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WATER USE EFFICIENCY IN C_3 CEREALS UNDER MEDITERRANEAN CONDITIONS: A REVIEW OF SOME PHYSIOLOGICAL ASPECTS

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SUMMARY – In this review we will discuss physiological traits of C_3 cereals related to water use efficiency (WUE) in Mediterranean environments, from leaf (instantaneous WUE) to crop level (water productivity). Carbon isotopic discrimination ($\Delta^{13}C$) is the main approach used to estimate instantaneous WUE in C_3 plants, and the negative correlation between both parameters has been confirmed by several works. However, the relationship between $\Delta^{13}C$ and grain yield is more complex, and may differ among environments. In Mediterranean irrigated conditions, a positive correlation between $\Delta^{13}C$ and grain yield is found in barley and wheat, whereas in 'stored-water' crops (such as in some regions of Australia), lower $\Delta^{13}C$ (i.e. higher WUE) is associated with higher grain yield, particularly in more stressful conditions. These apparent inconsistencies and their possible implications in plant breeding are discussed.

One physiological trait that has received minor attention is the role of the ear photosynthesis to improve instantaneous WUE. Several works carried out by our group (using gas exchange analysis and carbon isotopic discrimination) have reported that, ears of barley and durum wheat have a higher instantaneous WUE than the flag leaf, both in well watered as in drought conditions. The underlying causes for the higher WUE of ears are not completely known, but their refixation capacity (i.e., the capacity of re-assimilate respired carbon dioxide) could be important. Although the genotypic variability of this trait has not been extensively studied, some data support the idea that such variability does exist.

At the crop level, decreasing soil evaporation is a crucial factor to ameliorate the WUE in Mediterranean conditions, and, in this sense, a fast initial growth of the crop (early vigour) seems to be a relevant trait. A positive correlation between higher early vigour and grain yield was found in some studies. However, early vigour may not confer an advantage in other environments, and this issue is discussed.

Finally, because the water losses by the epidermal cuticle (residual transpiration) may be important in droughted plants, we will briefly comment on possible future research directions in this field, as a possible way to improve WUE in cereals.

Key words: barley, carbon isotope discrimination, cereals, ear photosynthesis, Mediterranean, water use efficiency, wheat

INTRODUCTION

Water is the main abiotic factor limiting plant production in several regions of the world, with crop growth and economic yield being severely affected by water availability (Araus et al. 2002). The water use (WU. i.e. the water consumed) and water use efficiency (WUE, in general terms, the efficiency of this consumed water to produce biomass or grain yield) are critical parameters where water is scarce, as in semi-arid regions with Mediterranean climate.

Mediterranean climate (e.g. Mediterranean basin in Europe, North Africa, West Asia, Western Australia), is characterised by relatively scarce and erratic precipitation, with wet winter and dry and hot summers (e.g. Acevedo. et al. 1999), and spring being the main growing season (Fischer and Turner 1978). The vegetative growth of C_3 cereals takes place at low vapour pressure deficit (VPD) and (eventually) with well soil moisture conditions. Grain filling, by contrast, may be affected by terminal water stress episodes, in particular associated with high irradiances and high temperatures (i.e. 'drought'). World cereal demand is growing at the present (for wheat, *ca.* 2% per year)

(Skovmand et al. 2001), and more water will be required in expanded agriculture of the future. In this context, saving water during the crop cycle through higher WUE may have strong impact at local and regional scale. In short, the use of less water to achieve high yield is a major objective of the modern agriculture (Richards et al 2001).

In this article we will focus the discussion to C_3 cereals, in particular durum (*Triticum turgidum* L. var *durum*) and bread (*T. aestivum* L.) wheat as well as barley (*Hordeum vulgare* L.)., three widely cultivated crops in Mediterranean regions. In the last decades, several morpho-physiological traits of these cereals associated with high (or eventually, low) WUE have been studied. We will discuss some of these issues in the present review.

WATER USE EFFICIENCY: ONE TERM FOR DIFFERENT CONCEPTS

In general terms, the efficiency of one process is the ratio between the obtained product (the numerator) and the energy or resource invested in the process (denominator). In the context of WUE the 'product' is the assimilated carbon and the 'inversion' is the used water (the resource). Numerator and denominator of this ratio may be considered at several levels, and consequently, different definitions of WUE can be made. At leaf scale, WUE may be defined as the net CO_2 assimilated by photosynthesis (*A*), divided by the water transpired in the same time period (symbolised as *E* or *T*), being an i*nstantaneous* definition of WUE ($WUE_{instantaneous}$) (v.g. Polley 2002) as measured by gas exchange analysis. Intrinsic water-use efficiency *($WUE_{intrinsic}$)*, on other hand, is the ratio between A and stomatal conductance (i.e. A/g). Since *$WUE_{intrinsic}$* is not influenced by VPD (the driving force of transpiration rate), this parameter is used in comparative studies where different evaporative demands could be present (e.g. Morgan et al. 1991; Johnson 1993; Abbad et al. 2004).

