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# Field Measurements of Canopy Spectra for Biomass Assessment of Small-Grain Cereals

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## 1. Introduction

Small-grain cereals are the food crops that are most widely grown and consumed in the world. Wheat and rice jointly supply more than 55% of total calories for human nutrition, occupying about 59% of the total arable land in the world (225 and 156 million ha, respectively). Global production is around 682 million metric tons for wheat and 650 million metric tons for rice (FAOSTAT, 2008). Wheat is a very widely adapted crop, grown in a range of environmental conditions from temperate to warm, and from humid to dry and cold environments. Demand for wheat and rice will grow faster in the next few decades, and yield increases will be required to feed a growing world population. Because land is limited and environmental and economical concerns constrain the intensification of such crops, yield increases will have to come primarily from breeding efforts aimed at releasing new varieties that provide higher productivity per unit area.

The most integrative plant traits responsible for grain yield increases in small-grain cereals are the total biomass produced by the crop and the proportion of the biomass allocated to grains, the so-called harvest index (Van den Boogaard et al., 1996). The product of these traits provides a framework for expressing the grain yield in physiological terms and for contextualizing past yield gains in small-grain cereals, particularly wheat and barley. Retrospective studies conducted with wheat frequently associate increases in yield with increases in partitioning of biomass to the grain, with small or negligible increases (Austin et al., 1980, 1989; Royo et al., 2007; Sayre et al., 1997; Siddique et al., 1989; Waddington et al., 1986), or even significant decreases (Álvaro et al., 2008a) in total biomass production. Increases in biomass have been reported in spring wheat (Reynolds et al., 1999; 2001), winter bread wheat (Shearman et al., 2005), and durum wheat (Pfeiffer et al., 2000; Waddington et al., 1987).

Since harvest index has a theoretical maximum estimated to be 0.60 (Austin, 1980), increases in grain yield of more than 20 percent cannot be expected through increasing the harvest index above the maximum levels reached currently by some wheat genotypes (Reynolds et al., 1999; Richards, 2000; Shearman et al., 2005). It is therefore generally believed that future improvements in grain yield through breeding will have to be reached by selecting genotypes with higher biomass capacity, while maintaining the high partitioning rate of photosynthetic products (Austin et al., 1980; Hay, 1995).

Total dry matter is mainly determined by two processes: i) the interception of incident solar irradiance by the canopy, which depends on the photosynthetic area of the canopy; and ii)

the conversion of the intercepted radiant energy to potential chemical energy, which relies on the overall photosynthetic efficiency of the crop (Hay & Walker, 1989). The relationship between above-ground biomass and yield has been demonstrated empirically in wheat. Positive associations ( $R^2=0.56$ ,  $P<0.05$ ) have been reported between biomass at maturity and yield in durum wheat (Waddington et al., 1987), and between biomass at anthesis and yield in bread wheat (Reynolds et al., 2005; Shearman et al., 2005; Singh et al., 1998; Tanno et al., 1985; Turner, 1997; Van der Boogaard et al., 1996), durum wheat (Royo et al., 2005), barley (Ramos et al., 1985) and rice (Turner, 1982). In a study conducted in Mediterranean conditions with 25 durum wheat cultivars, Villegas et al. (2001) found a strong association ( $R^2=0.75$ ,  $P<0.001$ ) of the biomass accumulated from the first node detectable stage with anthesis and yield. Vegetative growth before anthesis becomes particularly important when stresses during grain filling such as those caused by rising temperatures and falling moisture supply—usually occurring after anthesis in Mediterranean environments—limit the crop photosynthesis, forcing yield to depend greatly on the remobilization to the grain of pre-anthesis assimilates accumulated in leaves and stems (Álvaro et al., 2008b; Palta et al., 1994; Papakosta and Gagianas, 1991; Shepherd et al., 1987). The contribution of pre-anthesis assimilates to wheat grain yield and the efficiency of dry matter translocation to the filling grains seem to have increased in the last century as a consequence of breeding (Austin et al., 1980; Álvaro et al., 2008a,b).

Biomass assessment is thus essential not only for studies monitoring crop growth, but also in cereal breeding programs as a complementary selection tool (Araus et al., 2009). Tracking changes in biomass may also be a way to detect and quantify the effect of stresses on the crop, since stress may accelerate the senescence of leaves, affecting leaf expansion (Royo et al., 2004) and plant growth (Villegas et al., 2001).

Biomass assessment in breeding programs, in which hundreds of lines have to be screened for various agronomical traits in a short time every crop season, is not viable by destructive sampling because it is a time-and labor-intensive undertaking, it is subject to sampling errors, and samplings reduce the final area available for determining final grain yield on small research plots (Whan et al., 1991). Originally used in remote sensing of vegetation from aircraft and satellites, remote sensing techniques are becoming a very useful tool for assessing many agrophysiological traits (Araus et al., 2002). The measurement of the spectra reflected by crop canopies has been largely proposed as a quick, cheap, reliable and non-invasive method for estimating plant aboveground biomass production in small-grain cereals, at both crop level (Aparicio et al., 2000, 2002; Elliot & Regan, 1993; R.C.G. Smith et al., 1993) and individual plant level (Álvaro et al., 2007).

## 2. Growth patterns and biomass spectra

The growth cycle of small-grain cereals involves changes in size, form and number of plant organs. The external stages of cereal growth include germination, crop emergence, seedling growth, tillering, stem elongation, booting, inflorescence emergence, anthesis and maturity (Fig. 1). The classical monitoring of crop biomass requires destructive samplings of plants at different growth stages, counting of the number of plants contained in the sample and its weighing after oven-drying them. Crop biomass may be expressed as crop dry weight (CDW), which can be obtained from the plants sampled at a given stage as the product of average dry weight per plant ( $W$ , g) and the number of plants per unit area, and is frequently expressed as  $g\ m^{-2}$  (Villegas et al., 2001). The leaf area expansion of a cereal crop

may be monitored through changes in its leaf area index (LAI, a dimensionless value), which is the ratio of leaf green area to the area of ground on which the crop is growing. LAI may be calculated as the product of the mean one-sided leaf area per plant (LAP,  $\text{m}^2 \text{ plant}^{-1}$ ) and the number of plants per unit area in the sample ( $\text{plants m}^{-2}$ ). Changes in total green area of the crop may be described through the green area index (GAI, a dimensionless value), which is the ratio of total green area of the plants (leaves and stems, as well as spike peduncles and spikes when applicable) to the area of ground on which the crop is growing. It can be calculated as the product of total green area per plant (GAP,  $\text{m}^2 \text{ plant}^{-1}$ ) and the number of plants per unit area in the sample ( $\text{plants m}^{-2}$ ) (Royo et al., 2004).

