Breeding for high water-use efficiency

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Abstract

There is a pressing need to improve the water-use efficiency of rain-fed and irrigated crop production. Breeding crop varieties with higher water-use efficiency is seen as providing part of the solution. Three key processes can be exploited in breeding for high water-use efficiency: (i) moving more of the available water through the crop rather than it being wasted as evaporation from the soil surface or drainage beyond the root zone or being left behind in the root zone at harvest; (ii) acquiring more carbon (biomass) in exchange for the water transpired by the crop, i.e. improving crop transpiration efficiency; (iii) partitioning more of the achieved biomass into the harvested product. The relative importance of any one of these processes will vary depending on how water availability varies during the crop cycle. However, these three processes are not independent. Targeting specific traits to improve one process may have detrimental effects on the other two, but there may also be positive interactions. Progress in breeding for improved water-use efficiency of rain-fed wheat is reviewed to illustrate the nature of some of these interactions and to highlight opportunities that may be exploited in other crops as well as potential pitfalls. For C3 species, measuring carbon isotope discrimination provides a powerful means of improving water-use efficiency of leaf gas exchange, but experience has shown that improvements in leaf-level water-use efficiency may not always translate into higher crop water-use efficiency or yield. In fact, the reverse has frequently been observed. Reasons for this are explored in some detail. Crop simulation modelling can be used to assess the likely impact on water-use efficiency and yield of changing the expression of traits of interest. Results of such simulations indicate that greater progress may be achieved by pyramiding traits so that potential negative effects of individual traits are neutralized. DNA-based selection techniques may assist in such a strategy.

Key words: Carbon isotope discrimination, drought resistance, transpiration efficiency, wheat.

Introduction

Raising the water-use efficiency of both irrigated and rain-fed crop production is an urgent imperative (Hamdy et al., 2003). Of the world’s allocatable water resource, c. 80% is currently consumed by irrigated agriculture. This level of consumption by agriculture is not sustainable into the future. Projected population growth (another 2 billion people within 2–3 decades) will require that more of the available water resource be used for domestic, municipal, industrial, and environmental needs. The most realistic solution to the increased demand for water will be reallocation to these other purposes of some of the water currently used by agriculture. Even a modest reallocation to these other purposes of some of the water currently used by agriculture. Even a modest reallocation will not only need more water to satisfy these other purposes, it will also need to be fed and clothed. This will require substantially more efficient production from a smaller irrigation water resource. It will also require substantially

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Abbreviations: A, instantaneous rate of CO2 assimilation; A/T, instantaneous water-use efficiency of leaf gas exchange; Cao, atmospheric CO2 concentration; Ci, leaf internal CO2 concentration; Ci/Cao, the ratio of internal and atmospheric CO2 concentrations; \( \Delta^{13}C \), carbon isotope discrimination; E, evaporation from the soil; ET, evapotranspiration, the sum of soil evaporation and plant transpiration from a crop canopy (instantaneous or cumulative); G, stomatal conductance; HI, harvest index; T, instantaneous rate of transpiration from a single leaf, and transpiration from a crop canopy (instantaneous or cumulative); \( \text{T/ET} \), the ratio of transpiration to evapotranspiration; W, transpiration efficiency of crop biomass production; \( W_a \), atmospheric water vapour concentration; \( W_i \), leaf internal water vapour concentration.
higher water-use efficiency from rain-fed agriculture, which remains the primary means of food production in most countries and for most farmers. Several strategies will be required to improve the productivity of water use in irrigated and rain-fed agriculture (Wang et al., 2002). Breeding crop varieties that are more efficient in their water use is one such strategy. Others include better management of the water resource and changes in crop management. None of these strategies should be seen as operating in isolation. Rather, it is likely that the greatest gains will be obtained through complementary approaches involving each of them.

In this article, recent progress in breeding for high water-use efficiency will be reviewed and some possible avenues for making further advances will be outlined. As a starting point, the article will first establish a conceptual framework for considering ways by which crop water-use efficiency might be improved through breeding. The prospects for improving crop water-use efficiency by changing the water-use efficiency of leaf gas exchange will be considered in some detail. Other likely avenues will also be considered briefly where substantial gains in crop water-use efficiency could be made. The review will concentrate on cereals and particularly on wheat, a dominant food crop which is grown in irrigated and rain-fed production systems over a wide range of latitudes. Wheat is also a crop that has been the focus of a long-standing breeding effort for higher water-use efficiency (Richards et al., 2002). This effort has culminated in the recent release in Australia of two new commercial wheat varieties, ‘Drysdale’ and ‘Rees’, bred specifically for higher water-use efficiency.

**Water-use efficiency as a breeding target**

Breeding to address a specific objective implies first, that the objective has been well defined and, second, that heritable traits have been identified that can come some way towards achieving the breeding objective. ‘Water-use efficiency’ as a breeding target could be defined in many ways, depending on the scale of measurement and the units of exchange being considered. All potential definitions will have some measure of water being exchanged for some unit of production. For physiologists, the basic unit of production could be mole of carbon gained in photosynthesis \( A \) in exchange for water used in transpiration \( T \). Thus a physiological definition might equate, at its most basic level, to the instantaneous water-use efficiency of leaf gas exchange \( A/T \). For farmers and agronomists, the unit of production is much more likely to be the yield of harvested product achieved from the water made available to the crop through precipitation and/or irrigation, i.e. a farmer’s definition is one of agronomic water-use efficiency.

While agronomic water-use efficiency will be taken to be the ultimate breeding target, a major thrust of this article will be to place the physiological definition of water-use efficiency in the context of the farmer’s definition. To do this it is useful to consider crop yield as being constructed from a framework of relatively simple components (equation 1).

\[
Yield = ET \times T / ET \times W \times HI
\]

(1)

In this framework, grain yield is described as being a function of the amount of water used by the crop \((\text{evapotranspiration}, \ ET)\), the proportion of that water actually transpired by the crop \((T/ET)\), the transpiration efficiency of biomass production \((W)\), i.e. how much biomass is produced per millimetre of water transpired, and, lastly, how effectively the achieved biomass is partitioned into the harvested product, i.e. the ratio of grain yield to standing biomass termed the harvest index \((HI)\). This framework is not based on the notion of ‘drought resistance’, but rather on the broad processes by which crops actually achieve yield in water-limited environments (Passioura, 1977; Condon and Richards, 1993; Richards et al., 2002). None of the components of this yield framework is truly independent of the others (Condon and Richards, 1993), but each can be considered a target for genetic improvement. Leaf-level water-use efficiency, \(A/T\), is directly related to only one of these components, \(W\), the transpiration efficiency of biomass production. However, as will be discussed in following sections, \(A/T\) also has the potential to influence each of the other three components in the yield framework.

