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WATER USE EFFICIENCY

Water use efficiency in C_3 cereals under Mediterranean conditions: a review of physiological aspects

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Abstract

In this review, we will discuss physiological traits of C_3 cereals related to water use efficiency (WUE) in Mediterranean environments, from leaf ($WUE_{instantaneous}$) to crop level (WUE_{yield} or 'water productivity'). First, we analyse the $WUE_{instantaneous}$ and the possible trade-off between improving this parameter and growth/yield performance. Ways to ameliorate WUE without penalties are discussed. We also analyse in what cases breeding by high or low $WUE_{instantaneous}$ is a suitable criterion to maintain grain yield under drought (Mediterranean) conditions. This question is approached in the framework of carbon isotope discrimination, ($\Delta^{13}C$), the main indirect parameter used to integrate (at time and space scale) the $WUE_{instantaneous}$ in C_3 plants. A negative correlation between these two parameters has been confirmed by several studies. The relationship between $\Delta^{13}C$ and grain yield, however, is more complex, and may differ from one environment to another. In Mediterranean conditions with moderate or no water stress, 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 $WUE_{instantaneous}$) is associated with higher grain yield, particularly in more stressful conditions. These apparent inconsistencies and their possible implications for plant breeding are discussed. One physiological trait that has received minor attention in attempts to improve $WUE_{instantaneous}$ is the role of ear photosynthesis. Ears of barley and durum wheat have a higher $WUE_{instantaneous}$ than the flag leaf, both in well-watered and in drought conditions. The underlying causes of the higher $WUE_{instantaneous}$ of ears are not fully understood, but their refixation capacity (i.e. the capacity to 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 variation in refixation capacity may be attributable to genetic factors. At the crop level, decreasing soil evaporation is a crucial factor in efforts to improve the WUE_{yield} in Mediterranean conditions, and fast initial growth of the crop (i.e. early vigour) seems to be relevant. In wheat, modern varieties with dwarfing genes (giberellic acid – insensitive) have higher yields but, concomitantly, they have lower initial growth performance. Recently, semi-dwarf cultivars (giberellic acid – sensitive) with high grain yield and simultaneously high early vigour were found, opening new avenues to increase WUE_{yield} in wheat. The negative effects of futile water loss by cuticular and nocturnal transpiration are also commented. Finally, we discuss some agronomic practices (in particular, 'deficit irrigation' systems) linked to physiological traits that confer higher $WUE_{yield,}$, in particular, in the cases of Mediterranean regions.

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 assimilate carbon, produce biomass or grain yield; see below) are crucial parameters where water is scarce, as in semi-arid regions with Mediterranean climate (e.g. Mediterranean basin in south Europe, North Africa and West Asia as well as Western Australia and parts of South Africa and Chile). Mediterranean climate is characterised by relatively scarce and erratic precipitation, with wet winter and dry and hot summers (Acevedo *et al.*, 1999). In the case of C_3 cereals, although the vegetative growth takes place at low vapour pressure deficit (VPD) and (eventually) with well soil moisture conditions, the grain filling may be affected by terminal water stress episodes, in particular, associated with high irradiances and high temperatures (i.e. drought).

However, world cereal demand is growing at the present (for wheat, ca. 2% per year; Skovmand *et al.*, 2001), and more water will be required for agriculture in the future. In this context, producing more per unit of water during the crop cycle through higher WUE may have strong impact at local and regional scale, in particular, in semi-arid regions as West Asia–North Africa (WANA) and southern Europe. In short, the use of less water to achieve high yield is a major objective of the modern agriculture (Richards *et al.*, 2001; Araus, 2004). This study addresses some plant physiological aspects related to a better WUE in cereal crops. In particular, we will analyse the morphophysiological traits that have been identified as possible 'targets' to ameliorate WUE of C_3 cereals under Mediterranean conditions. We will focus the discussion to C_3 cereals, in particular, durum (*Triticum turgidum* L. var *durum*) and bread (*Triticum aestivum* L.) wheat as well as barley (*Hordeum vulgare* L.), three widely cultivated crops in Mediterranean regions.

We are aware that water management is a multifactorial issue, and plant physiology is only one aspect (for a detailed discussion of '*water crisis*' in the Mediterranean; see Araus, 2004; see also Parry *et al.*, 2005). However, the research of 'drought tolerance' in cereals is beyond the scope of this review (for a detailed analysis of this issue see Araus *et al.*, 2002; Parry *et al.*, 2005; Trethowan *et al.*, 2005). Phenological adjustment (flowering time), for instance, has been reported to be a crucial trait in the performance of cereals under drought conditions (Araus *et al.*, 2002). Although not analysed here, this and another traits also have a strong impact on WUE.

The main issues that are discussed in the present review may be summarised as follows: (a) ways to improve gas exchange WUE (i.e. photosynthesis : transpiration ratio) without compromising growth and/or agronomic yield, (ii) carbon isotope discrimination as a tool in cereal breeding: selecting by high or low WUE? (iii) Gas exchange in the ears: an opportunity to improve the WUE of cereals under Mediterranean conditions? (iv) Decreasing futile (i.e. non-productive) losses of water: the role of the early vigour and residual transpiration in semi-arid regions, and (v) can WUE be manipulated by agronomic practices?: deficit irrigation systems. In each section, we analyse the theoretical framework of the issue, but focus on the particular case of Mediterranean region.

The several means of the term 'Water Use Efficiency'

Before addressing the issues pointed above, in this section we define the several uses of the term 'water use efficiency'. Numerator (the obtained product) and denominator (water invested in the process) of the ratio WUE may be considered at several levels and temporal scales, and consequently, different definitions of WUE can be made. At photosynthetic organ (e.g. leaf) scale, WUE is 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 instantaneous definition of WUE ($WUE_{instantaneous}$) (Polley, 2002). Instantaneous measurements of WUE are carried out by gas exchange methods, i.e. infrared gas analyser and porometry (e.g. Tambussi *et al.*, 2004). One related parameter, the intrinsic water use efficiency ($WUE_{intrinsic}$), is the ratio between A and stomatal conductance (i.e. A/g; for the advantage of this parameter see 'Gas exchange WUE and their impact in crops').

