/BF00226108.
[26] C. Royo, M. Maccaferri, M. Moragues, C. Royo, F. Álvaro, M.C. Sanguineti, R. Tuberosa, F. Maalouf, L.F. García Del Moral, A. Demontis, S. Rhouma, M. Nachit, N. Nserallah, D. Villegas, Understanding the relationships between genetic and phenotypic structures of a collection of elite durum wheat accessions FAP-Farming with alternative pollinator project View project Understanding the relationships between genetic and phenotypic

structures of a collection of elite durum wheat accessions, 119 (2010) 91–105. doi:10.1016/j.fcr.2010.06.020.

[27] S. Fukai, M. Cooper, Development of drought-resistant cultivars using physiomorphological traits in rice, F. Crop. Res. 40 (1995) 67–86. doi:10.1016/0378-4290(94)00096-U.

[28] M.R. Bellon, Crop research to benefit poor farmers in marginal areas of the developing world: A review of technical challenges and tools, CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. 1 (2006). doi:10.1079/PAVSNNR20061070.

[29] E. Acevedo, P.Q. Craufurd, P. Pérez-Marco, R.B. Austin, Traits associated with high yield in barley in low-rainfall environments, J. Agric. Sci. 116 (1991) 23–36. doi:10.1017/S0021859600076115.

[30] S. Ceccarelli, S. Grando, Decentralized-participatory plant breeding: An example of demand driven research, Euphytica. 155 (2007) 349–360. doi:10.1007/s10681-006-9336-8.

[31] S. Ceccarelli, W. Erskine, J. Hamblin, S. Grando, Genotype by environment interaction and international breeding programmes, Exp. Agric. 30 (1994) 177–187. doi:10.1017/S0014479700024121.

[32] S. Gbegbelegbe, D. Cammarano, S. Asseng, R. Robertson, U. Chung, M. Adam, O. Abdalla, T. Payne, M. Reynolds, K. Sonder, B. Shiferaw, G. Nelson, Baseline simulation for global wheat production with CIMMYT mega-environment specific cultivars, F. Crop. Res. 202 (2017) 122–135. doi:10.1016/j.fcr.2016.06.010.

[33] J. Araus, J. Bort, P. Steduto, D. Villegas, C. Royo, Breeding cereals for Mediterranean conditions: ecophysiological clues for biotechnology application, Ann. Appl. Biol. 142 (2003) 129–141. doi:10.1111/j.1744-7348.2003.tb00238.x.

[34] A. Blum, Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress, F. Crop. Res. 112 (2009) 119–123. doi:10.1016/j.fcr.2009.03.009.

[35] R.A. Fischer, D. Rees, K.D. Sayre, Z.M. Lu, A.G. Condon, A. Larque Saavedra, Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies, Crop Sci. 38 (1998) 1467–1475. doi:10.2135/cropsci1998.0011183X003800060011x.

Supplementary Materials:

Table S1: Analysis of variance for nitrogen grain yield (GNY) for twelve durum wheat genotypes grown in each group of environments

Source of variation GNY	df	SS	SS (%)	F	*p-Value*
Combined					
Total	745	1260043			
E	20	693173.9	55.0	42.59	*.000*
G	11	18515.74	1.5	2.069	*.021*
G × E	220	128555.7	10.2	0.718	*.998*
Error	494	401991.5			
Coria					
Total	107	77345.1			
E	2	19385.1	50.1	75.80	*.000*
G	11	966.5	13.7	3.779	*.000*
G × E	22	433,2	12.1	1.694	*.050*
Error	72	255.7			
RF					
Total	317	367785.4			
E	8	103737.8	28.2	13.39	*.000*
G	11	6441.3	1.7	0.605	*.823*
G × E	88	47655.9	12.9	0.560	*.999*
Error	210	203236.7			
IR					
Total	320	509926.4			
E	8	32103.9	50.4	37.753	*.000*
G	11	1124.8	2.4	1.323	*.213*
G × E	88	686.4	11.8	0.807	*.875*
Error	212	850.4			

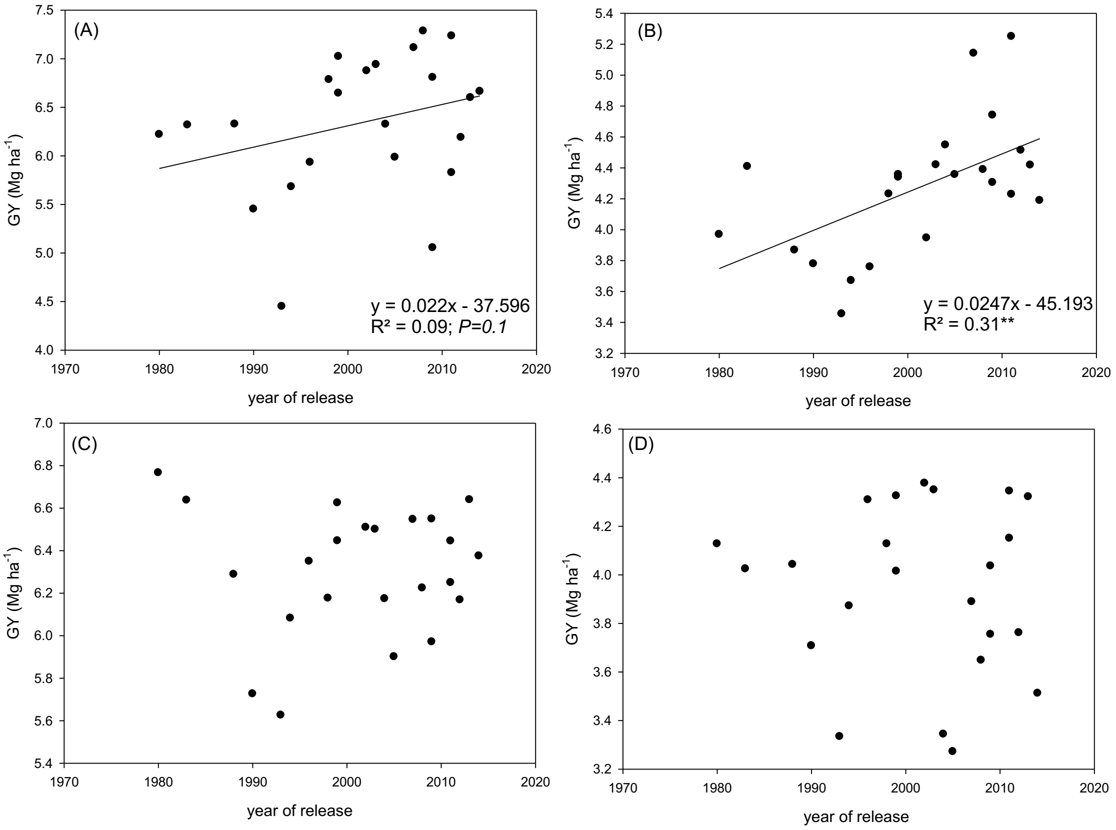

Figure S1: Relationship between grain yield and the year of cultivar release across the set of twenty three genotypes within eachof the four groups of environments A: Coria, B: Late, C: IR and D: RF. Each point represents GY average value of any of the twenty-three genotypes for each group of environments and during two growing seasons (2016-2018).

General Discussion

The ultimate objective of any breeding program is to develop outstanding genotypes in terms of yield, adaptation, resistance to biotic and abiotic stresses, and end use qualities. These improvements should be measurable through time and space. Determining the breeding progress or the rate of genetic gain helps to evaluate breeding programs regularly in order to improve their efficiency. Different approaches and tools can be used to measure the rate of breeding progress or genetic gain. The most common approach has involved retrospective studies consisting of a direct comparison of genotypes released in different eras as a result of previous breeding programs (Araus et al., 2002; Reynolds and Tuberosa, 2008). The advantage of this approach is that any trait that has been consistently (but frequently unconsciously) modified by breeders as they selected for yield (or any other target parameter) per se could be identified and eventually used as a selection criterion in further breeding.

Efficiency of durum wheat breeding in Spain

During the last 60 years, most of the progress in major cereals has been derived from empirical (conventional or traditional) breeding, which has taken yield as the main trait for selection. As a result of the Green Revolution, yield increases have been possible through the gradual (but rather fast) replacement of traditional tall cultivars by semi-dwarf and fertilizer-responsive varieties with superior harvest indices and less prone to lodging. In Spain, the introduction of semi-dwarf CIMMYT-derived durum wheat varieties took place in the 1970's (Royo and Briceño-Félix, 2013). During certain periods of the past decades, the CIMMYT varieties introduced (such as Mexa, Vitron and Gallareta -tested in this thesis: **Chapters 1 and 3** occupied the majority of the area devoted to durum wheat cultivation in Spain, for example, Mexa was cultivated on almost 90% of the durum wheat area during the mid-1980s (Royo,2005). Competitive Italian cultivars (such as Claudio and Simeto, also tested in this thesis: **Chapters 1 and 3** were introduced later but the area on which they are cultivated have been increasing in recent years (Royo and Briceño-Félix, 2013). Indeed, almost all the genotypes used in this study, and representing the cultivars most grown in Spain during the four decades after the first arrival of Green Revolution were basically from CIMMYT or from Italian origin (Chapter 1). The massive introduction of foreign varieties in Spain and their utilization in the local breeding has incited the reduction of durum wheat diversity in Spain. Indeed, Martos et al. (2005) demonstrated that Spanish durum germplasm grown before 1945 constitutes a completely different gene pool to that of the varieties released more recently.

