SPECTRAL REFLECTANCE INDICES AS A SELECTION CRITERION FOR YIELD IMPROVEMENT IN WHEAT

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Introduction

Since the genetic basis of yield improvement in wheat is not well established (Reynolds et al., 1999), the classical breeding approach for yield improvement still relies on an informed "numbers game" where many crosses are made among potentially complementary parents. Subsequently, large numbers of progeny from these crosses have to be assessed visually in segregating populations and entered into yield trials as advanced lines to identify suitable materials to test in the target environments (Jackson, 2001). Nonetheless, wheat breeders in the last several decades have been successful in improving yield potential using grain yield as a selection criteria, which involves the trial and error method of selecting for yield *per se* (Slafer and Andrade 1991; Loss and Siddique 1994), although the rate of genetic improvement in yield has varied with different environments.

In the typical breeding program, a large number of promising genotypes are evaluated against the best commercial cultivars to select the suitable genotypes for the target environments. Although, the large-scale field evaluation process is expensive in terms of time and money, it is still necessary. Often, additional yield evaluations are necessary in successive years and in different locations. Proper statistical procedures are needed to cope with the undesirable genotype-environment interactions, thereby reducing the chance of discarding a good genotype from the trials (Ball and Konzak, 1993). To avoid this laborious, time consuming, and cumbersome process, breeders need an easy, rapid and inexpensive indirect selection process to screen genotypes in a relatively short time before harvest (Reynolds et al., 1999). It would be really advantageous if the indirect selection tool could detect high yielding genotypes rapidly and efficiently from a large number of promising genotypes.

The use of morphological and physiological selection criteria to differentiate grain yield is an indirect breeding approach. The use of these physiological tools in breeding programs is limited because of the limited understanding of their relationship with yield, and frequently due to a complex evaluation procedure (Loss and Siddique, 1994; Richards 1996). Nonetheless, canopy temperature which can be sensed remotely using IR thermometry, has been shown to be well associated with yield of wheat cultivars (Reynolds et al., 1994; Fischer et al., 1998), as well as with the yield of recombinant inbred lines and advanced breeding materials (Reynolds et al., 1998; 1999) in irrigated, high radiation environments. Condon and Richards (1992) and Condon et al (2004) proved that the use of carbon isotope discrimination was a useful trait to improve grain yield potential in water-limiting environments.

Some recent studies suggest that spectral reflectance is one of the most promising remote sensing technique for screening genotypes for grain yield (Araus et al., 2001). Canopy light reflectance properties mainly based on the absorption of light at a specific wavelength are associated with specific plant characteristics. The spectral reflectance in the visible (VIS) wavelengths (400-700nm) depends on the absorption of light by leaf chlorophyll and associated pigments such as carotenoids and anthocyanins. The reflectance in the VIS is low because of the high absorption of light energy by these pigments. The reflectance in the near infrared (NIR) wavelengths (700-1300nm) is high because of the multiple scattering of light by different leaf tissues (Knipling, 1970). Spectral reflectance indices (SRIs) were developed on the basis of simple mathematical formulae such as ratios or differences between the reflectance at given wavelengths (Araus et al., 2001). Simple ratio (SR=NIR/VIS) and normalized difference vegetation index [NDVI=(NIR-VIS)/(NIR+VIS)] were the first SRIs developed, combining 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 (Tucker and Seller, 1986). SRIs based only on VIS have been developed, such as the photochemical reflectance index [PRI=(R_{531}nm-R_{570}nm)/(R_{531}nm+R_{570}nm)] 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}nm/R_{900}nm) to assess water status of the canopy (Peñuelas et al., 1993). SRIs have been widely reported by different authors to assess different physiological conditions of the canopy, including the estimation of total dry matter, leaf area index (LAI), photosynthetic capacity (Sellers, 1987), as well as green leaf area index and fractional photosynthetically active radiation absorption (Wiegand and Richardson, 1990; Baret and Guyot, 1991). SRIs have also proven to be useful in the assessment of early biomass and the vigor of different wheat genotypes (Elliot and Regan, 1993), water status in gerbera and barley (Peñuelas et al., 1993; Peñuelas et al., 1997), and different pigment concentrations in the leaves of soybean (Chappelle et al., 1992). The potential for using NDVI to predict in-season grain yield has also been reported in wheat under water - stressed environments (Raun et al., 2001).

Attempts have been made to evaluate the potential use of SRIs in plant breeding to differentiate genotypes for yield under well watered and/or moistures-stressed conditions in wheat (Hatfield, 1981; Ball and Kazak, 1993; Aparicio et al., 2000; Royo et al., 2003) and soybean (Ma et al., 2001). The studies under moisture-stressed conditions indicated potential for using spectral indices, but under well-watered conditions the association between yield and existing indices (Normalized Difference Vegetation Index, NDVI and simple ratio, SR) was weak.

A limited number of experiments in the past indicated the potential for using spectral reflectance measurements in breeding programs. Since the most widely used SRIs (NDVI and SR) are insensitive at leaf area index exceeding 3 (LAI > 3), it is necessary to investigate different areas of the spectrum to develop new indices, which might overcome these limitations. To date, studies have done mostly under water stressed conditions, and with one or a few indices. Therefore, comprehensive studies involving contrasting environments with diverse genetic materials and measurements of spectral reflectance at different growth stages are required to verify the potential of new reflectance indices for breeding for wheat yield and biomass production.

Using any parameter as a breeding tool, it is important for a breeder to know the genetic correlations with the performance (yield and biomass in this study), heritability, and genetic gain from selection (Reynolds et al., 2001, Jackson, 2001). The correlated response for a complex trait such as grain yield can be predicted when the heritability and the genetic correlation between the traits are known. Indirect selection is effective when the genetic correlation between the utilized characters is very strong and the heritability is much higher for the selected trait than for the unselected trait.

Thus, the goal of this study was to evaluate a broader range of spectral reflectance indices as potential screening tools in different moisture environments. The objectives of the present study were to i) evaluate the correlation of existing spectral indices with yield and agronomic traits of bread wheat genotypes under irrigated and water limiting conditions, ii) derive different

SR indices that distinguish among high yielding genotypes better than pre-existing indices, iii) determine the best growth stages to apply the spectral reflectance tool, iv) estimate broad-sense heritability within and between years for different SRIs, v) estimate expected selection response for the SRIs, vi) determine the efficiency of correlated selection response for grain yield estimated from SRIs, and vii) determine the efficiency of selecting superior genotypes for grain yield based on the best SRIs, as compared with the selection efficiency based on yield *per se*.

CHAPTER II

Spectral Reflectance Indices as a Potential Indirect Selection Criteria for Wheat Yield under Irrigation

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Abbreviations

Boot, Booting Stage

GF, Grainfilling Stage

GHIST, Global Historic Trials

GNDVI, Green Normalized Difference Vegetation Index

Hd, Heading Stage

LAI, Leaf Area Index

NIR, Near Infrared Radiation

NWI-1, Normalized Water Index-1

NWI-2, Normalized Water Index-2

PRI, Photochemical Reflectance Index

RLs1, Random Lines-1

RLs2, Random Lines-2

RNDVI, Red Normalized Difference Vegetation Index

SR, Simple Ratio

SRIs, Spectral Reflectance Indices

WI, Water Index

Abstract

The objectives of this study were to assess the potential of using spectral reflectance indices (SRIs) as an indirect selection tool to differentiate spring wheat genotypes for grain yield under irrigated conditions. This paper demonstrates only the first step in using the SRIs as indirect selection criteria. Genetic variation for SRIs, the effect of phenology and year on SRIs and their interaction with genotypes, and the correlations between SRIs and grain yield and yield components of wheat are presented in this paper. Three field experiments, GHIST (15 CIMMYT globally adapted genotypes), RLs1 (25 random sister lines), and RLs2 (36 random sister line) were conducted under irrigated conditions at the CIMMYT research station in northwest Mexico in three different years. Five previously developed SRIs (PRI, WI, RNDVI, GNDVI, SR), and two newly calculated SRIs (NWI-1 and NWI-2) were evaluated in the experiments. In general, genotypic variation for all the indices was significant. Near infrared radiation (NIR) based indices (WI, NWI-1, NWI-2) gave the highest levels of association with grain yield during the three years of the study. A clear trend for higher association between grain yield and the NIR based indices at heading and grainfilling than at booting was observed. Overall, NIR based indices were more consistent and differentiated grain yield more effectively compared to the other indices. The results demonstrated the potential of using SRIs as a tool in breeding programs for selecting for increased genetic gains for yield.

Introduction

Since the genetic basis of yield improvement in wheat is not well established (Reynolds et al., 1999), the classical breeding approach for yield improvement still relies on an informed "numbers game" where crosses are made among potentially complementary parents. Subsequently, large numbers of their progeny have to be assessed visually in early generations and in yield trials as advanced lines to identify suitable materials to test in the target environment (Jackson, 2001). Classical breeding programs consider grain yield *per se* as the main selection criterion for grain yield (Loss and Siddique, 1994), but due to the high genotype-by environment (GXE) interaction component of this trait, commonly used statistical procedures are frequently not powerful enough to accurately differentiate between genotypes (Bhatti et al., 1991), thereby increasing the risk of accidentally discarding good lines, or retaining inappropriate genotypes in trials (Ball and Konzak, 1993). Nonetheless, field evaluations are still necessary to effectively identify superior genotypes in a real-life setting, which is expensive in terms of time and financial resources especially when a large number of genotypes are being evaluated. Moreover, often additional evaluations are necessary in successive years and in different locations. To avoid or at least to reduce this laborious, time consuming, and cumbersome process, an easy, rapid and inexpensive selection tool may help breeders reliably screen large numbers of genotypes in a relatively short time before initiating expensive yield trials (Reynolds et al., 1999). It would be very advantageous if such a selection tool has higher heritability than grain yield, shows a strong correlation with grain yield, and could detect high yielding genotypes rapidly and efficiently from a large number of genotypes.

The use of morphological and physiological selection criteria to differentiate grain yield is an indirect breeding approach. Physiological tools have had limited utility in plant breeding programs (Jackson et al., 1996), partly because of the time consuming evaluation methods and the lack of association with yield (Loss and Siddique, 1994; Richards, 1996). Nonetheless, canopy temperature, which can be sensed remotely using IR thermometry, has been shown to be well associated with the yield of wheat cultivars (Reynolds et al., 1994; Fischer et al., 1998), as well with the yield of recombinant inbred lines and advanced breeding materials (Reynolds et al., 1998; 1999) in irrigated, high radiation environments.

