

# CHAPTER 5

## Recent Tools for the Screening of Physiological Traits Determining Yield

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This review presents practical guidelines for breeders of wheat (and other small grain cereals) interested in adopting a physiological approach to crop improvement. Some of the most promising tools for fast, reliable characterization of yield-determining traits will be discussed from an ecophysiological perspective. We will focus on the practical aspects and limitations of using relevant screening tools or selection criteria. The potential contributions of physiological research to plant breeding, as well as its inherent limitations, have been extensively reviewed from a breeding perspective (for example, Jackson et al., 1996). A theoretical framework for identifying yield determinants that are obvious candidates for evaluation has also been established (see below), although it has not been used in practical breeding.

The use of physiological traits as screening tools in breeding is still largely experimental—for different reasons. Sometimes the traits are very indirectly related to yield (Araus, 1996; Richards, 1996) or there is little ecophysiological understanding of the crop, especially when breeding for yield under stress. Nevertheless, breeding for crop escape has been very successful, and phenological changes have been the most important indirect factor in increasing wheat yields under Mediterranean conditions (Slafer et al.,

1993; Loss and Siddique, 1994). However, in breeding for crop resistance, the evaluated traits and screening tools are often related to tolerance, not avoidance (see definitions in Larcher, 1995).

An indirect (i.e., physiology-based) breeding strategy could fail to produce yield gains and might even lead to a decrease in yield. Improved plant tolerance, though it protects the crop, can limit yield potential. The target environment where selection is carried out must be defined *a priori*, and the possibility of a negative breeding effort should not be ignored. In fact, plants that show the **most tolerant response during screening may also be the most sensitive**, in terms of yield loss, for example, because they are unable to delay the effect of stress at the cellular level.

The most promising methods allow for quick screening of “integrative” physiological traits (Araus, 1996), so called because they integrate physiological processes either in time (i.e., during the plant cycle) or at the organization level (e.g., whole plant, canopy). Other quick screening methods for evaluating, for example, the photosynthetic performance of plants under stress conditions have been proposed—among them, chlorophyll fluorescence measurements on single

leaves. However, under field conditions fluorescence values may only reflect differences in phenology across genotypes. Nevertheless, remote sensing detection of fluorescence spectra at the canopy level could become a promising approach for breeding purposes (Lichtenthaler, 1996).

### Identifying Physiological Traits for Use As Selection Criteria

One approach to search for traits that could be used in breeding programs is to identify the physiological processes determining productivity. A crop’s yield potential (or harvestable part, Y) over a given period of time can be divided into three major processes (Hay and Walker, 1989). First, the interception of incident solar radiation by the canopy; second, the conversion of intercepted radiant energy to potential chemical energy (i.e., plant dry matter); and third, the partitioning of dry matter between the harvested parts and the rest of the plant. Whereas the first component depends on the canopy’s total photosynthetic area, the second relies on the crop’s overall photosynthetic efficiency (i.e., total dry matter produced per unit of intercepted radiant energy); the third is harvest index. Total biomass, which is the result

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of the first two components, can be physiologically defined as the result of canopy photosynthesis over time.

Other approaches may be followed, depending on agroecological conditions. For example, under water-limiting (e.g., Mediterranean) conditions, the most widely used framework, proposed by Passioura (1977), allows the study of indices that maximize yield per rainfall unit. Thus economic yield depends on the total water transpired by the crop, water use efficiency, and harvest index. Although these three components are not truly independent, Passioura's is a useful framework for searching for critical traits to improve yield under drought.

Yield can be divided into several integrative components or traits. Yield itself is the most integrative trait, because it is influenced by all factors (known and unknown) that determine productivity. However, there are many known limitations in a purely empirical breeding approach based only on yield. Therefore, any breeding strategy based on a physiological (i.e., analytical) approach should use screening tools or criteria to evaluate the integrative physiological parameters that determine harvestable yield with a single measurement. Although harvest index has been the most successful trait when modified to improve yield, the other two components of the above equations, which are responsible for total crop biomass, remain (basically) unchanged. In the following pages we will focus on tools used to evaluate physiological traits determining total biomass. We will discuss two different kinds of tools for screening integrative physiological traits: carbon isotope discrimination ( $\Delta$ ) and indices based on canopy spectral-reflectance.

The  $\Delta$  not only evaluates genotypic differences in water use efficiency, but can also be affected by the total amount of water transpired by the crop (the first component of Passioura's equation) or by photosynthetic activity (the second component of the yield potential equation). Keeping in mind developing country breeders, we have included under the generic title "surrogates" other screening criteria, such as ash accumulation in different plant parts or criteria related to leaf structure. Though these features are not  $\Delta$  surrogates in a strict sense, they are always related to yield and are both quicker and cheaper than  $\Delta$  determinations; furthermore, no large facilities or highly qualified technical support are necessary to use them.

Canopy spectral reflectance is one of the most promising remote sensing techniques (see also Araus, 1996). Although at present the equipment is very expensive, in a few years its cost should drop dramatically. Another remote sensing technique, canopy temperature, provides integrated information on the crop's stomatal conductance at the canopy level (see chapter by Reynolds) (Reynolds et al., 1994) and has the advantage of being low cost. However, its usefulness is limited in severely stressed environments, and genotypic differences in phenology and canopy architecture can further limit its validity.

## Carbon Isotope Discrimination

For  $C_3$  plants, discrimination ( $\Delta$ ) of the heavier ( $^{13}C$ ) stable carbon isotope over the lighter, more abundant ( $^{12}C$ ) form (99%) during photosynthetic  $CO_2$  fixation is an integrated measure of internal plant physiological properties

and external environmental conditions that influence photosynthetic gas exchange (Farquhar et al., 1989). In  $C_3$  cereals such as wheat,  $\Delta$  is positively related to  $CO_2$  levels in intercellular air spaces (Diagram 1) (Farquhar et al., 1982; Farquhar and Richards, 1984; Ehdaie et al., 1991) and, given a constant leaf-to-air vapor pressure difference, also negatively related to water use efficiency (WUE, measured either as net photosynthesis/transpiration, also called transpiration efficiency, or as plant biomass produced/water transpired) (Farquhar and Richards, 1984; Hubick and Farquhar, 1989). Plants with high WUE would be less able to discriminate against  $^{13}C$ , and thus would accumulate more of the heavy carbon isotope in their tissues than less efficient water users.

When measured in plant dry matter, D provides an (integrated) indication of WUE throughout plant growth (Farquhar et al., 1982, 1989). D has been proposed as a possible screening tool for identifying variations in WUE in wheat (Farquhar and Richards, 1984; Ehdaie et al., 1991; Condon and Richards, 1993) and barley (Hubick and Farquhar, 1989). In fact, the permanent relationships between WUE and D during treatment and the high broad-sense heritability of D in wheat, together with other considerations, indicate that D may be useful for modifying the WUE and yield of water-limited wheat crops (Condon et al., 1987; Condon and Richards, 1992, 1993).

## The relationship between $\Delta$ and water-use efficiency

Following on the model of Farquhar et al. (1982),  $\Delta$  in  $C_3$  plants may be defined in its simplest form as:

$$\Delta = a + (b - a)(p_i/p_a),$$

where  $a$  is the carbon-13 discrimination caused by diffusion in air (4.4 ‰),  $b$  is that caused by carboxylation by the

RuBP carboxylase enzyme (27 ‰), and  $(p_i/p_a)$  is the intercellular to atmospheric  $CO_2$  partial pressure ratio. Conversely,  $p_i/p_a$  may be defined as a function of  $\Delta$ :

$$p_i/p_a = (\Delta - 4.4)/2.6.$$

If we assume that the temperature of the leaf (or other photosynthetic organ) is close to air temperature, and if daytime relative humidity is also known, WUE (the net assimilation to transpiration ratio) may be defined as a function of  $p_i/p_a$ :

$$WUE = p_a(1 - p_i/p_a)/V(1 - RH)1.6,$$

where V is the saturated partial water vapor pressure at a given temperature, RH is relative humidity, and 1.6 is the

psychrometric constant (Farquhar et al., 1982). Thus, WUE can be estimated from  $\Delta$  values using the following equation:

$$WUE = p_a[1 - (\Delta - 4.4)/22.6]/V(1 - RH)1.6.$$

An agronomic estimation of WUE (considered as the ratio of dry matter accumulated to total water transpired) can be also be derived from  $\Delta$  using different equations (Hubick et al., 1986; Hubick and Farquhar, 1989; Craufurd et al., 1991; see also Araus et al., 1993).

### Methodological considerations

Carbon isotope analysis is performed by mass spectrometry. Although the equipment needed for such testing is expensive and often beyond the capability of many laboratories and research

stations, there are commercial firms that do the analyses reliably and at a reasonable price. Before sending the material to be analyzed, it must be ground very finely.

$^{13}C/^{12}C$  ratio values are expressed as carbon isotope composition ( $\delta^{13}C$ ) values, where

$$\delta^{13}C (\text{‰}) = [(R \text{ sample}/R \text{ standard}) - 1] \times 1000,$$

R being the  $^{13}C/^{12}C$  ratio. The standard for comparison is a secondary standard calibrated against PeeDee belemnite (PDB) carbonate. Test precision is usually lower than 0.10 ‰. The value of the discrimination ( $\Delta$ ) against  $^{13}C$  is calculated from  $\delta_a$  and  $\delta_p$ , where a refers to air and p to plant (Farquhar et al., 1989):

$$\Delta = (\delta_a - \delta_p) / (1 + \delta_p)$$

On the PDB scale, free atmospheric  $CO_2$  has a current deviation,  $\delta_a$ , of approximately -8.0 ‰ (Farquhar et al., 1989).

