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Spectral Reflectance to Estimate Genetic Variation for In-Season Biomass, Leaf Chlorophyll, and Canopy Temperature in Wheat

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ABSTRACT

Spectral indices as a selection tool in plant breeding could improve genetic gains for different important traits. The objectives of this study were to assess the potential of using spectral reflectance indices (SRI) to estimate genetic variation for in-season biomass production, leaf chlorophyll, and canopy temperature (CT) in wheat (*Triticum aestivum* L.) under irrigated conditions. Three field experiments, GHIST (15 CIMMYT globally adapted historic genotypes), RILs1 (25 recombinant inbred lines [RILs]), and RILs2 (36 RILs) were conducted under irrigated conditions at the CIMMYT research station in northwest Mexico in three different years. Five SRI were evaluated to differentiate genotypes for biomass production. In general, genotypic variation for all the indices was significant. Near infrared radiation (NIR)–based indices gave the highest levels of association with biomass production and the higher associations were observed at heading and grainfilling, rather than at booting. Overall, NIR-based indices were more consistent and differentiated biomass more effectively compared to the other indices. Indices based on ratio of reflection spectra correlated with SPAD chlorophyll values, and the association was stronger at the generative growth stages. These SRI also successfully differentiated the SPAD values at the genotypic level. The NIR-based indices showed a strong and significant association with CT at the heading and grainfilling stages. These results demonstrate the potential of using SRI as a breeding tool to select for increased genetic gains in biomass and chlorophyll content, plus for cooler canopies.

Significant progress in grain yield of spring wheat under irrigated conditions has been made through the classical breeding approach (Slafer et al., 1994), even though the genetic basis of yield improvement in wheat is not well established (Reynolds et al., 1999). Several authors have reported that progress in grain yield is mainly attributed to better partitioning of photosynthetic products (Waddington et al., 1986; Calderini et al., 1995; Sayre et al., 1997). The systematic increase in the partitioning of assimilates (harvest index) has a theoretical upper limit of approximately 60% (Austin et al., 1980). Further yield increases in wheat through improvement in harvest index will be limited without a further increase in total crop biomass (Austin et al., 1980; Slafer and Andrade, 1991; Reynolds et al., 1999). Though until relatively recently biomass was not commonly associated with yield gains, increases in biomass of spring wheat have been reported (Waddington et al., 1986; Sayre et al., 1997) and more recently in association with yield increases (Singh et al., 1998; Reynolds et al., 2005; Shearman et al., 2005). Thus, a breeding approach is needed that will select genotypes with higher biomass capacity, while maintaining the high partitioning rate of photosynthetic products.

Direct estimation of biomass is a time- and labor-intensive undertaking. Moreover, destructive in-season sampling involves large sampling errors (Whan et al., 1991) and reduces the final area for estimation of grain yield and final biomass. Regan et al. (1992) demonstrated a method to select superior genotypes of spring wheat for early vigor under rainfed conditions using a destructive sampling technique, but such sampling is impossible for breeding programs where a large number of genotypes are being screened for various desirable traits. Spectral reflectance indices are a potentially rapid technique that could assess biomass at the genotypic level without destructive sampling (Elliott and Regan, 1993; Smith et al., 1993; Bellairs et al., 1996; Peñuelas et al., 1997).

Canopy light reflectance properties based mainly on the absorption of light at a specific wavelength are associated with specific plant characteristics. The spectral reflectance in the visible (VIS) wavelengths (400–700 nm) depends on the absorption of light by leaf chlorophyll and associated pigments such as carotenoid and anthocyanins. The reflectance of the VIS wavelengths is relatively low because of the high absorption of light energy by these pigments. In contrast, the reflectance of the NIR wavelengths (700–1300 nm) is high, since it is not absorbed by plant pigments and is scattered by plant tissue at different levels in the canopy, such that much of it is reflected back rather than being absorbed by the soil (Knipling, 1970). Spectral reflectance indices were developed on the basis of simple mathematical formula, such as ratios or differences between the reflectance at given wavelengths (Araus et al., 2001). Simple ratio (SR = NIR/VIS) and the normalized difference vegetation

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Abbreviations: CT, canopy temperature; CTD, canopy temperature depression; GHIST, global historic; NDVI, normalized difference vegetation index; NIR, near infrared radiation; NWI-1, normalized water index-1; NWI-2, normalized water index-2; PSSRa, pigment specific simple ratio-chlorophyll a; RARSa, ratio analysis of reflectance spectra-chlorophyll a; RARSb, ratio analysis of reflectance spectra-chlorophyll b; RARSc, ratio analysis of reflectance spectra-carotenoids; RILs, recombinant inbred lines; SR, simple ratio; SRI, spectral reflectance indices; WI, water index.

index [NDVI = (NIR − VIS)/(NIR + VIS)] were the first developed SRI, and they combined information from the VIS and NIR wavelengths. These indices were used to predict different vegetative parameters, such as green biomass and green leaf area index. Spectral reflectance indices have been developed based only on VIS, such as the photochemical reflectance index [PRI = $(R_{531} - R_{570})/(R_{531} + R_{570})$] to assess radiation use efficiency by the plants (Peñuelas et al., 1995), and also only on NIR, such as the water index (WI = R_{970}/R_{900}) to assess water status of the canopy (Peñuelas et al., 1993).

Spectral reflectance indices have proven to be useful in the assessment of early biomass and vigor of different wheat genotypes (Elliott and Regan, 1993; Bellairs et al., 1996). Their studies were performed mainly under water-limiting conditions and with measurements taken at early growth stages to measure early vigor. The most widely used SRI, the NDVI and the SR, were not able to predict the variations in biomass successfully when estimated at later growth stages in durum wheat (Aparicio et al., 2000, 2002). Therefore, it is necessary to investigate the reflectance at other wavelengths to verify if there is a relationship between biomass and SRI, which may provide an indirect selection tool to differentiate spring wheat genotypes for biomass production.

Spectral reflectance indices based on visible wavelengths have also been developed to estimate the concentration of different leaf pigments such as chlorophyll and carotenoids. Chappelle et al. (1992) developed ratio analysis of reflectance spectra indices (RARS) to estimate chlorophyll-a, chlorophyll-b, and carotenoids based on the reflectance of light at different visible wavelengths (500, 650, 675, 700 nm) in soybean leaves. Later Blackburn (1998) used reflectance at 680 and 800 nm to estimate chlorophyll-a concentration, and showed that the combination of these wavelengths significantly improved the relationship with chlorophyll-a compared to the indices developed by Chappelle et al. (1992). A handheld portable SPAD chlorophyll meter has also been used to estimate chlorophyll concentration. SPAD chlorophyll meter readings have been shown to be strongly associated with extracted chlorophyll from the plants (Yadava, 1986; Dwyer et al., 1991). The relationship between spectral indices (RARS) and the SPAD chlorophyll meter values were never verified at the genotypic level. It is worthwhile to verify the relationship between the RARS indices and SPAD chlorophyll meter values in different growth stages at the genotypic level, so that breeders can quantitatively assess important physiological traits like stay-green to improve the productivity of the genotypes.

The WI, calculated from the reflected light at 900 and 970 nm, is an indicator of plant water status at both the leaf and canopy level (Peñuelas et al., 1993, 1997). It can assess the changes of relative water content, leaf water potential, stomatal conductance, and canopy temperature depression (CTD) when water stress becomes considerable (Peñuelas et al., 1993). Wheat genotypes with higher grain yield have been demonstrated to have a cooler canopy under irrigated conditions (Fischer et al., 1998). A significant positive correlation between WI and CTD was reported in spring wheat under irrigation by Gutierrez-Rodriguez et al. (2004), but the study was conducted with a small number of unrelated genotypes. The correlation between WI and CTD in different generations of the breeding population and with stable adapted genotypes may provide a true genetic relationship between these two physiological parameters, and the information could be useful for plant breeders in selecting genotypes with higher productive capacity.