**Breeding for greater leaf-level water-use efficiency**

The prospect of improving agronomic water-use efficiency by breeding for greater leaf-level water-use efficiency has long been an attractive one (Fischer, 1981). To assist in identifying ways this might be achieved, leaf-level water-use efficiency, \(A/T\), can be described mathematically by noting that, first, \(A\) is the product of stomatal conductance to \(\text{CO}_2\) \((G_a)\) and the gradient in concentration of \(\text{CO}_2\) between the outside \((C_o)\) and inside \((C_i)\) of the leaf (equation 2)

\[
A = G_a(C_o - C_i)
\]

(2)

and, second, that \(T\) is the product of stomatal conductance to water vapour \((G_w)\) and the gradient in concentration of water vapour from the inside \((W_i)\) to the outside \((W_o)\) of the leaf (equation 3)

\[
T = G_w(W_i - W_o)
\]

(3)

For \(\text{CO}_2\), the concentration is greater in the air outside the leaf, while the reverse is true for water vapour. The ratio \(A/T\) then becomes (equation 4)

\[
A/T = [G_a(C_o - C_i)]/[G_w(W_i - W_o)]
\]

(4)

which can be simplified even further (equation 5) by noting that the ratio of the diffusivities of \(\text{CO}_2\) and water vapour in air has a value of \(c. 0.6\). Thus,
Equation (5) indicates two possible routes for improving leaf-level water-use efficiency. One is to lower the value of \( \frac{C_i}{C_a} \), thereby increasing the value of \( (1 - \frac{C_i}{C_a}) \). The other is to make \( (W_1 - W_a) \) smaller, i.e. to make the gradient driving transpirational water loss smaller.

**Lowering the gradient in water vapour concentration**

The simplest and most influential means by which breeding has improved the transpiration efficiency of biomass production via greater \( A/T \) has been to change crop characteristics so as to lower the average evaporative gradient during the crop growth cycle (Tanner and Sinclair, 1983; Richards et al., 2002). Reflecting processes at the leaf level, crop water loss is driven by the gradient in water vapour concentration between the crop canopy and the atmosphere. This gradient is least in cool, humid regions and, in most regions, during the coolest months of the year. During the past century breeders of many crop species have exploited genetic variation associated with intrinsic earliness, response to photoperiod, and vernalization requirement to generate enormous variation in crop phenology. This phenological variation has allowed crops to be grown successfully in regions and at times of the year that lower the prevailing evaporative demand, thus raising \( A/T \) and boosting crop yield. As opportunities arise, every effort should be taken to exploit this simple route to improved crop water-use efficiency further. Even seemingly unrelated objectives, such as eliminating disease susceptibility so that crops can be grown reliably in cooler, more humid conditions may present opportunities to adjust sowing time and crop phenology for improved \( A/T \) and crop yield. A good example of what is possible is the doubling of yield achieved by improving the disease resistance of chickpea, transforming it from a spring-sown to an autumn-sown crop in northern Syria (Keatinge and Cooper, 1983).

**Changing the value of \( \frac{C_i}{C_a} \)**

Referring back to equation (5), another way that breeding could improve \( A/T \), and thereby improve the transpiration efficiency of biomass production, is to raise the value of the numerator \( (1 - \frac{C_i}{C_a}) \), i.e. to select genotypes for which the ratio \( C_i/C_a \) is small. A small value of \( C_i/C_a \) will reflect either a relatively low value of \( G \), a relatively high photosynthetic capacity (amount and activity of photosynthetic machinery per unit leaf area) or a combination of these two (Farquhar et al., 1989). The interrelationships between \( C_i/C_a \), photosynthetic capacity, and stomatal conductance are perhaps best appreciated in the context of the ‘\( A/C_i \)’ plot (Long and Bernacchi, 2003). In such a plot (Fig. 1), curved lines rising from near the origin describe the dependence of \( A \) on \( C_i \) as external CO2 concentration is varied experimentally. Variation in the initial slope of these curves equates to variation in photosynthetic capacity. In Fig. 1, the two curved lines are intersected by two straight lines originating at the ambient CO2 concentration, \( C_a \). Variation in the slope of these straight lines equates to variation in stomatal conductance. The intersections indicated by numerals represent the operating values of \( C_i \) (and thus \( C_i/C_a \), \( A \), and \( G \)) for three genotypes of a \( C_3 \) species. As shown in Fig. 1, lower operating values of \( C_i/C_a \) (say 0.6 compared with 0.7) may be achieved through higher photosynthetic capacity (genotype ‘2’ compared with genotype ‘1’), lower stomatal conductance (genotype ‘3’ compared with genotype ‘1’), or a combination of these two.

It is clear from Fig. 1 that it is not possible to infer, merely from measuring a change in \( C_i/C_a \), that such a change is due to a change in photosynthetic capacity or to a change in \( G \). Two other things are also clear from Fig. 1. If \( C_i/C_a \) is lowered as a result of an increase in photosynthetic capacity, then \( A \), the rate of photosynthesis per unit leaf area, will be greater. Conversely, if \( C_i/C_a \) is lowered as a result of a decrease in \( G \), then the value of \( A \) will be lowered.

There is likely to be another penalty, not revealed in Fig. 1, associated with a decrease in \( G \). Data for ‘\( A/C_i \)’ plots, and in fact most leaf gas-exchange data, are collected by inserting leaves into cuvettes in which the air is very well stirred and the leaf-air vapour concentration gradient is constrained to be approximately constant. Outside such cuvettes, this is unlikely to be the case, with the result that as \( G \) decreases leaf temperature will increase. An increase in leaf temperature will cause an increase in \( W_1 \) and therefore an increase in the gradient driving transpiration \( (W_1 - W_a) \), resulting in an increase in \( T \) per unit change in \( G \).

The net result is that the change in \( A/T \) as \( G \) decreases will not be as great as that predicted by equation (5).
The relationship between carbon isotope discrimination and $C_i/C_a$

Despite the advent of reliable, relatively-portable leaf gas-exchange systems, it is pertinent to note that $G$, photosynthetic capacity, and $A/T$ are still tedious to measure in large breeding populations. It is now accepted that relative differences in $C_i/C_a$, at least within $C_3$ species, may be evaluated indirectly by measuring the carbon isotope composition of plant dry matter (Farquhar et al., 1989). The stable isotope of carbon, $^{13}C$, makes up very close to 1% of the carbon in atmospheric CO$_2$. The proportion of $^{13}C$ in the dry matter of $C_3$ plants is fractionally less than in the atmosphere, primarily because $C_3$ species discriminate against $^{13}C$ during photosynthesis. Carbon isotope discrimination ($\Delta^{13}C$) is a measure of the $^{13}C/^{12}C$ ratio in plant material relative to the value of the same ratio in the air on which plants feed, and has been defined as follows (Farquhar and Richards, 1984):

$$\Delta^{13}C = [(R_a/R_p) - 1] \times 1000 \quad (6)$$

where $R_a$ is the value of the $^{13}C/^{12}C$ ratio in the atmosphere and $R_p$ is the value of $^{13}C/^{12}C$ in plant material. For convenience, units of $\Delta^{13}C$ are expressed as per thousand ($\%_o$), i.e. the fractional difference from unity is multiplied by 1000. The ratio $R_a/R_p$ has a value near 1.02 for $C_3$ plants, giving values of $\Delta^{13}C$ near 20$\%_o$.

