Universitat de Lleida

Grain yield determinants in a traditional and a modern wheat cultivar under a wide range of conditions in a Mediterranean region: source-sink relationships, components, resource capture and use efficiencies

Julia Cartelle Saball

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Source-sink relationships, components, resource capture and use efficiencies

Doctoral Thesis
Julia Cartelle Saball

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Universitat de Lleida
Departament de Producció
Vegetal i Ciència Forestal

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Julia Cartelle Saball

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December 2015

Para mis padres, Ester y Jorge

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Abstract

Wheat is one of the main crops grown in the Mediterranean region of Spain and its production is based in cultivars of Mexican (CIMMYT) and French origin. The traditional cultivars are generally chosen by the farmers over the modern ones in the poorest environments, assuming that their grain yield is more stable, while the modern ones are frequently sown under higher-yielding conditions. This selection between cultivars is based on a widespread belief, but there had been very few studies comparing directly the performance (and bases for the differential performance) of these two kinds of cultivars in the region. Therefore, quantifying and further understanding their differences in source-sink relationships, grain yield components, nitrogen use efficiency (NUE) water use efficiency (WUE) and root growth under Mediterranean conditions is relevant.

The main objective of the present thesis was to assess the physiological grain yield determinants of a traditional and a modern bread wheat cultivar in response to different water and nitrogen (N) availabilities under the Mediterranean crop area in north-eastern Spain. Within this general objective, the aim was (i) to establish if there exists source-limitation to grain growth under Mediterranean conditions, (ii) to analyse the main yield components and grain yield responsiveness, (iii) to compare N and water use and their use efficiencies and (iv) to study root growth and its relevance to resources uptake and use for the traditional and the modern cultivar respectively.

To fulfil these objectives three field experiments were carried out under contrasting levels of N and water availabilities using a traditional (Anza) and a modern (Soissons) wheat cultivar during the 2003-4; 2004-5 and 2005-6 growing seasons within the rainfed agricultural area of Catalonia. For the purposes of objective (i) sink size was modified, roughly doubling assimilates availability per grain by degraining the spikes *c.* 10 days after anthesis and an additional experiment was conducted during the first season using the same cultivars at different sowing dates within an irrigated area.

Across all the experimental conditions grain weight did not respond noticeably to the reduction in sink demand during the effective grain filling period, even under Mediterranean conditions (*i.e.* grain filling frequently exposed to terminal stresses). Grain yield varied widely from 0.78 to 7 Mg ha^{-1}and it was mainly determined by grain number per unit land area. There was not a clear trend for the traditional cultivar to outyield the modern one under low yielding conditions or *vice versa*.

N and water use and their efficiencies were related to variations in grain yield to the same extent both for the traditional and the modern cultivar, therefore neither of them supported the hypothesised advantage of choosing one cultivar over the other depending on the yielding conditions. N and water supply limited biomass, grain yield and resource use and use efficiency. N uptake occurred mainly along the pre-anthesis period, while grain N concentration was generated chiefly by translocation from the vegetative organs to the grain.

In general, the modern cultivar presented higher values of root length (RL), root dry weight (RDW) and root length density (RLD) than the traditional one. Nevertheless this superiority did not translate in consistent differences in grain yield. RL and RDW were determinants of N uptake and grain yield.

Keywords**:** wheat, Mediterranean, grain yield, source-sink relationship, grain number, N uptake; water use, NUE, WUE, roots.

Resumen

El trigo es uno de los principales cultivos en la zona mediterránea de España y su producción se basa en cultivares de origen mexicano (CIMMYT) y francés. Los agricultores generalmente prefieren cultivares tradicionales para los ambientes más pobres suponiendo que su rendimiento es más estable; mientras que eligen los más modernos para ambientes de alto rendimiento. Sin embargo han habido muy pocos estudios comparando directamente la performance de estos dos tipos de cultivares en la región. Por lo tanto, es relevante cuantificar y entender sus diferencias en la relación fuente-sumidero, componentes del rendimiento, eficiencia en el uso del nitrógeno (EUN) y del agua (EUA) y el crecimiento radical bajo condiciones mediterráneas.

El principal objetivo de la presente tesis fue evaluar los determinantes fisiológicos del rendimiento de un cultivar de trigo harinero tradicional y uno moderno en respuesta a diferentes disponibilidades de agua y nitrógeno (N) en la zona agrícola mediterránea del noreste de España. En el marco de este objetivo, el propósito fue (i) establecer si existe limitación por fuente al crecimiento del grano bajo condiciones mediterráneas, (ii) analizar los principales componentes y la respuesta del rendimiento, (iii) comparar el uso del N y el agua y sus eficiencias y (iv) estudiar el crecimiento radicular y su relevancia para la captura y uso de recursos para el cultivar tradicional y el moderno respectivamente.

Para ello, se llevaron a cabo tres experimentos de campo bajo niveles contrastantes de disponibilidad de N y agua, utilizando un cultivar de trigo tradicional (Anza) y uno moderno (Soissons) durante las campañas 2003-4; 2004-5 y 2005-6 en el área agrícola de secano de Cataluña. Para los fines del objetivo (i) el tamaño del sumidero fue modificado desgranando las espigas alrededor de 10 días después de antesis, virtualmente duplicando la disponibilidad de asimilados por grano y durante la primera campaña, se condujo un experimento adicional utilizando los mismos cultivares con diferentes fechas de siembra en una zona de regadío.

A través de todas las condiciones experimentales, el peso de grano no respondió notablemente a la reducción en la demanda del sumidero durante el periodo efectivo de llenado de grano, inclusive bajo condiciones mediterráneas (*i.e.* llenado de grano frecuentemente expuesto a estrés terminal). El rendimiento varió ampliamente, desde 0.78 a 7 Mg ha^{-1} y fue determinado principalmente por el número de granos por unidad de superficie. No se observó que el cultivar tradicional superara claramente en rendimiento al moderno bajo condiciones pobres o *vice versa*.

El uso de N y el agua y sus eficiencias estuvieron relacionadas con las variaciones en el rendimiento tanto para el cultivar tradicional como para el moderno, por lo tanto, ninguno de los dos respaldó la hipotética ventaja de elegir uno de los dos según las condiciones de rendimiento. Las disponibilidades de N y agua limitaron la producción de biomasa, el rendimiento, el uso y la eficiencia en el uso de recursos. La absorción de N ocurrió principalmente durante pre-antesis, mientras que la concentración de N en grano estuvo generada mayormente por translocación desde los tejidos vegetativos.

En general, el cultivar moderno presentó mayor tamaño radical que el tradicional. Sin embargo, esta superioridad no se tradujo en diferencias consistentes en rendimiento. La longitud y el peso de las raíces fueron determinantes de la absorción de nitrógeno y del rendimiento.

***Palabras clave*:** trigo, condiciones mediterráneas, rendimiento, relaciones fuente-sumidero, número de granos, absorción de N, uso del agua, EUN, EUA, raíces.

Resum

El blat és un dels principals cultius de la zona mediterrània d'Espanya i la seva producció es basa en cultivars d'origen mexicà (CIMMYT) i francès. Els agricultors generalment prefereixen cultivars tradicionals per a ambients més pobres suposant que el seu rendiment és més estable; mentre que els més moderns se solen sembrar en condicions d'alt rendiment. Aquesta elecció de cultivars se sustenta en una estesa creença, però hi han hagut molt pocs estudis comparant directament l'acompliment (i les bases del seu acompliment diferencial) d'aquest dos tipus de cultivars a la regió. Per tant, es rellevant quantificar i entendre les seves diferències en la relació font-embornal, components del rendiment, eficiència en l'ús del nitrogen (EUN) i de l'aigua (EUA) i el creixement de les arrels sota condicions mediterrànies.

El principal objectiu de la present tesi va ser avaluar els determinants fisiològics del rendiment d'un cultivar de blat fariner tradicional i un modern en resposta a diferents disponibilitats d'aigua i nitrogen (N) a la zona agrícola mediterrània del nord-est d'Espanya. En el marc d'aquest objectiu , el propòsit va ser (i) establir si existeix limitació per font al creixement del gra sota condicions mediterrànies , (ii) analitzar els principals components i la resposta del rendiment, (iii) comparar l'ús del N i el aigua i les seves eficiències i (iv) estudiar el creixement de les arrels i la seva rellevància per a la captura i ús de recursos per al cultivar tradicional i el modern respectivament.

Per a això, es van dur a terme tres experiments de camp sota nivells contrastants de disponibilitat de N i aigua, utilitzant un cultivar de blat tradicional (Anza) i un modern (Soissons) durant les campanyes 2003-4; 2004-5 i 2005-6 en l'àrea agrícola de secà de Catalunya. Per a les finalitats de l'objectiu (i) la mida de l’embornal va ser modificat desgranant les espigues al voltant de 10 dies després de antesi, virtualment duplicant la disponibilitat d'assimilats per gra i durant la primera campanya, es va conduir un experiment addicional utilitzant els mateixos cultivars amb diferents dates de sembra en una zona de regadiu.

A través de totes les condicions experimentals, el pes de gra no va respondre notablement a la reducció en la demanda de l’embornal durant el període efectiu d'ompliment de gra, inclusivament sota condicions mediterrànies (*i.e.* omplert de gra freqüentment exposat a estrès terminal). El rendiment va variar àmpliament, des 0,78-7 Mg ha^{-1} i va ser determinat principalment pel nombre de grans per unitat de superfície. No es va observar que el cultivar tradicional superés clarament en rendiment al modern sota condicions pobres o *vice versa*.

L'ús del N i l'aigua i les seves eficiències van estar relacionades amb les variacions en el rendiment igualment per al cultivar tradicional i el modern, per tant, cap dels dos va donar suport la hipotètica avantatge de triar un dels dos segons les condicions de rendiment . Les disponibilitats de N i aigua van limitar la producció de biomassa, el rendiment, l'ús i l'eficiència en l'ús de recursos. L'absorció de N va ocórrer principalment durant pre-antesi, mentre que la concentració de N en gra va estar generada principalment per translocació des dels teixits vegetatius.

En general, el cultivar modern va presentar major grandària radicular que el tradicional. No obstant això, aquesta superioritat no es va traduir en diferències consistents en rendiment. La longitud i el pes de les arrels van ser determinants de l'absorció de nitrogen i del rendiment.

***Paraules clau*:** blat, condicions mediterrànies, rendiment, relacions font-embornal, nombre de grans, absorció de N, ús de l'aigua, EUN, EUA, arrels.

Chapter I
General Introduction

Field experiment in Agramunt (wheat crop seedlings emerged)

Chapter I

1. General Introduction

1.1. Wheat crop in Mediterranean environments – The Ebro Valley Region

Wheat (*Triticum aestivum*) is one of the world's major commodity cereals along with maize and rice (FAOSTAT 2013). It is also one of the most widely adapted crops around the world and although it evolved in the Mediterranean climate of West Asia, it performs best in temperate wetter climates. Even so, wheat is one of the mains crops produced under Mediterranean type climates (Loss and Siddique, 1994; Schillinger *et al.*, 2008).

Mediterranean climate regions are distributed between parallels 30º to 40º in both hemispheres. They are: the Mediterranean basin (south-western Europe, west Asia and north Africa); southern Africa; California, central Chile and south and south-western Australia (Di Castri, 1991).

Wheat and other small grain cereals in this type of environments are usually grown in dryland systems. They are sown after autumn rains and the vegetative growth occurs during the frequently mild and wet winters; when the water input exceeds the crop evaporative demands due to the low temperatures and the consequently slow growth (Loss and Siddique, 1994). Aschmann (1973) quantified the main characteristics of the Mediterranean climates determining that at least 65% of the annual rainfall is concentrated in the winter months, varying from 250 to 900 mm with an average winter temperature below 15ºC with less than 3% of hours per year with temperatures minus 0ºC.

Unlike the vegetative and early reproductive phases, the late reproductive phase (that of stem elongation) begins with the increased temperatures and photoperiods of the spring and with increased likelihood of exposure to water deficits (as evapotranspiration capacity increases while rainfall tend to decrease) the crop reaches anthesis at mid-spring. Thereafter the grains are filled under more stressing (water and heat) conditions and under what it is generally referred as *terminal drought* reaching maturity in early summer (Loss and Siddique 1994). Regardless of the fact that water and thermal stresses tend to intensify during the last quarter of the growing season, yield variations seem more related with grain number than with the average size of the grains (*e.g.* Slafer *et al.*, 2014 and references reviewed therein). Therefore, any physiological stresses occurring during the critical stage for grain number and yield determination, between the beginning of stem elongation and anthesis, are also crucial to target agronomic practices and breeding strategies focus in Mediterranean environments (Savin et al., 2015).

Wheat is one of the major cereal crops in Spanish agricultural production. Around 2 million hectares were annually harvested throughout the last decade with an average yield of *ca.* 3 Mg ha^{-1} and a total production of *ca.* 6 million tons (FAOSTAT 2013).

The Ebro Valley area lies within the Mediterranean region of Spain and its wheat production is based in cultivars of Mexican (CIMMYT) and French origin that have been successfully cultivated in farms and experimental sites during the last 40 years (Acreche *et. al*, 2008). The French and the CIMMYT cultivars may have developed different physiological features that determine their productivity and might therefore be relevant to the GxE interaction on yield determination. However, the traditional agronomic practices applied to these materials in the area do not acknowledge the potential differences among them. Therefore further understanding of their comparative behaviour, particularly under Mediterranean conditions, is relevant to identify physiological traits affecting the crop growth and determining grain yield in each case.

In the present thesis, at the time experiments were designed, two bread wheat cultivars were chosen to represent CIMMYT and French origin and also traditional and a modern cultivars according to their release date. Anza is a semi-dwarf spring wheat cultivar released in 1971 (1974 in Spain) and was widely grown during decades by many local farmers; while Soissons, a winter wheat with putatively higher yield, was released in 1987 (1990 in Spain). The genotypes were chosen in order to contrast the most widely sown traditional and modern cultivars in the region at the time of the study. The traditional one, Anza, was generally sown in the poorest environments, under the assumption that it was more stable yield than the modern one, Soissons, which was frequently chosen under high yielding conditions due to its higher potential yield. Their popularity was likely based on the fact that for this region both Anza and Soissons were standard controls for the Evaluation of New Cereals Varieties in Spain (GENVCE, 1999-2003). Despite that at the time of starting this research there was a strong belief among wheat farmers and extension professionals that Anza would perform better in low-yielding conditions and Soissons would do so in higher-yielding conditions, there had been very few studies comparing directly performance (and bases for the differential performance) of these two cultivars in the region. Making such comparison is relevant. If the hypothetical behaviour of these cultivars is not evidenced in rigorous comparisons, the criterion for choosing cultivars may be different. If the hypothetical behaviour proofs to be in line with evidence, the analytical comparison may offer ideas on attributes that might be advantageous for breeding to improve yield in particular yielding conditions.

1.2. Yield determination in a traditional and a modern cultivar under Mediterranean conditions

To understand the bases for the differences in yield between treatments (in this case chiefly between a traditional and a modern cultivar) it is necessary to investigate yield into determinants. Grain yield determination in wheat (and in other grain crops) has been analysed mainly through two alternative and non-mutually-exclusive approaches (Fig. 1.1): (i) considering crop growth (as biomass accumulation) and partitioning (whose ultimate output is harvest index); and (ii) dividing it into numeric components, which are defined sequentially and partially overlapped along the growing season, being therefore negatively related among themselves (Slafer and Savin, 2006).

The two major numeric components of yield are grain number per unit land area and mean grain weight (Fig. 1.1). Potential grain number per unit land area depends on the development of spike number per unit land area and grains per spike, which can be in turn also decomposed into further numeric sub-components (Slafer and Savin, 2006).

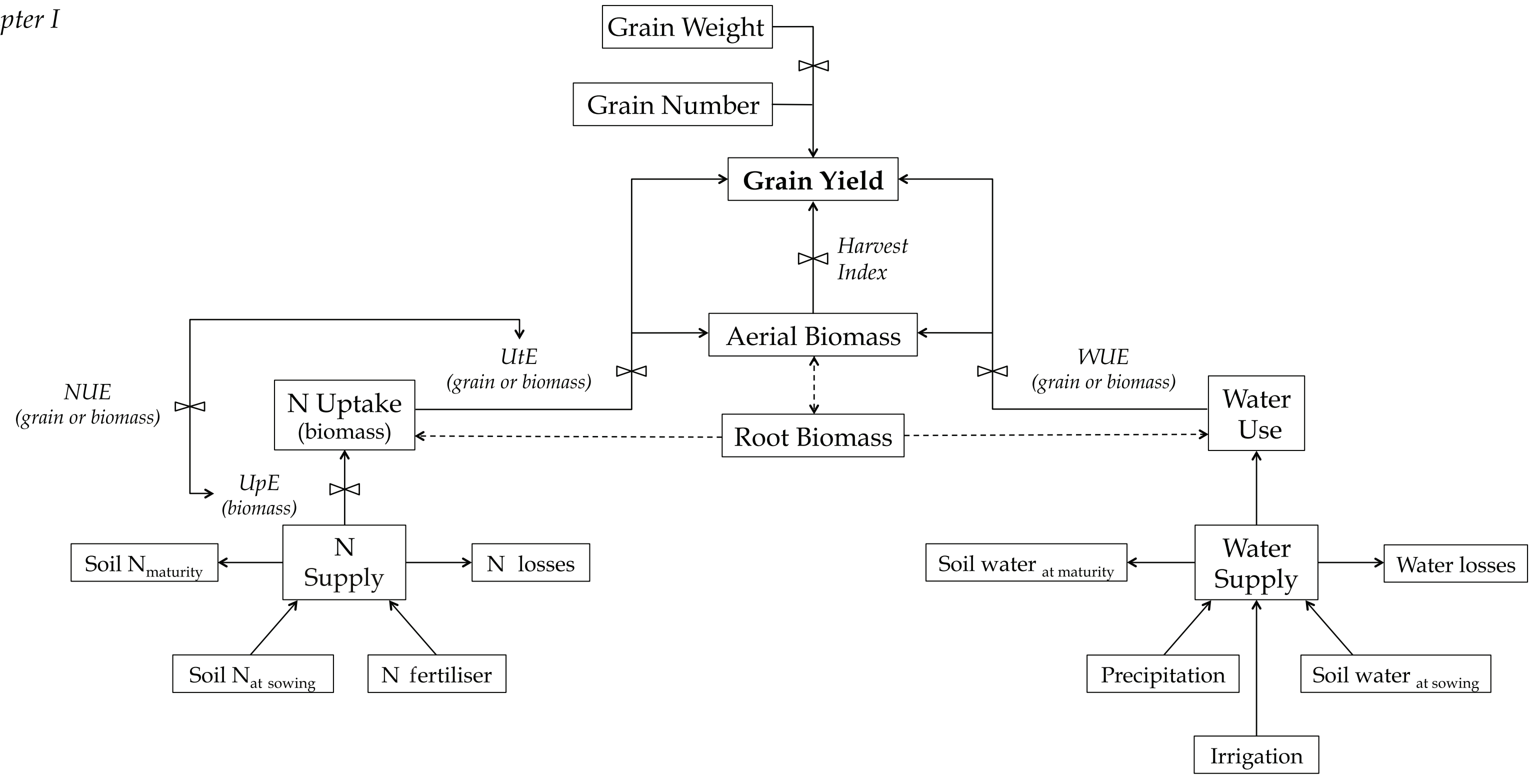

Figure 1.1. Right side: Water-related traits and processes involved in grain yield generation. Water use (WU) is the sum of soil water at sowing plus the growing season accumulated precipitation, plus irrigation when applied minus the soil water remaining at maturity. The grain yield or aerial biomass produced per unit of WU is the water use efficiency (WUE). Adapted from Passioura and Angus, 2010. Left side: Nitrogen-related traits and processes involved in grain yield generation: N uptake is the N absorbed by the aerial plant tissues. The ratio between aerial biomass N uptake and total N supply* at maturity is the N uptake efficiency (UpE). The ratio of grain yield or aerial biomass to aerial biomass N uptake at maturity is the N utilisation efficiency (UtE). The grain yield or aerial biomass produced by unit of N supplied (*i.e.* the product of UpE by UtE) is the Nitrogen use efficiency (NUE). Grain yield is partitioned into its two main numeric components: mean grain weight and grain number per unit land area. *In the present study N supply was calculated as the crop N uptake plus the remaining soil N at maturity, therefore, the soil N at sowing, the N incorporated by the fertiliser when applied and the possible N losses by volatilisation and deep drainage were already included in the calculation.

The growth/partitioning approach assumes that yield is simply a fraction of biomass; but whilst biomass is accumulated throughout the growing season, yield seems to be particularly sensitive to growth in some phases more than in others. The most critical period for grain yield determination occurs around anthesis, approximately from three weeks before flowering up to a few days afterwards (Fischer, 1985; Savin and Slafer, 1991; Calderini et al., 2001; Slafer and Savin, 2006). Thus, although sub-components of the number of grains per unit land area are produced during the whole pre-anthesis period, there is a short window of time from *c.* 20 days before to *c.* 10 days after anthesis when grain number is largely defined. Therefore, intrinsic phenology differences between modern and traditional cultivars may expose them to different resources availabilities scenarios, particularly in the erratic Mediterranean environmental conditions. Consequently, that may affect different components of their yield components and its final generation. Furthermore, modern small grain cereals cultivars selected under high yielding conditions have shown to be often more responsive to high inputs environments than older ones (*i.e.,* Abeledo *et al.*, 2003), that are expected to be more stable in terms of yield under the frequent stressful conditions of the Mediterranean regions, but not necessarily to outyield their modern counterparts (Calderini and Slafer, 1999; Acreche *et al.*, 2008).

It is well known from previous reports in different environments, that grain number per unit land area is the main component determining yield over individual grain weight (Prystupa *et al.*, 2004; Shearman *et al.*, 2005; Peltonen-Sainio *et al.*, 2007; Zhang *et al.*, 2007; Slafer *et al.*, 2014). In spite of these two yield components being negatively related, which can often be interpreted as a sign of competition for assimilates among grains, in most conditions they have proven to be independent, indicating a lack of source-limitation to grain growth. However, individual grain weight may also be a source of grain yield variation, especially under Mediterranean conditions due to the frequent thermal and water stresses during grain filling period. In fact, in north-eastern Spain, there were some evidences of increments in cereal grain size after modifying the source-sink relationship (*i.e.* degraining half of the spikes), particularly in the lowest yielding environments, suggesting some degree of source limitation or source-sink co-limitation to grain growth (Voltas *et al.*, 1997; Acreche and Slafer, 2009). Therefore, in order to increase yield through management and breeding strategies it is important to understand, in the first place, the physiology of yield determination under Mediterranean conditions. It would be especially useful to determine firstly, if the negative relationship between grain number per unit land area and mean grain weight is indicating competition for assimilates in semi-arid conditions, especially when comparing a traditional and a modern cultivar that is expected to be more source-limited due to its increased grain number per unit land area. Secondly, if focusing on grain number per unit land area would be as effective in producing yield gains as it has proven to be in other environments.

Apart from the numeric components approach to yield determination, biomass accumulation and partitioning is the other key process involved in it. Water and nitrogen (N) supply, when adequate to plant demand increase yields trough an increase in biomass, produced mainly for a greater LAI and thereby, grater PAR interception.

Generally, in Mediterranean environments the total evaporative demand along the crop growing season exceeds the water supply. Consequently, rainfed crop yields in such regions were considered to be mainly limited by water and have therefore, more variable and lower yields than those of humid regions. However, beyond the unquestionable relevance of water stress in determining low yielding conditions in rainfed Mediterranean systems, there are also other factors constraining or co-limiting yield in these environments, particularly N availability (Passioura 2002; Cossani *et al.*, 2010; Passioura and Angus, 2010; Sadras and Richards, 2014). Thus, a further understanding of the main physiological mechanisms and traits that are determining N and water-limited wheat yields in Mediterranean environments could be helpful to identify opportunities to improve them and design breeding and management strategies.

As a framework to analyse grain yield determination thought the biomass partitioning approach in Mediterranean environments (potentially water-limited), the identity proposed by Passioura (1977) was followed: Y = WU*WUE*HI; where Y is grain yield, WU is water use (*i.e.* crop evapotranspiration); WUE is water use efficiency (*i.e.* biomass/WU) and HI is harvest index, the proportion of grain from the aerial biomass (Fig. 1.1) and these three components are independent and therefore plausible to be improved on their own (Passioura and Angus, 2010). In general terms, WUE refers to biomass produced per unit of water used to generate that biomass. Water use can be referring to crop transpiration, evapotranspiration or even total water inputs to the system. Similarly, biomass can be expressed in terms of total crop biomass or grain yield. Hence, it is important to take into account the scale at which these variables are being used to calculate WUE when comparing results.

Similarly, for nitrogen use efficiency (NUE) several levels of complexity can be found in scientific literature. The more generally used are the parameters defined by Moll *et al.* (1982) where NUE can be partitioned into two components: N uptake efficiency (UpE) as the ratio between aerial biomass N uptake (*i.e.* N absorbed by the crop) and N supply (*i.e.* soil N availability or applied fertiliser) and N utilization efficiency (UtE) as aerial biomass or grain yield divided by the N uptake. Being NUE the product of the latter two (*i.e.* the ratio of aerial biomass or grain yield to N supply; Fig.1.1).

Hence, if considering N as a yield determinant, the identity would be: Y = N uptake*UtE*HI, where N uptake is the product of N availability and UpE, and estimated as the aerial biomass by its %N (*i.e.* N use) and UtE is nitrogen utilization efficiency (*i.e.* biomass/N uptake; Fig. 1.1).

Improving the efficiency with which N and water are used by the crop by identifying the processes related to variations in these efficiencies and their interaction is a useful approach to achieve higher yields, especially in Mediterranean areas (Araus, 2004). Besides, taking into account the phenological and morphological differences between a traditional and a modern cultivar might also contribute to the knowledge of NUE and WUE in Mediterranean environments.

At the time the present thesis experiments were conducted; there were previous results regarding bread wheat NUE in Mediterranean environments (*e.g.* López-Bellido and López-Bellido, 2001; López-Bellido *et al.*, 2005), as well as some evidences comparing traditional and modern cultivars (*i.e.* Guarda *et al.*, 2004), but understanding the differences between a traditional and a modern cultivar under Mediterranean conditions in terms of NUE and WUE simultaneously was almost inexistent.