### Implications for plant breeding

**What type of sample to take and when?** Considerable genotypic variations for  $\Delta$  have been found in bread wheat (Condon and Richards, 1992), barley (Romagosa and Araus, 1991; Acevedo, 1993), and durum wheat (Araus et al., 1993a), but environmental factors may cause even larger changes in the value of  $\Delta$ , which could compromise the effective use of  $\Delta$  in breeding programs. After studying wheat Condon and Richards (1992) concluded that assessing genotypic variation in  $\Delta$  would be most effective under well-watered conditions. In this regard, Richards and Condon (1993) pointed out that under adequate conditions,  $\Delta$  is highly heritable and exhibits substantial genetic variation and few genotype x environment interactions.

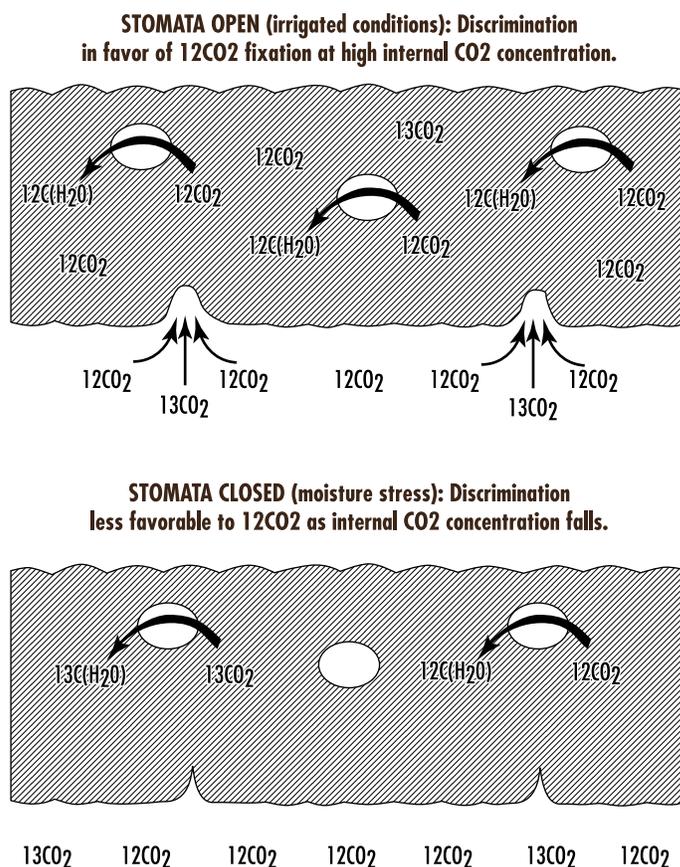


Diagram 1. Carbon isotope discrimination under irrigated and dry conditions.

As an alternative in rainfed environments, Condon and Richards (1992) proposed sampling for  $\Delta$  at early crop stages, when terminal stress is lacking. However, the information available on rainfed environments often does not support these expectations. Though correlation between  $\Delta$  and yield is usually weak or non-existent when dry material from seedlings is analyzed (Bort et al., 1998), it increases when upper plant parts are used in  $\Delta$  analyses (Figure 1). The best genetic correlations between  $\Delta$  and yield, together with the high broad sense heritability of  $\Delta$ , have also been reported for the upper parts of durum wheat (Araus et al., 1998b).

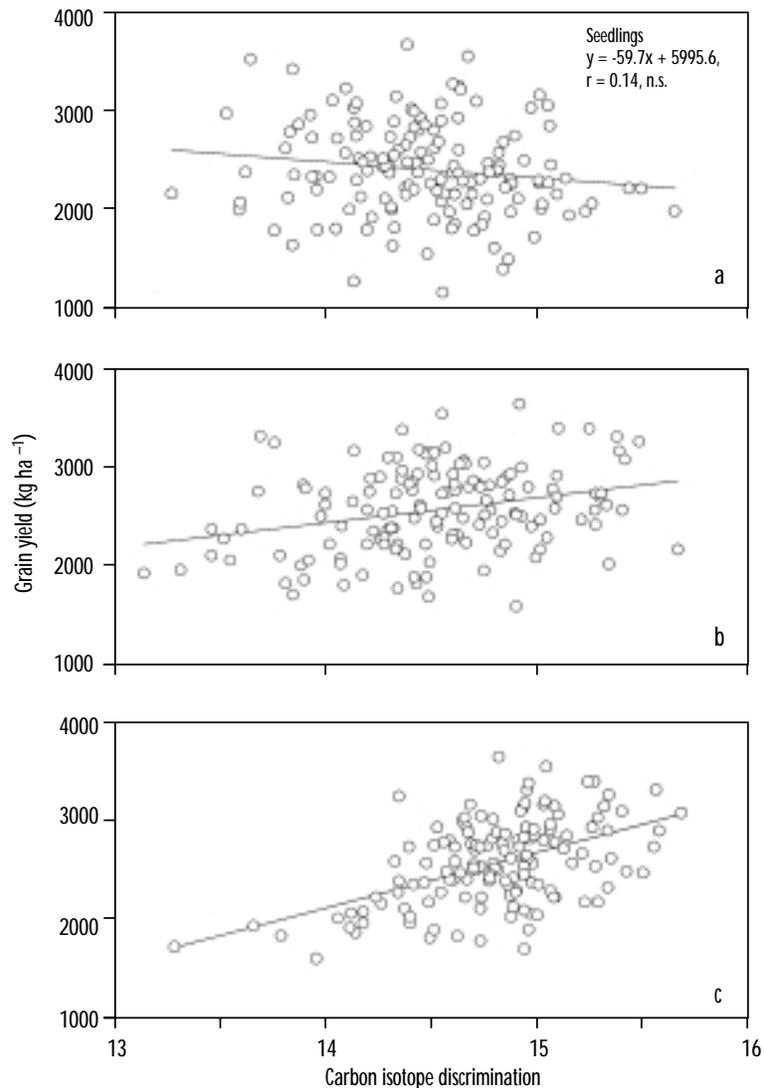
The correlation between yield and  $\Delta$  increases with plant age, perhaps due to the effects of progressive stress (particularly after anthesis) on yield. In fact,  $\Delta$  usually decreases from the oldest to the youngest plant parts, even under well-watered conditions (Hubick and Farquhar, 1989; Acevedo, 1993; see also Figure 1). Such a decrease may be attributed to stomatal closure in response to declining soil water and/or increasing vapor-pressure deficit during the last period of crop growth (Condon and Richards, 1992; Condon et al., 1992; Araus et al., 1993b). Thus, mature kernels could be the most adequate plant part to sample. Under Mediterranean conditions, for example, the  $\Delta$  of kernels rather than the  $\Delta$  of lower plant parts may provide more information on which genotype is less affected by stress during grain filling.

**Higher or lower carbon isotope discrimination?** In water-limited environments, genotypes with low  $\Delta$  should have greater biomass and hence potential for higher yields, assuming that all genotypes use the same amount of water for transpiration (Richards, 1996). In fact, selecting for high WUE (Passioura, 1977) or low  $\Delta$  (Craufurd et

al., 1991) has been proposed as an important alternative when defining plant breeding strategies under limited water conditions. However,  $\Delta$  values often correlate positively with grain yield and/or total biomass in wheat (Condon et al., 1987; Kirida et al., 1992; Araus et al., 1993c, 1997b; Morgan et al., 1993; Sayre et al., 1995) and barley

(Romagosa and Araus, 1991; Richards, 1996) under well-irrigated or rainfed conditions (Figure 1).

From an agronomic point of view, a positive relationship between  $\Delta$  and yield may exist if plants are not using all available soil water. Assuming the same phenology, a genotype with high  $\Delta$  will



**Figure 1.** Relationships between grain yield and carbon isotope discrimination ( $\Delta$ ) measured in dry matter of seedlings (a), in the penultimate leaf sampled around heading (b) and in mature kernels (c) for a set of 144 durum wheat genotypes grown under rainfed conditions in the Tel Hadya, northwestern Syria. For more details, see Araus et al. (1997b).

be able to sustain a high level of transpiration. Therefore,  $\Delta$  can be considered an indicator of WUE, but also depends on the water transpired by the crop, which is in fact the first parameter of Passioura's identity. The positive association between  $\Delta$  and yield also suggests that variations in stomatal conductance rather than in intrinsic photosynthetic capacity are predominant in determining  $\Delta$  (Romagosa and Araus, 1991; Condon et al., 1992). Higher  $\Delta$  is related to a higher level of  $\text{CO}_2$  in the cellular air spaces due to greater stomatal conductance (Farquhar and Richards, 1984), which leads to higher photosynthetic rates and, hence, higher yield even in the absence of stress. In this situation, WUE decreases (and  $\Delta$  increases) because stomatal limitation reduces transpiration more than photosynthesis, even when yield may be positively affected by low stomatal limitation on photosynthetic rate.