Agronomists and crop physiologists, however, 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 (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, weeks or months) than instantaneous photosynthesis and transpiration rate. Secondly, accumulated dry matter represents an integrative parameter at space scale, because it may include organs (leaves, stems, and eventually, roots) and process (e.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 this section).

In a broad sense, assimilated dry matter can be considered as the total biomass (commonly, aboveground parts) or, alternatively, as the accumulated dry matter partitioned the economical product (for cereals, the grains). Thus, it may be defined as WUE for the biomass ($\mathrm{WUE}_{\mathrm{biomass}}$) and the grain yield (symbolised here as $\mathrm{WUE}_{\mathrm{yield}}$, Hatfield *et al.*, 2001; Huang *et al.*, 2005 or '*water productivity*', Pereira *et al.*, 2002; Passioura, 2004) respectively. Although strongly linked, $\mathrm{WUE}_{\mathrm{biomass}}$ and $\mathrm{WUE}_{\mathrm{yield}}$ may indicate different concepts. As we will discuss later, whereas both may be severely affected by growth, $\mathrm{WUE}_{\mathrm{yield}}$ is also influenced by the partition of assimilated in the economical product, i.e. the harvest index (HI) of the crop.

However, the denominator (i.e. the consumed water) of the WUE can be considered in two ways. Water losses may include, despite water transpired by the plant, the direct evaporation from the soil (symbolised as E_s; Oweis *et al.*, 2000). Thus, the estimation of WUE in experiments carried out in pots (where soil evaporation is commonly eliminated) may be sensibly different with respect to plants grown in the field.

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 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 term equivalent to $\mathrm{WUE}_{\mathrm{instantaneous}}$; Araus *et al.*, 2002; Masle *et al.*, 2005), other researchers have used the same term to design the dry matter produced per unit transpiration (Angus & van Herwaarden, 2001; Richards *et al.*, 2001). To remove the confusion that arises from the misuse of the terminology in WUE, we will use the definitions summarised in Figure 1.

Ways to ameliorate $\mathrm{WUE}_{\mathrm{instantaneous}}$, without penalties

As mentioned above, gas exchange WUE includes $\mathrm{WUE}_{\mathrm{instantaneous}}$ and $\mathrm{WUE}_{\mathrm{intrinsic}}$. From a methodological viewpoint, both are similar, but $\mathrm{WUE}_{\mathrm{intrinsic}}$ is not influenced by VPD (the driving force of transpiration rate), and, consequently this parameter is used in comparative studies, where different evaporative demands could be present (Morgan & LeCain, 1991; Johnson, 1993).

A higher $\mathrm{WUE}_{\mathrm{intrinsic}}$ can be achieved either through lower stomatal conductance (Van den Boogaard *et al.*, 1997; Ashraf & Bashir, 2003) or higher photosynthetic capacity or a combination of both (Morgan & LeCain, 1991; see references in Condon *et al.*, 2002). As pointed out clearly by Parry *et al.* (2005), the amelioration of $\mathrm{WUE}_{\mathrm{intrinsic}}$ without penalties should implicate to obtain genotypes with modified photosynthesis versus stomatal conductance relationship. In short, the challenge in the research of WUE is to make 'higher photosynthetic rate at lower stomatal conductances'. 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 bread wheat cultivars have been reported, but at expenses of a parallel rise of stomatal conductance (Fischer *et al.*, 1998). At first, if a high assimilation rate is linked to a higher stomatal conductance, $\mathrm{WUE}_{\mathrm{instantaneous}}$ will not ameliorate (but rather the opposite will happen). Photosynthetic rate could be increased by other ways such as: (a) by CO_2 concentrating mechanism (C_4-like metabolism), (b) increased mesophyll conductance and (c) increased Rubisco specificity factor (for a detailed discussion of this issue see Parry *et al.*, 2005). The reduction of photorespiration of C_3 plants (e.g. increasing of affinity of the enzyme Rubisco by CO_2) has been postulated (see references in Parry *et al.*, 2005), but scarce success has been achieved until the present (Reynolds *et al.*, 2000). However, even if all the steps of C_4 metabolism are introduced in C_3 plants, WUE will not be ameliorated without anatomical changes (whose genetic basis is still not known; Parry *et al.*, 2005), and in fact biotechnology to produce functional C_4 photosynthesis in C_3 plants is not yet available.

One postulated way to increase $\mathrm{WUE}_{\mathrm{instantaneous}}$ is by a higher specific leaf weight (SLW; the ratio between weight and leaf area) because a higher SLW represents an increase in photosynthetic machinery per leaf area. A correlation between SLW and $\mathrm{WUE}_{\mathrm{instantaneous}}$ has been reported, although it seemed to be low (Morgan & LeCain, 1991). However, an increase of mesophyll conductance is linked to higher photosynthetic rates, without increasing stomatal conductance. In this situation, $\mathrm{WUE}_{\mathrm{instantaneous}}$ will be increased. In synthetic hexaploids, wheat-derived populations, mesophyll conductance accounted for 85% variation in photosynthetic rate (del Blanco *et al.*, 2000), suggesting that simultaneous increase in photosynthetic rate and $\mathrm{WUE}_{\mathrm{instantaneous}}$ are possible. If a high $\mathrm{WUE}_{\mathrm{instantaneous}}$ 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 accounts for most of variation of $\mathrm{WUE}_{\mathrm{instantaneous}}$ (Condon *et al.*, 2002 and references cited therein). As mentioned above, however, in many cases, under Mediterranean conditions, the increases in $\mathrm{WUE}_{\mathrm{instantaneous}}$ seem to be associated rather with the reduction of stomatal conductance, with a concomitant decrease in growth rate (Bolger & Turner, 1998). Thus, high $\mathrm{WUE}_{\mathrm{instantaneous}}$ can be associated with conservative