Agronomists define WUE rather from an *integrative* approach, i.e. the accumulated dry matter divided by the water used by the crop in the same period (e.g. Abbate et al. 2004). It must be noted that the term '*integrative*' has two components, thus, a temporal and spatial dimension. Firstly, compared with $WUE_{instantaneous}$, the dry matter accumulation takes place throughout a longer time (at least, several days or months) than instantaneous photosynthesis rate. Secondly, dry matter represent an integrative parameter at space scale, because may include organs (leaves, stems, roots) and process (v.g. respiration in heterotrophic tissues) at several plant levels. Thus, although gas exchange and integrative WUE can be related, we must keep in mind the different spatial and temporal scale of both concepts (see below in the section '*Scaling-up: from instantaneous to crop WUE*').

In broad sense, assimilated dry mater can be considered as the total biomass (commonly, above-ground parts) or, alternatively, as the accumulated dry matter partitioned the economical product (for cereals, the grains). Thus, it may be defined a WUE for the biomass (*$WUE_{biomass}$*) and for the grain yield (*WUE_{yield}*) (v.g. Hatfield et al. 2001; Huang et al. 2005). Although strongly linked, *$WUE_{biomass}$* and *WUE_{yield}* may indicate different concepts. As we will discuss later, whereas both may be severely affected by growth, *WUE_{yield}* is also influenced by the partition of assimilated, i.e. the harvest index of the crop.

On the other hand, the denominator of the WUE (i.e. the consumed water) can be considered in different ways. Water losses may include, despite of water transpired by the plant, the direct evaporation from the soil (symbolised as E_s; e.g. Oweis et al. 2000).

Because of the existence of several scales and uses of the term '*WUE*', this concept should be accurately defined in each particular study. For instance, whereas that many authors consider the term '*transpiration efficiency*' as CO_2 assimilated by photosynthesis, divided by the water transpired in the same time period (*i.e,* a equivalent term to *$WUE_{instantaneous}$*; e.g. Araus et al. 2002), another researchers have used the same term to design the dry matter produced per unit transpiration (for instance Angus and van Herwaarden 2001; Richards et al. 2001). In order to remove the confusion arises from the misuse of the term '*water use efficiency*', which may indicate different meanings, another terms has been introduced, such as *water productivity* (Pereira et al. 2002; Passioura 2004) equivalent to WUE_{yield}. In summary, from the above considerations in this review we will use the following definitions of WUE:

Gas exchange WUE	Integrative WUE
$WUE_{instantaneous}$ = A / T	$WUE_{biomass}$ = Dry Matter / (evapo)transpired water
$WUE_{intrinsic}$ = A / g	WUE_{yield} (‘*water productivity*’) = Grain Yield / (evapo)transpired water

Instantaneous and intrinsic WUE

A higher $WUE_{instantaneous}$ can be achieved either through lower stomatal conductance (e.g.; Van den Boogard 1997; Ashraf and Bashir 2003) or higher photosynthetic capacity or a combination of both (Morgan and LeCain 1991; see references in Condon et al. 2002). At first, if a high assimilation rate is linked to a higher stomatal conductance, WUE will not ameliorate. However, photosynthetic rate could be increased by another ways. The reduction of photorespiration of C_3 plants (e.g. increasing of affinity of the enzime Rubisco by CO_2) has been postulated, but scarce exit has been achieved until the present (Reynolds et al. 2000). No increases of the photosynthetic rate were found in association with breeding, at least in durum wheat (see references in Araus et al. 2002). Only recently, increases in photosynthetic rate in wheat cultivars have been reported, although at expenses of a parallel rise of stomatal conductance (Fischer et al. 1998).

One postulated way to increase $WUE_{instantaneous}$ is by a higher specific leaf weight (SLW, the ratio between weight and leaf area), since a higher SLW represents an increase in photosynthetic machinery per leaf area. The correlation between SLW and $WUE_{instantaneous}$ has been reported, although seem to be low (Morgan and LeCain 1991). On other hand, an increase of mesophyll conductance is linked to higher photosynthetic rates, without increasing stomatal conductance. In this situation, $WUE_{instantaneous}$ will be increased. In synthetic hexaploids wheat-derived populations, mesophyll conductance accounted the 85% of variation in photosynthetic rate (del Blanco et al. 2000), suggesting that simultaneous increase in photosynthetic rate and $WUE_{instantaneous}$ are possible. If a high $WUE_{instantaneous}$ is associated with a high photosynthetic capacity, positive correlation with growth rate could be found. This seems to be the case of some legumes (such as peanut), where variation in photosynthesis account for most of variation of $WUE_{instantaneous}$ (Condon et al. 2002 and references cited therein).

However, the increases in $WUE_{instantaneous}$ seem to be associated rather with de reduction of stomatal conductance. In this case, (as in several species in Mediterranean conditions; for instance Bolger and Turner 1998), the growth rate may be reduced. Thus, high $WUE_{instantaneous}$ can be associated with conservative water use (and consequently, a lower growth; Condon et al. 2002) and this may have penalties in terms of grain yield (see Araus et al. 2002). However, Van Den Boogaard et al. (1996), analysing two cultivars, of wheat reported that higher water use efficiency was independent of growth rate. In fact, *A/T* ($WUE_{instantaneous}$) ratio was positively correlated with LAR (leaf area ratio). Since a higher LAR is commonly associated with higher growth rates (Poorter 1989), the results reported by Van Den Boogaard (1996) indicate that a higher $WUE_{instantaneous}$ and high growth rate could not to be mutually excluding.