Finally, although it is well known that the root system is key to resources capture, there are not many evidences on its relationship with soil resources uptake, particularly in the more limited Mediterranean areas (Carvalho *et al.*, 2014). Thus, a better understanding of root growth dynamics in the field and ultimately the relationship with grain yield under Mediterranean conditions could provide scope for further improvement.

1.3. Objectives and outline of the present thesis

The general objective of the present thesis was to assess the physiological grain yield determinants of a traditional and a modern bread wheat cultivar (representing the most popular choice by farmers of the region to grow in relatively poor and less stressful environments, respectively) in response to different water and N availabilities under Mediterranean conditions, within the dryland crops area of the Ebro Valley in north-eastern Spain. Within this general objective, the aim was (i) to test if any of the chosen cultivars represent any advantage in terms of yield under contrasting environmental conditions, from stressful to high inputs environments, given their assumed differences in yield potential and stability for the Mediterranean region, and (ii) To identify physiological traits and processes related to N fertilization and water availability that can constitute management and breeding tools towards higher and sustainable yields in Mediterranean regions.

For this purpose, the following specific objectives were pursued:

(i) To study if there exists source-limitation to wheat grain growth under Mediterranean conditions and if so, (a) to quantitatively estimate the degree of limitation, and (b) to determine whether a traditional and a modern cultivar would differ in this condition (Chapter II*).

(ii) To analyse the main yield components and grain yield responsiveness of a traditional and a modern cultivar under a range of N and water availability (Chapter III).

(iii) To compare NUE and WUE between a traditional and a modern cultivar in Mediterranean environments and to study their relationship with grain yield, N and water supply and use (Chapter IV).

(iv) To study the root growth dynamics of a traditional and a modern cultivar and its relevance to resources uptake and as a yield determinant (Chapter V).

The present thesis is divided into six chapters. These chapters consist of the general introduction (Chapter I), four experimental research chapters (Chapters II*; III; IV and V) and a general discussion and main conclusions (Chapter VI).

*Chapter 2 is based on a paper published in a SCI journal.

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Chapter II

Grain weight responses to post-anthesis spikelet-trimming


Field experiment in Agramunt (wheat crop around tillering)

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Chapter II

2. Grain weight responses to post-anthesis spikelet-trimming

Abstract

Average grain weight is a major yield component contributing to its variation, especially in Mediterranean regions where grain weight is frequently exposed to terminal stresses affecting grain growth. Most of the literature agrees that wheat grain growth is hardly limited by the source. However, no source-sink ratios studies seem to have been conducted in the Mediterranean region (at the moment when the experiments were performed) to determine to what degree wheat grain growth is actually limited by the source in these particular regions. Two field experiments were conducted in Catalonia (north-eastern Spain), where a traditional cultivar (Anza) and a more recently released one (Soissons) were sown in a range of different nitrogen and water availabilities and sowing dates. This was to analyse the degree of source limitation for grain growth. Sink size was modified by removing half of the spikelets *c.* 10 days after anthesis, virtually doubling the availability of assimilates per grain effectively growing.

Trimming the spikes did not produce significant changes in grain growth rate or duration of grain filling. Consequently, grain weight did not respond noticeably to the reduction in sink demand and any eventual response has been far from representing a strong competition among grains during grain filling.

2.1. Introduction

It is well known that grain number per unit area is the yield component most strongly related to yield variations in wheat (*e.g.* Frederick and Bauer, 1999), as well as in most other grain-crops (Slafer, 1994; Egli, 1998). Despite this strong and consistent relationship, the average grain weight can be an important source of variation of grain yield (Calderini *et al.*, 2001), particularly so in cereal growing regions characterised by terminal stresses, such as those of the Mediterranean basin (Acevedo *et al.*, 1999). Therefore, understanding the causes of grain weight determination could be critical to plant breeders and agronomists aimed to increase yield or yield stability, particularly in the Mediterranean region.

In addition, number of grains per unit land area and the averaged grain weight are frequently negatively correlated (Slafer and Andrade, 1993); a fact often interpreted as a sign of the competition among grains for an insufficient assimilate availability during grain filling (*i.e.* whenever grain number is increased each grain can access less assimilates than those needed to maximise growth). If grains compete for limited growth resources during grain filling reducing sink size and, as a consequence, improving the source-sink ratio after anthesis would be important in general and critical in Mediterranean conditions. On the other hand, if grain weight was determined largely independently of post-anthesis source strength it would be critical to improve potential grain size.

A direct approach to determine whether (and to what degree) final grain size is consequence of competition among grains during grain filling has been to modify the source-sink relationship (*i.e.* defoliation, shading, degraining treatments imposed soon after anthesis before the onset of the effective grain filling period). In general, individual studies may have found some inconsistencies, but analysing them together clearly reveals that wheat grains do not or slightly respond to these kinds of source-sink manipulations (*e.g.* Slafer and Savin, 1994; Borrás *et al.*, 2004). In other words, that the capacity of the wheat canopy to provide assimilates to the growing grains is generally adequate to allow the grains to completely fill (Savin and Slafer, 1991; Richards, 1996).

Despite the fact that this generalised behaviour included some analyses of wheat grain size responses to post-anthesis source-sink ratios under stress (*e.g.* Slafer and Miralles, 1992), and the review by Slafer and Savin (1994); later updated and expanded by Borrás *et al.* (2004) were comprehensive, virtually no studies were conducted in the Mediterranean region.

In Mediterranean conditions grain size is recognised to be a yield component particularly vulnerable due to the frequent stresses to which grain growth is exposed in cereal production. Thus, results might not be straightforwardly extrapolated from other regions.

Under rain-fed Mediterranean conditions in north-eastern Spain, Voltas *et al.* (1997) have found average increments of *c.* 20% on barley grain size when degraining half of the spikes. Moreover, the greatest increments were found in those trials with the smallest control grain size, suggesting a major degree of source limitation in low-yielding environments, indicating that the stronger the stress in post-anthesis the more limited by the source was grain growth. However, Voltas *et al.* (1997) had imposed the treatments at anthesis and it was clearly shown a few years later, that removing florets or grains before the onset of grain growth may alter the potential size of grains (Calderini *et al.*, 2001). Then the reported increase in grain size may be the reflection of a sink rather than a source-limitation during grain filling (*i.e.* removing competitors during flowering and the very few days following it may have contributed to an increase in grain weight potential rather than an increase in assimilate availability per grain as the driving force for the observed increase in final grain weight due to the treatment). Clearly further studies on the grain size responsiveness to source-sink manipulations, exclusively during the actual grain growth period, in Mediterranean conditions are needed before concluding on likely differences in these environments compared with other, less stressful grain filling conditions.

As it has been shown that the degree of sink-limitation for yield during grain filling might have tended to be reduced with breeding (Kruk *et al.*, 1997; Shearman *et al.*, 2005), if there is a source-limited grain growth this limitation would be more clearly evidenced in modern than in traditional cultivars, as modern cultivars normally overyield their predecessors by increasing the number of grains per m^2 (Calderini *et al.*, 1999).

The aim of this study was to determine whether wheat grain growth under Mediterranean conditions is actually source-limited and if so whether a traditional and a modern cultivar would differ in their responsiveness.

2.2. Materials and methods

2.2.1. Site

Two field experiments were carried out in the 2003-2004 growing season. Experiment 1 was conducted at Agramunt (lat. 41° 47´17´´ N, long. 1° 5´59´´ E, altitude 337 m) in a Xerorthent soil. Experiment 2 was conducted at the experimental facilities of the Centre UdL-IRTA in Gimenells (lat. 41° 39´11´´ E, long. 0° 23´28´´, altitude 258 m) in a Calcixerept petrocalcic soil.

Both locations are some 80 km apart within the province of Lleida (Catalonia, north-eastern Spain). Agramunt is within the rainfed agricultural system while Gimenells is within an irrigated area.

Experiment 2 was also fertilized with nitrogen (N) to avoid its deficiencies and irrigated (345 mm along the growing season). Weeds, insects and diseased were controlled or prevented using conventional commercial pesticides applied following the recommendations from their manufacturers. Experiment 1 was sown on 21 November 2003 at a rate of 390 seeds m^{-2}. Experiment 2 was sown at different dates increasing seed rate accordingly (see below).

2.2.2. Treatments and experimental design

The common treatments in both experiments, needed to achieve the objective of the study, were a factorial combination of two cultivars and two levels of sink manipulation. The background under which these treatments were imposed included a range of environmental conditions that differed in each experiment. This range was produced by a factorial combination of 2 nitrogen fertilization levels and 2 irrigation levels in experiment 1; and by four different sowing dates in experiment 2.

The two genotypes chosen were the traditional cultivar Anza (released in 1971 and very widely grown in the past and still grown at the time this experiment was conducted by many farmers) and the later released (1987) French cultivar Soissons.

Sink size was modified by manipulating the number of spikelets per spike. For this purpose, at anthesis (50% of spikes extruded anthers in each experimental unit) 40 main-shoot spikes (having similar number of spikelets per spike), from the central rows of each plot, were tagged randomly. Ten days later all the spikelets from one side of 20 spikes, chosen randomly from the 40 spikes labelled before, were removed by hand (trimmed spikes), while the other 20 spikes remained unaltered as controls.

In experiment 1 environmental treatments consisted of a combination of two levels of nitrogen fertilizer, 0 kg_N ha^{-1} (N_0) and 200 kg_N ha^{-1} (N_1) applied at tillering (DC 2.0, Zadoks *et al.*, 1974) as urea (46-0-0), with rain-fed (RF) or irrigated conditions (IR), the latter consisted of a twice-weekly irrigation of *c.* 10 mm each starting at the beginning of stem elongation (DC 3.1). In this experiment the treatments were arranged in a split-block split-split-plot design with three replicates. Main plots consisted of the two cultivars, sown in strips across the two water regimes in the entire replication. The sub-plots consisted of the two nitrogen levels; while the source-sink manipulation treatment was assigned to sub-sub-plots. Sub-plots consisted of 22 rows, 0.135 m apart and 5 m long.

In experiment 2 environmental treatments consisted of four sowing dates: 17 December (S_1); 15 January (S_2); 16 February (S_3) and 15 March (S_4). The sowing densities increased with the delay in sowing (380, 410, 440 and 470 seeds m^{-2} for S_1, S_2, S_3 and S_4, respectively) in order to ensure a dense canopy able of maximising radiation interception during stem elongation. The cultivar Soissons in S_4 did not reach anthesis, (probably because of its vernalisation requirements) and therefore there is no data available for this particular case.

2.2.3. *Sampling and analysis*

In both experiments, from ten days after anthesis onwards, one control and one degrained spike randomly chosen from each sub-plot were harvested twice a week. These spikes were threshed and the grains were counted, dried (for at least two days at 65ºC) and weighed. As the average weight of all grains within the spike could mask the possible source-limitation of some particular position grains (related to their potential size), proximal grains from central spikelets were weighted separately.

Timing of physiological maturity (when final grain weight is reached) and final grain weight were estimated by fitting the grain weight data over time with the bi-linear model $y=a+bx$ when $x \leq c$; $y= a+bc$ when $x>c$)]; where y is grain weight (mg); a the intercept (mg); b the rate of grain growth (mg $^{\circ}Cd^{-1}$); x the thermal time after anthesis; and c the thermal time from anthesis to physiological maturity (ºCd). This model assumes a constant growing rate prior to physiological maturity, reached in a discontinuous way (Loss *et al.*, 1989). Although more advanced ways of analysing growth curves are available, (Yin *et al.*, 2003) this simple procedure offers biologically meaningful parameters with accuracy (as shown in results). For fitting the bilinear model, regressions were performed using an optimization technique that iteratively fits the data (Jandel, 1991), for more details see Miralles *et al.* (1996).

The base temperature used in the calculation of thermal units during grain filling was 8.2ºC (Slafer and Savin, 1991). Thermal units were calculated averaging daily air temperature, recorded by meteorological stations located near the experimental sites.

Averages, standard deviation and differences for each level of environmental treatments between days from sowing to anthesis, number of grains per spike and individual grain weight were analysed by analysis of variance using SAS.

2.3. Results

2.3.1. Weather conditions

Total rainfall throughout the growing season was 305 and 257 mm in experiments 1 and 2, respectively (Fig. 2.1). In addition, *c.* 120 mm (in the irrigated treatment of experiment 1) and 345 mm (in experiment 2) were irrigated.

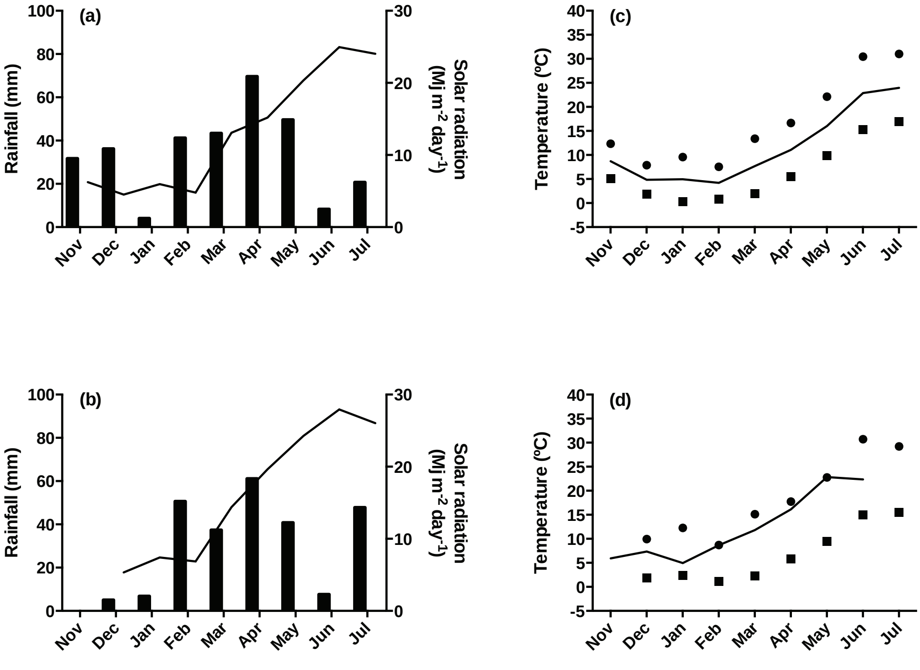

Figure 2.1. Left panels: monthly accumulated rainfall (bars) and monthly averaged daily solar radiation (lines) from sowing to maturity in experiments 1 (a) and 2 (b) respectively. Right panels: daily maximum (circles), minimum (squares) and average (lines) temperatures averaged over monthly periods from sowing to maturity in experiments 1 (c) and 2 (d) respectively.

Maximum and minimum temperatures averaged from anthesis to maturity were practically the same for Anza and Soissons in experiment 1 (*c.* 31 and 17ºC, respectively). They were also similar in experiment 2 both for the two cultivars and sowing dates (*c.* 30 ºC and 15 ºC).

2.3.2. *Fitting grain growth with the model*

The effect of treatments on grain weight and its components (rate of grain growth and grain filling duration) were derived from fitting the sequential data of grain weight taken from anthesis onwards with the optimization model programmed to fit a bi-linear curve. Figure 2.2 illustrates this kind of fitting for a particular treatment (Anza fertilized and irrigated in experiment 1, the first treatment later described in the Tables when presenting the specific results of the study) highlighting the three parameters derived from the model.

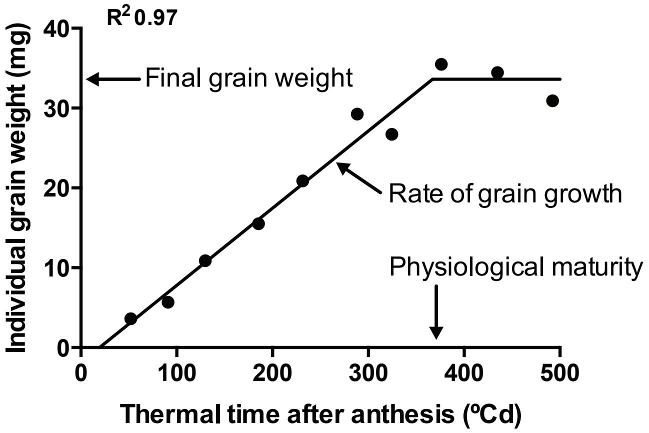

Figure 2.2. Dynamics of averaged grain weight after anthesis. For this illustrative example data are from cultivar Anza, grown under irrigation in fertilized plots of experiment 1. Arrows indicate parameters derived from curve fitting procedure.

In each of the 30 treatments analysed in this study the model fitted the data reasonably well (R^2 0.61-0.98; n=8-13). This reliability in the parameters of this simple model (parameters that are biologically meaningful more straightforwardly than those of the sigmoid curve) is in line with both theoretical and empirical evidences (Loss *et al.*, 1989).

2.3.3. *Genotype x environment background on which the spikelet-trimming was imposed*

In experiment 1 Anza reached anthesis earlier than Soissons, 181 and 187 days from sowing respectively. Availability of resources provided by the combination of fertilization and irrigation regimes did not affect noticeably phenological development. In experiment 2 anthesis date showed a significant cultivar x sowing date interaction; Anza flowered earlier than Soissons in all sowing dates (112 and 133 days on average respectively) except on S_2. Both cultivars differ among their selves across sowing dates. Soissons did not reach anthesis in the latest sowing of experiment 2 because of a clear lack of satisfaction of vernalisation requirements. Therefore it was not considered in comparisons (Table 2.1). The number of grains per spike tended to be larger in the modern cultivar, Soissons, than in the traditional one, Anza (Table 2.1). The spikes of Soissons had on average 21 and 13% more grains than those of Anza in experiments 1 and 2, respectively. There was cultivar x water level interaction in experiment 1 (P=0.05); Soissons had on average more grains per spike than Anza both under irrigation and rain-fed conditions (Table 2.1).

Table 2.1. Days from sowing to anthesis, number of grains per spike and their averaged weight for the two cultivars, Anza and Soissons under the different growing conditions of each experiment.

	Water regime	**Fertilization (kg$_N$ ha^{-1})**	**Cultivar**	**Days from sowing to anthesis**	**Number of grains (spike^{-1})**	**Averaged grain weight (mg)**
Experiment 1	Irrigated	200	Anza	181±0.58	37±9.76	33.61±6.10
			Soissons	187±0.58	48±9.10	32.55±6.07
		0	Anza	181±0.58	40±10.95	34.28±7.01
			Soissons	186±0.58	43±13.25	35.03±7.90
	Rainfed	200	Anza	181±1.00	40±9.19	31.47±5.86
			Soissons	187±0.58	52±12.89	31.14±4.08
		0	Anza	180±1.73	37±12.09	34.39±5.85
			Soissons	187±1.00	52±11.68	31.99±4.57
	Sowing date		**Cultivar**	**Days from sowing to anthesis**	**Number of grains (spike^{-1})**	**Averaged grain weight (mg)**
Experiment 2	17-Dec-03		Anza	146±2.08	48±11.00	37.83±11.07
			Soissons	157±1.73	49±9.83	31.93±9.11
	15-Jan-04		Anza	126±1.15	42±6.72	33.89±8.55
			Soissons	127±2.08	49±7.77	32.66±12.09
	16-Feb-04		Anza	97±2.08	38±5.26	38.57±10.9
			Soissons	115±2.08	50±9.49	28.14±8.84
	15-Mar-04		Anza	80±1.00	41±7.39	38.23±4.49
			Soissons	---	---	---

On the other hand, the modern cultivar had grains that, on average, were either equally-sized or lighter (though not significantly) than those of the traditional cultivar (Table 2.1). There were no clear differences in grain weight across environmental treatments either (P>0.13).

2.3.4. Grain weight responses to spikelet-trimming

Throughout the whole range of environmental background conditions generated in each of the two studies, trimming the spikes did not seem to have produced clear changes in the parameters of grain growth (Table 2.2). Neither the rate of grain growth nor the duration of grain filling was significantly affected by trimming the spikes in any treatments (and in fact there was not even a non-significant consistent trend to increase the rate or the duration with doubling the source-sink ratio by halving the sink size) (Table 2.2).

Table 2.2. Parameters obtained with the optimization model when regressing the weight of the grains against thermal time after anthesis. Parameters are the intercept (*a*), the rate of grain growth averaged for the whole grain filling period (*b*), and the thermal time after anthesis when physiological maturity was reached (*c*). The model was fitted to the combination of all treatments: the two cultivars, Anza and Soissons growing in a range of environmental conditions in each of the two experiments subjected to the two different source-sink ratios: unmodified spikes (control) and spikes with all the spikelets from one side removed by 10 days after anthesis (trimmed).

	Cultivar	**Water regime**	**Fertilzation (kg_N ha^{-1})**	**Spike treatment**	***a* (mg)**	***b* (mg °Cd^{-1})**	***c* (°Cd)**	**R^2**
Experiment 1	Anza	Irrigated	200	Control	-1.92±1.78	0.10±0.01	367.21±22.07	0.96
				Trimmed	-2.36±0.99	0.11±0.00	348.33±8.11	0.98
			0	Control	-2.11±3.04	0.12±0.02	310.20±27.87	0.90
				Trimmed	0.62±2.15	0.10±0.01	376.21±22.39	0.94
		Rainfed	200	Control	-1.11±1.12	0.11±0.01	308.18±11.22	0.98
				Trimmed	-4.48±1.61	0.13±0.01	312.95±10.60	0.98
			0	Control	0.39±1.45	0.10±0.01	343.09±16.27	0.96
				Trimmed	0.66±2.20	0.09±0.01	376.20±23.67	0.94
	Soissons	Irrigated	200	Control	0.57±2.90	0.11±0.02	295.25±25.95	0.92
				Trimmed	-3.90±3.92	0.13±0.02	285.48±20.24	0.92
			0	Control	-0.38±3.76	0.12±0.02	298.10±32.02	0.88
				Trimmed	3.64±3.45	0.14±0.02	278.53±15.39	0.94
		Rainfed	200	Control	-0.19±2.51	0.11±0.01	295.23±23.57	0.92
				Trimmed	-3.47±2.13	0.12±0.01	290.46±11.97	0.98
			0	Control	1.05±2.97	0.10±0.02	296.72±28.62	0.90
				Trimmed	-1.15±2.38	0.12±0.01	291.11±13.95	0.96

	Cultivar	**Sowing date**	**Spike treatment**	***a* (mg)**	***b* (mg °Cd^{-1})**	***c* (°Cd)**	**R^2**
Experiment 2	Anza	S_1	Control	-5.40±5.78	0.13±0.03	325.38±39.50	0.77
			Trimmed	-9.34±4.71	0.15±0.02	344.39±25.92	0.90
		S_2	Control	-7.85±7.35	0.15±0.04	261.83±31.12	0.79
			Trimmed	-0.46±4.43	0.11±0.02	348.05±23.63	0.88
		S_3	Control	-4.87±9.56	0.12±0.04	347.32±55.08	0.66
			Trimmed	-5.35±5.83	0.13±0.02	353.29±24.72	0.90
		S_4	Control	-7.10±5.07	0.13±0.02	331.19±28.18	0.88
			Trimmed	-11.90±2.86	0.14±0.01	365.80±12.40	0.98
	Soissons	S_1	Control	-4.26±9.49	0.13±0.05	273.65±52.84	0.62
			Trimmed	-0.39±6.28	0.12±0.03	315.75±30.57	0.83
		S_2	Control	-1.39±4.53	0.08±0.02	425.10±45.05	0.83
			Trimmed	-5.86±3.14	0.10±0.01	424.35±20.83	0.96
		S_3	Control	1.05±6.09	0.07±0.03	360.23±59.88	0.61
			Trimmed	-7.72±9.75	0.12±0.04	303.57±32.47	0.64

Consequently, final grain weight did not increase significantly in response to the drastic increase in the source-sink ratio in any of the two cultivars (Fig. 2.3). However, though differences between source-sink treatments were non-significant, there was a consistent trend to increase grain weight with trimming (with the exception of the third sowing date of cultivar Anza in experiment 2; Fig. 2.3). The relative increase in individual grain weight ranged between 5 and 15% in Anza and between 2 and 14% in Soissons. In addition, we found no relationship between the control grain weight size and the magnitude of the non-significant change (R^2 0.005 and 0.21, P>0.10, in experiments 1 and 2, respectively).

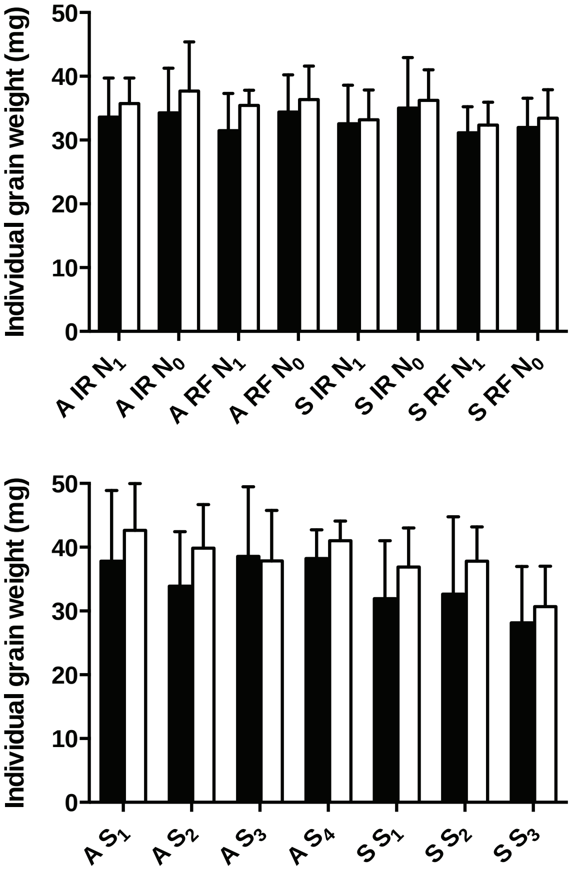

Figure 2.3. Comparison of grain weight in control and trimmed spikes (filled and open bars, respectively) across the range of treatments generated in both experiments for Anza (A) and Soissons (S). Segments on top of each bar stand for the standard deviation.

The same trend was evident when analysing the response of final grain weight of the proximal grains in central spikelets instead of the average of all grains (Fig. 2.4).