More recent studies suggest that spectral reflectance is another promising remote sensing technique for screening genotypes (Araus, 1996; Araus et al., 2001). Canopy light reflectance properties mainly based on the absorption of light at a specific wavelength are associated with specific plant characteristics. The spectral reflectance in the visible (VIS) wavelengths (400-700nm) depends on the absorption of light by leaf chlorophyll and associated pigments such as carotenoids and anthocyanins. The reflectance in the VIS is low because of the high absorption of light energy by pigments. The reflectance of the near infrared (NIR) wavelengths (700-1300nm) is high because of the multiple scattering of light by different leaf tissues (Knipling, 1970). Spectral reflectance indices (SRIs) were developed on the basis of simple mathematical formulae such as ratios or differences between the reflectance at given wavelengths (Araus et al., 2001). Simple ratio (SR=NIR/VIS) and normalized difference vegetation index [NDVI=(NIR-VIS)/(NIR+VIS)] were the first SRIs developed, combining information from the VIS and NIR wavelengths. These indices were used to predict different vegetation parameters, such as green biomass and green leaf area index (Tucker and Seller, 1986). SRIs have also been developed based only on VIS, including the photochemical reflectance index [PRI=(R_{531}nm-

R_{570}nm)/(R_{531}nm+R_{570}nm)] used to assess radiation use efficiency by the plants (Penuelas et al., 1995), and also only on NIR, such as the water index (WI=R_{900}nm/R_{970}nm) to assess water status of the canopy (Penuelas et al., 1993). SRIs have been widely reported by different authors to assess different physiological conditions of the canopy such as total dry matter, leaf area index, photosynthetic capacity (Sellers, 1987), as well as green leaf area index and fraction of photosynthetically active radiation absorption (Wiegand and Richardson, 1990; Baret and Guyot, 1991; Weigand et al., 1991). SRIs have also proven to be useful in the assessment of early biomass and vigor of different wheat genotypes (Elliot and Regan, 1993; Bellairs et al., 1996), water status in gerbera and barley (Penuelas et al., 1993; Penuelas et al., 1997), and different pigment concentrations in the leaves of soybean (Chappelle et al., 1992). The potential for using SRIs to predict in-season grain yield have also been reported in wheat (Raun et al., 2001) and in maize (Osborne et al., 2002) under water stressed environments.

Attempts have been made to evaluate the potential use of SRIs in plant breeding to differentiate genotypes for yield under well watered and/or moisture stressed conditions in wheat (Hatfield, 1981; Ball and Kazak, 1993; Aparicio et al., 2000; Royo et al., 2003) and soybean (Ma et al., 2001). The studies under moisture stressed conditions showed the potential of using spectral indices under such conditions, but under well-watered conditions the association between yield and existing indices (Normalized Difference Vegetation Index, NDVI, and simple ratio, SR) were weak.

The goal of this study was to evaluate a broader range of spectral reflectance indices as potential screening tools in irrigated, high yielding environments. Specific objectives of the present study were to i) evaluate the correlation of existing spectral indices with yield and agronomic traits of bread wheat genotypes under near optimum nitrogen and irrigation levels, ii) derive new improved spectral reflectance indices that distinguish among high yielding genotypes better than pre-existing indices, and iii) to determine the best growth stage to apply the spectral reflectance tool.

Materials and Methods

Three experiments were conducted under irrigated conditions in three cropping seasons (year 2001-02, year 2002-03, and year 2003-04) at the CIMMYT (International Maize and Wheat Improvement Center) experimental station near Ciudad Obregon in Sonora, Mexico (lat. 27.33^{o}N, long 109.09^{o}W, 38 m above sea level). The soil type at the experimental station is a 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. The experiments were planted in a bed planting system where each 5 m long plot consisted of two beds and the distance between bed centers was 80 cm. Plot area was 8.0 m^2 (1.6 m x 5 m). In the first two years, 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 a 20 cm inter-row spacing.

The seeding rate for each experiment was 78 kg ha^{-1} and the experiments were planted in the last week of November. Nitrogen and phosphorus were applied to the plots at rates of 200 kg ha^{-1} and 26 kg ha^{-1}, respectively. During the first two years, 150 kg nitrogen and all of the phosphorus were applied during land preparation, and 50 kg nitrogen 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 nitrogen was supplied at half the dosage during planting and half with the second supplementary irrigation. A total of five supplementary irrigations were applied in the first and third years, but in the second year only four supplementary irrigations were given. Folicur 250EW (25% Tebuconazole) was applied twice in every crop cycle, in the second week of February (early booting) and in the second week of March (just after flowering) at the rate of 500 ml ha^{-1} to protect the experimental materials from prevalent leaf rust.

Harvested area for grain yield was 4.8 m^2 (1.6 m x 3 m). Before harvesting, 100 tillers with spikes were cut at the ground level to estimate the various yield components. The collected 100 tillers were oven dried at 75^0 C for 48 hours. The weight of the oven dried 100 tillers was measured, and then the tillers were threshed to calculate the harvest index. Two hundred grains were randomly collected from the harvested plots to estimate thousand grain weight. Harvested grain yield was converted to grain yield in tons per hectare (t ha^{-1}). From harvest index, grain weight of 100 tillers, thousand-grain weight, and grain yield per unit area, other yield components, including spikes m^{-2}, grains $spike^{-1}$, and biomass at maturity were calculated.

Experimental materials

Experiment 1: In the first experiment, we used 15 worldwide-adapted spring bread wheat genotypes developed by the wheat-breeding program of CIMMYT. The genotypes represent the historical success achieved by the breeding program at CIMMYT. While all are high yielding in distinct regions around the world, they vary widely in morphological traits and in parentage. The genotypes were planted in a 5x3 alpha-lattice design with two replications. In this paper we will refer to this experiment as "GHIST", since it studies a global historical set of commercial genotypes.

Experiment 2: This experiment had 25 genotypes, comprising 23 random sister lines derived from a cross between the two parents SONALIKA and ATTILA. The sister lines were derived by planting 1000 F_2 seeds and harvesting the population in bulk. Approximately 500 F_3 seeds were space planted and random individual F_3 plants were selected. The selected F_3 plants were harvested separately and planted in separate F_4 small plots. The F_4 small plots were harvested separately (F_5 seed) and planted in individual F_5 yield plots that were harvested separately. The process was continued for the F_6 and F_7. The experiment was planted in a 5x5 alpha-lattice design with two replications. In this paper we will refer to this experiment as "RLs1".

Experiment 3: We used 36 genotypes, comprising 34 random sister lines and their two parents (BACANORA 88 and CNDO/R143//ENTE/MEXI_2/3/AE. SQ.(TAUS)/4/WEAVER). The random lines were developed in the same procedure as described for RLs1. The experiment was planted in a 6x6 alpha-lattice design with two replications. In this paper we will refer to this experiment as "RLs2".

Radiometric measurements

The spectral reflectance measurements were taken by a portable narrow-bandwidth Spectroradiometer (Model FieldSpec UV/VNIR, Analytical Spectral Devices, Boulder, Colorado) with a 25^o field of view. This instrument can detect reflected light from the canopy ranging from 350nm to 1100nm. Therefore, it covers visible and near infrared radiation (NIR). It gives 512 continuous bands with a sampling interval of 1.43 nm. The spectrometer was connected to a computer, which stored the individual scans for subsequent processing. Each reflectance measurement was the average of 10 scans (which was adjusted and calculated by the spectroradiometer) and the scanning area was approximately 18.94 cm^2. The sensor was mounted with the help of a pistol grip 40-50 cm above the canopy facing the center of the bed. The spectroradiometer was recalibrated against a white reference plate ($BaSO_4$) every 10 plots. The reflectance measurements were taken between 10:30 am to 2:00 pm under sunny conditions, and reflectance measurements were taken from four different places within each plot. The mean of the four readings was used to calculate spectral indices of each individual plot for statistical analysis. The average time required for the completion of the reflectance measurement from four different areas within the plot was approximately 40-45 sec/plot.

The spectral reflectance measurements were taken at booting (Zadoks' stage between 39 and 47), heading (Zadoks' stage between 55 and 69), and grain filling (Zadoks' stage between 75 and 83) in all experiments (Zadoks et al., 1974), except for RLs1 and RLs2 in the year 2001-02, where the reflectance measurements were taken only at the heading stage.

Calculation and selection of indices

Initially, different ratios and normalized indices were calculated based on a combination of visible and near-infrared wavelengths. From the combinations tested, two indices were selected for presentation in this paper. Those two indices combined information from 850nm, 900nm, and 970nm. The 970 nm has been reported as a weak water absorption band (Penuelas et al., 1993), and the other two bands (850nm and 900 nm) were used as reference bands. We have referred to these two indices as normalized water index-1 (NWI-1) and normalized water index-2 (NWI-2). Five other reference indices, including the most widely used NDVI and SR, were calculated (described below) and compared with the two new indices.

Using the notation R_i to indicate the reflectance of light at a wavelength of i nm. The different spectral reflectance indices calculated were:

Photochemical reflectance index, PRI = $(R_{531}-R_{570})/ (R_{531}+R_{570})$ (Penuelas et al., 1995), which is an indicator of radiation use efficiency by the plants.

Water index, WI = R_{970}/R_{900} (Penuelas et al., 1993), which indicates canopy water status.

Red normalized difference vegetation index, RNDVI = $(R_{780}-R_{670})/(R_{780}+R_{670})$ (Raun et al., 2001), which indicates canopy photosynthetic area.

Green normalized difference vegetation index, GNDVI = $(R_{780}-R_{550})/(R_{780+}R_{550})$ (Gitelson et al., 1996), which indicates canopy photosynthetic area.

$SR=R_{900}/R_{680}$ (Aparicio et al., 2000), which is also an indicator of canopy photosynthetic active area.

The above mentioned normalized water indices were calculated as follows:

Normalized water index-1, NWI-1 = $(R_{970}-R_{900})/(R_{970}+R_{900})$, and

Normalized water index-2, NWI-2 = $(R_{970}-R_{850})/(R_{970}+R_{850})$.

Statistical analysis

Alpha-lattice analyses for grain yield and spectral indices were carried out using PROC MIXED procedure of the SAS/STAT statistical package (SAS Inst., 1987). Data were analyzed in each individual growth stage within the same year and between the years. Combined analyses were carried out across different growth stages and different years (year 02-03 and year 03-04 for RLs1 and RLs2; and all three years for GHIST). Pearson correlation coefficients were used to estimate the relationships of yield and yield components with different spectral indices, and the relationships of indices at different growth stages within the same year and between years.