There are several processes that contribute to the value of $\Delta^{13}C$ measured in plant dry matter of $C_3$ species (Farquhar et al., 1989; Brugnoli and Farquhar, 2000). The following approximate expression (equation 7) was developed by Farquhar and Richards (1984) to account for the two dominant processes; fractionations associated with diffusion of CO$_2$ into the intercellular airspaces ($a=4.4\%_o$) and fractionations associated with carboxylation of CO$_2$ into the first products of photosynthesis by Rubisco ($b=28\%_o$).

$$\Delta^{13}C \approx a + (b - a) C_i/C_a \quad (7)$$

The value of 28$\%_o$ for $b$ is approximate because there are several additional potential sources of variation in $\Delta^{13}C$, such as those associated with subsequent metabolism and respiration, but these are ill-defined and in most situations are likely to be small in magnitude (Brugnoli and Farquhar, 2000). Substituting the numerical values indicated into equation (7) generates the following:

$$\Delta^{13}C \approx 4.4 + 23.6 C_i/C_a \quad (8)$$

This simple equation shows that $\Delta^{13}C$ is positively related to the ratio $C_i/C_a$. However, earlier it was noted that $A/T$ should be negatively related to $C_i/C_a$ (equation 5). Therefore $\Delta^{13}C$ and $A/T$ should also be negatively related.

Carbon isotope discrimination and water-use efficiency

The realization that $\Delta^{13}C$ could provide a relatively simple, indirect measure of variation in $A/T$ (Farquhar et al., 1982; Farquhar and Richards, 1984) gave renewed impetus to the prospect of exploiting variation in leaf-level water-use efficiency to improve agronomic water-use efficiency. Up to that time, it had been considered that there was little variation in $C_i/C_a$ within or among $C_3$ crop species, the only substantial difference being that between $C_3$ and $C_4$ species (Fischer, 1981; Tanner and Sinclair, 1983). Since the pioneering work of Farquhar and colleagues (Farquhar et al., 1982; Farquhar and Richards, 1984), it has subsequently been demonstrated, for several $C_3$ species, that variation in $\Delta^{13}C$ closely reflects variation in the ratio $C_i/C_a$.

One such example is shown in Fig. 2A, but there have been many similar studies (reviewed in Brugnoli and Farquhar, 2000) in which either $\Delta^{13}C$ was measured ‘on-line’ by sampling the air stream before and after it passed over a leaf, $\Delta^{13}C$ was measured in recent photosynthate extracted from leaves, or $\Delta^{13}C$ was measured in the dry matter of sink tissues.

Variation in $\Delta^{13}C$ among genotypes of $C_3$ species is large enough to, in theory, generate substantial variation in

![Fig. 2. (A). Relationship between $C_i/C_a$ measured on flag leaves and $\Delta^{13}C$ measured in dry matter of growing ears of wheat enclosed in the flag leaf sheath, for 13 genotypes. The growing ear is a major sink for $C$ assimilated by the flag leaf. The solid line is a linear regression fitted to the data points. The dashed line is the relationship $\Delta^{13}C=4.4+23.6 C_i/C_a$ (equation 8) (adapted from Condon et al., 1990 with permission from CSIRO Publishing.). (B). Relationship between plant water-use efficiency (g dry matter produced kg$^{-1}$ water transpired) and $\Delta^{13}C$ of plant dry matter, for 16 wheat varieties grown without water limitation in large pots sealed at the soil surface to eliminate soil evaporation. The solid line is a linear regression fitted to the data points (adapted from Condon et al., 1990 with permission from CSIRO Publishing.).]
A/T and potentially, substantial variation in water-use efficiency of dry matter production (Farquhar and Richards, 1984). This has been confirmed in numerous studies with pot-grown plants. Negative correlations between $\Delta^{13}C$ and plant water-use efficiency have been demonstrated in many species (Fig. 2B), as predicted by theory (reviewed in Farquhar et al., 1989; Hall et al., 1994; Condon and Hall, 1997).

Further, it has been shown that with adequate attention to sampling strategies, $\Delta^{13}C$ is a highly heritable trait that is relatively easy to manipulate in breeding populations (Condon and Richards, 1992; Rebetzke et al., 2002). Measurements of $\Delta^{13}C$ can be taken using freshly-sampled or stored plant material and they can be automated for relatively rapid throughput. All these observations point to $\Delta^{13}C$ as a potential candidate for use in breeding for greater agronomic water-use efficiency via greater A/T. However, significant challenges have arisen as attempts have been made to 'scale up' from associations between $\Delta^{13}C$ and water-use efficiency of leaves and single plants to associations between $\Delta^{13}C$ and water-use efficiency and yield of field stands. Some of these challenges were anticipated, others were not. The nature of these challenges and possible solutions are considered in the following sections.

**Relationships between grain yield and carbon isotope discrimination in wheat and barley**

The greatest challenge to using $\Delta^{13}C$ in breeding for greater agronomic water-use efficiency is the high level of inconsistency observed in the relationship between $\Delta^{13}C$ and yield. This inconsistency has been well-documented in numerous studies involving the cereals bread wheat ($T. aestivum$ L.), durum wheat ($T. durum$ var. durum L.) and barley ($Hordeum vulgare$ L.). From the negative association between $\Delta^{13}C$ and leaf-level A/T and the consistently negative associations observed between $\Delta^{13}C$ and water-use efficiency at the single plant level in many pot studies, it might be inferred that crop yield and $\Delta^{13}C$ might also be consistently negatively related. Yet for a large number of studies involving collections of cereal genotypes grown in rain-fed and irrigated environments in Australia (Condon et al., 1987, 1993, 2002; Condon and Richards, 1993; Condon and Hall, 1997), the Mediterranean region (Volta et al., 1999; Merah et al., 2001; Royo et al., 2002; Araus et al., 2003) and elsewhere (Sayre et al., 1995; Fischer et al., 1998), relationships reported between grain yield and $\Delta^{13}C$ have only infrequently been negative. Much more often these relationships have been either positive or 'neutral'. Many of the studies on associations between productivity and $\Delta^{13}C$ in cereals have used sets of genotypes in which there has been substantial variation not only in $\Delta^{13}C$, but in flowering date and height as well, two characteristics that could strongly influence yield independent of variation in $\Delta^{13}C$. Even in studies where flowering date and height have been tightly constrained, such as that shown in Fig. 3 (discussed below), associations between productivity and $\Delta^{13}C$ have still been variable.