Thus, the objectives of the present study were to (i) evaluate the correlation of the most widely used SRI, NDVI and SR, with the biomass production of a large number of bread wheat genotypes under near optimum N and irrigation levels at different stages of development; (ii) derive new SRI that better differentiate genotypes with higher biomass production capacity; (iii) calculate the association of chlorophyll concentration estimated by the SPAD chlorophyll meter and the spectral indices based on the visible wavelengths; and (iv) determine the relationship between CT and the WI at the genotypic level.

MATERIALS AND METHODS

One experiment (GHIST) was conducted for 3 yr (years 2001–2002, 2002–2003, and 2003–2004), and two experiments (RILs1 and RILs2) were conducted for 2 yr (years 2002–2003 and 2003–2004) under irrigated conditions at the CIMMYT (International Maize and Wheat Improvement Center) experimental station near Ciudad Obregon in Sonora, Mexico (27°33′ N, 109°09′ W, 38 m above sea level). The soil type at the experimental station is coarse sandy clay, mixed montmorillonitic type caliciorthid, low in organic matter and slightly alkaline (pH 7.7) in nature (Sayre et al., 1997). The weather is mostly sunny and dry during the winter cropping cycle (November–April). The experiments were planted on raised beds and each 5-m plot consisted of two beds. The width of the beds was 80 cm. Each plot area was 8.0 m^2 (1.6 by 5 m). In the first 2 yr, three rows were planted on each bed with 15-cm distance between rows. In the third year, two rows were planted on the beds with 20-cm row spacing.

The seeding rate for each experiment was 78 kg ha^{-1}. Nitrogen and P were applied to the plots at the rate of 200 kg ha^{-1} and 26 kg ha^{-1}, respectively. During the first 2 yr, 150 kg N and all of the P were applied during land preparation, and 50 kg N was applied in the second week of January, coinciding with the first node growth stage and the second supplementary irrigation. In the third year, the same procedure was followed, but half of the total dosage of N was applied before planting and half with the second supplementary irrigation. A total of five supplementary irrigations were applied in the first and third years, and four supplementary irrigations in the second year. Folicur 250EW (25% tebuconazole) was applied twice in every crop cycle, in the second week of February and in the second week of March at the rate of 0.5 L ha^{-1} to protect the experimental materials from leaf rust (caused by *Puccinia triticina* Eriks.).

Experimental Materials

Experiment 1 (GHIST): This experiment contained 15 widely adapted and unrelated spring bread wheat genotypes developed by the CIMMYT wheat-breeding program over the past 40 yr, released by national programs around the world, and widely grown by the farmers. The genotypes represent the historical success achieved by CIMMYT's breeding program. While all are

high yielding, they vary widely in morphological traits. The genotypes were planted in a 5 by 3 α-lattice design with two replications. In this paper, this experiment is referred to as "GHIST," since it studies a historical set of commercial genotypes.

Experiment 2 (RILs1): This experiment had 25 genotypes, comprising 23 RILs and the two parents ('Sonalika' and 'Attila'). The RILs were random F_3–derived F_6 and F_7 lines. All plants in the F_2 were bulked. The experiment was planted in a 5 by 5 α-lattice design with two replications. In this paper, this experiment is designated as "RILs1."

Experiment 3 (RILs2): This experiment consisted of a total of 36 genotypes, comprising 34 RILs and the two parents ('Bacanora88' and 'Cndo/R143//Ente/Mexi2/3/Ae. sq.(Taus)/4/Weaver). The RILs were developed in the same procedure as described for RILs1. The experiment was planted in a 6 by 6 α-lattice design with two replications. In this paper, this experiment is called "RILs2."

Radiometric Measurements

The spectral reflectance measurements were taken by a portable narrow-bandwidth Spectroradiometer (Model FieldSpec UV/VNIR, Analytical Spectral Devices, Boulder, CO) with 25° field of view. This instrument can detect reflected light from the canopy ranging from 350 to 1100 nm. Therefore, it covers the visible and part of the NIR portion of the spectrum. The Spectroradiometer gave 512 continuous bands with a sampling interval of 1.43 nm. The Spectroradiometer was connected to a computer, which stored the individual scans for subsequent processing. Each reflectance measurement was the average of 10 scans from an area of 18.94 cm^2 of the plot. The sensor was mounted with the help of a pistol grip 40 to 50 cm above the canopy facing the center of the bed. The Spectroradiometer was calibrated against a white reference plate ($BaSO_4$) after collecting spectral reflectance readings from every 10th plot. The reflectance measurements were taken between 1030 to 1330 h under sunny conditions, and from four different places randomly within each plot. The mean of those four readings were used to calculate the spectral indices.

The spectral reflectance measurements were taken at booting (Zadoks stage 39–47), heading (Zadoks stage 55–69), and grainfilling (Zadoks stage 75–83) in the GHIST experiment for 3 yr (years 2001–2002, 2002–2003, and 2003–2004) and in RILs1 and RILs2 for 1 yr (year 2003–2004) (Zadoks et al., 1974). For the RILs1 and RILs2 in year 2002–2003 the readings were only taken at booting and heading, and not at grainfilling. The genotypes varied among themselves for their growth stages. So the reflectance measurements were taken in different genotypes in different times coinciding with closest possible day to the respective growth stages.

Harvest of Biomass

For harvesting biomass, all the plants in a 0.5-m-long area were cut at soil level from one of the two beds of each plot, and harvested area for biomass was 0.4 m^2 (0.5 by 0.8 m). The spectral reflectance data were taken randomly before harvesting biomass. The biomass cut was done randomly from the middle 3 m of the plot (1-m areas were excluded from both ends of the plot to avoid any border effect) on the same or the closest possible day coinciding with the spectral reflectance measurements. After harvesting of biomass, total fresh weight was taken and a representative sample was oven-dried at 75°C for 48 h. The oven-dried weight of biomass was recorded and total dry weight of biomass was estimated from total fresh weight and converted into grams per square meter. Biomass was sampled three times at booting, heading, and grainfilling in the GHIST experiments in both years (2002–2003 and 2003–2004), and two times (booting and heading) and three times (booting, heading, and grainfilling) for the other two experiments (RILs1 and RILs2) in the years 2002–2003 and 2003–2004, respectively.

Chlorophyll Meter Measurements

A handheld portable SPAD-502 chlorophyll meter (Minolta, Tokyo, Japan) was used to estimate chlorophyll concentration. This instrument provides a convenient means of assessing relative leaf chlorophyll concentration. Fifteen flag leaves were used to take chlorophyll meter readings from each plot at three growth stages (booting, heading, and grainfilling) in the GHIST experiment for 3 yr, and the data presented are the means of the 15 readings for each plot. Chlorophyll meter data were taken on the same day or the closest possible day coinciding with the spectral reflectance measurements.

Calculation and Selection of Indices

Initially, different ratios and normalized SRI were calculated using a combination of different visible and NIR wavelengths. Based on these results, two indices (based on NIR) were selected for presentation in this paper. The two indices combined information from 850, 900, and 970 nm. The 970 nm has been reported as a weak water absorption band (Peñuelas et al., 1993), and the other two bands (850 and 900 nm) were used as reference bands to normalize 970 nm. We have referred to these two indices as normalized water index-1 (NWI-1) and normalized water index-2 (NWI-2). Seven other reference indices, including the most widely used NDVI and SR to assess different physiological parameters, were also calculated. The different SRI, which were calculated based on different references, are provided in the Table 1. The above mentioned normalized water indices were calculated as follows:

$$\text{NWI-1} = (R_{970} - R_{900})/(R_{970} + R_{900}), \text{ and}$$
$$\text{NWI-2} = (R_{970} - R_{850})/(R_{970} + R_{850}).$$

'R' and the subindex in the above formulae indicate the reflectance of light at that specific wavelength (in nm).