Results

Genotypic performance

Minimum, maximum, mean, LSD, and P-value of F-test within and across years for grain yield in three different experiments are presented in Table 1. The genotypic variations for grain yield in all three experiments within and across years were significant. The minimum and maximum mean SRIs values over different growth stages and years in the GHIST experiment were –0.054 to –0.027 (PRI), 0.838 to 0.875 (WI), 0.820 to 0.893 (RNDVI), 0.723 to 0.893 (GNDVI), 14.2 to 26.6 (SR), -0.088 to –0.067 (NWI-1), and –0.084 to –0.059 (NWI-2). The SRIs values in the RLs1 experiment ranged from –0.055 to –0.032 (PRI), 0.862 to 0.887 (WI), 0.854 to 0.904 (RNDVI), 0.754 to 0.798 (GNDVI), 12.3 to 19.3 (SR), -0.074 to –0.060 (NWI-1) and –0.069 to –0.052 (NWI-2). For RLs2, the range of SRIs was –0.0594 to -0.0333 (PRI), 0.836 to 0.874 (WI), 0.821 to 0.900 (RNDVI), 0.741 to 0.807 (GNDVI), 14.3 to 23.8 (SR),). -0.0893 to -0671 (NWI-1), and -0.0856 to -0.0610 (NWI-2). Genotypes showed significant variation for spectral reflectance indices in all three experiments (data not presented).

Effect of growth stages

Minimum, maximum, mean, LSD, and P-value for these SRIs at the three different growth stages (booting, heading and grainfilling) are presented in Table 2. The analysis of spectral indices at individual growth stages across the years revealed significant genotypic variation for the indices at all growth stages. In general, there was less variation among genotypes for the spectral indices at the booting stage than at the later growth stages. This was evidenced by the fact that when considering individual years, not all indices showed significant differences among genotypes when measured at booting (data not shown).

Regarding the main effect of growth stages on SRIs, the values based on NIR (WI, NWI-1 & NWI-2) tended to decrease from booting to heading, and then increased in the grainfilling stage (Table 2, Fig. 2). The only index based on visible wavelength, PRI, showed the highest value at the booting stage, with values decreasing as the growth cycle progressed (Table 2). The values of RNDVI, GNDVI and SR (based on red, green and near infrared radiation) were similar at the booting and heading stages, while their values decreased during grainfilling.

Genotype, growth stage and year interactions

Mean squares from the analysis of variance combined over growth stages and years for the GHIST experiment are presented in Table 3. The genotypic main effect in the combined analysis was significant for all indices. Genotypes and growth stages, and genotype by year interactions were mostly significant for all the indices. Similar trends for genotypic and interactions effects were also observed in two other experiments for different SRIs (data not shown). The associations of each SRI measured in different growth stages within the same year, and between the years are presented for the experiment GHIST in Tables 4 and 5. In general, a strong association was observed for all indices measured at heading and grainfilling in all three years (Table 4). The indices RNDVI, GNDVI and SR also showed a strong association between booting and heading, and booting and grainfilling in the year 02-03 and year 03-04, but not in the year 01-02. The associations between booting and heading, and booting and grainfilling for the three NIR-based indices (WI, NWI-1, and NWI-2) and for PRI were very low in all three years.

All indices showed the highest association between years at the grainfilling stage except for PRI and GNDVI between year 01-02 and year 02-03 (Table 5). RNDVI, GNDVI, and SR also showed a strong association between year 02-03 and year 03-04 at the booting stage, but not between year 01-02 and year 02-03. The NIR based indices showed low between-year correlations for that growth stage.

Association of grain yield with SRIs

The correlation of grain yield with spectral indices at (i) different growth stages for each year, (ii) averaged across growth stages for each year (mean†) and iii) averaged over growth stages and years (overall mean‡) are presented in Table 6. The association between mean SRIs (averaged over growth stages within an individual year) and the overall grain yield (mean of three years) are presented in Table 7. We calculated seventy-two different ratios and normalized indices by using different visible and NIR wavelength combinations, and also calculated twenty previously published SRIs which were indicative of different physiological conditions of plants. Initially, we selected five out of seventy two SRIs that were calculated, but later selected only two of them to present in this paper because of their high correlation with grain yield and consistent performance over years and different genetic backgrounds. Of the previously published SRIs, we selected five to present in this paper. RNDVI, GNDVI, and SR have been the most widely used by different authors to study the physiological conditions of plants, and all five SRIs (RNDVI, GNDVI, SR, WI and PRI) have been reported by various authors to differentiate genotypes for grain yield under water-stressed conditions in durum wheat, and well watered conditions in bread wheat and soybean.

Comparing all the indices, those based on NIR (WI, NWI-1, and NWI-2) demonstrated a higher level of association with grain yield compared with the other spectral indices (RNDVI, GNDVI, SR, and PRI) at the heading and grainfilling stages, except for GHIST in Y01-02. NIR based indices showed significant negative correlations with grain yield at heading and grainfilling stages in all the experiments in all three years. The other indices (i.e. PRI, RNDVI, GNDVI, SR) generally showed significant positive relationships with grain yield. Of all the indices calculated, PRI showed the least predictive capacity to differentiate genotypes for grain yield. When considering indices averaged over growth stages, NIR based indices gave the highest correlations with grain yield except for GHIST in Y01-02.

At booting, RNDVI, GNDVI, SR. and PRI were better correlated with grain yield than the NIR based indices. NIR based indices showed a very low level of association with grain yield at the booting stage, which was consistent across all the experiments and in all the years. In most cases, mean indices over three growth stages correlated better with grain yield than any single growth stage with a few exceptions. A similar trend has also been observed when the mean indices over two growth stages (heading and flowering) were used, and the correlation values were very close to the correlation values when the mean indices over three growth stages were used, with a few exceptions (data not shown). The correlations between the overall mean indices (across different growth stages and years) showed very strong correlations with the overall mean grain yield of genotypes (across different years) and the correlations values were higher than between SRIs and grain yield within an individual year.

The three NIR based indices showed a very strong association with overall mean grain yield (mean of three years), when the mean indices over three growth stages within an individual year were correlated with overall mean grain yield. The levels of association were consistently higher than the association between other SRIs and overall mean grain yield with a single exception (Table 7).

No single NIR based index showed any definite superiority over the others. Nonetheless, when we consider all the correlations between these three indices and grain yield within year, between indices measured in an individual year and overall mean grain yield, and between overall mean indices and overall mean grain yield, NWI-2 gave either similar or marginally better correlations than the other two NIR based indices (WI and NWI-1). For simplicity of the presentation in the regression model, we have used only NWI-2.

Attempts were made to determine the most suitable regression model to explain variability among the genotypes for grain yield across different years (Figs. 1) and different growth stages within the same year (Figs. 2). NWI-2 and RNDVI were compared in different regression models because of the performance of NWI-2, and because the RNDVI has been the most widely used SRI to study grain yield variations at the genotypic level, as well as in agronomic trials. In general, a simple linear model did not differ greatly from exponential and power regression models in explaining grain yield variation (except in GHIST). The exponential model explained 2-3% more of the variability for grain yields for the different indices in year 03-04. A simple linear model was equally applicable in explaining the phenological pattern of the relationship between grain yield and the indices (except for GHIST). The exponential model explained 2-3% more of the variability for RNDVI and NWI-2 when the readings were taken at heading and grainfilling stages in year 03-04. As the non-linear model was non-significant, the graphs have been presented in a simple linear model in the different figures for experiment RLs2.

We selected the 20 % top yielding genotypes for grain yield based on NDVI and NWI-2 in two populations (RLs1 and RLs2). First, we ranked the genotypes based on grain yield *per se*, and then ranked the genotypes based on the two indices. 60% to 80% and 57% to 86% of the top 20% highest yielding genotypes were selected based on NWI-2 in RLs1 and RLs2, respectively, in the three different years of study. NDVI selected 20% to 80% and 43% to 71% of the top 20% genotypes in RLs1 and RLs2 experiments, respectively, in the three-year period of study.

Association of SRIs with agronomic traits

The associations between SRIs and two agronomic traits, grains m^{-2} and biomass at maturity (BM), averaged over the three years are presented in Table 8. The correlations between SRIs and additional agronomic traits such as harvest index, spikes m^{-2}, grains $spike^{-1}$ and thousand-grain weight were also evaluated. In general, the associations between SRIs and these four agronomic parameters were low and inconsistent (not shown).

All indices showed significant association with grains m^{-2} and biomass at maturuity in all three experiments. However, the three NIR-based indices were better correlated with these two parameters compared to the other indices in RLs1 and RLs2.

Discussion

Genotypic performance

In this study, we observed a wide range of genetic variation for different spectral indices, as was also shown in earlier studies under irrigated conditions (Ball and Konzak, 1993; Aparicio et al., 2000) and moisture stressed conditions (Aparicio et al., 2000; Royo et al., 2003). For most indices, genotypes could not be distinguished from one another at the booting stage. This could be attributed to a more or less uniformly high leaf area index (LAI) at booting, making it difficult for genotypes to be differentiated. Variation among genotypes increased at heading and during grainfilling, quite likely due to a decrease in LAI and differing morphological characteristics of the spikes. Asrar et al. (1984) and Ahlrichs and Bauer (1983) showed that NDVI and NIR reflectance were sensitive to changes in leaf area up to values of LAI 3, after which they reached a plateau. Calderini et al. (1997) reported that wheat under irrigated conditions achieved maximum LAI between the stages of terminal spikelet and booting (LAI>5 for most of the genotypes), and then decreased towards anthesis. Hatfield (1981), in another study on wheat grown under irrigated conditions, observed only small differences among varieties in NDVI once 100 percent ground cover was reached, but variability among varieties again increased as the crop cycle progressed, and the highest variability was obtained at maximum head weight due to differences in spike size and/or morphology.

Effect of growth stages

In general, values of PRI, RNDVI, GNDVI, and SR decreased from heading to grainfilling (Table 2). Aparicio et al. (2000) also reported a similar trend of decreasing values for spectral indices (PRI, RNDVI, and SR) with the advancement of growth stages in durum wheat under irrigated conditions. As RNDVI, GNDVI, and SR are indices that combine red, green and

NIR wavelengths, a reduction in leaf area index would decrease the reflectance of NIR, but increase reflectance of visible wavelengths. The overall result would cause a decrease in the values of these three indices at the grainfilling stage. This is consistent with the observation that LAI commonly peaks at the booting stage and decreases as the growth cycle progresses (Calderini et al., 1997; Aparicio et al., 2000).

Values for the NIR-based indices WI, NWI-1, and NWI-2 decreased from booting to heading, and then increased at grainfilling. These indices are based on reflectance at 970 nm, which is a weak water absorbance band (Penuelas et al., 1993), and on reflectance at 900 nm and 850 nm, which is caused by multiple reflection and scattering of light in the spongy mesophyll structure (Knipling, 1970). The water index (WI) shows an inverse relationship with water status at both the canopy and leaf level (Penuelas et al., 1993; Penuelas et al., 1997). WI assesses the changes in relative water content, leaf water potential, stomatal conductance, and canopy temperature in plants (Penuelas et al., 1993). Based on the values of WI and the other NIR related indices, it appears that in general the plant canopy contains a higher total amount of water at heading compared to later growth stages. Assessment of fresh and dry biomass made at these growth stages on the above experiments indicated that there was 14-22% more water in the canopy at heading than at grainfilling (data not shown).