Relatively high transpiration levels may have implications for water-limited environments. For example, when water supply can be provided to the crop under drought stress, (e.g., by deep soil moisture extraction), the high-yielding (i.e., high-transpiring) genotype will have the most advantage (Blum, 1993, 1996). In fact, relatively low canopy temperatures resulting from high stomatal conductance and transpiration are typical of the more drought-resistant genotypes (Garrity and O'Toole, 1995; see also Blum, 1996). In addition, when grown at above-optimal temperatures, such as the maximum daily temperatures typical during grain filling, the positive correlation observed between stomatal conductance and yield may also be related to heat avoidance (Reynolds et al., 1994).

Alternatively, mechanisms that prevent water loss, such as inherently lower stomatal conductance, may limit yield

potential because of the intercellular levels of  $\text{CO}_2$ , thus decreasing photosynthesis. These genotypes will consistently show low  $\Delta$  values (Morgan et al., 1993). In fact, stomata that close only in response to severe water stress may be more useful in terms of yield than stomata that permanently show low conductance values (Jones, 1987). Moreover, selection for low  $\Delta$  (i.e., high WUE) may favor low-producing genotypes under drought conditions (i.e., drought-susceptible genotypes). Therefore, low  $\Delta$  may not be a good selection criterion for improving yields in dry environments. Plant production under drought conditions depends not only on WUE but largely on the genotype's capacity to sustain transpiration (Blum, 1993).

Blum (1996) pointed out that when soil moisture is very limited, the high-yielding genotype may be at a disadvantage because of its high stomatal conductance. In fact, it has been reported for wheat and barley that this (crossover) happens if yield is reduced to below 2-3 t ha<sup>-1</sup> (Ceccarelli and Grando, 1991; Blum, 1993). Other reports on durum wheat and barley do not support the existence of such a crossover in environments with a mean grain yield of 1.5 t ha<sup>-1</sup> or lower (Romagosa and Araus, 1991; Araus et al., 1998b). In these environments it may still be worth selecting for yield potential, particularly if deep extractable soil moisture is available to provide a yield above that of the crossover of genotype yields (Richards, 1996). Summarizing, the above results support the hypothesis that under Mediterranean conditions high-yielding genotypes, which sustain greater stomatal conductance and transpiration losses during grain filling, can provide higher yields in a wide range of environments with different levels of drought stress.

**Optimal yield conditions.** Carbon isotope discrimination has also been proposed as a useful trait to select for yield potential (Araus et al., 1993c; Sayre et al., 1995; Araus, 1996). The positive correlation between  $\Delta$  and yield would exist in the same context as above. A positive relationship between  $\Delta$  and growth has also been reported for seedlings grown under adequate water conditions (Febrero et al., 1992; Lopez-Castañeda et al., 1995), even when under field conditions cold stress (usual at this early stage) may obscure such a relationship (Bort et al., 1998). Nevertheless, increased early growth and leaf area development may be inherently linked to decreased WUE (Turner, 1993) and thus to higher  $\Delta$ . Blum (1996) pointed out that the data accumulated on carbon isotope discrimination and yield appears to support a consistent positive relationship between crop yield and photosynthetic capacity for various genetic materials of wheat and other crops (Hall et al., 1994). If selection for high photosynthetic capacity or higher crop productivity brings about an increase in stomatal conductance, then a concomitant increase in  $\Delta$  (or a decrease in crop WUE) is expected (Blum, 1996).

**Role of phenology in genotypical differences in  $\Delta$ .** In the absence of stress,  $\Delta$  in wheat is independent of phenological differences (Sayre et al., 1995). However, under non-optimal conditions the role of phenology in the relationship between  $\Delta$  and yield must be considered. Thus, as pointed out before, in Mediterranean environments phenology is the most important factor that accounts for increased wheat yields, as it affects assimilate partitioning, the pattern of water use, and other traits (see references in Slafer et al., 1993; Loss and Siddique, 1994). In addition, some of the genotypical differences in  $\Delta$ , as well as their positive association with

yield, can be due to phenology. Thus early flowering lines are more likely to have high  $\Delta$  than later-flowering lines due to the lower transpirative demand, which maintains higher stomatal conductances in the former (Ehdaie et al., 1991; Acevedo, 1993). Summarizing, under Mediterranean conditions early-flowering in wheat and other cereals is related to higher yields, which is in accordance with higher  $\Delta$  in the earlier genotypes. Alternatively, there is genotypical variability in  $\Delta$  that cannot be explained by phenology (Condon and Richards, 1993; Richards and Condon, 1993; Araus et al., 1998b) and is just due to differences in accumulated transpiration.

### $\Delta$ Surrogates

Given the cost and technical skills involved in carbon isotope analysis, different “surrogates” of  $\Delta$  have been investigated, such as mineral accumulation in vegetative plant parts (Figures 2 and 3) and leaf structure. Instead of being  $\Delta$  substitutes or surrogates, these selection criteria probably allow the evaluation of traits, other than WUE, that determine yield. Regarding the first criterion, if passive transport driven by transpiration is (at least partially) the mechanism of mineral accumulation in vegetative parts, then mineral content will also be an indicator of the first parameter of Passioura’s identity, i.e. total water transpired. The second trait corresponds to structural criteria that indicate the amount of photosynthetic tissue per unit leaf area and is therefore related to photosynthetic capacity. In addition, the mechanisms underlying the physiological association between  $\Delta$  and either mineral accumulation (Walker and Lance, 1991; Masle et al., 1992) or the amount of photosynthetic tissue (Araus et al., 1997a, b) are not fully understood. However, the empirical relationships of

these alternative criteria with  $\Delta$  and yield may justify their use on a routine basis. Such tools might be used during the early phases of a breeding program, which usually involve large populations. If the facilities or the funds are available, later selections could be based on more precise and accurate, yet expensive,  $\Delta$  analysis (Mayland et al., 1993). These two alternative criteria are discussed in the following paragraphs. Interestingly, these surrogates can be used in  $C_4$  crops (such as corn or sorghum), where  $\Delta$  is not as useful for evaluating WUE and yield itself as in  $C_3$  plants (Farquhar et al., 1989; Henderson et al., 1998).

### Mineral content in vegetative parts

Potassium, silicon, total mineral, or ash content accumulated in vegetative tissues have been proposed as surrogates of  $\Delta$  in cereals, forage crops, and soybean (Walker and Lance, 1991; Masle et al., 1992; Mayland et al., 1993; Mian et al., 1996). Masle et al. (1992) reported for all the herbaceous  $C_3$  species they assayed a positive linear relationship between total mineral content of vegetative tissues and the inverse of either WUE or  $\Delta$ . Therefore, the amount of minerals accumulated by plants in the glasshouse or in the field could be a potentially useful indicator of  $\Delta$  and WUE (Walker and Lance, 1991; Masle et al., 1992; Mayland et al., 1993).

In theory, total mineral and ash content seem to be better surrogates than the content of any single mineral, such as silicon or potassium (Masle et al., 1992; Mayland et al., 1993). Therefore, estimating plant mineral content, especially ash content, which requires only a muffle furnace, might be an attractive alternative to  $\Delta$  for preliminary screening of large, genetically diverse populations (Masle et al., 1992; Araus et al., 1998b).

**Methodological considerations regarding ash content.** Samples must be properly oven-dried and ground. Approximately 1 to 1.5 g of dry matter is placed in a pre-weighed porcelain crucible (empty crucible), the crucible with the sample is weighed (filled crucible), and the sample is burnt in a furnace at 450<sup>B</sup>C for 12 h. Then the crucible with mineral residue (burnt crucible) is weighed again. Ash content is expressed (on a concentration basis) as a percentage of sample dry weight as follows:

$$\text{Ash content (\%)} = \frac{(\text{burnt crucible weight} - \text{empty crucible weight})}{(\text{filled crucible weight} - \text{empty crucible weight})} \times 100$$

### Implications for plant breeding: Choice of environment and type of sample.

The positive correlation between ash content and  $\Delta$  may indicate that plants able to maintain higher stomatal conductance and transpiration will accumulate more ash in a transpirative organ, provided entry and accumulation of minerals in the plant take place (at least partially) through the transpirative stream.

Which are the best growing environments for using this surrogate? Mineral accumulation seems to be better related to  $\Delta$  and even to yield under well-watered conditions (Masle et al., 1992; Mayland et al., 1993), although these traits can be useful under drought conditions (Figure 2a; Araus et al., 1998b). Measurements taken on plants grown in the greenhouse can give contradictory results (Walker and Lance, 1991). Another question is the kind of sample to use. Later developed leaves (flag or penultimate leaves) are best. Ash accumulated in the flag leaf may be positively related to the  $\Delta$  of kernels (Araus et al., 1998b). Leaves must be mature to let minerals accumulate, but not senescent because minerals can remobilize to other plant parts. Thus, ash content measured at maturity in straw did not correlate with either  $\Delta$  of mature grains or yield. (Voltas et al., 1998).

