Recently, two new commercial wheat varieties were released for broad-acre dryland production in Australia, which were very different from any previous varieties. The varieties Drysdale (released in 2002) and Rees (released in 2003) carry a broad spectrum of disease-resistance genes and produce high-quality flour, as might be expected for new wheat varieties competing in a demanding market. But Drysdale and Rees are unique because they are the first varieties, of any crop species, specifically bred for performance in dry environments using carbon isotope discrimination as an indirect selection criterion.

Drysdale and Rees were bred by backcrossing the high transpiration efficiency trait, i.e., the highly efficient exchange of CO₂ for water through the stomata, into the widely grown, milling-quality variety Hartog. The backcrossing and selection process was based on the use of carbon isotope discrimination as a surrogate measure of transpiration efficiency, and resulted in a population of elite lines, including the two new varieties, that outyield the recurrent parent Hartog by 5 to 10 percent on average. The yield advantage tends to be greater in drier environments and less in wetter environments. These new wheats are the first of what we anticipate will be several varieties bred for high transpiration efficiency and yield using carbon isotope discrimination as a secondary trait.

These landmark releases come 20 years after it was first proposed that the isotopic composition of plant carbon in C₃ species should reflect differ-
ences in the transpiration efficiency of leaf gas exchange (Farquhar et al., 1982) and that carbon isotope analysis may therefore prove a useful tool in breeding for improved water-use efficiency and yield in dry environments (Farquhar and Richards, 1984). In this chapter, we discuss the philosophy and practice behind the development and use of carbon isotope discrimination as a secondary trait for cereal improvement and draw out some of the major conclusions and challenges that have emerged as the concept has progressed from biophysical theory to the reality of new wheat varieties in farmers' fields.

**CARBON ISOTOPE DISCRIMINATION: A PHYSIOLOGICAL MARKER FOR HIGH TRANSPIRATION EFFICIENCY**

In this section we present an overview of what is meant by the term carbon isotope discrimination, the basis for the association between carbon isotope discrimination and transpiration efficiency, and why carbon isotope discrimination might be considered a useful secondary trait for yield improvement of dryland crops. The treatment is not exhaustive. For a more complete coverage of the concepts covered here, the reader is referred to the early publications by Farquhar et al. (1982) and Farquhar and Richards (1984), and to more recent reviews such as those by Farquhar et al. (1989), Hall et al. (1994), Condon and Hall (1997), Brugnoli and Farquhar (2000), Condon et al. (2002), and Condon et al. (2004).

**Carbon Isotopes in the Biosphere**

Carbon accounts for approximately 40 percent of plant dry weight, and is assimilated into plants by the process of photosynthesis. During photosynthesis, CO\(_2\) from the atmosphere diffuses into the leaf interior through the thousands of tiny stomatal pores in the leaf epidermis. The CO\(_2\) is then assimilated to generate the simple sugars (carbon skeletons) that are a substrate for downstream synthesis of the multitude of organic compounds that are important for plant growth. The carbon in atmospheric CO\(_2\) and throughout the biosphere occurs as two stable (i.e., nonradioactive) isotopic forms. The most common form is \(^{12}\)C, which accounts for about 98.9 percent of the C in atmospheric CO\(_2\). The other stable isotope, \(^{13}\)C, makes up about 1.1 percent of atmospheric CO\(_2\). The proportion of \(^{13}\)C in the biosphere is sufficiently large that very small variations in the \(^{13}\)C/\(^{12}\)C ratio can be measured accurately.

Early measurements of the \(^{13}\)C/\(^{12}\)C ratio revealed that the C-isotope composition of plant dry matter was different from the composition of the atmosphere on which plants feed. Plants were found to contain fractionally less \(^{13}\)C and thus relatively more \(^{12}\)C than the atmosphere. It has also been known for some time that considerable variation exists in the \(^{13}\)C/\(^{12}\)C ratio of plant dry matter (Brugnoli and Farquhar, 2000). This variation has several implications for crop productivity, but before expanding on these, we first need to explore more closely the processes that cause variation in the C-isotope composition of plant dry matter. Before doing so, the principles involved in carbon isotope analysis and how the data are expressed are briefly summarized.

**Isotope Analysis and Terminology**

The isotopic composition of plant carbon is most often measured using isotope-ratio mass spectrometry (Preston, 1992). The technique first requires the production of a pure sample of CO\(_2\) gas from the plant material. This is usually achieved by combusting a small sample of dried, finely ground plant material at high temperature. The CO\(_2\) produced is then purified, for example by gas chromatography, and introduced into the mass spectrometer where the CO\(_2\) molecules are ionized at high voltage and focused into a fast-moving beam. The isotopes are separated on the basis of mass-charge ratio by passing the beam of ions through a strong magnetic field.

It is technically difficult to accurately measure the absolute isotopic composition of plant carbon because \(^{13}\)C is present in such low amounts (approximately 1.1 percent of total C at natural abundance). Nevertheless, differences in composition between samples can be measured with useful precision. The isotopic composition is therefore expressed by comparing the molar abundance ratio, \(^{13}\)C/\(^{12}\)C, of the plant sample (\(R_p\)) to the value of the molar abundance ratio in a standard (\(R_s\)). Carbon isotope composition (\(\delta^{13}\)C) is labor calculated as \(R_p / R_s - 1\). For historical reasons, the standard for carbon has been Pee Dee belemnite (PDB), a carbonaceous rock, although a synthetic replacement is now provided by the International Atomic Energy Agency (IAEA). Values of \(\delta^{13}\)C\(_{\text{PDB}}\) are often expressed as the value times 10\(^3\) or per mil so that they appear as whole numbers. The \(\delta^{13}\)C\(_{\text{PDB}}\) of plant material has a value that is small and negative, in the range of approximately 10 per mil to ~30 per mil. This is because the \(^{13}\)C/\(^{12}\)C ratio of plant material is less than that of PDB. The atmosphere has a value of \(\delta^{13}\)C\(_{\text{PDB}}\) that is also slightly negative, about ~8 per mil relative to PDB.

Carbon isotope composition (\(\delta^{13}\)C\(_{\text{PDB}}\)) has proven to be a useful empirical measure, but as understanding developed of the processes causing varia-
tion in isotopic composition of plants and the biological significance of 
these processes (described in the following sections), a change in terminol-
ogy was called for. Farquhar and Richards (1984) defined the term carbon 
iso t ope discrimination \( (\Delta^{13}C) \) as the molar ratio of \( ^{13}C/^{12}C \) in at-
ospher ic \( \text{CO}_2 \), the carbon source for plants, divided by the same ratio in the 
plant product (\( R_p \)), but expressed as a deviation from unity, i.e., \( \Delta^{13}C = \frac{R_p}{R_o} - 1 \). The value of \( R_p/R_o \) is a number only slightly greater than one, so 
the deviation from unity is used for numerical convenience to obtain values 
that are small but positive, in the range of approximately 2 per mil to 22 per 
mil for plant material grown in atmospheric air. The definition of \( \Delta^{13}C \) 
requires that values of both \( R_p \) and \( R_o \) are known. \( R_o \) can be measured as previously 
described. Fortunately, for samples taken from most field-grown plants, 
variation in the value of \( R_o \) is not usually of concern since the isotopic 
composition of the atmosphere is close to constant, although it is be-
coming very slowly more negative as more fossil fuels are burned. If plant 
samples being analyzed are from a situation in which the isotopic composi-
tion of the air is likely to be very different from —8 per mil, then the isotopic 
composition of the air should also be measured to allow calculation of \( \Delta^{13}C \),
or \( \delta^{13}C \) units should be used to express isotopic composition.

**Carbon Isotope Discrimination During Photosynthesis**

As noted earlier, plant dry matter contains relatively less \( ^{13}C \) than is found in the atmosphere. The major reason for the difference in C-isotope 
composition between atmospheric \( \text{CO}_2 \) and plant dry matter is that plants 
discriminate against \( ^{13}C \) during the process of photosynthesis. The discrim-
ination principally occurs at two sites, although some (usually minor) addi-
tional sources exist, which will be discussed briefly later. The first main site 
of discrimination is at the stomatal pores, during the physical diffusion of 
\( \text{CO}_2 \) into the leaf. The second is at the site of capture of the \( \text{CO}_2 \)-derived 
carbon during its initial incorporation into simple sugars.

The diffusion of \( \text{CO}_2 \) through the stomata in the leaf epidermis and into 
the substomatal cavities in the leaf mesophyll is driven by a \( \text{CO}_2 \)-concentration 
gradient between the atmosphere, where \( \text{CO}_2 \) is currently present at approx-
imately 370 ppm, and the substomatal cavities. Here the \( \text{CO}_2 \) concentra-
tion is considerably lower than in the atmosphere (at least during the 
day), most typically in the range of 150 to 280 ppm, depending on plant 
species and prevailing growing conditions. Because of their slightly smaller 
mass, molecules of \( \text{CO}_2 \) containing \( ^{13}C \) diffuse through the stomata a little 
more easily than the larger molecules that contain \( ^{12}C \).

Discrimination against \( ^{13}C \) during the initial capture of \( C \) in the bio-
chemical step of photosynthesis is the second major source of variation in 
\( ^{13}C/^{12}C \) ratio measured in plant dry matter. In plant species with \( \text{C}_3 \) (Calvin-
cycle) photosynthesis, such as wheat, barley, and rice, almost all the initial 
capture of \( C \), or carboxylation, is performed by the enzyme Rubisco. This 
enzyme has the potential for a relatively large amount of discrimination 
against \( ^{13}C \), of approximately 30 per mil. In \( \text{C}_4 \) species, a small amount of \( C \) 
capture by the enzyme PEP carboxylase also occurs. This enzyme discrimi-
nates slightly in favor of \( ^{13}C \). In contrast to \( \text{C}_3 \) species, PEP (phospho-
enolpyruvate) carboxylase is the enzyme responsible for most \( C \) capture 
in plants of \( \text{C}_4 \) species such as maize and sorghum. The full extent of net 
discrimination in \( \text{C}_3 \) species by Rubisco and PEP carboxylase is never 
expressed in plant tissue. This is because the \( \text{CO}_2 \) at the sites of carboxylation 
is impeded from exchanging freely with the atmosphere, due to the pres-
ence of the epidermis and stomatal pores. The extent of discrimination 
during carboxylation is thus dependent on how similar the \( \text{CO}_2 \) concentra-
tion inside the leaf \( (c_i) \) is to the \( \text{CO}_2 \) concentration outside, in the atmos-
phere \( (c_o) \). The closer the ratio \( c_i/c_o \) to unity, the greater the extent of discrimi-
nation during carboxylation, and vice versa.