Canopy Temperature

A handheld infrared thermometer (Model AG-42, Telatemperature Crop, Fullerton, CA), with a field of view of 2.5°, was used to measure CT (°C). The data were taken from the same side of each plot at 1-m distance from the edge and approximately 50 cm above the canopy at an angle of 30° to the horizontal. Readings were made between 1300 and 1500 h on sunny days. To avoid the effect of soil temperature on the CT, the data were taken when the infrared thermometer viewed no soil because of high leaf coverage areas. The CT measurements were taken at three different growth stages (booting, heading, and grainfilling) in the year 2003–2004, only at grainfilling in year 2001–2002 and year 2002–2003 in the GHIST experiment, and two times (booting and grainfilling) in RILs1 and RILs2 in the year 2002–2003 and 2003–2004. The data for each plot are the means of two to four sets of readings.

Statistical Analysis

Alpha-lattice analyses for biomass, different SRI, SPAD-chlorophyll meter readings, and RARS and pigment-specific simple ratio chlorophyll-a (PSSRa) indices (combined across different years) were performed using the MIXED procedure

Table 1. The formulae, functions, and references of different previously developed spectral reflectance indices, which were used in these studies.

Spectral reflectance indices	Formula†	Function	References
Normalized difference vegetation index (NDVI)	$(R_{780} - R_{670})/(R_{780} + R_{670})$	Estimation of canopy photosynthetic area	Raun et al., 2001
Simple ratio (SR)	R_{900}/R_{680}	Estimation of canopy photosynthetic area	Aparicio et al., 2000
Water index (WI)	R_{970}/R_{900}	Canopy water status	Peñuelas et al., 1993
Ratio analysis of reflectance spectra (RARSa)	R_{675}/R_{700}	Estimation of chlorophyll-a	Chappelle et al., 1992
Ratio analysis of reflectance spectra (RARSb)	$R_{675}/(R_{650}R_{700})$	Estimation of chlorophyll-b	Chappelle et al., 1992
Ratio analysis of reflectance spectra (RARSc)	R_{760}/R_{500}	Estimation of carotenoids	Chappelle et al., 1992
Pigment specific simple ratio (PSSRa)	R_{800}/R_{680}	Chlorophyll-a	Blackburn, 1998

† R and the subindex indicate the reflectance of light at that specific wavelength (in nm).

of the SAS software (SAS Institute, 2001). Phenotypic and genetic correlations were used to estimate the relationship between biomass and different spectral indices, between SPAD chlorophyll meter readings and RARS and PSSRa indices, and between CT and NIR-based indices at different growth stages within the year and averaged over different growth stages within the year. These correlations were estimated using the SAS software (SAS Institute, 2001).

RESULTS

Genotypic Variations

The means (±SE) of five different SRI at three different growth stages are presented in Table 2. Spectral reflectance indices based on red and near infrared radiation (NDVI and SR) had genotype effects at all growth stages in all three experiments and years. Genotypic effects also were significant for NIR-based SRI (WI, NWI-1, and NWI-2) in most of the growth stages and years.

Table 3 indicates that the genotypes varied for the SRI used to estimate chlorophyll and carotenoids concentration and SPAD values at all three growth stages across three different years in the GHIST experiment. Table 4 provides the mean fresh and dry biomass values (±SE) harvested at three different growth stages (booting, heading and grainfilling) in the three different experiments in 2 yr. Genotypic variation was significant for both fresh and dry biomass in all growth stages in all experiments.

Effects of Growth Stages

The NIR-based indices (WI, NWI-1, and NWI-2) (averaged across genotypes) decreased from the booting stage to the heading stage, and increased again at the grainfilling stage (Table 2 and Fig. 1a). The highest indices values were generally observed at the booting stage, and the lowest at the heading stage. The other two indices (NDVI and SR) generally gave similar values at the booting and heading stages, and then decreased with grainfilling. (Table 2 and Fig. 1a,1b).

The values for chlorophyll concentration (SPAD values) were similar at booting and heading stages, but decreased at the grainfilling stage (Table 3). The values for ratio analysis of reflectance spectra–chlorophyll-a (RARSa) increased as the growth stage advanced, while pigment specific simple ratio-chlorophyll-a showed a reciprocal effect, even though both indices measure chl-a concentration in the plant. The ratio analysis of reflectance spectra–chlorophyll-b (RARSb) and ratio analysis of reflectance spectra–carotenoids (RARSc) values decreased marginally from booting to heading, and then decreased sharply from heading to grainfilling (Table 3). The trend for changes in RARS and PSSRa values followed the changes in SPAD values at the different growth stages (Fig. 2).

The genotypic mean value for dry biomass increased as the growth cycle progressed from booting to grainfilling in all the experiments (Table 4). The mean bio-

Table 2. Mean (±SE) of normalized difference vegetation index (NDVI), simple ratio (SR), water index (WI), normalized water index-1 (NWI-1), and normalized water index-2 (NWI-2) at different growth stages in the three experiments in two different years.

			Spectral reflectance indices				
Experiments	Year	Growth stages	NDVI	SR	WI	NWI-1	NWI-2
GHIST	2003–2004	Booting	0.933 ± 0.005**	29.3 ± 2.9**	0.871 ± 0.007**	−0.068 ± 0.005**	−0.066 ± 0.004**
		Heading	0.930 ± 0.006**	28.3 ± 2.4**	0.830 ± 0.007**	−0.093 ± 0.004**	−0.092 ± 0.004**
		Grainfilling	0.809 ± 0.02**	10.6 ± 1.5**	0.855 ± 0.006**	−0.079 ± 0.003**	−0.067 ± 0.004**
	2002–2003	Booting	0.944 ± 0.004**	34.7 ± 2.9**	0.874 ± 0.006**	−0.067 ± 0.004**	−0.058 ± 0.004**
		Heading	0.913 ± 0.008**	22.1 ± 2.3**	0.861 ± 0.008**	−0.075 ± 0.005**	−0.070 ± 0.005**
		Grainfilling	0.868 ± 0.008**	14.6 ± 1.1**	0.869 ± 0.009**	−0.069 ± 0.005**	−0.062 ± 0.006**
RILs1	2003–2004	Booting	0.887 ± 0.011*	17.7 ± 1.9**	0.898 ± 0.008**	−0.053 ± 0.005**	−0.049 ± 0.005**
		Heading	0.909 ± 0.010**	21.5 ± 2.2**	0.839 ± 0.005**	−0.088 ± 0.006**	−0.083 ± 0.005**
		Grainfilling	0.754 ± 0.021**	7.5 ± 0.51**	0.877 ± 0.006**	−0.066 ± 0.004**	−0.054 ± 0.003**
	2002–2003	Booting	0.911 ± 0.006**	21.9 ± 1.7*	0.887 ± 0.01	−0.059 ± 0.005	−0.066 ± 0.004
		Heading	0.894 ± 0.025	18.3 ± 3.8	0.865 ± 0.013	−0.072 ± 0.006	−0.066 ± 0.006
RILs2	2003–2004	Booting	0.928 ± 0.005*	26.5 ± 1.8**	0.867 ± 0.005**	−0.071 ± 0.003**	−0.068 ± 0.003**
		Heading	0.924 ± 0.004**	25.2 ± 1.7**	0.815 ± 0.008**	−0.101 ± 0.005**	−0.099 ± 0.005**
		Grainfilling	0.713 ± 0.043**	6.61 ± 1.0**	0.877 ± 0.01**	−0.066 ± 0.006**	−0.050 ± 0.007**
	2002–2003	Booting	0.926 ± 0.006*	25.7 ± 2.3*	0.855 ± 0.014*	−0.078 ± 0.006*	−0.074 ± 0.006*
		Heading	0.910 ± 0.006**	21.1 ± 1.7**	0.854 ± 0.009**	−0.079 ± 0.004**	−0.079 ± 0.006**

* Significant at $P = 0.05$.
** Significant at $P = 0.01$.