Genotype, growth stage and year interactions

In this study, we observed significant interaction between growth stages and genotypes in regard to their index values (Table 3). The low association between booting and flowering, and booting and grainfilling for NIR based indices and PRI (Table 4), indicates that the ranking of genotypes changed between growth stages. On the other hand, RNDVI, GNDVI, and SR showed a higher correlation between booting and flowering, and between booting and grainfilling. The interactions of growth stages and indices indicate that care must be taken to identify a suitable growth stage at which the indices will be applied in order to discriminate most effectively among the genotypes in breeding trials. Aparicio et al. (2002) also reported a significant interaction between growth stages and spectral indices (NDVI and SR) in durum wheat.

Association of grain yield with SRIs

Several authors (Ball and Konzak, 1993; Penuelas et al., 1997; Aparico et al., 2000; Ma et al., 2001 and Roya et al., 2003) have reported on the potential use of different spectral indices (mostly in regard to NDVI and SR) to differentiate genotypes for grain yield under diverse environmental conditions. A large proportion of the variation in yield in barley under three salinity levels was explained by spectral indices (Penuelas et al., 1997), in soybean with three different planting densities and two different soil types under well-watered conditions (Ma et al., 2000), and in durum wheat under different moisture levels and at different locations (Royo et al., 2003). A large part of the variation being explained in these studies may be driven by the diverse environmental conditions to which the crops were exposed. Royo et al. (2003) reported a very high variability in grain yield of different durum wheat genotypes when SRIs were combined across environments in a stepwise regression model (nine different moisture levels, nine field experiments and nine spectral reflectance indices were considered together), but when individual environments were studied on their own, the amount of variation explained decreased considerably. Their study did not give any clear indication of which SRIs were suitable for use under different environmental conditions. However, Aparicio et al. (2000) did show a strong relationship between grain yield and spectral indices under rainfed conditions in durum wheat.

This study includes historical landmark genotypes developed by CIMMYT with high yield capacity and with considerable diversity in morphology, plus different sister lines (RLs) expressing a similarly large range of diversity. Strong, consistent phenotypic correlations were observed between the NIR based indices and grain yield in all experiments in three successive years. Also, high genotypic correlations between grain yield and the NIR based spectral indices at the heading and grainfilling stages were observed (genotypic correlation values ranged from –0.594 to –0.925 in the above mentioned three different experiments) (data not shown). The estimation of genotypic correlations was done on small genotypic sample sizes in our study and may not be completely reliable, but their magnitude should be reasonably true population values. This indicates that when using the NIR based indices, the high amount of variation explained is related to variability among genotypes, and not due to any large environmental effects.

The spectral indices based on NIR (WI, NWI-1, and NWI-2) generally showed negative correlations with yield, increasing in value with the advancement of the crop cycle in all experiments (Table 6). These indices all incorporate an indicator of water status in the canopy. With an increasing amount of water in the plant, a decreasing amount of energy at 970 nm is reflected. Hence, the negative sign of the correlations reflect the fact that an increasingly low water status is associated with decreasing yields. Similar results were reported by Penuelas et al. (1997) and Royo et al (2003), both under irrigated and water-stressed conditions in durum wheat and barley.

RNDVI, GNDVI, and SR gave significant positive correlations with grain yield at the heading and grainfilling stages in nearly all cases over the three years. Ball and Konzak (1993) and Royo et al. (2003) reported a significant positive correlation between grain yield and RNDVI at grainfilling for spring and durum wheat under well-watered conditions. On the other hand, Aparicio et al. (2000) found a significant correlation between grain yield and RNDVI only at the maturity stage, and not at the other growth stages (booting, heading, anthesis, and milk-grain) in durum wheat under irrigated conditions. We have observed that in most cases RNDVI, GNDVI, and SR showed an increasingly higher correlation with grain yield as growth progressed from booting to flowering or grainfilling (Table 6). Also Ma et al. (2001), when studying soybean, found a clear increasing trend in the correlation values between NDVI and grain yield from full flowering (R2) to seed formation (R5) at three different planting densities in two soil types under well-watered conditions.

All indices studied explained the largest amount of the variation when taken at the heading or grainfilling stages. Our results generally agree with the findings of Ma et al. (2001) in soybean and Royo et al. (2003) in durum wheat under well-watered conditions. In our study it is evident that the mean values over different growth stages gave higher correlations with grain yield, which was not reported by previous authors. The three NIR based indices measure the water status at the canopy level, while RNDVI, GNDVI and SR measure the greenness of the canopy, and PRI is an indicator of radiation use efficiency in plants. All the indices are indicative of healthy plant conditions in the field. The repeated measurements on the same genotypes at different growth stages basically accumulate information on the health or condition of the genotypes over a period of time. We hypothesize that the mean values of the indices for the different growth stages represent cumulative information on the health of the canopy, which translates into a higher correlation with final grain yield. This may also be an indication that the underlying genetic correlation in stronger than the phenotypic one.

Since the time required to take the SRI data in the field is just 40-45 seconds/plot, it would be desirable to take the readings more than once. At a minimum, one measurement at heading and another one at grainfilling might effectively differentiate genotypes for grain yield. Hence, those two stages appear to be the most appropriate time to apply these spectral indices if the objective is to discriminate genotypes for grain yield. Very strong correlations were observed between the overall mean of the SRIs and overall grain yield. This strong correlation is also evidence of improved association between SRIs and grain yield when more dates were averaged over years and growth stages, which was not reported before.

Our study also demonstrated a high efficiency of the SRIs to evaluate the yield performance of genotypes over a period of time, which might be one of the most critical questions for breeders in evaluating a genotype for a particular environment (Table 7). The three NIR based indices were more successful than others. Our study also showed a very high efficiency for SRIs to select superior genotypes for grain yield, and NWI-2 performed better than RNDVI in selecting the top yielding genotypes for grain yield. These results are a definitive indication of the efficiency of NIR based SRIs for selecting superior genotypes for grain yield production.

The performance of the three NIR based indices was very similar in explaining grain yield variability among genotypes within a year or over a period of time. Normalizing the water index (NWI-1, and NWI-2) did not significantly improve the relationship. 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. That was not demonstrated in our study. Nonetheless, NWI-2 showed a marginal superiority over the two indices.

Association of SRIs with agronomic traits

The association between the SRIs and yield components was evaluated with the objective of determining if any particular yield component was driving the association with yield. The specific SRIs that were best associated with yield (i.e. the NIR based indices) also showed a greater correlation with these yield components. This observation is consistent with the fact that in all experiments grain yield, grains m^{-2}, and biomass showed significant positive correlations among themselves (data not presented). Waddington et al. (1986), and Sayre et al. (1997) clearly demonstrated that grain yield is particularly well correlated with grains m^{-2} in irrigated spring wheat in studies executed in the same location as used in this study. Therefore, it could be hypothesized that the relationship between NIR based indices and grains m^{-2} may be the most important basis of the high relationship between NIR based indices and grain yield.

Future research goals

Using an indirect selection tool is appropriate if the genetic correlation between the selected and unselected traits is very high, the heritability is much higher for the selected trait than for the unselected trait, and the correlated response in the unselected trait based on the selected trait is higher than the direct response to selection of the unselected trait. In practice, this combination is rarely obtained. It is also important to consider the time and cost involved in using indirect selection tools compared to the use of grain yield *per se* as a selection criterion. A research project is currently underway to estimate heritability, expected genetic gain, correlated response to selection for grain yield estimated from the SRIs, and the efficiency of selecting superior genotypes for grain yield based on SRIs compared to the selection of superior genotype based on yield *per se* at different geographic locations and at different moisture conditions under the supervision of Oklahoma State University and International Maize and Wheat Improvement Center, Mexico. Efforts are ongoing to develop a new lightweight spectral sensor to take measurements for these NIR based indices in the field. The new sensor (cost approximate $US 4000 to 5000) will facilitate faster measurements in the field compared to the spectroradiometer (cost $US 30,000) used in the current studies and will bring down the current cost of equipment to a minimum level. These improvements should facilitate the adoption of this selection criterion by breeders and enhance their ability to discriminate genotypes for grain yield in the breeding trials.

Conclusions

Spectral reflectance indices have shown the potential to differentiate genotypes for grain yield in this study with different types of breeding lines of spring wheat under irrigated conditions. The best growth stages to apply the indices to differentiate genotypes for grain yield were heading and grainfilling. Comparing various indices, the indices based on NIR (WI, NWI-1, and NWI-2) demonstrated consistently higher levels of association and explained a higher proportion of the variability for grain yield compared with the other spectral indices (RNDVI, GNDVI, SR, and PRI). The NIR based index (NWI-2) showed a very high efficiency in selecting superior genotypes in different experiments. The correlations in random populations confirm a genetic basis or link between yield and the physiological characteristics indicated by SRIs.

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Tables

Table 1. Minimum, maximum, mean, and LSD of grain yield (t ha^{-1}) within and between years in three different experiments

	Y 01-02	Y 02-03	Y 03-04	Across Years
		GHIST		
Minimum	5.23	4.91	5.28	4.97
Maximum	7.32	7.40	7.07	7.50
Mean	6.49	5.92	5.86	6.09
LSD(5%)	0.51	0.96	0.58	0.73
P-value	*	*	**	**
		RLs1		
Minimum	4.16	4.17	3.68	3.62
Maximum	6.49	6.47	5.41	6.47
Mean	5.43	5.50	4.56	5.16
LSD(5%)	0.54	1.00	0.56	0.72
P-value	**	*	**	**
		RLs2		
Minimum	4.90	5.00	4.16	4.15
Maximum	7.91	7.26	5.92	7.91
Mean	6.37	6.23	5.29	5.96
LSD(5%)	0.73	0.93	0.80	0.86
P-level	**	**	**	**

* Significant at 0.05 probability.

** Significant at 0.01 probability.

Table 2: Minimum, maximum, mean and LSD of spectral indices at individual growth stages across years.