## Mineral content in mature kernels as a criterion to complement $\Delta$

A negative relationship between ash content (on a dry mass basis) in mature kernels and yield has been reported for barley and durum wheat, under both optimum and non-optimum (i.e., rainfed) conditions (Febrero et al., 1994; Araus et al., 1998b; Voltas et al., 1998). This may be explained by the fact that ash content on a kernel mass basis may be an indirect indicator of total reproductive sink per culm attained at maturity (Araus et al., 1998b). In fact, total kernel mass per spike is the product of two yield

components developed last during the crop cycle: number of kernels per spike and kernel mass. Thus, kernel ash has been proposed as a criterion complementary to kernel  $\Delta$  in assessing genotype differences in cereal yield under Mediterranean conditions (Febrero et al., 1994; Voltas et al., 1998). In theory (Diagram 2), the pattern of ash accumulation in kernels is different from that in vegetative tissues because, unlike mineral accumulation in vegetative tissues, grain filling does not take place via the xylem (driven by transpiration) (Slafer et al., 1993). Such differences in mineral accumulation could explain the complementarity of ash content and  $\Delta$  in

kernels as integrative traits predicting grain yield (Febrero et al., 1994; Voltas et al., 1998).

Summarizing, kernel ash combined with either kernel  $\Delta$  or leaf ash can be partially complementary (i.e. independent) parameters when assessing differences in grain yield (Febrero et al., 1994; Araus et al., 1998b; Voltas et al., 1998). Selecting for low ash content in kernels, combined with either high  $\Delta$  in kernels or, alternatively, high ash content in the flag leaf, deserves further attention in wheat breeding (Figure 3). This approach could be particularly interesting if it were coupled with a new analytical technique

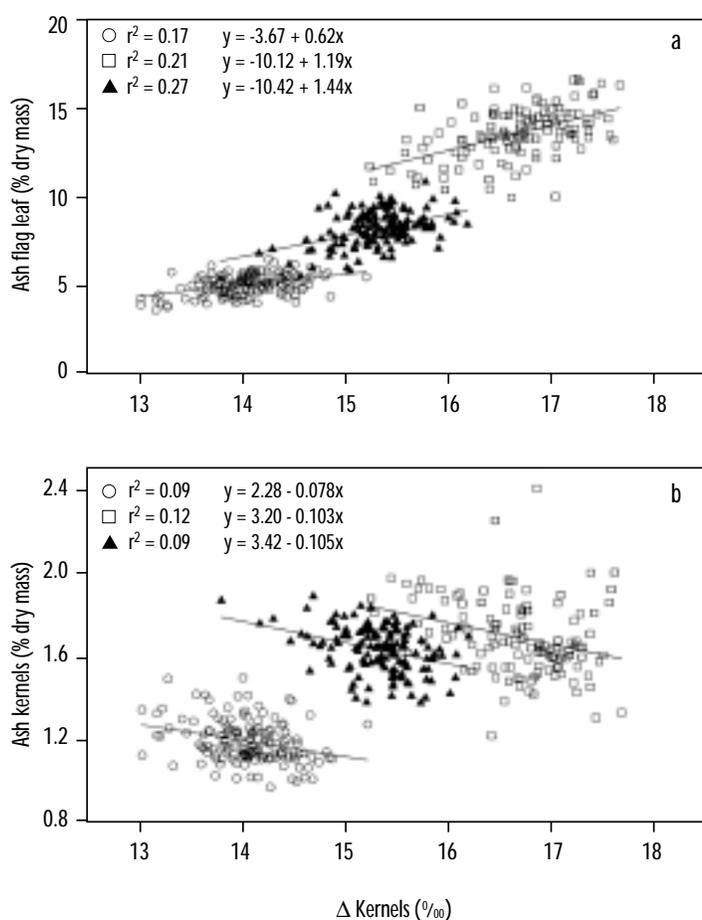


Figure 2. Relationship between carbon isotope discrimination ( $\Delta$ ) in mature kernels and ash content based on dry mass (a) of the flag leaf blades around three weeks after anthesis, and (b) in the same mature kernels. Plants were cultivated in three trials differing in water status: Breda, Tel Hadya rainfed, and Tel Hadya with supplementary irrigation.

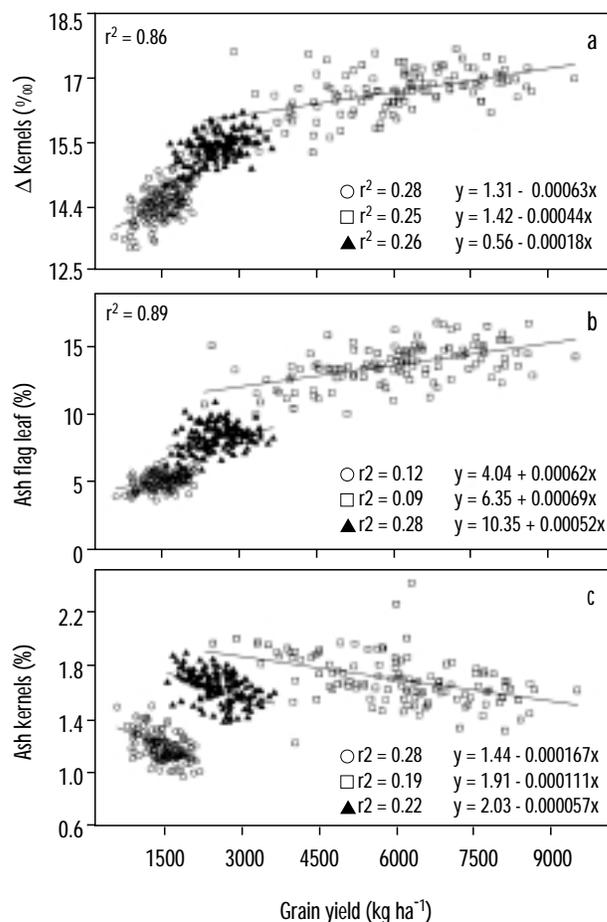


Figure 3. Relationship between grain yield and (a) carbon isotope discrimination ( $\Delta$ ) in mature kernels, (b) ash content (based on dry of the flag leaf around three weeks after anthesis), and (c) ash content of the same mature kernels.

such as near infrared reflectance spectroscopy (NIRS), which would allow a fast, reliable estimation of ash content and  $\Delta$  in intact kernels (see Araus, 1996).

### Leaf structural criteria

Changes in  $\Delta$  can derive from changes in the balance between leaf stomatal conductance and photosynthetic capacity. In wheat, genotypic variations in  $\Delta$  seem to derive from differences in both stomatal conductance and photosynthetic capacity, each contributing about the same (Condon et al., 1990; Condon and Richards, 1993; Morgan et al., 1993). If the intrinsic photosynthetic capacity of leaves is

increased,  $\Delta$  could decrease and WUE could be improved, without compromising yield potential (see above). Therefore, a negative relationship between  $\Delta$  and yield could be expected even in the absence of stress.

Genotypic differences in photosynthetic capacity may depend on the amount of photosynthetic tissue per unit leaf area. Thus, single structural parameters such as dry mass per unit leaf area (LDM, the reciprocal of specific leaf area, also termed specific leaf dry weight, or SLDW) or total nitrogen or chlorophyll content per unit leaf area may be good indicators of the strength of photosynthetic tissue (see references in

Araus et al., 1989; Nageswara Rao and Wright, 1994). For example, total chlorophyll content per leaf area may be evaluated in a fast, single and non-destructive way using a portable chlorophyll meter like the SPAD-502 (Soil-Plant Analysis Development Section, Minolta Camera Co., Ltd., Japan). Usually the leaf parameter that correlates negatively best with  $\Delta$  is LDM, followed by SPAD, which indicates that genotypes with thicker and/or more compact leaves have lower  $\Delta$ . The results suggest that LDM and SPAD measurements can be used as single, rapid indicators of  $\Delta$  in barley (Araus et al., 1997a) and durum wheat (Araus et al., 1997b) under optimal growing conditions (see also Lopez-Castañeda et al., 1995).

However, some of these correlations may exist under drought conditions and could be useful for breeding, but may be spurious in nature. In fact, growing conditions have a strong direct effect not only on  $\Delta$ , but also on leaf structure, which in turn could lead to spurious relationships (Araus et al., 1997b). The correlations between  $\Delta$  and leaf structure, rather than being sustained by a physiological relationship between the amount of photosynthetic tissue and  $\Delta$ , may sometimes be indirect associations caused by a parallel effect of water status and phenology on leaf structure, grain  $\Delta$ , and yield (Araus et al., 1997a, b). Summarizing, LDM should be used only in the absence of drought to determine segregating population differences in leaf  $\Delta$  based on internal photosynthetic capacity. It is worth selecting for higher kernel  $\Delta$  and grain yield based on higher LDM in rainfed trials, although there probably is no direct physiological basis behind such relationships (Araus et al., 1997b).