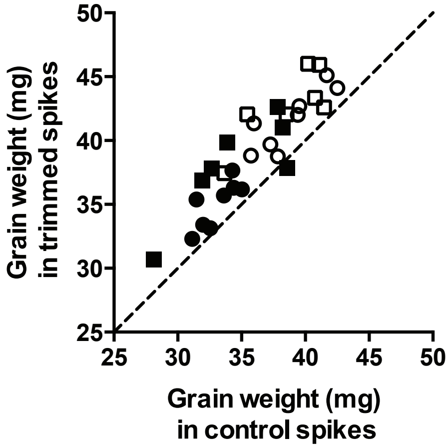

Figure 2.4. Averaged grain weight for all grains in the spikes (closed symbols) and for only the proximal grains in central spikelets (open symbols) in trimmed spikes plotted against the same variable in the control spikes for each background treatment in experiments 1 (circles) and 2 (squares). Dashed line represents the 1:1 ratio.

2.4. Discussion

Faster development rate causing an earlier anthesis in Anza might be the reason why it was not unusual to still find fields sown with this cultivar at the time the experiment was conducted, released more than 30 years before, particularly in the more drought-prone wheat growing areas of Spain. This is because in cases of severe terminal drought Anza would hypothetically have more stable yields due to a partial escape to that stress, as it may be derived from comparative retrospective analyses comparing trends in time to anthesis between countries with terminal or non-terminal drought (for instance between Argentina's Rolling Pampas with drought more frequently occurring during winter months and the western wheat belt of Australia characterised by Mediterranean weather, as illustrated by Araus *et al.* (2002). However, in the rainfed treatments of the present study Anza did not perform better than Soissons, which may be the reason why, unlike in the Mediterranean conditions of Western Australia, wheat breeding in Spain did not consistently reduce the length of the period to anthesis, as revealed by a recent study comparing performance of bread wheats released from the 1940's to the 1990's conducted in the area (Acreche *et al.*, 2008).

The fact that the range of resources provided by the fertilization and irrigation levels in experiment 1 did not affect phenology is in line with the evidences in the literature showing lack of consistent effects of availability of resources on wheat development (see review by Miralles and Slafer, 1999 and references quoted therein).

The more modern cultivar Soissons had higher number of grains per spike than the older one, Anza. This is consistent with what wheat breeders have done almost universally: selecting for improved yield has brought about increases in number of grains with slight reductions in averaged grain weight (*e.g.* Calderini *et al.*, 1999). This sort of opposite trends with breeding has been interpreted as a sign of competition among grains for limited resources, *i.e.* as number of grains is increased the more grains compete for assimilates during grain filling, resulting in smaller individual grains. Most evidence from the literature including many wheat growing regions, but none within wheat growing areas of the Mediterranean basin, are conflicting with this competitive-based interpretation (see discussion in Slafer, 2003).

So these background conditions produced by the combination of a traditional and a modern wheat grown in a wide range of environmental conditions in Mediterranean north-eastern Spain offered a situation suitable to test whether in this region grain growth is more limited by the source than in other regions, as inferred from the barley study of Voltas *et al.* (1997).

In total it seems that in the present study with Mediterranean wheats the results are well in line with those described in the literature for other regions: final grain weight is largely unresponsive to changes in sink size and any eventual response is far from representing a strong competition among grains during grain filling (*e.g.* Borrás *et al.*, 2004). This means that even in Mediterranean wheats photosynthetic capacity of the canopy during grain filling may be in excess of the demands of the grains to be filled at their maximum capacity to store assimilates (Savin and Slafer, 1991; Richards, 1996; Reynolds *et al.*, 2000; 2005).

Consequently the conflicting inferences made in the present study with those provided by Voltas *et al.* (1997), who reported a degree of source limitation during post-anthesis in barley grown in Catalonia may be due to intrinsic differential responsiveness of the two different species or to the difference in the approach used to impose the treatments. Although genotypic differences are possible, most literature in other temperate regions found in barley similar conclusions to those reported in wheat (that yield during grain filling is strongly limited by sink strength; *e.g.* Dreccer *et al.*, 1997). The contrasting results would have been a consequence of different experimental approaches used by the authors to modify the source-sink relationship. While in the present study we modified sink size 10 days after anthesis, Voltas *et al.*, (1997) applied a sink-reduced treatment at anthesis and during the few days immediately after anthesis when potential grain size is being determined in cereals (*e.g.* Calderini *et al.*, 2001). Thus, Voltas *et al.* (1997) might have involuntarily increased sink strength during the effective grain filling period by degraining at anthesis and then as yield would be sink-limited during grain filling, the treated plants with stronger sinks may have outyielded the controls.

Finally, the lack of a significant response of grain weight to trimming, both in central grains (higher potential weight) and the average of the whole spike was not due to a mixture of grain populations of different potential sizes but a true lack of any relevant competition processes among grains limiting their final size during the effective period of grain filling.

In conclusion, it have been shown under quite a wide range of Mediterranean conditions that final grain weight slightly and not significantly responds to the increase in the source-sink ratio. This is indicating that during grain filling period grain yield is mainly sink-limited both to the modern and the traditional cultivar, Anza and Soissons respectively.

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Chapter III
Grain yield determination

Field experiment in Agramunt (wheat crop during pre-anthesis)

Chapter III

3. Grain yield determination

Abstract

As in other cropping areas, in the Spanish Mediterranean region, modern cultivars (with improved potential yield but less stability) outyielded their predecessors under higher yielding environments though under poorer environmental conditions yield did not noticeably differ. However, farmers from the Ebro Valley in Catalonia (north-eastern Spain) tend to grow traditional cultivars over modern ones under the poorest environments. Three field experiments were conducted in the area, where a traditional cultivar (Anza) and a later released one (Soissons) were sown under a range of contrasting nitrogen (N) and water availabilities. The aim was to study grain yield determination and responsiveness to resources availability of a traditional and a modern wheat cultivar under Mediterranean conditions.

Across all the experimental conditions Anza had a shorter pre-anthesis period than Soissons with no consistent differences for the grain filling duration. Grain yield ranged from 0.78 to 7 Mg ha^{-1}, it was responsive to variations in N and water availabilities and strongly correlated with aerial biomass and grain number per unit land area, while unrelated to grain weight and harvest index. Number of grains was in turn associated with spike dry matter at anthesis. Grain yield was similar for Anza and Soissons in all experiments; the traditional cultivar did not consistently performed better than the modern one under low yielding conditions or *vice versa*.

3.1. Introduction

Improvement of wheat yield during the last century has been achieved mainly by increasing harvest index through raising the number of grains both per spikelet and per spike (*e.g.* Austin *et al.*, 1989; Siddique *et al.*, 1989b; Calderini *et al.*, 1999; Shearman *et al.*, 2005; Acreche *et al.*, 2008) with no major changes in above ground biomass (*e.g.* Calderini *et al.*, 1995). As grain number is the main component determining yield (Prystupa *et al.*, 2004; Slafer *et al.*, 2005; Peltonen-Sainio *et al.*, 2007; Zhang *et al.*, 2007; Slafer *et al.*, 2014) yield gains have been usually strongly associated with the number of grains per unit land area (*e.g.* Frederick and Bauer, 1999; Slafer *et al.*, 2005). This has been extensively reported for a relatively wide range of environments (Calderini *et al.*, 1999 and references quoted therein). However, less has been explored under Mediterranean conditions, where cereal yields are commonly restricted by water and heat stresses (that are both frequent and unpredictable) occurring mainly during the last part of the growing season, coinciding with grain filling (López *et al.*, 1996; Acevedo *et al.*, 1999; López-Bellido *et al.*, 2000; Rana and Katerji, 2000; Olesen and Bindi, 2002). This is particularly important as drought and high temperatures during grain filling can reduce grain weight (Loss and Siddique, 1994). Therefore, it could be hypothesized that under these conditions grain yield might be also determined by grain weight.

However, the results obtained in the experiments from the previous Chapter of the present thesis (Chapter II), showed that final grain weight only slightly and mostly not significantly responds to an increase in the source–sink ratio. This is indicating that even under Mediterranean conditions grain yield is mainly sink-limited during the grain filling period, in agreement with results for durum wheat (Pedro *et al.*, 2011). But, as indicated by Acreche and Slafer (2009); modern wheat lines tested in Mediterranean environments tended to be more responsive to increases in source availability (due to their increased number of grains per unit land area) than older ones; increasing moderately grain weight and indicating a sort of source-sink co-limitation. This is similar to what has been reported for other regions as well (Slafer and Savin, 1994; Kruk *et al.*, 1997; Borrás *et al.*, 2004). Therefore, it could be expected that grain yield in a modern wheat cultivar could be not only limited by the number of grains per unit land area but also to some extent determined by grain weight, in comparison with a traditional one.

Modern cereal cultivars with improved potential yield have shown more responsiveness to improved growing conditions but less yield stability (Slafer and Kernich, 1996; Abeledo *et al.*, 2003). However, despite of its higher stability old cultivars do not frequently outyield modern ones under low-yielding environments (Calderini and Slafer, 1999).

Similarly in the Spanish Mediterranean region modern cultivars yielded more than their predecessors when tested under higher yielding environments though under poorer environmental conditions yield did not noticeably differ (Acreche *et al.*, 2008), indicating that modern cultivars in Spain are also less stable due to its improved responsiveness to better environments rather than due to their lower yields under poor conditions. Despite the lack of clear evidence in the literature, farmers from the Ebro Valley area (within the semi-arid region in Catalonia, north-eastern Spain) tend to prefer growing traditional cultivars over modern ones under the poorest environments. Thus, at the time when the experiments were designed and carried out, the farmers with more aversion to the risk frequently chose Anza (a traditional, but semi-dwarf, cultivar) and those with less risk-aversion generally preferred Soissons (more modern and with a putatively higher potential yield than Anza) but nitrogen (N) fertilization management was similar for any of the cultivars sown (Jaume Gregory, Department of Agriculture of Catalonia at the Agramunt Office, personal communication; 2003). There is a lack of information from field experiments under Mediterranean conditions, such as those of the Ebro valley, regarding yield responsiveness when comparing these traditional and modern cultivars theoretically differing in yield stability.

Therefore, the main objective of the present chapter was to study grain yield responsiveness to the main agronomic management practices affecting the availability of resources (N fertilization and irrigation) of a traditional and a modern wheat cultivar under Mediterranean environmental conditions. In addition, another aim was to analyse the main yield components and their importance determining yield under this sort of environments in order to improve management and breeding strategies.

3.2. Materials and methods

3.2.1. Site

Three field experiments were carried out during the 2003-2004; 2004-2005 and 2005-2006 growing seasons (experiments 04; 05 and 06 respectively). All of them were located at Agramunt (lat. 41° 47´17´´ N, long. 1° 5´59´´ E, altitude 337 m) a county within the rainfed agricultural system of the Lleida province, Catalonia, north-eastern Spain. The experiments were always carried out in the most possible realistic conditions: The experiments were performed in a rented farm field and all the agronomic management, beyond the imposition of the treatments, were those used by, and with the machinery of the farmers. While this approach increases the experimental error compared with having plots in an experimental station, it does also increase noticeably the validity of the results for a more straightforward extrapolation to the real world. The soil was classified as a typical Xerorthent in experiments 04 and 05 and typical Xerofluvent in experiment 06 (SSS, 1999).

The previous crop was always wheat (the monoculture of cereals is by far the most common scenario of rainfed agriculture at the region, alike in other Mediterranean areas). Prior to sowing (November), the experimental fields were fertilized with phosphorus and potassium (0-7-14) at a rate of 700 Kg ha^{-1} to avoid deficiencies in these elements. Insecticides, fungicides and herbicides were applied when necessary to avoid biotic stresses, following the recommendations made by their manufacturers in all experiments.

3.2.2. Treatments and experimental design

Two bread wheat cultivars were used: a traditional one, Anza (A) a semi-dwarf wheat released in 1971 and Soissons (S), with putatively higher potential yield, released in 1987 (see full description in Chapter I). The genotypes were chosen aiming to contrast the most widely sown traditional and modern cultivars in the region.

The range of environmental conditions imposed in all the experiments consisted of a combination of two nitrogen fertilization rates and two water availabilities. Fertilization treatments were an unfertilized control (N_0) and a heavily fertilized condition (N_1). Irrigation treatments consisted of a rainfed treatment (RF) or a well-irrigated condition (IR). The aim of these treatments was to create very contrasting conditions of resource availability within each of the three growing seasons (which did also vary in availability of resources for the RF-N0 treatments), in order to study the performance of the cultivars and their responsiveness to widely variable yielding conditions, but not to determine N fertilization or irrigation best practices to establish recommendations.

In experiment 04, the watering treatment consisted of a twice-weekly irrigation of 10 mm each time. The source of N was urea (46-0-0) and the fertilizer was applied around tillering stage (DC 2.0; Zadocks *et al.*, 1974). In experiments 05 and 06, the irrigation treatment was applied once a week with 7-9 or 14-18 mm irrigated each time. The N source was ammonium nitrate (34.4-0-0) and its application was equally split between a first application at 2 leaves stage (DC 1.2) and the second application at the beginning of tillering (DC. 2.2) or at DC 3.1 in experiments 05 and 06, respectively.

In all experiments the irrigation treatment started each season at the beginning of stem elongation (DC 3.1) and continued to maturity. It was suspended in weeks in which it rained considerably. For this purpose we installed a drip irrigation system in the irrigated plots only. As the experiments were carried out in a typically rainfed area, the water was brought to the field in a water track and pumped into the main pipe connected to the lateral tubes of the drip irrigation system installed in the plots.

The detailed sowing data, initial soil water content, seasonal rainfall, irrigation water supply and N fertilization rates are summarized for each experiment in Table 3.1. In order to calculate the initial seeding rate in each experiment, the germination capacity of the material, the weight of the grains and the field soil conditions were considered, aiming to achieve a final plant density of approximately 250 plants m^{-2} (Table 3.1).

Table 3.1. Sowing dates and densities; water supply (soil water content at sowing, total rainfall from sowing to maturity and irrigation) and nitrogen treatments in experiments 04, 05 and 06.

Experiment	**Sowing date**	**Sowing density (viable seeds m^{-2})**	**Initial soil water content (mm)**	**Seasonal rainfall (mm)**	**Irrigation (mm)**	**Fertilization (Kg$_N$ ha^{-1})**
04	21-Nov-03	390	282	305	0/120	0/200
05	16-Nov-04	300	167	163	0/150	0/200
06	30-Nov-05	300	227	95	0/120	0/150

The experiments were arranged in a split-block, split-plot design with three replicates. Main plots consisted of the combination of the wheat cultivars, sown in strips across the two water regimes in the entire replication. The subplots of 22 rows, 0.135 m apart and 5 m long were assigned to the different nitrogen doses.

3.2.3. Sampling and analysis

Phenological stages were determined through frequent (at least once weekly) inspection of all plots, the duration of phases was expressed in thermal time units (ºCd) using a base temperatures of 0 ºC for pre-anthesis development (as standard in most agronomic studies; *e.g.* Acreche and

Slafer, 2009; Ferrante *et al.*, 2013) and 8.2 ºC for the grain filling period (Slafer and Savin, 1991).

In all experiments, aerial biomass dry matter production was determined at anthesis (DC 6.5) and maturity (DC 9.2). The samples were divided into leaf laminae, stems (including leaf sheaths) and spikes. In order to estimate dry matter production the whole material was oven-dried at 65ºC for 2 days.

At maturity, spikes were freshly counted and then threshed in order to assess grain yield and its main components, average individual grain weight (IGW) and grain number per unit land area. Harvest index (HI) was calculated as the ratio between grain yield and total aerial biomass. For assessing the determination of grain number we calculated the efficiency with which the available resources at anthesis were used to set grains, as the number of grains set per unit of spike weight at anthesis *(i.e.* fruiting efficiency; Gonzalez *et al.*, 2011a; Pedro *et al.*, 2011; Ferrante *et al.*, 2012). In experiment 06, spike dry weight at anthesis was estimated using the chaff dry weight at maturity.

The environmental index was calculated as the average yield of the two cultivars for every evaluated environment (Finley and Wilkinson, 1963) to compare the yield responsiveness of the two cultivars.

The gravimetric water content was measured in each block before sowing for a general field characterization (Table 3.1). For this purpose, soil samples were obtained for the layers 0-25, 25-50, 50-75 and 75-100 cm depth. From these soil samples 100 g were weighed fresh and later placed in a forced-air oven at 105ºC during a minimum of 2 days and weighed again to assess the humidity content. The volumetric water content was obtained using the gravimetric soil water content and soil bulk density at each depth.

Rainfall, incident global radiation and minimum and maximum temperature data were recorded daily at the nearest meteorological station of the experimental sites within the Catalonian Agrometeorological network. The phothermal quotient (Q) was calculated for the critical period for grain number determination, from 20 days before to 10 days after anthesis (Fischer 1985; Savin and Slafer 1991). Q is the ratio between intercepted daily radiation and mean temperature above a base temperature of 4.5 ºC (Fischer 1985).

For each trait analysed, analysis of variance (ANOVA) was performed for each year independently, using the General Linear Model (GLM) procedure of SAS by using the correct error term to evaluate each factor. Standard errors of the mean (SEM) were used to indicate variance of the measurements. Linear regression was used to assess the relationship between traits.

3.3. Results

3.3.1. Weather conditions

The distribution of rainfall through the three growing seasons responded to the typical Mediterranean pattern, being quite erratic but with rainfall scarcity in June and July, coinciding with the grain filling period for wheat. In experiment 04, 90% of rainfall was available for the crop vegetative and reproductive phases; 70 % up to jointing (DC 3.1) and 20% between jointing and anthesis (D6.5). The remaining 10% rained towards the end of grain filling period (*c.* DC 8.7; Fig. 3.1a). In experiment 05, almost 50% of the season rainfall occurred before jointing and the other half at the beginning of the grain filling period (DC 7.3), with virtually no precipitations between jointing and anthesis (Fig. 3.1b). Finally, in experiment 06; 88% of the rainfall was accumulated at the very beginning of the season, before jointing, being very scarce during the rest of the cycle (Fig. 3.1c).

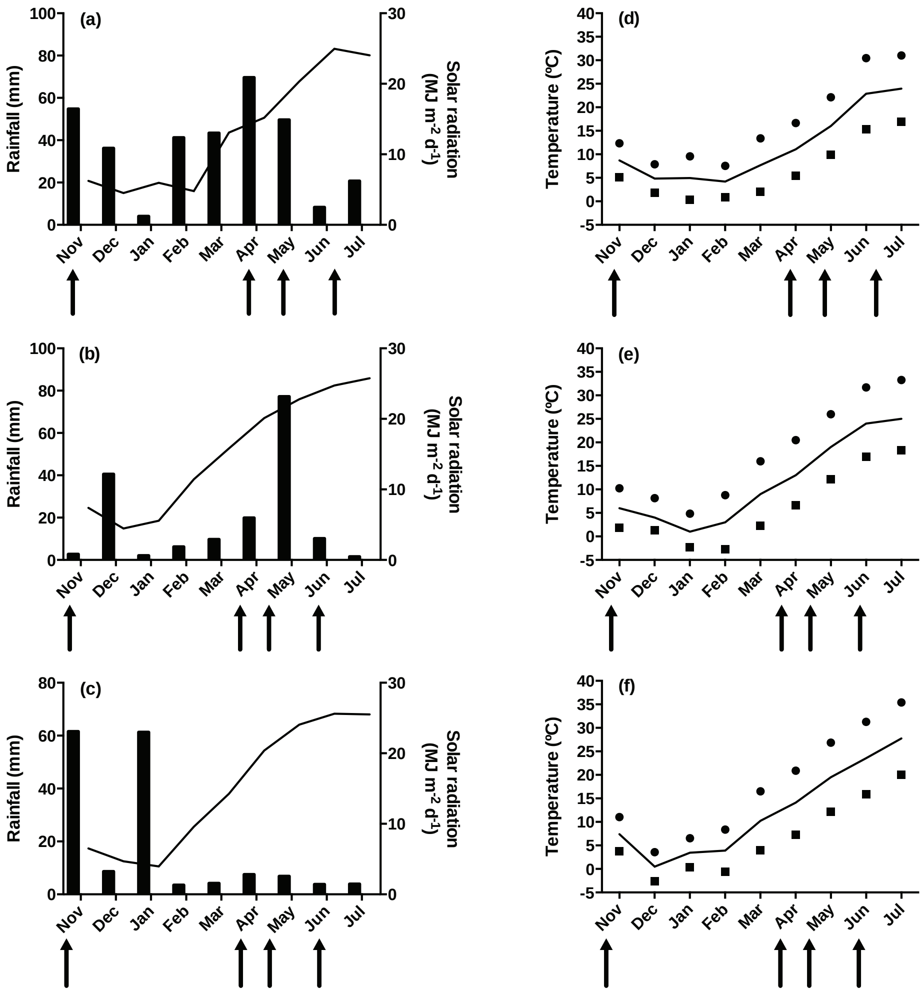

Figure 3.1. Left panels: monthly accumulated rainfall (bars) and monthly averaged daily solar radiation (lines) from sowing to maturity in experiments 04 (a), 05 (b) and 06 (c) respectively. Right panels: Daily maximum (circles), minimum (squares) and average (lines) temperatures averaged over monthly periods from sowing to maturity in experiments 04 (d), 05 (e) and 06 (f) respectively. Arrows at the bottom indicate average date for sowing, jointing (DC 3.1), anthesis (DC 6.5) and maturity (DC 9.2) for the two cultivars from left to right.

In addition, daily average global radiation was lower in experiment 04 than during the two following growing seasons, especially from February to June (Fig. 3.1) when the average for experiment 04 was *c.* 16 MJ m^{-2} d^{-1} against the *c.* 19 MJ m^{-2} d^{-1} reached for the same period in the following experiments.

There were no major differences in temperatures between growing seasons, the average, ranging from 0.5 to 28 °C; but during experiment 04 temperatures tended to be higher from sowing to around mid tillering (DC 2.4-2.5) and lower around anthesis compared to the other two growing seasons (Fig 3.1, right panels).

3.3.2. Crop phenology

The traditional cultivar (Anza) had a shorter thermal time to anthesis than Soissons along the three experiments (Fig. 3.2a). Anza pre-anthesis duration was 124; 110 and 78 °Cd shorter than Soissons in experiments 06, 04 and 05, respectively. Only in one out of the 12 cases, Anza and Soissons had similar pre-anthesis durations (experiment 05; fertilized and irrigated treatment) (Fig. 3.2a).

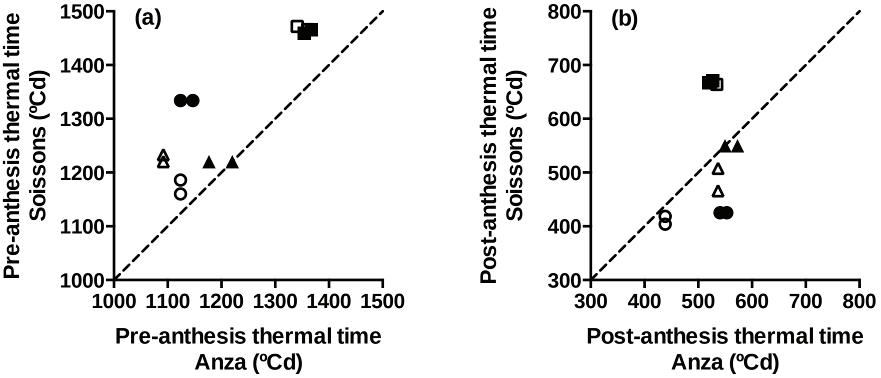

Figure 3.2. Relationship between Soissons and Anza thermal time to anthesis (a) and thermal time from anthesis to maturity (b) in experiments 04 (squares), 05 (triangles) and 06 (circles) respectively. Closed and open symbols indicate irrigated and rainfed treatments respectively. The dashed line represents de 1:1 relationship.

While in experiment 04, the differences in thermal time to anthesis were due to differences between cultivars; in the following two seasons there were also differences between the irrigation treatments. In experiment 05, Anza under rainfed conditions reached anthesis earlier than the irrigated treatment (1092 °Cd in RF and 1199 in IR; SEM 7.54; P<0.01). Meanwhile, in the third year the same trend was observed for Soissons (1173 °Cd in RF and 1334 °Cd in IR; SEM 12.78; P<0.01; Fig. 3.2a). Differences between cultivars were not consistent across the experiments for the grain filling duration (Fig. 3.2b).

3.3.3. *Grain yield*

Across experiments and treatments, grain yield ranged from 0.78 (Anza RF N_0 in experiment 05) to 7 Mg ha^{-1} (Soissons IR N_1 in experiment 04), reflecting the extremely contrasting conditions of the growing seasons (Fig. 3.3).

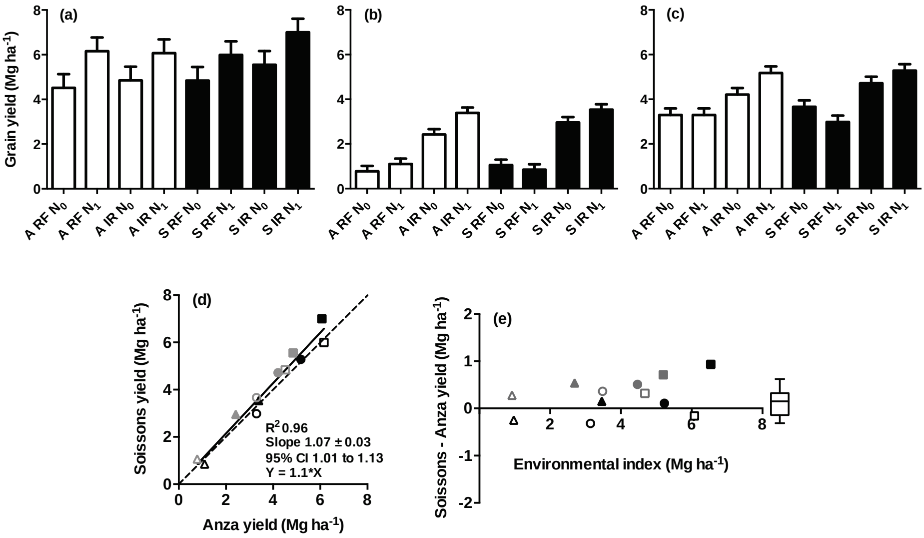

Figure 3.3. Top panel: grain yield for Anza (A, open bars) and Soissons (S, solid bars) for each combination of N and water availability: rainfed unfertilised (RF N_0), rainfed fertilised (RF N_1), irrigated unfertilised (IR N_0) and irrigated fertilised (IR N_1) in experiments 04 (a), 05 (b) and 06 (c) respectively. The error bars represent the SEM. Bottom panels: Soissons against Anza grain yield (d), the dashed line represents the 1:1 relationship; the black line and regression values correspond to the lineal regression forced through the origin with 95% confidence intervals (CI). Grain yield difference between Anza and Soissons against the environmental index (e), in experiments 04 (squares), 05 (triangles) and 06 (circles) respectively. The open and closed symbols represent RF and IR, grey and black N_0 and N_1 respectively. At the right, yield differences are plotted in a box and whisker graph. The box extends from the 25th to 75th percentiles, the line in the middle of the box is plotted at the median and the whiskers go down to the smallest value and up to the largest.