Gas exchange analysis carried out in bread wheat showed that there are genotypic difference in $WUE_{instantaneous}$ (Morgan and LeCain 1991; Abbad et al. 2004), although the greatest genetic variation were observed in the comparison of hexaploid wheat with early progenitors (see Morgan et al. 1993 and references cited therein).

Higher stomatal conductances have been reported in the progress of yield analysing cultivars of Northwest Mexico (Fisher al. 1998). Cultivars with higher transpiration rate and, thus, greater transpiration rate and canopy temperature depression (CTD), show higher grain yield. This positive correlation between CDT and grain yield has been reported elsewhere, in particular in warm regions, where heat tolerance associated to a cooler canopies could be important (Reynolds et al. 2001). Consequently, if the increase of $WUE_{instantaneous}$ is associated to a reduction of stomatal conductance, penalties in terms of heat tolerance (and virtually in grain yield) could take place in crops of warm regions.

Instantaneous WUE and high growth rate: are they mutually exclusive?

The influence of physiological traits on WUE depends on the balance between the effects on growth and WU. At first, plant traits that increase $WUE_{instantaneous}$ might have penalties in terms of growth rate (Van den Boogard et al. 1997). In other words, it has been argued that a high *WUE* could be genetically linked to a low growth rate. By one side, relative growth rate (*RGR*) is split into the net assimilation rate (*NAR*, the increase in plant weight per unit leaf area) and the leaf area ratio (*LAR*, the ratio between the total leaf area and the total plant weight) (Poorter 1989). On the other hand, plant transpiration rate is the product between the *LAR* and the transpiration rate per leaf area unit (*E*). Consequently, if a morphological or physiological plant trait increases the WUE affecting the *LAR*, the *RGR* could decrease. Moreover, a lower transpiration rate per unit leaf area could lead to a decrease in *NAR* if a lower stomatal conductance and photosynthetic rate area were implicated. However, some reports show no association between a higher A/T ratio (i.e. $WUE_{instantaneous}$) and slow growth (Van den Boogaard et al. 1997). In this study -where 10 cultivars of bread wheat were analysed- a positive correlation between *LAR* and the *A/E* ratio was found. Both assimilation rate and stomatal conductance showed negative correlation with the *LAR*, but the slope was lower in the former, leading to an increase of the *A/E* ratio at higher *LAR* (Van den Boogaard et al. 1997). In summary, an amelioration of the $WUE_{instantaneous}$ could be possible without a concomitant decrease of growth rate, in particular if *LAR* was not affected.

Instantaneous WUE and water stress

The broadest known effect of the water stress in plants is the stomatal closure and the increase of $WUE_{instantaneous}$. The increase of $WUE_{instantaneous}$ under water stress is a well documented phenomenon (e.g. Johnson 1993; Morgan et al. 1993; Rekika et al. 1998; Van den Boogaard et al. 1997), although the response of $WUE_{instantaneous}$ is dependent of the severity of the water stress. In bread wheat, for instance, a moderate water deficit, can lead to a relevant increase of $WUE_{instantaneous}$ (e.g. Morgan et al. 1993; Rekika et al. 1998; Van den Boogaard et al. 1997), but a decrease of $WUE_{instantaneous}$ has also been reported in plants subjected to severe stress (for instance El Hafid et al. 1998; Shanguan et al. 2000). This behaviour is not surprisingly, because at severe water deficit, photosynthesis may be decreased by metabolic causes (i.e. non-stomatal limitation Lawlor 2002). Thus, in spite of the decrease of transpiration rate when stomata are partially closed, the $WUE_{instantaneous}$ may drop at severe drought if photosynthetic capacity is affected. In the last years, the increase of $WUE_{instantaneous}$ under moderate drought is used in management systems where a 'deficit irrigation' or 'partial root drying' is imposed to the crops. This issue is discussed in section '*WUE and agronomic practices' (see below)*.

Scaling-up: from instantaneous to crop WUE

Several efforts has been conduced to understand mechanistic (genetical and physiological) basis of the stomatal response and development, in order to ameliorate the $WUE_{instantaneous}$ of crops (for a genetic approach, *see* Chaerle et al. 2005). However, in moving between scales, it is important to take into account that the water relations of plant canopy are distinctively different than would be predicted from lower organisation levels, such as individual leaves. Thus, differences observed in $WUE_{instantaneous}$ could not be reflected at canopy or yield levels. For instance, it has been pointed out that differences *ca.* 24% in instantaneous WUE, can drop down to 5% at canopy level (Lambers et al. 1998). Moreover, Bolger and Turner (1998), reported that, whereas significant differences in *A/g* ratio between Mediterranean annual pastures were observed in glasshouse experiments, these differences seem to be eliminated in the field. The subjacent causes of this phenomenon are diverse, but it may summarise in two points: **(a)** the dominance of 'boundary layer resistance' in transpiration rate and **(b)** the effects in thermal balance of the leaf associated to the lower stomatal conductance.