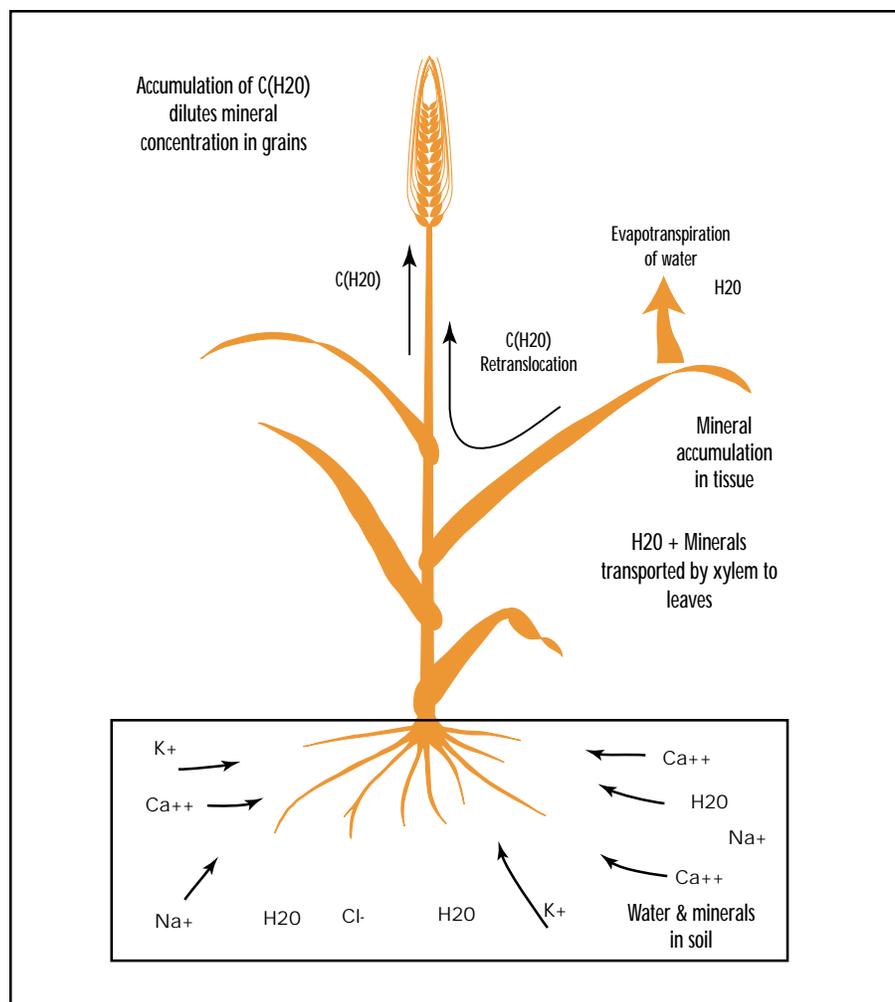


Diagram 2. Accumulation of ash in plants.

## Spectral Reflectance Methods

The pattern of light reflection on leaves at different wavelengths through the photosynthetically active radiation (PAR, 400-700 nm) and near infrared radiation (NIR, 700-1200 nm) regions of the electromagnetic spectrum is very different from that of soil and other materials (Diagram 3). Leaf pigments absorb light strongly in the PAR region but not in the NIR, thus reducing the reflection of PAR but not of NIR. Such a pattern of pigment absorption determines the characteristic reflectance signature of leaves (Figure 4). Similarly, the light spectrum reflected by a canopy (either natural or agricultural) differs from that reflected by the bare soil and varies in a way that can be related to the overall area of leaves and other photosynthetic organs in the canopy, as well as to their pigment composition and other physiological factors (Figure 5). Therefore, the measurement of spectra reflected by vegetation canopies provides information that can be used to estimate a large scope of parameters. Some of them are related to the green biomass of the canopy, its photosynthetic size (i.e., total area of leaves and other photosynthetic organs), the amount of PAR absorbed by the canopy, and its photosynthetic potential. Other parameters are more related to the canopy's physiological status at the time of measurement and can be used to assess the extent of some nutrient deficiencies and environmental stresses. The physiological parameters that can be estimated by spectral reflectance techniques include chlorophyll and carotenoid concentrations, photosynthetic radiation use efficiency (PRUE), and water content.

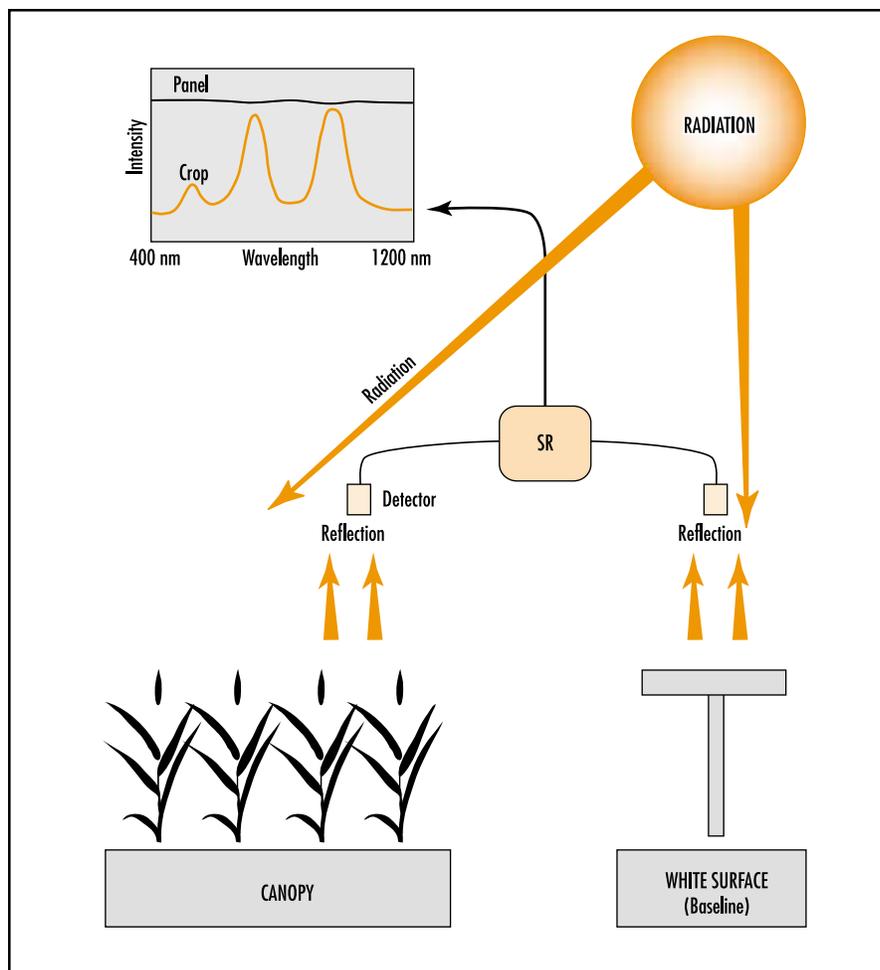


Diagram 3. Spectral reflectance from crop surfaces.

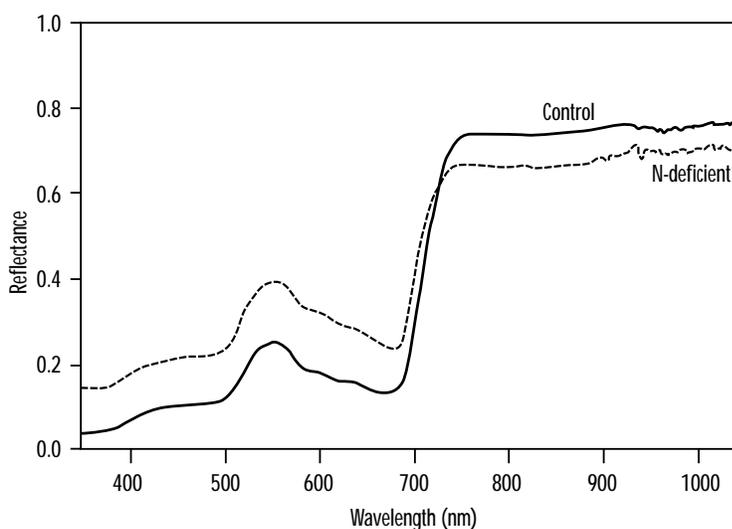
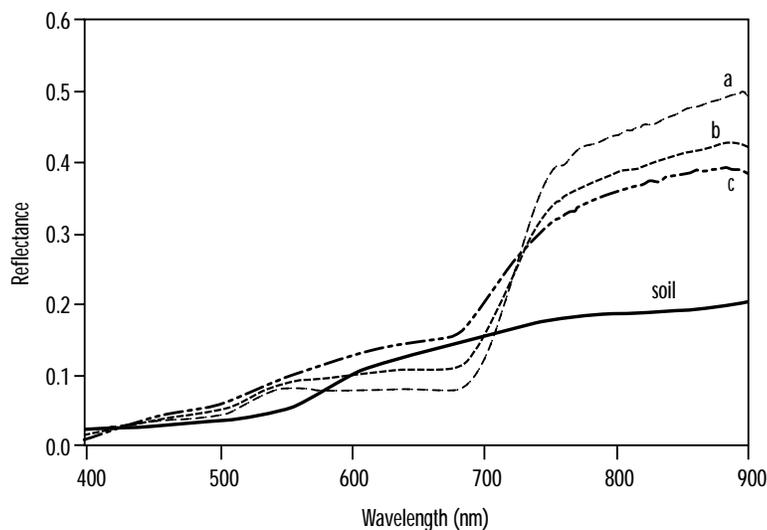


Figure 4. Reflectance signature of two wheat leaves differing in nitrogen status. Note the higher reflectance in the photosynthetically active radiation region of the nitrogen deficient leaf due to lower chlorophyll content in the leaf area.



**Figure 5. Changes in the pattern of canopy reflectance in a durum wheat plot.** Measurements were taken every three days (a, b, c), during the last week of grainfilling, coinciding with fast crop senescence. Note the decrease, during senescence, in the amplitude of the change in reflectance in the red-NIR (around 700 nm) edge. Note also the increase within the PAR region of the reflectance in the red compared to the blue band due to a relatively faster decrease during senescence in chlorophyll compared to carotenoids. Soil reflectance is included for comparison.