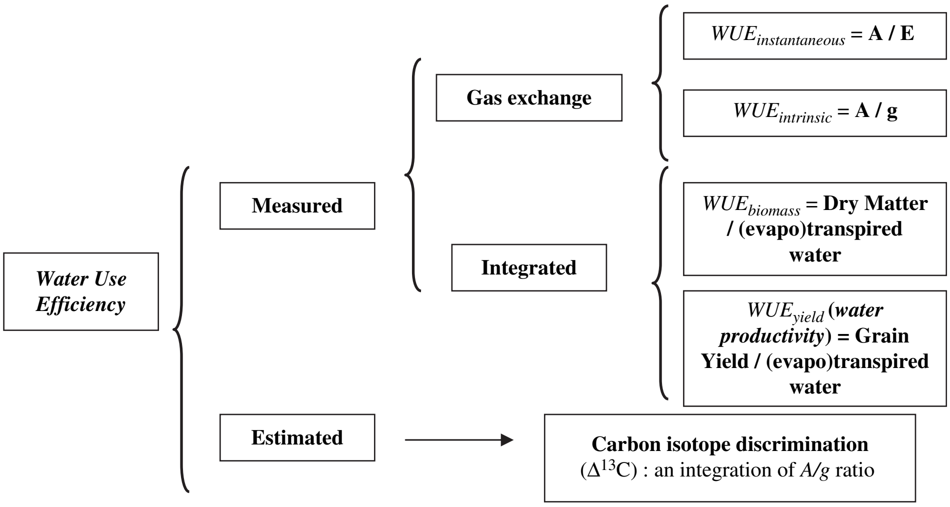

Figure 1 The several means of 'water use efficiency'. The scheme represents the several definitions of water use efficiency (WUE) used in the text. *A*, net photosynthetic rate expressed as μmol CO_2 m^{-1} s^{-1}; E, transpiration rate expressed as mmol H_2O m^{-2} s^{-1}; $\Delta^{13}C$, carbon isotope discrimination.

WU (and consequently, a lower growth; Condon *et al.*, 2002) and obviously this may have penalties in terms of grain yield (Araus *et al.*, 2002). We examine this question in the following sections. First, we will address the possible penalties of high WUE over relative growth rate (RGR).

In short, one important question to answer is if a high $WUE_{instantaneous}$ and high growth rate are mutually exclusive. The influence of physiological traits on $WUE_{instantaneous}$ depends on the balance between the effects on growth and (transpirative) WU. On the one hand, RGR (i.e. the increment in plant weight per unit plant weight) may be 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 negatively 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 a concomitant lower photosynthetic rate) were implicated. In this context, plant traits that increase $WUE_{instantaneous}$ might have penalties in terms of growth rate (Van den Boogaard *et al.*, 1997), thus, a high WUE could be genetically linked to a low growth rate. However, some reports show no association between a higher $WUE_{instantaneous}$ and slow growth (Van den Boogaard *et al.*, 1996, 1997). In this study, with 10 cultivars of bread wheat analysed, a positive correlation between LAR and the $WUE_{instantaneous}$ 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 $WUE_{instantaneous}$ 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 is not affected. This needs to be explored in the future.

At present, new tools (in particular molecular approaches) open opportunities to improve $WUE_{instantaneous}$. Reducing transpiration by partial stomatal closure is a possibility, although the control of stomatal density might also be optimised (Chaerle *et al.*, 2005). It has recently been reported that a gene (named *ERECTA*) regulates $WUE_{instantaneous}$ in *Arabidopsis* (Masle *et al.*, 2005). This gene – formerly known for its effects in inflorescence development and other traits – modifies the transpiration efficiency by acting on stomatal density, stomatal conductance and mesophyll development. Interestingly, this is considered to be the first gene to regulate $WUE_{instantaneous}$ in both components i.e. photosynthesis and transpiration rate. Homologues of this gene have been found in other species, opening a novel way to ameliorate $WUE_{instantaneous}$ in crops (Masle *et al.*, 2005).

Gas exchange in the ears: an opportunity to ameliorate WUE of cereals under Mediterranean conditions?

$WUE_{instantaneous}$ is commonly evaluated in leaves. However, compared with flag leaf, a higher $WUE_{instantaneous}$

of the ear of C_3 cereals has been reported in some studies, in particular, in Mediterranean regions (Teare *et al.*, 1972; Araus *et al.*, 1993; Bort *et al.*, 1994; Abbad *et al.*, 2004). The higher $\mathrm{WUE_{instantaneous}}$ of the ear has been estimated by different approaches, including instantaneous measurements of the photosynthesis : transpiration ratio (i.e. gas exchange analysis; Araus *et al.*, 1993; Bort *et al.*, 1994; Abbad *et al.*, 2004; Figs 2 and 3) and by isotope discrimination of ^{13}C ($\Delta^{13}C$; Araus *et al.*, 1992). The relationship between $\mathrm{WUE_{instantaneous}}$ and $\Delta^{13}C$ is well known (see below). As explained before, in C_3 plants, $\Delta^{13}C$ is positively related to CO_2 levels in intercellular spaces and (given a constant VPD) negatively related to $\mathrm{WUE_{instantaneous}}$ (Farquhar & Richards, 1984; Hubick & Farquhar, 1989). Triticale (× *Triticosecale*), for instance, has been reported to have a progressively higher ^{13}C isotope 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 durum wheat (Araus *et al.*, 1993) and barley (Hubick & Farquhar, 1989), suggesting a higher $\mathrm{WUE_{instantaneous}}$. This observation is supported by gas exchange analysis (Araus *et al.*, 1993; Abbad *et al.*, 2004; Fig. 2). Subjacent causes of the higher $\mathrm{WUE_{instantaneous}}$ are not clear, but might come from the capacity to recycle (i.e. refix) respired CO_2, a well-documented process in ear of wheats (Bort *et al.*, 1996; Gebbing & Schnyder, 2001). In addition, compared with flag leaf, ear photosynthesis is less affected by water deficit in durum wheat (Tambussi *et al.*, 2005, 2007), opening the opportunity to ameliorate $\mathrm{WUE_{yield}}$ under drought.