(a) If the boundary layer resistance is high, as it is the case of dense canopies of wheat and barley (Kang and Zhang 2004), the stomatal opening could exert a lower control over the transpiration rate. Considering that stomatal conductance is commonly measured by porometry -in which the leaf boundary layer is eliminated-, observed differences in stomatal conductances could have lower impact than expected in transpiration rate if the former is the dominant factor.

(b) The potential gain in $WUE_{instantaneous}$ at crop level may be lower than expected, for instance, if low stomatal conductance is linked to higher leaf temperature and, thus, increased transpiration per stomatal conductance unit (Condon et al. 2002). Additionally, the increase of leaf temperature could have penalties in the cases where the evaporative cooling effect of transpiration seems to be important (Reynolds et al. 2001).

In summary, both phenomena (i.e. boundary layer and the increase of leaf temperature) may limit the 'scaling-up' between $WUE_{instantaneous}$ of leaf and crop level. For instance, it has been pointed out that modern irrigation systems in which partial water stress is applied (improving WUE by partial stomatal closing) could have a lower effect than expected in crops with dense canopies (see below; Kang and Zhang 2004).

Decreasing futile losses of water: leaf temperature and residual transpiration

Considering that a leaf temperature is a component of the driving force of the transpiration rate, a lower leaf temperature might have a important impact in $WUE_{instantaneous}$. Low chlorophyll content has been associated with lower leaf temperatures in barley. Landraces adapted to Mediterranean dry conditions in Syria have leaves with pale colour, due to a decrease in chlorophyll content per unit leaf area. This reduction in chlorophyll did not cause any change in photosynthetic capacity (Tardy et al. 1998). In the Mediterranean landrace Tadmor, for instance, lower chlorophyll content is associated with lower leaf temperatures, in particular when stomata are closed (Havaux and Tardy 1999). Lower leaf temperature under water stress could mitigate the heat stress associate to drought, and concomitantly, reduce the losses of water across the cuticle and improve WUE.

The losses of water trough the cuticle (residual transpiration) are futile, because it is not paired with CO_2 influx into the leaf. Residual transpiration may be quantitatively important during the day, in particular in dry and hot climate and substantial genetic variability has been report for wheat (see references in Richards et al. 2001). For instance, measurements carried out on varieties and landraces from the Middle East, North Africa, INRA and CYMYT, Araus et al. (1991) and Febrero et al. (1991) found considerable differences (*ca*. 50%) in epidermal (residual) conductance among genotypes. Although the heritability of this traits is unknown (Richards et al. 2001), the results mentioned above suggest that WUE of cereals could be improved by this way. It must be noted that a better WUE linked to a lower cuticular transpiration do not have penalties in terms of photosynthetic rate, and consequently, in growth performance.

In addition, some transpiration occurs at night, through abnormal stomatal closure and the leaf cuticle. A recent report showed that night-time transpiration is not trivial in several C_3 and C_4 species, because it may achieve 20% respect diurnal rates in some cases (Snyder et al. 2003). In this work, nocturnal transpiration was positively correlated with diurnal rates, i.e. higher night time losses of water are found in species with the higher diurnal transpiration rates. The impact of this phenomenon has not been extensively analysed in crop. Warmer conditions at night (e.g. during the grain filling of cereals in semi arid- Mediterranean regions) could lead to significant water losses, decreasing the WUE of the crop.

Leaves versus ear photosynthesis: an opportunity to ameliorate $WUE_{instantaneous}$ in Mediterranean regions?

The WUE of the ear of C_3 cereals has been reported in some studies (Teare et al. 1972; Araus et al. 1993; Bort et al. 1994; Abbad et al. 2004). The higher WUE of the ear has been estimated by different approaches, including instantaneous measurements of the photosynthesis/transpiration ratio (*i.e.* gas exchange analysis) and by isotopic composition of ^{13}C or $\delta^{13}C$ (Araus et al. 1992). As it was mentioned above, the relationship between WUE and $\delta^{13}C$ is well known. In C_3 cereals, $\Delta^{13}C$ is positively related to CO_2 levels in intercellular spaces and (given a constant vapour pressure deficit), negatively related to WUE (Farquhar and Richards 1984; Hubick and Farquhar 1989). In fact, triticale (*x Triticosecale*) has been reported as having a progressively higher ^{13}C isotopic composition ($\delta^{13}C$, i.e. a lower $\Delta^{13}C$) from flag leaf to glumes and glumells (lemma plus palea) (Araus et al. 1992). Ear parts show lower $\Delta^{13}C$ compared with the flag leaf of bread wheat (Araus et al. 1993), suggesting a higher WUE (Hubick and Farquhar 1989). This observation is supported by gas exchange analysis (i.e., direct measurements of *A/T* ratio; Araus et al. 1993; Abbad et al. 2004). The higher $WUE_{instantaneous}$ in the ear might come from the capacity to recycle respired CO_2, which has been well documented (Bort et al. 1996; Gebbing and Schnyder 2001).