		GHIST			RLs1			RLs2		
		Boot	Hd	GF	Boot	Hd	GF	Boot	Hd	GF
PRI	Minimum	-0.039	-0.058	-0.105	-0.045	-0.055	-0.119	-0.039	-0.046	-0.117
	Maximum	-0.005	-0.015	-0.035	-0.007	-0.013	-0.036	-0.008	-0.017	-0.046
	Mean	-0.023	-0.028	-0.056	-0.024	-0.031	-0.088	-0.024	-0.029	-0.083
	LSD(5%)	0.004	0.005	0.008	0.008	0.015	0.014	0.004	0.007	0.013
	P-Level	*	**	**	*	**	**	*	**	**
WI	Minimum	0.826	0.774	0.831	0.838	0.795	0.832	0.865	0.819	0.853
	Maximum	0.899	0.885	0.900	0.886	0.881	0.915	0.908	0.883	0.921
	Mean	0.876	0.832	0.857	0.861	0.834	0.875	0.889	0.852	0.886
	LSD(5%)	0.027	0.024	0.022	0.024	0.026	0.023	0.026	0.021	0.025
	P-Level	*	**	**	*	**	**	*	**	**
RNDVI	Minimum	0.810	0.793	0.684	0.856	0.861	0.639	0.902	0.878	0.549
	Maximum	0.953	0.954	0.891	0.938	0.928	0.922	0.948	0.941	0.864
	Mean	0.900	0.894	0.834	0.903	0.902	0.823	0.927	0.917	0.742
	LSD(5%)	0.031	0.052	0.035	0.024	0.036	0.050	0.011	0.011	0.076
	P-Level	*	**	**	*	**	**	*	**	**
GNDVI	Minimum	0.712	0.673	0.619	0.723	0.753	0.625	0.781	0.769	0.554
	Maximum	0.869	0.874	0.793	0.837	0.833	0.824	0.851	0.847	0.767
	Mean	0.792	0.790	0.736	0.780	0.797	0.734	0.816	0.817	0.679
	LSD(5%)	0.029	0.071	0.032	0.028	0.043	0.046	0.019	0.018	0.052
	P-Level	*	**	**	*	**	**	*	**	**
SR	Minimum	15.9	10.0	5.6	12.7	14.1	3.1	18.9	15.0	3.5
	Maximum	40.3	40.2	17.0	30.7	26.3	13.8	37.2	31.9	13.3
	Mean	25.7	22.9	11.8	20.1	19.9	6.7	26.2	23.2	7.3
	LSD(5%)	3.55	5.66	2.12	3.92	5.83	1.91	4.02	3.31	2.23
	P-Level	**	**	**	*	**	**	*	**	**
NWI-1	Minimum	-0.097	-0.128	-0.093	-0.071	-0.100	-0.080	-0.088	-0.114	-0.091
	Maximum	-0.052	-0.059	-0.051	-0.048	-0.062	-0.040	-0.061	-0.064	-0.043
	Mean	-0.066	-0.092	-0.077	-0.059	-0.080	-0.060	-0.075	-0.090	-0.066
	LSD(5%)	0.012	0.009	0.009	0.011	0.009	0.011	0.011	0.011	0.009
	P-Level	*	**	**	*	**	**	**	**	**
NWI-2	Minimum	-0.094	-0.127	-0.084	-0.068	-0.096	-0.073	-0.084	-0.112	-0.082
	Maximum	-0.043	-0.052	-0.041	-0.045	-0.056	-0.023	-0.057	-0.065	-0.028
	Mean	-0.060	-0.087	-0.069	-0.055	-0.074	-0.049	-0.071	-0.089	-0.055
	LSD(5%)	0.012	0.009	0.011	0.012	0.011	0.014	0.011	0.011	0.012
	P-Level	*	**	**	*	**	**	*	**	**

Boot, Hd, and GF, Spectral indices calculated at booting, heading and grainfilling stages, respectively. Estimates were calculated based on three years (Year 01-02, Year 02-03 & Year 03-04) in GHIST and two years (Year 02-03 & Year 03-04) in RLs1 and RLs2.

* Significant at 0.05 probability.

** Significant at 0.01 probability.

Table 3. Mean squares of the analysis of variance for different spectral reflectance indices combined across different growth stages and three different years in GHIST.

		Pr>F						
Effect	DF	PRI	WI	RNDVI	GNDVI	SR	NWI-1	NWI-2
Year	2	0.0041**	0.0271**	0.1394**	0.1738**	1645.5**	0.0057**	0.0061**
Entry	14	0.0013**	0.0032**	0.0077**	0.0099**	166.1**	0.0006**	0.0007**
GS	2	0.0275**	0.0814**	0.1195**	0.0900**	4871.8**	0.0149**	0.0173**
Year*Entry	28	0.0001**	0.0004**	0.0005	0.0005	14.8**	0.0001**	0.0001**
GS*Entry	28	0.0003**	0.0008**	0.0025**	0.0018**	17.5**	0.0001**	0.0002**
Year*GS	4	0.0009**	0.0182**	0.0250**	0.0281**	662.5**	0.0031**	0.0033**
Year*GS*Entry	56	0.00003**	0.0003*	0.0004	0.0003	6.8*	0.00005*	0.0001**
Error	135							
Total	269							

GS, growth stages.

* Significant at 0.05 probability.

** Significant at 0.01 probability.

Table 4. The relationship of spectral indices among different growth stages within years in GHIST

	PRI		WI		RNDVI		GNDVI		SR		NWI-1		NWI-2	
	Hd	GF	Hd	GF	Hd	GF	Hd	GF	Hd	GF	Hd	GF	Hd	GF
Year 01-02														
Boot	0.451	0.500	0.290	0.059	0.178	0.145	0.404	0.144	0.548*	0.331	0.331	0.086	0.287	0.073
Hd		0.884**		0.886**		0.919**		0.896**		0.910**		0.885**		0.848**
Year 02-03														
Boot	0.481	0.501	0.094	0.204	0.677**	0.723**	0.792**	0.759**	0.599**	0.613*	0.097	0.257	0.149	0.318
Hd		0.869**		0.729**		0.716**		0.686**		0.780**		0.715**		0.731**
Year 03-04														
Boot	0.196	0.124	0.197	0.024	0.898**	0.771**	0.844**	0.616**	0.874**	0.715**	0.236	0.037	0.060	0.050
Hd		0.943**		0.922**		0.943**		0.919**		0.915**		0.933**		0.932**

Boot, Hd, and GF spectral indices calculated at booting, heading and grainfilling stages, respectively.

* Significant at 0.05 probability.

** Significant at 0.01 probability.

Table 5. The relationship of spectral indices between years at different growth stages in GHIST.

	PRI	WI	RNDVI	GNDVI	SR	NWI-1	NWI-2
Between Y01-02 and Y02-03							
Booting	0.508	0.305	0.326	0.465	0.614*	0.306	0.301
Heading	0.855**	0.726**	0.848**	0.873**	0.823**	0.722**	0.703**
Grainfilling	0.831**	0.803**	0.852**	0.816**	0.844**	0.767**	0.792**
Between Y02-03 and Y03-04							
Booting	0.507	0.581*	0.829**	0.825**	0.771**	0.571*	0.519 *
Heading	0.863 **	0.624*	0.731**	0.710**	0.771**	0.628*	0.658 **
Grainfilling	0.904 **	0.855 **	0.885**	0.912**	0.915**	0.848**	0.848 **

* Significant at 0.05 probability.

** Significant at 0.01 probability.

Table 6. Correlation coefficients between grain yield and different spectral indices at different growth stages in different years.

	Y01-02				Y02-03				Y03-04				
Indices	Boot	Hd	GF	Mean†	Boot	Hd	GF	Mean†	Boot	Hd	GF	Mean†	Overall Mean‡
GHIST													
PRI	0.580*	0.692**	0.785**	0.691**	0.451	0.662**	0.635*	0.721**	0.648**	0.645**	0.637*	0.709**	0.818**
WI	-0.139	-0.731**	-0.731**	-0.707**	-0.222	-0.719**	-0.739**	-0.740**	-0.438	-0.662**	-0.628*	-0.753**	-0.876**
RNDVI	0.518*	0.585*	0.707**	0.829**	0.750**	0.675**	0.590*	0.672**	0.639*	0.620*	0.595*	0.646**	0.787**
GNDVI	0.522*	0.751**	0.734**	0.814**	0.767**	0.747**	0.627*	0.753**	0.749**	0.646**	0.584*	0.698**	0.863**
SR	0.292	0.585*	0.692**	0.581*	0.825**	0.731**	0.628*	0.800**	0.629*	0.640**	0.643**	0.724**	0.812**
NWI-1	-0.136	-0.739**	-0.769**	-0.701**	-0.233	-0.758**	-0.740**	-0.751**	-0.464	-0.651**	-0.601*	-0.731**	-0.881**
NWI-2	-0.097	-0.676**	-0.709**	-0.703**	-0.092	-0.784**	-0.744**	-0.748**	-0.472	-0.687**	-0.708**	-0.802**	-0.872**
RLs1													
PRI		0.188			0.216	0.058	-0.067	-0.003	0.455*	0.455*	0.591**	0.568**	0.354
WI		-0.549**			-0.305	0.651**	-0.418*	-0.718**	-0.272	-0.799**	-0.834**	-0.814**	-0.861**
RNDVI		-0.219			0.309	0.299	0.303	0.368	0.612**	0.648**	0.762**	0.759**	0.546**
GNDVI		-0.115			0.431*	0.399*	0.401*	0.478*	0.530**	0.624**	0.709**	0.722**	0.506**
SR		-0.235			0.354	0.396*	0.135	0.411*	0.553**	0.611**	0.659**	0.648**	0.408*
NWI-1		-0.588**			-0.343	-0.654**	-0.381	-0.748**	-0.229	-0.778**	-0.845**	-0.797**	-0.863**
NWI-2		-0.607**			-0.345	-0.605**	-0.308	-0.728**	-0.222	-0.786**	-0.828**	-0.803**	-0.870**
RLs2													
PRI		0.262			0.507**	0.584**	0.279	0.456**	0.294	0.303	0.502**	0.479**	0.452**
WI		-0.578**			-0.554**	-0.605**	-0.569**	-0.667**	-0.419*	-0.716**	-0.723**	-0.773**	-0.743**
RNDVI		0.379*			0.111	0.434**	0.249	0.287	0.492**	0.557**	0.564**	0.598**	0.457**
GNDVI		0.551**			0.288	0.622**	0.250	0.408*	0.435**	0.579**	0.554**	0.589**	0.571**
SR		0.374*			0.119	0.439**	0.220	0.308	0.442**	0.551**	0.597**	0.582**	0.468**
NWI-1		-0.593**			-0.539**	-0.611**	-0.562**	-0.660**	-0.385*	-0.711**	-0.726**	-0.772**	-0.747**
NWI-2		-0.599**			-0.588**	-0.682**	-0.578**	-0.706**	-0.429**	-0.704**	-0.715**	-0.773**	-0.780**

Boot, Hd, and GF, spectral indices calculated at booting, heading and grainfilling stages, respectively.

* Significant at 0.05 probability

** Significant 0.01 probability.

† Correlation between yield and mean spectral indices averaged over three different growth stages within the same year.

‡ Correlation between mean yield and mean spectral indices averaged over years.

Table 7. The correlations between different spectral reflectance indices (averaged over growth stages within each individual year) and grain yield (mean of three years) in three different experiments under irrigated conditions, presented by year.