Table 3. Mean (±SE) of chlorophyll concentration (SPAD values), ratio analysis of reflectance spectra-chlorophyll a (RARSa), pigment specific simple ratio-chlorophyll a (PSSRa), ratio analysis of reflectance spectra-chlorophyll b (RARSb), and ratio analysis of reflectance spectra-carotenoids (RARSc) at three individual growth stages across 3 yr in the GHIST experiment.

Indices	Growth stages		
	Booting	Heading	Grainfilling
CHL(SPAD unit)	45.9 ± 1.59**	46.1 ± 1.37**	38.5 ± 2.02**
RARSa	0.374 ± 0.012*	0.429 ± 0.012**	0.565 ± 0.017**
PSSRa	27.5 ± 2.215*	23.3 ± 3.042**	12.5 ± 0.963**
RARSb	17.8 ± 2.04*	17.0 ± 1.91**	11.4 ± 1.11**
RARSc	24.4 ± 1.91*	19.8 ± 1.53*	10.2 ± 0.883**

* Significant at $P = 0.05$.
** Significant at $P = 0.01$.

mass at the three different growth stages in the GHIST experiment (year 2003–2004) were plotted against the values of the three different indices (NDVI, WI, and SR) in Fig. 1. This figure indicates that although the biomass accumulation increased with advanced growth stage, the values for NDVI and SR decreased. The value for WI decreased with the increase of biomass from booting to flowering, but increased at the grainfilling stage with the increase in biomass.

Correlations between Dry Biomass and SRI

Spectral reflectance indices showed a significant phenotypic correlation with dry biomass at the heading and grainfilling stages (Table 5). Near infrared radiation–based indices were negatively correlated with dry biomass, while NDVI and SR were positively correlated. NDVI and SR were uncorrelated with dry biomass at the booting stage, but NIR-based indices (WI, NWI-1, and NWI-2) were consistently correlated with dry biomass with a single exception.

Phenotypic correlations increased between the indices and dry biomass as the crop transitioned from vegetative to reproductive stages. While dry biomass and NIR-based indices were correlated at the booting stage, the coefficients were lower than at the heading and grainfilling stages, except for the GHIST experiment in the year 2003–2004. Correlation coefficients calculated between dry biomass and NIR-based indices increased using values averaged over growth stages rather than correlation between dry biomass and NIR-based indices in individual growth stages, with a single exception. Consequently, very strong correlation among genotypes (GHIST) and genetic correlation (RILs1 and RILs2) were obtained between dry biomass and all indices when those correlations were calculated from values averaged over three growth stages within each year.

The relationships (phenotypic) between dry biomass and NDVI, SR and one of the NIR-based indices (NWI-2) are presented in a simple linear model (Fig. 3), where correlation coefficients were calculated from the values averaged over all the growth stages and years for biomass and SRI. The NIR-based index (NWI-2, Fig. 3c) explained a greater proportion of the variability than the other two indices (NDVI and SR) (Fig. 3a,3b). Considering all the phenotypic and genetic correlations in all experiments in all growth stages, NIR-based indices gave a higher predictive capacity to differentiate genotypes for total dry biomass than either NDVI or SR.

Correlations between Dry Biomass and SPAD Values, RARSa, PSSRa, RARSb, RARSc

Chlorophyll concentration (SPAD values), PSSRa, RARSb, and RARSc gave positive phenotypic and genetic correlations, while RARSa showed negative phenotypic and genetic correlations with dry biomass (Table 6). The correlation coefficients increased with advancement in growth stage, and all indices had the greatest phenotypic correlations with dry biomass at the grainfilling stage. PSSRa, RARSb, and RARSc also had positive phenotypic correlations with biomass at the heading stage. When comparing all indices for their correlation with biomass (phenotypic correlation and correlation among genotypes), PSSRa, RARSb, and RARSc had a higher capacity to differentiate genotypes for biomass production than chlorophyll concentration (SPAD unit) and RARSa.

Correlation between SPAD Values and RARSa, PSSRa, RARSb, and RARSc

The phenotypic correlations and the correlation among genotypes between chlorophyll concentration (SPAD unit) and other spectral reflectance based indices to estimate the concentration of chlorophyll and carotenoids (RARSa, PSSRa, RARSb, and RARSc) are presented in Table 7. In general, PSSRa, RARSb and RARSc showed significant phenotypic correlations with the SPAD unit values at the heading and grainfilling stages. The highest level associations were observed at

Table 4. Mean (±SE) of fresh weight (FW) and dry weight (DW) of biomass at three individual growth stages in three different experiments in two different years.

Year	Growth stages	GHIST		RILs1		RILs2	
		FW	DW	FW	DW	FW	DW
				g m^{-2}			
2003–2004	Booting	2912 ± 122*	505 ± 24*	2281 ± 242**	392 ± 40**	2664 ± 317**	454 ± 49**
	Heading	3946 ± 313**	915 ± 62**	2861 ± 287**	740 ± 68**	3826 ± 253**	946 ± 56**
	Grainfilling	3471 ± 148**	1247 ± 48**	2716 ± 278**	1075 ± 77**	3060 ± 330**	1224 ± 132**
2002–2003	Booting	2133 ± 220*	433 ± 48*		291 ± 53*		412 ± 33*
	Heading	2820 ± 162**	1058 ± 100**	2909 ± 229*	587 ± 53*	3322 ± 220**	666 ± 54**
	Grainfilling	2875 ± 256**	1403 ± 89**				

* Significant at $P = 0.05$.
** Significant at $P = 0.01$.

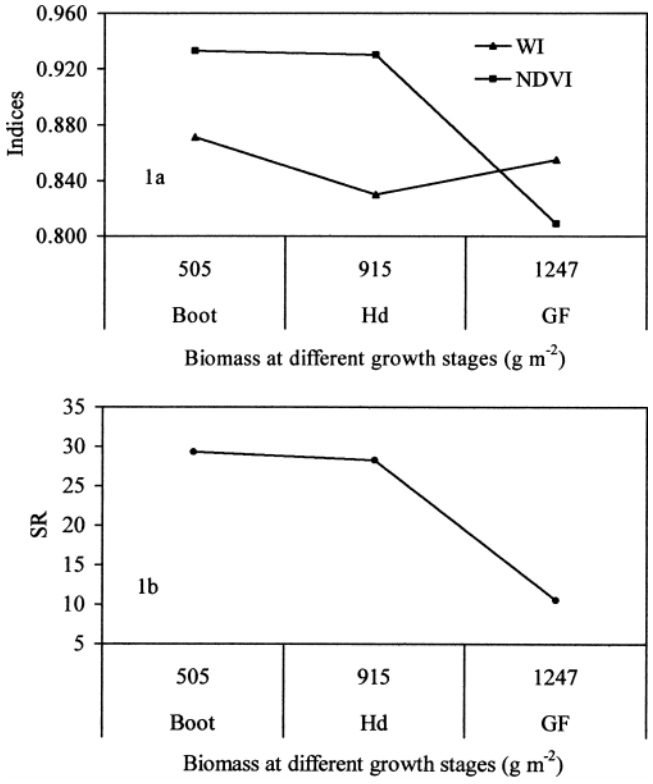

Fig. 1. The changes in values of normalized difference vegetation index (NDVI), water index (WI), and simple ratio (SR) with an increase in biomass and with the advancement of growth stages in experiment GHIST in year 2003–2004. Boot, Hd, and GF indicate the biomass and spectral indices were measured at booting, heading, and grainfilling.

the grainfilling stage. The combined correlation analysis between SPAD values and RARSa, PSSRa, RARSb, and RARSc also demonstrated that the association was higher at the generative growth stage than at the vegetative growth stage. Strong correlation among genotypes was also observed between the SPAD unit and PSSRa, RARSb, and RARSc, when the correlations were calculated from the values averaged over three growth stages. Linear relationships (phenotypic) were significant between the SPAD unit and PSSRa (Fig. 4a), RARSb (Fig. 4b), and RARSc (Fig. 4c) across three different years at the grainfilling stage. The model explained more than 50% of the variation between SPAD unit and PSSRa, RARSb, and RARSc. RARSa demonstrated poor phenotypic correlation and correlation among genotypes with chlorophyll concentration (SPAD values).