The genetic gain between pre- and post-Green Revolution durum wheat genotypes (released between 1930 and 1975) in Spain was reported, 0.63% y^{-1} in terms of yield per plant (Royo et al., 2007). However, the genetic gain after the Green Revolution reported in this thesis evaluated between 1980 and 2009 was 0.44% y^{-1} (24 kg ha^{-1}) in terms of grain yield per hectare between 1980 and 2003 with no clear additional improvements thereafter **(Chapter 1)**. The lack of increase in wheat during the last decades seems to be a constant in several countries (Beche et al., 2014; Brisson et al., 2010; Graybosch and Peterson, 2010). However, the increase in yield in the first period studied (1980-2003), was mainly due to an increase in the number of grains per m^{-2} (117 kernels m^{-2} y^{-1}) mostly caused by an increase in the number of kernels per spike (0.24 kernels $spike^{-1}$ y^{-1}), while no changes in the number of spikes per unit area and the harvest index were found **(Chapter 1 and 2).** This was paralleled with an increase in biomass during the whole period studied (42 kg ha^{-1} y^{-1}), in the same sense, an increase in biomass was reported in bread wheat studied between 1972 and1995 (104 kg ha^{-1} y^{-1}) in UK (Shearman et al., 2005) and between 1966 and 2009 in northwest Mexico (6.7 g m^{-2} y^{-1}).The increase in the number of kernels per spike is almost universally recognized as the main cause of yield improvement since the Green Revolution (Royo et al., 2007; Sayre et al., 1997).

In general, the lack of genetic advance, particularly during the last decades, in the cultivars studied could be explained by the development of the varietal structure in Spain. As commented above, most, if not all modern cultivars grown across the country during the last 30 years have been developed using foreign germplasm (from CIMMYT and Italy) (Royo and Briceño-Félix, 2013), while a breeding program developing own germplasm and exploding the genotypic variability existing on Spanish durum wheats (and therefore well adapted material to the specific conditions of the country) has been not evidenced . However other causes that might explain the low genetic gain in Spain, such as the susceptibility of the new cultivars to major diseases, together with the emergence of new more virulent strains, appear to be less evident. The same applies for the predisposition to lodging (particularly under high-yielding irrigated conditions). In fact, during the past decades the breeding effort for durum wheat in Spain has been focused on improving lodging tolerance and restricting the number of non-productive tillers characteristic of the old varieties, while enhancing flour quality (Royo and Briceño-Félix, 2013). Moreover, even when genetic advance has been minor or even absent there is genotypic variability for grain yield across the varieties cultivated in Spain through the past decades. These two aspects are evidenced in the results of **Chapter 2**, where the least productive genotype (Don Sebastian) was more modern and exhibited taller stature and lower HI than the most productive genotypes, which can be considered as a step backward in genetic improvement for grain yield in Spain. Even so, the least productive genotypes exhibited higher TKW and nitrogen concentration in kernels than the most productive genotypes **(Chapter 2).** This confirms that for some varieties grain quality has been a key breeding objective, even if this has been at the cost of lower yield.

Moreover, the future breeding strategies to increase yield potential and also stability should be taken in a number of directions, including defining proper ideotypes and potential relevant secondary traits rather than only depending on just the innovative use of both germplasm and crossing strategies, followed by empirical selection for grain yield at multiple locations (Araus et al. 2008; Reynolds et al. 2011).

Identifying Physiological Traits

The water deficit is the main environmental constraint limiting cereal production worldwide, and particularly in the Mediterranean basin; a problem likely to worsen in the future (Slafer et al., 2005). The morphological, physiological and metabolic traits associated with improved cereal plant performance under limited water conditions may have a major role in future breeding advance (Passioura 1996, Slafer & Araus 1998, Richards 2000). Specifically, traits related to yield should have a positive impact on water use (WU), (agronomical) water use efficiency (WUE) or biomass distribution to reproductive organs (Passioura, 1977, 1996, Richards, 1996, 2000, Araus et al., 2002b, 2008; Blum 2009).

Phenology is an important attribute of the crop that can contribute to a better agronomical performance under stressful conditions. Indeed, phenological adaptation (e.g. shortening crop cycle) change allows the crop to escape stress under Mediterranean conditions (Araus et al. 2002). In fact, phenology has been extensively exploited for genotypic adaptation and Mediterranean areas but at least further shortening the duration of the crop cycle does not appear feasible. In agreement with that, in this Thesis, phenology did not constitute a key element in the difference between the response of genotypes under the wide range of Spanish environments. The genotypes of this Thesis have a significant phenotypic variability (**Chapter 1 and 3)** but this was not reflected in yield, as no relationship between grain yield and days to heading nor relationship with the year of genotype release was been seen. Moreover, to minimize any potential interference of phenology on yield, in **Chapter 2** six genotypes have been chosen, from the set of the 20 durum wheat genotypes, that represent the variability in terms of yield of the complete set, and with a relatively narrow range of variability in the number of days from planting to heading.

In wheat, directional selection (Chapman et al., 2012) has been used to breed varieties that respond consistently to the target environment and management practices. Whilst this approach has been successful in achieving yield gains in some tested environments, strong G×E interactions mean that it is difficult to identify genotypes responding consistently and positively in a range of environments, even for a single physiological trait (Lopes et al., 2012a; Reynolds et al., 2012). In the **Chapter 3**, two different strategies of semi-dwarf durum wheat response have been observed. In the low yielding environments, the advantage is given to the cultivars exhibiting a clearer trend to a survival strategy, with a higher water use efficiency (WUE) as inferred by a higher $\delta^{13}C$ in mature kernels and a positive phenotypic relationship between GY vs $\delta^{13}C$ (Araus et al., 2003; Blum, 2009). By contrast under optimum conditions, cultivars exhibiting a drought avoidance response, with a higher water use (in spite of a lower WUE), as inferred by a lower $\delta^{13}C$ in mature kernels and a negative phenotypic relationship between GY vs $\delta^{13}C$ (Araus et al. 2003, Blum, 2009) are the best performers. That confirm the trend found towards a crossover in the graphic model of Finlay and Wilkinson (Finlay and Wilkinson, 1963) that placed this crossover in the most stressful growth conditions, when analyzing the $\delta^{13}C$ in **Chapter 2**.

This, suggests that the best performing genotypes are those able to uptake more water by maintaining greater stomatal opening and therefore greater rates of transpiration (Fig. 6). In agreement with this hypothesis the most productive genotypes in addition to having a low value of $\delta^{13}C$, exhibited a higher value of CTD (cooler canopy). Cool canopy temperatures have been associated with increased plant access to water as a result of deeper roots (Li et al., 2019; Lopes and Reynolds, 2010), more open stomata (Araus et al., 2002) and higher transpiration and probably reflects a higher effective use of water by the crop (Blum 2009).

Additionally, a positive correlation was found between length of the flag leaf blade and $\delta^{13}C_{Kernels}$, while the leaf length was negatively correlated with GY. These results suggest that the architecture of the plant affects to some extend $\delta^{13}C_{Kernels}$ and therefore the GY. Thus, a large, prostrated, flag leaf, as in the case of low yielding genotypes, may suffer water stress and then close its stomata earlier than genotypes with smaller and more erect flag leaves. In addition, no difference between harvest biomass was found between the low and high yielding genotypes, and with the difference with leaf size, that implies that high-yielding genotypes with small leaves have more leaves per unit of surface. Genotypes with erect small leaves may keep a better aeration, and therefore exhibit cooler canopies which a lower evapotranspirative demand and as consequence better water status and a lower value of $\delta^{13}C_{Kernels}$.

In addition, maintaining green leaf area late during grain filling (stay-green) was proposed as a trait to increase grain yield in humid Mediterranean environments (Araus et al., 2003). In this thesis, in contrary, the low yielding genotypes with high $\delta^{13}C_{Kernels}$ maintain better stay-green attitude, assessed through different vegetation indices, than the higher yielding cultivars. In fact, stay-green interacts with environmental conditions; it seems relevant as a trait for conditions where rainfalls are expected at some point during the reproductive phase of the crop than conditions where terminal drought occurs (Ludlow & Muchow, 1990; Mahalakshmi & Bidinger, 2002), which is not usually the case in Spain. Therefore, staygreen is useful for environments where there is a high probability of rainfall during grain filling (Richards et al., 2002) . On the other hand, stay-green may be also an indicator of low yield, with low yielding genotypes having better balance between N source and N sink. Thus, a high source of N for remobilization (large flag leaf) together with a low harvest index (and therefore low sink for N accumulation) in low yielding genotypes may contribute to delay senescence (Sanchez-Bragado et al., 2017; **Chapter 2**).