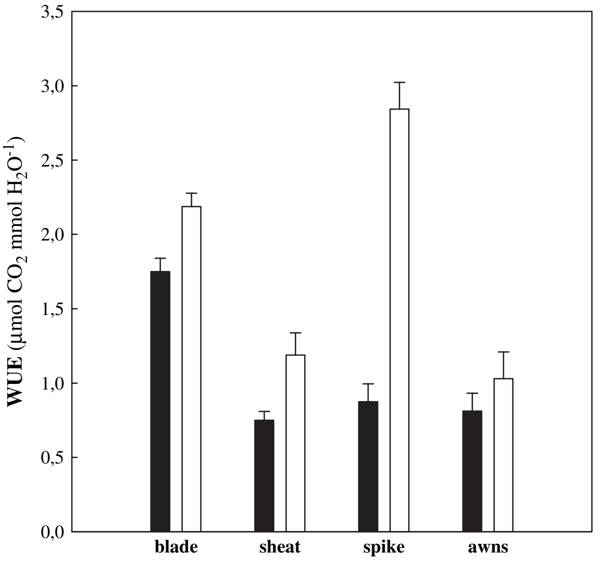

Figure 2 The WUE of the ear versus the flag leaf. Water use efficiency ($\mathrm{WUE_{instantaneous}}$) of different parts of durum wheat 2 weeks after anthesis. Separate measurements of gas exchange (IRGA) of ears with distal portions of the awns outside the chamber (referred as spikes) and the awns protruding beyond the apical spikelets (awns). Closed bars, the denominator of $\mathrm{WUE_{instantaneous}}$ was calculated using the net photosynthesis; Open bars, the denominator was corrected using net photosynthesis plus dark respiration. Values represented are means ± SEM from 10 genotypes (redrawn from Araus *et al.*, 1993).

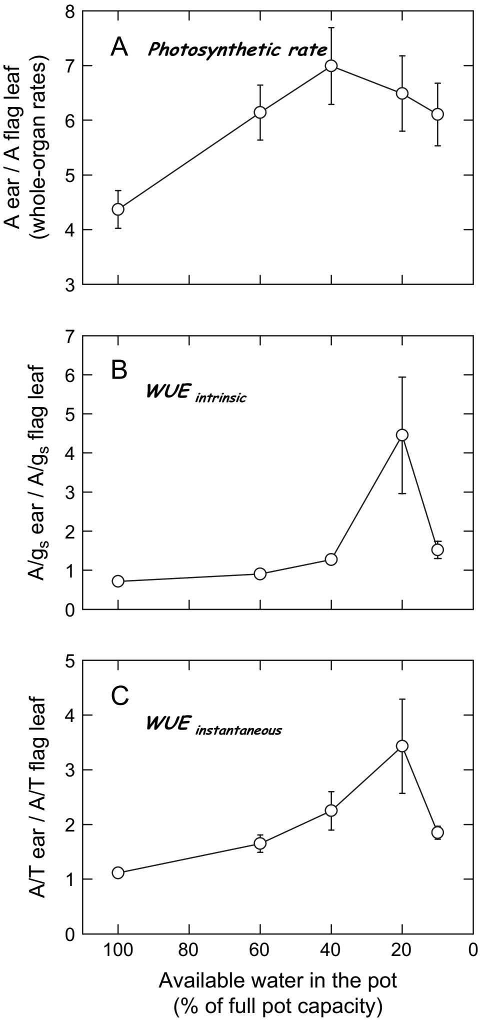

Figure 3 Photosynthesis and the WUE of the ear of durum wheat under water stress. Evolution of the ear-to-leaf ratio for the whole organ photosynthesis (A), $\mathrm{WUE_{intrinsic}}$ (B) and $\mathrm{WUE_{instantaneous}}$ (C) at different levels of available water in the pot substrate. Gas exchange measurements were performed by infrared gas analyser at anthesis (redrawn from Abbad *et al.*, 2004). Values represented are means ± SEM from six genotypes. Note that $\mathrm{WUE_{intrinsic}}$ and $\mathrm{WUE_{instantaneous}}$ of the ear increase under water stress with respect to the flag leaf.

Although genotype variability (and their possible implication in breeding) of the ear $\mathrm{WUE_{instantaneous}}$ has been scarcely analysed, there are reports that suggest that some variability exists (see table VII in Abbad *et al.*, 2004). These authors analysed the performance of flag leaf and the ear in photosynthetic rate and $WUE_{instantaneous}$ in six cultivars of durum wheat, either in well-watered or water-stressed treatments. Under both conditions, significant differences in $WUE_{instantaneous}$ between cultivars were found, suggesting that this trait could be explored in future research. In another work in durum wheat and barley, Bort *et al.* (1996) showed that refixation capacity of the ear (a parameter possibly linked to higher ear $WUE_{instantaneous}$) seem to be genetically fixed.

Since the occurrence of post-anthesis water deficit in many Mediterranean environments – where the photosynthetic contribution of the ear is quantitatively important (e.g. Araus *et al.*, 1993) – the improvement of $WUE_{instantaneous}$ (and eventually, WUE_{yield}) in this way could be feasible. Future research should explore this field, in particular, the ear traits (such as the refixation capacity) related to a higher photosynthetic rate and $WUE_{instantaneous}$.