In experiment 04 the effect of N fertilization in biomass production and grain yield was remarkable and statistically significant ($P<0.01$; Fig. 3.3a). As the season started with *c.* 300 mm of water stored in the soil and was rather rainy (Table 3.1), the irrigation did not result in clear yield differences. Average grain yield was 6.31 and 4.94 Mg ha^{-1} for N_1 and N_0 respectively (SEM 0.31).

In contrast, in experiment 05, the most significant differences both in aboveground biomass and grain yield were due to irrigation ($P<0.01$). Although less significant, there were also yield increments for the N_1 treatments ($P<0.05$; Fig. 3.3b). Both N_0 and N_1 treatments yielded more under IR than under RF regimes ($P<0.001$). However, only under IR, N_1 outyielded N_0, averaging 7.88 and 5.67 Mg ha^{-1} of total biomass (SEM 0.20) and 3.46 and 2.69 Mg ha^{-1} of grain yield respectively (SEM 0.17; Fig. 3.3b).

In Experiment 06 the only differences in grain yield were due to the irrigation treatments ($P< 0.1$), the irrigated crop yielded 1.46 Mg ha^{-1} more than under RF (SEM 0.31; Fig. 3.3c).

When compared, Anza and Soissons grain yields, despite their putative differences in yield potential and yield stability, they did not differ consistently (Fig. 3.3d), there was not a clear trend for Soissons to outyield Anza in high yielding conditions, nor for Anza to outyield Soissons under low yielding conditions. However, overall the conditions Soissons grain yield tended to be marginally higher than Anza in all the experiments (Fig. 3.3e).

3.3.4. Yield components

In experiment 04, yield components, grain number per unit land area and grain weight presented differences in response to the fertilization treatment ($P<0.01$ and $P<0.05$ respectively). It was particularly significant in the case of grain number per unit land area that increased from *c.*15 to 22 grains m^{-2} (SEM 1.15; Fig. 3.4a and 3.4b). The response explored in the average weight of the grains was much smaller and unrelated to yield (Fig. 3.4c). Dry matter partition to the grain presented no significant differences across the treatments, averaging 33.4 % (Fig. 3.4g).

In experiment 05, grain number followed the same trend; increasing from *c.* 3 to 10 grains m^{-2} (under RF and IR conditions, respectively) ($P<0.01$; SEM 0.47; Figs. 3.4a and 3.4b), while grain weight did not present significant relationship with yield in this experiment either (Fig.3.4c). Although unrelated to yield, there was a significant difference between cultivars in average grain weight; 28.04 *vs.* 32.46 mg grain^{-1} in Soissons and Anza, respectively ($P<0.05$; SEM 0.38; Fig. 3.4d). As in experiment 04, the average HI remained quite stable across the treatments (Fig. 3.4g).

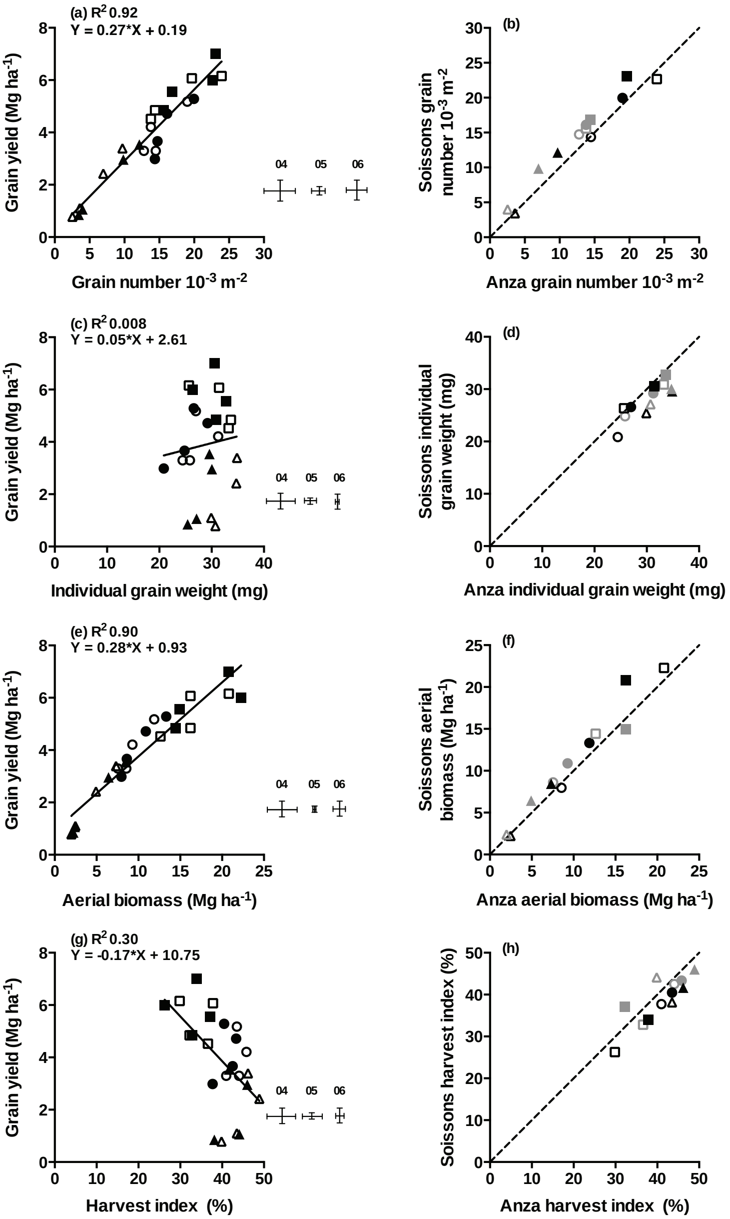

Figure 3.4. Left panels: grain yield regressed against (a) grain number per unit land area, (c) individual grain weight, (e) aerial biomass production (g) and harvest index (g) for Anza (open symbols) and Soissons (closed symbols) in experiments 04 (squares); 05 (triangles) and 06 (circles) respectively. The error bars represent the SEM values for experiments 04, 05 and 06 respectively. Right panels: Anza *vs*. Soissons grain number per unit land area (b), individual grain weight (d), aerial biomass production (f) and harvest index (h). The open and closed symbols represent rainfed and irrigated, grey and black unfertilised and fertilised treatments respectively. The dashed line accounts for the 1:1 relationship.

In experiment 06, average grain number did not change significantly for either cultivar or environmental treatment. Individual grain weight varied mainly with the irrigation ($P<0.05$) and N levels ($P<0.001$) ranging from 24.00 to 28.51 mg grain^{-1} for RF and IR and from 24.71 to 27.79 N_1 and N_0 respectively (SEM 0.4) (Figs. 3.4c and 3.4d). Finally, HI was not affected by the environmental treatments (Fig. 3.4g); however, Anza tended to have higher HI than Soissons throughout most conditions (Fig. 3.4h).

Considering all experiments and treatments together, grain yield was strongly and positively correlated with grain number per unit land area (R^2 0.92; $P<0.0001$; Fig. 3.4a) and completely unrelated to the average weight of the grains (R^2 0.008; $P>0.1$; Fig. 3.4c). Grain yield was also closely related to total aerial biomass at maturity (R^2 0.90; $P<0.001$; Fig. 3.4e), while no relationship was found with HI (R^2 0.30; $P>0.01$; Fig. 3.4g); indicating that yield responsiveness was more related to biomass production and the environmental factors affecting the resource capture more than to partitioning of the biomass to the grains.

Number of grains was positively and significantly associated with spike dry matter at anthesis both for Anza and Soissons. There were no clear differences in spike dry weight at anthesis between the traditional and the modern cultivar (Fig. 3.5a). Grain number per unit land area was also explained by the photothermal quotient (Q) during the critical period for its determination (20 days prior and 10 days after anthesis), which did not vary clearly between cultivars either (Fig. 3.5b).

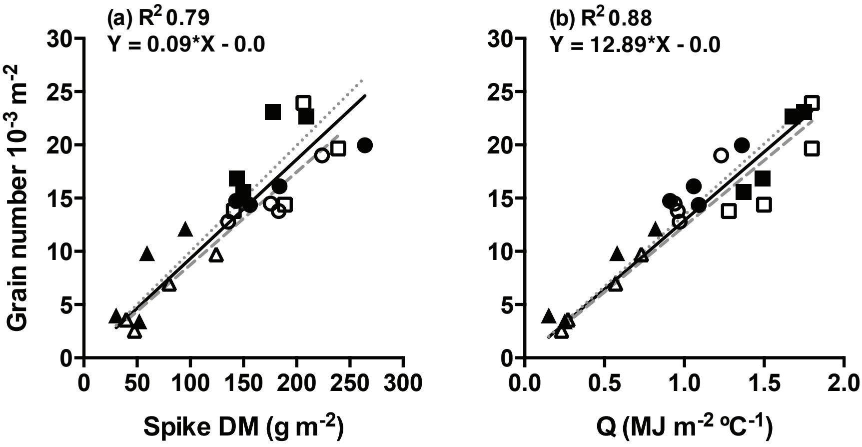

Figure 3.5. Grain number per unit land area against (a) spike dry matter at anthesis (estimated by the chaff weight at maturity in experiment 06) and (b) photothermal quotient (Q) for Anza (open symbols) and Soissons (closed symbols) in experiments 04 (squares), 05 (triangles) and 06 (circles) respectively. The dashed grey line represents Anza linear regression; the dotted grey line Soissons's and the black line and regression values correspond to the pooled data regression forced through the origin.

On average, Soissons had higher fruiting efficiency (116 grains g_{spike}^{-1}) than Anza (90 grains g_{spike}^{-1}) for all the experiments (Fig. 3.6). There were no significant effects of the environmental treatments for this trait.

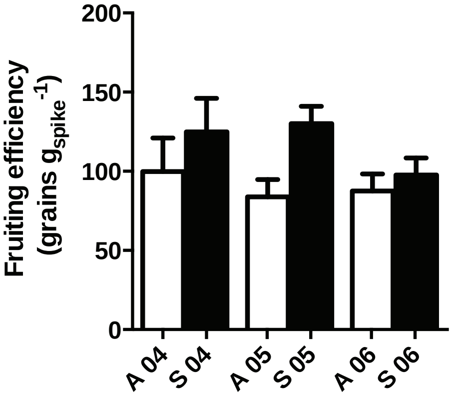

Figure 3.6. Average fruiting efficiency for Anza (A, white bars) and Soissons (S, black bars) in experiments 04, 05 and 06. The error bars represent the SEM.

3.4. Discussion

Anza, the traditional cultivar, was constitutively earlier-flowering than the modern cultivar. As this is an expected pattern of cultivar behaviour, in this rainfed Mediterranean area of Catalonia, the less risky farmers tend to choose the traditional cultivar over the modern one because its shorter pre-anthesis period may result in avoiding thermal stress during grain filling period. However, grain yield resulted similar between Anza and Soissons in all experiments (Fig. 3.3d), although there was great inter-annual yield variability due to environmental conditions (years x treatments), which produced a wide range in grain yield in the two wheat cultivars (Figs. 3.3a, b and c). Therefore, the data of this study cannot confirm the hypothetically better performance of the traditional cultivar over the modern one under more stressful environments under Mediterranean environments. In fact, this was also tested by examining other studies available in the area using the same cultivars (Fig. 3.7).

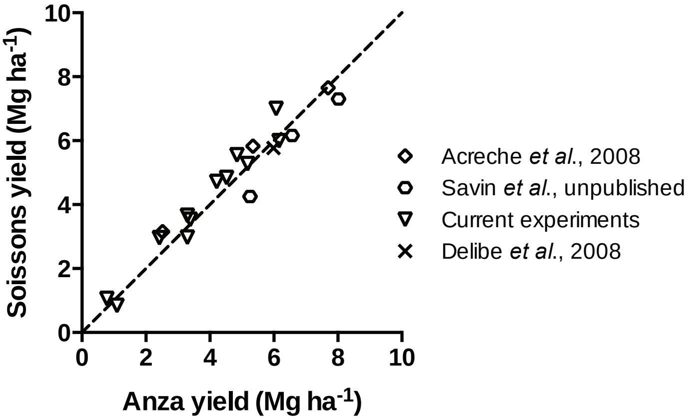

Figure 3.7. Soissons against Anza grain yield for the current experiment and other studies in Spain: Acreche *et al.*, 2008; Savin *et al.*, unpublished and Delibe *et al.*, 2008. The dashed line represents the 1:1 relationship.

Across the wide range of conditions explored in the study, the number of grains per m^2 mainly determined grain yield. Although this relationship is a commonplace in wheat science (Slafer, 2003; Fischer, 2011; Sadras and Slafer, 2012), a stronger relationship with IGW could have been expected in this case in which grain filling is exposed to varying degrees of stresses, particularly for the modern cultivar, which is constitutively later-flowering than the traditional one. Moreover, not only grain weight was not correlated to grain yield; but also it was not negatively correlated to grain number, as it is frequently the case (*e.g.* Acreche and Slafer, 2006). This reinforces the idea discussed in the previous chapter: that even in Mediterranean conditions (*i.e.* grain filling frequently exposed to terminal stresses), grain weight is not limited by resource availability during grain filling and there is no strong competition for assimilates between the growing grains.

In turn, grain number per unit land area has been largely explained by the spike dry weight at anthesis to the same extent in both cultivars, as previously reported by other authors (Prystupa *et al*, 2004; Pedro *et al*, 2011; Ferrante *et al.*, 2012). However, fruiting efficiency has been higher for Soissons, as expected for a modern cultivar, showing also some limited degree of trade-off with the grain size (Fischer, 2011): although not always significant, Soissons tended to have lighter grains than Anza, supporting the notion that the difference in grain size between cultivars may be more constitutive than related to sink strength, as seen in Ferrante *et al.* (2012). Besides, modern cultivars, have increased grain number by increasing the proportion of grains with lighter potential weight (*i.e.* tiller's spike and distal grains, Acreche and Slafer, 2006), reducing the mean grain weight, a fact that is as well backing up the concept that assimilates availability generally exceeds the sink demand.

3.5. References

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Chapter IV

Nitrogen and water use and use efficiency

Field experiment in Agramunt (wheat crop during post-anthesis)

Chapter IV

4. Nitrogen and water use and use efficiency

Abstract

In semi-arid regions water availability is one of the main factors limiting crop grain yield and its response to nitrogen (N). Thus, a better understanding of the relationships among N, water and the main physiological traits related to grain yield under Mediterranean environments, would be useful to management and breeding to achieve higher yields. Three field experiments were conducted to compare N and water use and use efficiencies (NUE and WUE respectively) and their impact on yield determination in a traditional (Anza) and a modern (Soissons) wheat cultivar under Mediterranean conditions.

N and water use and their efficiencies presented similar range of values both for the traditional and the modern cultivar and were limited by N and water supply. N uptake occurred mainly along the pre-anthesis period, while grain N concentration was generated chiefly by translocation from the vegetative organs to the grain. Pre-anthesis N uptake, water use, NUE and WUE were positively related to grain yield. Both uptake (UpE) and utilization efficiency (UtE) explained changes in NUE, whilst grain yield variations were more linked to UpE than to UtE.

Grain yield per unit of water used in all the tested cases fell below the maximum WUE upper limit attainable for semi-arid conditions indicating that there is scope to further improvements in yield-related traits under Mediterranean environments.

4.1. Introduction

In semi-arid regions water availability is considered to be the main factor limiting crop growth and yield response to nitrogen (N) application depending on the given agronomic management and environmental conditions (Garabet *et al.*, 1998; Asseng *et al.*, 2001; Angás *et al.*, 2006), being N one of the most important nutrients related to cereal production which plays a key role in achieving high yields. However, Passioura (2002) showed that in semi-arid environments of Australia, the response to N was more limited by other factors such as root diseases, weeds and nutrients scarcity than by water availability. In this environment the increments in wheat yields were achieved by fertilising with N, after root diseases were controlled by rotations with broad-leaf break crops (Angus, 2001). In a later comprehensive data review, Passioura and Angus (2010) showed that actual yields achieved by farmers were below the expected water-limited benchmark yield, indicating that there are indeed many other agronomical factors determining them and that there is scope to further improvement in water use efficiency (WUE). Thus, a better understanding of the relationships among N, water and the main physiological traits related to grain yield, in the always-erratic Mediterranean environments, would be a useful approach to design management and breeding strategies.

It is widely accepted that about 80% of the total N uptake in cereals occurs before anthesis in most cropping areas, indicating that grain N accumulation during grain filling is obtained mainly by translocation, particularly under water-limited conditions. In line with this, Smith *et al.* (2000) showed that for both fertilised and non-fertilised wheat the accumulation of N ceased before anthesis. On the other hand, post-heading N uptake can be stimulated under favourable rainfall conditions (*e.g.* Delogu *et al.*, 1998). Under optimal growth conditions there were both post-anthesis N uptake as well as N remobilization related to higher yields in durum wheat (Arduini *et al.*, 2006). Whereas, in experiments carried out in northern European conditions, wheat N content at maturity was more linked with high N uptake post-anthesis than with N translocation from vegetative parts to the grains when comparing with others winter cereals as barley and oats (Muurinen *et al.*, 2007). However, there are few evidences about N uptake during grain filling in Mediterranean environments and further investigation is needed to explore the source of N determining the yield in semi-arid regions characterized by frequent post-anthesis stresses.

It has been widely proven for winter cereal crops that there is no additional grain yield response to N fertilizer supply beyond certain point (*e.g.* Sylvester-Bradley and Kindred, 2009; Pask *et al.*, 2012). Previous works in Mediterranean areas found higher cereal yields in response to N fertilization but, as expected, no extra increments when increasing N rates above certain point (*e.g.* López-Bellido and López-Bellido, 2001; Cossani *et al.*, 2009); and no response in years when water becomes severely limiting (López-Bellido *et al.*, 2000; Garrido-Lestache *et al.*, 2004; Garrido-Lestache *et al.*, 2005). The trend between N and grain yield may be suggesting N luxury consumption that naturally, leads to a negative relationship between nitrogen use efficiency (NUE) and N rates (López-Bellido and López-Bellido, 2001; Angás *et al.*, 2006); NUE diminished as N fertilizer rates increase (and dry matter increases).

NUE can be partitioned into two mains components; uptake efficiency (UpE) as the ratio of crop N uptake to the N supply and utilization efficiency (UtE) as the ratio of grain yield to the N uptake (Moll *et al.*, 1982). Ortiz-Monasterio *et al.* (1997) found that UpE was the predominant component explaining NUE when N was low, whereas the importance of UtE increased as the N rate did. In contrast, Gaju *et al.* (2011) reported that UtE was the component more relevant to changes in NUE under both low and high N conditions. However, further understanding of the driving force of NUE is required for semi-arid regimes. Cossani *et al.* (2012) found, in the same area of the present study, that NUE was equally related to both components.

N uptake and translocation dynamics along the crop growing season could affect the relative contribution of UpE and UtE to NUE and as a consequence, the management and selection criteria associated to it.

In several studies worldwide, genetic gains in NUE were explained by variations in UpE (under low N supply), by UtE (under high N supply) or approximately equally by both (Foulkes *et al.*, 2009 and references quoted therein), but again, there is a lack of evidence in semi-arid conditions.

Further knowledge of the crop NUE and WUE efficiencies under semi-arid conditions is critical for designing management practices, for identifying outstanding traits to improve them and for developing or selecting more efficient cultivars. There are previous results on Mediterranean regions comparing barley and wheat (Albrizio *et al*, 2010; Cossani *et al.*, 2012) and bread and durum wheat (Marti and Slafer, 2014). However, as far as we are aware there are almost no previous results showing differences in NUE and WUE between a traditional and a modern bread wheat cultivar under Mediterranean conditions.

Consequently, the aim of the this work was to compare the nitrogen and water use and use efficiencies in an traditional and a modern wheat cultivar as an approach to unfold their impact on yield determination under Mediterranean conditions.

4.2. Materials and methods

4.2.1. Site, treatments and experimental design

Three field experiments were carried out during 2003-4, 2004-5 and 2005-6 growing seasons (experiments 04; 05 and 06 respectively). The site, treatments and experimental design were the same as described in chapter III.

4.2.2. Sampling and analysis

Phenological stages determination, aerial biomass production at the beginning of stem elongation (DC 3.1), anthesis (DC 6.5) and physiological maturity (DC 9.2), grain yield and its main components were also assessed as developed in chapter III.

Aerial biomass sampled at anthesis and physiological maturity in all experiments and also at the beginning of stem elongation in experiments 05 and 06 was grounded to analyse for N concentration (%) on stems + sheath, leaf blades, spikes (at anthesis) and grains (at maturity) by near infrared reflectance (NIR).

Plants N uptake (*i.e.* N use) was calculated as the product of the aerial biomass and its N%. Each season, some samples were selected to determine N content by the Dumas combustion Method in order to calibrate with NIR method.

Mineral content of soil N (NO_{-3}) was measured at maturity to determine the remaining soil N using the Nitracheck reflectometer instrument. In order to contrast the applied method, in every experiment, a subset of soil samples was sent to a soil analysis laboratory (Applus Agroambiental, S.A.) where N content was determined by a segmented flux auto-analyser.

The Nitracheck was later calibrated by regressing its determination against the laboratory ones. The determination coefficient was in all experiments higher than 0.9.

Nitrogen use efficiency parameters were calculated following Moll *et al.* (1982) as:

•***N uptake efficiency (UpE)*** as the ratio between aerial biomass (bm) N uptake at maturity to total N supply. The N supply was calculated as the crop N at maturity plus the soil remaining NO_{-3} at maturity (Kg_{Nbm} $ha^{-1}/Kg_{Nsupply}$ ha^{-1}).

•***N utilization efficiency (UtE)*** as the ratio of grain yield or aerial biomass at maturity to aerial biomass N uptake at maturity (Kg_{grain} ha^{-1}/Kg_{Nbm} ha^{-1}; Kg_{bm} ha^{-1}/Kg_{Nbm} ha^{-1}).

•***N use efficiency (NUE)*** as the product of UpE by UtE (*i.e.* the ratio of grain yield or aerial biomass to total N supply (Kg_{grain} $ha^{-1}/Kg_{Nsupply}$ ha^{-1}; Kg_{bm} $ha^{-1}/Kg_{Nsupply}$ ha^{-1}).

Seasonal water use (WU) in each experiment was calculated as the initial soil water content (mm) + accumulated precipitation (mm) + accumulated irrigation (mm) – soil water content at harvest (mm).

Water use efficiency (WUE) was calculated as the ratio between grain yield or total aerial biomass at maturity and water use.

Gravimetric water content was measured before sowing and again at harvest in order to calculate WU and WUE, following the methodology described in chapter III.

Statistical analysis was performed as described in chapter III.

4.3. Results

4.3.1. N concentration and N uptake

Across the whole range of environmental conditions explored in the 3 experiments at jointing, anthesis and maturity, N concentration (N%) and N uptake (Kg_N ha^{-1}) were, in general, similar for Anza and Soissons (Fig.4.1).

N concentration remained approximately the same from anthesis to maturity in experiment 04 (0.99 % and 0.91% respectively; SEM 0.04) while in experiments 05 and 06 decreased significantly along jointing, anthesis and maturity (Figs. 4.1a; b and c).

N uptake did not varied significantly after anthesis in experiments 04 and 06, suggesting that N uptake occurred mainly during pre-anthesis (Figs.4.1d; e and f). However, experiment 05 was the only case where there was a significant post-anthesis N uptake (Figs. 4.1e and f).

In general, N% and N uptake for both Anza and Soissons increased significantly in response to N fertilization in almost every stage of development. However, the magnitude of the response was higher for N uptake than for N concentration that remained more stable across the environmental treatments in experiments 05 and 06 and totally irresponsive in experiment 04. During the second and third seasons, N concentration increased significantly at anthesis and maturity in response to fertilization, while N uptake was higher under fertilization at every stage in the three experiments. In the second season N uptake was also significantly enhanced by irrigation from anthesis onwards (Figs. 4.1g and h).

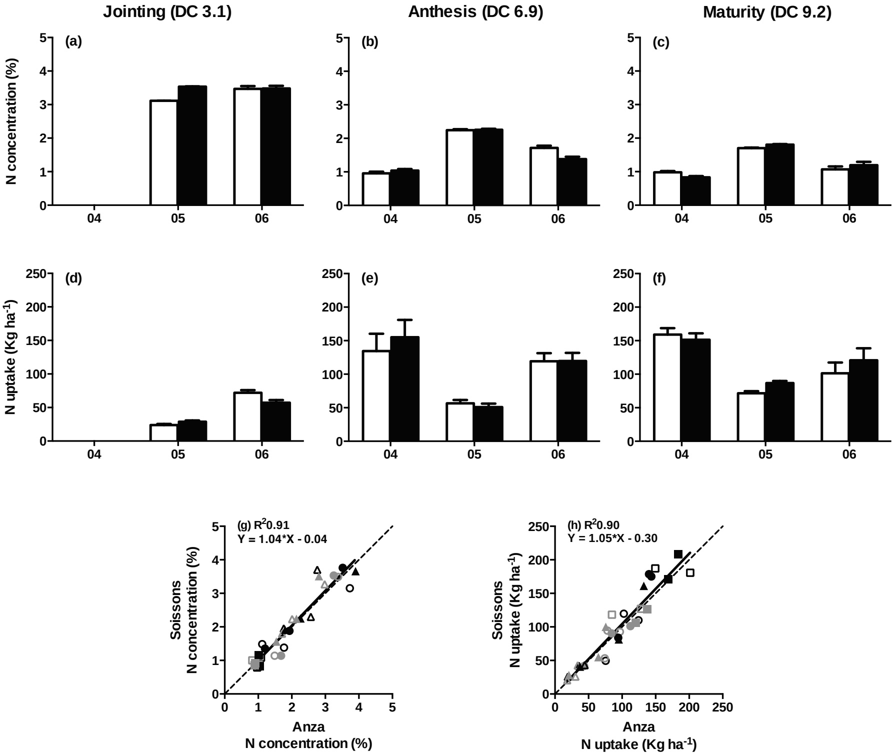

Figure 4.1. Top panel: aerial biomass N concentration averaged across N and water availabilities at jointing (a) in experiments 05 and 06 and at anthesis (b) and maturity (c) in experiments 04, 05 and 06 respectively; middle panel: aerial biomass N uptake averaged across N and water availabilities at jointing (d) in experiments 05 and 06 and at anthesis (e) and maturity (f) in experiments 04, 05 and 06 respectively for Anza (open bars) and Soissons (solid bars). The error bars represent the SEM. Bottom panel: Soissons *vs.* Anza N concentration (g) and aerial biomass N uptake (h) for each combination of N and water availabilities at the same stages as above. Open and closed symbols represent rainfed and irrigated; grey and black unfertilised and fertilised treatments respectively. The dashed line accounts for the 1:1 relationship and the solid line for the linear regression.