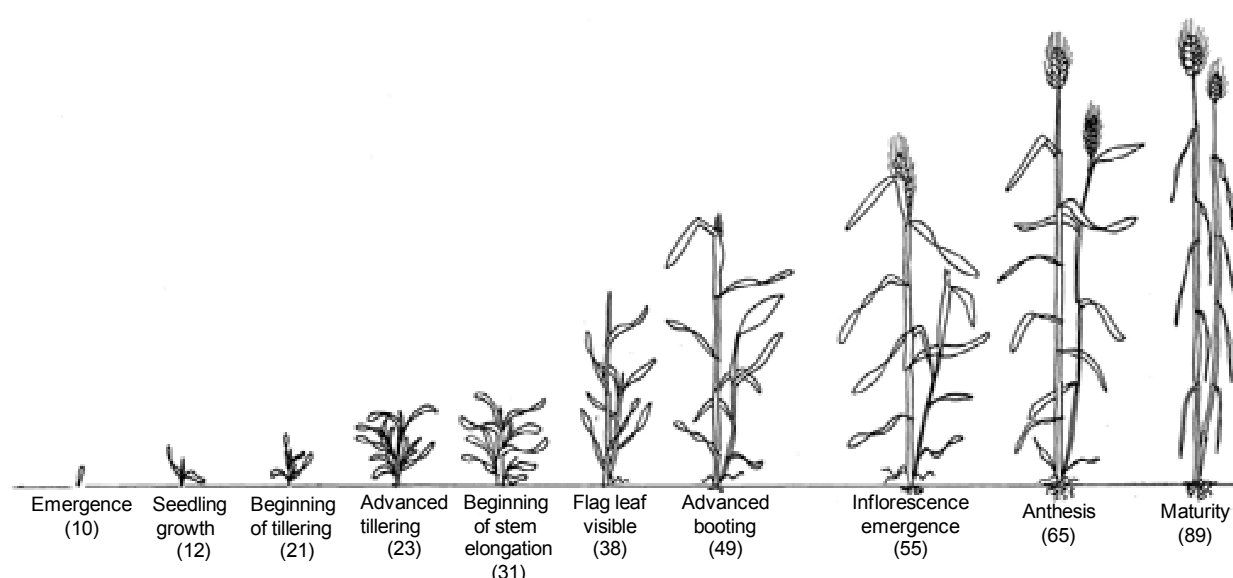


Fig. 1. Growth stages of small-grain cereals. Numbers correspond to the Zadoks scale (Zadoks et al., 1974)

Raw data from destructive sampling can be fitted to mathematical models, usually empirically based, to describe the growth pattern during the crop cycle. The logistic model of Richards (Richards, 1959), the expolinear equation of Goudriaan & Monteith (Goudriaan & Monteith, 1990), and the asymmetric logistic peak curve first used by Royo and Tribó (Royo & Tribó, 1997), have been used to describe the growth of crops. This last model has been useful for monitoring the biomass and leaf area expansion of triticale (Royo & Blanco, 1999) and durum wheat (Royo et al., 2004; Villegas et al., 2001). The mathematical models present the variation in dry matter production, leaf area or green area expansion over time, allowing variations between species (Fig. 2), genotypes, years and environmental conditions to be assessed (Fig. 3). Similarly to the case of grain yield, variability induced by the genetic background in the growth pattern of small-grain cereals has been found to be lower than the environmental variation caused by either year or site effects (Royo et al., 2004; Villegas et al., 2001).

Crop growth conditions can be monitored by measuring the spectra reflected by crop canopies in the visible (VIS,  $\lambda=400\text{-}700 \text{ nm}$ ) and near-infrared (NIR,  $\lambda=700\text{-}1300 \text{ nm}$ ) regions of the electromagnetic spectrum (Fig. 4). Given that the amount of green area of a canopy determines the absorption of photosynthetic active radiation by photosynthetic organs, spectral reflectance measurements can provide an instantaneous quantitative assessment of

the crop’s ability to intercept radiation and photosynthesize (Ma et al., 1996). Therefore, the absorption by the crop canopy of very specific wavelengths of electromagnetic radiation is associated with certain morphological and physiological crop attributes related to the development of the total photosynthetic area of the canopy.

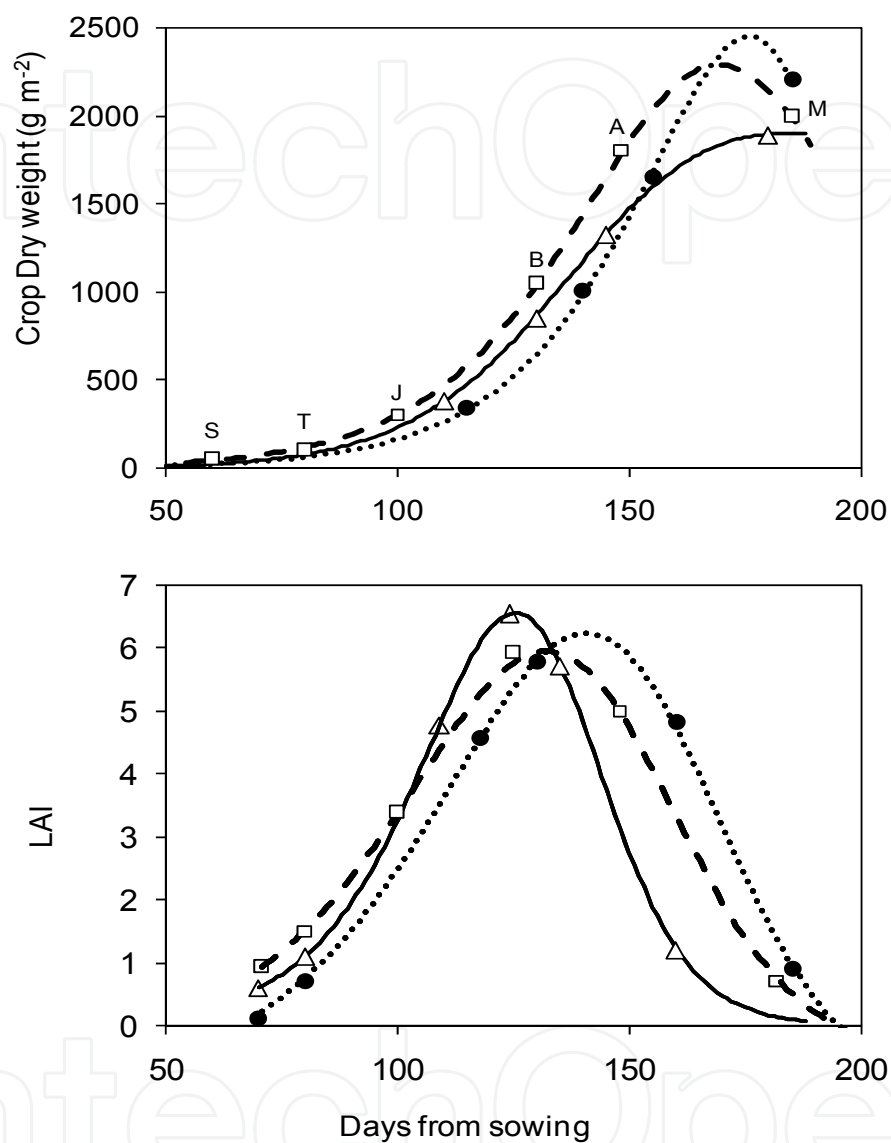


Fig. 2. Illustration of the differences between the patterns of biomass accumulation and leaf area expansion of barley ( $\Delta$ ), spring triticale ( $\square$ ), and winter triticale ( $\bullet$ ) from experiments conducted in 4 Mediterranean environments. Samples were taken at seedling (S), tillering (T), beginning of jointing (J), booting (B), anthesis (A), and physiological maturity (M). Biomass increased continually from anthesis to maturity in barley, but in triticale the peak of biomass took place between anthesis and maturity. The maximum LAI was reached at the booting stage in barley, but a little later in triticale. Adapted from Royo & Tribó (1997)

The reflectance spectra of a healthy crop-canopy shows a relative maximum around 550 nm, a relative minimum around 680 nm and an abrupt increase around 700 nm, remaining fairly constant beyond this point (Fig. 4). The spectral reflectance in the VIS wavelengths depends on the absorption of incident radiation by leaf chlorophyll and associated pigments such as

carotenoid and anthocyanins. Crop reflectance is very low in the blue (400-500 nm) and red (600-700 nm) regions of the spectrum, because they contain the peaks of chlorophyll absorbance. Beyond 700 nm the reflectance of the NIR wavelengths is high since it is not absorbed by plant pigments and is scattered by plant tissues at different levels in the canopy (Knippling, 1970).