Genotype variability (and their possible implication in breeding) of the ear photosynthesis and WUE has been scarcely analysed. However, there are reports that suggest that some variability could exist (see Table VII in Abbad et al. 2004). In this study, the authors analyse the performance of flag leaf and the ear in photosynthetic rate and the *A/T* ratio (i.e., $WUE_{instantaneous}$) in six cultivars, either in well-watered or water-stressed treatments. Under both conditions, significant differences in *A/T* ratio between cultivars were found, suggesting that this trait could be explored in future research. In another work in bread wheat and barley, Bort et al. (1996) showed that refixation capacity of the ear (a parameter possibly linked to ear WUE) seem to be genetically fixed.

In summary, future investigations should be carried out in order to explore this field research, in particular the ear traits (e.g. refixation capacity) related to a higher photosynthetic rate and $WUE_{instantaneous}$.

Decreasing soil evaporation: early vigour in Mediterranean conditions

Soil evaporation is an important component of total water losses, in particular in sparse canopies at the beginning of the crop growth (e.g. Kato et al. 2004). In semi arid region (for instance Northern Syria), soil evaporation may be a major component of total water use, (value >50% are reported; e.g. Corbeel et al. 1998), in particular in non-fertilised crops (Gregory et al. 2000). A better seedling emergence and a more early vegetative growth (i.e. early or seedling vigour) has been reported as an important trait in terms of WUE in cereals in Mediterranean conditions (Richards et al. 2001; Richards et al. 2002). Evaporation from the soil is negatively correlated with fractional area of shaded soil (e.g. Passioura 2004). For instance, compared with wheat, barley achieve higher leaf area at early stages of the crops (e.g. López-Castañeda et al. 1995; Rebetzke et al. 2004), decreasing the loss of water by soil evaporation. The presence of a canopy can decrease soil evaporation by three main mechanisms (Gregory et al. 2000) (*a*) reduction of net radiation absorbed by the soil (*b*) humidification of the air, increasing the aerodynamic resistance to the transfer of water vapour from the soil and (*c*) the uptake of water from the roots near the soil surface reduces the hydraulic conductance (Gregory et al. 2000). In wheat canopies, the evaporation from the soil is negatively correlated with the fractional shaded area (Passioura 2004) and it is well documented that is lower in barley than wheat (Siddique et al. 1990). In this study, cultivars with higher SLA and better early vigour reduce soil evaporation. Moreover, compared with old cultivars, modern cultivars of wheat had lower soil evaporation rates early in the growing season despite that transpiration efficiency for dry matter production were similar for all cultivars. Moreover, the growth took place under low VPD, decreasing the total transpired water by the crop, and consequently, increasing the WUE (López-Castañeda et al. 1995). However, it has been reported that a closed canopy increases the water losses by interception and evaporation from the canopy (Leuning et al. 1994).

In summary, the fast growth of leaf area has little benefit in regions where soil evaporation is a small component of total crop water use (Condon et al. 2004). Similar consideration may be pointed out in areas where evaporation is high, but it is limited by the movement of water in the soil (and non by canopy density; Gregory et al. 2000; Yunusa et al. 1993). According to estimations derived from simulation models, the reduction of soil evaporation by a higher SLA and early vigour seem to occur only in Mediterranean-type environments and when high nitrogen doses are applied (Asseng et al. 2003).

Finally, it must be mentioned that an early growth may be achieved by applying some agronomic practices. Early sowing date (Oweis et al. 2000; Richards et al. 2002), non-tillage management (Klein et al. 2002) or small irrigation at the first stages of the crops (Tavakkoli and Oweis 2004), for instance, ensure early germination, seedling establishment and a fast growth. In addition, sedding pattern may be relevant. The use of narrow row spacing and adequate plant population would help conserve water and hence increase the WUE. Works carried out in semi-arid region of Morocco, for instance, revealed that WUE were increased when row spacing was reduced (Karrou 1998).

Although the study of the early vigour has received considerable attention in Mediterranean climate, it could be relevant in other dry regions. In the Loess Plateau in China (a semi-arid region), the use of straw mulch (to reduce soil evaporation) has been proposed to improve WUE in bread wheat crops (Huang et al. 2005). In this context, thus, a fast growth at early stage may replace or complement the mulching practice.

Early vigour in 'non-competitive' modern cultivars

In wheat, the widely use of *semi-dwarf* cultivars (GA -giberellic acid- insensitive) which it has increased the harvest index of modern cultivars with lower plant height (Austin 1999), is associated with short coleoptiles (Richards et al. 2002). Tall cultivars have longer and wider leaves and produce higher biomass than semi-dwarf genotypes (v.g. *Rht*) at early stage (Rebetzke et al. 2004). These traits of semi-dwarf genotypes lead a poor seedling establishment and, consequently, higher soil evaporation at the beginning of the crop. At first, it could suggest that a high potential yield and high early vigour are mutually exclusive, i.e. that modern cultivars are 'non-competitive ideotype' (Blum 1996). However, a recent report showed the existence of dwarfing genes that promote short shoot but no small coleoptiles, opening the possibility to explore in this way (see references in Passioura 2004). Researches carried out by Richards' group found that GA-sensitive lines have good partitioning characteristic (i.e. high harvest index) and, at the same time, long coleoptiles (Richards et al. 2002). Consequently, obtain wheat varieties with high yield potential and high WUE at early stage of the crops is feasible.