Total nitrogen uptake (*i.e.* N content at maturity) was the highest in experiment 04, intermediate in experiment 06 and the lowest in experiment 05 (Fig. 4.1f). In experiment 04, it was significantly increased by nitrogen fertilization, accounting for 180.51 and 129.79 Kg_N ha^{-1} for N_1 and N_0 respectively ($P<0.05$; SEM 9.45; Fig. 4.1h). During the second season; the IR plots presented the highest N uptake; being irrigation the main factor affecting its variability: under IR, N uptake was on average 117.43 Kg_N ha^{-1} *vs.* 40.93 Kg_N ha^{-1} for RF ($P<0.01$; SEM 3.84); although slightly less significant, there was again response to N fertilization (95.23 and 63.12 Kg_N ha^{-1} for N_1 and N_0 respectively; $P<0.01$; SEM 2.91; Fig. 4.1h). In experiment 06 fertilization increased N uptake from 86.92 to 135.27 Kg_N ha^{-1} ($P<0.05$; SEM 12.65; Fig. 4.1h).

As for the grain N content, there were no clear differences between Anza and Soissons's post-anthesis N uptake (Fig. 4.2d). There was a significant negative relationship between grain N uptake and the rest of the plant tissues N content during the post-anthesis period (Fig. 4.2d) indicating that the grain N uptake driver was mainly the N translocation; particularly from the leaf blade and the non-grain spike (Figs. 4.2a and c) and not the post-anthesis N absorption (Fig. 4.2e).

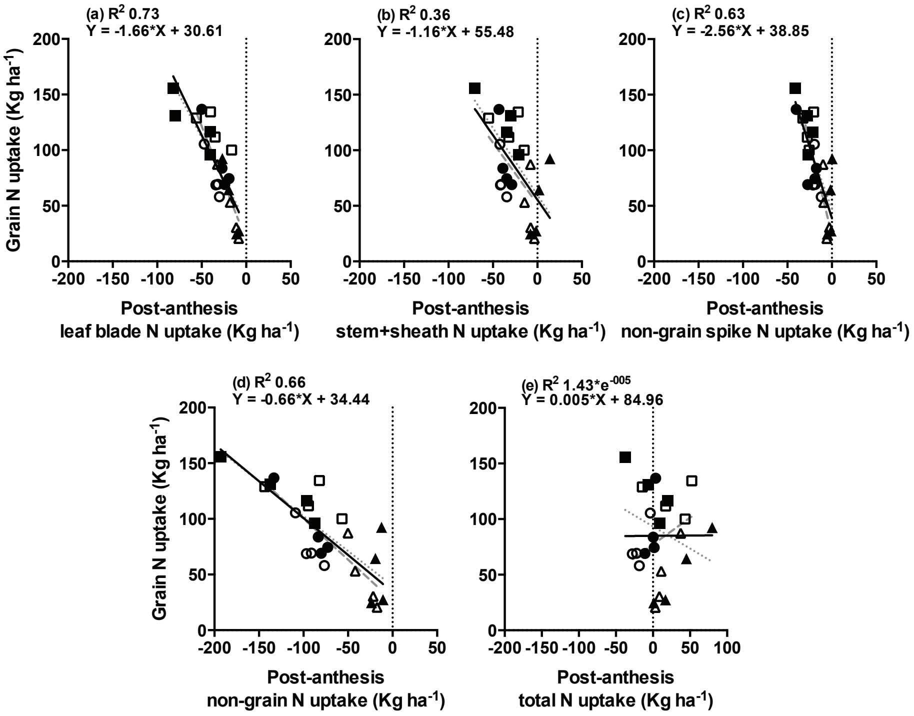

Figure 4.2. Linear regressions for grain N uptake *vs.* post-anthesis N uptake in leaf blades (a); stems+sheaths (b); non-grain spike (spike at anthesis + chaff at maturity; c); the sum of the 3 latter (d) and the post-anthesis total aerial biomass N uptake (e) for Anza (open symbols) and Soissons (closed symbols) in experiments 04 (squares); 05 (triangles) and 06 (circles) respectively. The dashed grey line represents Anza linear regression; the dotted grey line Soissons's and the black line and regression values correspond to the pooled data regression.

Taking into account all the experiments, phenological stages and treatments, as expected, the N concentration diluted during the growth cycle, while N uptake increased as biomass accumulated (Fig. 4.3).

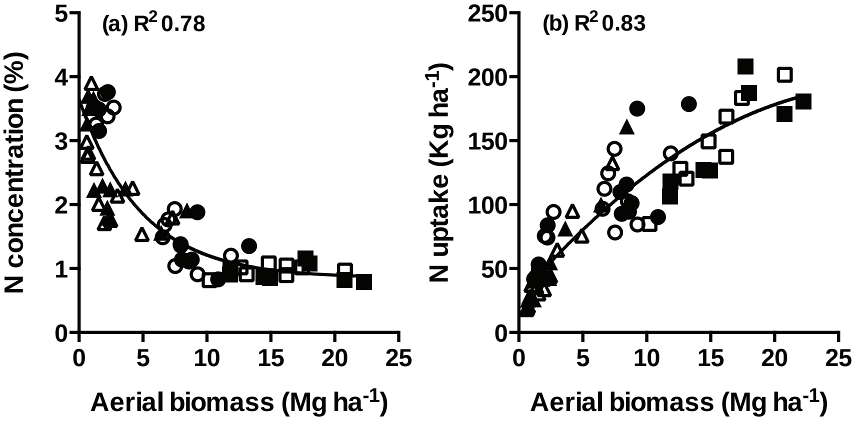

Figure 4.3. (a) Nitrogen concentration and (b) total N uptake as a function of accumulated aerial biomass for Anza (open symbols) and Soissons (closed symbols) at anthesis (DC 6.5) and maturity (DC 9.2) in experiment 04 (squares); and also at jointing (DC 3.1) in experiments 05 (triangles) and 06 (circles) respectively.

In the three experiments, differences in grain number and yield were associated with the total crop N accumulated particularly at anthesis (Figs. 4.4a and c). As previously shown (Fig. 4.1), N uptake occurred mainly during pre-anthesis, therefore; grain yield was related to the pre-anthesis N uptake while there was no relationship with the post-anthesis N uptake (R^2 0.02, data not shown). In the case of experiment 05 there was N uptake after anthesis and the relationship with grain yield was significant (R^2 0.72; Fig. 4.4b).

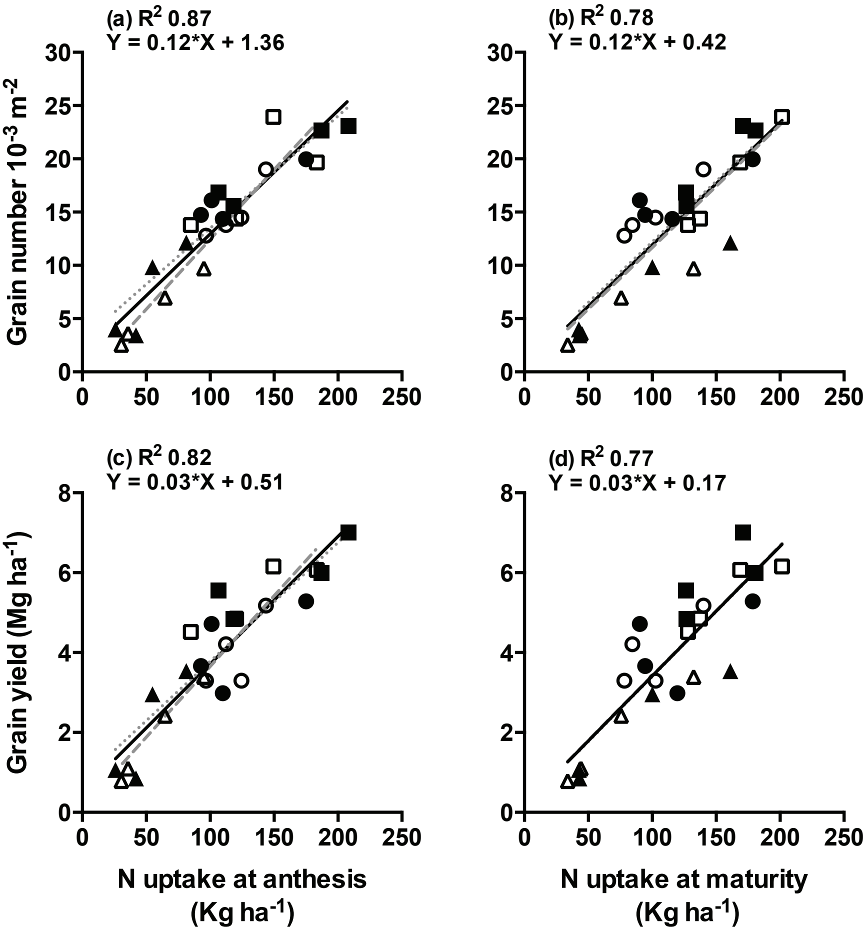

Figure 4.4. Grain number per unit land area regressed against aerial biomass N uptake at anthesis (a) and maturity (b) and grain yield regressed against aerial biomass N uptake at anthesis (c) and maturity (d) for Anza (open symbols) and Soissons (closed symbols) in experiments 04 (squares), 05 (triangles) and 06 (circles) respectively. The dashed grey line represents Anza linear regression; the dotted grey line Soissons's and the black line and regression values correspond to the pooled data regression.

4.3.2. Nitrogen use efficiency and its components

Since aerial biomass and grain NUE were linearly related (R^2 0.89; $P<0.001$) only results for grain yield are reported and illustrated. Across the range of environmental conditions imposed by the treatments and the seasonal variability, grain yield was positively related to NUE (Fig. 4.5a). In turn, both UpE and UtE were positively related with changes in NUE (Figs. 4.5d and e). However, grain yield was more related to UpE than to UtE (Figs. 4.5b and c).

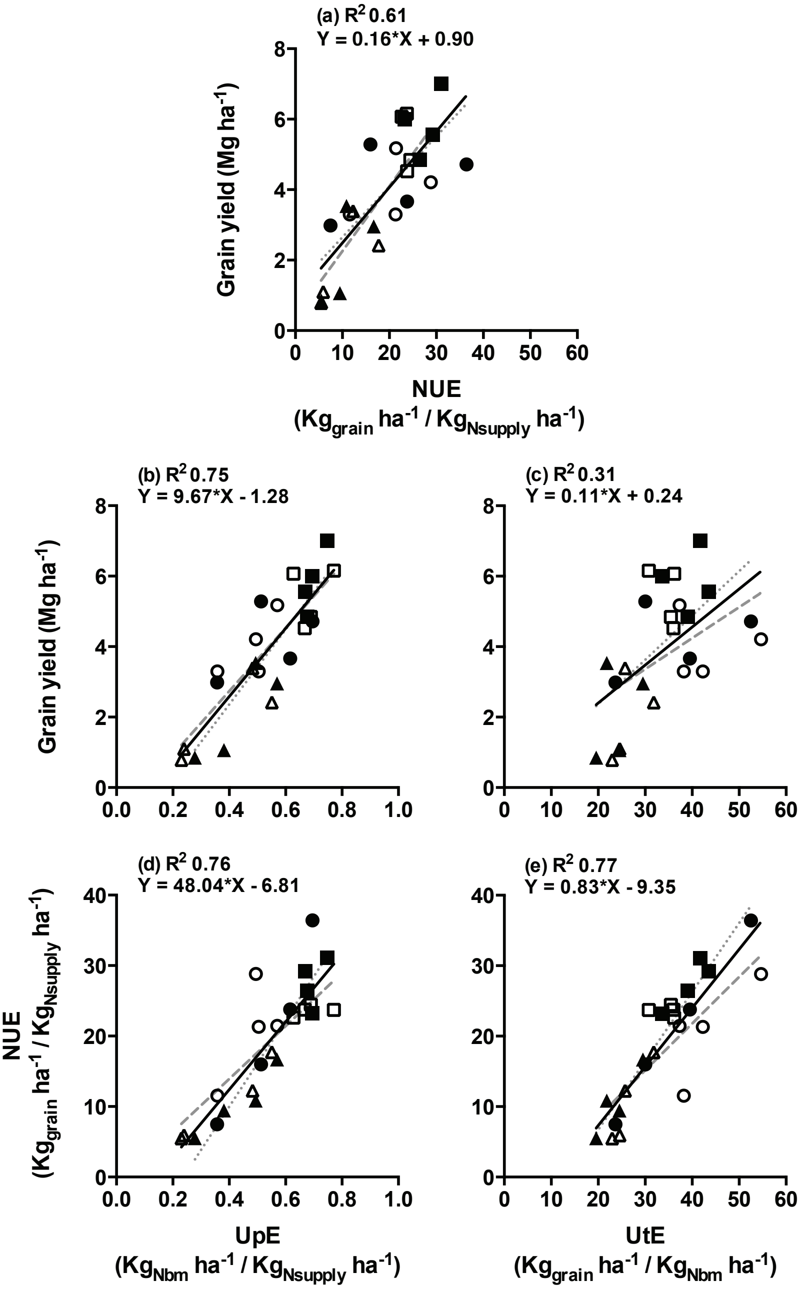

Figure 4.5. Grain yield regressed against NUE (a), UpE (b) and UtE (c) and NUE regressed against UpE (d) and UtE (e) for Anza (open symbols) and Soissons (closed symbols) in experiments 04 (squares), 05 (triangles) and 06 (circles) respectively. The dashed grey line represents Anza linear regression; the dotted grey line Soissons's and the black line and regression values correspond to the pooled data linear regression.

Anza and Soissons presented similar UpE and UtE and NUE range of values across all treatments and experiments (Figs. 4.5 and 4.6); the apparent differences between the slopes of Anza and Soissons regressions in Fig. 4.5 are not statistically significant.

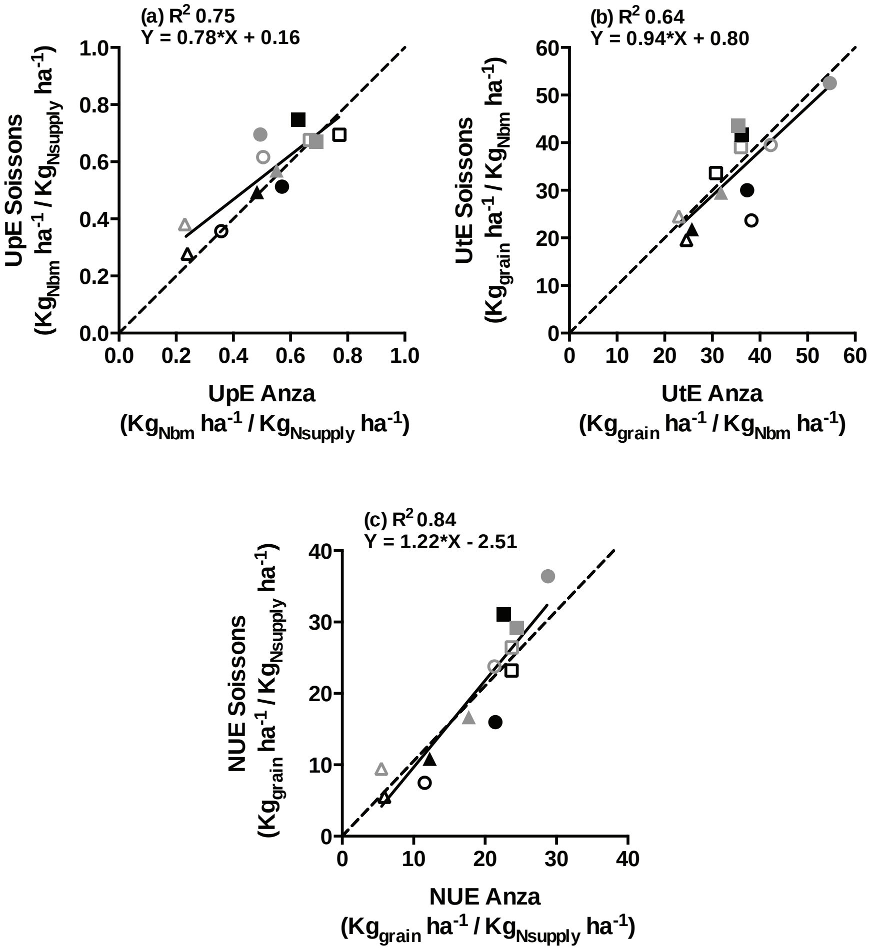

Figure 4.6. Soissons *vs.* Anza UpE (a); UtE (b) and NUE (c). Open and closed symbols represent rainfed and irrigated, grey and black unfertilised and fertilised treatments respectively. The dashed line accounts for the 1:1 relationship and the solid line for the linear regression.

Similar trends as the reported above were found when calculating UtE and NUE on the base of biomass, as expected considering the direct relationship between grain yield and biomass production (as described in Chapter III).

NUE ranged from 5.49 kg_{grain} $kg_{Nsupply}^{-1}$ in experiment 05 for Anza RF N_0 to 36.43 kg_{grain} $kg_{Nsupply}^{-1}$ in experiment 06 for Soissons IR N_0. In experiments 05 and 06, NUE was significantly higher in the N_0 treatment compared with the N_1. Instead, in experiment 04, NUE did not differ for any of the treatments. Only during the second experiment IR increased NUE over RF (Fig. 4.6c).

UpE was similar in most cases; except in experiment 05 where it was increased by irrigation from 0.28 to 0.52 kg_{Nbm} $kg_{Nsupply}^{-1}$ ($P<0.05$; SEM 0.03; Fig. 4.6a).

On the contrary, UtE was more affected by the fertilization treatment (Fig.4.6b). In experiment 04, there were no differences for UtE, whilst in experiment 05 it decreased under fertilization from 27.21 to 22.92 Kg_{grain} Kg_{Nbm}^{-1} ($P<0.01$; SEM 0.96). Finally, in experiment 06, UtE ranged from 32.31 to 47.25 Kg_{grain} KgN_{bm}^{-1} for N_1 and N_0 respectively. ($P<0.01$; SEM 1.66).

4.3.3. Grain N concentration

Grain N concentration ranged from 1.47% for Soissons RF N_1 in experiment 06 to 2.95% for Soissons IR N_0 in experiment 05 (Fig.4.7). Except in the first season, where there was an unusual water input from rainfall (Table 3.1; Fig. 3.1), grain N concentration was increased by N fertilization, from 2.41 to 2.73% in experiment 05 ($P<0.01$; SEM 0.04) and from 1.73 to 2.36% in experiment 06 ($P<0.01$; SEM 0.06) for N_0 and N_1 respectively.

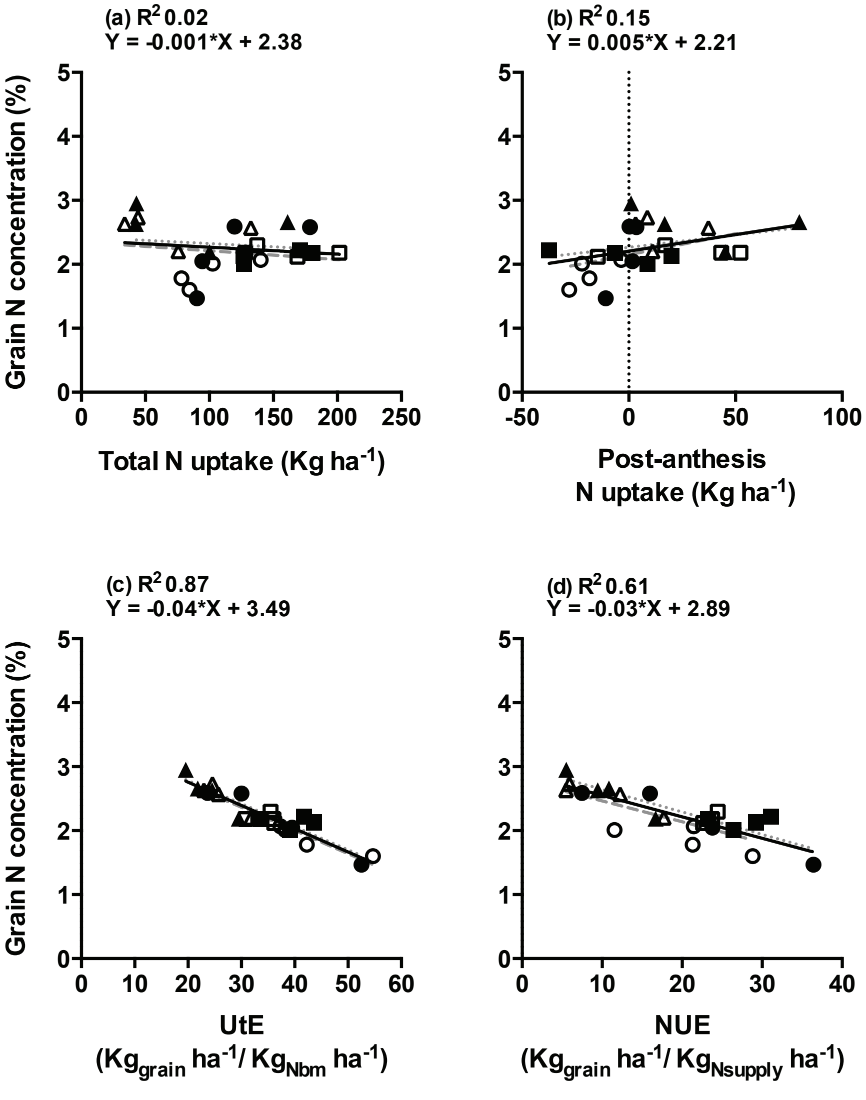

Figure 4.7. Grain N concentration regressed against aerial biomass total N uptake (a); aerial biomass post-anthesis N uptake (b); UtE (c) and NUE (d) for Anza (open symbols) and Soissons (closed symbols) in experiments 04 (squares); 05 (triangles) and 06 (circles) respectively. The dashed grey line represents Anza linear regression; the dotted grey line Soissons's and the black line and regression values correspond to the pooled data linear regression.

As well as grain N uptake, grain N concentration did not changed either with total or post-anthesis N uptake (Figs. 4.2e and 4.7a and b), indicating that, under the tested conditions, the driving force of grain N grain acquisition was mainly translocation from vegetative organs during post-anthesis and there was no additional increment in grain N concentration due to post-anthesis uptake. There was a negative relationship between the grain N concentration and UtE and NUE (Figs. 4.7c and d) driven by the dilution effect occurred whenever grain biomass was increased (Fig. 4.3).

4.3.4. Water use and water use efficiency

Water use varied greatly across the experiments, from *c.* 565 mm in experiment 04 to *c.* 220 mm in experiment 06 in response to the differences in initial soil water content and rainfall (chapter III). The highest values of water use occurred in experiment 04, while they were similar in experiments 05 and 06.

As expected, water use was lower for the RF treatments in each of the seasons (Fig. 4.8d), although this difference was statistically significant only in experiments 04 and 06, with a clear difference between the N_1 and N_0 treatments, ranging from: 512.65 mm to 496.23 ($P<0.01$; SEM 3.40) in experiment 04 and from 298.66 to 287.54 ($P<0.01$; SEM 1.29) in experiment 06, for N_1 and N_0 respectively (Fig. 4.8d).

Differences in grain number and grain yield could be explained by water use (Fig. 4.8a and b) although not in the same extent as by N uptake did (Fig 4.4). Grain yield achieved for every unit of water used (*i.e.* evapotranspiration) in the different backgrounds explored by the environmental treatments and seasonal conditions was in every case bellow the upper limit for dry environments calculated by Sadras and Angus (2006). As expected, N uptake and water use were positively related (Fig. 4.8c). Following the same trend as for N uptake, there were no differences between Soissons and Anza in water use either (Fig. 4.8d).

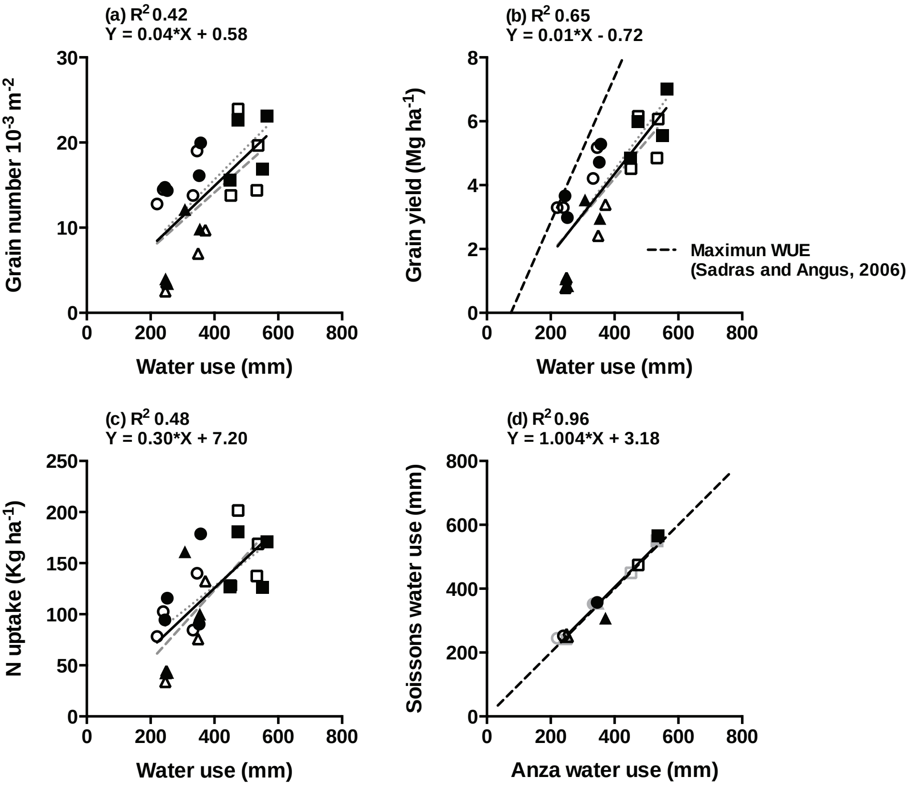

Figure 4.8. Grain number; grain yield and N uptake regressed against water use (a; b and c respectively) for Anza (open symbols) and Soissons (closed symbols) in experiments 04 (squares); 05 (triangles) and 06 (circles) respectively. The dashed grey line represents Anza linear regression; the dotted grey line Soissons's and the black line and regression values correspond to the pooled linear regression. The dashed line in (b) represents the boundary WUE (grain yield/water use) for dry environments (slope=0.022 Mg_{grain} $ha^{-1}mm^{-1}$ accounting for the maximum WUE and X intercept=60 mm accounting for soil evaporation) as proposed by Sadras and Angus 2006. (d) Soissons *vs.* Anza water use; open and closed symbols represent rainfed and irrigated; grey and black unfertilised and fertilised treatments respectively. The dashed line accounts for the 1:1 relationship and the solid line for the linear regression.