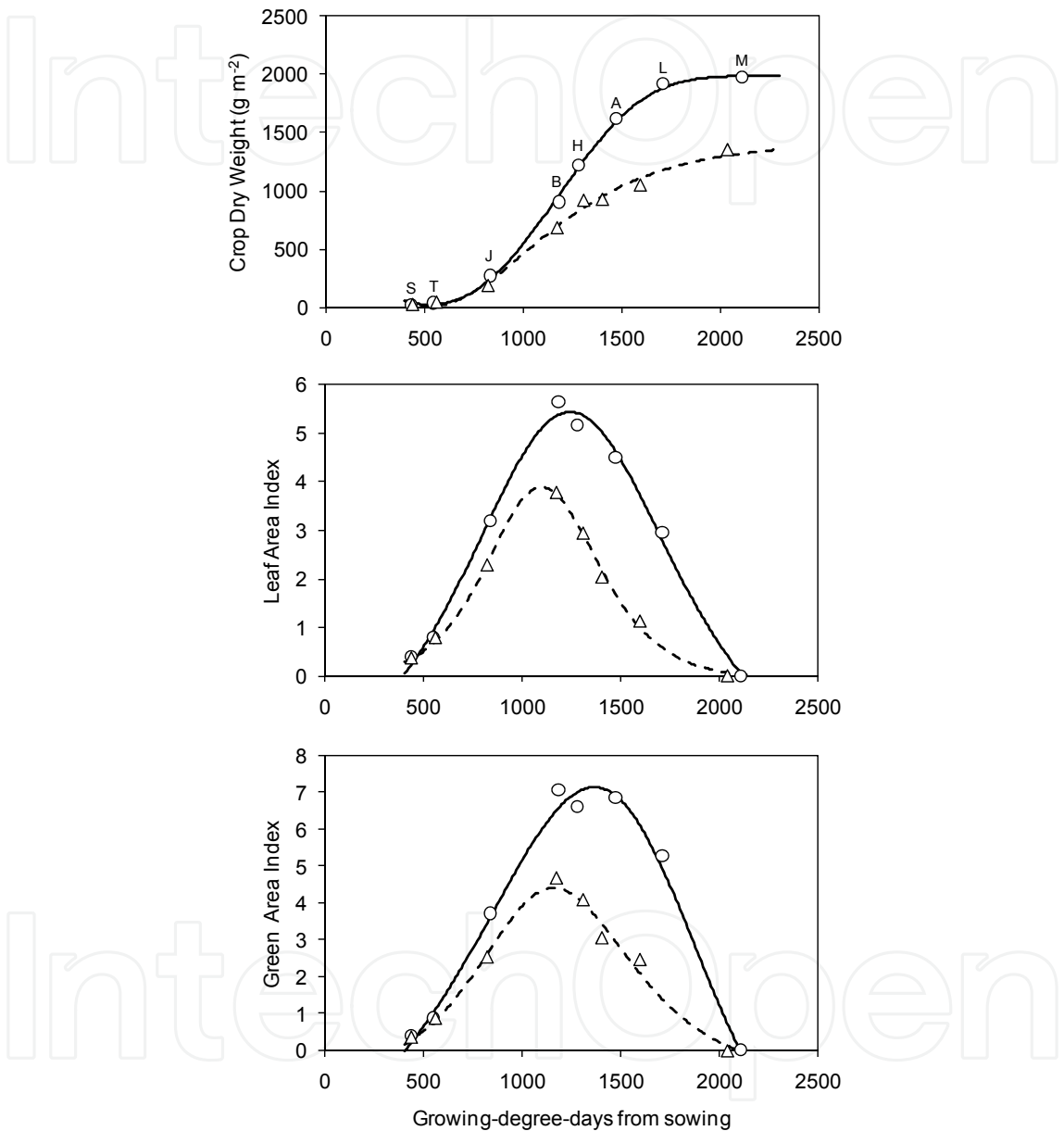


Fig. 3. Illustration of the effect of water input on the pattern of biomass accumulation (CDW), leaf area index (LAI), and green area index (GAI) of durum wheat grown under irrigated (○) and rainfed conditions (Δ). Data are means of 25 durum wheat cultivars grown in 1998 under Mediterranean conditions. The crop received 384 and 194 mm of water under irrigated and rainfed conditions, respectively. Samples were taken at seedling (S), tillering (T), beginning of jointing (J), booting (B), heading (H), anthesis (A), milk grain stage (L), and physiological maturity (M). Upper figure adapted from Villegas et al. (2001). LAI and GAI figures adapted from Royo et al. (2004)

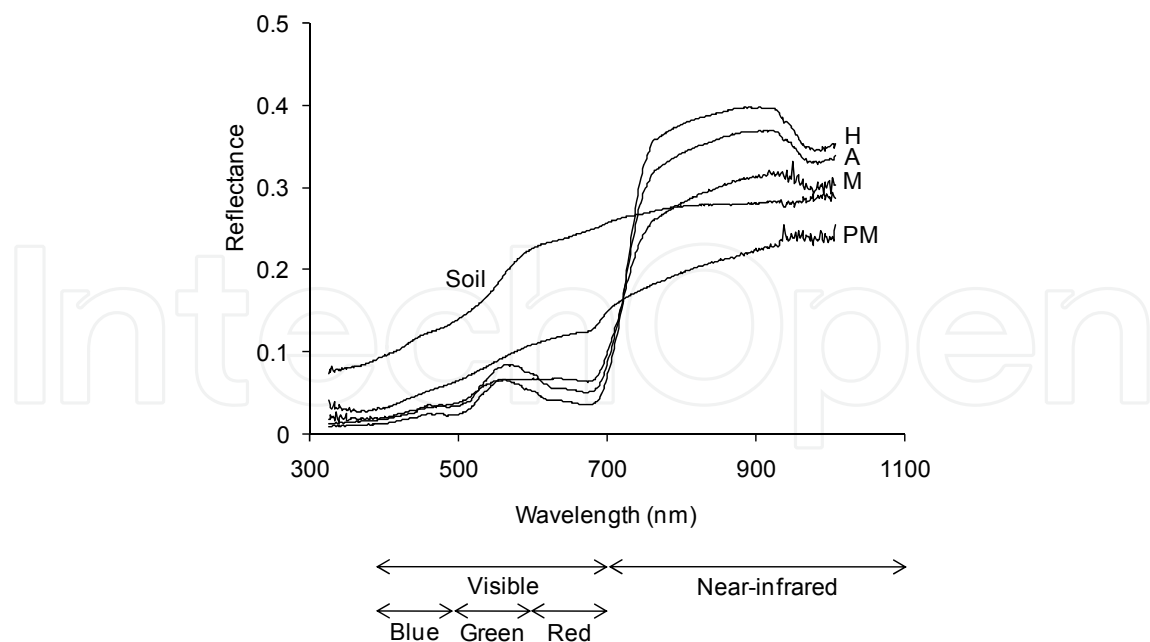


Fig. 4. Variation of the reflectance spectra of a healthy wheat canopy at different growth stages compared with the bare soil spectrum. H, heading; A, anthesis; M, milk-grain stage; PM, physiological maturity. The magnitude of the increase in reflectance at around 700 nm indicates differences in biomass

### 3. Methodology for capturing spectra

#### 3.1 Field equipment

High spectral resolution devices have recently improved in sensitivity, decreased in cost, and increased in availability. The equipment for field measurements consists of a portable spectroradiometer, which measures the irradiance at different wavelengths with a band width of about 1-2 nm through the VIS and NIR regions of the spectrum. This unit is connected to a computer, which stores the individual scans, a fore-optics sensor for capturing the radiation, and some complements such as reference panels and supports (Fig. 5). The sensor appraises the radiation reflected by the crop canopy, delimiting the field of view to a given angle, generally between 10° and 25°, which limits the area of the crop scanned to 20-100 cm<sup>2</sup>. The angle of incident light and the angle of observation of the sensor determine the proportion of elements in the observation field. The sensor is usually mounted on a fixed or hand-held tripod, which allows all measurements to be taken at the same angle and distance from the surface of the crop —usually from 0.5 m to around 1.0 m above the canopy facing the center of the plot. A fiber optic cable transmits the captured radiation to the spectrum analyzer. To convert captured spectra to reflectance units the spectra reflected by the crop canopy must be calibrated against light reflected from a commercially available white reference panel of BaSO<sub>4</sub> (Jackson et al., 1992). Each measurement takes around 1-2 s and between 5 and 10 scans are usually averaged per measurement.