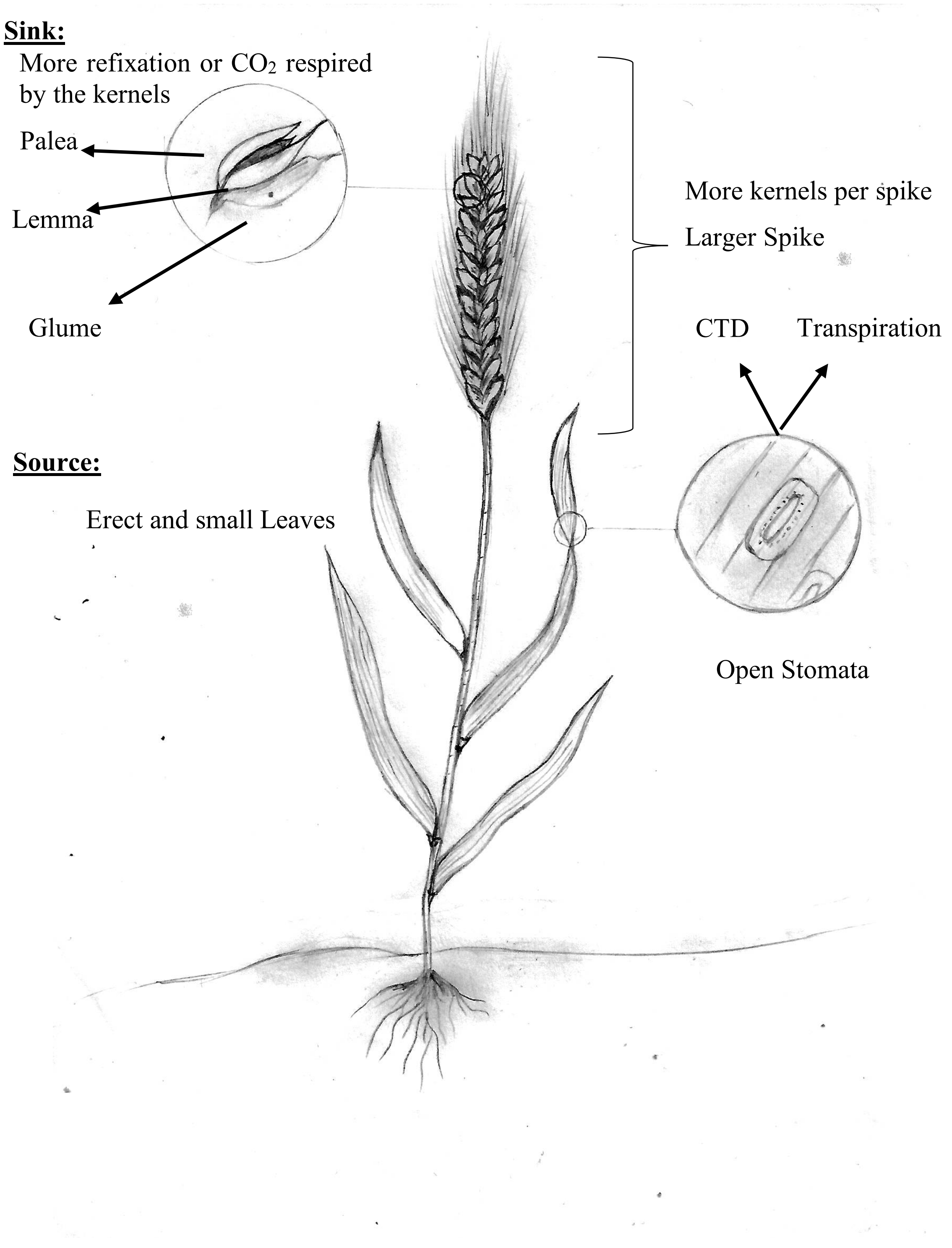

Figure 10: A wheat ideotype, designed to give a high grain yield in Spanish environments

Correlation between $\delta^{13}C$ of different plant parts and GY suggests that the flag leaf play a minor role in the grain filling and the photosynthetic contribution of the non-laminar parts appears to be more important. Indeed, and for most of the environmental conditions tested in **Chapter 2** no correlation was found between $\delta^{13}C$ of the leaf and GY, whereas the non-laminar photosynthetic organs were in general more correlated with GY. It has been observed that the organ that most differs in terms of water-soluble fraction $\delta^{13}C$ from the most extreme grain yield genotypes of Chapter 2 is the palea. The palea is the photosynthetic ear tissue closet to the grain and the furthest from the atmospheric air, so the $\delta^{13}C$ of the palea seems to originate from the refixation of CO_2 respired by the grain rather than from assimilating atmospheric CO_2. These results suggest the contribution of the non-laminar photosynthetic organs of the ear to grain yield is not just through the assimilation of carbon from the surrounding air but also refixing CO_2 respired by the grains (Bort et al., 1996).

Adaptation

From the present Thesis, a global image of a low genetic advance achieved for durum wheat released in Spain after the Green Revolution may be acquired. However, the scenario is far more complex. In fact, a large variability in the relative genetic advance for grain yield was observed depending on the climatic characteristics of the environment in which the varieties were tested; these differences exceed 350% (**Chapter 1**). In the present scenario of climate change, the improvement of the adaptation of varieties seems more important than ever. The present work showed, that a relatively small change in the maximum average temperature can largely determine the success of the varieties. For example, in **Chapter 1** it was shown that a difference in the maximum average daily temperature of 3ºC, such as that between Zamadueñas environments in 2016 and Aranjuez in 2016, contributed to the difference in absolute yield gain between the two environments being more than 139%.

The highest absolute gains were obtained in environments with higher maximum average temperatures, indicating that, at least in relation to this climatic variable, the most modern varieties seem to be better adapted to an increase in temperature than the old ones. In fact the genetic advance was higher for warm and well-watered conditions (either cultivation in south Spain or through a late planting) resembling those prevalent in the areas where germplasm was first screened (e.g. in the case of CIMMYT, the Yaki valley in NE Mexico or in the case of Italy, Foggia, southern Italy and Sicily (Scarascia Mugnozza, 2005) Apparently, breeding in Spain has produced little progress in low yielding (rainfed) environments and non-warm irrigated environments from continental (i.e. cool) areas of central Spain (**Chapters 1 and 3**). Therefore, the adaptation of semi-dwarf durum in Spain has shown a tendency to specific adaptation rather than large-scale adaptation.

Implications for Future Durum Wheat Breeding in Spain

Satisfying future Spanish demand for durum wheat will imply increasing potential yield and adaptation to stresses. Therefore, future breeding strategies to increase yield potential and stability should be taken in a number of directions, including defining proper ideotypes and potential relevant secondary traits rather than only depending on just the innovative use of both germplasm and crossing strategies, followed by empirical selection for grain yield at multiple locations (Araus et al. 2008; Reynolds et al. 2011). In spite of the fact that durum wheat performance in Spain was characterized by a specific adaptation and based on the physiological and agronomical traits investigated in this thesis a conceptual model adapted to the Mediterranean environment in Spain has been built (Fig. 6). According to the results of this thesis, increasing yield potential has been mainly achieved through:

A higher sink size, through a higher number of kernels, which resulted mainly from more grains per ear (Chairi et al., 2019, 2018), and attributed to the improvement of the number of grains per spikelet (Álvaro et al., 2008) with an increase in the number of fertile florets per spike (Álvaro et al., 2008).

A small erect flag leaf, firstly, which allows greater light penetration and more homogeneous distribution into the canopy (Monneveux et al., 2004; Reynolds et al., 2000) which translate into a higher photosynthetic active radiation of the canopy. Moreover , this plant architecture may reflect a diminution of the interplant competition therefore, favoring the communalism (Reynolds et al., 1994). Finally, erect-small leaves allow a better canopy ventilation which helps to reduce temperature.

A higher nutrient uptake and efficient use of water as a result of deeper roots (Blum 2009; Li et al., 2019; Lopes and Reynolds, 2010).

A higher contribution of the ear parts of the plants in providing assimilates during grain filling through the assimilation of carbon from the surrounding air (Sanchez-Bragado et al., 2014b), and also refixing CO_2 respired by the grains (Bort et al., 1996; Chairi et al., 2019).

Conclusions

Conclusions

1. Average genetic gain in grain yield at the country level was estimated on 24 kg ha^{-1} y^{-1} (0.44% y^{-1}) between 1980 and 2003 with no clear additional improvements thereafter.

2. Yield improvement was due to increases in the number of kernels per m^{-2} (117 kernels m^{-2} y^{-1}) and the number of kernels per spike (0.24 kernels spike^{-1} y^{-1}), while grain weight remained unchanged.

3. Aboveground biomass at maturity was increased with year of release with no change of the HI.

4. However, estimation of genetic gains of semi-dwarf durum wheat grown in Spain from 1980 to 2009 depended on the testing environment, particularly maximum daily temperatures.

5. Breeding in Spain has made genetic progress in warm environments with optimum conditions (optimum water input); therefore, environments that resemble those from where the original germplasm (mostly advanced lines) was selected and current breeding is driving adaptation patterns more towards specific adaptation

6. Two different patterns of selection have been reported due the G×E interaction and change in the ranking of genotypes: in the high yielding environments plant favors more water uptake whereas, in low yielding environments plant favors a higher WUE.

7. While the kernel is the most effective plant part for $\delta^{13}C$ assessment as a phenotypic trait to test durum wheat performance, the non-laminar parts of the plants play a key role in providing assimilates during grain filling. Moreover, there is no specific plant part that accounts for the majority of the assimilates moving to the grains, but rather the contributors are several.

8. Under Spanish Mediterranean conditions the best genotypes were those with a stature around 85 cm with a relatively small erect flag leaf, and higher water use combined with better water status in terms of a cooler canopy, more open stomata, higher transpiration of the flag leaf and more negative $\delta^{13}C$ in kernels together with a proper balance between N source and N sink during grain filling and a higher capacity to re-fix CO_2 respired by the kernel.

Resumen General

El trigo es, junto con el maíz y el arroz, uno de los cultivos más importantes del mundo y el primer alimento básico estratégico para la mayoría de las poblaciones del mundo. La producción anual del trigo se estima en alrededor de 771 millones de toneladas, lo que la convierte en la segunda cosecha más grande del mundo detrás del maíz (FAOSTAT, 2019). Se cultiva en unos 219 millones de hectáreas en todo el mundo (FAOSTAT, 2019). La demanda mundial de trigo está aumentando y las predicciones sugieren que la producción de granos debe aumentar anualmente en un 2.4% para satisfacer las necesidades humanas en 2050 (Ray et al., 2013). Sin embargo, los aumentos actuales en la productividad mundial del trigo son solo del 0.9% por año, lo que hace imperativo encontrar formas de aumentar el aumento en la producción de trigo (Ray et al., 2013).

La Introducción de la Tesis examina las evidencias en todo el mundo de las recientes ganancias genéticas en el rendimiento del trigo contadas después de la Revolución Verde y, en los casos en que se ha registrado el avance genético, qué entornos objetivo, así como los rasgos agronómicos y morfofisiológicos han estado involucrados. Además, la Tesis desarrolla el caso específico del trigo duro en España, a través de tres capítulos que representan los resultados de la investigación (utilizando el formato de papel científico) logrados utilizando un panel posterior a la Revolución Verde cultivado en España a través de varios años, sitios y condiciones de cultivo. Finalmente, una discusión general se enfoca en las perspectivas futuras para identificar rasgos para aumentar el rendimiento con referencia particular al trigo duro cultivado en España.

1. Introducción

Durante la Revolución Verde de los años sesenta y setenta, los aumentos de rendimiento de los primeros cultivares semienanos se asociaron con una reducción en la estatura y un aumento concomitante en el índice de cosecha, junto con una reducción en el alojamiento y, por lo tanto, una mayor capacidad de respuesta a la alta fertilidad del suelo. e irrigación (Foulkes et al., 2011). Si bien el salto en el rendimiento de grano asociado con la Revolución Verde ha representado más que duplicar los rendimientos de grano durante los años sesenta e incluso setenta del siglo pasado, las nuevas tendencias en ganancia genética, una vez que Los principales beneficios de los nuevos genotipos y las prácticas de gestión de cultivos asociadas se han realizado, siguen siendo menos evidentes.