### Spectral reflectance indices

Spectral reflectance indices are formulations based on simple operations between reflectances at given wavelengths, such as ratios, differences, etc., which are widely used to quantitatively relate changes in reflectance spectra to changes in physiological variables. These indices have the advantage of summing up in a few numbers the large amount of information contained in a reflectance spectrum with narrow waveband resolution.

Originally used in remote sensing by aircraft and satellites, reflectances measured at the ground level are very useful for assessing agrophysiological traits. These traits can be evaluated simultaneously in each sample, at a rate of up to one thousand samples per day, which is much more tedious and time consuming with other methods. This makes spectroradiometric indices ideal for screening for yield potential or for resistance to different stresses.

### Sample applications

Perhaps the most widespread application of reflectance indices is for assessing parameters related to canopy greenness. These parameters are related to the canopy's photosynthetic size and include green biomass, leaf area index (LAI) (total one-side leaf area of the crop relative to soil area), green leaf area index (GLAI) (similar to LAI, but includes only functional green leaves), and green area index (GAI) (similar to GLAI, but includes other photosynthetic organs such as green stems). The amount of green area in a canopy determines PAR absorption by photosynthetic organs, which in turn determines the canopy's potential production. The fraction of the incident PAR that is absorbed by the canopy (fPAR) can be estimated from LAI-related parameters or directly from reflectance measurements. Cumulative PAR absorption, which is one of the parameters determining total biomass and thus final yield (see the beginning of

this chapter), can be assessed by measuring reflectance periodically during the growth cycle.

Some physiological parameters can also be quantified by spectral indices. Leaf pigments can be detected and quantified based on reflectance spectra and can be used as indicators of several physiological processes. Thus, the canopy's nutritional state can be evaluated through pigment concentration, as chlorophyll (Chl) concentration in leaves is (usually) closely correlated to its nitrogen content. Indices that are good indicators of Chl are (usually) also good indicators of N-content. In addition, plants with low N usually have a high carotenoid (Car) to Chl ratio, which can also be assessed by reflectance indices (Figure 5).

Pigment remote sensing can also be used for assessing the crop's phenological stage (Figure 5) and the occurrence of several stress factors (Blackburn, 1998; Peñuelas, 1998). For example, the Car to Chl ratio can be associated with senescing processes that result from the plant's natural ontogeny pattern or are triggered by different stresses. Also, phenological stages can be associated with different Car/Chl values. Several indices related to changes in pigment composition have been developed and can be used for the remote detection of nutrient deficiencies, environmental stresses, pest attacks, etc. In such contexts, by periodically assessing leaf area, leaf area duration (LAD) can also be used as an indicator of resistance to certain environmental stresses.

The photosynthetic capacity of a canopy can be estimated by using vegetation indices that correlate to the photosynthetic size of the canopy or indices related to the amount of chlorophyll. However, actual photosynthesis may not match

photosynthetic capacity due to the variability of photosynthetic use efficiency of the absorbed radiation, especially when plants are exposed to unfavorable conditions. The photochemical reflectance index (PRI) was developed to detect pigment changes in the xanthophyll cycle associated with changes in PRUE (Filella et al., 1996). PRI has been shown to track the changes in PRUE induced by factors such as nutritional status and midday reduction, across different species and functional types.

Another potential application of reflectance indices is remote detection of relative water content (RWC) of plants. Different levels of water stress can be detected indirectly through their effects on vegetation indices related to leaf area, pigment concentration, or photochemical efficiency. In addition, specific indices have been developed for the direct assessment of RWC.

## Measurement techniques

**Instruments.** The instruments required for measuring reflectance spectra are: 1) a field spectroradiometer that analyzes the spectrum of sampled radiation, 2) foreoptics that capture the radiation reflected by a given target, and 3) reference panels, supports, and levels for repeated sampling of incident radiation and radiation reflected by the canopy.

Modern narrow-band spectroradiometers measure the irradiance at different wavelengths with a bandwidth of about 2 nm through the PAR and NIR regions of the spectrum. Most spectral indices use specific wavebands in the 400-900 nm range; only a few use longer wavelengths, such as the water index, which uses 970 nm (Peñuelas et al., 1993). The use of spectroradiometers with narrow band resolution allows the calculation of several parameters obtained from the first and second

derivative of the reflectance spectra against wavelength, which can be used to complement the reflectance indices.

Radiation reflected by the canopy in the PAR and NIR regions is sampled by a foreoptic that limits the field of view to a given solid angle, usually between 10 and 25°. Sampled radiation is conveyed to the spectrum analyser through a fiber optic cable. Light reflected by the canopy is measured with the foreoptic held 1-2 m above the canopy on a fixed or hand-held support, such as a boom (Picture 1), and with the help of the required levels or protractors to ensure that all measurements are taken at the same angle between the foreoptics and the sampled surface.

In order to cross-reference the intensity of reflected radiation at each wavelength to the intensity of incident radiation at the same wavelength, all sampled

spectra must be converted to reflectance units, i.e., the ratio between the absolute spectrum reflected by the canopy and the absolute spectrum incident on the canopy. Regular measurements of the spectra incident on the canopy are then made. Incident spectra are measured by aiming the foreoptic at a white reference panel in the same orientation to the sun and to the foreoptic as the canopy. Reference panels are commercially available under the name Spectralon (Labsphere, PO Box 70, North Sutton, NH 03260) or they can be made of barium sulphate (Jackson et al., 1992).

**Factors affecting the estimation of canopy parameters by spectroradiometrical methods.** In addition to canopy variables estimated using spectroradiometrical methods, other factors related to the canopy or external to it will affect the measured



Picture 1. How to place the foreoptic while measuring radiation reflected by a wheat canopy.

reflectance spectra. Variation in canopy structure (such as changes in leaf erectness or appearance of reproductive organs), as well as in the angles between sun, sensor, and target surface, will affect the amount of shadow and/or soil background appearing in the field of view; this can cause non-desired variation in the measured spectra.

There are no standard methods to cope with the variability introduced by interference; most researchers using spectroradiometry adapt the details of their experimental protocols to the particular traits and objectives of their experiments. It is important to fix the measuring conditions used to obtain the spectra. Viewing angle and viewing height, row orientation, and time of day have to be determined when designing an experiment. Disturbance by factors beyond the researcher's control has to be considered when interpreting the results. Not all indices are equally affected by these factors, and indices also differ in their sensitivity to the parameter being measured. Some indices may be more appropriate than others, depending on the aims of the study, canopy characteristics, and measurement conditions.

To minimize the variability induced by sun position, it is preferable to take all measurements at about noon. Nevertheless, the angle of the sun is most important in canopies with low LAI (Kollenkark et al., 1982; Ranson et al., 1985). As for the viewing angle, nadir (sensor looking vertically downward) is perhaps the most commonly used set-up. This is because it has a lower interaction with sun position and row orientation, and delays the time at which spectra become saturated by LAI. On the other hand, nadir viewing is more affected by the soil background. When an off-nadir

viewing angle is used, variability due to changes in solar elevation or sensor elevation is minimized if the angle between the sensor azimuth and the sun azimuth is 0-90° (Wardley, 1984).

In a row canopy with low soil cover, the amount of shadow within the canopy varies during the day, depending on the angle between sun azimuth and row orientation. Such angular changes can produce variation in the measured reflectance as great as 100% in red and lower in NIR wavelengths (Kollenkark et al., 1982). Peak variability occurs when the sun is shining down the rows (when sun azimuth equals row orientation), lightening the soil surface and thus giving a higher reflectance reading. For that reason, if reflectance is measured at about noon, rows oriented east to west are more appropriate than rows oriented south to north, especially if soil cover is poor.

Ratio indices are usually less sensitive to changes in viewing geometry and tend to cancel the effects for angular changes (Wardley, 1984). However, they can also be altered because at some wavelengths (such as in red) reflectance is (usually) more intensely altered than at other wavelengths (such as in NIR). Light incident on shaded leaves is poor in the wavelengths that have been absorbed by upper leaves, and their reflected spectra is even poorer. For that reason, the higher the number of shaded leaves that appears in the field of view, the larger the differences in the canopy's reflectance spectra between regions where radiation is absorbed by photosynthetic pigments and regions where it is not. If due to external factors such as viewing angle, sun angle, or wind, the number of shaded leaves in the field of view increases, this will lead to an increase in indices related to green biomass.

The relationship between indices and estimated canopy parameters has been reported to be disturbed by phenological changes that affect crop structure, such as those associated with anthesis in maize (Andrieu and Baret, 1993) or head emergence in wheat (Shibayama et al., 1986). Leaf erectness can also affect canopy reflectance. Model calculations and test results show that radiation reflected perpendicularly from plant canopies is considerably greater from planophile canopies than from erectophile canopies (Jackson and Pinter, 1986). The vertical elements of an erectophile canopy trap reflected radiation within the canopy, while in a more planophile canopy, more radiation is reflected vertically. These structural effects can alter indices used for estimating the same canopy parameter in a different way. For example, Jackson and Pinter (1986) observed that although indices SR and PVI (see later in this chapter) are both used for estimating GLAI, SR was higher in erectophile canopies of wheat, while PVI was higher in planophile canopies. Optical differences in the surface of plant organs, such as different glaucousness (Febrero et al., 1998), can also have some effect on the canopy's reflectance spectra.