As summarized in Equation (6.1) (Farquhar and Richards, 1984), plants 
of \( \text{C}_3 \) species have C-isotope ratios that contain a contribution from 
discrimination at the stomata and (principally) from Rubisco:

\[
\Delta^{13}C = a + (b - a) \cdot \frac{c_i}{c_o}
\]  
(6.1)

The equation shows a positive, linear relationship between \( \Delta^{13}C \) and the 
ratio \( c_i/c_o \). The parameters \( a \) and \( b \) account for the discrimination at the 
stomata and the net discrimination during carboxylation. The measured 
value for \( a \) is 4.4 per mil, and \( b \) has a practical value often close to 28 per 
il, this value for \( b (= b_2 - d) \) being the numerical difference between discrime-
nation by Rubisco \( (b_2 = 30 \text{ per mil}) \) and net discrimination by PEP 
carboxylase, diffusion to the sites of carboxylation, and other processes 
during carboxylation \( (d = 2 \text{ to } 5 \text{ per mil}, \text{depending on assimilation rate}) \) 
(Brugnoli and Farquhar, 2000). Inserting these values for \( a \) and \( b \) into Equa-
tion 6.1 yields:

\[
\Delta^{13}C = 4.4 + 23.6 \cdot \frac{c_i}{c_o}
\]  
(6.2)

which states simply that for plants of \( \text{C}_3 \) species less discrimination exists 
against \( ^{13}C \) as \( c_i/c_o \) gets smaller (Figure 6.1).
Equations 6.1 and 6.2 represent an essentially instantaneous process of discrimination. It is known that the value of $c_i/c_o$ is not constant and neither, therefore, is the amount of discrimination. Rather, discrimination responds to numerous short-term and long-term environmental influences, fluctuating depending on the extent that the stomatal step dominates or the carboxylation step dominates. These processes are also subject to some level of genetic control. Measuring $\Delta^{13}C$ in dry matter of C3 species provides an assimilation-weighted average value of $c_i/c_o$ over the life of the plant material being analyzed.

Carbon isotope discrimination by plants of C4 species is small compared with that by plants of C3 species (Figure 6.1) and reflects to a much larger extent the discrimination that takes place at the stomata. A small amount of variation exists in C-isotope composition of C4 plants resulting from discrimination during C-fixation. This variation may be of significance for plant productivity and will be considered in more detail toward the end of the chapter. Because of the greater potential for variation in C3 plants, discrimination by C3 species has attracted far more attention and forms the basis for most of what follows.

**FIGURE 6.1.** Relationships for C3 and C4 plant species between carbon isotope discrimination during photosynthesis and the ratio of intercellular and atmospheric concentrations of CO2 ($c_i/c_o$). The boxes represent the range of typical data points obtained in leaf gas exchange studies in which carbon isotope discrimination was determined “online” by measuring the carbon isotope composition of the gas stream before and after it passed over the leaf. Source: Adapted from Brugnoli and Farquhar, 2000.

**Application of Carbon Isotope Discrimination in Cereal Improvement**

**Variation in Carbon Isotope Composition Among Metabolites**

The values of $\Delta^{13}C$ measured in plant dry matter principally reflect discrimination during photosynthesis, yet in all plants some potential for additional small changes in C-isotope composition exists as C flows through biochemical pathways downstream from photosynthesis to form various metabolites. For example, lipids tend to be depleted in $^{13}C$ compared with bulk plant dry matter, whereas the reverse is true for carbohydrates (Figure 6.2). The reasons for these differences in C-isotope composition among metabolites are primarily due to fragmentation fractionations (Tcherkez et al., 2004) associated with transfer of C atoms during the biochemical processes that generate these various compounds. These processes will not be discussed in detail here, however, Brugnoli and Farquhar (2000) provide a useful summary. It is possible that further, additional fractionation of C isotopes may take place during processes associated with transport of metabolites and respiration since these processes also often entail some rearrangement of organic molecules.

**FIGURE 6.2.** Carbon isotope discrimination in various metabolites in C3 species. The boxes represent the range of variation found in the literature, with the vertical bars being the mean values for each compound. The dashed line represents $\Delta^{13}C$ for bulk dry matter. Source: Adapted from Brugnoli and Farquhar, 2000.
Transpiration Efficiency and Its Association with Carbon Isotope Discrimination

The association of transpiration efficiency and Δ13C comes about because of independent associations of both transpiration efficiency and Δ13C with the ratio $c_i/c_a$. The association between Δ13C and $c_i/c_a$ has already been described. The association between transpiration efficiency and $c_i/c_a$ arises as follows: Transpiration efficiency at the leaf level can be defined as the instantaneous ratio of the rates of CO2 assimilation ($A$) and transpiration ($T$). Both $A$ and $T$ can be described by very similar, simple equations. $A$ is the product of stomatal conductance to CO2 ($g_c$) and the concentration gradient of CO2 between the outside ($c_a$) and the inside ($c_i$) of the leaf:

$$A = g_c (c_a - c_i)$$  \hspace{1cm} (6.3)

$T$ is the product of stomatal conductance to water vapor ($g_w$) and the concentration gradient of water vapor from the inside ($w_i$) to the outside ($w_a$) of the leaf:

$$T = g_w (w_i - w_a)$$  \hspace{1cm} (6.4)

For CO2, the concentration is greater outside the leaf, whereas the reverse is true for water vapor. Equations 6.3 and 6.4 can be combined to yield a mathematical description of transpiration efficiency:

$$A/T = [g_c (c_a - c_i)]/[g_w (w_i - w_a)]$$  \hspace{1cm} (6.5)

This equation can be further simplified by noting, first, that both CO2 and water vapor diffuse over the same pathways through the stomata and, second, that the relative diffusivities of CO2 and water vapor in air has a value of $c_a/0.6$. Thus:

$$A/T = 0.6 c_a (1 - c/c_a) (w_i - w_a)$$  \hspace{1cm} (6.6)

If the gradient in water vapor concentration is assumed to be an independent variable, then Equation 6.6 indicates that $A/T$ should be negatively related to the ratio $c_i/c_a$ (Farquhar and Richards, 1984).

The value of $c_i/c_a$ is determined by the balance between two factors: the supply of CO2 to the leaf interior, which is governed by the stomatal conductance to CO2, and the demand for CO2 consumption, which is governed by the capacity (amount and activity) of the photosynthetic machinery. The value of $c_i/c_a$ is typically near 0.7 for unstressed plants of C3 species (Farquhar, Ehleringer, and Hubick, 1989). Lower values of $c_i/c_a$ and hence higher $A/T$ could be achieved either through lower stomatal conductance, higher photosynthetic capacity, or a combination of these two.

Equation 6.6 describes transpiration efficiency as being negatively related to $c_i/c_a$ but it has already been noted in Equation 6.2 that Δ13C is positively related to $c_i/c_a$. Accordingly, through their independent associations with $c_i/c_a$, it would be expected that, for plants of C3 species, $A/T$ and Δ13C should be negatively correlated. The realization that Δ13C, through its relationship to $c_i/c_a$, could provide an indirect measure of variation in $A/T$ (Farquhar et al., 1982; Farquhar and Richards, 1984) sparked renewed enthusiasm for the prospect of exploiting differences in $A/T$ to improve the yield of water-limited C3 crops.

Transpiration Efficiency As a Yield-Enhancing Trait

With respect to crop yield improvement in dry environments, interest in $A/T$ (and therefore Δ13C) lies in the extent to which greater $A/T$ contributes to greater crop water-use efficiency and ultimately to greater yield. The relationships between crop transpiration efficiency, crop water-use efficiency, and crop yield can be summarized as:

$$Y = ET·T/ET·W_T·HI$$  \hspace{1cm} (6.7)

where the grain yield of a water-limited crop ($Y$) is equal to the product of the total amount of water used by the crop (evapotranspiration, $ET$); the proportion of that water actually transpired by the crop ($T/ET$); the transpiration efficiency of biomass production ($W_T$), i.e., how much biomass is produced per millimeter of water transpired; and, last, 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). As noted by Passioura (1977), for Equation 6.7 to be applied most usefully, there should be no strong negative interactions between any of the components on the right-hand side of the equation. In reality, none of the components of this yield framework is truly independent of the others (Condon and Richards, 1993; Condon et al., 2002). Nonetheless, each component could be considered a potential target for genetic improvement, provided negative interactions with other components can be minimized. Leaf-
level transpiration efficiency, \( A/T \), is directly related to only one component, \( W_T \), the transpiration efficiency of biomass production, but 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.

\section*{VARIATION IN \( \Delta^{13}C \) OF C\(_3\) CEREALES}

\subsection*{Genotypic Variation in \( \Delta^{13}C \)}

Substantial genotypic variation in \( \Delta^{13}C \) has been observed in many \( C_3 \) cereal species. Numerous studies have shown variation in \( \Delta^{13}C \) of at least 2 per mil, occasionally closer to 3 per mil, in bread wheat (Condon et al., 1987; Condon et al., 1990; Eldebe et al., 1991; Sayre et al., 1995), durum wheat (Araus et al., 1998; Merah et al., 1999; Royo et al., 2002), and barley (Hubick and Farquhar, 1989; Craufurd et al., 1991; Azevedo et al., 1993; Vollas et al., 1998), and in various species of rangeland (Johnson et al., 1991; Johnson and Bassett, 1991) and turf grasses (Eldon et al., 1999). Considerable genotypic variation is known to exist in \( \Delta^{13}C \) within rice (Dingkuhn et al., 1991; Condon et al., 1991), and no obvious reasons explain why similar variation in \( \Delta^{13}C \) should not be present in other cereals such as oats, rye, and triticale.

For healthy, unstressed plants of \( C_3 \) species, average values of \( c_1/c_0 \) are usually close to 0.7 (Farquhar, Ehleringer, and Hubick, 1989). This is equivalent to an average value of \( \Delta^{13}C \) of ca. 21 per mil (from Equation 6.2). Given this average value of \( \Delta^{13}C \), what does a range of \( \pm 1 \) per mil genotypic variation in \( \Delta^{13}C \) mean in terms of potential variation in \( A/T \)? This can be calculated by making further use of Equation 6.2 (which relates variation in \( \Delta^{13}C \) to variation in \( c_1/c_0 \)) and Equation 6.6 (which relates variation in \( A/T \) to variation in \( c_1/c_0 \)). From these it can be determined that, in a relative sense, \( A/T \) of a genotype with a \( \Delta^{13}C \) value of 20 per mil could be approximately 1.3 times greater than \( A/T \) of a genotype with a \( \Delta^{13}C \) of 22 per mil. Thus large potential gains in \( A/T \) are associated with relatively low values of \( \Delta^{13}C \). However, various reasons explain why it is unlikely that all of these gains will be realized in crops grown in the field. These reasons, discussed in detail in following sections, relate to a series of complications that arise as processes of exchange of \( CO_2 \) for water are scaled up from the level of instantaneous fluxes at the stomata to crop growth and water use in field canopies. Many of these complications relate to the physiological basis of variation in \( \Delta^{13}C \) and \( A/T \).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Genotype & Stomatal conductance\(^a\) (mol\(-m^{-2}\cdot s^{-1}\)) & Photosynthetic capacity\(^b\) (mmol\(-m^{-2}\cdot s^{-1}\cdot Pa^{-1}\)) & \( \Delta^{13}C \) (\%) \\
\hline
Veery 3 & 0.55 & 1.66 & 21.0 \\
Sunstar & 0.56 & 2.06 & 20.4 \\
Hartog & 0.49 & 1.56 & 20.2 \\
K1056 & 0.46 & 1.60 & 19.5 \\
Quarrion & 0.48 & 1.68 & 19.5 \\
M3844 & 0.43 & 1.79 & 19.3 \\
\hline
\end{tabular}
\caption{Variation in stomatal conductance, photosynthetic capacity, and carbon isotope discrimination among representative Australian semidwarf wheat genotypes.}
\end{table}

\textit{Source: Adapted from Condon et al., 1990}

\(^a\) Stomatal conductance and photosynthetic capacity (initial slope of the relationship between \( A \) and \( c_1 \)) were measured on flag leaves of well-watered and fertilized plants.