A nivel mundial, el aumento del rendimiento del trigo de 1961 a 1990 fue del 2,6% anual, pero de 1990 a 2007 el aumento promedio del rendimiento fue de solo el 0,52% anual (Alston et al., 2010). Desde 1961 hasta el presente, las ganancias genéticas reportadas en los ambientes de alto rendimiento (particularmente áreas irrigadas o áreas de alta precipitación junto con prácticas de manejo adecuadas) han sido del orden de 1.2% a 0.5%. Por lo tanto, para las condiciones del CIMMYT en el noreste de México, los valores oscilan entre 0.59% y 1% (Aisawi et al. 2015, Lopes et al. 2012, Manès et al. 2012), mientras que en el caso de China estos valores oscilan entre 0.48% y 1.05% (Zhou et al. 2007; Zheng et al. 2011; Xiao et al. 2012), y los valores del Reino Unido (Shearman et al., 2005; Pennacchi et al. 2018) variaron entre 1.1% y 1.2%. En Chile, la ganancia genética alcanzó 63.4 kg $ha^{-1}y^{-1}$ (0.67% y^{-1}) (Pozo et al., 2019). Sin embargo, también hay algunos casos en los que el progreso genético parece estar mostrando una meseta, por ejemplo, el trigo de primavera de Brasil entre 1999 y 2009 (Beche et al., 2014). En estos estudios, la ganancia genética ha aumentado en 0.45%. Además, Brisson et al. (2010) encontraron que el progreso genético en el potencial de rendimiento en Francia había sido contrarrestado desde 1990 en adelante por el cambio climático (particularmente por el estrés por calor) y en España Chairi et al. (2018) encontraron un aumento en el rendimiento de grano con el año de liberación de cultivares a una tasa de 0.44% y^{-1} de 1980 a 2003, sin mejoras adicionales claras a partir de entonces.

Del párrafo anterior, se evidencia que el avance genético actual del trigo, incluso en los mejores casos, está claramente por debajo de la tasa anual de 2.4% requerida para satisfacer las necesidades humanas en 2050 (Ray et al., 2013). Además, en los entornos menos productivos, donde el estrés abiótico limita el rendimiento real del grano, las evidencias de avance genético durante las décadas posteriores a la Revolución Verde parecen aún menos evidente (Acreche et al., 2008; Alston et al., 2010). Por ejemplo, El trigo de invierno en las grandes llanuras de América del Norte entre 1984 y 2008 osciló entre el 0,75% en los viveros regionales de rendimiento del sur y el 0,85% en los viveros regionales de rendimiento del norte (Graybosch y Peterson, 2010), en Argentina, el progreso genético en términos de rendimiento fue del 0,18% después de 1999 (Lo Valvo et al., 2017) y en Australia del Sur, bajo condiciones de lluvia, la tasa anual de aumento en el rendimiento de grano fue de 30 kg ha^{-1} entre 1973 y 2007 (Sadras y Lawson, 2011)

Como consecuencia, de la Revolución Verde, se ha informado que las ganancias de rendimiento están asociadas con aumentos en HI, mientras que ha habido pocos cambios en la biomasa aérea total. Alternativamente, también se puede demostrar que la ganancia genética asociada con la Revolución Verde fue básicamente la consecuencia de un aumento en los granos m^{-2} (Calderini et al., 1995; Reynolds et al., 1999) debido a un aumento en el número granos por espiga (Royo et al., 2007; Sayre et al., 1997). Otros estudios han encontrado que los cultivares de trigo semi enano mostraron floretes más fértiles por espiga como consecuencia del aumento de la partición asimilada en la espiga durante el período previo a la floración (Calderini et al., 1995; Miralles et al., 1998). En cuanto a la densidad de espiga y los informes individuales de peso del grano indican cambios menores (Shearman et al., 2005; Sayre et al., 1997), incluso cuando para el peso del grano hay resultados contradictorios sobre los cambios ocurridos después de Revolución verde. Por lo tanto, donde hay estudios que concluyen que se ha producido un aumento con el peso del grano (Aisawi et al., 2015; Calderini et al., 1995a, Lopes et al., 2012), otros informes concluyen que se ha producido una tendencia hacia la reducción del peso del grano para cultivares liberados antes de 1980 (Calderini et al., 1995a; M. Sanchez-Garcia et al., 2012). El aumento en el número de granos por espiga y, por lo tanto, en HI causado por la Revolución Verde se debió a la introducción de genes del nanismo (Rht) que disminuyeron la altura de la planta sin alterar su biomasa final. El HI máximo alcanzable parece situarse alrededor de 0.6 (Austin et al., 1980). Sin embargo, estudios recientes han mostrado poca o ninguna mejora en el índice de cosecha alcanzado durante las últimas décadas del siglo XX, que parece haberse estancado en valores cercanos a 0.50-0.55 en los entornos más óptimos (Aisawi et al. , 2015; Sadras y Lawson, 2011; Sayre et al., 1997). A pesar del límite fisiológico teórico, el desarrollo de variedades que exceden un índice de cosecha de 0,50 sigue siendo difícil en muchos entornos (Fischer y Edmeades, 2010). Estas limitaciones aparentes contribuyen a considerar el aumento de la biomasa del cultivo en la madurez, sin alterar el índice de cosecha, como una de las principales estrategias que se promoverán en futuros programas de mejora (Parry et al., 2011). Los estudios posteriores a la Revolución Verde informan resultados contradictorios sobre el cambio en la biomasa. Por ejemplo, se informó un aumento en México entre 1966 y 2009 (Aisawi et al., 2015), en Australia entre 1980 y 2008 (Sadras y Lawson., 2011) y en el Reino Unido entre 1983 y 1995 (Shearman et al., 2005), aunque no se informaron cambios en España entre 1940 y 1998 (Acreche et al., 2008; Royo et al., 2007) e Italia entre 1930-1992 (Royo et al., 2007), y en el caso de Argentina se informó una disminución entre 1999 y 2011 (Lo Valvo et al., 2017).

Un aumento en el rendimiento depende de una miríada de rasgos morfofisiológicos que contribuyen a una absorción más eficiente y un mayor uso de los recursos que necesita la planta (luz, agua y nutrientes). Así, por ejemplo, la adquisición de agua y nutrientes y la eficiencia de un dosel para capturar la luz, y la eficiencia de la fotosíntesis, la fuerza del sumidero y la translocación, son algunos de los factores que contribuyen en gran medida al rendimiento del cultivo (Parry y Hawkesford, 2012). Si consideramos la luz como el recurso principal, entonces la biomasa y el rendimiento pueden formularse en términos de fotosíntesis del cultivo y dependerán de (i) la capacidad del dosel para interceptar y capturar la luz; (ii) la duración de la captura de luz; y (iii) la capacidad fotosintética y la eficiencia del dosel. Por ejemplo, cuando el agua es el principal recurso que limita el rendimiento, la productividad del cultivo dependerá de (i) la capacidad de extraer agua del suelo; (ii) la duración de la extracción de agua y (iii) la eficiencia con la que se utiliza el agua.

Pocos estudios han informado un avance genético de la tasa fotosintética en genotipos semi-enanos. Shearman et al. (2005) encontraron que el progreso genético en GY en trigo de invierno del Reino Unido de 1972 a 1995 se basó principalmente en la mejora de la biomasa de la cosecha que se asoció con una mayor eficiencia de uso de radiación (RUE) durante el período de alargamiento del tallo, lo que sugiere las interacciones sumidero versus origen también están involucradas. Por lo tanto, una fase de alargamiento del tallo más larga significa que el crecimiento del cultivo acumulado durante el alargamiento del tallo sería mayor, ya que el dosel (Slafer et al., 2005) interceptarían más radiación. En la provincia de Henan, en China, un aumento en el rendimiento de grano en los genotipos liberados entre 1981 y 2008 se correlacionó positivamente con la tasa fotosintética neta de la hoja de bandera durante el llenado del grano, con los cultivares más recientes que exhiben una fotosíntesis más alta junto con una mayor conductancia estomática y tasas de transpiración (Zheng et al. , 2011). Un estudio para los trigos liberados en la provincia de Shandong (China) sugirió que se lograron ganancias genéticas en el rendimiento de los granos mejorando la fotosíntesis de los cultivos en y después del rumbo, y la fuente para el llenado de granos puede haberse beneficiado del aumento de WSC del tallo en los tallos en antesis (Xiao et al., 2012).

Los estudios en condiciones de riego en México sugieren que las mejoras en la fotosíntesis por unidad de área foliar ya pueden haber ocurrido con un aumento en la conductancia estomática (por ejemplo, Fischer et al., 1998; Reynolds et al., 1999), aunque aparentemente esto no se ha traducido a una mayor biomasa total en campo. De hecho, los informes sobre trigo y otros cultivos sugieren un aumento en la conductancia estomática como uno de los factores detrás del avance genético en los cultivares modernos (Roche 2015). Los resultados de la firma de isótopos de carbono estables también respaldan la contribución de una mayor conductancia estomática, aumentando la fotosíntesis y, finalmente, el rendimiento. La interdependencia entre el agua y el CO2 no se limita a los estomas y su subcavidad, ya que parece que estas dos moléculas comparten vías de difusión también a través del mesófilo (Ferrio et al., 2012). Por lo tanto, no es sorprendente que las tasas de fotosíntesis y conductancia estomática se hayan correlacionado estrechamente (Wong et al., 1979). La depresión de la temperatura del dosel (Lopes y Reynolds 2010), junto con la discriminación contra el isótopo de carbono estable más pesado (^{13}C), son dos rasgos fisiológicos que ayudan a estimar qué genotipos extraen más agua que otros Araus et al., 2003; Slafer et al., 2005). De hecho, los genotipos que tienen una temperatura de dosel más baja al mediodía tienen un estado de agua relativamente mejor (Blum et al., 1982). Las temperaturas frescas del dosel se han asociado con un mayor acceso de las plantas al agua, como resultado de raíces más profundas (Li et al., 2019; Lopes y Reynolds, 2010) y, por lo tanto, con una mejor conductancia estomática. Se ha propuesto que una conductancia estomática más alta se asocia con un avance genético en el potencial de rendimiento (Fischer et al., 1998), así como en condiciones de estrés moderado a medio (Roche, 2015). Esto se ve respaldado por las correlaciones fenotípicas positivas que generalmente se encuentran entre el rendimiento y la discriminación de isótopos de carbono (Δ^{13}C) o las correlaciones negativas con la composición de isótopos de carbono (δ^{13}C) dentro de los ambientes mediterráneos (Araus et al., 1998, 2003, 2013, 1998; Merah et al., 2001; Monneveux et al., 2006), lo que significa que los genotipos que pueden mantener un mayor uso del agua (incluso a costa de un WUE más bajo) son los más productivos (Araus et al., 2013, 2008) De hecho, el uso efectivo del agua (EUW) y no del WUE es el objetivo de la mejora del rendimiento de los cultivos incluso bajo estrés por sequía (Blum, 2009). Varios estudios previos mostraron correlaciones fenotípicas entre CTD y ganancias genéticas en el rendimiento de grano entre las liberaciones de trigo del CIMMYT (Aisawi et al., 2015; Fischer et al., 1998; Lopes et al., 2012b).