WATER USE EFFICIENCY, GRAIN YIELD AND ISOTOPIC DISCRIMINATION: BREEDING FOR HIGH O LOW WUE?

The Passioura equation as conceptual model

In water limited environments, grain yield could be modelled by Passioura equation, which it has been largely discussed in several works (e.g. Blum 2000; Araus et al. 2002):

$$GY = W \times WUE_{biomass} \times HI$$

where *W* is the water used by the crops (evapotranspiration) and *HI* is the harvest index. Accordingly with this model, grain yield could be increased by (a) the capacity of capture more water, (b) the efficiency for producing dry matter per unit of used water and (c) the ability to devote more assimilates to the grains (Araus et al. 2002). Although at first view this model is attractive, - and *sensu* Passioura (2004) the three components of the equation are sufficiently independent to make it worthwhile considering them one by one- the terms are not independent and their interrelationship may be complex. A higher WUE may be related with a lower water use (and virtually, lower growth and grain yield) under drought conditions. In addition, although harvest index may be drought-independent in some cases, drought-dependent HI is often a function of post-anthesis W (Richards et al. 2002 and references cited therein; see references in Araus et al. 2002).

In short, is the WUE a suitable parameter to use in breeding programs? The answer is complex and not universal, because it seems to depend on the target environment. As it was pointed out by Blum, WUE is therefore a misleading parameter when applied to plant breeding for water-limited environments where soil water extraction capacity is important (Blum 2001). Those traits that could confer a higher water extraction capacity, and then, a higher water use, such as osmotic adjustment (Blum et al. 1999), could have the opposite effect in WUE if higher stomatal conductances were involved. We discuss this point in the following section.

Carbon isotope discrimination and their relationship with WUE and grain yield

Discrimination of the stable isotope ^{13}C ($\Delta^{13}C$) has been widely accepted as an indicator of WUE (see review of this issue in Araus et al. 2001; Pate 2001). In short, $\Delta^{13}C$ in C_3 plants is determined by the following equation:

$$\Delta^{13}C = a + (b-a).(ci/ca)$$

where a = 4.4 ‰ represents the isotope fractionation associated with differential diffusivities of ^{13}C *versus* ^{12}C, b = 27 ‰ is the fractionation by Rubisco carboxilation and ci and ca are the intercellular and ambient CO_2 concentration respectively (Pate 2001). The ci/ca ratio is determined by the balance between stomatal conductance and photosynthetic rate, thus, related to the A/g ratio (the demand and supply of CO_2 respectively). Since $\Delta^{13}C$ and ci/ca are partially determined by the A/g ratio, measurements of Δ^{13} C provide a relative index of $WUE_{intrinsic}$ or $WUE_{instantaneous}$ for given VPD conditions (e.g. Pate 2001). In fact, a negative correlation between $\Delta^{13}C$ and WUE has been contrasted in several works (e.g. Hubick and Farquhar 1989; Morgan et al. 1993; Johnson 1993). It must be noted that from a mechanistic point of view, $\Delta^{13}C$ is related with instantaneous A/E ratio (or rather, with A/g ratio), but it can provide a time and spatially integrated estimate of water use efficiency. However, the correlation of $\Delta^{13}C$ with WUE at other scales could be lower (for instance, Shaheen et al. 2005).

Although the negative relationship between $\Delta^{13}C$ and WUE is widely consistent throughout several studies (*see references above*), the sign and magnitude of the correlation with grain yield in C_3 cereals is complex, and may be strongly influenced by several factors. In Mediterranean conditions, numerous studies reported a positive correlation between kernel $\Delta^{13}C$ and grain yield of bread wheat (Morgan et al. 1993) durum wheat (Merah et al. 1999; Merah et al. 2001, Araus et al. 1997; 2003; Fischer et al. 1998; Clay et al. 2001; Royo et al. 2002) and barley (Voltas et al. 1998). In a recent study Monneveux et al. (2005) confirms these previous finding, although a consistent positive correlation between grain $\Delta^{13}C$ and yield was observed only under post-anthesis water stress conditions. Under pre-anthesis and limited residual moisture stress, the correlation was weaker. At full irrigation, by contrast, no correlation was found (Monneveux et al. 2005). Summarising the former considerations, Royo et al. (2002) suggests that selecting for higher $\Delta^{13}C$ (lower WUE) in breeding programs in Mediterranean basin could take advantage only under wet or irrigation conditions.

A high $\Delta^{13}C$ in the grains may involve different phenomenon, such as (*a*) a greater access to soil water (for instance, related to a deeper root systems) or higher water extraction capacity (e.g. occurrence of osmotic adjustment) (*b*) higher remobilization of stems reserves of pre-anthesis assimilates which may have a lower isotope signature and (*c*) an earlier flowering (see Condon et al. 2002 and references cited therein). For these reasons, it has been pointed out that kernel $\Delta^{13}C$ may be a more cryptic parameter than leaf $\Delta^{13}C$ (Condon et al. 2004). However, the correlation between leaf $\Delta^{13}C$ and grain yield are poor in some cases (Merah et al. 2001).