Years	PRI	WI	RNDVI	GNDVI	SR	NWI-1	NWI-2
				GHIST			
Y01-02	0.750**	-0.825**	0.719**	0.702**	0.625**	-0.822**	-0.827**
Y02-03	0.809**	-0.893**	0.813**	0.869**	0.880**	-0.801**	-0.792**
Y03-04	0.827**	-0.833**	0.802**	0.857**	0.756**	-0.818**	-0.861**
				RLs1			
Y01-02†	-0.189	-0.500*	-0.212	-0.124	-0.220	-0.535**	-0.573**
Y02-03	0.201	-0.706**	0.131	0.247	0.404*	-0.739**	-0.753**
Y03-04	0.456*	-0.797**	0.677**	0.682**	0.590**	-0.794**	-0.799**
				RLs2			
Y01-02†	0.375	0.514**	0.447**	0.578**	0.415*	-0.549**	-0.563**
Y02-03	0.407*	-0.717**	0.395*	0.479**	0.418*	-0.716**	-0.761**
Y03-04	0.420*	-0.583**	0.377	0.463**	0.395*	-0.575**	-0.573**

* Significant at 0.05 probability.

** Significant at 0.01 probability.

† Correlation between the different SRIs measured only at heading stage and grain yield (mean of three years) in two experiments in a particular year

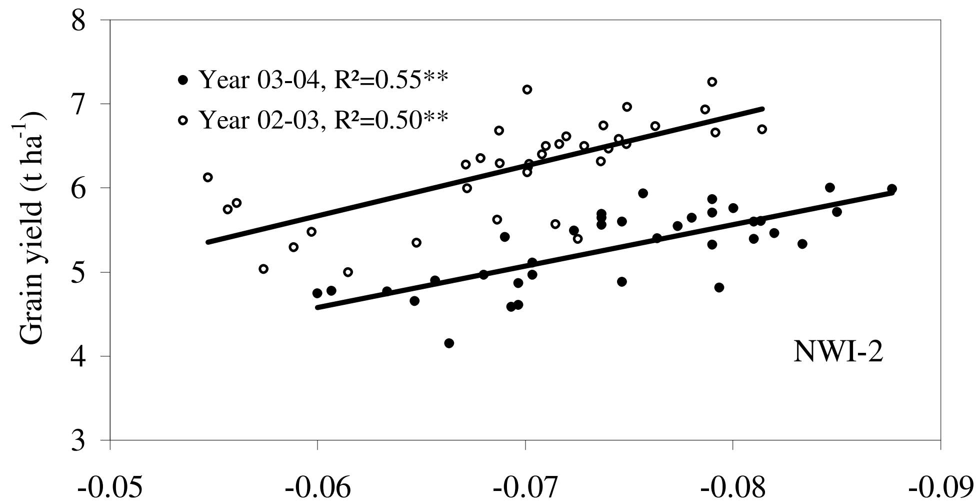
Table 8. Relationship between spectral indices and grains m^{-2} and biomass at maturity (BM) averaged over three years.

	GHIST		RLs1		RLs2	
Indices	Grains m^{-2}	BM	Grains m^{-2}	BM	Grains m^{-2}	BM
PRI	0.754**	0.408	0.433*	0.438*	0.308	0.503**
WI	-0.624*	-0.576*	-0.688**	-0.816**	-0.625**	-0.775**
RNDVI	0.749**	0.609*	0.624**	0.612**	0.423*	0.523**
GNDVI	0.759**	0.581*	0.562**	0.549**	0.478**	0.565**
SR	0.673**	0.659**	0.581**	0.580**	0.442**	0.477**
NWI-1	-0.633*	-0.582*	-0.704**	-0.820**	-0.628**	-0.781**
NWI-2	-0.573*	-0.587*	-0.727**	-0.847**	-0.626**	-0.821**

* Significant at 0.05 probability.

** Significant at 0.01 probability.

Figures



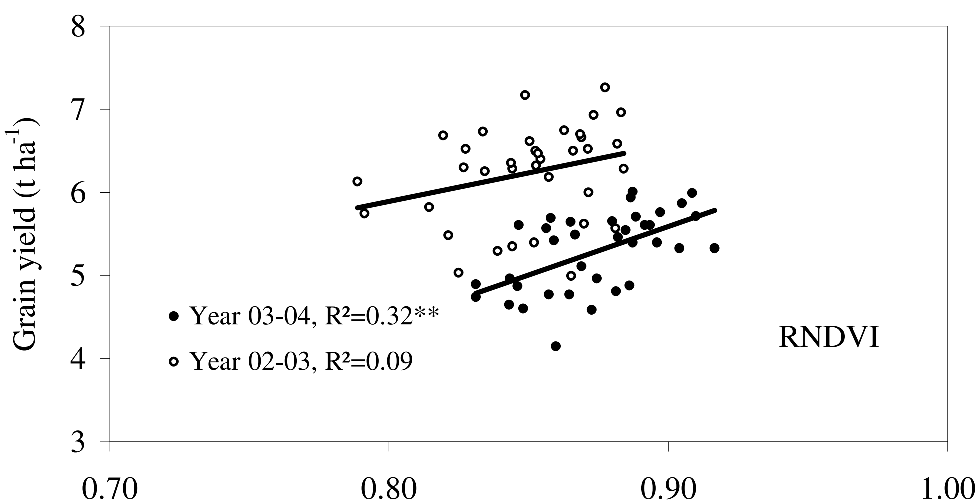
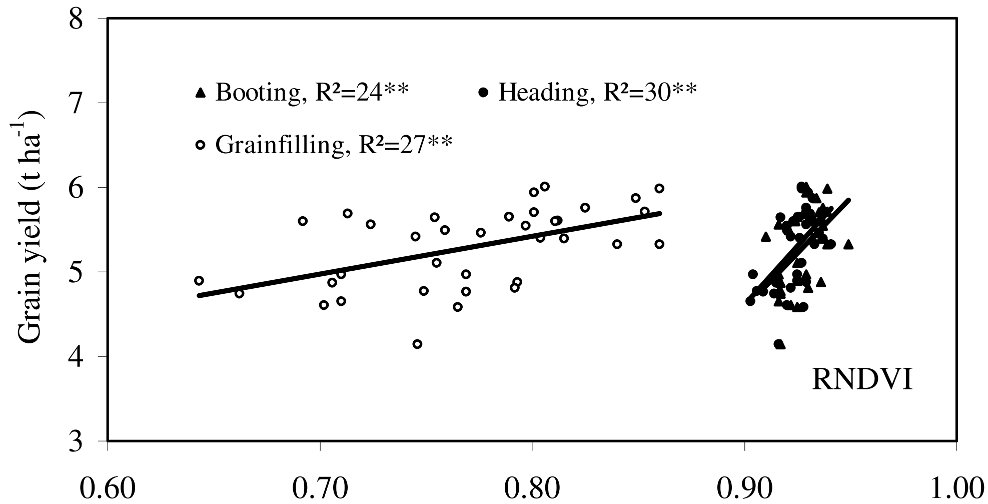


Fig 1. Relationship between grain yield and normalized water index-2 (NWI-2) and red normalized difference vegetation index (RNDVI) in RLs2 experiments in two different years where yields of the genotypes were plotted against mean values of the indices of three different growth stages within the same year. ** Significant at 0.01 probability.



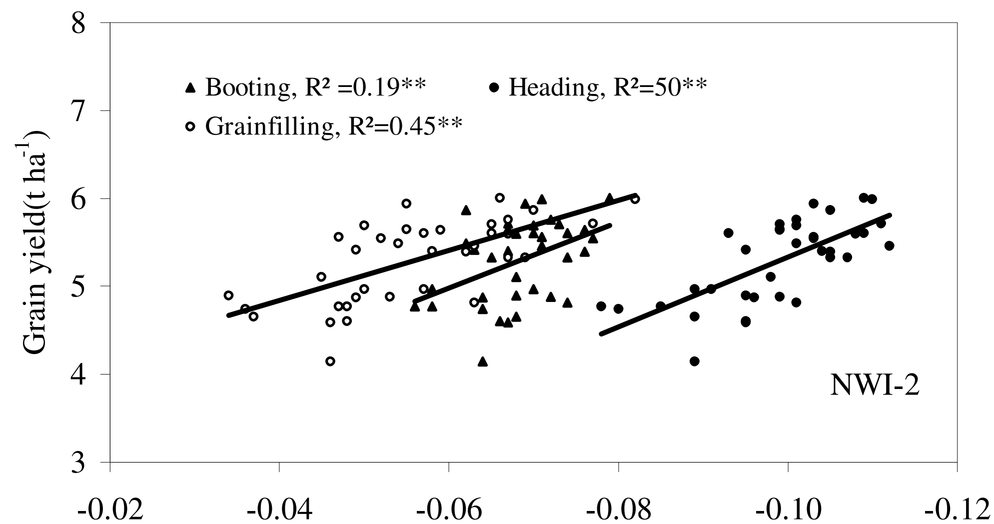


Fig 2. The relationship between grain yield and normalized water index-2 (NWI-2) and red normalized water index (RNDVI) at three different growth stages in year 03-04 in RLs2 experiment. ** Significant at 0.01 probability.

CHAPTER III

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

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Abbreviations

CT, Canopy Temperature

CTD, Canopy Temperature Depression

GHIST, Global Historic Trial

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

RILs1, Recombinant Inbred Lines-1

RILs2, Recombinant Inbred Lines-2

NDVI, Normalized Difference Vegetation Index

SR, Simple Ratio

SRIs, Spectral Reflectance Indices

WI, Water Index

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 to estimate genetic variation for in-season biomass production, leaf chlorophyll and canopy temperature in wheat under irrigated conditions. Three field experiments, GHIST (15 CIMMYT globally adapted historic genotypes), RILs1 (25 RILs), and RILs2 (36 RILs) were conducted under irrigated conditions at the CIMMYT research station in northwest Mexico in three different years. Five spectral reflectance indices 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 spectral reflectance indices also successfully differentiated the SPAD values at the genotypic level. The NIR based indices showed a strong and significant association with canopy temperature at the heading and grainfilling stages. These results demonstrate the potential of using SRIs as a breeding tool to select for increased genetic gains in biomass and chlorophyll content, plus for cooler canopies.

Introduction

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; Caldirini 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 not commonly associated with yield, increases in biomass of spring wheat have been reported (Waddington et al. 1986; Sayre et al., 1997; Singh et al., 1998). 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 (400-700nm) 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 near infrared (NIR) wavelengths (700-1300nm) 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 (SRIs) 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 index [NDVI=(NIR-VIS)/(NIR+VIS)] were the first developed SRIs, 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. SRIs have been developed based only on VIS, such as the photochemical reflectance index [PRI=(R_{531}nm-R_{570}nm)/(R_{531}nm+R_{570}nm)] 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}nm/R_{900}nm) 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 (Elliot and Regan, 1993; Bellairs et al., 1996). Their studies were carried out mainly under water-limiting conditions and with measurements taken at early growth stages in order to measure early vigor. The most widely used spectral reflectance indices, the normalized difference vegetation index (NDVI) and the simple ratio (SR), were not able to predict the variations in biomass successfully when estimated at later growth stages in durum wheat (Aparicio et al., 2000; Aparicio et al., 2002). Therefore, it is necessary to investigate the reflectance at other wavelengths in order to verify if there is a relationship between biomass and spectral reflectance indices, which may provide an indirect selection tool to differentiate spring wheat genotypes for biomass production.