Los sistemas de raíces juegan un papel importante en el rendimiento del cultivo. Las raíces son importantes para el anclaje y la absorción de agua y nutrientes de la solución del suelo (Foulkes et al., 2011). Por ejemplo, se ha demostrado que el enraizamiento más profundo es un rasgo importante para capturar el agua almacenada en profundidad. Esto es especialmente importante durante las etapas de crecimiento posteriores cuando se usa agua para el llenado de granos, ya que impacta directamente en el rendimiento de los granos (Lopes y Reynolds, 2012). Se ha informado que bajo una sequía moderada, el acceso a agua adicional del subsuelo durante el llenado de granos puede contribuir a un aumento en el rendimiento del trigo de hasta 0.62 Mg ha^{-1} (Kirkegaard et al., 2007). En áreas secas, Aziz et al. (2017) informaron que las reducciones en la biomasa de la raíz con los cultivares de trigo liberados a lo largo del tiempo dieron como resultado mayores rendimientos de grano y que la relación raíz: brote, que depende de la división del carbono del fotosintato, y la proporción de biomasa total asignada a las raíces fueron mayores en variedades lanzadas temprano (entre 1958 y 1989) que las lanzadas entre 1994 y 2007.

Comprender los mecanismos involucrados en la adaptación de variedades al medio ambiente, así como identificar las principales variables climáticas que determinan la sensibilidad de las variedades al medio ambiente, es crucial para el desarrollo de nuevas variedades, más adaptadas y con un rendimiento más alto y más estable.

La mayoría de los estudios publicados hasta la fecha en todo el mundo sobre la mejora genética del trigo han centrado su atención en el rendimiento y, finalmente, en sus componentes agronómicos y / o morfofisiológicos. Sin embargo, hay pocos trabajos que aborden el estudio de los cambios producidos por la mejora en los patrones de adaptación de germoplasma. Los obtentores han seguido varias estrategias para favorecer la adaptación de las variedades al medio ambiente. En el CIMMYT, la selección se lleva a cabo en entornos de alto potencial con el objetivo de obtener variedades con amplia adaptación, lo que lleva a mejoras significativas en el rendimiento en una amplia gama de condiciones agroecológicas (Braun et al., 1997). Estudios recientes han demostrado que en entornos limitantes, la mejora en el rendimiento se debe a la selección de variedades con baja interacción con el medio ambiente y alto rendimiento potencial (Cattivelli et al., 2008), mientras que en entornos de alto potencial, las variedades más exitosas de los cereales han mostrado una tendencia hacia una adaptación más específica (Royo et al., 2008; Sánchez-Garcia, 2012).

La adaptación al medio ambiente puede estar detrás de los informes contradictorios sobre la existencia o la falta de avance genético después de la Revolución Verde. En general, el mejoramiento para una adaptación específica tiende a implicar mayores ganancias genéticas para estas condiciones ambientales particulares, pero a cambio de mayores costos en términos de mejoramiento en relación con los de una estrategia de adaptación amplia. Los costos relativamente altos pueden deberse al aumento de las pruebas de campo en lugar de la duplicación de las estaciones de reproducción, ya que las operaciones de cruce e hibridación pueden centralizarse en una sola estación nacional que proporciona a cada subregión germoplasma novedoso para la selección local (y, posiblemente, recursos genéticos para locales prueba para identificar material padre de interés específico) (Annicchiarico, 2002).

El hecho de que el trigo duro sea comparativamente el hermano pequeño del trigo integral, junto con la percepción de que la variabilidad genética disponible para el trigo duro es menor que para el trigo integral hexaploide, hace que la ausencia de estudios sobre el trigo duro sea una cuestión de hecho, particularmente aquellos se centraron en las décadas posteriores al salto en el rendimiento de granos debido a la Revolución Verde. Esto es importante en el caso de España que, después de Italia, es uno de los principales productores de trigo duro a nivel mundial.

1. Objetivos

El objetivo general de esta Tesis es evaluar si ha habido cambios agronómicos y fisiológicos asociados con la mejora genética del trigo duro cultivado en España después de la Revolución Verde y las condiciones ambientales donde las ganancias de mejoramiento han sido mayores. El estudio se basó en una colección de 20 variedades comerciales, cultivadas en España entre principios de los años 70 del siglo pasado y el presente. El conjunto fue readaptado durante los últimos dos ciclos de cultivo y aumentó a un total de 23 cultivares, incluidos los más recientes, lanzados durante la presente década. Los cultivares se compararon durante años consecutivos en condiciones de cultivo de amplio rango aseguradas por el cultivo en tres sitios diferentes que difieren en latitud y temperatura, junto con la implementación de riego de apoyo y diferentes tiempos de siembra. Junto con el rendimiento de grano, se estudió un conjunto de características agronómicas y fisiológicas relevantes relacionadas con la productividad y adaptación del cultivo. El objetivo final de la Tesis es generar información que pueda ayudar a acelerar la eficiencia de los programas de mejora genética del trigo. Este objetivo general se especifica en los siguientes objetivos específicos:

1- Cuantifique la ganancia genética del rendimiento del trigo duro en España después de la Revolución Verde.

2- Diseccionar los parámetros agronómicos y fisiológicos relacionados con el progreso genético del trigo duro en España y proponer un ideotipo de alto rendimiento y mejor adaptado al entorno mediterráneo español.

3- Estudiar los cambios causados por la mejora en los patrones de adaptación del trigo a las principales áreas de cultivo del país, a través del estudio de la interacción genotipo por ambiente (GxE).

2. Capitulo 1

El primer articulo aborda la cuestión de si ha habido alguna ganancia genética en el rendimiento del trigo duro liberado en España después de la Revolución Verde y evalúa los rasgos agronómicos y fisiológicos asociados con la evolución del cultivo durante este tiempo. Los experimentos de campo se llevaron a cabo con una amplia gama de cultivares de trigo duro (lanzados en España desde 1980 hasta 2009) y se llevaron a cabo en diferentes sitios que abarcan una amplia gama de temperaturas de crecimiento y regímenes de agua en Aranjuez y Zamadueñas durante tres temporadas consecutivas (2013 / 14, 2014/15, 2015/16) bajo riego de secano y suplementario y en Coria por dos temporadas consecutivas (2014/15 y 2015/16) solo bajo condiciones de secano. El rendimiento del grano aumentó con el año de liberación de los cultivares a una tasa de 24 kg ha^{-1} año $^{-1}$ (0.44% año $^{-1}$) desde 1980 hasta 2003, sin mejoras adicionales claras a partir de entonces. La mejora moderada del rendimiento de grano de 1980 y 2003 se asoció con los granos m^{-2} y los granos por espiga, con un aumento de 117 granos m^{-2} año $^{-1}$ y 0.24 granos por espiga año $^{-1}$, respectivamente. Además, la biomasa aérea en la cosecha y el rendimiento de nitrógeno del grano aumentaron con el año de liberación de los cultivares durante todo el período. Sin embargo, no se encontraron diferencias para el peso de mil granos, número de espigas m^{-2}, días de partida, altura de la planta, índice de cosecha, depresión de la temperatura del dosel, discriminación de isótopos de carbono o concentración de nitrógeno en el grano. En general, estos resultados indicaron que la tasa de progreso genético en el rendimiento del trigo duro en España después de la Revolución Verde ha sido baja e incluso se ha detenido durante la última década, mientras que no hay una tendencia clara en algunos rasgos de calidad del grano (TKW y concentración de N de grano) fue registrado. Sin embargo, las ganancias genéticas absolutas y relativas estimadas para el rendimiento se asociaron positivamente con las temperaturas medias y máximas diarias desde la siembra hasta la cosecha del sitio de prueba, lo que sugiere que la reproducción se ha realizado en entornos de alta temperatura.