The classical spectroradiometers measure about 250-500 bands, evenly spaced from a wavelength of 350 to 1110 nm, so a wide range of spectral reflectance indices can be calculated or the complete VIS/NIR reflectance spectra can be used. Cheaper units, such as Green Seeker™, which give only the basic spectroradiometric indices of green biomass, such

as the normalized difference vegetation index (NDVI) and the simple ratio (SR, see section 4), have been designed more recently for diagnosing nitrogen status and biomass assessment (Li et al., 2010b). The methodology allows sampling at a rate of up to 1000 samples per day.



Fig. 5. Measurements of spectral reflectance on field plots and layout of the tube used by Álvaro et al. (2007) to capture the spectra of individual plants

### 3.2 Factors affecting the reflectivity of the canopy surface

Measurements of the reflectance spectra of crop canopies are affected by both sampling conditions and canopy features. The most important are detailed in the following sections.

#### 3.2.1 Sensor position

The angles between sun, sensor and canopy surface may lead to the appearance of shadow or soil background in the field of view of the apparatus, causing disturbing effects in the spectra measured (Aparicio et al., 2004; Baret and Guyot, 1991; Eaton & Dirmhirn, 1979). The angle of the sun is more important in canopies with low LAI (Kollenkark et al., 1982; Ranson et al., 1985). Variability in reflectance due to variation in the sensor view angle has been reported to depend on the stage of development of the crop (J.A. Smith et al., 1975), the structure of the vegetative canopy (Colwell, 1974) and the leaf area index (Aparicio et al., 2004). Angles between the sensor azimuth and the sun azimuth of between  $0^\circ$  and  $90^\circ$  minimize the variability caused by changes in the elevation of the sensor or the sun (Wardley, 1984). However, when off-nadir view angles are used, the analysis of the remote sensing data could be complicated due to the non-Lambertian characteristics of vegetation (unequal reflection of incident light in all directions and reflection depending on the wavelength) (Ranson et al., 1985). The degree of canopy cover captured by the sensor is minimum at nadir position, and increases with the angle of observation. The effect of angle

is particularly important in crops arranged in rows, which may have different orientations in relation to the solar angle and the observation angle (Ranson et al., 1985; Wanjura & Hatfield, 1987). The nadir position of the sensor (sensor looking vertically downward) is the most widely used, because it has a low interaction with sun position and row orientation and delays the time at which spectra become saturated by LAI (Araus et al., 2001).

### 3.2.2 Environmental conditions

Environmental factors can cause undesired variation in the captured spectra. Light intensity, sun position, winds or nebulosity may interfere with the way in which the interaction between solar irradiation and crop is captured (Baret & Guyot, 1991; Huete 1987; Jackson 1983; Kollenkark et al., 1982). Green biomass may be overestimated when measurements are taken on cloudy days because the increased diffuse radiation improves the penetration of light into the canopy. Brief changes in canopy structure caused by winds may also induce variations in the captured spectra (Lord et al., 1985). The presence of people or objects near to the target view area should be avoided, since they can cause alterations in the measured spectra by reflecting radiation. The instruments should be painted a dark color and people should preferably wear dark clothes (Kimes et al., 1983). As a means of minimizing the variability induced by sun position, it has also been recommended that measurements be taken at about noon on rows oriented east to west.

### 3.2.3 Canopy attributes

The reflectivity of a crop canopy may be affected by a number of internal and external factors. The crop species, its nutritional status, the phenological stage (Fig. 4), the glaucousness, the geometry of the canopy and the spatial arrangement of its constitutive elements greatly affect the optical properties of the canopy surface. Under severe nitrogen deficiencies, chlorosis in leaves causes plants to reflect more in the red spectral region (Steven et al., 1990). The presence of non-green vegetation or non-leaf photosynthetically active organs (such as spikes and leaf sheaths of cereals) and changes in leaf erectness can also affect the spectral signature of the canopy (Aparicio et al. 2002; Bartlett et al., 1990; Van Leeuwen & Huete, 1996); for high LAI values, the reflectivity decreases with greater leaf inclination in both the VIS and the NIR wavelengths (Verhoef & Bunnik, 1981). Radiation reflected perpendicularly from plant canopies has been reported to be greater for planophile than for erectophile canopies (Jackson & Pinter, 1986; Zhao et al., 2010).

### 3.2.4 Soil interferences

When the crop canopy does not cover the entire soil surface, the target view area may include measurements of soil background, which may disturb the spectra measurements. Soil reflectances in the red and NIR wavelengths are usually linearly related (Hallik et al., 2009). As shown in Fig. 4, reflectance of bare soil differs from that of the crop canopy, because green vegetation reduces the values of red reflectance and increases the values of NIR reflectance when compared with those of the soil background. A number of studies on the effect of the soil reflectivity on the crop reflectance (Colwell, 1974; Huete et al., 1985), concluded that the most important factors are the chemical composition and water content of the soil. Greater discrimination power between wheat plots differing in biomass has been found on dark soils than on light soils (Bellairs et al., 1996).

In an attempt to minimize the variability induced by external factors, reflectance values recorded by the spectroradiometer are seldom taken directly but rather used to calculate

different indices —usually formulas based on simple operations between reflectances at given wavelengths.

#### 4. Traditional and new spectral reflectance indices for biomass appraisal

Spectral reflectance indices were developed using formulations based on simple mathematical operations, such as ratios or differences, between the reflectance at given wavelengths. Most spectral indices use specific wavebands in the range 400 to 900 nm and their most widespread application is in the assessment of plant traits related to the photosynthetic size of the canopy, such as LAI and biomass.

The most widespread vegetation indices (VI), for measurements not only at ground level but also at aircraft and satellite level (Wiegand & Richardson, 1990) are the normalized difference vegetation index ( $NDVI = (R_{NIR} - R_{RED}) / (R_{NIR} + R_{RED})$ ) and the simple ratio ( $SR = R_{NIR} / R_{RED}$ ) (see Table 1 for their definition). The ratio between the reflectances in the near-infrared (NIR) and red (RED) wavelengths is high for dense green vegetation, but low for the soil, thus giving a contrast between the two surfaces. For wheat and barley a wavelength ( $\lambda$ ) of around 680 nm is the most commonly used for  $R_{RED}$ , and one of 900 nm for  $R_{NIR}$  (Peñuelas et al., 1997a). These indices have been positively correlated with the absorbed photosynthetically active radiation (PAR), the photosynthetic capacity of the canopy and net primary productivity (Sellers, 1987). According to Wiegand & Richardson (1984, as cited in Wiegand et al., 1991), the fraction of the incident radiation used by the crops for photosynthesis (FPAR) may be derived from vegetation indices through their direct relationship with LAI, according to Equation (1):

$$FPAR(VI) = FPAR(LAI) \times LAI(VI) \quad (1)$$

For this reason, vegetation indices have proven to be useful for estimating the early vigor of wheat genotypes (Bellairs et al., 1996; Elliot & Regan, 1993), monitoring wheat tiller density (J.H. Wu et al., 2011), and assessing green biomass, LAI and the fraction of radiation intercepted in cereal crops (Ahlrichs & Bauer, 1983; Aparicio et al., 2000, 2002; Baret & Guyot, 1991; Elliott & Regan, 1993; Gamon et al., 1995; Peñuelas et al., 1993, 1997a; Price & Bausch, 1995; Tucker 1979; Vaesen et al., 2001). They tend to minimize spectral noise caused by the soil background and atmospheric effects (Baret et al., 1992; Collins, 1978; Demetriades-Shah et al., 1990; Filella & Peñuelas, 1994; Mauser & Bach, 1995).