**Low-$\Delta^{13}C$ cereals tend to have ‘conservative’ crop growth**

Why have relationships between grain yield and $\Delta^{13}C$ been so variable in so many studies? There appear to be several reasons, but a critical one is that for cereals low-$\Delta^{13}C$ (high A/T) is a ‘conservative’ trait in terms of water use and, perhaps more importantly, in terms of crop growth rate. Put simply, in the absence of soil water deficit, low-$\Delta^{13}C$ genotypes tend to grow slower than high-$\Delta^{13}C$ genotypes, resulting in lower total biomass production and grain yield (Condon and Richards, 1993; Condon et al., 1993, 2002; Lopez-Casteneda et al., 1995). The data in Fig. 3 for isomorphic recombinant inbred lines grown in the favourable 1993 season illustrate this well. In this instance, positive associations of $\Delta^{13}C$ with biomass production and grain yield were sustained by substantial regular rainfall events throughout the growing season.

An obvious, widely-cited reason why low-$\Delta^{13}C$ could be associated with ‘conservative’ crop growth rate in cereals is...
if differences in Δ\(^{13}\)C in the absence of soil water deficit are the result of differences in stomatal conductance. Genotypes with lower stomatal conductance will tend to have higher A/T and lower Δ\(^{13}\)C, all else being equal, but in this case higher A/T is likely to be associated with lower photosynthetic rate per unit leaf area (Fig. 1, ‘genotype’ 3) and, consequently, a slower rate of crop growth.

Low conductance may not be the only reason why low-Δ\(^{13}\)C is associated with ‘conservative’ crop growth rate. In cereals, variation in Δ\(^{13}\)C can also result from variation in photosynthetic capacity (Condon et al., 1990; Morgan and LeCain, 1991). If low-Δ\(^{13}\)C is the result of high photosynthetic capacity, the expectation might be a higher rate of photosynthesis per unit leaf area (Fig. 1, ‘genotype’ 2) and thus faster crop growth rate. However, crop growth rate may again be slower because, in cereals, substantial increases in photosynthetic capacity are most readily achieved by concentrating N into smaller leaves that intercept less light. This may slow the rate of crop growth until full light interception is achieved (Condon and Richards, 1993; Condon and Hall, 1997). If full light interception is not achieved or achieved only briefly, as often occurs in drier cropping environments, then high photosynthetic capacity may not result in greater growth. The data in Fig. 4 indicate that, in fact, the reverse may occur, i.e. that cereal genotypes with low photosynthetic capacity may actually achieve faster crop growth. In this study, biomass production to anthesis was positively correlated with Δ\(^{13}\)C (Fig. 4A), as has been frequently observed, but the three genotypes with the highest Δ\(^{13}\)C values did not have greater G (Fig. 4B). Instead, the inference, from similar values of G but higher values of Δ\(^{13}\)C, was that they had lower photosynthetic capacity. This inference was confirmed in studies on glasshouse-grown plants (Condon et al., 1990).

So, in cereals, higher photosynthetic capacity may not necessarily be associated with faster crop growth rate. A similar conclusion can be drawn from the study by Fischer et al. (1998) on physiological changes in bread wheats released by CIMMYT from 1962 to 1988. The more recent of these wheats had substantially higher grain yields under irrigation, but this was not associated with greater biomass production. The yield gain, however, reflected higher harvest index for the more recent wheats. Among this ‘historic’ collection of CIMMYT wheats there was no change in total biomass production despite the more recent wheats having both higher photosynthetic capacity and higher G, together generating substantially higher values of A on a leaf area basis.

**Interactions between growth and water use**

Irrespective of its physiological basis, ‘conservative’ crop growth by low-Δ\(^{13}\)C cereal genotypes has important implications for agronomic water-use efficiency. The most consistently positive relationships between Δ\(^{13}\)C and yield have been found in environments or seasons where supplemental irrigation or regular rainfall events throughout the growing season have maintained a high soil water status. In these environments the faster growth of high-Δ\(^{13}\)C genotypes has usually translated directly into higher final biomass production and grain yield (Condon et al., 1987, 2002; Sayre et al., 1995; Fischer et al., 1998). Low-Δ\(^{13}\)C genotypes have achieved less biomass and lower yields. It is also likely that they have also left more water behind in the soil profile at maturity (Condon et al., 2002).

In less favourable environments, variation in the extent and timing of any water limitation may interact with the ‘conservative’ growth and water use of low-Δ\(^{13}\)C genotypes to generate complex relationships between yield and Δ\(^{13}\)C. This complexity is also illustrated in Fig. 3. In the drier 1992 season, high-Δ\(^{13}\)C was associated with lower biomass production and grain yield in the lines from one cross (Rosella×Matong), but for lines from the second cross (Quarrion×Cranbrook) there were no associations...
between productivity and $\Delta^{13}C$. Soil water status at flowering is likely to have been higher for the second cross because lines from this cross flowered, on average, one week earlier than lines from the first cross. The results for these two sets of lines grown in these two seasons indicate that the amount of rainfall is an important variable contributing to variation in grain yield. They also indicate that the timing of development of soil water deficit, with respect to the critical flowering phase, is also an important variable.

If high-$\Delta^{13}C$ genotypes exhaust the available soil water too quickly, before flowering, there is likely to be a yield penalty (Fig. 3C; Fischer, 1981). However, the penalty, in terms of crop water use and yield, associated with faster growth of high-$\Delta^{13}C$ cereals is not always as great as might be expected. In fact, the faster growth of high-$\Delta^{13}C$ cereal genotypes has often been shown to be of benefit in seasons or environments in which frequency of rainfall events was high early in the season, but was not sustained during later stages of growth, typical Mediterranean-type environments (Condon et al., 1993; Condon and Hall, 1997; Voltas et al., 1999; Merah et al., 2001; Royo et al., 2002; Araus et al., 2003). In this sort of environment, evaporation from the soil surface can account for as much as 50% of the growing season rainfall (Cooper et al., 1987). Studies where water use has been carefully partitioned between plants and soil have shown that the more ‘profligate’ transpiration associated with high-$\Delta^{13}C$ actually resulted in little difference in total water use to anthesis, despite substantially more growth by high-$\Delta^{13}C$ genotypes at this critical stage of development (Condon et al., 1993, 2002; Lopez-Castaneda et al., 1995). This was because the fast early growth of high-$\Delta^{13}C$ genotypes meant that they shaded the soil surface quickly. They were thus much more successful at restricting soil evaporation when rain events were frequent than were slower-growing, low-$\Delta^{13}C$ genotypes. Because total water use to anthesis was similar, the greater anthesis biomass of high-$\Delta^{13}C$ genotypes translated into higher grain number and higher yield.