3. Capitulo 2

El conocimiento de los rasgos agronómicos y fisiológicos asociados con las ganancias genéticas en el rendimiento es esencial para mejorar la comprensión de los factores limitantes del rendimiento y para informar las futuras estrategias de mejoramiento. El objetivo de este trabajo es diseccionar los rasgos agronómicos y fisiológicos relacionados con la ganancia genética y proponer un ideotipo con alto rendimiento que se adapte mejor a los ambientes mediterráneos españoles. Seis genotipos de trigo duro semienano (es decir, moderno) se cultivaron en una amplia gama de condiciones de cultivo en España durante dos años sucesivos. Se evaluaron diversos rasgos agronómicos, fisiológicos y morfológicos foliares. Granos por espiga fue el componente de rendimiento más afectado por la ganancia genética. Si bien no existía interacción entre el genotipo y las condiciones de crecimiento para el rendimiento de grano, los genotipos más productivos se caracterizaron por una altura de planta de alrededor de 85 cm, pequeñas hojas de bandera erectas, estomas más abiertos, un mejor equilibrio entre N fuentes y N sumideros y una mayor capacidad para volver a fijar el CO_2 respirado por el grano. Además, en general, las partes no laminares de las plantas juegan un papel clave en el suministro de asimilados durante el llenado del grano. La alta heredabilidad de la mayoría de los parámetros estudiados permite su consideración como rasgos para fenotipar el trigo duro mejor adaptado a una amplia gama de condiciones mediterráneas

4. Capitulo 3

Este estudio evalúa los cambios causados por la reproducción en la interacción genotipo x ambiente (G × E) de las variedades de trigo duro más cultivadas en España después de la Revolución Verde. Se probó un conjunto de 12 cultivares en 27 ambientes, entendidos como la combinación de diferentes sitios, años y tratamientos (régimen de agua y fechas de siembra) representativos de las condiciones de cultivo de trigo duro en España con rendimientos promedio de grano que varían entre 2.8 y 9.1 Mg ha^{-1}. Los factores ambientales más importantes que afectaron la interacción G × E para el rendimiento fueron la temperatura máxima y media durante todo el ciclo. Se ha informado una mejora en el rendimiento genético en ambientes cálidos y en condiciones óptimas de agua, ambientes similares a aquellos de donde se origina o se origina la procedencia del germoplasma. La adaptación del durum semi-enano en España ha mostrado una tendencia a la adaptación específica en lugar de la adaptación a gran escala. Se han informado dos patrones diferentes de selección debido a la interacción G × E y el cambio en la clasificación de los genotipos: en los entornos de alto rendimiento (GY> 5 Mg h-1), la planta favorece una mayor absorción de agua, con más transpiración y más estomas abiertos (mayor valor negativo de δ^{13}C y mayor depresión de la temperatura del dosel (CTD)), mientras que, en entornos de bajo rendimiento (GY <5 Mg ha-1), la planta cierra los estomas y favorece una mayor eficiencia en el uso del agua (valor positivo de δ^{13}C y menor valor de CTD) .

5. Conclusiones

1. La ganancia genética del rendimiento en grano a nivel de país se estimó en 24 kg ha^{-1} año^{-1} (0.44% año^{-1}) entre 1980 y 2003, sin mejoras adicionales claras a partir de entonces.

2. La mejora del rendimiento se debió a aumentos en el número de granos por m^{-2} (117 granos m^{-2} año^{-1}) y el número de granos por espiga (0.24 granos por espiga año^{-1}), mientras que el peso del grano permaneció sin cambios. .

3. La biomasa aérea en la madurez se incrementó con el año de liberación sin cambio del HI.

4. Sin embargo, la estimación de las ganancias genéticas del trigo duro semi enano cultivado en España desde 1980 hasta 2009 dependió del entorno de prueba, particularmente de las temperaturas máximas diarias.

5. La cría en España ha progresado genéticamente en ambientes cálidos con condiciones óptimas (aporte óptimo de agua); por lo tanto, los entornos que se parecen a aquellos de donde se seleccionó el germoplasma original (en su mayoría líneas avanzadas) y la reproducción actual está impulsando los patrones de adaptación más hacia una adaptación específica

6. Se han informado dos patrones diferentes de selección debido a la interacción G × E y el cambio en la clasificación de los genotipos: en los entornos de alto rendimiento, la planta favorece una mayor absorción de agua, mientras que en entornos de bajo rendimiento, la planta favorece una mayor WUE.

7. Mientras que el grano es la parte más efectiva de la planta para la evaluación de $\delta^{13}C$ como un rasgo fenotípico para evaluar el rendimiento del trigo duro, las partes no laminares de las plantas juegan un papel clave en el suministro de asimilados durante el llenado del grano. Además, no hay una parte específica de la planta que represente la mayoría de los asimilados que se trasladan a los granos, sino que los contribuyentes son varios.

8. En condiciones mediterráneas españolas, los mejores genotipos fueron aquellos con una estatura de alrededor de 85 cm con una hoja de bandera erecta relativamente pequeña, y un mayor uso del agua combinado con un mejor estado del agua en términos de un dosel más frío, estomas más abiertos, mayor transpiración de la hoja bandera y más $\delta^{13}C$ negativo en los granos junto con un equilibrio adecuado entre la fuente de N y el sumidero de N durante el llenado del grano y una mayor capacidad para re-fijar el CO_2 respirado por el grano.

References

References

Acevedo, E., Craufurd, P.Q., Austin, R.B., Pérez-Marco, P., 1991. Traits associated with high yield in barley in low-rainfall environments. J. Agric. Sci. 116, 23–36. doi:10.1017/S0021859600076115

Acreche, M., Brice, G., Mart, J.A., Slafer, G.A., 2008. Physiological bases of genetic gains in Mediterranean bread wheat yield in Spain. Eur. J. Agron. 28, 162–170. doi:10.1016/j.eja.2007.07.001

Aisawi, K.A.B., Reynolds, M.P., Singh, R.P., Foulkes, M.J., 2015. The physiological basis of the genetic progress in yield potential of CIMMYT spring wheat cultivars from 1966 to 2009. Crop Sci. 55, 1749–1764. doi:10.2135/cropsci2014.09.0601

Alston, J.M., Beddow, J.M., Pardey, P.G., 2010. Global Patterns of Crop Yields and Other Partial Productivity Measures and Prices. Shifting Patterns Agric. Prod. Product. Worldw. 39–61.

Álvaro, F., Isidro, J., Villegas, D., García del Moral, L.F., Royo, C., 2008. Old and modern durum wheat varieties from Italy and Spain differ in main spike components. F. Crop. Res. 106, 86–93. doi:10.1016/j.fcr.2007.11.003

Annicchiarico, P., 2002. Genotype x environment interactions: challenges and opportunities for plant breeding and cultivar recommendations.

Araus, J., Bort, J., Steduto, P., Villegas, D., Royo, C., 2003. Breeding cereals for Mediterranean conditions: ecophysiological clues for biotechnology application. Ann. Appl. Biol. 142, 129–141. doi:10.1111/j.1744-7348.2003.tb00238.x

Araus, J.L., Cabrera-Bosquet, L., Serret, M.D., Bort, J., Nieto-Taladriz, M.T., 2013. Comparative performance of δ13C, δ18O and δ15N for phenotyping durum wheat adaptation to a dryland environment. Funct. Plant Biol. 40, 595–608. doi:10.1071/FP12254

Araus, J.L., Slafer, G.A., Reynolds, M.P., Royo, C., 2002. Plant breeding and drought in C3 cereals: What should we breed for? Ann. Bot. 89, 925–940. doi:10.1093/aob/mcf049

Araus, J.L., Slafer, G.A., Royo, C., Serret, M.D., 2008. Breeding for Yield Potential and Stress Adaptation in Cereals. CRC. Crit. Rev. Plant Sci. 27, 377–412. doi:10.1080/07352680802467736

Araus, J.L., Villegas, D., Aparicio, N., del Moral, L.F.G., El Hani, S., Rharrabti, Y., Ferrio, J.P.P., Royo, C., Garcıa del Moral, L.F., Hani, S. El, Rharrabti, Y., Ferrio, J.P.P., Royo, C., 2003. Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. Crop Sci 43, 170–180. doi:10.2135/cropsci2003.1700

Araus, J.L.., Amaro, T., Voltas, J., Nakkoul, H., Nachit, M.M., 1998. Chlorophyll fluorescence as a selection criterion for grain yield in durum wheat under Mediterranean conditions. F. Crop. Res. 55, 209–223. doi:10.1016/S0378-4290(97)00079-8

Austin, R.B., Bingham, J., Blackwell, R.D., Evans, L.T., Ford, M.A., Morgan, C.L., Taylor, M., 1980. Genetic improvements in winter wheat yields since 1900 and associated physiological changes. J. Agric. Sci. 94, 675. doi:10.1017/S0021859600028665

Aziz, M.M., Palta, J.A., Siddique, K.H.M., Sadras, V.O., 2017. Five decades of selection for yield reduced root length density and increased nitrogen uptake per unit root length in Australian wheat varieties. Plant Soil 413, 181–192. doi:10.1007/s11104-016-3059-y

Beche, E., Benin, G., da Silva, C.L., Munaro, L.B.B., Marchese, J.A., Lemes da Silva, C., Munaro, L.B.B., Marchese, J.A., 2014. Genetic gain in yield and changes associated with physiological traits in Brazilian wheat during the 20th century. Eur. J. Agron. 61, 49–59. doi:10.1016/j.eja.2014.08.005

Becker, H., breeding, J.L.-P., 1988, undefined, n.d. Stability analysis in plant breeding. Wiley Online Libr.

Biometrika, K.G.-, 1971, undefined, n.d. The biplot graphic display of matrices with application to principal component analysis. academic.oup.com.