Moving between organisation levels: the trouble of scaling-up in WUE

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. Several efforts have been taken to understand the mechanistic (genetical and physiological) basis of the stomatal response and development, to ameliorate the $WUE_{instantaneous}$ of crops (for a genetic approach, see Chaerle *et al.*, 2005). However, differences observed in $WUE_{instantaneous}$ could not be reflected at canopy or (even more) 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 & 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 summarised in two points: (a) the dominance of boundary layer resistance: if the boundary layer resistance is high, as is the case of dense canopies of wheat and barley (Kang & 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 boundary layer is the dominant factor. (b) The effects in leaf thermal balance: reduced stomatal conductance decreases transpiration but also increases leaf temperature (see references in Van den Boogaard *et al.*, 1996). The potential gain in $WUE_{instantaneous}$ at crop level may be lower than expected, for instance, if a low-stomatal conductance is linked to higher leaf temperature and, thus, increasing the transpiration per stomatal conductance unit (Condon *et al.*, 2002). Additionally, the increase of leaf temperature could have penalties (in grain yield and eventually in WUE_{yield}) in the cases where the evaporative cooling effect of transpiration seems to be important (Reynolds *et al.*, 2001).

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, and this should be taken into account by researchers. For instance, it has been pointed out that the modern irrigation systems in which partial water stress is applied (improving $WUE_{instantaneous}$ by partial stomatal closing; see above) could have a lower effect than expected in crops with dense canopies as wheat (see below; Kang & Zhang, 2004). However, the penalties of a higher $WUE_{instantaneous}$ mentioned above (e.g. a lower growth or lower water extraction capacity under water deficit) are also causes of possible failure in the scaling-up between leaf and crop level.

WUE and grain yield in cereals: selecting by high or low $WUE_{biomass}$?

In water-limited environments (as in Mediterranean semi-arid regions), grain yield could be modelled by Passioura identity (Passioura, 1977; for more details on this topic, see Blum, 2000; Araus *et al.*, 2002).

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

where WU 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 to 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 identity 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 $WUE_{biomass}$, for instance, may be related to a lower WU (and virtually, lower growth and grain yield) under drought conditions. In addition, although HI may be drought independent in some cases, drought-dependent HI is often a function of post-anthesis WU (Araus *et al.*, 2002; Richards *et al.*, 2002 and references cited therein). Improved WUE_{yield} in modern cultivars was associated with the increase of HI, in addition to a faster development, earlier flowering and improved canopy structure (Siddique *et al.*, 1990). The $WUE_{biomass}$, by contrast, seems to be similar between old and modern cultivars (Richards *et al.*, 1993). Because the HI seems to achieve the maximum (at least in wheat; Austin 1999; Reynolds *et al.*, 2000), further increments in WUE_{yield} should implicate the rise of $WUE_{biomass}$. Although we do not discuss this issue in this review, we are aware that the amelioration of HI under water-stress conditions (i.e. drought) is an important issue.

The main question that we approach here and in the following sections is whether WUE is a suitable parameter to be used in breeding programmes. The answer depends on the target environment. As pointed out by Blum (2000), WUE is therefore a misleading parameter when applied to plant breeding for water-limited environments where soil water extraction capacity is important. Those traits that could confer a higher water extraction capacity, and then, a higher WU, such as osmotic adjustment (Blum *et al.*, 1996), could have the opposite effect on $WUE_{instantaneous}$ if higher stomatal conductances were involved (as discussed above). The carbon isotope discrimination is a suitable framework to discuss this crucial aspect of WUE in crops.

Carbon isotope discrimination as a tool in cereal breeding

Discrimination of the stable isotope ^{13}C ($\Delta^{13}C$) has been widely accepted as an indicator of $WUE_{intrinsic}$ (see review of this issue in Araus *et al.*, 2001; Pate 2001) and it can be used as a suitable framework to analyse this question. 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, A/g ratio (the demand and supply of CO_2 respectively: $WUE_{intrinsic}$). As $\Delta^{13}C$ 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 (Pate 2001). In fact, a negative correlation between $\Delta^{13}C$ and gas exchange WUE has been widely reported (Hubick & Farquhar, 1989; Johnson, 1993; Morgan *et al.*, 1993). However, the sign and magnitude of the correlation of $\Delta^{13}C$ 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 (Araus *et al.*, 1997, 1998, 2003b; Fischer *et al.*, 1998; Merah *et al.*, 1999, 2001; Clay *et al.*, 2001; Royo *et al.*, 2002; Fig. 4) and barley (Voltas *et al.*, 1998). In a recent study, Monneveux *et al.* (2005) confirmed 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 preanthesis and limited residual moisture stress, the correlation was weaker. At full irrigation, by contrast, no correlation was found (Monneveux *et al.*, 2005).

The positive correlation between $\Delta^{13}C$ and yields under Mediterranean conditions may also be explained by phenology. Genotypes with fewer days from sowing to flowering show higher $\Delta^{13}C$ values (Richards, 1996; Araus *et al.*, 1998) probably because they attain grain filling with more water in the soil, whereas the evapotranspirative demand is lower. In summary, positive relationships between $\Delta^{13}C$ and grain yield are mostly found under moderate to well-watered conditions, whereas for severely stressed environments (yields below 2.0 Mg ha^{-1}) such relationships are absent or negative (Voltas *et al.*, 1999; Araus *et al.*, 2003).