Correlations between Canopy Temperature and NIR-Based Indices

Table 8 demonstrates the phenotypic correlations between CT and NIR-based indices (WI, NWI-1, and NWI-2), and the phenotypic correlation and genetic correlations between CT and NIR-based indices (with correlation coefficients calculated based on the values averaged over years and growth stages). All the NIR-based indices gave significant positive phenotypic correlations with CT when the relationship was tested at the heading and grainfilling stages. The relationship was much stronger at the grainfilling stage than at the booting stage. A high level of genetic correlation (RILs1 and RILs2) and correlation among the genotypes (GHIST) also existed between CT and the NIR-based indices.

DISCUSSION

Genotypic Variations

Statistically significant genotypic variation for different SRI have been reported previously in spring wheat under well-watered conditions at the grainfilling stage (Ball and Konzak, 1993), and in durum wheat under rainfed conditions at different growth stages (Aparicio et al., 2002). Both studies were conducted by using advanced, unrelated breeding genotypes. In this study, we used 15 widely adapted and unrelated CIMMYT wheat genotypes, and 25 and 36 RILs from two different crosses. The globally adapted genotypes expressed high variation for morphological traits such as green leaf area duration, height, plant type, heading and maturity dates, and yield potential. While the genotypic variations within the GHIST experiment were relatively large, the SRI were predictive of the performance of the various genotypes. The other two sets of experimental materials (RILs) were derived from single crosses and had less variation for morphological traits than that possessed by the genotypes in GHIST experiment. The genotypic variation for SRI found among RILs and its association with yield signify that these indices can be used to differentiate among breeding materials despite relatively low levels of morphological variation, and hence may have application in culling within early generations before breeding materials reach the expensive yield trial stage. The SRI that were developed based on NIR wavelengths expressed nonsignificant variation on some occasions. This result was accentuated by poor germination and a lack of soil coverage in one of the experiments, namely RILs1 in 2002–2003, which caused

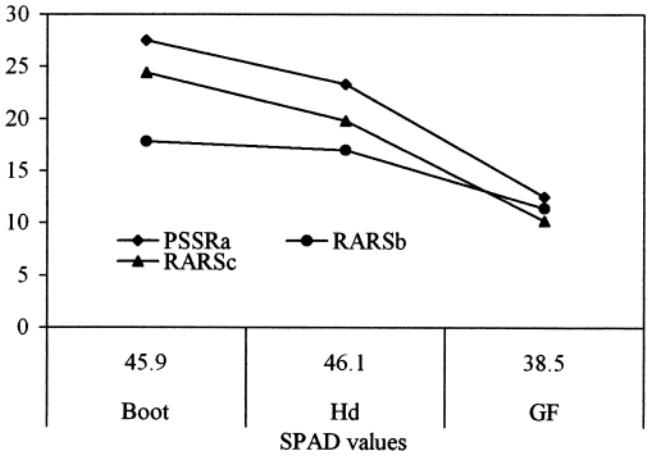

Fig. 2. The trends of changes in pigment specific simple ratio-chlorophyll-a (PSSRa), ratio analysis of reflectance spectra-chlorophyll-b (RARSb), and ratio analysis of reflectance spectra-carotenoids (RARSc) with the changes of values of SPAD in three different growth stages. Boot, Hd, and GF indicate the measurements were taken at booting, heading, and grainfilling.

Table 5. The phenotypic (r_p) and genetic correlations (r_g) between biomass and different spectral reflectance indices at three different growth stages in different experiments over a two-year period.

		Year 2003–2004					Year 2002–2003				
		r_p				r_g	r_p				r_g
Experiment	Indices†	Booting	Heading	Grainfilling	Mean‡	Mean§	Booting	Heading	Grainfilling	Mean£	Mean‡‡
GHIST	NDVI	0.526	0.534*	0.662**	0.788**	0.777**	−0.165	0.578*	0.681**	0.657**	0.836**
	SR	0.494	0.596*	0.622*	0.862**	0.897**	−0.097	0.614*	0.693**	0.721**	0.898**
	WI	−0.700**	−0.592*	−0.597*	−0.903**	−0.932**	−0.552*	−0.631**	−0.736**	−0.757**	−0.821**
	NWI-1	−0.712**	−0.585*	−0.600*	−0.903**	−0.938**	−0.559*	−0.632**	−0.738**	−0.760**	−0.825**
	NWI-2	−0.801**	−0.604*	−0.563*	−0.883**	−0.921**	−0.629**	−0.566*	−0.800**	−0.737**	−0.806**
RILs1	NDVI	0.187	0.787**	0.654**	0.763**	0.843**	0.060	−0.133		0.218	0.385
	SR	0.079	0.791**	0.697**	0.843**	0.880**	0.073	−0.020		0.343	0.529**
	WI	−0.531**	−0.713**	0.704**	−0.762**	−0.706**	0.007	−0.269		−0.158	−0.023
	NWI-1	−0.531**	−0.696**	0.691**	−0.764**	−0.708**	0.100	−0.258		−0.009	−0.234
	NWI-2	−0.588**	−0.713**	0.692**	−0.764**	−0.715**	0.102	−0.170		−0.047	−0.013
RILs2	NDVI	0.216	0.504**	0.453*	0.445*	0.414*	0.111	0.530**		0.512**	0.497**
	SR	0.158	0.487**	0.464*	0.487**	0.561**	0.095	0.514**		0.489**	0.435*
	WI	−0.541**	−0.697**	−0.562**	−0.700**	−0.744**	−0.315*	−0.680**		−0.706**	−0.867**
	NWI-1	−0.517**	−0.694**	−0.564**	−0.699**	−0.751**	−0.310*	−0.679**		−0.695**	−0.837**
	NWI-2	−0.563**	−0.700**	0.599**	−0.693**	−0.733**	−0.330*	−0.694**		−0.729**	−0.887**

* Significant at $P = 0.05$.
** Significant at $P = 0.01$.
† NDVI, normalized difference vegetation index; SR, simple ratio; WI, water index; NWI-1, normalized water index-1; NWI-2, normalized water index-2.
‡ Phenotypic correlation coefficients calculated from the values averaged over growth stages within year.
§ Genetic correlation coefficients calculated from the values averaged over growth stages within year.