SRIs based on visible wavelengths have also been developed to estimate the concentration of different leaf pigments such as chlorophyll and carotenoids. Chapelle 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 800nm 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 Chapelle et al. (1992). A hand-held 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 water index, calculated from the reflected light at 900 and 970nm, is an indicator of plant water status at both the leaf and canopy level (Peñuelas et al., 1993; Peñuelas et al., 1997). It can assess the changes of relative water content, leaf water potential, stomatal conductance, and canopy temperature depression 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 water index and canopy temperature depression was reported in spring wheat under irrigation by Gutiereg-Rodriguez et al. (2004), but the study was conducted with a small number of unrelated genotypes. The correlation between water index and canopy temperature depression 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 spectral reflectance indices, NDVI and SR, with the biomass production of a large number of bread wheat genotypes under near optimum nitrogen and irrigation levels at different stages of development, ii) derive new spectral reflectance indices 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 canopy temperature and the water index at the genotypic level.

Materials and Methods

One experiment (GHIST) was conducted for three years (year 2001-02, year 2002-03, and year 2003-04), and two experiments (RILs1 and RILs2) were conducted for two years (year 2002-03 and year 2003-04) under irrigated conditions at the CIMMYT (International Maize and Wheat Improvement Center) experimental station near Ciudad Obregon in Sonora, Mexico (lat. 27.33° N, long 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-meter plot consisted of two beds. The width of the beds was 80 cm. Each plot area was 8.0 m^2 (1.6 m x 5 m). In the first two years, 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 phosphorus were applied to the plots at the rate of 200 kg ha^{-1} and 26 kg ha^{-1}, respectively. During the first two years, 150 kg nitrogen and all of the phosphorus were applied during land preparation, and 50 kg nitrogen 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 nitrogen was applied prior to 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 500 ml ha^{-1} to protect the experimental materials from leaf rust.

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 years, 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 list of genotypes is given by Babar et al. (2005). The genotypes were planted in a 5x3 alpha-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 recombinant inbred lines (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 5x5 alpha-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 recombinant inbred lines (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 6x6 alpha-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, Colorado) with 25^{o} field of view. This instrument can detect reflected light from the canopy ranging from 350nm to 1100nm. Therefore, it covers the visible and part of the near infrared (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 40-50 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 10^{th} plot. The reflectance measurements were taken between 10:30 am to 1:30 pm 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 three years (year 2001-02, year 2002-03, and year 2003-04) and in RILs1 and RILs2 for one year (year 2003-04) (Zadoks et al., 1974). For the RILs1 and RILs2 in year 2002-03 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 0.5m 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 m x 0.8 m). The spectral reflectance data were taken randomly prior to harvesting biomass. The biomass cut was done randomly from the middle three meters of the plot (one meter 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 75^0 C for 48 hours. The oven-dried weight of biomass was recorded and total dry weight of biomass was estimated from total fresh weight, and converted into g m^{-2}. Biomass was sampled three times at booting, heading and grainfilling in the GHIST experiments in both years (year 2002-03 and year 2003-04), and two times (booting and heading) and three times (booting, heading and grainfilling) for the other two experiments (RILs1 and RILs2) in the year 2002-03 and year 2003-04, respectively.

Chlorophyll meter measurements

A hand-held 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 three years, 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 spectral reflectance indices 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 850nm, 900nm, and 970nm. The 970 nm has been reported as a weak water absorption band (Peñuelas et al., 1993), and the other two bands (850nm 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 spectral reflectance indices, which were calculated based on different references, are provided in the Table 1. The above mentioned normalized water indices were calculated as follows:

Normalized water index-1, NWI-1 = $(R_{970}-R_{900})/(R_{970}+R_{900})$, and

Normalized water index-2, NWI-2 = $(R_{970}-R_{850})/(R_{970}+R_{850})$.

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

Canopy temperature (CT)

A hand-held Infrared Thermometer (Model AG-42, Tela-temperature Crop, Fullerton, CA), with a field of view of 2.5^o, was used to measure canopy temperature (^{o}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^o to the horizontal. Readings were made between 1:00-3:00 pm on sunny days. To avoid the effect of soil temperature on the canopy temperature, the data were taken when the infrared thermometer viewed no soil because of high leaf coverage areas. The canopy temperature measurements were taken at three different growth stages (booting, heading, and grainfilling) in the year 2003-04, and only at grainfilling in year 2001-02 and year 2002-03 in the GHIST experiment, and two times (booting and grainfilling) in RILs1 and RILs2 in the year 2002-03 and 2003-04. The data for each plot are the means of two to four sets of readings.

Statistical analysis

Alpha-lattice analyses for biomass, different spectral reflectance indices, SPAD-chlorophyll meter readings, and RARS and PSSRa indices (combined across different years) were carried out using the MIXED procedure of the SAS software (SAS Inst., 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 canopy temperature 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 Inst., 2001).

Results

Genotypic variations

The means (±SE) of five different spectral reflectance indices 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 near infrared radiation (NIR) based spectral reflectance indices (water index, NWI-1, and NWI-2) in most of the growth stages and years.

Table 3 indicates that the genotypes varied for the spectral reflectance indices 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 two years. 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 (water index, 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 and 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 RARSa increased as the growth stage advanced, while PSSRa showed a reciprocal effect, even though both indices measure chl-a concentration in the plant. The RARSb and 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 biomass at the three different growth stages in the GHIST experiment (year 2003-04) were plotted against the values of the three different indices (NDVI, water index, 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 water index 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 SRIs

Spectral reflectance indices showed a significant phenotypic correlation with dry biomass at the heading and grainfilling stages (Table 5). NIR 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 (water index, 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 was correlated at the booting stage, the coefficients were lower than at the heading and grainfilling stages, except for the GHIST experiment in the year 2003-04. 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 SRIs. 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 and Fig 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), RARSa, 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 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 canopy temperature and NIR based indices (water index, NWI-1, and NWI-2), and the phenotypic correlation and genetic correlations between canopy temperature 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 canopy temperature 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 canopy temperature and the NIR based indices.

Discussion

Genotypic variations

Statistically significant genotypic variation for different spectral reflectance indices 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 recombinant inbred lines (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 SRIs 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 SRIs 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 SRIs that were developed based on NIR wavelengths expressed non-significant 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 2002-03, which caused interference in the spectral reflectance measurements (Table 5). Elliot 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 (Aparicio et al., 2002).

Effect of growth stages

Younger wheat plants generally absorb more photosynthetically active radiation, and therefore, reflect more NIR radiation. 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. NDVI 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. Fisher 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 (water index, NWI-1 and NWI-2) were calculated combining information from 850nm, 900nm and 970 nm. The 970nm wavelength is a water absorption band and the sensitivity of this band depends on the 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. NIR 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 radiation 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 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 (chl-a). Therefore, chl-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 SRIs

The spectral indices based on NIR (water index, 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 (Arlchis 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 ($r = 0.708$ to 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-base indices was indistinguishable. Normalizing the water index (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^o) was used throughout the experimental cycle and the data were taken in the middle of the day (10:30 am to 1:30 pm) 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 900nm and 680nm to develop the normalized difference vegetation index (NDVI) and the simple ratio (SR).

NDVI 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 grain filling 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 Elliot 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 loose 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 nm 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 nm 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 hand-held 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 nm and 940 nm to estimate leaf chlorophyll concentration (Yadava, 1986; Dwyer et al., 1991), and thus monitors nitrogen 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 (550nm, 650nm, 710nm) under different nitrogen levels in corn (Blackmer et al., 1994; Schepers et al., 1996). RARSa, RARSb, and RARSc were developed to estimate chl-a, chl-b and carotenoids in soybean (Chapelle et al., 1992), and PSSRa was developed to estimate chl-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 470nm, 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 canopy temperature depression (canopy temperature 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 recombinant inbred lines 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 water index 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 water index and canopy temperature depression was well correlated under irrigated conditions across different salinity gradients at the genotypic level. Babar et al. (2005) found a strong association between grain yield of wheat and water index under the same environmental conditions where the present study was conducted.

In this study, strong positive correlations were observed between canopy temperature and NIR based indices when the data were taken at heading and at the grain filling stages. These strong positive correlations indicate that genotypes with higher water content (represented by lower NIR based indices values) had lower canopy temperature. 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 canopy temperature depression. 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 (water index, 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 spectral reflectance indices and SPAD chlorophyll values to estimate chlorophyll and carotenoids in wheat at the genotypic level. The NIR based indices showed a strong association with canopy temperature 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 spectral reflectance indices, and between other physiological traits indicated by SPAD values, RARS indices, and canopy temperature.

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Tables

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 (chl-a)	Chapelle et al. (1992)
Ratio analysis of reflectance spectra (RARSb)	$R_{675}/(R_{650}*R_{700})$	Estimation of Chlorophyll-b (chl-b)	Chapelle et al. (1992)
Ratio analysis of reflectance spectra (RARSc)	R_{760}/R_{500}	Estimation of Carotenoids (car)	Chapelle et al. (1992)
Pigment specific simple ratio (PSSRa)	R_{800}/R_{680}	Chlorophyll-a (chl-a)	Blackburn (1998)

'R' and the sub-index in all of the above formulae indicate the reflectance of light at that specific wavelength (in nm).

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.

Experiments	Years	Growth stages	Spectral reflectance indices				
			NDVI	SR	WI	NWI-1	NWI-2
GHIST	Year	Booting	0.933±0.005**	29.3±2.9**	0.871±0.007**	-0.068±0.005**	-0.066±0.004**
	03-04	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**
	Year	Booting	0.944±0.004**	34.7±2.9**	0.874±0.006**	-0.067±0.004**	-0.058±0.004**
	02-03	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	Year	Booting	0.887±0.011*	17.7±1.9**	0.898±0.008**	-0.053±0.005**	-0.049±0.005**
	03-04	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**
	Year	Booting	0.911±0.006**	21.9±1.7*	0.887±0.01	-0.059±0.005	-0.066±0.004
	02-03	Heading	0.894±0.025	18.3±3.8	0.865±0.013	-0.072±0.006	-0.066±0.006
RILs2	Year	Booting	0.928±0.005*	26.5±1.8**	0.867±0.005**	-0.071±0.003**	-0.068±0.003**
	03-04	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**
	Year	Booting	0.926±0.006*	25.7±2.3*	0.855±0.014*	-0.078±0.006*	-0.074±0.006*
	02-03	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 three years in the GHIST experiment.

	Growth stages		
Indices	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 $P = 0.01$.

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.

Years	Growth stages	GHIST FW ($g\ m^{-2}$)	GHIST DW ($g\ m^{-2}$)	RILs1 FW ($g\ m^{-2}$)	RILs1 DW ($g\ m^{-2}$)	RILs2 FW ($g\ m^{-2}$)	RILs2 DW ($g\ m^{-2}$)
Year 03-04	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**
Year 02-03	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 $P = 0.01$.