Yield responsiveness to N was similar between cultivars and its variations were well explained by WU. Soissons responded negatively to fertilization in experiments 05 and 06 under water scarcity conditions (Fig. 4.9).

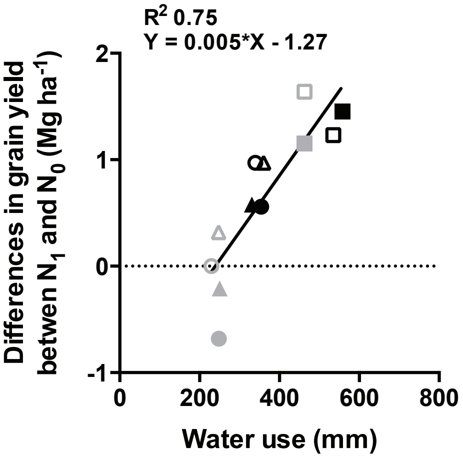

Figure 4.9. Differences in grain yield between fertilized and unfertilized treatments (grain yield N_1 – grain yield N_0) in relationship with water use (averaged for N_1 and N_0) for Anza (open symbols) and Soissons (closed symbols) under rainfed (grey symbols) and irrigation (black symbols) treatments in experiments 04 (squares), 05 (triangles) and 06 (circles) respectively.

Aerial biomass WUE and grain WUE were linearly related (R^2 0.86; $P<0.001$), thus only results for grain yield were reported and illustrated.

Across all the explored conditions Anza and Soissons presented similar WUE values (Fig. 4.10). WUE ranged from 3.42 to 15.03 kg_{grain} ha^{-1} mm^{-1} for Anza RF N_0 in experiment 05 and Soissons RF N_0 in experiment 06 respectively (Fig. 4.10c). N fertilization enhanced WUE 23% on average, although the effect was only significant ($P< 0.05$) in experiments 04 and 05 (Fig. 4.10c). In all cases, IR increased WUE, although not significantly ($P>0.1$). Changes in WUE explained 51% of grain yield variations (Fig. 4.10a), however they were not directly correlated with NUE (Fig. 4.10b).

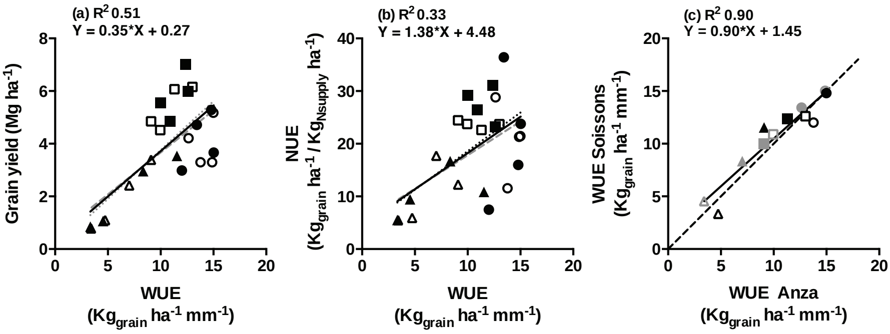

Figure 4.10. Grain yield and NUE regressed against WUE (a and b respectively) for Anza (open symbols) and Soissons (closed symbols) in experiments 04 (squares), 05 (triangles) and 06 (circles) respectively. The dashed grey line represents Anza linear regression; the dotted grey line Soissons's and the black line and regression values correspond to the pooled data linear regression. (c) Soissons *vs.* Anza WUE; open and closed symbols represent rainfed and irrigated; grey and black unfertilised and fertilised treatments respectively. The dashed line accounts for the 1:1 relationship and the solid line for the linear regression.

4.4. Discussion

Through selecting for high yields, wheat breeding has indirectly increased grain N content (through an increase in N partitioning to the grain) and reduced grain N concentration, with no major changes in the total amount of N uptaken by the crop of the modern cultivars compared to the old ones (Calderini *et al.*, 1995; Guarda *et al.*, 2004). However this was not directly reflected in Anza, as an example of a traditional cultivar and Soissons, as a modern one, that presented similar grain N uptake and concentration (Figs. 4.2d and 4.7). Whilst, in line with Calderini et al. (1995), under the range of conditions explored by the three experiments, total N uptake did not differ between the traditional and the modern cultivar (Fig. 4.1h).

As widely reported, N uptake took place chiefly during pre-anthesis and it was positively related to grain yield for both cultivars (Fig. 4.4). However experiment 05 was the only case where there was a significant post-anthesis N uptake (Figs. 4.1e and f). This might have been an exception due to the rainfall accumulated in May (Fig. 3.1b), after anthesis (but beyond the critical period for grain yield determination) that might have increased the soil N uptake during this stage.

Other indication that N uptake occurred mainly before anthesis was the lack of relationship between post-anthesis total N uptake with grain N uptake and grain N concentration (Figs. 4.2e and 4.7b respectively). This was in turn showing that grain N uptake during post-anthesis was driven by translocation from the vegetative organs (Fig. 4.2a, b, c and d) and there was no additional increment due to post-anthesis uptake.

Palta *et al*. (1994) found that under post-anthesis water deficits (a very common event in Mediterranean environments) total wheat grain N uptake was not affected because of greater translocation. Instead, Tahir and Nakata (2005) reported for a wide range of bread wheat cultivars, that N remobilisation efficiency from the shoots and leaves was negatively affected by drought, resulting in lower grain yield. In the tested conditions, where water scarcity did occurred during the second and the third experiments (Table 3.1 and Fig. 3.1), grain N accumulated mainly by N translocation, particularly from the leaf blades and the (non-grain) spike tissues (Figs. 4.2a and c). However, in experiment 05, intermediately dry but the worst year in terms of rainfall distribution along the growing cycle (Fig. 3.1), post-anthesis N content in vegetative organs was the highest while grain N uptake was the lowest (triangles in Fig. 4.2) among the three experiments, particularly under RF; reinforcing the idea that N translocation to the grain might be reduced under drought.

Regarding NUE, Ortiz–Monasterio *et al* (1997) found that when water availability was no limiting, UpE was the component better explaining the differences in NUE under low N rates, and that UtE became more important as the N rates increased. The same was observed more recently by Pask *et al*. (2012) in UK field experiments under non-restricted water conditions, while Gaju *et al*. (2011), under similar environmental conditions, found that both under low and high N conditions, UtE was the component better explaining variations in NUE. However, in the more limiting Mediterranean environment, both uptake and utilization efficiency seemed to be the leading force behind the NUE rather independently from water and N availability variations. As previously reported in the same region of study (Cossani *et al*., 2012), NUE was well explained both by UpE and UtE (Figs. 4.5d and e) for Anza and Soissons across the environmental conditions tested by the treatments and inter-season variability.

In line with the results of the present thesis (Fig. 4.6), previous findings in Mediterranean conditions (*e.g.* López-Bellido and López-Bellido, 2001; Angás *et al*., 2006; Giambalvo *et al*., 2010; Cossani *et al*., 2012) have shown that NUE was inversely related to N rates. This was also extensively proven in literature, including a wider range of conditions beyond the semi-arid areas (*e.g.* Gaju *et al*., 2011, Pask *et al*., 2012). UtE was also found to be inversely related to N availability (Sylverter-Bradley and Kindred, 2009; Barraclough *et al*., 2010; Cossani *et al*., 2012; Pask *et al*., 2012) indistinctly in semi-arid and non semi-arid regimes.

Both for Anza and Soissons, changes in grain yield, were better explained by UpE than by UtE (Fig. 4.5b and c), since, as seen in chapter III, its variation was more affected by biomass production than by harvest index. Previously, Van Ginkel *et al*. (2001) have reported similar findings, where grain yield and biomass improvements were more correlated with UpE than UtE both under low and high levels of N. In contrast, Barraclough *et al*. (2010) found that UtE was the predominant component explaining yield under a wide range of N rates.

As expected, variations in grain yield were also related to water use. Nevertheless, in spite of the quite wide range of conditions explored by the present experiments, WUE was in every case below the maximum WUE attainable for dry environments (Sadras and Angus, 2006) and both Anza and Soissons reached similar yield levels for each unit of water used by the crop (Fig. 4.8b). However, WUE values in experiment 06, were closer to the boundary WUE, particularly in the RF treatments. WUE was the furthest apart from the upper limit in experiment 04, probably due to the unusual water availability occurred that season (Fig. 4.8b). The positive correlation of the grain yield residuals (grain yield$_{\mathrm{data}}$ – grain yield$_{\mathrm{max.WUE}}$) with water use might be suggesting that there is scope to further improvements in WUE in Mediterranean environments (Fig. 4.11).

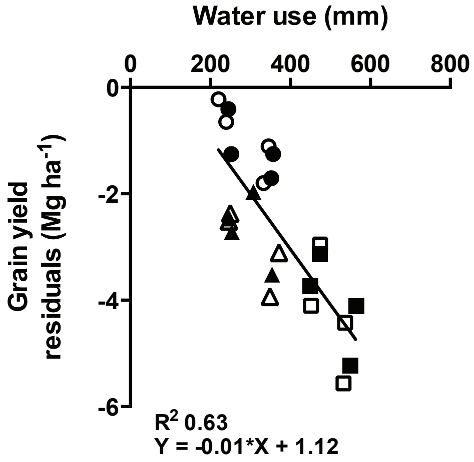

Figure 4.11. Grain yield residuals to the water-limited benchmark yield proposed by Sadras and Angus (2006) for dry environments regressed against water use for Anza (open symbols) and Soissons (closed symbols) in experiments 04 (squares), 05 (triangles) and 06 (circles) respectively.

Besides, as seen by Passioura and Angus (2010), there are also other physiological traits that can be improved to approach actual grain yield to the maximum attainable in semi-arid conditions. In experiments 04 and 05, NUE and UtE explained the differences between actual and maximum attainable yield (Fig. 4.12) as well as WU. In contrast, in experiment 06 grain yield residuals presented no correlation with NUE or any of its components, probably because it was the closest to the boundary yield.

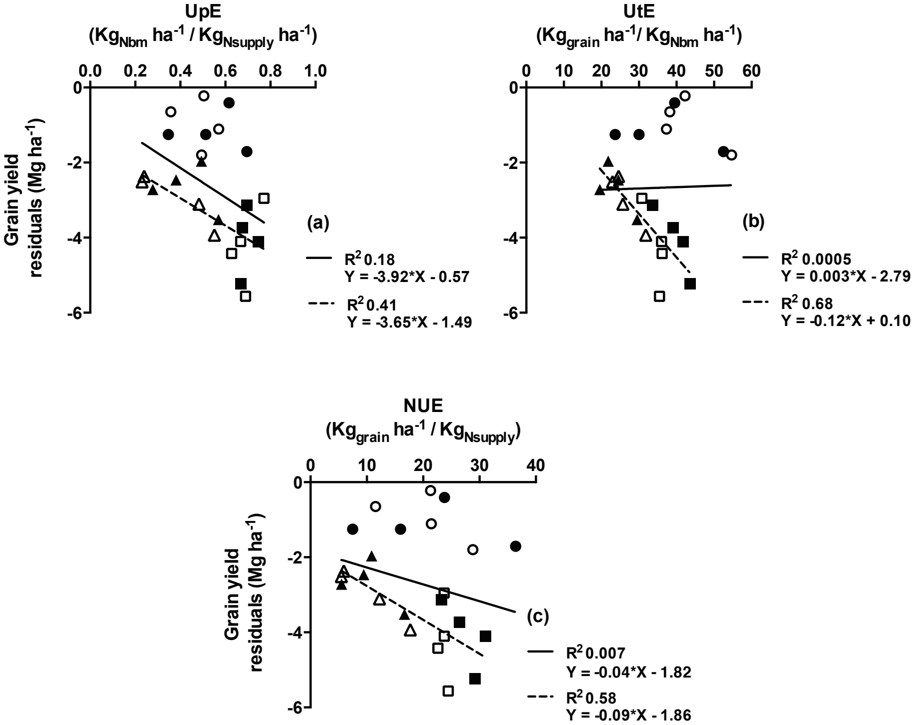

Figure 4.12. Grain yield residuals to the water-limited benchmark yield proposed by Sadras and Angus (2006) for dry environments regressed against UpE (a); UtE (b) and NUE (c) for Anza (open symbols) and Soissons (closed symbols) in experiments 04 (squares); 05 (triangles) and 06 (circles) respectively. The solid line represents the pooled data linear regression and the dashed line corresponds to experiments 04 + 05 linear regressions.

In conclusion, under the relatively wide range of environmental conditions explored by the three experiments, Anza and Soissons were able to use nitrogen and water with similar efficiency.

Differences in N content were more affected by the environmental treatments than N%, and they were mainly observed during pre-anthesis, indicating that N uptake occurs more frequently during this stage, whilst grain N is chiefly accumulated by translocation from the vegetative organs to the grain and unlikely increased by uptake during post-anthesis.

Pre anthesis N uptake directly affected grain yield. Increases in N uptake resulted in higher number of grains per unit land area and therefore higher yields, suggesting that under Mediterranean environments pre-anthesis N uptake is a key determinant of grain yield. Grain yield variations were also explained by WU, although not in the same extent as by NU. In turn, N uptake was enhanced by WU.

As UpE seemed as relevant to NUE as UtE under the Mediterranean conditions analysed, focusing on traits related both to N accumulation and partitioning could be important in order to improve grain yield through NUE. However, since UpE is more related to grain yield variations than UtE and it is very unlikely to further improve HI, UpE would be a more useful trait to focus on than UtE.

Finally, there seems to be scope for further improvement in both NUE and WUE aiming to reduce the gap between actual and attainable yield in Mediterranean conditions.

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Chapter V
Root growth


Field experiment in Agramunt (wheat crop at maturity)

Chapter V

5. Root growth

Abstract

Under Mediterranean environments, studying root growth is relevant to identify opportunities for further improving the capture of resources. To this purpose, root length (RL), root dry weight (RDW), root length density (RLD) and root to shoot dry weight ratio (R:S) (up to 100 cm depth) were measured along the growing cycle for a traditional (Anza) and a modern (Soissons) wheat cultivar from two field experiments in a Mediterranean region (north-eastern Catalonia).

The maximum root growth (RL and RDW) was reached around anthesis and between the topsoil and 25 cm depth. In general, the modern cultivar presented a larger root system in terms of RL, RDW, RLD and R:S than the traditional one. Nevertheless this superiority did not translate in consistent differences in grain yield, probably because both cultivars under the different water and N conditions were able to achieve the critical RLD threshold. Therefore, these results indicated that the traditional genotype was able to produce similar biomass and yield with less root growth. RL and RDW were determinants of N uptake, water use in a lesser extent and grain yield.

5.1. Introduction

Under semi-arid environments, such as the Mediterranean Basin, yield penalties are related to reductions in growth, therefore identifying opportunities for further improving the capture of resources may prove critical. In this environment, where yields are typically limited by terminal droughts, and also by other relevant agronomic factors directly affecting root capacity to capture soil resources (*e.g.* frosts, heat, weeds, roots diseases; Passioura and Angus, 2010 and references quoted therein) is relevant to study root growth.

If compared with wheat (or other cereals) aerial canopy, knowledge about the root system is far behind (Izzi *et al.*, 2008) but undoubtedly necessary to a more comprehensive understanding of wheat productivity determinants (which in turn is essential for designing more effective management or breeding strategies). Wheat root system plays a key role in resources capture, yet there is a limited knowledge about it and its relationship to soil resources uptake, particularly in the more limited Mediterranean environments (Carvalho *et al.*, 2014) and especially under realistic field conditions. This is so because field studies of root characteristics are scarce and not conclusive. The former may be due, at least partly, to the fact that root sampling in field experiments and its further processing and analysis in the laboratory are very demanding in terms of time costs (Zuo *et al.*, 2004 and Izzi *et al.*, 2008 and Ayad *et al.*, 2010); and the latter to the fact that errors associated to determinations are normally very large. There seems to be no easy way out to these two drawbacks and therefore it is important to add new evidences to help building up a cohesive body of evidences that will help to reach sounder conclusions.

Under field conditions, it has been observed that the fastest growth rate of roots occurred during pre-anthesis, being this stage more sensitive to water deficit (Asseng *et al.*, 1998). Maximum root length (RL) and dry weight (RDW) seem to be achieved around anthesis (Barraclough and Leigh, 1984; Gregory *et al.*, 1992; Asseng *et al.*, 1998).

In response to water deficits, a very common event in Mediterranean areas, the wheat root system growth can respond by reducing RL and RDW in absolute terms, although increasing the root to shoot ratio (R:S) and root length density (RLD) in deeper layers of the soil (Asseng *et al.*, 1998; Li *et al.*, 2010; Carvalho *et al.*, 2014). Under water stress, RLD was found to be reduced in the top layers of the soil while presenting a compensatory increment in the subsoil (> 30 cm depth) (Asseng *et al.*, 1998; Li *et al.*, 2010), provided that N fertilizer was applied (Barraclough *et al.*, 1989).

RDW and RLD in field grown winter cereals typically decrease (exponentially) with soil depth, with the majority of root mass concentrated in the top 25 cm (Lampurlanés *et al.*, 2001, Izzi *et al.*, 2008; Carvalho *et al.*, 2014).

When comparing old and new varieties of wheat in a Mediterranean environment, Siddique *et al.* (1990) found that both new and old wheats RDW reached their maximum values also at anthesis, while shoots growth continued almost until maturity. In that study, the old variety had significantly higher RDW and RLD at anthesis than the modern one, but anthesis occurred later than in the modern one; which presented increased yield and WUE. In contrast, in many other countries wheat breeding programmes during the last century did not consistently modify time to anthesis (*e.g.* in Spain; Acreche *et al.*, 2008) and therefore it is not possible to extrapolate these results to other regions.

Thus, in the above context, it is relevant to find out if traditional and modern cultivars widely grown in the Mediterranean differed in their root system growth. Therefore, the main objectives of this study were to investigate root growth attributes of a traditional and a modern wheat cultivar under a Mediterranean environment and to ascertain the importance of the root system for resources capture and yield. For this purpose, field experiments were installed under two contrasting water and nitrogen (N) availabilities during two consecutives growing seasons.

5.2. Material and Methods

5.2.1. Site, treatments and experimental design

Root samples were taken from experiments 05 and 06. Site, treatments and experimental design are detailed in chapter III.

5.2.2. Sampling and analysis

In both experiments, a direct estimation of the root system was done through a destructive root sampling method. Soil cores of each plot were taken at four soil depths: 0-25 cm; 25-50 cm; 50-75 cm and 75-100 cm at three developmental stages: jointing (DC 3.1); anthesis (DC 6.5) and maturity (DC 9.2). The samples were taken using a soil hand corer 3 cm diameter next to a central row within each plot. They were collected in plastic bags, frozen and kept at -5 ºC until processed.

In experiment 05, due to the experimental site soil characteristics it was not possible to take samples beneath 50 cm depth but in experiment 06 soil samples were taken up to 100 cm depth.

To extract the roots from the samples, soil was carefully washed off from roots with running water above a fine mesh sieve. Active wheat roots (white and light brown) were separated from debris and dead roots (grey, black or dark brown) collected using tweezers under a lighted magnifying glass. Each sample was immediately processed for image acquisition and further analysis.

In order to enhance contrast with the background, the clean roots were stain with methylene blue (1% concentration) during 5 minutes and carefully rinsed. The washed roots were spread uniformly, avoiding overlapping, in a transparent acrylic tray (size A4) with a thin layer (*c.* 3 mm) of tap water and scanned to an image of 300 dpi resolution, stored as a TIFF file for its analysis.

Total root length of each sample was calculated using an image analysis software; WinRHIZO (Regent In-strument Inc.) that provides with accurate measurements of root length (Bouma, 2000 and Himmelbaeur *et al.* 2004).

Finally, each sample was placed in a labelled Petri dish and dried in a stove at 65ºC during 48 hours, after which, root dry weight was recorded.

Root length density (RLD; cm cm^{-3}) was calculated as the ratio between the sampled root length and soil volume for each depth interval. Root length per unit land area (RL; Km m^{-2}) and root dry weight per unit land area (RDW; Mg ha^{-1}) were also assessed for each depth interval.

Averaged RLD was obtained as the mean throughout the soil layers analysed. Total RL and RDW were calculated as the sum of layers. The root to shoot ratio (R:S) was calculated as the relationship between total RDW and aerial biomass dry weight at each of the samples taken. Aerial biomass dry matter production, grain yield and phenological stages were assessed as described in chapter III.

N uptake, water use (WU) and water use efficiency (WUE) were assessed as described in chapter IV.

Statistical analysis was performed as described in chapter III.

5.3. Results

The general RL and RDW growth pattern during crop growing cycle was, in most cases, similar for both cultivars (Anza and Soissons) and environmental treatments (Fig. 5.1). In general, RL and RDW increased from the beginning of stem elongation up to anthesis, but it remained the same or decline from then to maturity, indicating that the maximum root growth was reached around anthesis and net root senescence might begin afterwards (Fig. 5.1). The minimum and maximum values of RL and RDW range at anthesis were reached for the rainfed and unfertilized controls (Fig.5.1).

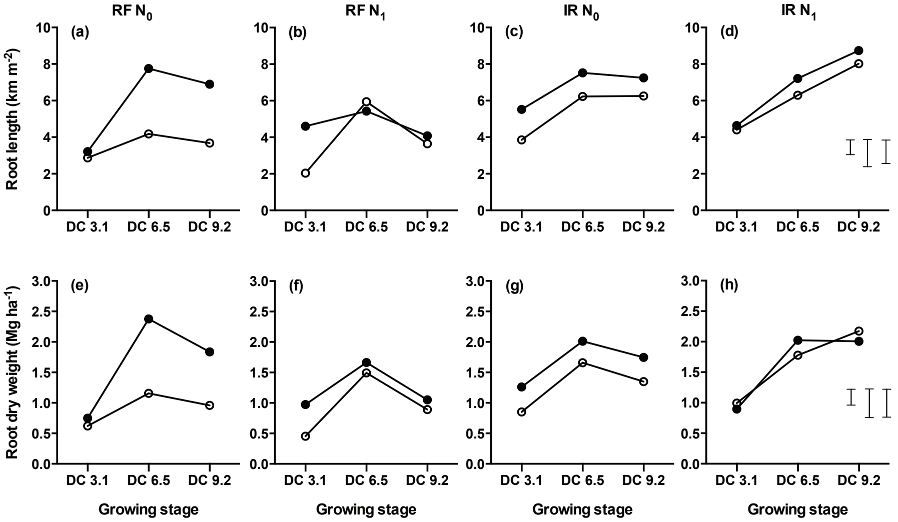

Figure 5.1. Top panel: root length for each combination of N and water availability: (a) rainfed unfertilised (RF N_0), (b) rainfed fertilised (RF N_1), (c) irrigated unfertilised (IR N_0) and (d) irrigated fertilised (IR N_1); bottom panels: root dry weight for: (e) RF N_0, (f) RF N_1, (g) IR N_0 and (h) IR N_1 averaged across experiments 05 and 06 at jointing (DC 3.1), anthesis (DC 6.5) and maturity (DC 9.2) for Anza (open symbols) and Soissons (closed symbols). The error bars represent, from left to right, SEM values for DC 3.1; 6.5 and 9:2 respectively.

Along the growing season, in the majority of field conditions explored by water and N treatments, the modern cultivar Soissons tended to present larger root systems (>RL and >RDW) than the traditional one, Anza. This genotypic effect was more significant than the environmental ones, particularly during pre-anthesis (Fig. 5.1). Only at maturity, the effect of water availability level was noticeable in both traits, and tended to outyield (not significantly) in root size the rainfed ones (Fig. 5.1).

Throughout the soil profile (1 m deep) Soissons also tended to exceed Anza for RL, RDW and RLD in the three growing stages analysed. Root growth reached the maximum values between the soil surface and 25 cm depth and was generally lower in the deepest soil layers (Fig. 5.2). Roots, measured in terms of RL, RDW and RLD, concentrated on the top layer of the soil in all stages, though the difference tended to equilibrate a little bit with phenology progress: *c.* 63, 45 and 39% of the total root system was located in the soil top 25 cm at jointing, anthesis and maturity respectively.