In other environments high-$\Delta^{13}C$ genotypes have achieved greater anthesis biomass at the expense of substantially greater soil water depletion at anthesis (Condon et al., 1993). Yet despite achieving very little growth after flowering compared with low-$\Delta^{13}C$ genotypes that had been more conservative in their water use, the high-$\Delta^{13}C$ genotypes were still able to yield more. This was because high-$\Delta^{13}C$ genotypes achieved a larger grain number (associated with higher anthesis biomass) and were then able to fill these grain, probably by translocating large amounts of stored assimilate. In the same environments low-$\Delta^{13}C$ genotypes failed to use stored assimilates as effectively. They may have had fewer reserves, due to less anthesis biomass, or the stored assimilates may not have been necessary because the low-$\Delta^{13}C$ genotypes had sub-soil moisture in reserve to sustain higher photosynthesis during grain filling (Condon and Hall, 1997). One outcome of achieving high yield, despite relatively little post-anthesis growth, is that high-$\Delta^{13}C$ genotypes tend to have a higher harvest index ($HI$).

Finally, there have been a small number of studies conducted in environments where there was relatively little rainfall during the growing season and crops relied heavily on soil moisture reserves from substantial rains before sowing or very early in crop development (Condon and Richards, 1993; Condon and Hall, 1997; Condon et al., 2002). It has been in these few studies, where there was relatively little soil evaporation and a strong reliance on metering-out soil-water reserves before anthesis, that negative associations between yield and $\Delta^{13}C$ have been most consistently observed.

To summarize these complex associations between $\Delta^{13}C$, growth, water use, and yield in cereals, the ‘conservative’ growth associated with low-$\Delta^{13}C$ and high $A/T$ has been found to interact with the extent and timing of water limitation to generate confounding effects on all of the components of the yield framework introduced earlier (equation 1). In some studies, high crop $W$ (reflecting high leaf-level $A/T$) has been offset by less total crop water use. High-$\Delta^{13}C$ genotypes yielded more and dried the soil profile more fully. Low-$\Delta^{13}C$ genotypes have also been found to lose more water by evaporation from the soil surface. In combination, these two factors of less soil-water extraction and greater losses to evaporation have meant that low-$\Delta^{13}C$ genotypes have sometimes been found to transpire considerably less water than high-$\Delta^{13}C$ genotypes (Condon et al., 1993, 2002). Thus there has often been no biomass gain associated with high $A/T$ and, frequently, less biomass production and lower grain yield. Greater biomass production by high-$\Delta^{13}C$ genotypes has been most marked at anthesis, this difference at anthesis being subsequently reflected in higher grain number and yield of high-$\Delta^{13}C$ genotypes even when they may have shown little biomass growth after anthesis. With respect to grain yield, the positive associations between biomass production and $\Delta^{13}C$ have been amplified in some studies by positive associations between $HI$ and $\Delta^{13}C$, perhaps partly due to greater translocation of stored reserves by high-$\Delta^{13}C$ genotypes.

*Defining the target environment using simulation modelling*

The confounding effects arising from the conservative growth of low-$\Delta^{13}C$ genotypes indicate that the potential yield benefit from breeding cereals with high $A/T$ may be limited, and is certainly likely to vary substantially with growing environment (Condon and Richards, 1993; Richards and Condon, 1993; Condon et al., 2002; Araus et al., 2003). This conclusion was tested more closely by examining the potential outcome of breeding for high $A/T$ using a wheat crop growth simulation model (Condon et al., 2002). The ‘SIMTAG’ model (Stapper and Harris, 1979)
was run for three representative, low-rainfall cropping environments in eastern Australia which were similar in average annual rainfall receipts (450–600 mm), but which varied in average seasonal distribution of rainfall, from winter-dominant to summer-dominant. In the first instance, for each location, the growth and yield of a ‘standard’, autumn-sown genotype was simulated using several decades of historic weather data. The model was then run again, for each location, to simulate the likely long-term average effect on yield of growing a genotype incorporating improved $A/T$, a genotype with faster early leaf area growth (to restrict soil evaporation), and a genotype combining both these traits. The principal results of the simulations were as follows (Fig. 5): (i) Incorporating higher $A/T$ resulted in an average yield advantage of 11% in the northern, summer-dominant rainfall environment where the crop relies strongly on metering out stored subsoil moisture. There was almost no increase in average yield in the southern, winter-dominant rainfall environment nor in the central region with highly variable rainfall. (ii) Faster early leaf area growth was most important in the winter-dominant, Mediterranean-type rainfall environment in southern Australia. It had little benefit in the northern region, where soil evaporation is a relatively small component of total crop water use. Long-term average yield gains ranged from 11% in the south to only 1% in the north. (iii) Combining high $A/T$ with faster early leaf area growth gave consistently large average yield gains across all three environments. Average yield gains ranged linearly from c. 17% in the south to 14% in the north. Combining the two traits provided the greatest advantage in the central region. In this region the rainfall distribution is uniform on average, but highly variable in amount and distribution from year to year.

Given the results of the many field experiments summarized in the previous section, the first two outcomes of this simulation study were not particularly surprising. They indicated that breeding for higher $A/T$ is probably a reasonable objective for the northern, summer-dominant rainfall environment and that breeding for greater early vigour is probably a reasonable objective for the southern, winter-dominant rainfall region. The large yield responses in all regions from combining the two traits was more of a surprise. This outcome probably reflects the fact that even though there are large differences between regions in the long-term average distribution of rainfall, there is substantial year-to-year variation in rainfall total and rainfall distribution in all three regions. Is it possible to combine these two traits by breeding? In the previous section it was emphasized that, for cereals, low $\Delta^{13}C$ (high $A/T$) is typically associated with relatively slow growth. So it may be difficult, physiologically, to combine high $A/T$ and fast early leaf area growth. But there may be ways to boost early growth that are independent of changes in $\Delta^{13}C$. These will be discussed in a later section.

**Yield response to breeding for high $A/T$**

Experience over many growing seasons at a range of locations in eastern Australia, supported by the outcomes of the simulation study, indicated that breeding for higher $A/T$ could have a large average benefit in the northern cropping region of eastern Australia. In this region the wheat crop relies on moisture stored from summer rains and $T$ makes up a large proportion of total crop $ET$. A backcrossing programme was initiated to improve $A/T$ (i.e. lower the $\Delta^{13}C$) of the relatively high-$\Delta^{13}C$ variety Hartog, widely-grown in Australia’s sub-tropical northern region. Briefly, Hartog was crossed with a low-$\Delta^{13}C$ donor, the lowest-$\Delta^{13}C$ $F_3$ families were selected and these were back-crossed with Hartog twice more, without selection between these two rounds of crossing.