Positive and significant correlations of SR and NDVI with LAI (Fig. 6), GAI and biomass (either on a linear or a logarithmic basis) have been reported in bread wheat and barley (Bellairs et al., 1996; Darvishzadeh et al., 2009; Fernández et al., 1994; Field et al., 1994; Peñuelas et al., 1997a). In a study conducted with 25 bread wheat genotypes, NDVI explained around 40% of the variability found in biomass (Reynolds et al., 1999). Studies involving 20-25 durum wheat genotypes have demonstrated a strong association between SR and NDVI and biomass under both rainfed and irrigated field conditions (Aparicio et al., 2000, 2002; Royo et al., 2003). Spectral reflectance measurements are also being used increasingly as a tool to detect the canopy nitrogen status and allow locally adjusted nitrogen fertilizer applications during the growing season (Mistele & Schmidhalter, 2010). Since grain yield is closely associated with crop growth and the vegetation indices are sensitive to canopy variables such as LAI and biomass that largely determine this growth, spectral data have also been proposed as suitable estimators in yield-predicting models (Aparicio et al., 2000; Das et al., 1993; Ma et al., 2001; Royo et al., 2003).

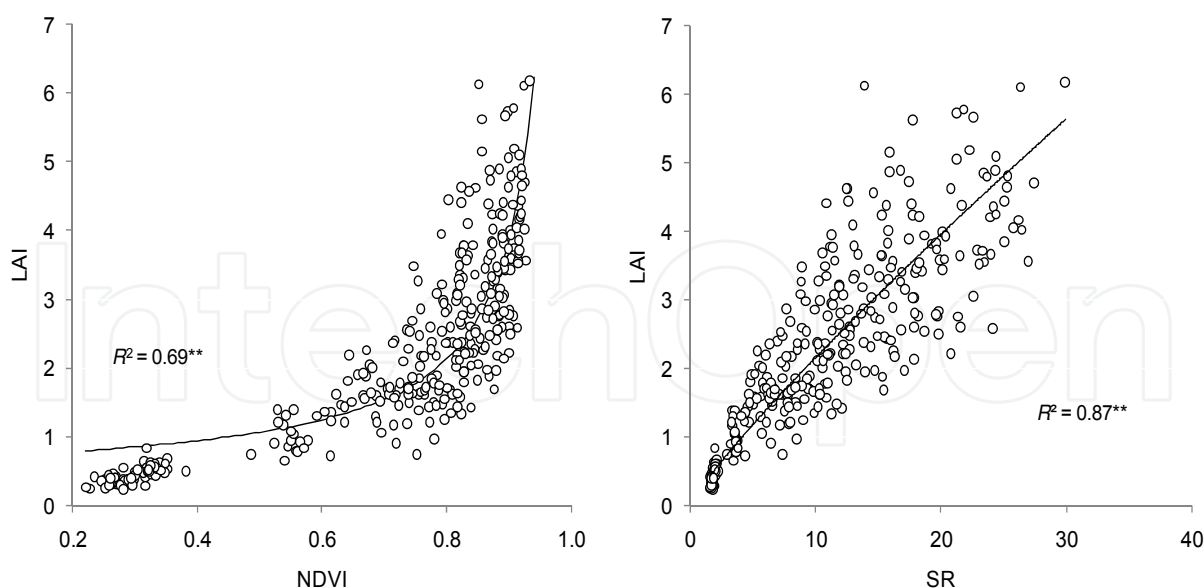


Fig. 6. Patterns of the relationships of leaf area index (LAI) with the normalized difference vegetation index (NDVI) and the simple ratio (SR). Data correspond to 7 field experiments involving 20-25 durum wheat genotypes and conducted under contrasting Mediterranean conditions for 2 years, with spectral reflectance measurements done at anthesis and milk-grain stage. Each point corresponds to the mean value of a genotype, experiment and growth stage. Adapted from Aparicio et al. (2002)

Another way to formulate the relationship between biomass and VI is to use the light use efficiency ( $\epsilon$ ) model (Kumar & Monteith, 1981) based on the fact that the growth rate of a crop canopy is almost proportional to the rate of interception of radiant energy. Thus, the crop dry weight of a crop canopy at a given moment ( $t$ ) may be expressed as a function of the incident radiation ( $I_0$ ), the fraction of the radiation intercepted by the crop canopy (FPAR), and the radiation use efficiency ( $\epsilon$ ), as follows:

$$CDW = \int_0^t I_0 \times FPAR(LAI) \times \epsilon \, dt \quad (2)$$

Small increases in biomass in a small period (expressed as days or thermal units) may then be calculated as a function of LAI from the derivative of Equation (2)

$$\frac{\delta CDW}{\delta t} = I_0 \times FPAR(LAI) \times \epsilon \quad (3)$$

The incident radiation ( $I_0$ ) may be obtained from meteorological stations or, alternatively, it can be estimated from air temperatures (Allen et al., 1998). FPAR(LAI) may be calculated from vegetation indices on the basis of the linear relationship existing between vegetation indices and the FPAR of green canopies (Daughtry et al., 1992), and particularly between NDVI and FPAR (Bastiaansen & Ali, 2003). Radiation use efficiency ( $\epsilon$ ) is assumed to be constant during the crop growing season (Casanova et al., 1998). Values of radiation use efficiency have been summarized by Russell et al. (1989) for different crops and environmental conditions; moreover,  $\epsilon$ -values can also be derived for a particular species

and environment from the slope of the relationship between total aboveground biomass and absorbed PAR energy (Liu et al., 2004; Serrano et al., 2000).

An example of use of Kumar & Monteith's model to assess the pattern of changes in biomass from the LAI estimated from spectral reflectance measurements is shown in Fig. 7. In the example, LAI and CDW values were calculated from destructive samplings, and a comparison is made between the pattern of changes in CDW derived from the mathematical model and that assessed by destructive samplings (Fig. 7b). The model requires frequent reflectance measurements to accurately assess the pattern of changes in LAI over time (Christensen & Goudriaan, 1993), and proper estimations of the incident radiation.