In summary, a higher $\Delta^{13}C$ 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 stem reserves of pre-anthesis assimilates, which may have

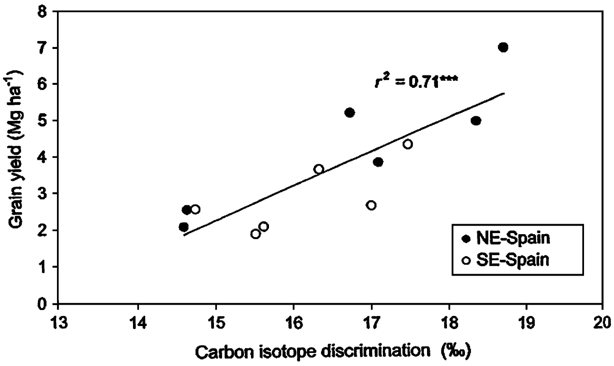

Figure 4 The relationship between carbon isotope discrimination and grain yield under Mediterranean conditions. Relationship across the whole set of trials between the carbon isotope discrimination ($\Delta^{13}C$) of mature grains and grain yield. Each point represents a rainfed or irrigated trial (composed of 25 genotypes and four replicates per genotype) grown in north-east Spain or south-east Spain (redrawn from Araus *et al.*, 2003).

a lower isotope signature and (c) an earlier flowering (Condon *et al.*, 2002 and references cited therein).

Some authors consider that kernel $\Delta^{13}C$ may be a more cryptic parameter than leaf $\Delta^{13}C$ (Condon *et al.*, 2004). Several works carried out by CSIRO group (Condon, Richards and coworkers) 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 cultivars produced using $\Delta^{13}C$ (low values in seedlings) as a breeding trait were released during 2002 and 2003 (e.g. *Drysdale* and *Rees* cultivars; see http://www.csiro.au; Condon *et al.*, 2004). 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, declines at higher mean yield, where higher seasonal rainfall is present (Rebetzke *et al.*, 2002). The apparent discrepancies between studies carried out in the Mediterranean basin versus some regions of Australia with Mediterranean climates seem to arise from the source of water used during crop cycle. In Mediterranean zone, rainfall (even if scarce) is present during the crop growth. In this context, genotypes with higher water extraction capacity will have higher grain yield and higher $\Delta^{13}C$. On the contrary, in some regions of Australia with summer rainfall, water stored prior to planting is a main part of the total water used by the crop. In those environments, rainfall is scarce after seeding, and the water saved could be a critical factor to avoid terminal severe water stress (Passioura, 2004). Cultivars with conservative strategy may have advantage over 'water spender' ones. The negative correlation between the advantages 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 of incorporating higher $WUE_{instantaneous}$ (low $\Delta^{13}C$) showed that the advantage was significant in environments where stored water dominates (with summer rainfall). In environments with typical Mediterranean climate (winter–spring rainfall), by contrast, improved $WUE_{instantaneous}$ did not confer advantage in yield (Condon *et al.*, 2004). In this case, early vigour seems to be more important (see the following section). In summary, in cases where additional water (i.e. after planting) is not available to the crop, to increase $WUE_{instantaneous}$ (i.e. low $\Delta^{13}C$) appears to be an alternative strategy (Araus *et al.*, 2002 and references therein).

One question to answer is the range of genotypic variability in $\Delta^{13}C$ (i.e. $WUE_{instantaneous}$) in cereals. This range is around 4‰ among wheat cultivars (Zaharieva *et al.*, 2001). Gas exchange analysis carried out in bread wheat confirms that there are intergenotypic differences in $WUE_{instantaneous}$ (Morgan & LeCain, 1991; Abbad *et al.*, 2004), although the greatest genetic variation was observed in the comparison of hexaploid wheat with early progenitors (Morgan *et al.*, 1993 and references cited therein). At least in wheat, variability is relatively lower compared with other related species. In *Aegilops geniculata* (a wild relative of hexaploid *T. aestivum*) the range of $\Delta^{13}C$ seem to be higher (ca. 7‰), suggesting that $WUE_{instantaneous}$ of wheat could be improved by introgression in hybridisation programmes (Zaharieva *et al.*, 2001). For a given VPD, a 1‰ variation in $\Delta^{13}C$ may represent ca. 15% in variation in $WUE_{intrinsic}$ (Morgan *et al.*, 1993) and WUE_{biomas} (Hubick & Farquhar, 1989).

Decreasing futile losses of water

Non-productive water losses from the leaf: the impact of leaf temperature

Considering that the leaf temperature is a component of the driving force of the transpiration rate (affecting the VPD), a lower leaf temperature has an important impact on $WUE_{instantaneous}$. At first, if we consider no changes in $WUE_{intrinsic}$, a lower leaf temperature will lead to amelioration in $WUE_{instantaneous}$. Several morphological traits have been linked to a lower leaf temperature, such as low chlorophyll content and a vertical position of the leaf. Low chlorophyll content, for instance, moreover to prevent photoinhibition has been associated with lower leaf temperatures in barley. Landraces like Tadmor, adapted to Mediterranean dry conditions of Syria, have leaves with pale colour because of 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), but is associated with lower leaf temperatures, in particular when stomata are closed (Havaux & Tardy, 1999). Lower leaf temperature under water stress could mitigate the heat stress associated with drought, and reduce respiration as well as the losses of water across the cuticle, thereby improving $WUE_{instantaneous}$. However, causal relationship between leaf temperature and chlorophyll content should be taken with caution. In *Aegilops* (a genus closely related to *Triticum)*, positive and negative correlations were observed between chlorophyll content and canopy temperature depression (CTD), depending on the origin of the accession of the trial (Zaharieva *et al.*, 2001). A lower transpiration rate may increase leaf temperature (by the decrease of evaporative cooling), leading to a loss of chlorophyll content (Zaharieva *et al.*, 2001 and references therein). In this context, a positive correlation between CTD and chlorophyll content could be found. Consequently, researchers must keep in mind this complexity in the analysis of genotypic variability of chlorophyll content. In order to avoid paradoxical results, this analysis should be carried out without the presence of abiotic stress factors (i.e. where chlorophyll degradation could be implicated).

Residual transpiration

The losses of water through the cuticle are obviously futile because it is not paired with CO_2 influx into the leaf. Stomatal closure may lower leaf conductance by around 94% (but not 100%) in several herbaceous species (Kerstiens, 1996). The 'residual transpiration' may be quantitatively important during the day, in particular, in dry and hot climate and substantial genetic variability has been reported for wheat (Dhanda & Sethi, 1998; see references in Richards *et al.*, 2001). For instance, in measurements carried out on varieties and landraces from the Middle East, North Africa, Institut National de la Recherche Agronomique (INRA) and Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), 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 trait is unknown (Richards *et al.*, 2001), the results mentioned above suggest that WUE ($WUE_{biomass}$ and WUE_{yield}) of cereals could be improved in 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 eventually in growth performance.