\(^b\) \( \Delta^{13}C \) was measured on sink tissue for \( C \) assimilated by the flag leaf, i.e., growingears enclosed in the flag leaf sheath.

\textit{Causes of Genotypic Variation in \( \Delta^{13}C \) in \( C_3 \) Cereals}
ductance and capacity to covary, such that $c_i/c_o$ is maintained close to a value of 0.7, at least in leaves of healthy, unstressed plants (Farquhar, Ellerisinger, and Hubick, 1989). This tendency for conductance and capacity to covary is illustrated in Table 6.2, which summarizes data on $\Delta^{13}C$ and related photosynthetic parameters obtained on a diverse collection of 41 rice genotypes grown under permanently flooded conditions in southeast Australia. Among all 41 genotypes a range in $\Delta^{13}C$ of recently grown leaf material from 19.2 per mil to 21.1 per mil existed. Although substantial variation in leaf conductance (measured in this study as leaf porosity using a viseous-flow porometer) was seen, very little relationship was apparent between $\Delta^{13}C$ and conductance. In fact, for the bulk of the 41 genotypes, considerable genotypic variation in conductance existed that was reflected in almost no change in $\Delta^{13}C$. This was because conductance and capacity (measured as leaf N content) tended to covary. When genotypes were arbitrarily split into three groups on the basis of N content, which is a robust measure of photosynthetic capacity in rice (Peng et al., 1995), they showed no difference in average $\Delta^{13}C$ of leaves. Rather, differences in average N content among the groups were reflected in parallel differences in leaf conductance, canopy temperature depression (CTD), and $\delta^{18}O$ composition ($\delta^{18}O$) (Table 6.2).

Similar observations of covariation of stomatal conductance and photosynthetic capacity were made by Fischer et al. (1998) on a "historic series" of semidwarf bread wheats released by CIMMYT. In this study, the genotypic variation in stomatal conductance exceeded the variation in photosynthetic capacity, with the result that more recent, high capacity, high conductance genotypes had higher values of $\Delta^{13}C$ than earlier, low conductance, low capacity genotypes. The range in $\Delta^{13}C$ among the wheats studied by Fischer et al. (1998) was 1 per mil.

### Consequences of Variation in $\Delta^{13}C$ Associated with Variation in Stomatal Conductance

The measurement of $\Delta^{13}C$ provides an integrated estimate of genotypic variation in $c_i/c_o$ and, potentially, $A/T$. Unfortunately, $\Delta^{13}C$ provides no information on whether $c_i/c_o$ is varying due to variation in stomatal conductance or due to variation in photosynthetic capacity. This information may be important for several reasons. One reason is that it is unlikely that the simplistic relationship between $\Delta^{13}C$ and $A/T$ described by Equation 6.6 will fully apply in situations in which variation in $\Delta^{13}C$ reflects variation in stomatal conductance. This is because any genotypic difference in stomatal conductance will usually also be reflected in a difference in leaf temperature. Genotypes with lower conductance will have warmer leaves (e.g., Table 6.2) and therefore larger vapor pressure gradients driving water from the leaves (Farquhar, Hubick, et al., 1989). As a result, transpiration per unit conductance will also be greater (Equation 6.4). Ultimately this means that the term $(w_o - w_d)$ in Equation 6.6 is not an independent variable, and any gain in $A/T$ will be less than expected from the lower value of $\Delta^{13}C$ that results from the lower conductance. For individual leaves, this situation will become worse the less the air is stirred around the leaves. This is because the air in the unstirred boundary layers around the leaves of a low-conductance genotype will be warmer and drier than the air in the boundary layers around the leaves of a higher-conductance genotype. The situation is predicted to become worse still when leaves form extensive, dense canopies, such as in a well-fertilized cereal crop. This is largely because even less stirring of air around leaves will occur in such a canopy, although additional reasons for this exist that are explored more fully elsewhere (Cowan, 1988; Jones, 1993). Despite the complications of elevated leaf temperature, in most situations genotypes with low $\Delta^{13}C$ resulting from lower stomatal conductance will still tend to have higher $A/T$, but the gain in $A/T$ will be diluted somewhat.

Of course another consequence of low $\Delta^{13}C$ associated with low stomatal conductance exists. Lower $\Delta^{13}C$ and higher $A/T$ resulting from low stomatal conductance are likely to be associated with lower photosynthetic rate per unit leaf area and possibly a slower rate of crop growth. Low $\Delta^{13}C$ associated with low stomatal conductance may therefore result in lower productivity if no strong limitation to growth exists from lack of water.

### TABLE 6.2. Summary of variation in photosynthetic traits among 41 diverse rice genotypes grown under continuous flooding in southeast Australia, 1999.

<table>
<thead>
<tr>
<th>N-content group</th>
<th>N content (%)</th>
<th>$\Delta^{13}C$ (%)</th>
<th>Conductance (per second)</th>
<th>CTD (°C)</th>
<th>$\delta^{18}O$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High N (n=14)</td>
<td>3.40</td>
<td>20.3 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td>5.6 ± 0.2</td>
<td>25.6 ± 0.2</td>
</tr>
<tr>
<td>Intermediate N</td>
<td>3.03(3-40)</td>
<td>20.2 ± 0.1</td>
<td>1.4 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>25.8 ± 0.1</td>
</tr>
<tr>
<td>Low N (n=14)</td>
<td>3.03</td>
<td>20.3 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>4.9 ± 0.2</td>
<td>26.0 ± 0.1</td>
</tr>
</tbody>
</table>

Source: Condon et al., 1999.

a Mean values of photosynthetic traits (± standard error) are shown for three equally sized groups, separated on the basis of leaf N content.

b $\Delta^{13}C$, $\delta^{18}O$ (oxygen isotope composition with respect to standard mean ocean water (SMOW)) and stomatal conductance were measured on upper canopy leaves. Stomatal conductance was measured using a viscous-flow porometer (Rebetzke et al., 2004), CTD (canopy temperature depression) using an infrared thermometer.
The complications associated with low stomatal conductance are not anticipated if low $\Delta^{13}C$ is associated with high photosynthetic capacity (greater draw-down of $c_i$ per unit conductance). In this case no change in leaf temperature should occur, so the response of $\Delta T$ to a change in $\Delta^{13}C$ should fully correspond to that given in Equation 6.6. Also, the expectation would be for greater photosynthetic rate per unit leaf area and, therefore, probably a faster rate of crop growth, although as discussed in a following section, the latter may not necessarily follow.

**Effects of Environment on $\Delta^{13}C$**

The values of $\Delta^{13}C$ measured in dry matter sampled from plants of $C_3$ cereals under stress are almost invariably lower than $\Delta^{13}C$ values measured on unstressed plants. Abiotic stresses such as soil-water deficit (e.g., Farquhar and Richards, 1984; Ehdie et al., 1991; Condon et al., 1992; Merah et al., 1999), soil compaction (Masle and Farquhar, 1988), soil salinity (Isla et al., 1998; Rivelli et al., 2002), and low humidity (Condon et al., 1992) all result in lower values of $\Delta^{13}C$ because they result in some degree of stomatal closure, causing $c_i/c_a$ to be lower. In some circumstances, abiotic stresses such as salinity may be severe enough to cause damage to the photosynthetic apparatus, especially if they are accompanied by conditions of high light (e.g., James et al., 2002). In such cases, a tendency toward a slightly higher value of $c_i/c_a$ may exist due to lower photosynthetic capacity, but a concurrent tendency toward lower $c_i/c_a$ will probably exist due to stomatal closure. In practice it is difficult to resolve these opposing influences on $c_i/c_a$ and $\Delta^{13}C$, even using sophisticated gas-exchange measurements.

It might be expected that N starvation would be reflected in higher values of $\Delta^{13}C$ since N starvation should result in lower photosynthetic capacity, but experiments conducted with wheat and barley found virtually no difference in $\Delta^{13}C$ between N-sufficient and N-starved plants (Condon et al., 1992; Kang et al., 1996; Robinson et al., 2000). This is probably because N starvation was applied early in development, with two outcomes. First, the plants adjusted leaf area, through smaller leaves and fewer tillers, so that leaf N content was maintained at relatively high levels (certainly less different than the difference in applied N). Second, it is likely that stomatal conductance fell to balance the photosynthetic capacity of the leaves and constrain $c_i/c_a$ near 0.7. Relatively low stomatal conductance is a common observation for N-starved cereals (Wong et al., 1979).

Values of $\Delta^{13}C$ measured on unstressed plants of $C_3$ cereals are typically in the vicinity of 19 to 22 per mil. For plants subjected to soil-water deficit, $\Delta^{13}C$ values measured on organs that have grown when plants were under severe stress have been found to be as low as 12 to 14 per mil. Many of the lowest values of $\Delta^{13}C$ have been measured on grain of temperate cereals grown in field experiments in very low rainfall environments or in container experiments in which soil water deficit became gradually more severe (e.g., Condon et al., 1992; Voltas et al., 1999; Botwright et al., 2002). It is not surprising that a gradual decline in the values of $\Delta^{13}C$ measured usually occurs on recently formed dry matter of temperate cereals grown in rainfed environments, since it is usual for soil water availability to decline and the vapor pressure deficit of the air to increase as the growing season progresses. Both of these stresses will result in stomatal closure and lower values of $\Delta^{13}C$ (Condon et al., 1992; Figure 6.3).

The very low values of $\Delta^{13}C$ measured in grain have prompted hypotheses on recycling of respired CO$_2$ within the grain-enclosing structures of the ear (Araus et al., 1993; Gebbing and Schnyder, 2001) and suggestions that an enhanced contribution to CO$_2$-fixation from PEP carboxylase present in bracts and glumes may exist (Bort et al., 1995). Whereas any enhanced contribution from PEP carboxylase remains moot, it is highly likely that some recycling of respired CO$_2$ does take place within the ear. The effect of this
renewing the $\Delta^{13}C$ value of grain is difficult to resolve since the carbon in the grain may be derived from numerous sources, including current photosynthesis from leaves and ear structures and assimilates stored earlier in the stems and leaf sheaths. Each of these C sources may have quite different $\Delta^{13}C$ signatures that largely derive from the environmental conditions prevailing at the time the C was acquired.

$\Delta^{13}C$ values are not always very different from leaf $\Delta^{13}C$ values. Mean grain $\Delta^{13}C$ values of approximately 19 per mil have been measured in field experiments on durum wheat given supplemental irrigation (Merah et al., 2001; Royo et al., 2002). In a study of 11 advanced breeding lines of rice grown under flooded padi conditions in southeast Australia (Condon et al., 1999), the mean $\Delta^{13}C$ value of grain harvested at maturity (mid-April) was 19.5 per mil (genotype range 18.6 to 20.3 per mil). This was very close to the mean $\Delta^{13}C$ value of 19.8 per mil measured on recently formed leaves sampled in early January (genotype range 19.4 to 20.2 per mil). The slightly lower values for grain $\Delta^{13}C$ than leaf $\Delta^{13}C$ could easily be attributed to the high starch content of the grain as compared with the relatively high lipid content of the leaves. Starch is known to have a lower $\Delta^{13}C$ value than lipids (Figure 6.2). Indeed, given the difference in chemical composition between leaves and grain, it is surprising that the difference in $\Delta^{13}C$ was not greater.