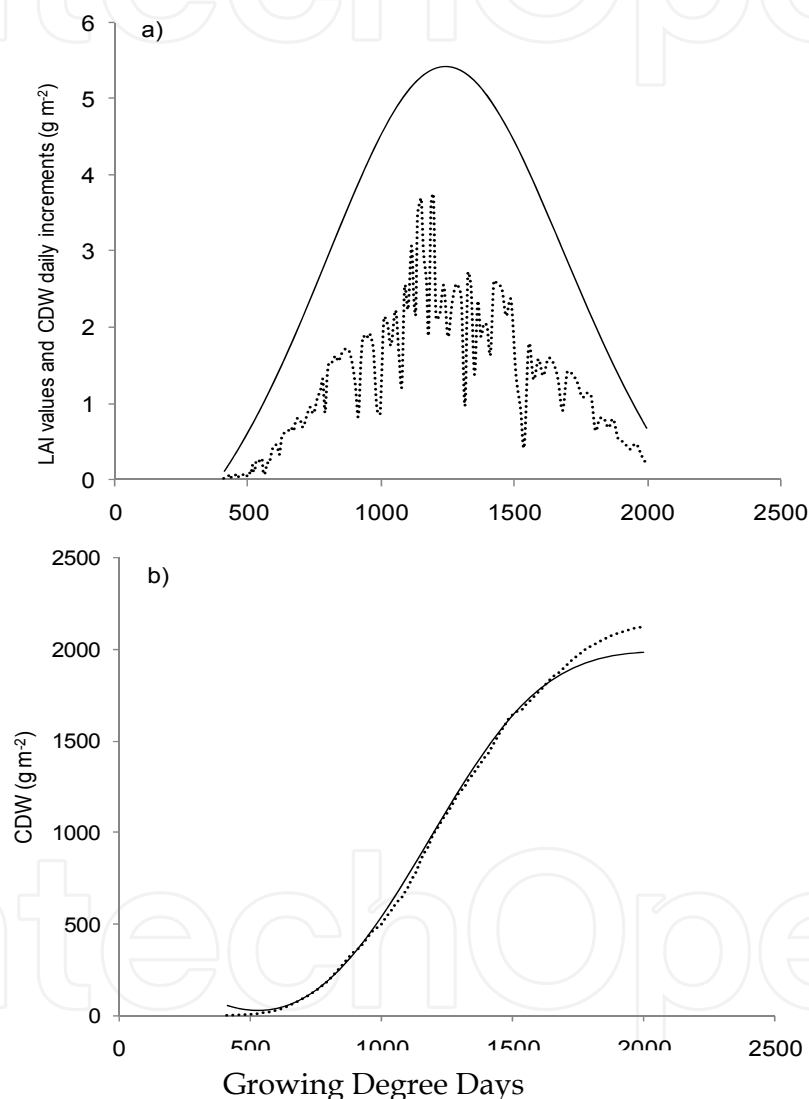


Fig. 7. Estimation of CDW from LAI data through the light use efficiency model (Kumar & Monteith, 1981). Fig. 7a. The solid line represents the mean pattern of changes in LAI of 25 durum wheat cultivars grown in 1998 under irrigated conditions, assessed through destructive biomass sampling (see Fig. 3). The discontinuous line shows daily increments in CDW, calculated from Eq. (3). Fig. 7b. The solid line shows the pattern of changes in CDW calculated from destructive sampling (see Fig. 3), while the discontinuous line represents the CDW values calculated from the integration of the daily CDW increments represented in Fig. 7a

Studies conducted in bread wheat (Asrar et al., 1984; Serrano et al., 2000; Wiegand et al., 1992) and durum wheat (Aparicio et al., 2002) have demonstrated that SR increases linearly with increases in LAI, while NDVI shows a curvilinear response (Fig. 6). When the LAI of wheat canopies exceeds a certain level, the addition of more leaf layers to the canopy does not entail great changes in NDVI (Aparicio et al., 2000; Sellers, 1987), because the reflectance of solar radiation from the underlying soil surface or lower leaf layers is largely attenuated when the ground surface is completely obscured by the leaves (Carlson & Ripley, 1997). The consequence is that for LAI values higher than 3, NDVI becomes relatively insensitive to changes in canopy structure (Aparicio et al., 2002; Curran, 1983; Gamon et al., 1995; Serrano et al., 2000; Wiegand et al., 1992), which constitutes an important limitation for the use of NDVI to estimate LAI. In this context the linearity of the relationship between SR and LAI is not advantageous, because SR may be directly derived from NDVI as  $SR = (1 + NDVI) / (1 - NDVI)$ , thus leading to similar statistical significances of both indices when LAI values are predicted (J.M. Chen & Cihlar, 1996). Because of the sensitivity of NDVI and SR to external factors—particularly the soil background at low LAI values—and the developments in the field of imaging spectrometry, a set of new vegetation indices have been developed in order to minimize the effect of disturbing elements in the capturing of the spectra (Baret & Guyot, 1991; Broge & Mortensen, 2002; Gilabert et al., 2002; Meza Diaz & Blackburn, 2003; Rondeaux et al., 1996).

In order to compare the suitability of the classical vegetation indices and the new ones mentioned in the literature as being appropriate for estimating growth traits in wheat and other cereals (P. Chen et al., 2009; Haboudane et al., 2004; Li et al., 2010a; Prasad et al., 2007), 83 hyperspectral vegetation indices were tested using durum wheat data from our own research. The indices were calculated from spectral reflectance measurements taken at different growth stages in 7 field experiments each involving 20-25 durum wheat genotypes, conducted under contrasting Mediterranean conditions for 2 years. Principal component analysis performed with the complete set of vegetation indices and LAI, GAI and CDW revealed that the vegetation indices most closely correlated with durum wheat growth indices were the 29 shown in Table 1. The correlation coefficients between growth traits and the selected indices are shown in Fig. 8. The results show that the majority of indices explained more than 50% of variation in LAI, GAI and CDW when determined at anthesis and milk grain stages, most correlation coefficients being statistically significant at  $P < 0.001$ . However, the correlation coefficients were significant only for a small number of indices when measurements were taken at physiological maturity. From these results we can conclude that despite the large number of vegetation indices described to improve the appraisal of growth indices given by NDVI and SR, this objective was attained in only a few cases.

Fig. 8 shows that some indices changed from positive values determined at milk-grain to negative ones determined at physiological maturity, confirming that the utility of vegetation indices to assess growth traits decreases drastically when the crop starts to senesce (Aparicio et al., 2000). Young wheat plants normally absorb more photosynthetically active radiation and therefore reflect more NIR. As the plants progress in growth stage, new tissues are formed but older green tissues lose chlorophyll concentration, turning chlorotic and then necrotic. These senescent tissues increase reflectance at the visible wavelengths and decrease reflectance at the NIR wavelengths, causing a decrease in the values of the vegetation indices compared with that obtained at earlier growth stages. Aparicio et al. (2002) concluded that genotypic differences were maximized in durum wheat when growth traits were determined by spectral reflectance measurements taken at anthesis and milk-grain stage.