The effect on yield of divergent selection at the $BC_2$ stage, based on measurements of $\Delta^{13}C$ was tested by growing 60 BC$_2$ lines with very similar height and flowering time in nine rain-fed environments in eastern Australia and five in Western Australia. Thirty of the $BC_2$ lines had high $A/T$ (low $\Delta^{13}C$) and the other 30 had low $A/T$ (high $\Delta^{13}C$). The results are summarized in Fig. 6A, which shows the relative average yield advantage of the low-$\Delta^{13}C$ selections at each location. The environments sampled in eastern Australia varied widely in the amount and seasonal distribution of rainfall and environment-mean yields ranged from 1.3 to 6.3 t ha$^{-1}$. High-$A/T$ lines out-yielded low-$A/T$ lines in all of these environments (Fig. 6; Rebetzke et al., 2002). The relative yield advantage of high-$A/T$ lines over low-$A/T$ lines increased linearly as site-mean yield declined. The relative yield advantage was c. 10% in the

![Fig. 5](image-url)  
**Fig. 5.** Summary of results of wheat crop growth simulations using the ‘SIMTAG’ crop growth model and long-term weather data (30–50 years) showing the average change in yield, in three environments in eastern Australia, of breeding for either higher $A/T$, greater early vigour, or a combination of higher $A/T$ and greater early vigour (Condon and Stapper, 1995; AG Condon et al., unpublished results). Simulations were done for autumn-sown crops grown in (i) a northern, sub-tropical environment where crops have a strong reliance on sub-soil moisture stored from summer rainfall, (ii) a southern environment with a winter-dominant rainfall pattern where the crop relies heavily on within-season rainfall, and (iii) an environment between these two, where the average rainfall distribution is even but rainfall is highly variable in amount and seasonal distribution.
lowest-yielding environments and c. 3% in the highest-yielding environments. At the sites in Western Australia, with Mediterranean-type rainfall patterns, high-A/T lines had a small (c. 3%) yield advantage over low-A/T lines. Yield levels at these sites were restricted to a small range, 1.9–2.6 t ha\(^{-1}\). High-yielding low-\(^{13}\)C breeding lines that also combined good quality and disease resistance were submitted for more comprehensive evaluation, resulting in the recent release of two of these lines as commercial cultivars. It is interesting that in the backcross population from which Drysdale and Rees were identified, selecting for high A/T carried no yield penalty in the Mediterranean-type environments of Western Australia nor at sites with higher yield levels of c. 6 t ha\(^{-1}\) in eastern Australia. This appears contrary to the results of many of the studies reviewed earlier. The reason for this is not clear, but results indicate that in this case low \(^{13}\)C was not associated with slower growth (AG Condon and GJ Rebetzke, unpublished data). This may partly be explained by the fact that the average difference in leaf \(^{13}\)C between the two sets of backcross lines was relatively small, only c. 0.8\%oo, but still large enough, in theory, to generate 15% greater A/T in low-\(^{13}\)C genotypes. It may be that the difference in \(^{13}\)C arose from two components: one component resulting from a small reduction in stomatal conductance, having very little effect on A (Fig. 1), and a second component resulting from a small increase in photosynthetic capacity that did not penalize the rate of crop growth but countered any potential effect on A. The net outcome of such a scenario would be a gain in A/T from both components and very little, if any, effect on crop growth rate in the absence of soil water deficit.

**Advantages of measuring \(^{13}\)C in leaves of well-watered plants**

In the backcrossing programme just described, genotypic variation in \(^{13}\)C was assessed using leaf material sampled early in crop growth from well-watered plants. This may appear paradoxical, given that the objective was to increase grain yield in environments where water limitation tends to increase as the growing season progresses. Why wasn’t screening for variation in \(^{13}\)C done during post-anthesis stress using the grain, for instance? There were two ‘practical’ reasons. One is that \(^{13}\)C measured early in the season has been shown to provide the highest repeatability and heritability (Condon and Richards, 1992). Nearer to anthesis, substantial differences in the extent of soil water depletion may develop among genotypes (Condon and Richards, 1993; Condon et al., 1993) and therefore substantial differences in stress-induced stomatal closure that lead to genotype\(\times\)environment (\(G\times E\)) effects on \(^{13}\)C. High \(G\times E\) reduces heritability. Another reason for using early-formed leaf material is that it allows the use as parents in backcrossing, plants sampled for \(^{13}\)C just a few weeks earlier. This saves time going through to the
next generation, and speed is often vital in backcrossing programmes (Simmonds, 1979).

There is another important reason for using unstressed leaf material. For cereals, processes in the weeks before flowering are often much more important for yield than processes during grain filling (Passioura, 1977; Fischer, 1981). This is because variation in grain yield is more strongly related to variation in grain number than grain size. Grain number is effectively set in the period leading up to anthesis. In Australia and many other rain-fed cereal cropping regions, it is common for soil-water depletion to start during the phase before anthesis and for substantial soil-water deficits to have developed by anthesis that may limit grain number. Sampling for variation in early-leaf $\Delta^{13}$C provides an estimate of variation in A/T at the start of the phase of rapid crop growth and water use leading up to anthesis. Variation in A/T will be most influential during the early part of this phase because this is when plants are relatively unstressed, the stomata of all genotypes are more open and a large proportion of final C is assimilated by the crop.

Advantages of measuring $\Delta^{13}$C of grain

For cereals such as wheat or barley grown in Mediterranean-type environments, higher yields have often been associated with high $\Delta^{13}$C, even in relatively dry locations (Voltas et al., 1999; Merah et al., 2001; Araus et al., 2003). The $\Delta^{13}$C of grain rather than of leaves was measured in many of these studies. This association between grain $\Delta^{13}$C and yield may well prove useful in selecting for higher yield in Mediterranean-type environments (Royo et al., 2002; Araus et al., 2003), but it is not clear how to interpret these relationships. Grain $\Delta^{13}$C is a much more ambiguous measurement than $\Delta^{13}$C measured on unstressed leaves.