Blum, A., 2009. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. F. Crop. Res. 112, 119–123. doi:10.1016/j.fcr.2009.03.009

Blum, A., Ebercon, A., 1981. Cell Membrane Stability as a Measure of Drought and Heat Tolerance in Wheat. Crop Sci. 21, 43. doi:10.2135/cropsci1981.0011183X002100010013x

Blum, A., Mayer, J., Gozlan, G., 1982. Infrared thermal sensing of plant canopies as a screening technique for dehydration avoidance in wheat. F. Crop. Res. 5, 137–146.

doi:10.1016/0378-4290(82)90014-4

Bort, J., Brown, R.H., Araus, J.L., 1996. Refixation of respiratory CO_2 in the ears of C_3 cereals. J. Exp. Bot. 47, 1567–1575. doi:10.1093/jxb/47.10.1567

Braun, H.J., Atlin, G., Payne, T., 2010. Multi-location testing as a tool to identify plant response to global climate change., in: Climate Change and Crop Production. CABI, Wallingford, pp. 115–138. doi:10.1079/9781845936334.0115

Brisson, N., Gate, P., Gouache, D., Charmet, G., Oury, F.X., Huard, F., 2010. Why are wheat yields stagnating in Europe? A comprehensive data analysis for France. F. Crop. Res. 119, 201–212. doi:10.1016/j.fcr.2010.07.012

Busey, P., 1983. Management of Crop Breeding, in: Crop Breeding. American Society of Agronomy, Crop Science Society of America, pp. 31–54. doi:10.2135/1983.cropbreeding.c3

Calderini, D.F., Dreccer, M.F., Slafer, G.A., 1995. Genetic improvement in wheat yield and associated traits. A re-examination of previous results and the latest trends. Plant Breed. 114, 108–112. doi:10.1111/j.1439-0523.1995.tb00772.x

Carmo-Silva, E., Andralojc, P.J., Scales, J.C., Driever, S.M., Mead, A., Lawson, T., Raines, C.A., Parry, M.A.J., 2017. Phenotyping of field-grown wheat in the UK highlights contribution of light response of photosynthesis and flag leaf longevity to grain yield. J. Exp. Bot. 68, 3473–3486. doi:10.1093/jxb/erx169

Ceccarelli, S., Grando, S., 1996. Drought as a challenge for the plant breeder. Plant Growth Regul. 20. doi:10.1007/BF00024011

Chairi, F., Sanchez-Bragado, R., Serret, M.D., Aparicio, N., Nieto-Taladriz, M.T., Luis Araus, J., 2019. Agronomic and physiological traits related to the genetic advance of semi-dwarf durum wheat: the case of Spain. Plant Sci. 110210. doi:10.1016/j.plantsci.2019.110210

Chairi, F., Vergara-Diaz, O., Vatter, T., Aparicio, N., Nieto-Taladriz, M.T., Kefauver, S.C., Bort, J., Serret, M.D., Araus, J.L., 2018. Post-green revolution genetic advance in durum wheat: The case of Spain. F. Crop. Res. 228, 158–169. doi:10.1016/j.fcr.2018.09.003

Chapman, S.C., Chakraborty, S., Dreccer, M.F., Howden, S.M., 2012. Plant adaptation to climate changeopportunities and priorities in breeding. Crop Pasture Sci. 63, 251–268. doi:10.1071/CP11303

Coplen, T.B., 2008. Explanatory glossary of terms used in expression of relative isotope ratios and gas ratios. IUPAC Recommendations 2008. (International Union of Pure and Applied Chemistry Inorganic Chemistry Division, Commission on Isotopic Abundances and Atomic Weights: Research Triangle Park, NC, USA). CanadaAustriaChina/Beijing); Natl. Represent. J. R. Laeter (Australia.

Crossa, J., Fox, P.N., Pfeiffer, W.H., Rajaram, S., Gauch, H.G., 1991. AMMI adjustment for statistical analysis of an international wheat yield trial. Theor. Appl. Genet. 81, 27–37. doi:10.1007/BF00226108

De Vita, P., Mastrangelo, A.M., Matteu, L., Mazzucotelli, E., Virzì, N., Palumbo, M., Storto, M. Lo, Rizza, F., Cattivelli, L., 2010. Genetic improvement effects on yield stability in durum wheat genotypes grown in Italy. F. Crop. Res. 119, 68–77. doi:10.1016/j.fcr.2010.06.016

Del Pozo, A., Matus, I., Serret, M.D., Araus, J.L., Pozo, A., Matus, I., Serret, M.D., Araus, J.L., Del Pozo, A., Matus, I., Serret, M.D., Araus, J.L., 2014. Agronomic and physiological traits associated with breeding advances of wheat under high-productive Mediterranean conditions. The case of Chile. Environ. Exp. Bot. 103, 180–189. doi:10.1016/j.envexpbot.2013.09.016

Donald, C.M., Hamblin, J., 1976. The Biological Yield and Harvest Index of Cereals as Agronomic and Plant Breeding Criteria. Adv. Agron. 28, 361–405. doi:10.1016/S0065-2113(08)60559-3

Ferrio, J.P., Pou, A., Florez-Sarasa, I., Gessler, A., Kodama, N., Flexas, J., Ribas-Carbó, M., 2012. The Péclet effect on leaf water enrichment correlates with leaf hydraulic conductance and mesophyll conductance for CO 2. Plant, Cell Environ. 35, 611–625. doi:10.1111/j.1365-3040.2011.02440.x

Finlay, K.W., Wilkinson, G.N., 1963. The analysis of adaptation in a plant-breeding programme. Aust. J. Agric. Res. 14, 742–754. doi:10.1071/AR9630742

Fischer, R.A., Edmeades, G.O., 2010. Breeding and cereal yield progress, Crop Science. Crop Science. doi:10.2135/cropsci2009.10.0564

Fischer, R.A., Rees, D., Sayre, K.D., Lu, Z.M., Condon, A.G., Larque Saavedra, A., 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Sci. 38, 1467–1475. doi:10.2135/cropsci1998.0011183X003800060011x

Foulkes, M.J., Slafer, G.A., Davies, W.J., Berry, P.M., Sylvester-Bradley, R., Martre, P., Calderini, D.F., Griffiths, S., Reynolds, M.P., 2011. Raising yield potential of wheat.

III. Optimizing partitioning to grain while maintaining lodging resistance. J. Exp. Bot. 62, 469–486. doi:10.1093/jxb/erq300

Fukai, S., Cooper, M., 1995. Development of drought-resistant cultivars using physiomorphological traits in rice. F. Crop. Res. 40, 67–86. doi:10.1016/0378-4290(94)00096-U

García Del Moral, L.F., Rharrabti, Y., Elhani, S., Martos, V., Royo, C., 2005. Yield formation in Mediterranean durum wheats under two contrasting water regimes based on path-coefficient analysis. Euphytica 146, 203–212. doi:10.1007/s10681-005-9006-2

Gauch, H., Zobel, R., 1996. AMMI analysis in yield trials. KANG, MS, GAUCH, HG (Ed) Genotype by environment interaction.

Gauch, H.G.. J., 1992. Statistical analysis of regional yield trials: AMMI analysis of factorial designs. Stat. Anal. Reg. yield trials AMMI Anal. factorial Des.

Graybosch, R.A., Peterson, C.J., 2010. Genetic Improvement in Winter Wheat Yields in the Great Plains of North America, 1959–2008. Crop Sci. 50, 1882–1890. doi:10.2135/cropsci2009.11.0685

Guzmán, C., Autrique, J.E., Mondal, S., Singh, R.P., Govindan, V., Morales-Dorantes, A., Posadas-Romano, G., Crossa, J., Ammar, K., Peña, R.J., 2016. Response to drought and heat stress on wheat quality, with special emphasis on bread-making quality, in durum wheat. F. Crop. Res. 186, 157–165. doi:10.1016/J.FCR.2015.12.002

Hawkesford, M.J., Araus, J.L., Park, R., Calderini, D., Miralles, D., Shen, T., Zhang, J., Parry, M.A.J., 2013. Prospects of doubling global wheat yields. Food Energy Secur. 2, 34–48. doi:10.1002/fes3.15

Kirkegaard, J.A., Lilley, J.M., Howe, G.N., Graham, J.M., 2007. Impact of subsoil water use on wheat yield. Aust. J. Agric. Res. 58, 303–315. doi:10.1071/AR06285

Li, X., Ingvordsen, C.H., Weiss, M., Rebetzke, G.J., Condon, A.G., James, R.A., Richards, R.A., 2019. Deeper roots associated with cooler canopies, higher normalized difference vegetation index, and greater yield in three wheat populations grown on stored soil water. J. Exp. Bot. doi:10.1093/jxb/erz232

Lo Valvo, P.J., Miralles, D.J., Serrago, R.A., 2017. Genetic progress in Argentine bread wheat varieties released between 1918 and 2011: Changes in physiological and numerical yield components. F. Crop. Res. 221, 0–1. doi:10.1016/j.fcr.2017.08.014

Lopes, M.S., Reynolds, M.P., 2010. Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. Funct. Plant Biol. 37, 147–156. doi:10.1071/FP09121

Lopes, M.S., Reynolds, M.P., Jalal-Kamali, M.R., Moussa, M., Feltaous, Y., Tahir, I.S.A., Barma, N., Vargas, M., Mannes, Y., Baum, M., 2012a. The yield correlations of selectable physiological traits in a population of advanced spring wheat lines grown in warm and drought environments. F. Crop. Res. 128, 129–136. doi:10.1016/j.fcr.2011.12.017

Lopes, M.S., Reynolds, M.P., Manes, Y., Singh, R.P., Crossa, J., Braun, H.J., 2012b. Genetic yield gains and changes in associated traits of CIMMYT spring bread wheat in a “Historic” set representing 30 years of breeding. Crop Sci. 52, 1123–1131. doi:10.2135/cropsci2011.09.0467

Manès, Y., Gomez, H.F., Puhl, L., Reynolds, M., Braun, H.J., Trethowan, R., 2012. Genetic yield gains of the CIMMYT International semi-arid wheat yield trials from 1994 to 2010. Crop Sci. 52, 1543–1552. doi:10.2135/cropsci2011.10.0574

Martos, V., Royo, C., Rharrabti, Y., Garcia Del Moral, L.F., 2005. Using AFLPs to determine phylogenetic relationships and genetic erosion in durum wheat cultivars released in Italy and Spain throughout the 20th century. F. Crop. Res. 91, 107–116. doi:10.1016/j.fcr.2004.06.003

Maydup, M.L., Antonietta, M., Guiamet, J.J., Tambussi, E.A., 2012. The contribution of green parts of the ear to grain filling in old and modern cultivars of bread wheat (Triticum aestivum L .): Evidence for genetic gains over the past century. F. Crop. Res. 134, 208–215. doi:10.1016/j.fcr.2012.06.008

Mckeand, S., Li, B., Hatcher, A., Weir, R., 1990. Stability parameter estimates for stem volume for loblolly pine families growing in different regions in the southeastern United States. For. Sci. 36, 10–17.