**Genotype × Environment Interaction for $\Delta^{13}C$**

Genotype × environment ($G \times E$) interactions for $\Delta^{13}C$ are potentially large because environmental influences can have a very large effect on $\Delta^{13}C$ values measured in plant dry matter, especially under conditions of declining soil water and rising evaporative demand (see previous section for details), and the $\Delta^{13}C$ value of all genotypes may not respond in the same manner or at the same time to these environmental perturbations. For example, the value of $\Delta^{13}C$ may fall faster in genotypes that use water faster or that have shallow root systems or that have stomata that are more sensitive to increasing vapor pressure deficit of the air. Among the eight wheat genotypes grown in the study summarized in Figure 6.3, genotypic variation in the slope of the seasonal decline in $\Delta^{13}C$ was strongly correlated with the proportion of profile water consumed at anthesis by each genotype. The rate of decline of $\Delta^{13}C$ was faster for genotypes that had used relatively more of the available soil water at anthesis (Condon et al., 1992).

Broad-sense heritability is a measure of the extent to which phenotypic variation in a trait can be attributed to genotypic differences, rather than the effects of environment, $G \times E$, and sampling. Thus it reflects the extent to which the range in genotypic values is repeatable, and therefore it also reflects the potential for progress in selection during breeding. Estimates of broad-sense heritability for $\Delta^{13}C$ can be high, in the order of 90 percent on a genotype-mean basis and 80 percent on a single-plot basis (Condon and Richards, 1992), but substantially lower values are not uncommon (Ehdaie and Waines, 1994; Araus et al., 1998; Merah et al., 2001). Different estimates of broad-sense heritability for $\Delta^{13}C$ may reflect the different tissues sampled for $\Delta^{13}C$ measurements. For temperate cereals, values of heritability for $\Delta^{13}C$ are often lowest for plant material sampled near anthesis, such as flag leaves, the rachis, and peduncle. This is when any genotypic differences in soil-water depletion are likely to be greatest. Heritabilities may be higher for grain, but this is not always the case across contrasting soil moisture regimes (Condon and Richards, 1992). Heritabilities have been found to be highest for dry matter laid down before or during early stem elongation, when plants are essentially unstressed and repeatability greatest (Condon and Richards, 1992).

Values of narrow-sense heritability for $\Delta^{13}C$ of bread wheat reported by Rebetzke, Condon, et al. (2002) were also high: 93 percent on a genotype-mean basis and 63 percent on a single-plot basis. In the same study, the corresponding narrow-sense heritabilities for grain yield were 55 and 14 percent, respectively. The high values of heritability for $\Delta^{13}C$ were obtained by employing dry matter sampling strategies that minimized the impact of potentially large $G \times E$ for $\Delta^{13}C$. Measurements of $\Delta^{13}C$ were done on vegetative leaf material laid down early in the standard growing season when plants were effectively unstressed, as recommended by Condon and Richards (1992).

**Genetic Control of $\Delta^{13}C$**

Narrow-sense heritability reflects the portion of the phenotypic variance that is transmitted from parent to progeny. Thus, it reflects breeding value. High narrow-sense heritabilities reported by Rebetzke, Condon, et al. (2002) for $\Delta^{13}C$ of field-grown plants were consistent with high narrow-sense heritabilities for $\Delta^{13}C$ measured for progeny from a range of wheat crosses evaluated in the glasshouse and field (Rebetzke et al., 2006). Furthermore, these high narrow-sense heritabilities were consistent with high heritabilities for transpiration efficiency measured on bread (Malik et al., 1999) and durum wheat (Solomon and Labuschagne, 2004) plants evaluated under water-limited and well-watered conditions in the glasshouse. Genetic studies in wheat have shown a preponderance of additive gene effects for $\Delta^{13}C$ (Rebetzke et al., 2006) and transpiration efficiency (Malik et al., 1999; Solomon and Labuschagne, 2004). Although small, some evi-
dence exists for nonadditive gene action including additive-based epistasis for $\Delta^{13}C$ in bread wheat (Rebetzke et al., 2006) and dominance for transpiration efficiency in durum (Solomon and Labuschagne, 2004). Although early generation selection should be highly effective in genetic gain for these traits, some non-additive gene action suggests the need to delay selection until after some inbreeding (e.g., among $F_2$- or $F_3$-derived families).

Genetic control of $\Delta^{13}C$ measured in unstressed plants is not expected to be simple, because variation in $\Delta^{13}C$ reflects variation in stomatal conductance and photosynthetic capacity. Both stomatal conductance and photosynthetic capacity are understood to be under the control of many genes in wheat (e.g., Carver et al., 1989; Rebetzke et al., 2001; Rebetzke et al., 2003). Consequently, $\Delta^{13}C$ is also likely to be under the control of many genes. Preliminary evidence from QTL mapping studies indicates as many as six independent genomic regions associated with genotypic variation in stomatal conductance and $\Delta^{13}C$ of vegetative tissue in different wheat mapping populations (Rebetzke et al., 2005). Despite this apparent complexity, genotypic variation in $\Delta^{13}C$ of unstressed plants is highly heritable in cereals. The high heritability of $\Delta^{13}C$ may arise because $\Delta^{13}C$ (or at least $c_l/c_a$) functions as a kind of set point of gas-exchange activity in plants (Ehltringer, 1993). As noted by Wong et al. (1979), unstressed plants of C$_3$ species tend to operate at a value of $c_l/c_a$ close to 0.7, and will alter stomatal conductance to restore this set point if, for example, photosynthetic capacity is changed by experimental manipulations. It is likely that the $c_l/c_a$ set point varies among genotypes of C$_3$ species such that some genotypes function with set points somewhat above or somewhat below 0.7. Genes associated with variation in $\Delta^{13}C$ may therefore reflect genes controlling variation in stomatal conductance alone (CO$_2$ supply), genes controlling variation in photosynthetic capacity alone (demand for CO$_2$), and genes that determine the particular balance between supply and demand that achieves a given $c_l/c_a$ set point.

Genetic control of $\Delta^{13}C$ measured in stressed plants is likely to be even more complicated than in unstressed plants (e.g., Ehdaiie and Waines, 1994). Additional genetic factors influencing stomatal response to stress are likely to exist. These factors may not necessarily act directly on stomata. For instance, they may influence root growth and access to water. Furthermore, if $\Delta^{13}C$ is being measured on sink tissues growing in stressed conditions, such as the grain, then some genetic factors may influence the contribution of different sources of C to the value of $\Delta^{13}C$ measured in the sink tissue.

**Water-Use Efficiency in the Glasshouse**

Theory developed by Farquhar et al. (1982) predicted that genotypic variation in $\Delta^{13}C$ should reflect genotypic variation in leaf-level $A/T$ because of the independent relationships of $\Delta^{13}C$ with $c_l/c_a$ and of $A/T$ with $(1 - c_l/c_a)$. This theory should extend to plant transpiration efficiency, $W_p$ (plant dry matter produced per unit water transpired) provided no association exists between $\Delta^{13}C$ and the proportion of C fixed during photosynthesis that is subsequently lost due to respiration during plant growth. Experiments on pot-grown bread wheat by Farquhar and Richards (1984) confirmed a negative association between $\Delta^{13}C$ measured in leaves and $W_p$. Since then, the negative association between $\Delta^{13}C$ and $W_p$ has been observed for a large range of C$_3$ species, both cereals and noncereals, grown in pots in the glasshouse and outdoors (reviewed in Hall et al., 1994). An example of such a relationship is shown in Figure 6.4a, in this case for bread wheat breeding lines from a cross between two parents with high and low $\Delta^{13}C$.

Studies on the relationship between $W_p$ and $\Delta^{13}C$ have been conducted with plants kept well watered and with plants subjected to varying degrees of soil-water limitation. Negative associations between $\Delta^{13}C$ and $W_p$ have been observed in almost all cases. Mean values of $\Delta^{13}C$ measured on water-limited plants have been smaller than for well-watered plants, and mean values of $W_p$ have been greater, reflecting the effects of lower average values of stomatal conductance and lower $c_l/c_a$ in water-limited plants (Figure 6.4b).

Another feature common to almost all the experiments done with plants grown in pots, including those grown in the studies represented in Figure 6.4, is that evaporation from the soil surface was effectively eliminated by covering the soil surface so that only transpiration was included in determining $W_p$. Any soil evaporation that did occur was usually subtracted after estimating water loss from pots without plants. Such procedures are not possible in field plots.

**Water-Use Efficiency in the Field**

For crops grown in the field, water use comprises two components: evaporation from the soil surface ($E_s$) and transpiration by the crop canopy. In some situations $E_s$ may account for a surprisingly large proportion of total
transpiration efficiency (g.kg⁻¹) is an important issue in evaluating the utility of Δ¹³C. Those studies on cereals that have been published (Condon et al., 1993; Lopez-Castaneda and Richards, 1994; Condon et al., 2002) all confirm the importance of $E_3$ as a key determinant of crop $W_{ET}$.

Importantly, each of these studies indicates that $E_3$ tends to be greater and $T/ET$ smaller for cereal genotypes with low values of Δ¹³C. The study summarized by Condon et al. (2002) illustrates this well (Table 6.3). In this study, season-long water use, growth, and yield at two sites were measured for two bread wheat genotypes with very similar height and flowering time. Water use was partitioned between $E_3$ and $I$. The two genotypes differed in Δ¹³C by ca. 2 per mil under well-watered conditions, largely because the conductance of the high-Δ¹³C genotype, Matong, was about 50 percent greater than that of the low-Δ¹³C genotype, Quarrier. At both sites, season-long, crop-scale $W_T$ of the genotype with lower conductance was approximately 15 percent greater than that of the genotype with higher conductance. However, the difference in crop-scale $W_{ET}$ was inconsistent. This was because $T/ET$ was lower for the low-Δ¹³C genotype. The wetter of the two sites (Wagga Wagga in 1989) had 60 mm less crop $T$ by the low-Δ¹³C genotype. This resulted in less biomass production and lower grain yield at this site for the low-Δ¹³C genotype, despite its greater crop $W_T$. At the drier site.

**TABLE 6.3.** A comparison of measures of productivity, water use, and water use efficiency at two sites in southeast Australia for two bread wheat genotypes differing in carbon isotope discrimination grown in “plots” of 1.5 to 5 ha.

<table>
<thead>
<tr>
<th>Site</th>
<th>Wagga Wagga, 1989</th>
<th>Condobolin, 1990</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Quarrier</td>
<td>Matong</td>
</tr>
<tr>
<td>Δ¹³C</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Conductance</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>ET (mm)</td>
<td>378</td>
<td>402</td>
</tr>
<tr>
<td>Biomass (t ha⁻¹)</td>
<td>13.1</td>
<td>14.3</td>
</tr>
<tr>
<td>Grain yield (t ha⁻¹)</td>
<td>5.5</td>
<td>5.9</td>
</tr>
<tr>
<td>Harvest index</td>
<td>0.42</td>
<td>0.41</td>
</tr>
<tr>
<td>T/ET</td>
<td>0.57</td>
<td>0.68</td>
</tr>
<tr>
<td>T (mm)</td>
<td>215</td>
<td>273</td>
</tr>
<tr>
<td>$W_{ET}$ (kg ha⁻¹ mm⁻¹)</td>
<td>34.6</td>
<td>35.5</td>
</tr>
<tr>
<td>$W_t$ (kg ha⁻¹ mm⁻¹)</td>
<td>50.9</td>
<td>52.3</td>
</tr>
<tr>
<td>Grain yield / T (kg ha⁻¹ mm⁻¹)</td>
<td>25.6</td>
<td>21.6</td>
</tr>
</tbody>
</table>

*Source: Adapted from Condon et al., 2002.*
(Condobolin in 1990), crop T was much more similar for the two genotypes and a difference in crop \( W_{ET} \) largely reflected the genotypic difference in crop \( W_T \). Because \( T \) was similar, the genotype with high crop \( W_T \) and low \( \Delta^{13}C \) had greater biomass production and higher yield.