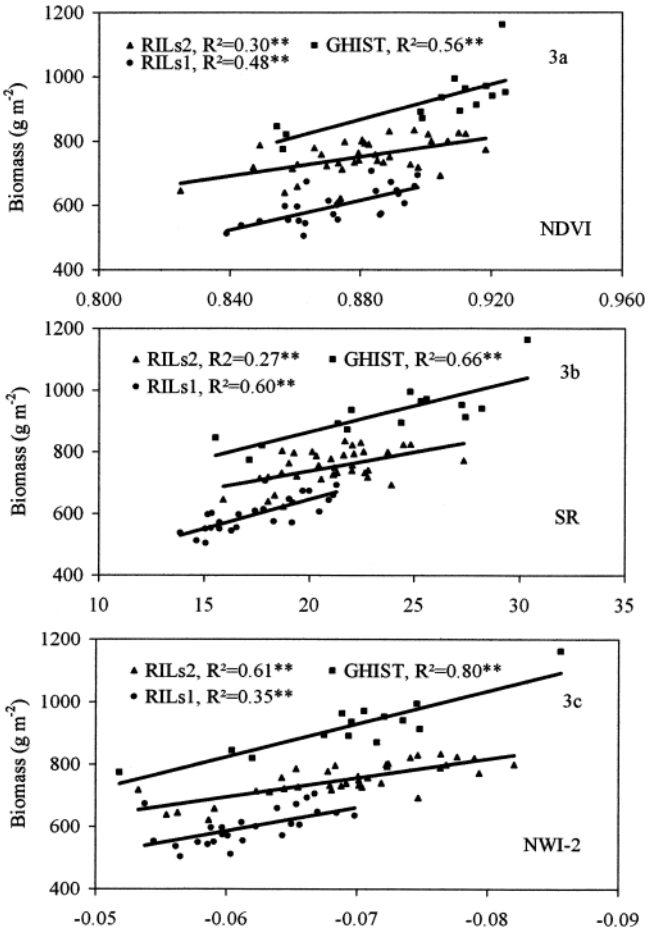

Fig. 3. The relationship between biomass and normalized difference vegetation index (NDVI), simple ratio (SR), and normalized water index-2 (NWI-2) in three different experiments, with the correlation coefficients calculated from the averages over 2 yr for biomass and spectral reflectance indices. ** Significant at $P = 0.01$.

interference in the spectral reflectance measurements (Table 5). Elliott and Regan (1993) previously reported that NIR wavelengths might be affected by soil reflectance due to reduced soil coverage.

Large genotypic variation for biomass production, which is evident in our study at various growth stages, has also been reported by other authors in spring wheat (Calderini et al., 1997) and in durum wheat (*Triticum turgidum* L. var. *durum*) (Aparicio et al., 2002).

Effect of Growth Stages

Younger wheat plants generally absorb more photosynthetically active radiation, and therefore, reflect more NIR. As the plants progress in growth stage, new tissue is formed (until all tillers have reached flag leaf emergence stage), but also older green tissue loses chlorophyll concentration, turns chlorotic and then necrotic. The latter increases the reflectance of the visible wavelengths and decreases reflectance at the NIR wavelengths. Normalized difference vegetation index and SR, which were developed based on the red (visible) and NIR wavelengths, give higher values at the early growth stages, but their values decrease with the advancement in growth cycle because the plants are losing photosynthetically active plant parts. This phenomenon is evident in our study and has also been reported by other researchers (Aparicio et al., 2000). Modern wheat genotypes reach maximum leaf area index at booting under irrigated conditions (Calderini et al., 1997) and subsequently the lower leaves senesce because of chlorophyll loss. Fischer et al. (1998) also found a decreasing photosynthetic rate with advanced growth stage under the same environmental conditions where the present study was conducted.

The NIR-based indices (WI, NWI-1 and NWI-2) were calculated combining information from 850, 900, and 970 nm. The 970-nm wavelength is a water absorption band and the sensitivity of this band depends on the

Table 6. The phenotypic correlation (r_p) and correlation among genotypes (r_g) between biomass and chlorophyll concentration (SPAD values), ratio analysis of reflectance spectra-chlorophyll a (RARSa), pigment specific simple ratio-chlorophyll a (PSSRa), ratio analysis of reflectance spectra-chlorophyll b (RARSb), and ratio analysis of reflectance spectra-carotenoids (RARSc) at three different growth stages in two different years in experiment GHIST.

		Growth stages				
		r_p				r_g
Year	Indices	Booting	Heading	Grainfilling	Mean†	Mean‡
2003–2004	CHL	0.271	0.503	0.672**	0.641**	0.547*
	RARSa	−0.052	−0.480	−0.594*	−0.675**	−0.753**
	PSSRa	0.377	−0.547*	0.661**	0.785**	0.801**
	RARSb	0.619*	0.385	0.558*	0.863**	0.899**
	RARSc	0.410	0.566*	0.643**	0.829**	0.843**
2002–2003	CHL	0.057	0.184	0.532*	0.273	0.318
	RARSa	0.319	−0.256	0.087	−0.250	−0.214
	PSSRa	0.167	0.418	0.646**	0.662**	0.681**
	RARSb	0.058	0.597*	0.612*	0.757**	0.864**
	RARSc	−0.099	0.658**	0.601*	0.703**	0.732**

* Significant at $P = 0.05$.
** Significant at $P = 0.01$.
† Phenotypic correlation calculated from the values averaged over three growth stages within a year.
‡ Correlation among genotypes was calculated from the values averaged over three growth stages within a year.

extent of penetration of radiation into the canopy, thus reflectance is more dependent on the total water content (Bull, 1991). Moreover, WI is influenced by leaf area index, and therefore by the total biomass (Royo et al., 2003). Jackson and Pinter (1986) found that the architecture of the plant canopy affects the direction of reflection of incident radiation. Near infrared radiation reflectance decreases with a change in leaf orientation, predominantly from horizontal to vertical at certain stages in the growth cycle (Jackson and Erza, 1985). The three NIR-based indices used in this study incorporated the water status of the canopy, and the index values decreased from booting to heading and increased again from heading to grainfilling. The lower the value, the higher the amount of water that is retained by the canopy. The NIR penetrated more into the canopy at the heading stage because of the fully extended flag leaf. Moreover because of the higher total water content (fresh weight minus dry weight, Table 4) at that growth stage, more energy was absorbed from the radiation that penetrated into the canopy. The combination of these two factors contributed to the reduction in indices values from the booting to the heading stage. The indices values increased as the total water content decreased (Table 4), but the penetration of the NIR wavelength was still high because of the fully extended flag leaf at

Table 7. The phenotypic correlation (r_p) and correlation among genotypes (r_g) between SPAD values and ratio analysis of reflectance spectra-chlorophyll a (RARSa), pigment specific simple ratio-chlorophyll a (PSSRa), ratio analysis of reflectance spectra-chlorophyll b (RARSb), and ratio analysis of reflectance spectra-carotenoids (RARSc) at three different growth stages in three different years in the experiment GHIST.

			Indices			
Year		Growth stages	RARSa	PSSRa	RARSb	RARSc
2003–2004	r_p	Booting	0.317	0.274	0.521*	0.239
		Heading	−0.359	0.777**	0.610*	0.760**
		Grainfilling	−0.841**	0.936**	0.823**	0.928**
		Combine1†	−0.820**	0.834**	0.619**	0.821**
		Combine2‡	−0.885**	0.872**	0.858**	0.878**
		Mean§	−0.601*	0.895**	0.811**	0.780**
	r_g	Mean¶	−0.660**	0.947**	0.906**	0.825**
2002–2003	r_p	Booting	0.473	−0.020	0.278	0.014
		Heading	0.054	0.185	0.350	0.153
		Grainfilling	0.034	0.669**	0.381	0.444
		Combine1†	0.438**	−0.258	0.194	−0.264
		Combine2‡	0.068	0.302	0.313	0.252
		Mean§	−0.033	0.373	0.429	0.383
	r_g	Mean¶	−0.02	0.371	0.470	0.395
2001–2002	r_p	Booting	0.341	0.078	0.225	−0.004
		Heading	0.187	0.595*	0.670**	0.471
		Grainfilling	0.106	0.881**	0.876**	0.831**
		Combine1†	−0.475**	0.707**	0.279	0.681**
		Combine2‡	−0.361*	0.884**	0.413*	0.828**
		Mean§	0.117	0.751**	0.672**	0.606*
	r_g	Mean¶	0.268	0.608*	0.787**	0.563*

* Significant at $P = 0.05$.
** Significant at $P = 0.01$.
† Phenotypic correlation across three (booting, heading, and grainfilling) growth stages.
‡ Phenotypic correlation across two (heading and grainfilling) growth stages.
§ Phenotypic correlation calculated from values averaged over three growth stages within a year.
¶ Correlation among genotypes calculated from values averaged over three growth stages within a year.