It is likely that the association between high grain $\Delta^{13}$C and high yield in Mediterranean environments is due to a combination of several factors. If high grain $\Delta^{13}$C values mirror high values of $\Delta^{13}$C at early stages of growth, then high grain $\Delta^{13}$C and high yield may reflect faster growth rate throughout crop development (Condon et al., 1993). But often the correlation between grain and leaf $\Delta^{13}$C values has been found to be relatively weak (Condon and Richards, 1992; Merah et al., 2001), so other factors may also be important. It may be that high grain $\Delta^{13}$C values reflect greater reliance for grain filling on pre-anthesis stem reserves laid down earlier in the season when plants were less stressed. It is likely that, to fill their grain, genotypes that have used more of the soil water store at anthesis will be more reliant on reserves laid down before anthesis, when $\Delta^{13}$C values were relatively high (Condon and Hall, 1997; Condon et al., 2002). Within an environment, high grain $\Delta^{13}$C may indicate greater access to soil moisture during grain filling because of more extensive rooting (White et al., 1990; Condon et al., 1993), or because of earlier flowering, or it may reflect the ability to maintain stomata more open after anthesis despite increasing soil and atmospheric water stress (Condon et al., 1993). Whatever the cause of high grain $\Delta^{13}$C, any of these characteristics (fast crop growth rate, ability to remodelize stored reserves, earlier flowering, better water extraction, stomatal insensitivity to water deficit) are likely to be useful for cereals in Mediterranean-type environments.

Opportunities to improve yield by breeding for high A/T in other species

Exploiting high A/T in breeding for greater agronomic water-use efficiency is complicated for cereals by an association between high A/T and slow crop growth rate. This seems not to be the case for groundnut (Arachis hypogaea L.). For this species, field studies in both well-watered and water-limited environments consistently show greater biomass production to be associated with higher A/T (Wright et al., 1993; Naultiyal et al., 2002). In groundnut, variation in photosynthetic capacity accounts for a large proportion of the variation in A/T (Nageswara Rao et al., 1995). Importantly, high photosynthetic capacity in this species does not appear to be associated with a slower rate of leaf area growth. Effective selection for high A/T in groundnut is achieved via selection for low specific leaf area (SLA), a less expensive alternative to $\Delta^{13}$C. However, one complication still remains, which is a tendency for low SLA (high A/T) to be associated with low HI (Wright et al., 1993). The problem is being overcome by applying concurrent selection pressure for low SLA and high HI (Nigam et al., 2001).

There may be species in addition to groundnut in which high photosynthetic capacity is associated with faster crop growth and higher A/T. Likely candidates may be other grain legumes because they have the capacity for symbiotic nitrogen fixation (Condon and Hall, 1997). Limited screening of cowpea (Vigna unguiculata (L.) Walp.) (Ashok et al., 1999) and common bean (Phaseolus vulgaris L.) (Ehleringer et al., 1991) germplasm indicates that variation in stomatal conductance may be the dominant source of variation in A/T for these species. More extensive screening is warranted in these and other legumes.

Identifying species or genotypes of species in which high photosynthetic capacity is associated with faster crop growth and higher A/T could be done by combining measurements of $\Delta^{13}$C with measurements of conductance and/or photosynthetic capacity. There are techniques available for detecting genotypic variation in conductance directly and rapidly using viscous-flow porometers (Rebetzke et al., 2001) or indirectly using oxygen isotope composition of dry matter (Farquhar et al., 1997) or canopy temperature (Fischer et al., 1998). Measurements of specific leaf area (Nageswara Rao et al., 1995) or leaf chlorophyll concentration (Rao et al., 2001) may be effective means of characterizing variation in photosynthetic capacity. Of course there may also be cropping environments for some crop species
where the slow crop growth rate and high A/T associated with low stomatal conductance is desirable because soil water is conserved for the critical flowering phase. The use of crop simulation models may be useful in identifying such circumstances.

For sunflower (Helianthus annuus L.), early research indicated that photosynthetic capacity was the major source of variation in A/T (Virgona and Farquhar, 1996), but more recent evidence indicates large genotypic variation in stomatal conductance as well (Lambrides et al., 2004). The same research group has also been evaluating the effect on hybrid sunflower yield of breeding and selection for low $\Delta^{13}$C. Progeny contrasting for $\Delta^{13}$C were selected from a cross between high-$\Delta^{13}$C and low-$\Delta^{13}$C parents. These high-$\Delta^{13}$C and low-$\Delta^{13}$C progeny were then crossed with the same male tester lines and the resulting low-$\Delta^{13}$C and high-$\Delta^{13}$C pools of hybrids were evaluated for $\Delta^{13}$C and yield in rain-fed trials in southern Queensland, Australia. The low-$\Delta^{13}$C pool of hybrids significantly out-yielded the high-$\Delta^{13}$C pool of hybrids in three of the four environments. The average yield advantage over all four environments was 25% (CJ Lambrides, SC Chapman, R Shorter, unpublished data).

Analysing large numbers of breeding lines for variation in $\Delta^{13}$C is an expensive exercise. These costs could be reduced by making use of DNA-assisted screening techniques. There have been some studies that have identified potential QTLs for $\Delta^{13}$C in various species (Specht et al., 2001; Thumma et al., 2001; Teulat et al., 2002). The majority of these studies has been done on plants subjected to soil water deficit, so it is difficult to determine whether the QTLs are associated with ‘constitutive’ or ‘inducible’ variation in $\Delta^{13}$C. Variation in $\Delta^{13}$C may be caused by variation in stomatal conductance or photosynthetic capacity. QTL analysis might also be usefully applied specifically for these traits, since variation in conductance and capacity may have different impacts on crop growth and yield, depending on the species. QTLs associated with variation in stomatal conductance have been identified in species such as rice (Price et al., 1997; Ishimaru et al., 2001).

Greater early vigour to improve agronomic water-use efficiency

To this point, this review has focused on improving agronomic water-use efficiency by manipulating leaf-level water-use efficiency, primarily through the use of $\Delta^{13}$C. However, there are other strategies that also offer promise (reviewed by Richards et al., 2002). As discussed earlier, an important strategy to improve agronomic water-use efficiency in cropping environments where the soil surface is frequently rewetted is to restrict water lost by evaporation from the soil surface. This maximizes crop transpiration and improves the ratio $T/JET$ (Condon and Richards, 1993; Richards et al., 2002). For cereals, indeed any crop, a reduction in soil evaporation is most easily achieved through the rapid development of leaf area to shade the soil surface from direct solar radiation. Good stand establishment and vigorous early plant growth will both contribute to rapid leaf area development.

Good stand establishment is best achieved by plants that reach the soil surface quicker if seed is sown relatively shallow, and that reach the soil surface much more consistently if seed is sown deep, such as when farmers are seeding into moisture below a dry topsoil. In wheat, the widely-used GA-insensitive dwarfing genes $Rht-B1b$ ($Rht1$) and $Rht-D1b$ ($Rht2$) have had a major impact on agronomic water-use efficiency by improving $HI$ and crop standability. The latter feature has been most important in irrigated cropping systems, but the same genes strongly inhibit the expression of long coleoptiles that may be an important attribute for rain-fed systems (Ellis et al., 2004). Alternative, GA-sensitive dwarfing genes exist in wheat that allow the expression of much longer coleoptiles (from tall wheats) in plants with semi-dwarf stature and high $HI$ (Rebetzke et al., 1999).