\( \Delta^{13}C \) and Productivity in the Field

Inconsistency observed in the relationship between \( \Delta^{13}C \) and grain yield is the greatest challenge to using \( \Delta^{13}C \) as an indirect trait in breeding cereals for rainfed environments. The grain yield data in Table 6.3 are a good example of this inconsistency. At the wetter of the two sites (Wagga Wagga 1989), the yield of the low-\( \Delta^{13}C \) genotype Quarrion was about 8 percent less than that of the high-\( \Delta^{13}C \) genotype Matong. At the drier location the situation was reversed.

This inconsistency in the relationship between \( \Delta^{13}C \) and grain yield has been well documented in numerous studies involving bread wheat, durum wheat, and barley genotypes grown in rainfed and irrigated environments in Australia (Condon et al., 1987; Condon and Richards, 1993; Condon et al., 1993; Condon and Hall, 1997; Condon et al., 2002), the Mediterranean region (Voltas et al., 1999; Merah et al., 2001; Royo et al., 2002; Araus et al., 2003), and in other environments (Sayre et al., 1995; Fischer et al., 1998). Despite the negative association between \( A/T \) and \( \Delta^{13}C \), relationships between grain yield and \( \Delta^{13}C \) have only infrequently been found to be negative. Much more often these relationships between grain yield and \( \Delta^{13}C \) have been either positive or neutral.

Many of the studies on associations between yield and \( \Delta^{13}C \) in cereals have used diverse sets of genotypes in which substantial variation has existed not only in \( \Delta^{13}C \) but also in known potentially confounding factors such as flowering time and height. In many studies involving diverse cereal germplasm, low \( \Delta^{13}C \) has been associated with later flowering, which itself is frequently associated with lower yield in dry environments and also with low harvest index. This suggests that a better assessment of the utility of selection for low \( \Delta^{13}C \) might be achieved by working within breeding populations from parents contrasting for \( \Delta^{13}C \). Yet even this has resulted in inconsistent results. Associations between productivity and \( \Delta^{13}C \) were still highly variable in studies in eastern Australia on two sets of breeding lines from biparental crosses for which flowering date and height were tightly constrained (Condon and Hall, 1997). In contrast, a more recent study with BC\(_2\) breeding lines (Rebetzke, Condon, et al., 2002) found low \( \Delta^{13}C \) to be consistently associated with higher yield in dry environments, with no effect of \( \Delta^{13}C \) in the highest yielding environments. The results from this latter study indicate that it may indeed be possible to achieve consistent yield gains in dry environments by selecting for high \( A/T \) using \( \Delta^{13}C \), but the earlier results with biparental breeding lines indicate that much still needs to be learned about how selection for low \( \Delta^{13}C \) affects growth and water use to influence yield.

**Growth Rate of Low-\( \Delta^{13}C \) Cereals versus High-\( \Delta^{13}C \) Cereals**

Several reasons may explain why relationships between grain yield and \( \Delta^{13}C \) have been so variable, but a critical one is that low-\( \Delta^{13}C \) cereal genotypes may grow more slowly than high-\( \Delta^{13}C \) genotypes. Slower growth would certainly be the expectation if low \( \Delta^{13}C \) was the result of lower stomatal conductance. If low-\( \Delta^{13}C \) genotypes do grow more slowly it is likely to have important effects on crop \( T \) and the ratio \( T/ET \), as discussed in the previous section. Another important implication, if slower growth is associated with low-\( \Delta^{13}C \) in cereals, is that yield potential is likely to be constrained in circumstances in which water supply does not impose a strong limitation on crop growth. High-\( \Delta^{13}C \) genotypes have consistently shown higher final biomass production and grain yield in studies in which supplemental irrigation or regular rainfall events throughout the growing season maintained a high soil water status (Condon et al., 1987; Sayre et al., 1995; Condon and Hall, 1997; Fischer et al., 1998; Condon et al., 2002). Low-\( \Delta^{13}C \) genotypes achieved less biomass and lower yields. It is also likely that they left more water behind in the soil profile at maturity (Condon et al., 2002; Table 6.3).

In less favorable environments the amount of rainfall is an important factor contributing to variation in grain yield. The timing of development of soil-water deficit, with respect to the critical flowering phase, is also important because of the influence of water deficit on grain number (Fischer, 1981; Passioura, 2004). Low-\( \Delta^{13}C \) cereal genotypes may tend to use soil water more slowly because of higher \( A/T \), and this may favor the retention of a larger proportion of potential grain number if a marked soil-water deficit exists at flowering. Some rainfed environments exist in which crop growth is strongly reliant on moisture stored in the soil profile from rainfall events, before sowing. In such environments, water transpired by the crop is not replaced by frequent rainfall events, and soil evaporation is a relatively small component of total crop-water use. In these environments, and in drier current-rainfall environments, associations between yield and \( \Delta^{13}C \) have tended to be negative or neutral rather than positive. This is probably because the faster water use by high-\( \Delta^{13}C \) genotypes resulted in excessive depletion of the soil water store at anthesis. In these environments, the
slower water use and perhaps more conservative growth of low-$\Delta^{13}$C genotypes may be advantageous in sustaining higher grain number and grain yield. Relationships between yield and $\Delta^{13}$C may be particularly variable in Mediterranean-type environments if low $\Delta^{13}$C is associated with slow early growth. This is because in rainfed Mediterranean-type environments any difference in transpiration between high-$\Delta^{13}$C and low-$\Delta^{13}$C genotypes may not be reflected in a similar difference in total crop-water use to anthesis if soil evaporation is greater from under the slower-growing canopies of low-$\Delta^{13}$C genotypes (Condon et al., 1993; Condon et al., 2002).

**Why Would Low-$\Delta^{13}$C Cereals Grow More Slowly?**

As stated in the previous section, an obvious reason why low $\Delta^{13}$C could be associated with relatively slow crop-growth rate in cereals is if low $\Delta^{13}$C in the absence of soil-water deficit is the result of low stomatal conductance. Genotypes with lower stomatal conductance will tend to have higher $A/T$ and lower $\Delta^{13}$C, all else being equal, but higher $A/T$ is likely to be associated with lower photosynthetic rate per unit leaf area and consequently a slower rate of crop growth.

Low conductance may not be the only reason why low $\Delta^{13}$C is associated with a slow crop-growth rate. Variation in $\Delta^{13}$C in cereals can also result from variation in photosynthetic capacity (Condon et al., 1990; Morgan and LeCain, 1991). If low $\Delta^{13}$C is the result of low photosynthetic capacity, the expectation might be a higher rate of photosynthesis per unit leaf area and thus faster crop-growth rate. However, crop growth rate may actually be slower because, in cereals, substantial increases in photosynthetic capacity are most readily achieved by concentrating N into smaller leaves that have greater mass per unit area and that intercept less light per unit N. This may slow the rate of crop growth until full light interception is achieved. If full light interception is achieved only briefly, as often occurs in drier cropping environments, or not achieved at all, then high photosynthetic capacity may not result in greater growth. In fact, the reverse may occur: cereal genotypes with low photosynthetic capacity may actually achieve faster crop growth. The eight genotypes shown in the study depicted in Figure 6.5 (Condon et al., 1993) provide an example. Biomass production to anthesis was positively correlated with $\Delta^{13}$C (Figure 6.5a), but the three genotypes with the highest $\Delta^{13}$C values did not have greater stomatal conductance (Figure 6.5b). Instead, the inference, given they had higher values of $\Delta^{13}$C (ca. 1 per mil higher) at similar values of conductance, was that these three genotypes had lower photosynthetic capacity. This inference was confirmed in studies on glasshouse-grown plants (Condon et al., 1990).

**Application of Carbon Isotope Discrimination in Cereal Improvement**

**FIGURE 6.5.** (a) The relationship between aboveground biomass production to anthesis and leaf $\Delta^{13}$C, and (b) the relationship between leaf $\Delta^{13}$C and stomatal conductance, for eight wheat genotypes grown at Moombaoolool, SE Australia in 1985. Values of $\Delta^{13}$C are average values for leaves from well-watered plants sampled well before anthesis. Values of stomatal conductance are the average of data obtained at five sampling times before anthesis. Least-squares linear regressions are shown for statistically-significant relationships ($P < 0.05$). Open symbols indicate genotypes with low photosynthetic capacity. Sources: Adapted from Condon et al., 1990; Condon et al., 1993.

The eight genotypes grown in this study were very diverse, and other factors may have been associated with low photosynthetic capacity and high $\Delta^{13}$C that were also associated with higher anthesis biomass. For example, just as substantial genotypic variation in dry matter and N partitioning appears to have existed within the leaves, affecting photosynthetic capacity, genotypic variation in factors influencing within-plant partitioning may have also existed that could also have influenced crop growth rate. Detailed studies on much more closely related genotypes have been initiated to resolve associations between factors contributing to variation in $\Delta^{13}$C and factors contributing to variation in crop growth rate, factors influencing biomass partitioning at leaf and plant levels, and the extent to which such associations are due to pleiotropic effects or genetic linkage.

**CASE STUDY: APPLICATION OF CARBON ISOTOPE ANALYSIS IN WHEAT BREEDING FOR AUSTRALIA**

**Wheat Production in Australia**

Wheat production in Australia is a major component of a highly mechanized, commodity-driven agricultural system. The vast bulk of the wheat
crop is produced without supplemental irrigation in environments with relatively low rainfall, so average yield per hectare is also relatively low. For the five years to 2001-2002, for example, Australian wheat production averaged 22.5 million tons at 1.9 t·ha⁻¹. Drought of varying intensity or duration occurs in many parts of the production zone in most years. In 2002 and 2003, severe and widespread drought reduced national production to 9.4 million tons at only 0.9 t·ha⁻¹.

For Australian growers, higher yields with minimal added input costs are key objectives to obtain in order to remain commercially viable. The best way to achieve this is through higher-yielding varieties. Yield progress from empirical breeding for drier areas in Australia has been slowing by high year-to-year variation in rainfall receipts and marked regional differences in seasonal rainfall distribution. Targeting specific physiological traits that should contribute to improved yield may be one means of making greater yield gains (Richards et al., 2002). Traits that contribute to improved crop Wp, such as high A/T, may have a place, but what are the prospects of yield gains from targeting higher A/T given the inconsistencies in relationships between Δ¹³C and grain yield found in numerous studies with cereals, and what is the expense of screening for high A/T using Δ¹³C analysis?