| Identification        | Definition                                        | Equation                                                                                                                                       | Reference                        |
|-----------------------|---------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
| NDVI                  | Normalized difference vegetation index            | $(R_{900}-R_{680})/(R_{900}+R_{680})$                                                                                                          | Peñuelas et al. (1993)           |
| SR                    | Simple ratio                                      | $R_{900}/R_{680}$                                                                                                                              | Peñuelas & Filella (1998)        |
| CI                    | Canopy index                                      | $R_{415}/R_{695}$                                                                                                                              | Read et al. (2002)               |
| CIG                   | Green chlorophyll index                           | $(R_{800}/R_{550})-1$                                                                                                                          | C.Y. Wu et al. (2010)            |
| DD                    | Double difference index                           | $(R_{750}-R_{720})-(R_{700}-R_{670})$                                                                                                          | Le Maire et al. (2004)           |
| MCARI [705,750]       | Modified chlorophyll absorption ratio index       | $[(R_{750}-R_{705})-0.2\times(R_{750}-R_{550})]\times(\frac{R_{750}}{R_{705}})$                                                                | C.Y. Wu et al. (2008)            |
| MCARI/OSAVI [705,750] | MCARI[705,750]/OSAVI[705,750]                     | $\frac{[(R_{750}-R_{705})-0.2\times(R_{750}-R_{550})]\times(\frac{R_{750}}{R_{705}})}{(1+0.16)\times(R_{750}-R_{705})/(R_{750}+R_{705}+0.16)}$ | C.Y. Wu et al. (2008)            |
| MCARI2                | Modified chlorophyll absorption ratio index 2     | $\frac{1.5[2.5(R_{800}-R_{670})-1.3(R_{800}-R_{550})]}{\sqrt{(2R_{800}+1)^2-(6R_{800}-5\sqrt{R_{670}})-0.5}}$                                  | Haboudane et al. (2004)          |
| mSR705                | Modified simple ratio 705                         | $(R_{750}-R_{445})/(R_{705}-R_{445})$                                                                                                          | Sims and Gamon (2002)            |
| MTVI                  | Modified transformed vegetation index             | $1.2\times[1.2\times(R_{800}-R_{550})-2.5\times(R_{670}-R_{550})]$                                                                             | Haboudane et al. (2004)          |
| ND705                 | Normalized difference vegetation index 705        | $(R_{750}-R_{705})/(R_{750}+R_{705})$                                                                                                          | Sims & Gamon (2002)              |
| NDI1                  | Normalized difference index 1                     | $(R_{780}-R_{710})/(R_{780}-R_{680})$                                                                                                          | Datt (1999)                      |
| NDI2                  | Normalized difference index 2                     | $(R_{850}-R_{710})/(R_{850}-R_{680})$                                                                                                          | Datt (1999)                      |
| NDVI2                 | Normalized difference vegetation index 2          | $(R_{800}-R_{600})/(R_{800}+R_{600})$                                                                                                          | Ma et al. (1996)                 |
| NWI-1                 | Normalized water index -1                         | $(R_{970}-R_{900})/(R_{970}+R_{900})$                                                                                                          | Prasad et al. (2007)             |
| NWI-2                 | Normalized water index -2                         | $(R_{970}-R_{850})/(R_{970}+R_{850})$                                                                                                          | Prasad et al. (2007)             |
| NWI-3                 | Normalized water index -3                         | $(R_{970}-R_{920})/(R_{970}+R_{920})$                                                                                                          | Prasad et al. (2007)             |
| NWI-4                 | Normalized water index -4                         | $(R_{970}-R_{880})/(R_{970}+R_{880})$                                                                                                          | Prasad et al. (2007)             |
| OSAVI                 | Optimal soil adjusted vegetation index            | $(1+0.16)\times(R_{800}-R_{670})/(R_{800}+R_{670}+0.16)$                                                                                       | Rondeaux et al. (1996)           |
| OSAVI [705, 750]      | Optimal soil adjusted vegetation index [705, 750] | $(1+0.16)\times(R_{750}-R_{705})/(R_{750}+R_{705}+0.16)$                                                                                       | C.Y. Wu et al. (2008)            |
| PSNDc                 | Pigment specific normalized difference c          | $(R_{800}-R_{470})/(R_{800}+R_{470})$                                                                                                          | Blackburn (1998)                 |
| R780/R740             | R780/R740                                         | $R_{780}/R_{740}$                                                                                                                              | Misteale and Schmidhalter (2010) |
| RI                    | Ratio index                                       | $R_{810}/R_{560}$                                                                                                                              | Xue et al. (2004)                |

|      |                                      |                                                                                  |                                                     |
|------|--------------------------------------|----------------------------------------------------------------------------------|-----------------------------------------------------|
| RM   | Red-edge model index                 | $(R_{750}/R_{720})-1$                                                            | Gitelson et al. (2005)                              |
| RR   | Reflectance ratio                    | $R_{740}/R_{720}$                                                                | Vogelmann et al. (1993)                             |
| RTVI | Red-edge triangular vegetation index | $(100(R_{750}-R_{730})-10(R_{750}-R_{550}))\times\sqrt{\frac{R_{700}}{R_{670}}}$ | P. Chen et al. (2009)                               |
| SRPI | Simple ratio pigment index           | $R_{430}/R_{680}$                                                                | Peñuelas et al. (1994) as read in Li et al. (2010a) |
| TVI  | Transformed vegetation index         | $0.5\times[120\times/R_{750}-R_{550})-200\times(R_{670}-R_{550})]$               | Broge & Le Blanc (2000)                             |
| VI   | Vegetation index                     | $R_{750}/R_{550}$                                                                | Gitelson et al. (1996)                              |
| WI   | Water index                          | $R_{900}/R_{970}$                                                                | Peñuelas et al. (1997b)                             |

Table 1. Definition of some of the spectral reflectance indices most closely associated with growth traits of small-grain cereals.  $R_n$  = reflectance at the wavelength (in nm) indicated by the subscript

Though a large number of studies demonstrate the utility of vegetation indices for assessing growth traits in small-grain cereals when there is a wide range of variability involved in the experimental data, the results indicate that the value of the indices decreases drastically when the range of variation caused by the environment or the crop canopies is low (Aparicio et al., 2002; Royo et al., 2003). In such cases the success of the indices at tracking changes in growth traits becomes much more experiment-dependent (Babar et al., 2006; Christensen & Goudriaan, 1993). Nevertheless, as stressed above, one of the practical applications of spectral reflectance may be its use as a routine tool for screening germplasm in breeding programs, when measurements are taken on a genotype basis, usually in one or a reduced number of experiments. Moreover, vegetation indices are more appropriate for assessing LAI than for estimating biomass (Aparicio et al., 2000, 2002; Serrano et al., 2000), particularly when measurements are taken with low variability backgrounds.

5. Field measurements of growth traits in individual plants

Biomass assessment of individual plants by conventional methodologies involves destructive sampling, which is inappropriate for studies aiming to monitor the growth of specific individuals during their growth cycle, or when the grain produced by the plant has to be harvested at ripening, as in breeding programs. In such cases growth traits such as dry weight per plant (W), green area per plant (GAP) and leaf area per plant (LAP) may be properly estimated through vegetation indices.

Since the devices commercially available at present only allow measurements at canopy level, spectral reflectance measurements of individual plants require some adaptation of common equipment to avoid background effects. In studies conducted with wheat by Casadesus et al. (2000) and with four cereal species by Álvaro et al. (2007), the plants were covered by a tube of reflecting walls provided by an artificial source of light (Fig. 5). In order to provide a homogeneous background, aluminum foil was placed around the base of each plant, covering the entire tube base. The spectroradiometer was fitted to a receptor for diffuse spectral irradiance, centered at the top of the tube. The spectra obtained were standardized with the spectrum previously sampled in the empty tube with the soil covered