Early vigour (fast leaf area development) is an important adaptation of barley and durum wheat to terminal drought in Mediterranean environments (Van Oosterum and Acevedo, 1992; Lopez-Castaneda and Richards, 1994; Villegas et al., 2000) because it improves the ratio $T/JET$ and encourages growth when evaporative demand is low, giving higher $A/T$. Traits important for vigorous early growth in cereals were identified by comparing barley with bread wheat (Lopez-Castaneda et al., 1995). This comparison showed that large embryo size, high SLA, and growth of a large coleoptile tiller were important attributes of barley, which is characterized by very high early vigour, but these traits were lacking in semi-dwarf wheats. Extensive screening of a collection of tall wheats revealed excellent sources of each of these traits (Richards and Lukacs, 2002). In a targeted breeding programme, these were combined to produce a new parental line with early leaf area growth double that of current Australian semi-dwarf varieties (Richards et al., 2002). High-vigour backcross lines with this parent as the vigour donor out-yielded low-vigour lines from the same population by up to 13% in favourable Mediterranean-type environments (c. 280–450 mm in-season rainfall), but there was no difference in yield in drier environments with less than 250 mm in-season rainfall (Table 1; Botwright et al., 2002). The sets of vigour backcross lines grown by Botwright et al. (2002) were still much less vigorous than the tall vigour donor, probably because the recurrent parent contained a GA-insensitive dwarfing gene. The high vigour traits of large embryo, large coleoptile tillers, and greater SLA are now being combined in GA-sensitive semi-dwarf backgrounds so as to achieve early vigour much more like barley (Richards et al., 2002). Field-based screening for early leaf area growth is subject to large genotype×environment interaction and hence poor heritability (Annicchiarico and...
Table 1. Growth and yield of two sets of BC2:F5-derived wheat breeding lines contrasting for early vigour and sown at two locations in Western Australia in 1999 (from Botwright et al., 2002)

<table>
<thead>
<tr>
<th>Location</th>
<th>Vigour class</th>
<th>Leaf area index 50 DASa</th>
<th>Anthesis biomass (g m⁻²)</th>
<th>Final biomass (g m⁻²)</th>
<th>Grain yield (g m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wongan Hillsb</td>
<td>High vigour</td>
<td>0.37</td>
<td>453</td>
<td>678</td>
<td>337</td>
</tr>
<tr>
<td></td>
<td>Low vigour</td>
<td>0.32*</td>
<td>408*</td>
<td>573**</td>
<td>293***</td>
</tr>
<tr>
<td>Merredinb</td>
<td>High vigour</td>
<td>0.39</td>
<td>511</td>
<td>634</td>
<td>266</td>
</tr>
<tr>
<td></td>
<td>Low vigour</td>
<td>0.30**</td>
<td>527n.s.</td>
<td>574**</td>
<td>247**</td>
</tr>
</tbody>
</table>

a DAS, days after sowing.  
b May-November rainfall 453 mm at Wongan Hills, 274 mm at Merredin.  
c *P <0.05; ** P <0.01; *** P <0.001; n.s., not significant (for contrasts within locations).

Pecetti, 1998; Rebetzke and Richards, 1999). To counter this, Richards et al. (2002) advocated selection using plants grown in more repeatable conditions and the use of leaf width as an indirect selection tool for embryo size. This is because of the high heritability of leaf width and its strong association with early leaf area.

Combining greater early vigour and higher A/T

The outcomes of the simulation study summarized earlier (Fig. 5) indicated that combining greater early vigour and higher A/T may give substantially greater average yield gains in many rain-fed environments, but especially in environments with large temporal variability in rainfall. Is such a combination of traits possible? High early vigour in cereals appears to be partly associated with high SLA (Lopez-Castaneda et al., 1995; Rebetzke et al., 2004). To the extent that high SLA is reflected in low photosynthetic capacity (via less N per unit leaf area), then high SLA may also be reflected in higher Δ¹³C. This suggests that if high vigour and low-Δ¹³C are to be successfully combined, then a tendency to higher SLA will need to be avoided during selection. This may be desirable for other reasons. SLA has relatively low heritability in cereals (Rebetzke et al., 2004), so its value as a selection tool for high early vigour may be limited. Also, simulation modelling of the impact of selecting for higher SLA in wheat indicates that maintaining N supply may be an important prerequisite for sustaining yield gains achieved via this trait (Asseng et al., 2003). The associations between early vigour in cereals and traits other than high SLA, such as embryo size and coleoptile tiller appearance (Liang and Richards, 1994), indicate that there may be other ways to achieve higher early vigour that could allow co-selection for lower values of Δ¹³C.

Conclusion

There is now growing evidence that targeting specific traits in a breeding programme may lead to higher agronomic water-use efficiency. It is also clear that the effects of any one trait must be considered in the context of the environment in which the crop is to be grown. A particular trait, such as high A/T, may be associated with higher yield in one type of environment but may have no effect or even be detrimental in other environments. Breeding for high A/T using low-Δ¹³C measured in the leaves of unstressed wheat plants has resulted in the release of new, higher-yielding varieties for eastern Australia. The new varieties result from a backcrossing programme targeting environments where stored soil moisture needs to be metered out from relatively early in the cropping season so as to maximize seed set and sustain seed growth. By contrast, for cereals growing in Mediterranean-type cropping regions and irrigated environments, higher yield appears to be associated with high-Δ¹³C of grain. For Mediterranean-type regions this association may, in part, be a result of an association between high-Δ¹³C and fast crop growth rate. Fast crop growth rate is reflected in the vigorous development of leaf area to shade the soil surface, a key trait for cropping environments where the soil surface is frequently rewetted and large gains in T/ET can be made. Effective selection protocols for faster leaf area growth have been devised and shown to be successful for yield improvement in Mediterranean-type environments. It is possible that even greater yield gains may be achievable in these and other rain-fed environments if fast crop growth rate can be combined with high A/T. Breeding has been initiated to combine these two traits in bread and durum wheat, but this may prove difficult if an association between high vigour and high-Δ¹³C cannot be broken.

A strategy that sets out deliberately to target a combination of high early vigour and high A/T in wheat may not be necessary in other species. It will depend strongly on the extent and timing of any water limitation in relation to developmental phases critical for yield determination, and whether there is an association between high A/T and slow crop growth rate. Such an association is likely to depend on whether variation in A/T is due to variation in stomatal conductance or photosynthetic capacity and the effects of these two components on crop growth rate. Relatively simple techniques are now available for characterizing variation in stomatal conductance and photosynthetic capacity. Other interactions may also come into play. In groundnut, A/T is positively associated with crop biomass production, but negatively associated with HI. Breeding progress is being made in this species by applying concurrent selection pressure for high A/T and high HI. Progress may be more rapid if the reasons for the association between A/T and HI were better understood.

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