Several works carried out by CSIRO' group (Condon et al.) showed that breeding by low leaf $\Delta^{13}C$ increased the grain yield under rainfed conditions in Australia (Rebetzke et al. 2002). In fact, the first genotypes with low $\Delta^{13}C$ and high grain yield were released during the 2002 and 2003 (v.g. *Drydale* and *Rees* cultivars; see web page of CSIRO: www.csiro.au; Condon et al. 2004). In according with this studies, the advantage of low carbon isotope discrimination is higher at low environment mean yields (i.e., when water stress is more severe). The improvement of low $\Delta^{13}C$ selection, however, decline at higher mean yield, where higher season rainfall are present (Rebetzke et al. 2002).

The apparent discrepancies between studies carried out in Mediterranean climate (e.g. in Southern Europe) *versus* some regions of Australia seem to arise from the source of water used during crop cycle. In Mediterranean zone, rainfall (although scarce) is present during the crop growth and stored water may be a minor component of the water used by the crop. In this context, genotypes with higher water extraction capacity will have higher grain yield and higher $\Delta^{13}C$. On other hand, in some regions of Australia with summer rainfall, stored water is a main part of the total water used by the crop. In those environments, rainfall are scarce after seeding, and the saving water could be a critical factor to avoid terminal severe water stress (Passioura 2004). In this context, cultivars with conservative strategy may have advantage respect 'water spender' ones. The negative correlation between the advantage in grain yield of low $\Delta^{13}C$ varieties and rainfall (mentioned above) support this idea (Rebetzke et al. 2002). Simulation of the effect on yield incorporating higher instantaneous WUE (low $\Delta^{13}C$) showed that advantage was significant in environments where dominate stored water (with summer rainfall). In environments with Mediterranean climate (i.e. winter rainfall), by contrast, improved WUE did not confer advantage in yield (Condon et al. 2004). In this case, early vigour seems to be more important (see above).

Araus et al. (2003) -analysing the environmental factors determining $\Delta^{13}C$ in durum wheat under Mediterranean conditions in several trials with 25 genotypes- found that the correlation between kernel $\Delta^{13}C$ and grain yield was steady a positive when mean yield of the trial above 2500 kg ha^{-1}. By contrast, trials with mean yield below 2000 kg ha^{-1} showed low correlation between $\Delta^{13}C$ and grain yield. Thus, in more stressful environments, $\Delta^{13}C$ may be a poor indicator of grain yield, as it was suggested by previous reports (e.g. Royo et al. 2002). Where additional water is not available to the crop, to increase WUE (selecting by low $\Delta^{13}C$) appears to be an alternative strategy (Araus et al. 2002 and references therein).

Cereals yield progress and WUE

In a retrospective study of wheat Siddique et al. (1990) found that WUE_{yield} increased substantially from old to modern cultivars, with little difference among modern cultivars. $WUE_{biomass}$, by contrast, was similar between cultivars. Improved WUE_{yield} in modern cultivars was associated with faster development, earlier flowering, improved canopy structure and higher harvest index (Siddique et al. 1990). More recently, in a study of several cultivars of bread wheat released in Mexico, Fischer et al. (1998) found an increase in photosynthetic rate and stomatal conductance in modern varieties: the rise in the last parameter (g) was higher than the former (A), leading to a decrease in $WUE_{intrinsic}$ of modern cultivars (calculated from date of Fischer et al. 1998).

One question to answer is the range of genotypic variability in WUE in cereals. The range in carbon isotope discrimination among cereals cultivars is around 4‰. However, in *Aegilops geniculata* (closely related to *Triticum*) the range seem to be higher (*ca.* 7‰), suggesting that WUE of wheat could be improved by introgression in hybridisation programs (Zaharieva et al. 2001).

WUE AND AGRONOMIC PRACTICES: IRRIGATION AND FERTILIZATION

Agronomic options for improving rainfall-use in dry land regions were extensively reviewed by Turner (2004): at least, half of the increase of rainfall-use efficiency may be attributed to improved agronomic management. The adoptions of practices such as minimum tillage, appropriate fertilization, timely planting, in conjunction with new cultivars, has the potential to increase rainfall use efficiency of dry land (such as Mediterranean) crops. Here, we will discuss irrigation and fertilization practices in relation to physiological aspects of WUE.

Irrigation

The amount, timing and frequency of irrigation have strong impacts in $WUE_{biomass}$ and WUE_{yield}. In fact, WUE may drop (e.g. Huang et al. 2005) or increase (Brandypadhyay et al. 2003) at higher irrigation levels. Irrigation may increase the WUE_{yield} without any effect in $WUE_{biomass}$, due to the increase of postanthesis water use, which results in a higher harvest index and better grain yield (Zhang et al. 1998). This author pointed out that the lack of an effect in $WUE_{biomass}$ could result from two counteracted forces: the decrease of *Es/T* ratio (related with early growth) may be counterbalanced by the decrease of 'transpiration efficiency' (in this context, the ratio between dry matter and plant transpiration), which could result from a greater leaf area and higher stomatal conductance in irrigated treatments (Zhang et al. 1998).