Merah, O., Monneveux, P., Deléens, E., 2001. Relationships between flag leaf carbon isotope discrimination and several morpho-physiological traits in durum wheat genotypes under Mediterranean conditions. Environ. Exp. Bot. 45, 63–71. doi:10.1016/S0098-8472(00)00081-2

Miralles, D.J., Katz, S.D., Colloca, A., Slafer, G.A., 1998. Floret development in near isogenic wheat lines differing in plant height. F. Crop. Res. 59, 21–30. doi:10.1016/S0378-4290(98)00103-8

Monneveux, P., Rekika, D., Acevedo, E., Merah, O., 2006. Effect of drought on leaf gas exchange, carbon isotope discrimination, transpiration efficiency and productivity in field grown durum wheat genotypes. Plant Sci. 170, 867–872. doi:10.1016/j.plantsci.2005.12.008

Monneveux, P., Reynolds, M.P., González-Santoyo, H., Peña, R.J., Mayr, L., Zapata, F., 2004. Relationships between grain yield, flag leaf morphology, carbon isotope discrimination and ash content in irrigated wheat. J. Agron. Crop Sci. 190, 395–401. doi:10.1111/j.1439-037X.2004.00116.x

Pacheco, A., Vargas, M., Alvarado, G., Rodríguez, F., 2015. GEA-R (genotype environment analysis with R for Windows) Version 4.0. CIMMYT.

Parry, M.A.J., Hawkesford, M.J., 2012. An Integrated Approach to Crop Genetic Improvement. J. Integr. Plant Biol. doi:10.1111/j.1744-7909.2012.01109.x

Parry, M.A.J., Reynolds, M., Salvucci, M.E., Raines, C., Andralojc, P.J., Zhu, X.-G., Price, G.D., Condon, A.G., Furbank, R.T., 2011. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. J. Exp. Bot. 62, 453–467. doi:10.1093/jxb/erq304

Pennacchi, J.P., Carmo-Silva, E., Andralojc, P.J., Feuerhelm, D., Powers, S.J., Parry, M.A.J., 2018. Dissecting wheat grain yield drivers in a mapping population in the UK. Agronomy 8, 1–15. doi:10.3390/agronomy8060094

Pozo, A., Ruf, K., Castillo, D., Mar, A., 2019. Genetic Advance of Durum Wheat Under High Yielding Conditions : The Case of Chile 2011–2015.

Ray, D.K., Mueller, N.D., West, P.C., Foley, J.A., 2013. Yield Trends Are Insufficient to Double Global Crop Production by 2050. PLoS One 8, 1–2. doi:10.1371/journal.pone.0066428

Reynolds, M., Foulkes, J., Furbank, R., Griffiths, S., King, J., Murchie, E., Parry, M., Slafer, G., 2012. Achieving yield gains in wheat. Plant, Cell Environ. 35, 1799–1823. doi:10.1111/j.1365-3040.2012.02588.x

Reynolds, M., Tuberosa, R., 2008. Translational research impacting on crop productivity in drought-prone environments Article in Current Opinion in Plant Biology · Genetic analysis of physiological traits to increase grain partitioning in high biomass cultivars in wheat View project FP7 DROPS View project. doi:10.1016/j.pbi.2008.02.005

Reynolds, M.P., Acevedo, E., Sayre, K.D., Fischer, R.A., 1994. Yield potential in modern wheat varieties: its association with a less competitive ideotype. F. Crop. Res. 37, 149–160. doi:10.1016/0378-4290(94)90094-9

Reynolds, M.P., Rajaram, S., McNab, A., International Maize and Wheat Improvement Center., 1996. Increasing yield potential in wheat : breaking the barriers : proceedings of a workshop held in Ciudad Obregón, Sonora, Mexico. CIMMYT.

Reynolds, M.P., Rajaram, S., Sayre, K.D., 1999. Physiological and genetic changes of irrigated wheat in the post-green revolution period and approaches for meeting projected global demand, in: Crop Science. pp. 1611–1621. doi:10.2135/cropsci1999.3961611x

Reynolds, M.P., van Ginkel, M., Ribaut, J., 2000. Avenues for genetic modification of radiation use efficiency in wheat. J. Exp. Bot. 51, 459–473. doi:10.1093/jexbot/51.suppl_1.459

Richards, R.A., Rebetzke, G.J., Condon, A.G., Van Herwaarden, A.F., 2002. Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. Crop Sci. 42, 111–121. doi:10.2135/cropsci2002.0111

Roche, D., 2015. Stomatal Conductance Is Essential for Higher Yield Potential of C_3 Crops. CRC. Crit. Rev. Plant Sci. 34, 429–453. doi:10.1080/07352689.2015.1023677

Royo, C., Álvaro, F., Martos, V., Ramdani, A., Isidro, J., Villegas, D., García del Moral, L.F., 2007. Genetic changes in durum wheat yield components and associated traits in Italian and Spanish varieties during the 20th century. Euphytica 155, 259–270. doi:10.1007/s10681-006-9327-9

Royo, C., Briceño-Félix, G.A., 2013. Spanish wheat pool, in: Journal of Chemical Information and Modeling. pp. 1689–1699. doi:10.1017/CBO9781107415324.004

Royo, C., Maccaferri, M., Moragues, M., Royo, C., Álvaro, F., Sanguineti, M.C., Tuberosa, R., Maalouf, F., García Del Moral, L.F., Demontis, A., Rhouma, S., Nachit, M., Nserallah, N., Villegas, D., 2010. Understanding the relationships between genetic and phenotypic structures of a collection of elite durum wheat accessions FAP-Farming with alternative pollinator project View project Understanding the relationships between genetic and phenotypic structures of a collection of elite durum wheat accessions 119, 91–105. doi:10.1016/j.fcr.2010.06.020

Royo, C., Martos, V., Ramdani, A., Villegas, D., Rharrabti, Y., García Del Moral, L.F.,

2008. Changes in yield and carbon isotope discrimination of Italian and Spanish durum wheat during the 20th century. Agron. J. 100, 352–360. doi:10.2134/agronj2007.0060

Sadras, V.O., Lawson, C., 2011. Genetic gain in yield and associated changes in phenotype, trait plasticity and competitive ability of South Australian wheat varieties released between 1958 and 2007. Crop Pasture Sci. 62, 533–549. doi:10.2135/cropsci2005.0175

Sanchez-Bragado, R., Elazab, A., Zhou, B., Serret, M.D., Bort, J., Nieto-Taladriz, M.T., Araus, J.L., 2014a. Contribution of the ear and the flag leaf to grain filling in durum wheat inferred from the carbon isotope signature: Genotypic and growing conditions effects. J. Integr. Plant Biol. 56, 444–454. doi:10.1111/jipb.12106

Sanchez-Bragado, R., Molero, G., Reynolds, M.P., Araus, J.L., 2014b. Relative contribution of shoot and ear photosynthesis to grain filling in wheat under good agronomical conditions assessed by differential organ δ^{13}C. J. Exp. Bot. 65, 5401–5413. doi:10.1093/jxb/eru298

Sanchez-Bragado, R., Serret, M.D., Araus, J.L., 2017. The Nitrogen Contribution of Different Plant Parts to Wheat Grains: Exploring Genotype, Water, and Nitrogen Effects. Front. Plant Sci. 7. doi:10.3389/fpls.2016.01986

Sánchez-Garcia, M., 2012. Genetic gains and changes in the pattern of adaptation of bread wheat varieties grown in Spain during the 20th century 173.

Sanchez-Garcia, M., Álvaro, F., Martín-Sánchez, J.A., Sillero, J.C., Escribano, J., Royo, C., 2012. Breeding effects on the genotype × environment interaction for yield of bread wheat grown in Spain during the 20th century. F. Crop. Res. 126, 79–86. doi:10.1016/J.FCR.2011.10.001

Sanchez-Garcia, M., Royo, C., Aparicio, N., Martín-Sánchez, J.A., Álvaro, F., 2012. Genetic improvement of bread wheat yield and associated traits in Spain during the 20th century. J. Agric. Sci. 151, 105–118. doi:10.1017/S0021859612000330

Sayre, K.D., Rajaram, S., Fischer, R.A., 1997. Yield potential progress in short bread wheats in northwest Mexico. Crop Sci. 37, 36–42. doi:10.2135/cropsci1997.0011183X003700010006x

Scarascia Mugnozza, G.T., 2005. The contribution of Italian wheat geneticists : From Nazareno Strampelli to Francesco D ’ Amato. Proc. Int. Congr. “In Wake Double Helix From Green Revolut. to Gene Revolution” 53–75.

Shearman, V.J., Sylvester-Bradley, R., Scott, R.K., Foulkes, M.J., 2005. Physiological processes associated with wheat yield progress in the UK. Crop Sci. 45, 175–185.

Slafer, G.A., Araus, J.L., Royo, C., García, L.F., Moral, D., 2005. Promising eco-physiological traits for genetic improvement of cereal yields in Mediterranean environments, Annals of Applied Biology.

Subira, J., Álvaro, F., García del Moral, L.F., Royo, C., 2015. Breeding effects on the cultivar×environment interaction of durum wheat yield. Eur. J. Agron. 68, 78–88. doi:10.1016/j.eja.2015.04.009

Thomas, H., Howarth, C.J., Thomas, H., Howarth, C.J., 2000. Five ways to stay green. J. Exp. Bot. · 51, 329–333. doi:10.1093/jexbot/51.suppl

Thomas, H., Smart, C.M., 1993. Crops that stay green. Ann. Appl. Biol. 123, 193–219. doi:10.1111/j.1744-7348.1993.tb04086.x

Vijayalakshmi, K., Fritz, A.K., Paulsen, G.M., Bai, G., Pandravada, S., Gill, B.S., 2010. Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature. Mol. Breed. 26, 163–175. doi:10.1007/s11032-009-9366-8

Wong, S.C., Cowan, I.R., Farquhar, G.D., 1979. Stomatal conductance correlates with photosynthetic capacity. Nature 282, 424–426. doi:10.1038/282424a0

Zheng, T.C., Zhang, X.K., Yin, G.H., Wang, L.N., Han, Y.L., Chen, L., Huang, F., Tang, J.W., Xia, X.C., He, Z.H., 2011. Genetic gains in grain yield, net photosynthesis and stomatal conductance achieved in Henan Province of China between 1981 and 2008. F. Crop. Res. 122, 225–233. doi:10.1016/J.FCR.2011.03.015