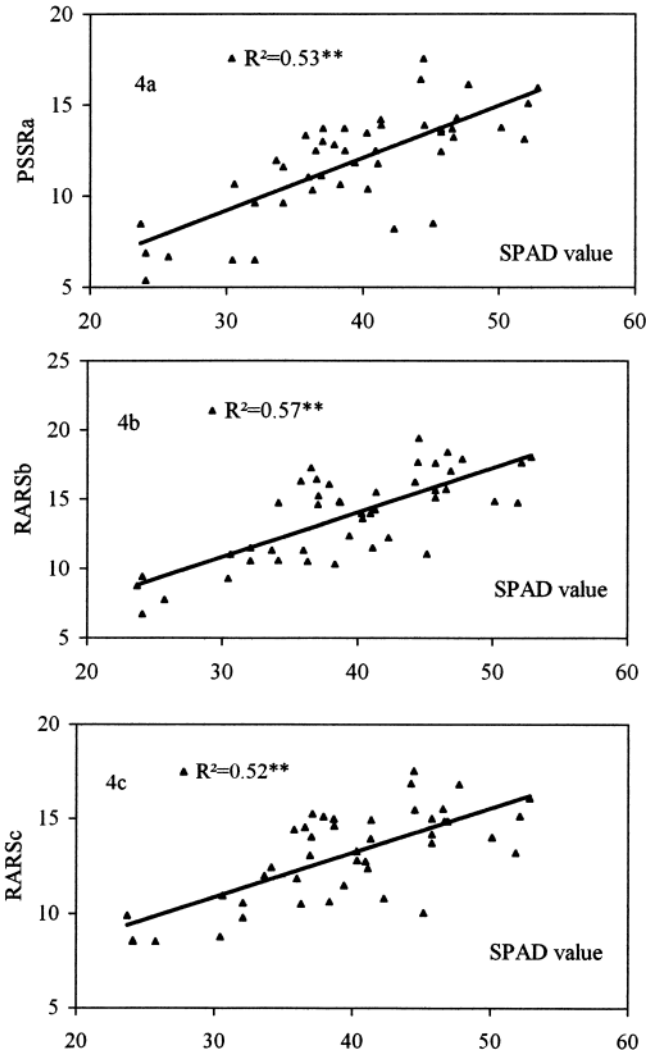

Fig. 4. Relationship between chlorophyll content (SPAD values) and pigment specific simple ratio-chlorophyll a (PSSRa), ratio analysis of reflectance spectra-chlorophyll b (RARSb), and ratio analysis of reflectance spectra-carotenoids (RARSc) across 3 yr in experiment GHIST at grainfilling stage. ** Significant at $P = 0.01$.

the grainfilling stage, which contributed to a lower value for the NIR-based indices at the grainfilling stage compared to the booting stage.

The SPAD and other visible wavelength based spectral indices (PSSRa, RARSb and RARSc) gave the highest values at the booting stage and maintained a similar value at the heading stage. However, the values decreased sharply at the grainfilling stage. RARSa gave the lowest value at the booting stage and the index was calculated by using the formula $R_{675}/R_{700.}$ Because of the division of R_{675} by R_{700}, lower values reflect a high concentration of chlorophyll-a. Therefore, chlorophyll-a values decrease with plant development. Modern wheat varieties possess the highest leaf area index at or near the booting stage (Calderini et al., 1997), and afterward the lower leaves start to senesce. The decrease in indices values (based on different leaf pigments absorption bands) after booting is largely due to the loss of green plant tissue.

Biomass accumulation increased with advances in the growth cycle. The maximum accumulated dry biomass was at the grainfilling stage in all experiments. The increase in biomass under irrigated conditions as growth stage progresses is logical and was also reported by Jackson and Pinter (1986) in spring wheat and Aparicio et al. (2000) in durum wheat.

Correlation between Dry Biomass and SRI

The spectral indices based on NIR (WI, NWI-1, and NWI-2) generally showed a negative significant correlation with the dry biomass, and the correlation coefficients increased in value with an advance in growth stage. The highest correlation occurred either at the heading or the grainfilling stage. These indices are indicators of water status in the canopy; the lower the values, the greater the amount of water retained by the canopy. Therefore, negative correlations indicate a canopy with higher amounts of water and this corresponds to higher biomass production. The strong genetic correlation (RILs1 and RILs2) and correlation among genotypes (GHIST) between dry biomass production and the NIR-based indices (averaged over three growth stages) indicate that this relationship can be mainly attributed to the genotypes, and not to environmental factors.

A further correlation analysis was done to better understand the relationship between biomass and NIR-based indices. Canopy water content was estimated by deducting dry biomass from fresh biomass values (Ahlrichs and Bauer, 1983). The correlations between water content and dry biomass, and between water content and the NIR-based indices were subsequently calculated (data not presented). In general, a strong correlation was found between water content and dry biomass ($r = 0.708$–0.952 in the different experiments). The correlation between water content and NIR-based indices ranged from -0.582 to -0.904 when estimated at heading and at grainfilling in the different experiments. Generally, the relationship between water content and NIR-based indices was higher at the reproductive growth stages than at the vegetative growth stages. These three-way associations between water content and NIR-based indices, and between water content and dry biomass has contributed indirectly to the association between biomass and NIR-based indices, and to the higher association in the reproductive growth stages than in the vegetative growth stages.

The superiority in performance among these three NIR-based indices was indistinguishable. Normalizing the WI (NWI-1 and NWI-2) did not improve the relationship. However, Tucker (1979) showed the superiority of a normalized index over a ratio index under water-stressed conditions. The normalization partially removed the disturbance caused by external factors such as soil interference, position of sun, illumination, and angle of view. Our study was done under irrigated conditions where the plots were more uniform and complete ground cover was achieved in the early growth stages, which minimized the interference from the soil. A constant field of view (25°) was used throughout the experimental cycle and the data were taken in the middle

Table 8. The phenotypic (r_p) and genetic (r_g) correlations between canopy temperature and water index (WI), normalized water index-1 (NWI-1), and normalized water index-2 (NWI-2) at three different growth stages in three different experiments in two different years.

				NIR-based indices		
Experiment		Year	Growth stage	WI	NWI-1	NWI-2
GHIST	r_p	2003–2004	Booting	−0.246	−0.238	−0.141
			Heading	0.732**	0.715**	0.728**
			Grainfilling	0.902**	0.896**	0.895**
		2002–2003	Grainfilling	0.821**	0.825**	0.785**
		2001–2002	Grainfilling	0.807**	0.815**	0.763**
		Mean†		0.775**	0.768**	0.741**
	r_g	Mean‡‡		0.849**	0.836**	0.824**
RILs1	r_p	2003–2004	Booting	0.233	0.203	0.189
			Grainfilling	0.756**	0.763**	0.794**
		2002–2003	Booting	0.489*	0.348	0.282
			Grainfilling	0.292	0.283	0.295
		Mean†		0.601**	0.596**	0.576**
	r_g	Mean‡‡		0.329	0.368	0.508**
RILs2	r_p	2003–2004	Booting	−0.265	−0.256	−0.192
			Grainfilling	0.670**	0.676**	0.634**
		2002–2003	Booting	0.498**	0.490**	0.500**
			Grainfilling	0.699**	0.699**	0.700**
		Mean†		0.646**	0.647**	0.669**
	r_g	Mean‡‡		0.766**	0.762**	0.804**

* Significant at $P = 0.05$.
** Significant at $P = 0.01$.
† Phenotypic correlation calculated from values averaged over years and growth stages.
‡‡ Genetic correlation calculated from values averaged over years and growth stages.

of the day (1030 to 1330 h) under cloud-free conditions. These circumstances resulted in a minimal advantage for the normalized value over the ratio index, and a high correlation with all three indices made it difficult to determine that any specific one was superior. Similarly Royo et al. (2003) reported similar correlation values between grain yield and NDVI and SR under irrigated conditions in durum wheat when the spectral reflectance data were taken at the grainfilling stage by using 900 and 680 nm to develop the NDVI and the SR.