Clouds increase the proportion of indirect radiation (i.e., diffuse) to total radiation incident on the canopy; this improves the penetration of light into the canopy. As a result, a greater proportion of incoming radiation is absorbed by photosynthetic pigments; this increases the vegetation indices and leads to an overestimation of green biomass. Wind during the measurements can momentarily alter canopy structure and disturb the relationship between the reflectance spectra and the canopy parameters to be estimated from the spectra (Lord et al., 1985).

Nearby objects, including instruments and the people operating them, can alter the measured spectra by reflecting radiation on the target surface. For that reason, they should be kept as far as possible from the field of view; the instruments should be painted a dark color, and people should wear dark clothes (Kimes et al., 1983).

**Taking measurements.** Systematic measurements of incident radiation must be made before and during the measurement of reflected radiation to account for possible variation in the incident spectra caused by atmospheric conditions or sun position.

Reference panels should be Lambertian surfaces, that is, they reflect the incident light equally in all directions and for all wavelengths. However, they are not perfect and the intensity of the reflection changes in an important way when panel orientation changes. For that reason, care must be taken to make all incident measurements keeping the panel at the same angle with the foreoptics and with the sun. Changes in the distance from the panel to the foreoptics are less important. This distance is set to ensure that the entire field of view is covered by the panel. Then the reflectance of the canopy samples can be measured making sure that the field of view of the instrument is covered with weed-free canopy and a uniform background, and with plant material homogeneous in structure (Bellairs et al., 1996).

## Use of Canopy Reflectance Indices

### Assessing the photosynthetic size of canopies using vegetation indices

Vegetation indices (VI) estimate parameters related to the photosynthetic size of a canopy based on the reflectances in the red and near infrared

regions. Green biomass, LAI, GAI, GLAI, fPAR, etc., can be estimated through their positive correlation (either linear or logarithmic) with vegetation indices (Wiegand and Richardson, 1990a, b; Baret and Guyot, 1991; Price and Bausch, 1995). Measuring vegetation indices periodically during the crop growing cycle allows the estimation of LAD (which can be used as an indicator of environmental stress tolerance) and the total PAR absorbed by the canopy, which is one of the most important factors for predicting yield (Wiegand and Richardson, 1990).

Vegetation indices take advantage of the great differences in reflectance at red and NIR caused by vegetation. The most widely used VI are the simple ratio (SR) and the normalized difference vegetation index (NDVI), which are defined as:

$$SR = R_{NIR} / R_{Red}, \text{ with a range of } 0 \text{ to } \infty,$$

where  $R_{NIR}$  is the reflectance at NIR and  $R_{Red}$  is the reflectance at red.

$$NDVI = (R_{NIR} - R_{Red}) / (R_{NIR} + R_{Red}), \text{ with a range of } -1 \text{ to } 1.$$

SR and NDVI were originally used with the wide wavebands of former radiometers (for example, 550-670 nm for red and 710-980 nm for near infrared in AVHRR radiometers in satellites of NOAA series). With the high spectral resolution of today's radiometers, wavebands can be much narrower. Hall et al. (1990) used a waveband centered at 770 nm for NIR and another at 660 nm for red, while Peñuelas et al. (1997b) used 900 nm and 680 nm for NIR and red, respectively.

Some authors have reported improvements in NDVI performance after changing the wavebands used in the index. Carter (1998) describes an improved correlation with leaf photosynthetic capacity when using a

modified NDVI where R701 (+/-2nm) and R520 (+/-2nm) were used for NIR and red, respectively.

Variations of these indices have been proposed to compensate for the effect of soil background. Thus the soil adjusted vegetation index (SAVI) was defined by Huete (1988) as:

$$SAVI = [(RNIR - RRed) / (RNIR + RRed + L)] (1 + L),$$

where the parameter L was adjusted to minimize noise caused by soil for a large range of soil covers. For most crop conditions L=0.5, while for very low soil covers L=1 would be more appropriate, and L=0.25 would be appropriate for very high covers (Huete, 1988).

Other indices include parameters obtained from the soil's reflectance spectrum. One of them is the transformed soil adjusted vegetation index (TSAVI) which was defined by Baret and Guyot (1991) as:

$$TSAVI = a(R_{NIR} - aR_{Red} - b) / [R_{Red} + a(R_{NIR} - b) + 0.08(1+a^2)],$$

where a is the slope and b is the intercept of the linear equation

$$R_{NIRsoil} = a * R_{Redsoil} + b.$$

An important drawback in estimating LAI by VI is the saturation of the VI with LAI. Saturation of NDVI starts at about LAI=1, and beyond LAI=2 it becomes insensitive to further increases in LAI (Gamon et al., 1995). Perpendicular vegetation index (PVI) partly overcomes the saturation problem inherent to NDVI (Richardson and Wiegand, 1977):

$$PVI = \{ (R_{Red,soil} - R_{Red,vegetation})^2 + (R_{NIR,vegetation} - R_{NIR,soil})^2 \}^{1/2}$$

Although PVI is more sensitive than NDVI to changes in the viewing

geometry, PVI does not become as clearly saturated as NDVI with changes in GLAI (Shibayama et al., 1986).

Examples of assessing LAI-related parameters by VI can be found in the literature (Baret and Guyot, 1991; Field et al., 1994; Price and Bausch, 1995). Ground level measurement of VI has been used successfully as a tool for assessing early biomass and vigor of different wheat genotypes (Elliott and Regan, 1993; Bellairs et al., 1996). Under experimental conditions of a wheat breeding program, Bellairs et al. (1996) reported young wheat canopies where LAI was less than 1.5, a coefficient determination ( $r^2$ ) of 0.90-0.95 between biomass and NDVI. As for assessing the intensity of different plant stresses, Peñuelas et al. (1997b) showed that NDVI was a useful tool for measuring agronomic responses of barley to salinity.

A practical use of vegetation indices is for making yield predictions. Yield can be predicted from successive VI measurements taken during the growing season, based on the following assumptions (Wiegand et al., 1991): 1) plant stands integrate the growing conditions experienced and express net assimilation achieved through the canopy, 2) stresses severe enough to affect economic yield will be detectable through their effects on crop development and the persistence of photosynthetically active tissue in the canopy, 3) high economic yields cannot be achieved unless plant canopies fully utilize available solar radiation as the plants enter the reproductive stage, and 4) vegetation indices calculated from remote observations in appropriate wavelengths effectively measure the photosynthetic size of the canopy. Wiegand and Richardson (1990b) reported an  $r^2$  of 0.5 for predicting wheat grain yield from PVI measured on four

dates during vegetative growth. Similarly, Rudorff and Batista (1990) reported an  $r^2$  of 0.66 between wheat yield and integrated VI from booting to completely senesced plants. If most uncertainty in yield prediction by VI is site-dependent, then calibrations of yield vs. VI across good and poor growing conditions within production areas can describe the results of past and future growing seasons acceptably (Wiegand et al., 1991).

## Remote sensing of pigments

### *Estimating chlorophyll concentration.*

Several indices have been developed for estimating Chl concentration using canopy reflectance methods. The simplest indices are just reflectance at 675 and 550 nm. Reflectance at 675 nm (R675) is very sensitive to changes in Chl content. However, the relationship becomes saturated at relatively low Chl values (around  $10 \mu\text{g cm}^{-2}$ ) and is a good indicator of chlorophyll content only at very low concentrations. Absorption by Chl at 550 nm is lower than at 675 nm; therefore, the reflectance at this wavelength (R550) is less sensitive to changes in Chl content but is not saturated at such low concentrations, thus covering a range of higher Chl values (Thomas and Gausman, 1977; Jacquemoud and Baret, 1990; Lichtenthaler et al., 1996).

Both R675 and R550 are non-normalized indices that can be affected by external factors (Curran, 1983). Other indices use more than one wavelength. Analyzing wavelengths that were more sensitive to changes in Chla, Chlb, and Cars in soybean leaves grown at different N levels, Chapelle et al. (1992) developed the ratio analysis of reflectance spectra (RARS) indices, RARSa, RARSb and RARSb, which optimized the estimation of Chla, Chlb, and Cars, respectively, in soybean leaves.

RARSa =  $R_{675} / R_{700}$  showed a determination coefficient of 0.93, with Chla ranging from 0.4 to  $27 \mu\text{g cm}^{-2}$ ; RARSb =  $R_{675} / (R_{650} * R_{700})$  showed an  $r^2$  of 0.82, with Chlb ranging from 1 to  $7 \mu\text{g cm}^{-2}$ ; and RARSb =  $R_{760} / R_{500}$  showed an  $r^2$  of 0.94, with Cars ranging from 1.5 to  $6 \mu\text{g cm}^{-2}$  (Chapelle et al., 1992). Blackburn (1998) reported that using R680 and R800, instead of R675 and R700, in RARSa significantly improved the prediction of Chla in a range of leaves from different species with different degrees of senescence. Other reflectance indices that can be used for estimating pigment concentration are summarized in Table 1.