Using Simulation Modeling to Evaluate Breeding for Higher A/T

One way to test the likely outcome of breeding for improved A/T could be through the use of a crop-simulation model. This was done for three representative low-rainfall wheat production environments in eastern Australia by using the SIMTAG wheat crop growth model (Stapper and Harris, 1989). The three environments for which wheat growth has been simulated have similar average annual rainfall (450-600 mm) but vary in seasonal rainfall distribution, from summer-dominant in the subtropical north (southeast Queensland), to winter-dominant in the south (northwest Victoria). The third region (central New South Wales) lies between these two extremes. It has a uniform average rainfall distribution, but rainfall is highly variable in amount and distribution from year to year. Apart from water supply, another key but variable constraint to wheat production in all regions is late frost at or near flowering, which can have a devastating effect on yield. So, within a region, commercial varieties are tuned to flower in a narrow window that meets the balance between avoiding the risk from frost and minimizing the risk from drought and heat as the crop matures into the summer. In each environment spring wheat is typically sown at the end of autumn and grows slowly through the relatively mild winter. Crop growth accelerates as temperatures begin to warm in the spring and the crop is harvested late spring to early summer, depending on the latitude and seasonal rainfall.

For each of the three environments, the SIMTAG model was run to “grow” a standard wheat variety using several decades of rainfall records. The model was then run again with parameters altered to simulate the effects of a 25 percent increase in A/T (equivalent to a reduction in Δ¹³C of ca. 1.5 per mil). Changes to the model parameters also included a 10 percent reduction in crop-growth rate in the absence of soil-water deficit with increased A/T. This growth reduction appears relatively strong and perhaps reflects a worst-case scenario, but such a growth reduction may be likely if the higher A/T and lower Δ¹³C are the result of much lower stomatal conductance. Furthermore, although the 10 percent reduction in crop growth rate appears relatively strong, this scale of reduction was needed to obtain model outputs consistent with observations on biomass production and yield made in field studies using cultivars divergent for Δ¹³C (Condon et al., 1993; Condon et al., 2002) and sets of breeding lines varying for Δ¹³C but with very similar flowering time and plant height (Condon and Hall, 1997).

The long-term effect of greater A/T combined with slowed crop-growth rate was determined by calculating the average long-term change in yield of the high A/T genotype compared with the standard genotype for each of the three environments. The simulations with SIMTAG (Condon et al., 2002; Condon et al., 2004) predicted that the effect on long-term average yield of breeding for a 25 percent improvement in A/T would have been close to zero in the winter-dominant rainfall environment and in the central region with a highly variable rainfall regime (Figure 6.6). In contrast, the simulations predicted an average 11 percent yield gain in the northern environment with summer-dominant rainfall. These outcomes indicated the northern region as a strong target environment for breeding for greater A/T. In this region, any potential yield penalty in favorable seasons resulting from the slower early growth that may be associated with high A/T was exceeded, on average, by the benefits of conservative growth and water use in drier seasons.

It is clear that the outcome of breeding for higher A/T may depend on how strongly or even if high A/T is associated with slower crop-growth rate in the absence of stress. To test the impact of this association, simulations were also run for the same 25 percent increase in A/T without any reduction in crop growth rate in the absence of water stress. Under this scenario, model outputs were very similar to those shown on the right-hand side of Figure 6.6, with uniformly large average yield gains in all regions. Recent field studies indicate that this scenario appears to have been achieved with one particular set of bread wheat backcross progeny (Condon et al., 2002;
Rebetzke, Condon, et al., 2002). The results for this particular population will be discussed in more detail in following sections.

**Simulating the Effects of Breeding for Greater Early Vigor**

A large proportion of wheat in Australia is grown in regions with winter-dominant rainfall where breeding simply for higher A/T seems unlikely to provide a yield benefit, particularly if high A/T is associated with slow crop-growth rate. For these regions, fast early growth is viewed as a key trait to improving crop WET. This is because of the benefits of restricting soil evaporation to improve T/ET and promoting growth early in the season, when evaporative demand is low (Richards et al., 2002). It was possible to alter parameters in the SIMTAG model to test the likely effects of breeding for high early vigor. By using the model it was also possible to ask what would be the likely effect on yield of combining selection for greater early vigor with selection for higher A/T, assuming such a combination could be achieved in practice. To simulate the effects of breeding for increased vigor with no change in A/T, the model was configured by doubling the size of the first leaf, which is in fact very similar to the goal of the breeding effort for greater early vigor described in Richards et al. (2002). As noted earlier, high A/T may be associated with relatively slow early growth, so it was considered reasonable to anticipate some penalty on crop-growth rate associated with combining higher A/T and early vigor. To achieve this, first-leaf size was only increased by 85 percent when simulations were run combining the two traits.

Simulating only an increase in early vigor predicted an average 11 percent yield gain in the environment with winter-dominant rainfall (Figure 6.6), confirming conclusions from many field studies (Richards et al., 2002). The prediction for the summer-dominant rainfall environment was that essentially no effect on average yield would be seen. In the third environment simulated, in which rainfall timing and amount is highly variable, the yield gain from more vigorous early growth was predicted to be about 3 percent on average—higher in years with consistent rainfall, and lower when rainfall petered out early. Interestingly, the simulations predicted that if it was possible to combine the two traits of greater early vigor and high A/T (in effect, a best-case scenario for high A/T), then a synergistic effect would occur, the combination of traits generating considerably higher average yields (14 to 17 percent) in all three environments simulated.

The results of the simulations with the SIMTAG model indicated, first, that it should be possible to confidently target specific traits for specific environments—high A/T for summer-dominant rainfall environments in which crops are reliant on moisture stored in the profile, and greater early vigor for winter-dominant rainfall environments in which it is important to maximize T/ET. Second, they indicated that because of year-to-year variation in rainfall totals and rainfall distribution, pyramiding these traits to obtain a best-case scenario for A/T may provide the best outcome in all environments.

**A Backcrossing Program to Improve A/T of Australian Wheats**

Based on the results of previous field studies of crop growth and water use in stored-moisture environments and the outcomes of the simulation study using SIMTAG, a backcross breeding program was initiated to improve A/T of wheats for Australia’s northeastern wheat-growing region. In this region the wheat crop relies on moisture stored from summer rains, and ET makes up a large proportion of total crop ET. The variety Hartog was chosen as the recurrent parent because it was grown extensively in Australia’s northeast and had a relatively high $\Delta^{13}$C. The variety Quarrion was used as
the donor of the low-$\Delta^{13}$C trait. Quarriion has some vernalization requirement and so has a longer season than most Australian spring wheats. Quarriion also carries the Sr26 stem-rust resistance translocation from *Thiophrum elongatum* L. In the absence of disease, this translocation has been associated with an average yield penalty in Australia of about 9 percent (The et al., 1988). However, Quarriion has the lowest $\Delta^{13}$C value of any Australian wheat surveyed. The range in $\Delta^{13}$C of other Australian wheats extends up to 2.5 per mil greater than Quarriion (Condon et al., 1990; Condon et al., 1992). Further details of the breeding and selection process are given in the next section.

Divergent selection based on $\Delta^{13}$C was made among progeny at the BC$_2$ stage. To test the effect of this selection on yield, 30 random low-$\Delta^{13}$C (high $A/T$) and 30 random high-$\Delta^{13}$C (low $A/T$) BC$_2$ lines, with similar phenology and height, were grown from 1995 to 1998 in environments in eastern Australia that varied in the extent to which crops relied on stored soil moisture, and in Western Australia, where the lines grew almost entirely on within-season rainfall. The average yield of the low-$\Delta^{13}$C selections and high-$\Delta^{13}$C selections was compared for each environment (Figure 6.7a). The average yield of the low-$\Delta^{13}$C selections was greater than that of the high-$\Delta^{13}$C selections in all 13 environments in which the comparison was made. The relative yield advantage of the low-$\Delta^{13}$C selections was greatest (approximately 10 percent) in low-yielding, 1 to 3 t ha$^{-1}$ environments in eastern Australia. The relative yield advantage was small at yield levels of 5 to 7 t ha$^{-1}$ in eastern Australia and at yields of 2 to 3 t ha$^{-1}$ in Mediterranean Western Australia (Condon et al., 2002; Rebetzke, Condon, et al., 2002).

Selecting for high $A/T$ in this population carried no yield penalty at sites with higher yield levels of approximately 6 t ha$^{-1}$ in eastern Australia, nor in Mediterranean-type environments in Western Australia. These results appear at odds with the results of many of the studies on diverse sets of germplasm reviewed earlier. The population of BC$_2$ lines grown in this study shares a much more uniform genetic background than the diverse sets of germplasm used in several earlier studies. In addition, the BC$_2$ lines were constrained to be of uniform flowering time and height, to minimize potential extraneous effects on yield of these two traits. Importantly, more recent studies with the selected lines indicate that in the progeny from this cross, low $\Delta^{13}$C is not associated with slower vegetative growth. The reason for this is not clear and is the subject of ongoing research. The average difference in leaf $\Delta^{13}$C between the two sets of backcross lines was ca. 0.8 per mil. This is not a particularly large difference but, in theory, it is still large enough to generate 15 percent greater $A/T$ in the low-$\Delta^{13}$C selections. It is feasible that, among lines from this population, lower values of $\Delta^{13}$C may have arisen from two components: one component resulting from a relatively small reduction in stomatal conductance that had very little effect on $A$ and crop-growth rate but caused some increase in $A/T$, and a second component resulting from a relatively small increase in photosynthetic capacity that countered any potential effect on crop-growth rate of the small reduction in stomatal conductance but that also caused an increase in $A/T$. The outcome of such a scenario could be a reasonable net gain in $A/T$ from both components that would be beneficial in dry environments but that would have very little, if any, effect on crop growth rate in the absence of soil water deficit, and thus no yield penalty at higher yield levels.
Low-$\Delta^{13}$C breeding lines with high yield, good quality, and disease resistance were selected from the population and submitted for more comprehensive evaluation. Two of the lines have been released as commercial cultivars in eastern Australia: Drysdale (in October 2002) and Rees (in October 2003). The yield in southeast Australia in the dry 2003 season of Drysdale, relative to that of a current commercial cultivar recommended for the same region, is shown in Figure 6.7b. The yield advantage of Drysdale was clear at the lower range of yields typical for this region.

Some Practicalities of Breeding for Low $\Delta^{13}$C in Wheat

The high-$A/T$ trait is being backcrossed into several more, widely grown Australian bread wheat and durum wheat backgrounds using $\Delta^{13}$C as a measure of relative differences in $A/T$. Our breeding protocol currently starts by crossing Quarriion, as the female parent and donor of high $A/T$, with a target recurrent parent. We use Quarriion as the female parent because some evidence suggests that it has a small maternal influence on $\Delta$, perhaps acting through the chloroplast genome (Rebetzke, Richards, et al., 2002). To screen the progeny for variation in $\Delta^{13}$C we sow $F_{2:3}$ (or later generation) families in replicated short rows. These are sown at the standard time for our environment. Plant material grown with minimal water stress is sampled at full tillering, before stem elongation, by cutting along the row at about 5 cm above the soil surface. The plant tops (mainly leaf material with minimal sheath or stem) are oven dried, ground, and then analyzed for C-isotope composition. Because they are cut above the apex, the plants are able to recover, and plant height, flowering time, and disease reactions can be assessed. If $\Delta^{13}$C analysis is completed before flowering then it is possible to identify those families with the lowest $\Delta^{13}$C values. These may be used as females in back- or topcrossing. Two backcrosses with the recurrent parent are often made, with variation for $\Delta^{13}$C being assessed after the second backcross, using the same process just described. A low-$\Delta^{13}$C subset of families is then advanced into the preliminary stages of field testing to identify those lines with the most suitable agronomic type. If required, single-head selections are taken from low-$\Delta^{13}$C families to accelerate progress toward homozygosity.