Normalized difference vegetation index and SR gave significant positive correlations with dry biomass when estimated at the heading and grainfilling stages in almost all occasions. Aparicio et al. (2000) reported a positive significant correlation between crop dry matter and NDVI and SR in durum wheat under irrigated conditions at the grainfilling stage. Correlation values generally increased with growth stage, which was also supported by Aparicio et al. (2000) and Aparicio et al. (2002) in durum wheat.

All indices studied explained the largest amount of variation when taken at the heading and grainfilling stages. Hence, those two stages appear to be the most appropriate time to utilize the spectral indices to discriminate the genotypes for biomass production, and thus crop productivity.

Correlations between Dry Biomass and SPAD Values, RARSa, PSSRa, RARSb, RARSc

Estimation of green biomass from the measurements of spectral reflectance at the visible wavelengths is mainly based on the absorption of electromagnetic wavelengths by chlorophyll and associated pigments. A strong negative correlation between visible wavelengths and dry biomass was reported by Ahlrichs and Bauer (1983) and Elliott and Regan (1993) in wheat under water-limiting conditions at low levels of biomass production. The light at visible wavelengths is highly absorbed by the green vegetation and at a very high concentration of pigments, the absorption of visible wavelengths (especially red light) is saturated by the pigments (Gitelson et al., 1996). In this study a low correlation was noted between indices based on visible wavelengths and SPAD values with biomass at the early growth stages. This may have been caused by a very high concentration of different pigments at the early growth stages, which made the indices insensitive to any differences among genotypes for biomass production. The correlation levels increased with advanced growth stages, when the plants started to lose their green photosynthetically active parts, thereby increasing the variability among the genotypes for the reflection of light at visible wavelengths, which was more effectively detected by the indices. The strong correlation among genotypes between biomass and the visible wavelength–based indices indicate the higher contribution of genotypic effects rather than environmental effects to these associations.

Even though RARSa and PSSRa are indicators of the chlorophyll-a concentration in the plant, a differential performance was observed between these indices to distinguish genotypes for biomass production. Blackburn (1998) developed the PSSRa index by using 680- and 800-nm wavelengths, which significantly improved the capacity to predict chlorophyll-a concentration over the RARSa index that was developed by Chappelle et al. (1992) using the 675 and 700 nm wavelengths. PSSRa combined information from both the red and NIR wavelengths, while RARSa combines information only from the red bands. Combining the information from the NIR wavelengths may have contributed to the

improved correlation between biomass and PSSRa over RARSa. This study demonstrates the effectiveness of using PSSRa over RARSa at the genotypic level as well. It also demonstrates the possibility of using indices based on visible wavelengths to differentiate genotypes with high biomass production levels under irrigated conditions at reproductive growth stages when the accumulated biomass contributes to the total yield by partitioning assimilates to the grains.

Correlation between SPAD Values and RARSa, PSSRa, RARSb, and RARSc

The SPAD-502 chlorophyll meter is a handheld portable instrument, which estimates chlorophyll, based on the amount of transmitted light in the chlorophyll absorption region of the spectrum. The SPAD chlorophyll meter uses light transmittance at 650 and 940 nm to estimate leaf chlorophyll concentration (Yadava, 1986; Dwyer et al., 1991), and thus monitors N status in corn leaves (Blackmer et al., 1994; Blackmer and Schepers, 1995). SPAD chlorophyll meter readings have been reported to be well correlated with different wavelengths (550, 650, 710 nm) under different N levels in corn (Blackmer et al., 1994; Schepers et al., 1996). RARSa, RARSb, and RARSc were developed to estimate chlorophyll-a, chlorophyll-b, and carotenoids in soybean (Chappelle et al., 1992), and PSSRa was developed to estimate chlorophyll-a in a range of plant species (Blackburn, 1998). Blackburn (1998) showed the optimum individual wavelengths for the estimation of different leaf pigments concentration such as chlorophyll-a, chlorophyll-b, and carotenoid were 680, 635, and 470 nm, respectively.

The SPAD chlorophyll meter uses single leaves to estimate chlorophyll concentration, but reflectance measurements can be made using the entire canopy. The advantage of reflectance measurements is that when made from above the canopy, they represent a more representative area relative to a single leaf. In this study, we have observed strong relationships between SPAD readings and PSSRa, RARSb, and RARSc when estimated at the heading and grainfilling stages, but the relationship was poor at booting. The relationship of these indices at booting was not well understood. At the later growth stages when the plants started to loose their pigments, both devices (chlorophyll meter and spectrometer) were equally effective, especially at the grainfilling stage. Our results verify the "greenness" as a factor in biomass production and the ability to accurately measure these differences should have an important impact on breeding progress where green leaf area duration (also known as "stay-green") is considered an important empirical trait in improving the productivity of genotypes.

Correlation between Canopy Temperature and NIR-Based Indices

Canopy temperature or CTD (CT minus air temperature), which is sensed remotely using an infrared thermometer, has been shown to be closely associated with grain yield of wheat cultivars (Reynolds et al., 1994; Fischer et al., 1998), as well as that of RILs and advanced breeding materials (Reynolds et al., 1998, 1999) in irrigated, high radiation environments. Leaf temperature is depressed below air temperature when water evaporates from the surface of leaves, thus keeping the canopy cooler. The WI assesses canopy water status and depends on the absorption of light by water at certain NIR wavelengths. The higher the water content of the tissue, the greater the absorption, and consequently the lower the reflectance. Peñuelas et al. (1997) showed that WI and CTD was well correlated under irrigated conditions across different salinity gradients at the genotypic level. Babar et al. (2006) found a strong association between grain yield of wheat and WI under the same environmental conditions where the present study was conducted.

In this study, strong positive correlations were observed between CT and NIR-based indices when the data were taken at heading and at the grainfilling stages. These strong positive correlations indicate that genotypes with higher water content (represented by lower NIR-based indices values) had lower CT. Thus, the canopy with higher water content are indicative of genotypes with higher biomass resulting from larger rates of carbon fixation associated with greater stomatal conductance and therefore, cooler canopies. Gutierrez-Rodriguez et al. (2004) suggested that the WI could be an alternative to CTD. Our results have reconfirmed that finding across a broad spectrum of genotypes. The combined information on these two traits can help plant breeders to select genotypes with higher water content and canopies with lower temperatures, thus identifying genotypes with higher productive capacity.

CONCLUSIONS

Spectral reflectance indices have shown the potential to differentiate genotypes for biomass production with different types of breeding lines of spring wheat under irrigated conditions. The best growth stages to apply the indices to differentiate genotypes for biomass production were heading and grainfilling. When comparing all the indices, those based on NIR (WI, NWI-1, and NWI-2) consistently demonstrated higher levels of association and explained a higher proportion of the variability compared with the other spectral indices (NDVI and SR). Our study has demonstrated the relationship between SRI and SPAD chlorophyll values to estimate chlorophyll and carotenoids in wheat at the genotypic level. The NIR-based indices showed a strong association with CT at the genotypic levels under irrigated conditions. The associations were stronger at the later growth stages compared to early growth stages for these physiological parameters. The correlations in RILs populations confirmed the genetic basis or link between biomass and SRI, and between other physiological traits indicated by SPAD values, RARS indices, and CT.

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