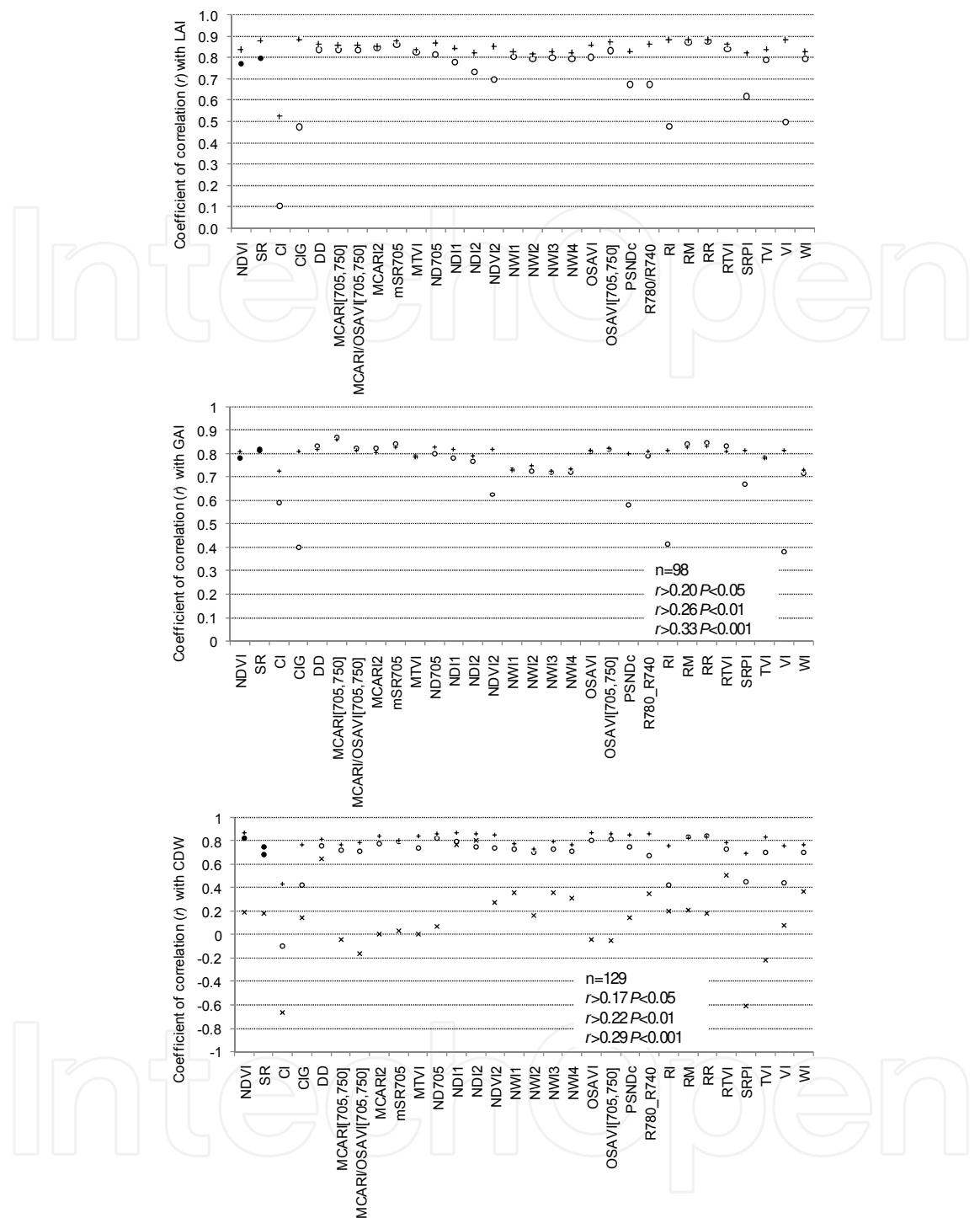


Fig. 8. Pearson correlation coefficients of some hyperspectral vegetation indices (see Table 1 for index definition)with the following durum wheat growth traits: a) leaf area index (LAI), b) green area index (GAI), and c) crop dry weight (CDW) considering pooled data of 7 field experiments involving 20-25 durum wheat genotypes, and conducted under contrasting Mediterranean conditions for 2 years. Destructive samples of biomass and reflectance measurements were taken at anthesis (○), milk-grain (+) and physiological maturity (x). Full symbols correspond to the classical vegetation indices, NDVI and SR. Unpublished data from Rojo and Villegas

with a homogeneous white reflecting surface. This method allows measurements to be taken at any time of the day, regardless of the environmental conditions (sun light angle and intensity, weather conditions, etc.), while avoiding background disturbances such as soil color. In this case each spectral reflectance measurement takes 20-30 s and five scans per plant are sufficient to obtain reliable results.

Consistent associations of NDVI and SR with W ( $R^2=0.91$ ,  $P<0.001$ ), GAI ( $R^2=0.88-0.89$ ,  $P<0.001$ ) and LAP ( $R^2=0.66-0.69$ ,  $P<0.001$ ) measured on spaced plants (Álvaro et al., 2007) have been reported. The accuracy of reflectance measurements to detect differences between individual plants seems to be comparable to that obtained by destructive measurements of growth traits (Álvaro et al., 2007), so this methodology is a promising tool for assessing growth traits in spaced individual plants. However, the time needed to prepare the plants and to take measurements may constrain its extensive use.

## 6. Limitations and future challenges of using spectral reflectance field measurements for biomass assessment

Despite the possibilities that spectral reflectance measurements offer for monitoring growth traits in plots and individual plants (e.g. in breeding programs), their use until now has been very limited. One of the main reasons is that a wide range of variability must exist for the target growth traits within the experimental units to be detected by the apparatus (Royo et al., 2003). The strongest associations between growth traits and spectral reflectance indices have been found in studies in which a wide range of variability is induced by experimental treatments, such as rates of seed or nitrogen fertilizer, varying levels of water availability or soil salinity, or the combined analysis of data recorded at different plant stages. However, when the range of variation is low, particularly when the differences are only in the genetic background, and the predictive ability of vegetation indices is tested in specific environments and growth stages, the value of spectral reflectance measurements for estimating growth traits has proven to be much more limited (Aparicio et al., 2002; Royo et al., 2003). The fact that the pattern of changes in biomass is quite similar among modern wheat varieties (Villegas et al., 2001) may be an additional obstacle to the implementation of remote sensing techniques as a screening tool in breeding programs.

Another limitation to the extensive use of spectral reflectance measurements to track changes in biomass derives from the huge number of indices reported in the literature and their misleading use (Araus et al., 2009). In addition, the lack of equipment specially designed to take measurements at individual plant level restricts the use of spectral reflectance in breeding programs, where selection in early segregating generations involves the screening of thousands of individual plants or small plots, and only reliable, fast, and cheap screening tools may be helpful. Prediction models are not of general use and need to be developed for specific situations, such as in farmer's fields, where evidence indicates a decrease in the performance of classical and newly identified indices (Li et al., 2010b). Other great challenges are the development of functions to calculate sensor-specific spectral signal-to-noise ratios for a number of different conditions, which would allow the models to include the effects of sensor-related noise (Broge & Leblanc, 2000), and the development of new sensors more adapted to practical applications.

## 7. Conclusions

The use of spectral reflectance measurements for the assessment of growth traits in small-grain cereals offers several benefits. Their non-destructive nature allows repetitive

measurements to be taken over time on the same plot or plant, so the grain produced on the measured plants is available at the end of their growth cycle. In addition, the method avoids the errors associated with destructive samplings of biomass, and is fairly quick. However, the use of canopy spectra for biomass assessment requires a thorough knowledge of the conditions of use and the constraints imposed by the measurement-related noise caused by the sensor system, the canopy structure, and the environment, which should be carefully taken into consideration in order to obtain reliable results.

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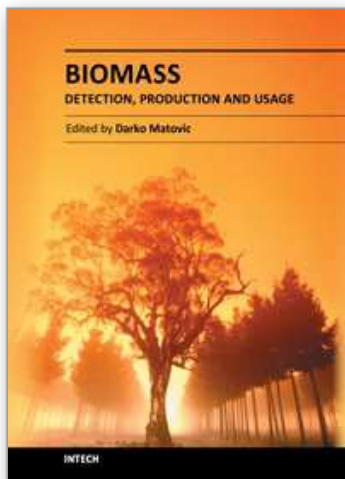
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Biomass has been an intimate companion of humans from the dawn of civilization to the present. Its use as food, energy source, body cover and as construction material established the key areas of biomass usage that extend to this day. Given the complexities of biomass as a source of multiple end products, this volume sheds new light to the whole spectrum of biomass related topics by highlighting the new and reviewing the existing methods of its detection, production and usage. We hope that the readers will find valuable information and exciting new material in its chapters.

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