Leaf chlorophyll content can also be assessed through its relationship with parameters derived from the position of the red edge. The red edge position (REP) is the wavelength in the 680-780 nm range where the change in reflectance when increasing the wavelength from red to NIR reaches its maximum. The REP shifts to slightly longer wavelengths as Chla values increase (Curran et al., 1990; Filella et al., 1995). By obtaining the first and second derivatives of the spectra in this area, several parameters that are good indicators of Chl content can be calculated. Among these parameters are the wavelength of the red edge ( $\lambda_{re}$ ), the maximum amplitude in the first derivative of the reflectance spectra ( $dR_{re}$ ), and the sum of amplitudes between 680 and 780 nm in the first derivative of the reflectance spectra ( $\Sigma dR_{680-780}$ ). These REP-related parameters are suitable indicators of chlorophyll content in a wider and higher range of concentration than R675 and R550, with the additional advantage that they are less affected by external factors such as the geometry, incident intensity, and soil background (Filella and Peñuelas, 1995).

In addition to the wide variety of indices related to absolute Chl concentration, the normalized phaeophytinization index (NPQI) can be used to detect chlorophyll degradation.

$$NPQI = (R_{415} - R_{435}) / (R_{415} + R_{435})$$

(Peñuelas et al., 1995c)

NPQI was introduced as an indicator of pest attacks on apple trees (Peñuelas et al., 1995c). In some cases it also seems to indicate different phenological states in wheat (Casadesús and Araus, unpublished data).

One practical approach for estimating Chl concentration using reflectance indices is to test the performance of more than one index and choose the one most appropriate for the experiment. Another approach is to pool the information contained in a number of indices. In this sense, Filella et al. (1995) were able to assign different reflectance spectra to different N-status classes using a discriminant analysis based on R430,

R550, R680,  $\lambda_{re}$ ,  $dR_{re}$ , and NDPI (defined later in this chapter). Non-destructive portable chlorophyll meters based on absorbance measurements through the leaf provide fast and easy determinations of chlorophyll content and are commercially available at a relatively low price. For example, the SPAD-502 mentioned above calculates the ratio of absorbances at 650 nm  $\lambda$  (chlorophyll absorbance peak) and at 940 nm (non-chlorophyll absorbance) (Monje and Bugbee, 1992). Estimates of chlorophyll using canopy spectral reflectance methods are in general closely related to the amount of chlorophyll per soil area calculated from the reading of portable chlorophyll meters multiplied by the LAI (Filella et al., 1995). Chlorophyll assessment using canopy reflectance methods has the advantage that it directly integrates the chlorophyll content of all the leaves in the canopy. It also offers additional information such as canopy size and content of pigments other than chlorophyll.

### Carotenoid to chlorophyll ratios.

Estimating the Car: Chl ratio by reflectance indices can be useful for assessing the extent of some plant stresses, given that increases in Cars concentration relative to Chl are often observed when plants are subjected to stress (Young and Britton, 1990).

Both Chl and Car absorb in the blue, but only Chl absorbs in the red. Indices that are combinations of the reflectance in these two regions are correlated to the Car : Chl ratio. The simplest indices are pigment simple ratio (PSR) and normalized difference pigment index (NDPI), which are formulated in an analog way to SR and NDVI and defined to estimate the ratio of total pigments to Chla (Peñuelas et al., 1993):

$$PSR = R_{430} / R_{680}, \quad NDPI = (R_{680} - R_{430}) / (R_{680} + R_{430})$$

Both PSR and NDPI are affected by disrupting effects introduced by leaf surface and structure. A new index was developed to avoid such problems: the structural independent pigment index (SIPI), which was defined by Peñuelas et al. (1995a) as:

$$SIPI = (R_{800} - R_{435}) / (R_{415} + R_{435})$$

SIPI uses wavelengths showing the best semi-empirical estimation of the Car : Chla ratio, and its formulation minimizes the disrupting effects of leaf surface and mesophyll structure (Peñuelas et al., 1995a). R800 is used as a reference where neither Cars nor Chl absorb and are only affected by the structure. The best fit between the Cars : Chla ratio and SIPI for a variety of plants with Chla ranging from 0.06 to 54  $\mu\text{g cm}^{-2}$  and Cars from 1 to 16  $\mu\text{g cm}^{-2}$  was exponential, with an  $r^2$  of 0.98 and the form, Cars : Chla =  $4.44 - 6.77 \exp^{-0.48 SIPI}$  (Peñuelas et al., 1995a).

**Table 1. Reflectance indices for estimating pigment concentration.**

Pigment	Definition	Reference
Chl	$R_{675}$	Jacquemoud and Baret, 1990
	$R_{550}$	Jacquemoud and Baret, 1990
	$R_{750/550}$	Lichtenthaler et al., 1996 Gitelson and Merzlyak, 1997
	$R_{750/700}$	Lichtenthaler et al., 1996 Gitelson and Merzlyak, 1997
	$NDVI_{green} = (R_{NIR} - R_{540-570}) / (R_{NIR} + R_{540-570})$	Gitelson and Merzlyak, 1997
	$\lambda_{re}, dR_{re}$ and $\Sigma dR_{680-780}$	Filella et al., 1995
Chla	$RARSa = R_{675} / R_{700}$	Chapelle et al., 1992
	$RARSa^* = R_{680} / R_{800}$	Blackburn, 1998
	$PSSRa = R_{800} / R_{675}$	Blackburn, 1998
Chlb	$RARSb = R_{675} / (R_{650} * R_{700})$	Chapelle et al., 1992
	$PSSRb = R_{800} / R_{650}$	Blackburn, 1998
Cars	$RARSc = R_{760} / R_{500}$	Chapelle et al., 1992
Cars/Chla	$SIPI = (R_{800} - R_{435}) / (R_{415} + R_{435})$	Peñuelas et al., 1992

Indices related to the Cars : Chl ratio change during the crop growing cycle. They are low during vegetative growth and start to increase before the beginning of senescence (Filella et al., 1995). They can be used in assessing the nutritional state of a crop (Filella et al., 1995), shown by high values of the indices when N is low, and for detecting pest attacks (Peñuelas et al., 1995c).

### Assessing radiation use efficiency by PRI

Canopy photosynthesis can be roughly estimated based on the estimation of the canopy's photosynthetic size or Chl concentration. However, these parameters are associated with potential canopy photosynthesis, which does not always correspond to actual photosynthesis, especially for plants growing in stressful environments. While VI are correlated with PAR absorption by the canopy (a slowly varying trait, in a range of days to weeks), the photochemical reflectance index (PRI) is correlated with photosynthetic radiation use efficiency (PRUE) of absorbed PAR, a rapidly varying process, in a range of hours.

Part of the PAR absorbed by Chl cannot be used for photosynthesis and is lost mainly through heat dissipation, which is linked to the xanthophyll-de-epoxidation cycle (Demmig-Adams and Adams, 1996). PRI reflects changes in reflectance of around 531 nm, which have been associated with pigment changes in the xanthophylls cycle (Gamon et al., 1992).

PRI was originally defined as physiological reflectance index (Gamon et al., 1992) but later the definition was slightly modified (its sign was changed) and the name of the

index was revised as photochemical reflectance index (Peñuelas et al., 1995b). Here PRI refers to the second definition.

$$PRI = (R_{531} - R_{570}) / (R_{531} + R_{570})$$

(Peñuelas et al., 1995b)

PRI is correlated with the de-epoxidation stage of the xanthophylls cycle, with zeaxanthin, and with radiation-use efficiency (Filella et al., 1996). Higher PRI values indicate greater efficiency.

PRI has been shown to track changes in photosynthetic radiation use efficiency induced by different factors such as nutritional state and midday reduction, across different species and functional types (Gamon et al., 1997). However, it does not properly track changes in PRUE if there are structural changes in the canopy associated with stress, such as leaf wilting (Gamon et al., 1992). Also, this index is valid only for fully illuminated canopies and does not perform properly across wide ranges of illumination from shade to sun (Gamon et al., 1997).

### Directly assessing plant water status

Some bands of radiation absorption by water exist in the 1300-2500 nm region, but due to its high absorbance in this region, reflectance becomes saturated (i.e., it does not respond to further increases in RWC) even in a canopy with low water content. In the 950-970 nm region, there is some weak absorption of radiation by water that is not saturated for a moderately dry canopy. The reflectance at 970 nm has been used in the definition of the water index (WI).

$$WI = R_{900} / R_{970} \text{ (Peñuelas et al., 1993, 1997)}$$

In WI, reflectance at 970 nm is taken as a wavelength sensitive to water content, while reflectance at 900 nm is taken as a reference which is similarly affected by canopy and leaf structures but with null absorption by water.

WI has been used to track changes in RWC, leaf water potential, stomatal conductance, and foliage minus air temperature differences when plant water stress is well developed (RWC < 0.85) (Peñuelas et al., 1993). Peñuelas et al. (1997a) reported a correlation coefficient of around 0.55 between WI and RWC for a range of species measured at different times of the year in their natural Mediterranean environment. However, WI appears to be quite insensitive until the drying process is well advanced. For that reason, WI can be useful for assessing wild fire risk but has less utility in irrigation scheduling. As for stress detection, Peñuelas et al. (1997b) showed that WI was a good indicator of water status in response to salinity.

NDVI is also affected by the drying process and by structural and color changes in the plants. The ratio of WI and NDVI has a better correlation with RWC increases, especially in species that undergo important changes in NDVI throughout the year (Peñuelas et al., 1997a).

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