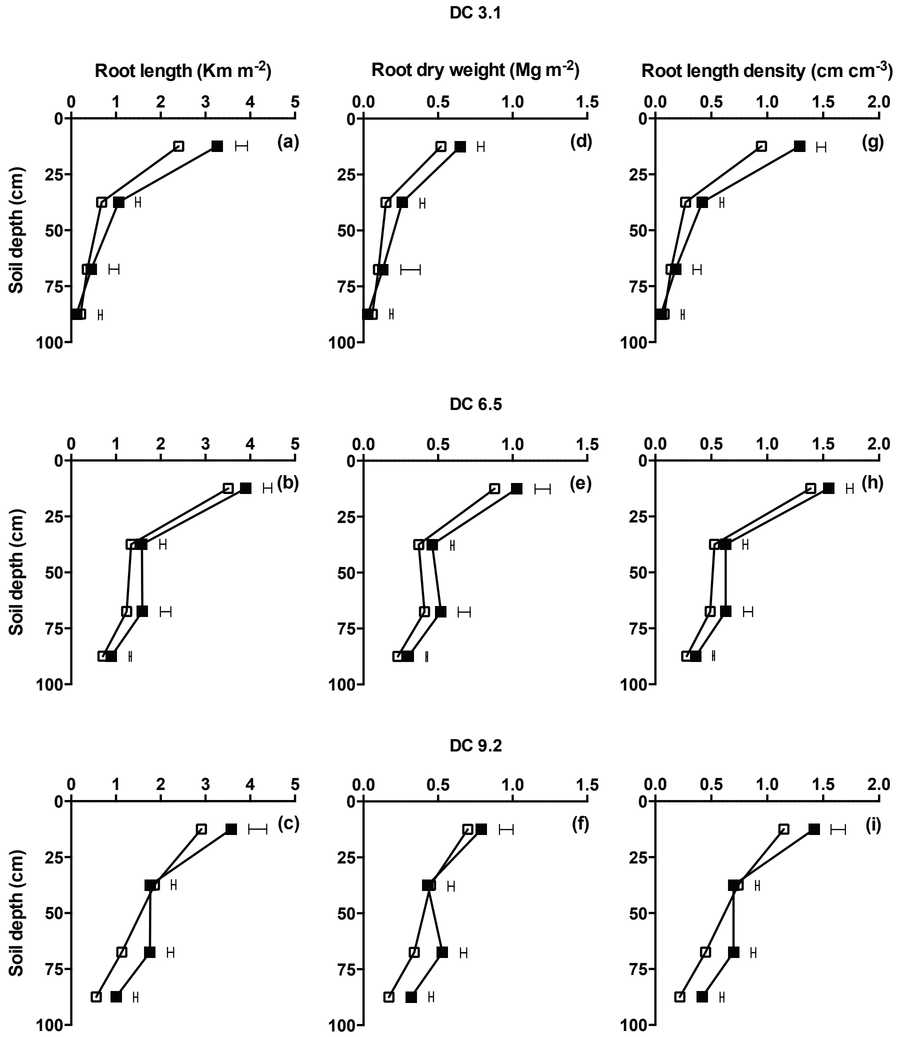

Figure 5.2. Left panels: root length averaged across experiments at (a) jointing (DC 3.1), (b) anthesis (DC 6.5) and (c) maturity (DC 9.2) respectively; middle panels: root dry weight at (d) DC 3.1, (e) DC 6.5 and (f) DC 9.2 respectively; right panels: root length density at (g) DC 3.1, (h) DC 6.5 and (i) DC 9.2 respectively averaged across experiments 05 and 06 for Anza (open symbols) and Soissons (closed symbols) by soil depth layers of 25 cm, up to 1 m depth. The error bars represent SEM values for each soil layer.

The superiority of Soissons over Anza in RL, RDW, RLD and R:S in both experiments along the growing cycle and environmental backgrounds was more consistent in the unfertilised environments, especially under rainfed conditions (Fig. 5.3).

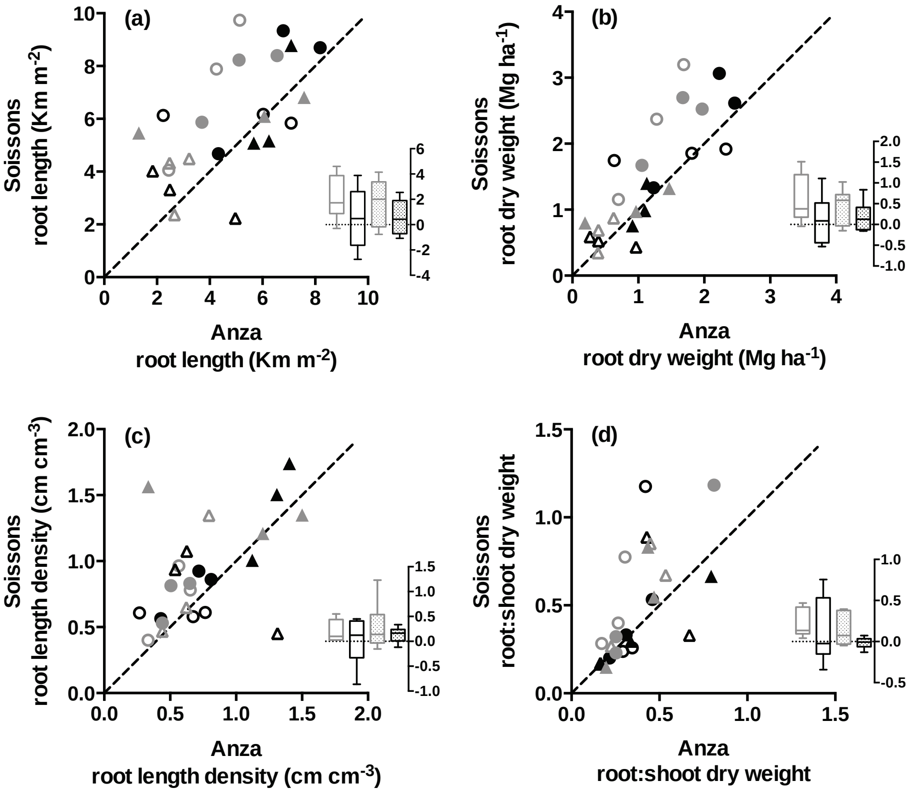

Figure 5.3. Soissons against Anza root length (a), root dry weight (b), root length density (c) and root to shoot dry weight ratio (d) at jointing, anthesis and maturity in experiments 05 (triangles) and 06 (circles). Open and closed and open symbols represent rainfed and irrigated, grey and black unfertilised and fertilised treatments respectively. Insets within each graph are the residuals from the 1:1 relationship (Soissons – Anza) for rainfed unfertilised (open grey bars), rainfed fertilised (open black bars), irrigated unfertilised (patterned grey bars) and irrigated fertilised (patterned black bars) from left to right plotted in a box and whisker graph. The box extends from the 25th to 75th percentiles, the line in the middle of the box is plotted at the median and the whiskers go down to the smallest value and up to the largest. The dashed line accounts for the 1:1 relationship.

Overall the three phenological stages analysed, N uptake was correlated with RL and RDW (Fig. 5.4) with differences between cultivars being not significant, however there was no relationship with RLD or R:S. Analysing each stage separately, except RLD, the rest of the traits had a positive relationship with N uptake at anthesis ($R^2>0.40$; $P<0.01$).

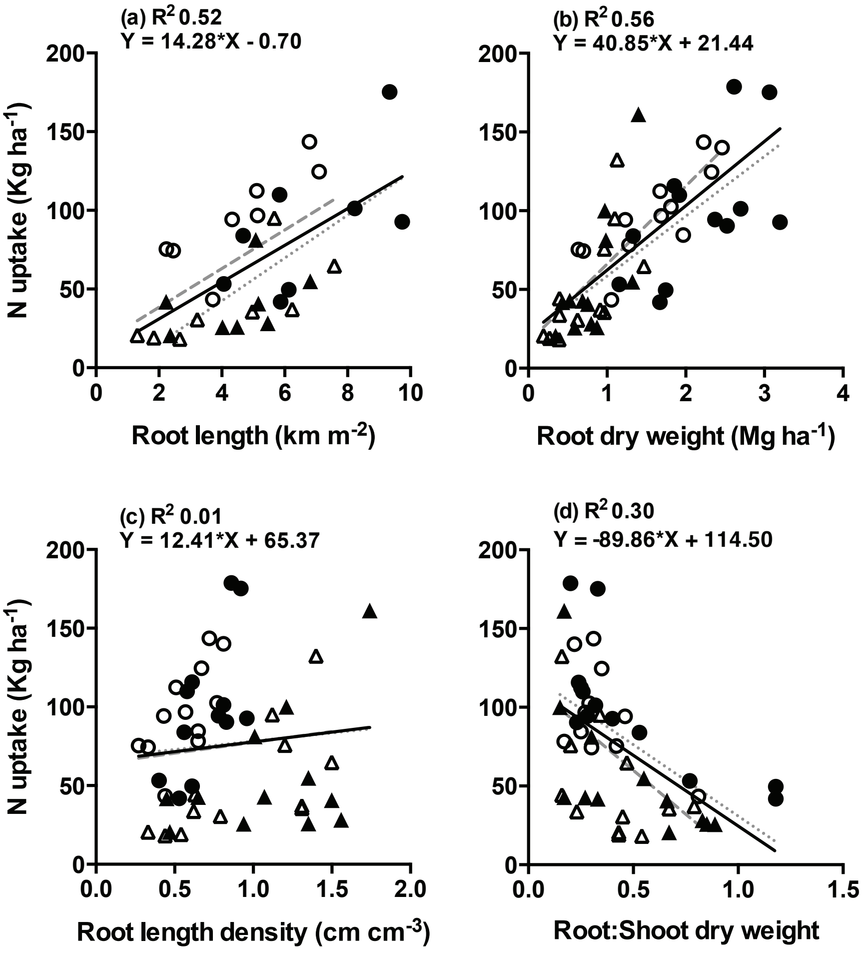

Figure 5.4. N uptake regressed against root length (a), root dry weight (b), root length density (c) and root:shoot dry weight ratio (d) for Anza (open symbols) and Soissons (closed symbols) at jointing, anthesis and maturity in experiments 05 (triangles) and 06 (circles) respectively. The dashed grey line represents Anza linear regression; the dotted grey line Soisson's and the black line and regression values correspond to the whole data linear regression.

RL explained a 42% of variation in WU ($P<0.01$), however, there was not a clear trend between WU and the rest of the root traits analysed (Fig. 5.5). Both RL and RDW were related with WU for each experiment separately ($R^2>0.57$; $P<0.03$; Figs. 5.5a and b).

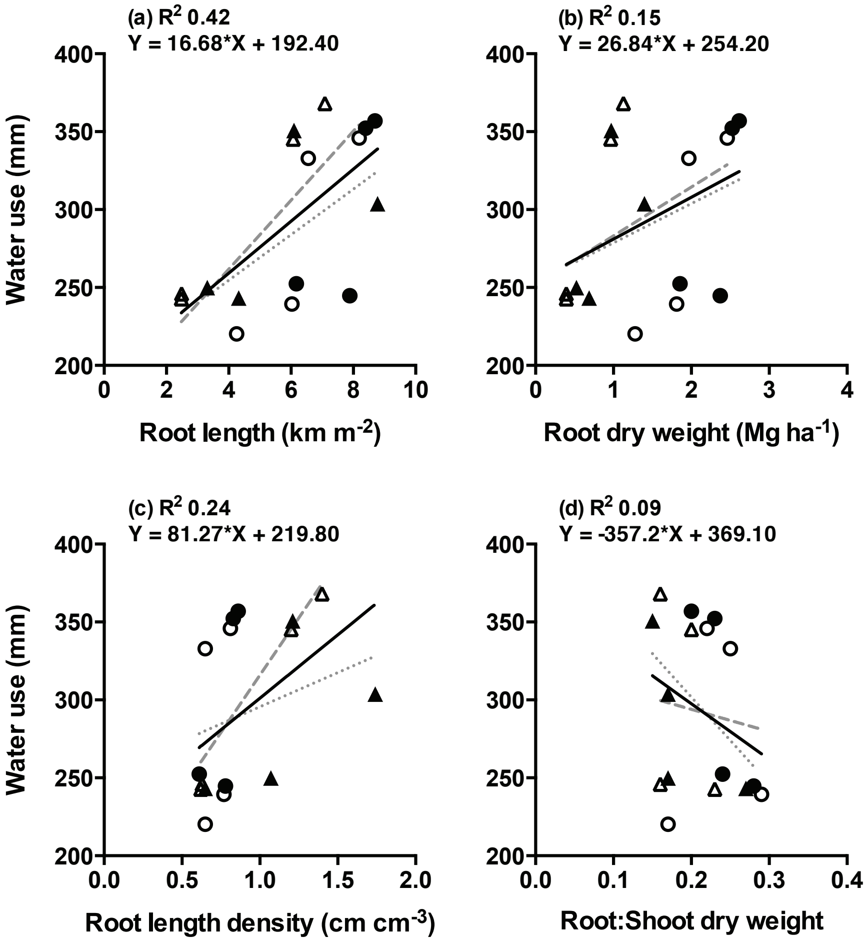

Figure 5.5. Water use regressed against root length (a), root dry weight (b), root length density (c) and root:shoot dry weight ratio (d) for Anza (open symbols) and Soissons (closed symbols) at maturity in experiments 05 (triangles) and 06 (circles) respectively. The dashed grey line represents Anza linear regression; the dotted grey line Soissons's and the black line and regression values correspond to the whole data linear regression.

Both total aerial biomass production and grain yield were positively associated with RL and RDW at anthesis ($R^2>0.51$; $P<0.005$; not shown); however they were best explained by RL and RDW at maturity (Fig. 5.6) and shown no relation at all at jointing. In most cases, Soissons had higher RL and RDW than Anza that did not translate in higher yields.

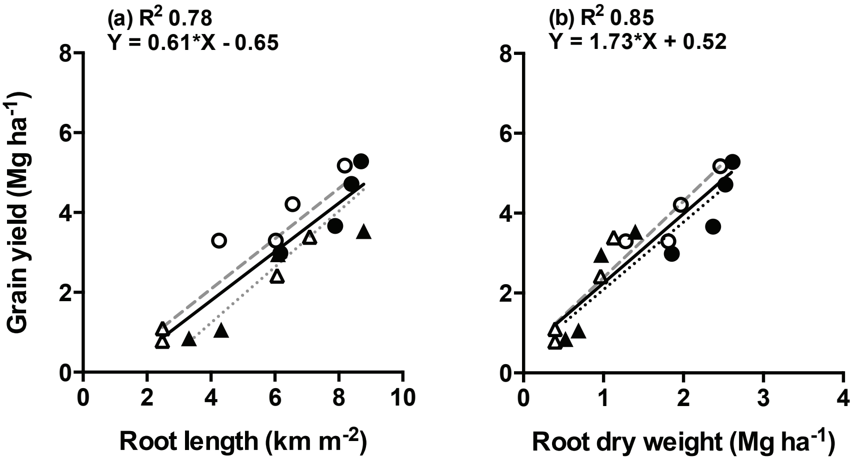

Figure 5.6. Grain yield regressed against total root length (a) and root dry weight (b) at maturity for Anza (open symbols) and Soissons (closed symbols) in experiments 05 (triangles) and 06 (circles) respectively. The dashed grey line represents Anza linear regression; the dotted grey line Soissons's and the black line and regression values correspond to the whole data linear regression.

5.4. Discussion

A wide range in RL, RDW, RLD can be found in the literature (Table 5.1). RL and RLD absolute values in both experiments were within the same order observed under similar growing conditions, in semi-arid areas (*e.g.* Lampurlanés *et al.*, 2002; Carvalho *et al.*, 2010; Morell *et al.*, 2011) and generally lower than those reported in other regions of the world (*e.g.* Barraclough and Leigh, 1984; Savin *et al.*, 1994; Asseng *et al.*, 1998).

In contrast, RDW values were within the range of those reported in Mediterranean and no Mediterranean environments (Table 5.1). Total depth explored in our study was 50 cm in experiment 05 and reached 100 cm in experiment 06. This might have been due to partially compacted soil layers at the experimental site. This kind of limitation to root sampling in field conditions have been observed in previous studies (Asseng *et al.*, 1998, Lampurlanés *et al.*, 2001, Lampurlanés *et al.*, 2002). However, in a recent field study the average maximum root depth have been found at 150 cm (Rasmussen, 2015).

Table 5.1. Range of values for root length (RL); root dry weight (RDW) and root length density (RLD) observed in literature and the present thesis. Values were estimated from graphs or taken from tables. *RLD range includes values from the deepest to the topsoil layers explored. **values per plant were transformed into values per unit land area assuming a plant density of 250 plants m^{-2}.

Reference	Mediterranean /Semi-arid	Species	Sampling method	Environmental Treatments	Phenological Stage	RL (km m^{-2})	RDW (Mg ha^{-1})	RLD (cm cm^{-3})*
Allard *et al*., 2013	No (France)	Wheat	Soil core 30 cm depth	N supply levels	Anthesis Maturity		1-3 1-2	
Asseng *et al*., 1998	No (USA)	Wheat	Minirizotron 110 cm depth	Water supply levels	Maturity	22-27		0-13
Ayad *et al*., 2010	Yes (Jordan)	Wheat, barley	Soil core 100 cm depth	Water and N supply levels	Maturity		<1-2	<1-6
Barraclough and Leigh, 1984	No (UK)	Wheat	Soil core 100 cm depth	Sowing dates	Anthesis	20-32	1-2	
Barraclough *et al*., 1989	No (UK)	Wheat	Soil core 180 cm depth	Water and N supply levels	Anthesis	22-35	<1-1	
Carvalho *et al*., 2014**	Reproduced (UK, glasshouse)	Durum wheat, barley	PVC columns 150 cm depth	Water supply levels	Maturity	2-42	<1-2	<1-2
Izzi *et al*., 2008	Yes (Syria)	Wheat, durum wheat	Soil core 75 cm depth	Water supply levels	Jointing Anthesis Grain filling		<1-1 1-3 3	
Lampurlanés *et al*., 2001	Yes (Spain)	Barley	Soil core 100 cm depth	Soil type, tillage levels	Jointing Anthesis Maturity		2-3 5-10 6-16	<1-2 <1-4 <1-2
Lampurlanés *et al*., 2002	Yes (Spain)	Barley	Soil core 100 cm depth	Soil type, tillage levels	Jointing Anthesis Maturity			1-4 <1-5 <1-2
Li *et al*., 2010	Yes (China)	Wheat	Soil core up to 180 cm depth	Water supply levels	Jointing Anthesis Maturity			<1-6 <1-5 <1-4
Morell *et al*., 2011	Yes (Spain)	Barley	Soil core 100 cm depth	N and tillage levels	Jointing Anthesis Maturity	<1-9		<1-1 <1-2 <1-1
Savin *et al*., 1994	No (Argentina)	Wheat	Soil core up to 140 cm depth	Water supply levels	Jointing Anthesis Grain filling		<1 3 2	<1-3 <1-5 <1-5
White *et al*., 2015	No (UK)	Wheat	Soil core 100 cm depth	Farm conditions	Jointing Anthesis Grain filling	9-15 5-18 3-8		<1-3
Xue *et al*., 2003	Yes (US)	Wheat	Soil core 140 cm depth	Water supply levels	Jointing Anthesis Grain filling		1-2 3-4 2-3	1-2 3-4 2-3
Current Experiments	Yes (Spain)	Wheat	Soil core 100 cm depth	Water and N supply levels	Jointing Anthesis Maturity	1-6 2-10 2-9	<1-2 <1-3 <1-3	<1-2

Following the general pattern of root growth throughout the crop cycle, roots cease to grow or there was even a decline in RLD and RDW during the post anthesis period (Fig. 5.1), as previously reported by other authors (Asseng *et al*., 1998; Foulkes *et al*., 2009; Morell *et al*., 2011), reinforcing the idea that the maximum root growth is reached around anthesis and that some degree of net root senescence can be noticed afterwards.

RL, RDW and RLD clearly decreased with depth, concentrating the majority of the roots in the top soil layer (Fig. 5.2). This is in agreement with previous findings (Barraclough and Leigh, 1984; Barraclough *et al*., 1989; Xue *et al*., 2003; Izzi *et al*., 2008; Carvalho *et al*., 2014; White *et al*., 2015).

In general, the modern cultivar tended to show larger root systems than the traditional one along the growing season and under the whole range of environmental conditions tested (Figs. 5.1 and 5.3) and into the soil profile explored (Fig 5.2) for RL, RD, RLD and R:S. However, the difference between Soissons and Anza did not necessarily mean higher resources capture by the modern cultivar, probably because by anthesis both Anza and Soissons had reached the critical value of RLD of 1 cm/cm^3 for 90% extraction of the soil available water and N suggested for barley and wheat (King *et al*., 2003; Foulkes *et al*., 2009; Carvalho *et al*., 2013). Nonetheless, this threshold was only reached in the topsoil layer (Fig. 5.2), being Soissons slightly higher in RLD than Anza. As formerly pointed out by White *et al*. (2015), the fact that the critical RLD value of 1 cm cm^{-3} has only been reached in the topsoil, might be indicating that there is no enough root growth in the deeper soil horizons to capture available water, or that the threshold may be lower in lower layers of the soil.

In agreement with Asseng *et al*. (1998); Ayad *et al*. (2010); Li *et al*. (2010) and Carvalho *et al*. (2014), R:S tended to be slightly increased in rainfed conditions (Fig. 5.3); indicating that under water scarcity, biomass partitioning to the roots in post-anthesis and/or root growth in pre-anthesis could have been favoured. In the present study, this trend was observed mainly after anthesis; therefore biomass partitioning seemed to be the main response mechanism (since post-anthesis root growth was not observed). In contrast, there was no compensatory root growth at the deeper layers of the soil (Fig. 5.2) under water scarcity or rainfed conditions as previously reported in other studies (Asseng *et al*., 1998; Ayad *et al*., 2010). However, RL, RDW and RLD also tended to be increased under RF conditions, particularly in the unfertilized controls (insets in Fig. 5.3), as also found in Asseng *et al*. (1998). Thus root growth may also be enhanced under environmental stressful conditions during pre-anthesis.

All in all, the environmental effect over root growth was less significant in the present study than the observed in other Mediterranean regions (*e.g.* Ayad *et al*, 2010).

RL and RDW reached at maturity were correlated with accumulated aerial biomass and grain yield (Fig. 5.6). They also were reasonably well correlated at anthesis, but with a lower correlation coefficient (R^2>0.51). Even though Soissons tended to have higher root growth and dry matter allocation than Anza across the seasons and the environments explored by the present study (Fig. 5.3), it did not imply a higher grain yield (Fig. 3.3d, chapter III). Thus, Anza root system might be more efficient in resource capture than Soissons's. Barraclough and Leight (1984) have previously reported that similarly yielding crops could have different root system size.

Overall the growing stages and experiments, variations in N uptake were well explained by RL and RDW (Fig. 5.4). In contrast, WU at maturity was only related to RL, explaining less than half of its variation. A relationship between root biomass and water use has been previously found (*e.g.* Izzi *et al*., 2008). However, in line with Carvalho *et al*. (2014), results from the present experiments did not show a clear trend between these two traits. There was a correlation between root biomass and WUE (not shown) that was probably more driven by the positive relation between roots mass and grain yield than by water use. All in all, root growth (*i.e.* RL and RDW) seems to be related to N uptake, to water use in a lesser extent and thus to grain number and yield.

Then, the results obtained in these studies indicated that root growth throughout the whole crop cycle was similar between traditional and modern wheat cultivars. Higher values of RL, RDW, RLD and R:S were obtained for the modern cultivar. However, these differences were not translated into higher biomass or yield productivity, probably because both cultivars under the different water and N conditions were able to achieve the critical RLD threshold (King *et al.*, 2003). Therefore, these results indicated that the traditional genotype was able to produce similar biomass and yield with less root growth.

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Chapter VI
General discussion and conclusions

Field experiment in Agramunt (wheat crop at maturity)

Chapter VI

6. General discussion and conclusions

The general discussion of the present thesis is divided into four sections aiming to provide an overall background to further understand some of the most relevant traits and processes involved in grain yield determination in a traditional and a modern wheat cultivar under a wide range of N and water availabilities in a Mediterranean region.

The first section highlights the main results and findings throughout the experimental chapters, aiming to integrate them and underline their implications in agronomy and breeding. Differences between the traditional and the modern cultivar were analysed in terms of source-sink relationships, grain yield and resource use and resource use efficiencies.

The second section adds some additional elements to the discussion aside from the thesis core theme that are relevant to the results interpretation, strengths, weaknesses and extrapolation.

The third section recaps briefly the conclusions reached after fulfilling the main objectives of the present thesis (outlined in the first chapter).

Finally, the fourth section offers ideas on potential opportunities for future research based on the present thesis findings that could be worthwhile pursuing.

6.1. Do a traditional and a modern wheat cultivar differ in their grain yield determinants under Mediterranean conditions?

As discussed in chapter II of the present thesis, the main two numeric yield components, grain number per unit land area and mean individual grain weight (IGW), are frequently negative related in cereals. This feedback interaction is expected to be higher in modern than in older cultivars because of their increased grain number, and may be interpreted as competition among grains for limited resources (*i.e.* source limitation for grain filling). This general possible interpretation could be even stronger for Mediterranean environments, subjected to frequent physiological stresses during the grain filling period and where grain size may also determine grain yield variations. Therefore, it was critical to analyse the physiological evidences behind the grain number and weight relationship for a traditional and a modern cultivar under Mediterranean conditions as the first approach to indicate which component would represent a more relevant opportunity to improve productivity.

In the study, average IGW and grain number per unit land area were independent. The results based in a source-sink manipulation experiment (as described in Chapter II) indicated that from all the scenarios analysed throughout two experiments in two different locations, covering a wide range of water and N availabilities as well as thermal conditions during grain filling, grain weight only varied from 2 to 15% in response to a 100% increment in the resources availability per grain by halving the spikes (Fig. 6.1) both for the traditional and the modern cultivar. Although Soissons, being the modern cultivar, tended to have higher number of grains both per spike and per unit land area with lower IGW (Table 2.1 and Fig. 3.4b and d), it did not present a higher response to the increment in assimilates availability.

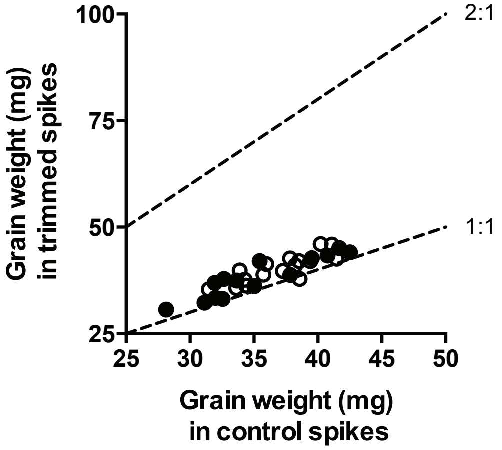

Figure 6.1. Averaged grain weight for all grans in the spikes and for only the proximal grains in central spikelets in trimmed spikes plotted against the same variable in the control spikes for Anza (open symbols) and Soissons (closed symbols) for each background treatment in experiments 1 and 2 as described in chapter II. Dashed lines represent the 1:1 and 2:1 ratios respectively.