Why Backcrossing?

The wheat breeding programs in Australia work very hard at developing wheats that yield well, have the appropriate maturity, satisfy the standards for a host of quality attributes, and have high levels of protection against a broad spectrum of biotic stresses. It is tedious work assembling all these genes. We use backcrossing to improve the $A/T$ of Australian wheats so as to also take best advantage of the other genes already assembled. These may include gene complexes that contribute to yield in the target environment independently of $A/T$. Yield is not just a process of acquiring carbon (or, for that matter, exchanging carbon for water). It is also a process of allocating carbon (and other nutrients) in a way that maximizes grain number and the capacity to fill those grains. Conventional, empirical selection for yield using multilocation testing identifies those genotypes in which the coadaptative gene complexes available to maximize yield have been assembled most effectively. Backcrossing favors the combining of genes for high $A/T$ with the other gene complexes that contribute to yield.

Backcrossing is easiest with a highly heritable, simple trait (Simmonds, 1979). In the case of $\Delta^{13}$C we are fortunate. Although $\Delta^{13}$C is undoubtedly a polygenic trait, it has a very high heritability, at least in our hands (Condon and Richards, 1992; Rebetzke, Condon, et al., 2002), so we are able to retrieve most of the genes that contribute to low $\Delta^{13}$C relatively easily but not completely. Only rarely have we been able to identify progeny with $\Delta^{13}$C values as low as the donor parent Quarriion. On the other hand, transgressive segregation for $\Delta^{13}$C values higher than the recurrent, high-$\Delta^{13}$C parent is routine (Rebetzke, Condon, et al., 2002).

The backcrossing approach we have taken with bread wheat in Australia is the best approach for us given the nature of the production system, rain-fall environments, and prevailing breeding practice. This is not to say that other approaches are not valid; it will depend on the nature of the target environment and the maturity of the breeding program.

Why Measure $\Delta^{13}$C on Leaves Growing under Nonstressed Conditions?

Given that the objective of our backcrossing program is to increase grain yield in water-limited environments, it may appear paradoxical that we screen for variation in $\Delta^{13}$C using leaf material sampled early in crop growth from well-watered plants. Why not screen for $\Delta^{13}$C during post-anthesis stress, using the grain, for instance? There are several reasons.

First, from a practical point of view, we have found that $\Delta^{13}$C measured early in the season provides the highest repeatability and heritability (Condon and Richards, 1992; Rebetzke, Condon, et al., 2002). Nearer anthesis, substantial genotypic differences in the extent of soil-water depletion may develop and therefore substantial differences in stress-induced stomatal
growth and 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. In these environments the slower crop-growth rate in the absence of soil-water stress often associated with low Δ13C may not limit grain yield. Some dryland winter or spring cropping environments exist in temperate North America and Asia and in subtropical regions of south Asia, South America, and Africa that fall into this category.

**High Grain Δ13C and Higher Yields in Mediterranean-Type Environments**

In Mediterranean-type environments, higher yields of wheat and barley have often been associated with high Δ13C in all but the driest locations (Araus et al., 1998; Merah et al., 1999; Voltas et al., 1999). In most of these studies the Δ13C of grain rather than of leaves has been measured. When the low-Δ13C and high-Δ13C backcross lines were compared in Western Australia (Figure 6.7a), the low-Δ13C lines had a small average yield advantage at all five sites. This result differs from what is often observed in Mediterranean-type environments. This could reflect that selection for Δ13C was done using early-formed leaf material rather than grain, or it could reflect the relatively low average yield levels in the environments encountered (1.8 to 2.6 t·ha⁻¹).

Because of high year-to-year variation in rainfall, a trait that is “neutral” in dry years but “positive” in wetter years may still be useful since it should be associated with higher long-term average yield. This may be the case for grain Δ13C in many Mediterranean-type environments. Associations between high grain Δ13C and high yield in Mediterranean environments could be due to a combination of several factors. High grain Δ13C values could to some extent mirror high values of Δ13C at early stages of growth. If this is the case, then high grain Δ13C and high yield may reflect faster growth rate up to anthesis, leading to higher grain number and higher yield (Condon et al., 1993). Alternatively, or in addition, high grain Δ13C values could reflect greater reliance for grain filling on stem reserves laid down when plants were less stressed and Δ13C values were higher. Genotypes that have used more of the soil water store at anthesis may be more reliant on reserves laid down before anthesis to fill the grain (Condon and Hall, 1997; Condon et al., 2002). So, grain Δ13C may be a poor reflection of variation in Δ13C during grain filling, but a good indicator of reliance on or ability to remobilize stored reserves. High grain Δ13C could also reflect greater access to soil moisture during grain filling because of more extensive rooting (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). Any of these characteristics associated with high grain $\Delta^{13}$C (fast crop growth rate, ability to remobilize stored reserves, earlier flowering, better water extraction, stomatal insensitivity to water deficit) would be useful for cereals in Mediterranean-type environments.

**Carbon Isotope Discrimination in C\textsubscript{4} Cereals**

Can variation in $\Delta^{13}$C to breed for improved performance of C\textsubscript{4} species in water-limited agriculture be exploited? This may be possible, but both theory and available data indicate that progress with C\textsubscript{4} species is likely to be even less straightforward than with C\textsubscript{3} species.

Some variation in $\Delta^{13}$C has been observed within C\textsubscript{4} species (e.g., Hubick et al., 1990; Mortlock and Hammer, 1999), but the extent of this variation in C\textsubscript{4} species is much less than in C\textsubscript{3} species. In addition, the relationship between transpiration efficiency and $\Delta^{13}$C is different in C\textsubscript{4} species. This is because of the substantial differences between C\textsubscript{3} and C\textsubscript{4} species in the processes of C\textsubscript{}-assimilation. In C\textsubscript{4} species C is fixed into 4-carbon compounds (malate or aspartate) in mesophyll cells by the enzyme PEP carboxylase. With respect to gaseous CO\textsubscript{2}, this enzyme actually discriminates a little in favor of $^{13}$C, in contrast to the C\textsubscript{3} enzyme Rubisco, which has a relatively large discrimination against $^{13}$CO\textsubscript{2}. After the initial carboxylation step in C\textsubscript{4} photosynthesis, the 4-carbon compounds are then transported to the bundle-sheath cells surrounding the vascular bundles. Here, after a decarboxylation step, CO\textsubscript{2} enters the C\textsubscript{3} pathway and is fixed by Rubisco. The bundle sheath is very close to being gas-tight, and this has two implications: The first is that it allows the CO\textsubscript{2} concentration to be held much higher than in the outside air, so carboxylation by Rubisco is much more efficient than in C\textsubscript{3} plants. Second, little opportunity exists for CO\textsubscript{2} to escape from the bundle sheath, and so little opportunity exists for any discrimination against $^{13}$CO\textsubscript{2} by Rubisco to be expressed. Discrimination by Rubisco can only be expressed if CO\textsubscript{2} can escape to the atmosphere. In reality the bundle sheath is not absolutely gas-tight, so some discrimination by Rubisco occurs.

These complexities of discrimination during C\textsubscript{4} photosynthesis can be expressed in a simple mathematical description relating $\Delta^{13}$C to $c_{i}/c_{o}$, as follows (Farquhar, 1983; Henderson et al., 1992; Brugnoli and Farquhar, 2000):

$$\Delta^{13}C = a + (b^* - a) \cdot c_{i}/c_{o}$$  (6.8)

In this equation, $a$ is the fractionation during diffusion through the stomata (4.4 per mil) and $b^* = [b_3 + \Phi \cdot (b_3 - s)]$, where $b_3$ is the discrimination by PEP carboxylase (5.7 per mil), $b_3$ is the discrimination by Rubisco (30 per mil), $s$ is discrimination during leakage from the bundle sheath (1.8 per mil) and the factor $\Phi$ accounts for the proportion of CO\textsubscript{2} released by decarboxylation that leaks out of the bundle sheath (Brugnoli and Farquhar, 2000). It should be noted that the value of $\Phi$ is not constant. It can also be noted that Equation 6.8 has the same form as Equation 6.1, which described discrimination during C\textsubscript{3} photosynthesis, with the greater complexities of C\textsubscript{4} photosynthesis captured in the term $b^*$ in Equation 6.8, analogous to the term $b$ in Equation 6.1.

Compared to C\textsubscript{3} species, for C\textsubscript{4} species relatively little variation in $\Delta^{13}$C associated with the primary processes of C\textsubscript{}-assimilation exists. This is because the value of the term $(b^* - a)$ is close to zero, whereas the term $(b - a)$, from Equation 6.1, has a value of ca. 24 per mil. In fact, for C\textsubscript{4} photosynthesis, the dependence of $\Delta^{13}$C on $c_{i}/c_{o}$ may be positive or negative depending on whether the value of $b^*$ is greater or less than then value of $a$, and this will depend on the amount of leakage from the bundle sheath. (Using the parameter values cited, $b^* = a$ at $\Phi = 0.36$, and no dependence of $\Delta^{13}$C on $c_{i}/c_{o}$ would exist.) In practice, the value of $\Phi$ has been usually found to be a little less than 0.3. Consequently, the relationship between $\Delta^{13}$C and $c_{i}/c_{o}$ for C\textsubscript{4} species is usually negative, but with a small slope (Figure 6.1). For C\textsubscript{3} plants the relationship between $\Delta^{13}$C and $c_{i}/c_{o}$ is positive, with a large slope.

Discrimination during C\textsubscript{4} photosynthesis may therefore vary with variation in $c_{i}/c_{o}$ or with variation in $\Phi$ (or both $c_{i}/c_{o}$ and $\Phi$). In practice, it is difficult to distinguish between these two influences, and both of them will be subject to genetic and environmental effects. The value of $c_{i}/c_{o}$ would be expected to respond to changes in stomatal conductance, which is under genetic and environmental influences as found for C\textsubscript{3} species. The value of $\Phi$ is subject to several poorly understood influences. Genetic variation in $\Phi$ has been indicated from the results of comparisons of C\textsubscript{4} species that differ in aspects of their biochemistry and anatomy (Ehleringer and Pearcy, 1983). In some C\textsubscript{4} species, the value of $\Phi$ has been observed to change in response to drought and salinity stresses. These changes have been attributed to changes in the activity of Rubisco relative to PEP carboxylase (Bowman et al., 1989; Saliendra et al., 1996). Hubick et al. (1990) attributed variation in $\Delta^{13}$C among sorghum (Sorghum bicolor L.) genotypes to variation in either or both $c_{i}/c_{o}$ and $\Phi$, whereas Henderson et al. (1992) found $\Phi$ to be relatively constant at 0.2 and variation in $\Delta^{13}$C to be related to variation in $c_{i}/c_{o}$.

Farquhar (1983) concluded that radiation-use efficiency may be higher if $\Phi$ was low, and that this may be reflected in productivity. Likewise, $A/T$
should be greater at lower values of $e_t/c_{p}$, (usually higher $\Delta^{13}C$ for $C_4$ species) and this may also be reflected in productivity (with the same caveats as for $C_4$ species with respect to conductance). Hammer et al. (1997) observed significant variation in plant transpiration efficiency ($W_p$) ranging from 6.0 to 7.7 g DM·kg$^{-1}$·H$_2$O among 45 diverse cultivars of sorghum grown in pots, but no relationship existed between $\Delta^{13}C$ and $W_p$. Subsequently, Henderson et al. (1998) observed significant but weak positive correlations between $\Delta^{13}C$ and $W_p$ for ca. 30 sorghum lines, whereas Mortlock and Hammer (1999) found $W_p$ to be negatively correlated with $\Delta^{13}C$ for a range of sorghum genotypes grown in pots under well-watered conditions. No relationship was observed for plants subjected to mild water stress, even though substantial variation in $W_p$ existed. Thus little evidence for variation in $W_p$ within genotypes of $C_4$ species can be found that can consistently be associated with variation in $\Delta^{13}C$.