Oweis et al. 2000 reported that WUE_{yield} of bread wheat under Mediterranean conditions was higher at 2/3 of irrigation requirements (compared with at full irrigation, where WUE_{yield} was lower). Similar results are reported by Tavakkoli and Oweiss (2004). As these authors pointed out, the common practice of supplemental irrigation is not the most efficient in terms of WUE_{yield} for Mediterranean environments. Considering that water is the main limiting resource in dry areas, the loss of grain yield due to deficit irrigation may be negligible compared with the saving in water (Oweis et al. 2000). '*Deficit irrigation*' is a strategy under which crops are deliberately allowed to sustain some degree of water deficit and yield reduction (Pereira et al. 2002). In Northern Syria, for instance, the maximum in water productivity of wheat are achieved with some grain yield reduction (see references in Pereira et al. 2002). In fact, increases of WUEyield under water limitation are reported in several studies and climatic conditions (e.g. Abbate et al. 2004 and references cited therein).

In the last years, considerable attention have received the irrigation systems where a part of root is subjected to dry conditions (known as 'CAPRI' or 'Controlled Alternate Partial Root Irrigation'). This technique is based in two assumptions (a) fully irrigated plants usually have widely opened stomata and (b) roots in the drying soil can respond by sending a root signal to the shoots, where the stomata may be partially closed, increasing $WUE_{instantaneous}$ (Kang and Zhang 2004). Although these systems could be implemented in several crops, their use in cereals could be more doubtful. As it was mentioned earlier, in dense canopies (such as cereals crops) boundary layer resistance may be high, and exert a main control over the transpiration. Additionally, the increase of leaf temperature coupled with lower transpiration might eliminate any advantage of stomatal close. However, the advantages of 'deficit irrigation' systems (see above) suggest that stomatal control is a useful feature to improve WUE at a crop level.

In summary, WUE_{yield} in Mediterranean regions may be incremented with some irrigation, in particular at the beginning of the crops, which improves early growth and decreases soil evaporation. Additionally, some degree of water deficit may improve WUE_{yield}, which it could be explained to some extent by stomatal control on the transpiration (Abbate et al. 2004).

Fertilization

Fertilizer use has a remarkable effect on crop yield and WUE. There are several studies reporting the increase of cereal WUE with N application (e.g. Zhang et al. 1998). At first, nitrogen fertilization may increase the CO_2 assimilation rate capacity, i.e. WUE_{yield} may be ameliorated due to the improvement in $WUE_{nstantaneous}$. In addition, and probably more importantly, fertilization increases the early growth and the crop cover, protecting the soil from evaporation and, consequently, increasing the proportion of transpired water by the plant (*see references above*). Additionally, WUE is also ameliorated because more crop growth takes place with lower VPD in early spring (Zhang et al. 1998). Nitrogen and phosphorous nutrition have been shown to increase the early growth in Mediterranean environments (see above; references in Turner 2004). In wheat, nitrogen fertiliser input reduced soil evaporation, increasing the WUE_{yield} (Asseng et al. 2001; Sadras 2002).

For barley in Mediterranean conditions, higher $WUE_{biomass}$ and WUE_{yield} were achieved by nitrogen application (Cantero-Martínez et al. 2003). According to this study, the higher WUE was explained by an increase of preanthesis WUE. WUE was increased by nitrogen fertilization only when water was not limiting: an excess of nitrogen may increase the water use, without improving WUE (Cantero-Martínez et al. 2003). Gregory et al. 2000 also reported an increase in $WUE_{biomass}$ in fertilised barley. Fertilization with N and P increased the shoot dry weight, the $T/(Es + T)$ ratio and the $WUE_{biomass}$. Total water use $(Es + T)$ was not modified, but the fertilization treatment increased the WUE changing the partitioning between Es and T (Gregory et al. 2000).

Furthermore, although there are several studies that showed the correlation between vigorous growths for fertilization (at early stages) and grain yield (see above), this seem not to be the case in some environments. In dry regions of Australia, a higher vegetative growth can lead to a reduction of yield (phenomenon named as '*haying off*') in particular when vigorous growth is followed by a terminal severe water stress (Angus and van Herwaarden 2001). Although at first this yield reduction could be explained by the lack of soil water during grain filling -because more water was used in producing additional vegetative material- another causes have been proposed. For instance, the decrease of soluble carbohydrate available for retranslocation associated with nitrogen availability (see Angus and van Herwaarden 2001 and references cited therein). In spite of the subjacent causes, the association between more vigorous growth by nitrogen fertilization and higher WUE seem to be not universal, and peculiarities of the environment target must be considered.

Finally, it must be noted that there is a 'trade-off'' between a higher $WUE_{instantaneous}$ and photosynthetic nitrogen-use efficiency, because negative correlations between both parameters have been reported (e.g. Van Den Boogaard 1997 and references cited therein). As it was pointed out by this author, improving $WUE_{instantaneous}$ may be suitable in environments where water is the limiting factor, but the cost of a less efficient use of nitrogen should be considered.

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