These results are pointing out that grain weight is mainly sink-limited during grain filling, even under Mediterranean environments subjected to frequent stresses during this period (*e.g.* Serrago *et al.*, 2013 and references quoted therein) and that even when wheat breeding was consistently increasing sink strength, the realisation of yield during the effective period of grain filling remains essentially sink-limited; a conclusion in line with that reached by Kruk *et al.* (1997) that still holds even when more than a decade of breeding elapsed since then. Consequently, in line with the fact that wheat grain yield is almost universally correlated to grain number per unit land area (Slafer *et al.*, 2014) and that grain growth is mostly sink-limited (although some advanced breeding lines may exhibit a certain degree of co-limitation, Acreche and Slafer, 2009), increasing grain number per unit land area could be relevant to further increase grain yield. However, the complexity of grain number determination along the growing cycle needs to be taken into account, since there are negative feedback processes involved in its numerical sub-components determination. A path to increase grain number implies increasing spike fertility: this may be achieved through increases in canopy photosynthesis (*e.g.* Araus *et al.*, 2008; Reynolds *et al*,

2012) or by further increasing dry matter partition to the juvenile spikes before anthesis and/or the efficiency with which spike dry matter at anthesis is used to set grains (fruiting efficiency; Slafer *et al.*, 2015). The last two traits (partitioning and fruiting efficiency) are further discussed in the next section.

Besides, from a yield components point of view, there is virtually no overlapping between the number of grains per unit land area and grain weight generation (Miralles and Slafer, 1999), reinforcing the idea that even if the relationship between them were negative, there would not be a negative feedback between these two components. So, its negative relationship seems to be explained by the increase in grains with lighter potential weight (*i.e.* grains in more distal positions of the spikes and in more secondary tillers) whenever grain number is increased (*e.g.* Acreche and Slafer, 2006).

Throughout the present thesis, a traditional (Anza) and a modern (Soissons) wheat cultivar were compared in terms of grain yield, resource use and resource use efficiencies under different environmental backgrounds representative of the Mediterranean erratic conditions, generated mainly by N and water availabilities and seasonal variability. The main results obtained are presented graphically in Figure 6.2 using the framework scheme discussed in Chapter I (Fig. 1.1), while the average difference between the modern and traditional wheat is also presented in another scheme (Fig. 6.3). The wide range of environmental conditions explored in all experiments resulted in the wide range of values obtained in all traits (Fig. 6.2). The comparison explored 24 cases with a grain yield ranging from 0.78 to 7 Mg ha^{-1}. Based on the experimental data, it can be concluded that the traditional cultivar did not consistently outyield the modern one under the more stressful conditions, nor did the modern cultivar consistently outyield its older counterpart whenever the conditions were less stressful. Nevertheless, Soissons yield was marginally higher than Anza's in 9 out of 12 N and water availabilities combinations (Fig. 6.3).

Concerning the two major numerical components of grain yield, grain number per unit land area was the main component explaining grain yield variation (*e.g.* Fischer, 2008; Fischer, 2011; Slafer *et al.*, 2014), even under the frequently stressing Mediterranean conditions during post-anthesis (Fig. 6.2) as previously found in this thesis (Chapter II) and other works performed after the experiments planned in this thesis under Mediterranean environments (Acreche *et al.*, 2008; Cossani *et al.*, 2009; Albrizio *et al*. 2010; Ferrante *et al.*, 2012; Marti and Slafer, 2014). In the present study the range of grain number per unit land area explored a wide variation from *c.* 2500 to 24000 grains per m^2 (Fig 6.2) and explained *c.* 90% of variation in grain yield for both cultivars (Chapter III). This conclusion reinforces the idea that further physiological understanding of grain number per unit land area and its complex determination along the pre-anthesis period is needed to continue increasing grain yield (Fischer, 2008; Sadras and Slafer, 2012; Slafer *et al.*, 2014).

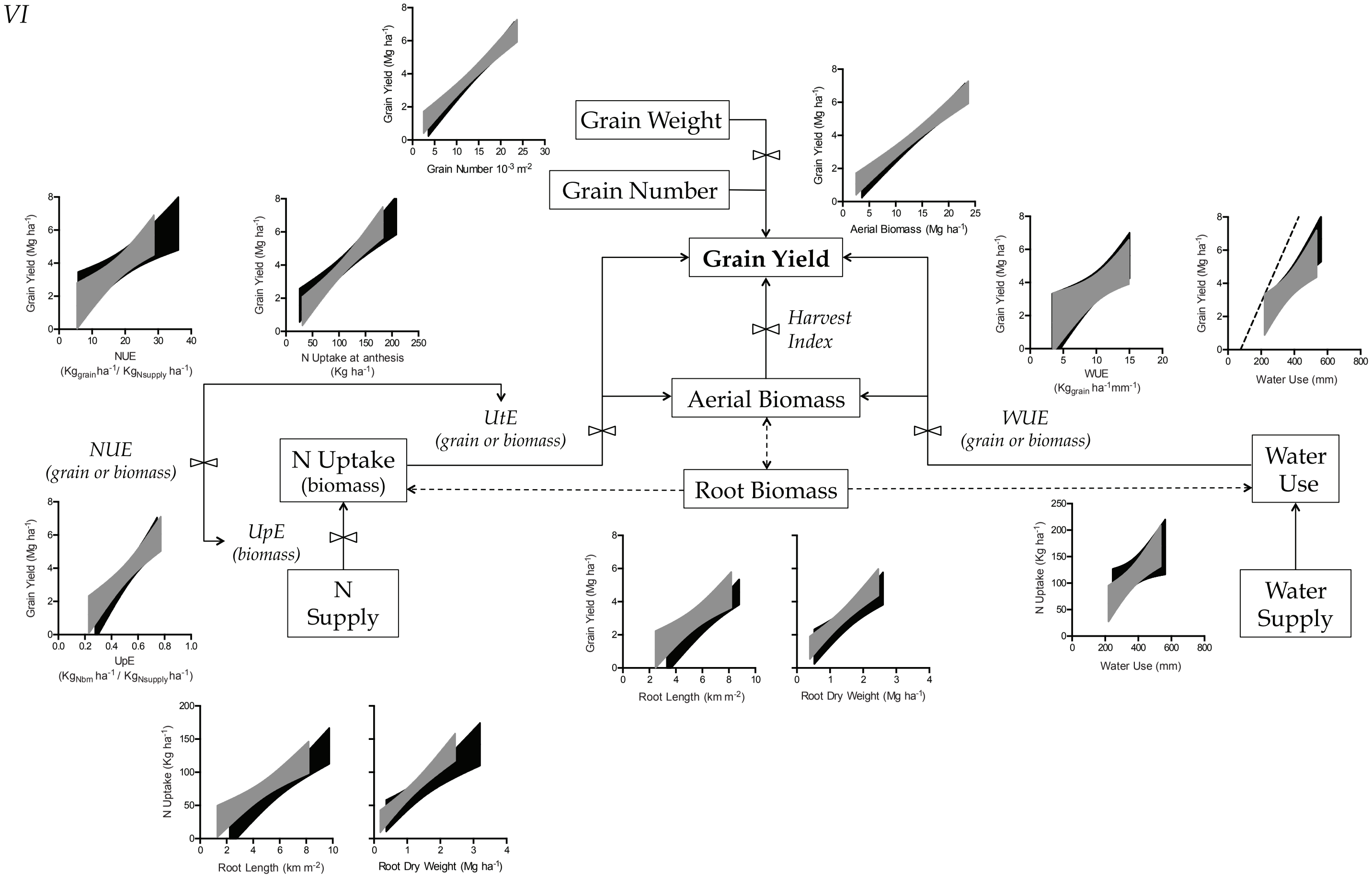

Figure 6.2. Water and nitrogen–related traits and processes involved in grain yield generation and relevant relationships among them. The grain yield or aerial biomass produced per unit of water use is the water use efficiency (WUE). Adapted from Passioura and Angus, 2010. The ratio between aerial biomass N uptake to total N supply at maturity is the N uptake efficiency (UpE). The ratio of grain yield or aerial biomass to aerial biomass N uptake at maturity is the N utilisation efficiency (UtE). The grain yield or aerial biomass produced by unit of N supplied (*i.e.* the product of UpE by UtE) is the Nitrogen use efficiency (NUE). Grain yield is partitioned into its two main numeric components: mean grain weight and grain number per unit land area. Relationships among traits are plotted as Anza (grey surface) and Soissons (black surface) linear regressions with their 95% confidence interval bars.

According to the experimental results of this thesis, grain yield greatly vary with total aerial biomass production (Fig. 6.2) while no relationship was found with harvest index (HI). Thus, variations in grain yield were more related to biomass accumulation, and therefore related to resource capture than to biomass partitioning to the grain.

As for resource use as grain yield determinants, N uptake was positively related to grain number per unit land area and to grain yield both for the traditional and the modern cultivar, again with no consistent differences between them in terms of N uptake (Figs. 6.2 and 6.3) and throughout the whole range of environmental conditions explored by the treatments and experiments across the growing seasons. However, the relationship was stronger at anthesis, indicating that N uptake took place mainly during pre-anthesis, whilst during post-anthesis, that N absorbed before was translocated to the grains, with no further N uptake. Similarly, there was no additional increment in the grain N concentration due to post-anthesis N uptake.

Changes in grain number per unit land area and grain yield were also explained by WU with no differences between cultivars. WU was in turn related to variations in N uptake (Fig. 6.2), suggesting that water evapotranspirated by the crop increases N uptake and consequently so do grain number and grain yield. Naturally, N and water use tended to be increased with higher soil N and water availabilities. In general, across the backgrounds explored in the study, the predominant limiting factor was N. Similar results in terms of resources use have been reported by other authors in Mediterranean environments (Garabet *et al.*, 1998; Albrizio *et al*. 2010, Cossani *et al*., 2012). Regarding N and water use differences between cultivars, no clear advantages of the traditional over the modern one were found under the poorest conditions.

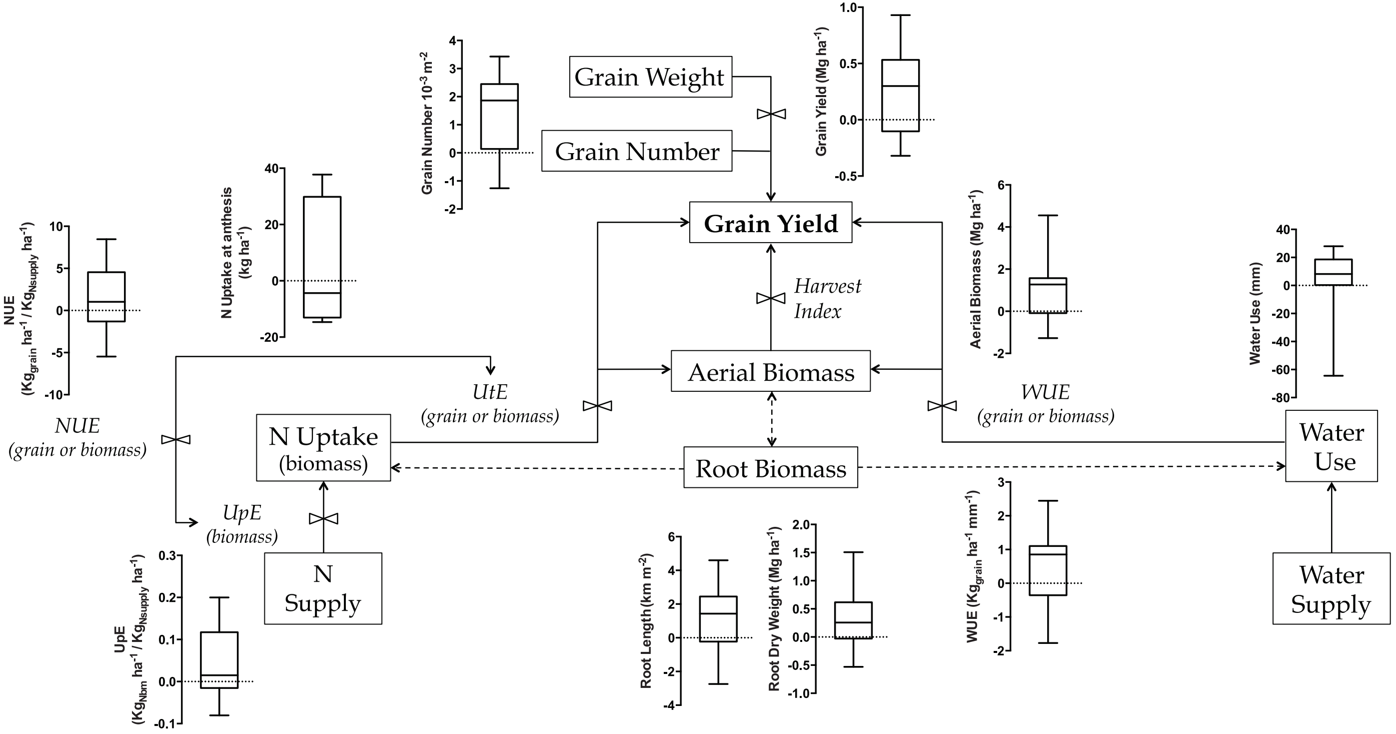

Figure 6.3. Differences between Soissons and Anza average values within treatments and experiments in the present thesis on water and nitrogen–related traits and processes involved in grain yield determination. Grain yield or aerial biomass produced per unit of water use is the water use efficiency (WUE) (adapted from Passioura and Angus, 2010). The ratio between aerial biomass N uptake to total N supply at maturity is the N uptake efficiency (UpE). The ratio of grain yield or aerial biomass to aerial biomass N uptake at maturity is the N utilisation efficiency (UtE). The grain yield or aerial biomass produced by unit of N supplied (*i.e.* the product of UpE by UtE) is the Nitrogen use efficiency (NUE). Grain yield is partitioned into grain weight and grain number per unit land area main numeric components. Grain yield differences between Soissons and Anza for each trait are plotted in a box and whisker graph. The box extends from the 25th to 75th percentiles, the line in the middle of the box is plotted at the median and the whiskers go down to the smallest value and up to the largest.

The two components of N use (i) uptake efficiency and (ii) utilisation efficiency equally limited NUE in the two studied cultivars, which presented inconsistent differences between them for these traits. NUE was positively related to grain yield (Fig. 6.2), nonetheless, UpE better explained grain yield (Fig. 6.2) than UtE, which seems reasonable, since grain yield was further limited by biomass production than by HI. N and water supply also affected NUE and its components. As previously reported NUE and UtE decreased with soil N availability (Gaju *et al*., 2011; Pask *et al*., 2012; Cossani *et al*., 2012), therefore, improving NUE would not only improve yield but also reduce fertiliser needs (Gaju *et al*., 2011; Pask *et al*., 2012), which in turn would reduce simultaneously production costs and likelihood of contamination; improving sustainability. UpE remained more stable across the environmental treatments and was only increased by irrigation during the second experiment, as well as NUE. However, no consistent difference between the modern and the traditional cultivars was observed (Fig. 6.3).

Regarding WUE, again, there seemed to be no consistent advantages for either cultivar over the other in terms of WUE (Fig. 6.3). In general, WUE was increased by N fertilization, as previously reported by others authors in Mediterranean regions (Delogu *et al*., 1998; Cossani *et al*., 2012). Grain yield was positively related to increases in WUE (Fig. 6.2), although it was independent from NUE.

Roots related traits (*i.e.* root dry weight, total root length, root length density…) remain the less studied from all grain yield determinant, particularly under field conditions, due to the difficulty of sampling and measure under such conditions (Ayad *et al*., 2010). To the best of our knowledge, there are some other works developed in Mediterranean field conditions for small grain cereals (Lampuralanés *et al*, 2001; Lampurlanés *et al*., 2002; Izzi *et al*., 2008; Ayad *et al*., 2010; Morell *et al*., 2011; Carvalho *et al*., 2014), but none of them contrasted a traditional and a modern wheat cultivar. In this context, a contribution of the present thesis is the in-field sampling of the rooting system and ulterior analysis, which although the implicit high level of data dispersion, may contribute to a more realistic approach (and therefore extrapolation) to the root system and its relationship with resource capture of a traditional and a modern cultivar under field conditions.

In the tested conditions of the present thesis, the modern cultivar surpassed the traditional one in terms of RL, RDW, RLD and R:S (Fig. 6.3). Nevertheless, both cultivars were able to achieve similar levels of biomass and grain yield production, suggesting that the traditional cultivar might be more efficient in terms of resources capture than the modern one. In fact, by anthesis both Anza and Soissons had reached the suggested RLD critical value of 1 cm cm^{-3} (King *et al*., 2013) for soil available water use in the first 25 cm of soil.

Variations in N uptake were directly related with root growth (RL and RDW; Fig. 6.2), while there was not a clear trend between root traits and WU. Grain yield was well explained by both RL and RLD at maturity (Fig. 6.2).

Considering that RL and RDW seems to be determinants of N use and grain yield, focusing on improving these traits could be useful to further increase grain yield. However, the lack of difference between the two studied cultivars grain yield could be suggesting that once a threshold root system size has been achieved there is no additional advantage in investing more resources to root growth. Further analysis using a wider genotypic background would be required to determine whether intrinsic differences between cultivars rooting system and/or environmental factors have a direct impact in resource use and yield.

6.2. Additional remarks

Regarding crop phenology, Anza reached anthesis earlier than Soissons in all experiments and the availability of resources provided by the environmental treatments did not affect noticeably this period duration in either cultivar. In contrast, there were no significant differences in grain filling duration between Anza and Soissons. However, when maturity was estimated visually using Zadoks scale, as in experiment 04, (Fig. 3.2; Chapter III) Soissons presented a higher thermal time from anthesis to maturity than Anza. Nonetheless, when calculated according to the model used in chapter II to accurately estimate the duration of the grain filling period, Soissons reached physiological maturity earlier than Anza (300 ºCd and 350 ºCd in average respectively). This apparent conflict highlights the importance of a good method to determinate physiological maturity in order to study the genotypic and environmental effects on the grain filling period, as well as the coarse approach of using a constant and universal base temperature for all cultivars, when genotypic differences can be identified when studied (*e.g.* Slafer and Savin, 1991). Moreover, this difference suggests that the physiological maturity was reached much earlier than the green color loss used as an indicator in the visual determination. Furthermore, this would reinforce the idea of the lack of source limitation to the grain growth.

Grain number per unit land area, has been extensively proven to be related to progress and variations in grain yield (Slafer *et al.*, 2014) and it has more recently been found to be positively correlated to the spike dry matter at anthesis (*e.g.* Prystupa *et al.*, 2004; Fischer 2011; Pedro *et al.*, 2011; Ferrante *et al.*, 2012). This positive relationship was also clearly observed in this thesis (Chapter III) under the wide range of environments explored. However, the relationship between grain number and spike N content at anthesis did not improve the previous one (Fig. 6.4), as also found in Prystupa *et al.* (2004) for barley and Ferrante *et al.* (2012) for durum wheat. This findings are in agreement with Fischer (2008 and 2011) that reported that even under restricted N conditions, grain number is more directly related to the spike dry weight than to its N content at anthesis, indicating the grain number determination is not directly related to N uptake by the spike (that depends in turn on the crop N status). Fischer (2012) as well as Ferrante *et al.* (2012) also noted a constitutive trade-off between fruiting efficiency and potential grain weight. The same trend was observed in this thesis, Soissons presented higher fruiting efficiency than Anza and tended to have lighter grains, more noticeably in experiment 05 where differences between cultivars were significant for both traits.

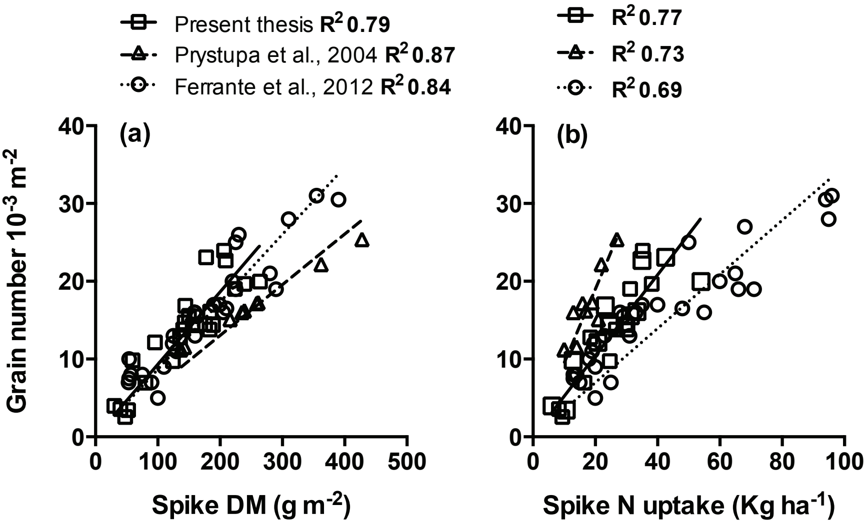

Figure 6.4. Grain number per unit land area against (a) spike dry matter at anthesis and (b) spike N uptake at anthesis for the pooled data from all the experiments in the present thesis (squares); Prystupa *et al.* (2004) (triangles) and Ferrante *et al.* (2012) (circles) respectively. The solid line represents this thesis data linear regression, the dashed line Prystupa *et al.* (2004) and the dotted line corresponds to Ferrante *et al.* (2012) data regression, all three forced through the origin.

6.3. Main conclusions

Wheat grain yield has proven to be mainly sink-limited during grain filling under a wide range of environmental conditions, even for rainfed crops in Mediterranean regions and grain number per unit land area remains the main component explaining its variation; both for the traditional and the modern cultivar of putatively higher potential yield. Thus, in order to further increase grain yields, it would be useful to identify traits that would lead to increase this component, *e.g.* spike fertility.

The traditional and the modern wheat cultivar did not consistently differ in grain yield across the wide range of explored environments. Neither the traditional cultivar outyielded the modern one under the most stressful conditions nor the modern one yielded regularly more under high yielding conditions; however it did tend to yield marginally more across all the environments explored in the present thesis (Fig. 6.3).

Variations in grain number per unit land area, and therefore in grain yield, were related to N uptake and water use both for the traditional and the modern cultivar, none of them presented a clear advantage in resource capture over the other under low or high yielding conditions. N and water use were limited by N and water supply.

N uptake occurred mainly along the pre-anthesis period, while grain N content was acquired chiefly by translocation from the vegetative organs to the grain and exceptionally by further N uptake during post-anthesis.

Both the traditional and the modern cultivar presented similar resource use efficiencies (NUE and WUE) and they were related to their variability in grain yield.

NUE was equally explained by its two components: UpE and UtE both for the traditional and the modern cultivar. However, variations in grain yield were more related to UpE than to UtE, since grain yield changes were better explained by biomass accumulation than by partition to the grain. There were no consistent differences between cultivars for UpE and UtE.

In most of the analysed cases, the modern cultivar presented higher values of root traits (RL, RDW, RLD and R:S) than the traditional one. In turn, N uptake and grain yield were related to RL and RDW across environments, but difference between cultivars did not translate in consistent differences in grain yield.

6.4. Opportunities for future research

The opportunities for future research suggested in this section are broad extrapolations based partially in the experimental results of the present thesis and in the references reviewed for their interpretation and discussion that arise topics and hypothesis that would require further research.

Noticeable wheat grain yield improvement during the last half of last century has mainly been achieved through the introduction of the semi-dwarf cultivars leading to an increased HI with slight changes in accumulated biomass (Calderini and Slafer, 1999; Foulkes *et al.*, 2011; Reynolds *et al.*, 2012; Richards *et al.*, 2014) and by increased grain number (Shearman *et al.*, 2005; Acreche *et al.*, 2008). Both for the traditional and the modern cultivar, HI in this study reached up to *c.* 45-50% reported as the current maximum achieved for wheat (Passioura and Angus, 2010; Fischer, 2011). As remarked by Fischer (2011) considering that both crop height and HI are close to their optimal ranges to maximise yield, biomass is a useful trait to breed for, as long as HI is maintained. The way to maintain it would be to keep increasing spike fertility, through either further increasing partitioning to the spike during stem elongation (Slafer *et al.*, 2005; Richards *et al.*, 2014) or by improving fruiting efficiency (Slafer *et al.*, 2015). Since spike dry weight at anthesis and grain number are positively associated (Ferrante *et al.*, 2012), further exploring this relationship could be fruitful (Fischer, 2011) to continue increasing yield through dry matter accumulation and grain number. Fruiting efficiency, the efficiency with which the resources allocated to the spikes are used to produce a certain number of grains (Slafer *et al.*, 2015 and references quoted therein), has not been consistently used in breeding so far and is a promising alternative.

As resource use is concern, further research on what is the stronger driving force behind NUE, N absorption (UpE) or utilisation (UtE) both in high and low-yielding conditions would help to decide which N related trait is worthwhile improving either through management and/or breeding. As pointed out in Gaju *et al.* (2011), genetic gains in NUE were not unequivocally correlated with either of its two components, the results therein quoted greatly differed with cultivars, environments and N supply levels. In general terms, under low N supply NUE tends to be either more related to UpE or to both UpE and UtE, while, as N availability increases NUE is better explained by UtE (Ortiz Monasterio *et al.*, 1997; Pask *et al.*, 2012). In the present study, as well as in others carried out under Mediterranean conditions (*e.g.* Cossani *et al.*, 2012), NUE appears to be equally related to both components, but further work would be required to support the present findings with a wider range of cultivars. Taking into account that improved cultivars are often bred under high yielding conditions along with the increasing need of expanding cropping soil use to semi-arid environments, a special emphasis in testing for these traits under low-yielding environments, such as most of the Mediterranean cropping area around the world, would be very useful.

As previously discussed, there is still scope to improve grain yield optimizing the ability of the crop to capture resources. Compared to the extent of information available for resource use and resource use efficiency and physiological yield determinants in general for the areal plant system, there is still a long road ahead to investigate in the roots compartment. In the present thesis, the larger root system of the modern cultivar did not translate in increased resource capture and therefore significant differences in yield; however, it could be useful to corroborate these results for instance, under contrasting conditions of less mobile nutrients, such as phosphorus, where the magnitude of the genotypic differences observed in the present thesis could be relevant. Identifying genetic variability for traits such as RLD as well as architectural traits such as root axis number or vertical distribution (Foulkes *et al.*, 2009) in a wider genotypic and environmental base would be relevant to grain yield improvement, particularly limited environments such as Mediterranean areas.

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