A further complicating factor for $C_4$ species is that, in comparison with $C_3$ species, the opportunity exists for a much greater proportion of variation in $\Delta^{13}C$ measured in plant dry matter to be associated with downstream, postphotosynthetic fractions that favor retention of either $^{12}C$ or $^{13}C$. Because of the relatively small amount of variation in $\Delta^{13}C$ associated with $C_4$ photosynthesis, it is conceivable that genotypic variation in chemical composition (e.g., variation in lipid or starch content; see Figure 6.2) could be reflected in variation in $\Delta^{13}C$ that is at least as great as that associated with $e_t/c_{p}$ or $\Phi$. It is also possible that such variation in chemical composition may itself be associated in some way with variation in productivity under certain circumstances. In summary, it seems that the numbers of uncertainties associated with interpreting the values of $\Delta^{13}C$ measured in plant dry matter of $C_4$ plants will make it difficult to reliably exploit genetic variation in $\Delta^{13}C$ to improve performance of $C_4$ species in water-limited agriculture.

FUTURE DIRECTIONS

Pyramiding High A/T with Greater Early Vigor

Progress in applying $\Delta^{13}C$ in breeding for improved yield of $C_4$ cereals in water-limited environments has been slowed by marked inconsistency in the relationship between $\Delta^{13}C$ and yield. This inconsistency has been most obvious in studies with widely divergent accessions, and may be the result of a tendency for low $\Delta^{13}C$ (high A/T) to be associated with slower crop-growth rate. This tendency may restrict the yield of low-$\Delta^{13}C$ genotypes in favorable sites or seasons. It may also be part of the reason for positive associations between grain $\Delta^{13}C$ and yield observed in rainfed Mediterranean-type environments, yet even in these latter environments the associations of yield with high $\Delta^{13}C$ are not so consistent that selection for high grain $\Delta^{13}C$ is unequivocally advocated (Araus et al., 2002; Royo et al., 2002).

Overcoming any possible association between low $\Delta^{13}C$ and slow crop-growth rate would allow the association between low $\Delta^{13}C$ and high A/T to be exploited more universally for yield improvement. But is this possible, given that the leaf-gas-exchange processes that link low $\Delta^{13}C$ with high A/T in cereals may also link low $\Delta^{13}C$ with slow crop growth rate? One way forward may be to bypass the link between leaf-level gas-exchange and crop-growth rate by combining selection for low $\Delta^{13}C$ with selection for larger seedling leaves that intercept more light, thereby promoting faster crop growth. The simulation study using SIMTAG (Figure 6.6) indicated that combining greater early vigor and higher A/T could give substantially greater average yield gains in many rainfed environments. The advantage from combining the two traits was greatest in the environment with large temporal variability in rainfall, but average yields were also higher in the stored-moisture and current-rainfall environments.

Is such a combination of traits possible? Studies on breeding populations from crosses between low-$\Delta^{13}C$ and high-$\Delta^{13}C$ parents (Figure 6.8) confirmed phenotypic and genetic associations between faster early leaf area growth and higher $\Delta^{13}C$. However, the same studies also indicated that the associations were not so strong that it was not possible to identify several

![Figure 6.8](image.png)

**FIGURE 6.8.** Relationship between early plant leaf area and leaf carbon isotope discrimination among field-grown progeny from a three-way cross between a low-$\Delta^{13}C$ and two higher-$\Delta^{13}C$ parents. Progeny are indicated as open triangles. **Source:** Sirault et al., 1999.
progeny that combined low Δ^{13}C and fast leaf area growth (Sirault et al., 1999). Studies by Richards and colleagues (Liang and Richards, 1994; Lopez-Castaneda et al., 1995; Richards et al., 1997; Richards and Lukacs, 2002) have identified several key traits that can be manipulated to dramatically boost early leaf area growth of temperate cereals. The most critical trait appears to be embryo size. Other contributing traits include appearance of a large coleoptile tiller and higher specific leaf area (SLA). In addition, it is likely that to achieve a substantial increase in early leaf area in wheats with semidwarf stature. GA-sensitive dwarfing genes may need to be used (Richards et al., 1997; Richards et al., 2001; Ellis et al., 2004), since they should allow much better expression of traits contributing to early vigor.

Among the traits contributing to greater early vigor may be an association between high SLA and higher Δ^{13}C if high SLA is reflected in low photosynthetic capacity (via less N per unit leaf area) and no parallel reduction in stomatal conductance exists. However, it is incorrect to conclude that any of the other early-vigour traits should be mechanistically linked to higher Δ^{13}C. This suggests that if high vigor and low Δ^{13}C are to be successfully combined, selection for higher vigor should concentrate on selection for large embryo size and presence of a large coleoptile tiller. A tendency to higher SLA may need to be avoided. SLA has relatively low heritability in cereals (Rebetzke, Botwright, et al., 2004), so its value as a selection tool for high early vigor may be limited anyway. The associations between early vigor in cereals and traits other than high SLA, such as embryo size and coleoptile tiller appearance, indicate that ways to achieve higher early vigor that could allow coselection for lower values of Δ^{13}C may exist.

**Marker-Assisted Selection for Δ^{13}C and Component Traits**

Indirect selection of transpiration efficiency via Δ^{13}C using phenotypic screening is effective because Δ^{13}C is highly heritable and under strong additive genetic control. However, analyzing large numbers of breeding lines for variation in Δ^{13}C is relatively expensive and can be slow. Costs could be reduced by selection of molecular markers chromosomally linked to genes associated with transpiration efficiency, assuming marker-assisted selection is already being used in a breeding program. The additional cost of genotyping molecular markers for Δ^{13}C would be marginal if complementing selection for other traits, and if the parents were known to differ significantly for Δ^{13}C.

Among the earliest reported plant mapping studies, Martin et al. (1989) used restriction fragment length polymorphism (RFLPs) to identify three additive QTLs for Δ^{13}C in leaf tissue from an interspecific cross of cultivated tomato (*Lycopersicon esculentum* Mill.) and the drought-tolerant wild relative *L. pennelli* (Cor.). Since then, QTL have been reported for Δ^{13}C in a range of monocotyledonous and dicotyledonous species (e.g., Specht et al., 2001; Thumma et al., 2001; Brendel et al., 2002; Teulat et al., 2002). A number of these studies have been undertaken on plants subjected to soil-water deficit or salt-induced osmotic stress (e.g., Ellis et al., 1997), and it is difficult to determine whether the QTL are associated with constitutive variation in Δ^{13}C and component traits stomatal conductance and photosynthetic capacity, or whether the QTL are associated with other traits causing pleiotropic variation in Δ^{13}C.

Variation in Δ^{13}C may be caused by variation in stomatal conductance or photosynthetic capacity. QTL analysis might also be useful applied specifically for these traits since variation in conductance and capacity may have different impacts on crop growth and yield and it may be useful to be able to manipulate conductance and photosynthetic capacity independently in breeding populations. QTL associated with variation in stomatal conductance have been identified in species such as rice (Ishimaru et al., 2001), while QTL for photosynthetic capacity have been identified in maize (Jompuk et al., 2005) and sunflower (Herve et al., 2001).

As with the Δ^{13}C QTL studies just reported, these mapping studies are useful in genetic and physiological dissection of a trait but are less useful in selection until validated in other breeding populations. Furthermore, evidence in *Stylosanthes scabra* (Thumma et al., 2001) and wheat (Rebetzke et al., 2005) suggests that Δ^{13}C is under control of many QTL of small effect, thus selection for altered Δ^{13}C in a breeding program would require sampling of large numbers of progeny in populations in order to identify those few families that have accumulated all or most of the desired Δ^{13}C alleles. Small increases in the numbers of markers under selection substantially increase the population sizes required to confidently identify combinations of independent genes (Bonnet et al., 2005). Therefore, selection should be made for carriers (homozygous or heterozygous for the target markers) in early generations or delayed until after some inbreeding in which combinations of the target homozygotes are likely to occur at higher genotypic frequencies. Nonetheless, given that other important genetic markers are likely to be under selection (e.g., disease resistance and grain quality), it is probable that only one to two Δ^{13}C QTLs of largest genetic effect can be accommodated in a commercial breeding program.

**CONCLUSION**

Strong evidence now suggests that measurements of Δ^{13}C can be successfully applied in breeding higher-yielding wheats for water-limited envi-
Environment. Selection for high A/T using low Δ¹³C measured in the leaves of unstressed plants has resulted in the release of new, higher-yielding wheat varieties for Australia. The new varieties are the result of a backcrossing program targeting environments in which soil moisture needs to be metered out relatively early in the cropping season in order to maximize seed set and sustain seed growth.

The utility of selection for low Δ¹³C in cereals in other rainfed environments may be hampered by marked year-to-year and site-to-site inconsistency that has been observed in the relationship between grain yield and Δ¹³C. This inconsistency may partly reflect that the majority of field studies have been done using diverse genotypes that vary for many other factors apart from Δ¹³C. A clear need exists for studies using more closely related genotypes, such as breeding populations segregating for Δ¹³C, in which variation in other yield-influencing traits such as flowering time is tightly constrained so that the effects of selection based on Δ¹³C can be more readily assessed.

Inconsistency in the relationship between grain yield and Δ¹³C may also to some extent be the result of an association between high Δ¹³C and faster crop-growth rate. Higher yield is frequently associated with high Δ¹³C in well-watered environments, and even for many rainfed Mediterranean-type cropping environments higher yield appears to be often associated with high Δ¹³C in the grain, although this association may be reversed in drier Mediterranean-type environments. Detailed studies on closely related genotypes are needed to resolve associations between factors contributing to variation in Δ¹³C and factors contributing to variation in crop-growth rate, including factors influencing biomass partitioning at the leaf and whole-plant levels. A need to determine whether associations between factors influencing crop growth rate or dry matter partitioning and factors influencing Δ¹³C are due to pleiotropic effects or genetic linkage also exists.

Results of computer simulation studies indicate that higher and more consistent yield gains may be achievable in many drought-prone cereal-growing environments if fast crop-growth rate can be combined with high Δ¹³C, i.e., low Δ¹³C. This combination of traits may not be easy to achieve because of the possible tendency for low Δ¹³C to be associated with slower crop-growth rate in cereals. Such a tendency is easiest to explain if low Δ¹³C is the result of low stomatal conductance, but relatively slow crop-growth rate may also occur if low Δ¹³C is the result of high photosynthetic capacity. Effective selection protocols for fast crop-growth rate independent of leaf-gas-exchange characteristics, via greater early vigor, have been devised and shown to be successful for yield improvement in Mediterranean environments. These selection protocols may facilitate selection for greater early vigor concurrently with selection for lower Δ¹³C. Accordingly, breeding and selection has been initiated in Australia to combine fast crop-growth rate and low Δ¹³C in bread wheat and in durum wheat.

REFERENCES


DROUGHT ADAPTATION IN CEREALS