One characteristic of plant cuticle linked to water losses is the glaucousness (the waxy covering over the plant cuticle). Qariani *et al.* (2000) analysed two isolines of durum wheat differing in this trait. They found that isolines with low glaucousness have higher residual transpiration, lower $WUE_{biomass}$ and lower grain yield, in particular, under water deficit. In barley grown under Mediterranean conditions in Spain, Febrero *et al.* (1998) reported a higher grain yield in glaucousness isolines, but the differences were not explained by residual (cuticular) conductance. Glaucousness might ameliorate $WUE_{instantaneous}$ by other ways, for instance, decreasing leaf temperature. In fact, plants with well-developed wax layer showed lower leaf temperature, reduced transpiration rate and, eventually, higher $WUE_{instantaneous}$ (Barnes & Cardoso-Vilhena, 1996).

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% of diurnal rates in some cases (Snyder *et al.*, 2003). These authors reported that 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 crops. Transpiration could exceed 0.5 mm per night in unstressed crops of wheat and this value could be considerably reduced by selection of genotypes with both low-cuticular and low-stomatal transpiration (Rawson & Clarke, 1988). Warmer conditions at night (e.g. during the grain filling of cereals in semi-arid Mediterranean regions as WANA) could lead to significant decreases in the WUE of the crop.

Decreasing water losses from the soil: the role early vigour in Mediterranean regions

In semi-arid regions of Mediterranean basin (for instance, northern Syria), soil evaporation may be a major component of total WU (value >50% are reported), in particular in non-fertilised crops (Gregory *et al.*, 2000). A better seedling emergence and an earlier vegetative growth (i.e. early or seedling vigour) have been pointed out as an important trait in terms of WUE in cereals under Mediterranean conditions (Richards *et al.*, 2001, 2002). 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) reduction of the hydraulic conductance of the soil during the uptake of water from the roots near the surface. In crop 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, when early growth occur, biomass accumulation took place under low VPD, decreasing the total transpired water by the crop, and consequently, increasing the WUE (López-Castañeda *et al.*, 1995). Although it has been reported that a closed canopy increases the water losses by interception and evaporation from the canopy (Leuning *et al.*, 1994), this phenomenon has not been extensively investigated.

The fast growth of leaf area has little benefit in regions where soil evaporation is a small component of total crop WU (Condon *et al.*, 2004). Similar consideration may be pointed out in areas where evaporation is potentially high, but is limited by the movement of water in the soil (and not 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). Although early vigour has been pointed out as a relevant factor in WUE in general terms, the particular characteristics (e.g. climatic and edafic factors) of the sites where this trait has a strong impact should be more clearly identified.

Finally, an early growth may be enhanced 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 to ensure early germination, seedling establishment and a fast growth (Tavakkoli & Oweis, 2004). In addition, seeding 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, showed that WUE (at biomass and yield level) were increased when row spacing was reduced (Karrou, 1998).

In wheat, the wide use of *semi-dwarf* cultivars (giberellic acid-insensitive), which has increased the HI of modern cultivars with lower plant height (Austin, 1999), is associated with short coleoptiles and low early vigour (Richards *et al.*, 2002). Tall cultivars, by contrast, have longer and wider leaves and produce higher biomass than semi-dwarf genotypes (e.g. *Rht*) at early stage (Rebetzke *et al.*, 2004). These traits of semi-dwarf genotypes lead to 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. 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 and coworkers found that GA-sensitive lines have good partitioning characteristic (i.e. high HI) and, at the same time, long coleoptiles (Richards *et al.*, 2002; Botwright *et al.*, 2005 and references cited therein). Consequently, wheat varieties with high yield potential and high WUE at early stage of the Mediterranean crops seems feasible.

As genotypic variability of early vigour in wheat seems to be low, another way to increase this trait could be by introgression with related species. Quantitatively trait loci (QTL) of early vigour have been recently identified in *Aegilops tauschii*, opening the possibility that favourable alleles of this species may be introgressed in wheat (ter Steege *et al.*, 2005).

Can $WUE_{instantaneous}$ be manipulated by agronomic practices?: deficit irrigation systems

In spite of the amelioration of WUE of genetic resources (i.e. obtaining cultivars with higher WUE_{yield}), agronomic practices (e.g. tillering management) can contribute in this sense. At least half of the increase of rainfall-use efficiency may be attributed to improved agronomic management (for a detailed discussion see Turner, 2004). In conjunction with new cultivars, the adoption of practices such as minimum tillage, appropriate fertilization and timely planting, has the potential to increase rainfall-use efficiency of dryland (such as Mediterranean) crops. As agronomic practices are beyond the scope of this review, we will briefly discuss irrigation practices in relation to physiological aspects of WUE, with special emphasis on water-deficit treatments.

The broadest known effect of water deficit in plants is the stomatal closure (e.g. Lawlor, 2002) and the concomitant increase of $WUE_{instantaneous}$ is a well-documented phenomenon (e.g. Johnson, 1993; Morgan *et al.*, 1993; Van den Boogaard *et al.*, 1997; Rekika *et al.*, 1998). However, the response of $WUE_{instantaneous}$ is dependent on the severity of the water stress. In bread wheat, for instance, a moderate water deficit, can lead to a sensible increase of $WUE_{instantaneous}$ (Morgan *et al.*, 1993; Van den Boogaard *et al.*, 1997; Rekika *et al.*, 1998), but a decrease of $WUE_{instantaneous}$ has also been reported in plants of several species subjected to severe stress (El Hafid *et al.*, 1998; Anyia and Herzog, 2004). This behaviour is not surprising, because at severe water deficit, photosynthesis may be decreased by metabolic causes (i.e. non-stomatal limitations; 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. However, in most circumstances, WUE based on transpiration alone (i.e. without taking into accountsoil evaporation) is consistently increased under crescent drought (Abbate *et al.*, 2004).

The increase of $WUE_{instantaneous}$ under moderate drought is used in management systems, where 'deficit irrigation' (i.e. below the full water requirement of the crop) is imposed on the crops. Oweis *et al.* (2000) reported that WUE_{yield} of bread wheat under Mediterranean conditions was the highest with a deficit irrigation consisting of two-thirds of the water required at full irrigation (i.e. WUE_{yield} at full irrigation was lower). Similar results were reported by Tavakkoli & Oweis (2004) and Zhang *et al.* (2006) (Fig. 5). As the former 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 this dry area, the loss of grain yield because of deficit irrigation may be negligible compared with the saving in water (Oweis *et al.*, 2000). For instance, in a semi-arid region of Zimbabwe, wheat yield decreases only by ca. 10% when three-quarters of

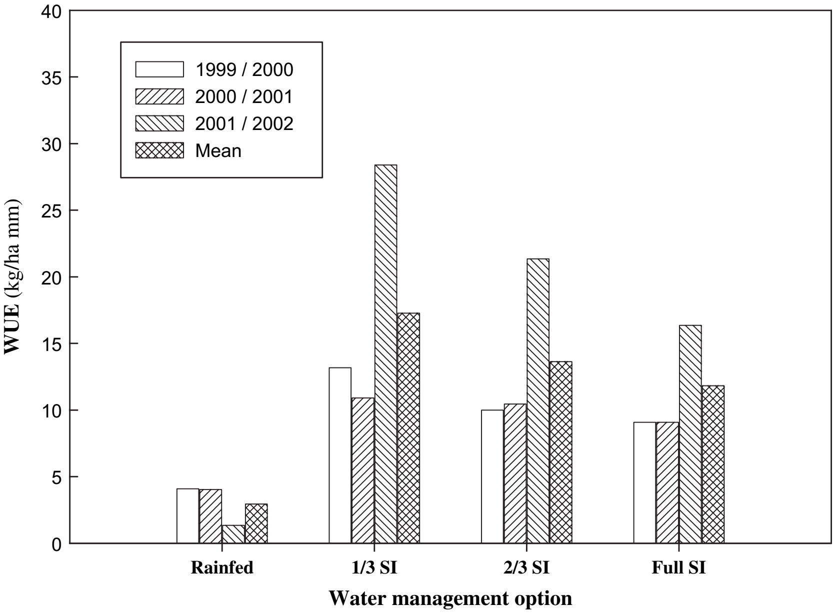

Figure 5 Water management and water productivity. The WUE_{yield} (water productivity) of bread wheat under Mediterranean conditions with full or deficit irrigation system. SI, supplemental irrigation (redrawn from Tavakkoli & Oweis, 2004).

water requirements were applied (Mugabe & Nyakatawa, 2000). In short, the '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). Thus, penalties in terms of grain yield could occur. 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 WUE_{yield} under water limitation are reported in several studies and climatic conditions (Abbate *et al.*, 2004 and references cited therein). However, there are other reports in wheat where no increase in WUE (neither WUE_{yield} nor $WUE_{biomass}$) was found under water-deficit treatments (Xue *et al.*, 2003). The beneficial effects and possible penalties of this practice should be further researched.

It must be noted that deficit irrigation may be complex at farmer scale, and this issue is not analysed in the present review. As pointed out in an FAO document (2003), 'the farmers need to know the deficit that can be allowed at each of the growth stages and the level of water stress that already exists. They need to have control over the timing and amount of irrigations. Thus, deficit irrigation carries considerable risk for the farmers where water supplies are uncertain' (for more details, see the document of FAO 2003).

Partial root drying, a particular type of deficit irrigation practice, has received considerable attention in the last years. This practice (known as Controlled Alternate Partial Root Irrigation or 'CAPRI' and Partial Root Drying or PRD) consists of subjecting part of the root system to dry conditions. It is based on 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 & Zhang 2004). In grapevines under Mediterranean conditions, for instance, both deficit irrigation and partial root drying lead to an increase in $WUE_{intrinsic}$ (de Souza *et al.*, 2005). Although these systems could be implemented in several crops, their use in cereals could be more doubtful. As 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 (or decline) any advantage of stomatal closure.

In summary, WUE_{yield} in Mediterranean regions may be incremented with some irrigation, in particular, at the beginning of the crop cycle, which improves early growth and decreases soil evaporation. Additionally, some degree of water deficit may improve WUE_{yield}, which could be explained to some extent by stomatal control on the transpiration. Future research should focus on the actual impact (including penalties) of this practice and feasibility at the farmers' scale.

Conclusions

We have discussed different issues regarding physiological traits of WUE in cereals grown under Mediterranean conditions, which can be summarised as follows: (a) the amelioration of $WUE_{intrinsic}$ by the increase of photosynthetic rate is only feasible if changes (increments) in stomatal conductance are not implicated; (b) the ear of C_3 cereals seem to have higher $WUE_{instantaneous}$ than the flag leaf, and genotypic variability has been observed in this trait; (c) breeding for high $WUE_{intrinsic}$ (evaluated by carbon isotope discrimination) may have a positive or negative impact on grain yield, depending on the environment target – in growing conditions – from moderate stress to absence of water stress, there is a positive correlation between carbon isotope discrimination and grain yield; (d) in order to improve $WUE_{biomass}$, early vigour is a desirable trait in regions with high soil evaporation and rainfall expected during the crop cycle; (e) an increase in $WUE_{intrinsic}$ (by partial stomatal closure) as well as in WUE_{yield} may be achieved by appropriate management practices like deficit irrigation.

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