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. NDVI, normalized difference vegetation index; SR, simple ratio; WI, water index; NWI-1, normalized water index-1; NWI-2, normalized water index-2.

		Year 03-04					Year 02-03				
		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 $P = 0.01$.

£ 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.

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
Years	Indices	Booting	Heading	Grainfilling	Mean£	Mean‡‡
Year 03-04	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**
Year 02-03	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.

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			
Years		Growth stages	RARSa	PSSRa	RARSb	RARSc
Year 03-04	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**
Year 02-03	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
Year 01-02	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.

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		
Experiments		Years	Growth stage	WI	NWI-1	NWI-2
GHIST	r_p	Year 03-04	Booting	-0.246	-0.238	-0.141
			Heading	0.732**	0.715**	0.728**
			Grainfilling	0.902**	0.896**	0.895**
		Year 02-03	Grainfilling	0.821**	0.825**	0.785**
		Year 01-02	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	Year 03-04	Booting	0.233	0.203	0.189
			Grainfilling	0.756**	0.763**	0.794**
		Year 02-03	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	Year 03-04	Booting	-0.265	-0.256	-0.192
			Grainfilling	0.670**	0.676**	0.634**
		Year 02-03	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**

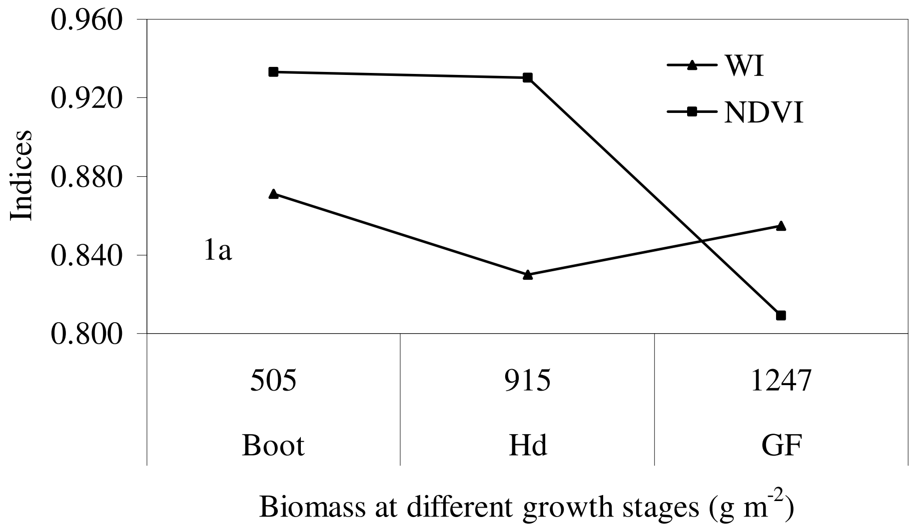
* Significant at $P = 0.05$.

** 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.

Figures



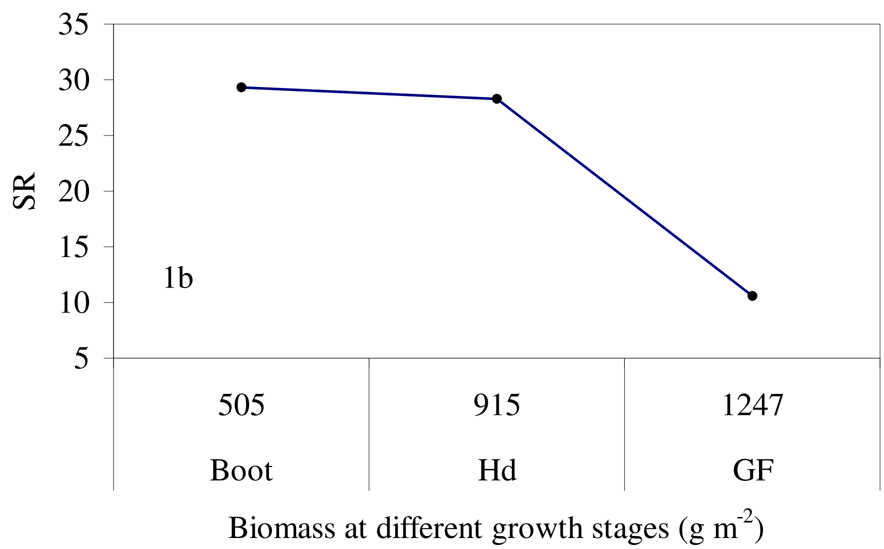


Fig 1. The changes in values of normalized difference vegetation index (NDVI), water index (WI), and simple ratio (SR) with an increase in biomass and with the advancement of growth stages in experiment GHIST in year 2003-04. Boot, Hd, and GF indicates the biomass and spectral indices were measured at booting, heading and grainfilling.

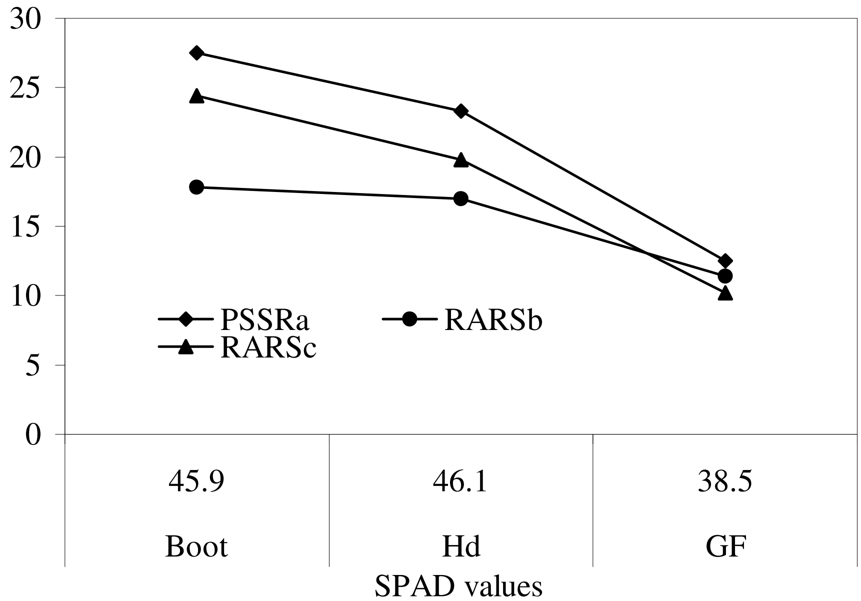


Fig 2. The trends of changes in pigment specific simple ratio-chlorophyll a (PSSRa), ratio analysis of reflectance spectra-chlorophyll b (RARSb), and ratio analysis of reflectance spectra-carotenoids (RARSc) with the changes of values of SPAD in three different growth stages. Boot, Hd, and GF indicates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

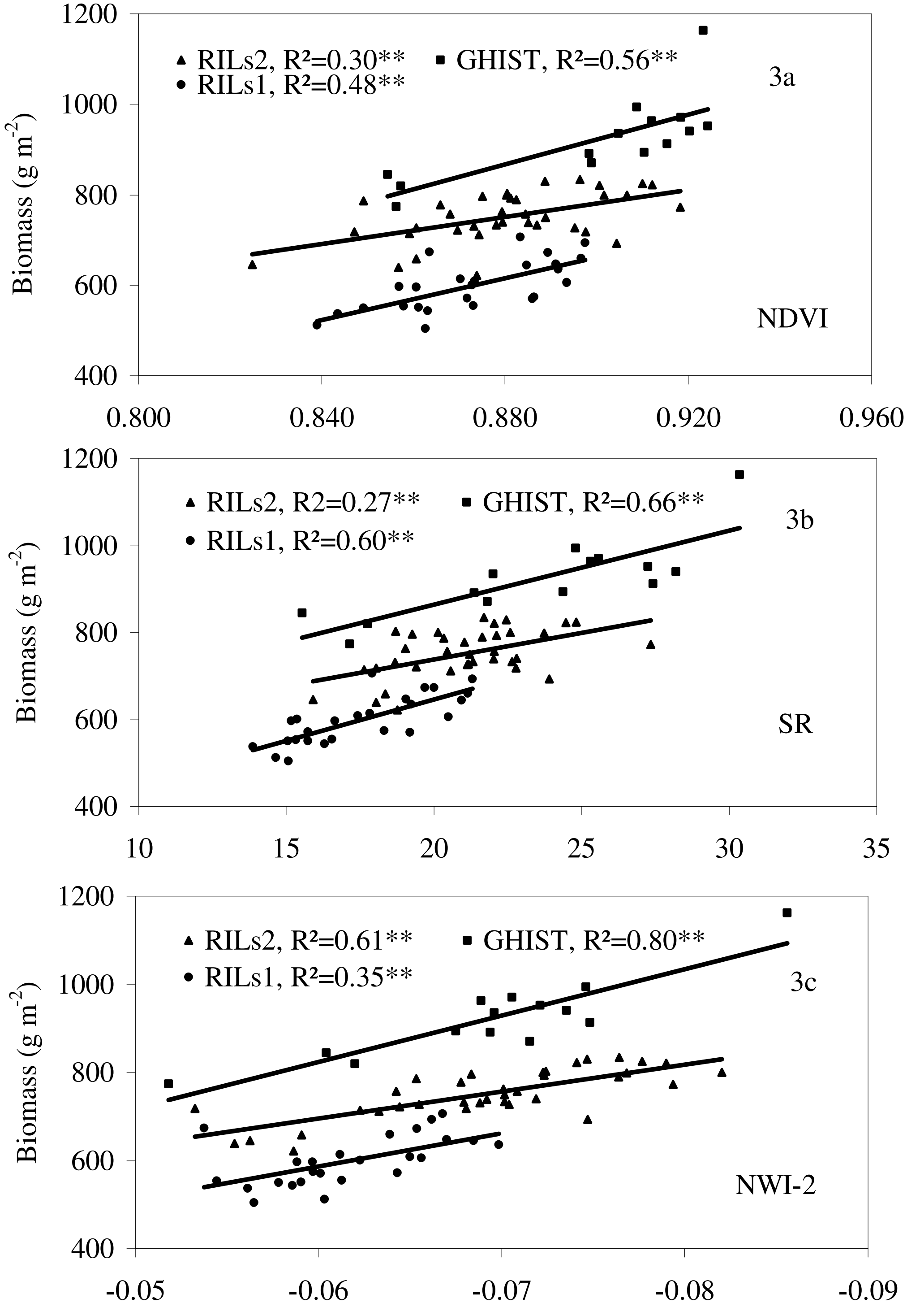


Fig 3. The relationship between biomass and normalized difference vegetation index (NDVI), simple ratio (SR), and normalized water index-2 (NWI-2) in three different experiments, with the correlation coefficients calculated from the averages over two years for biomass and spectral reflectance indices.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

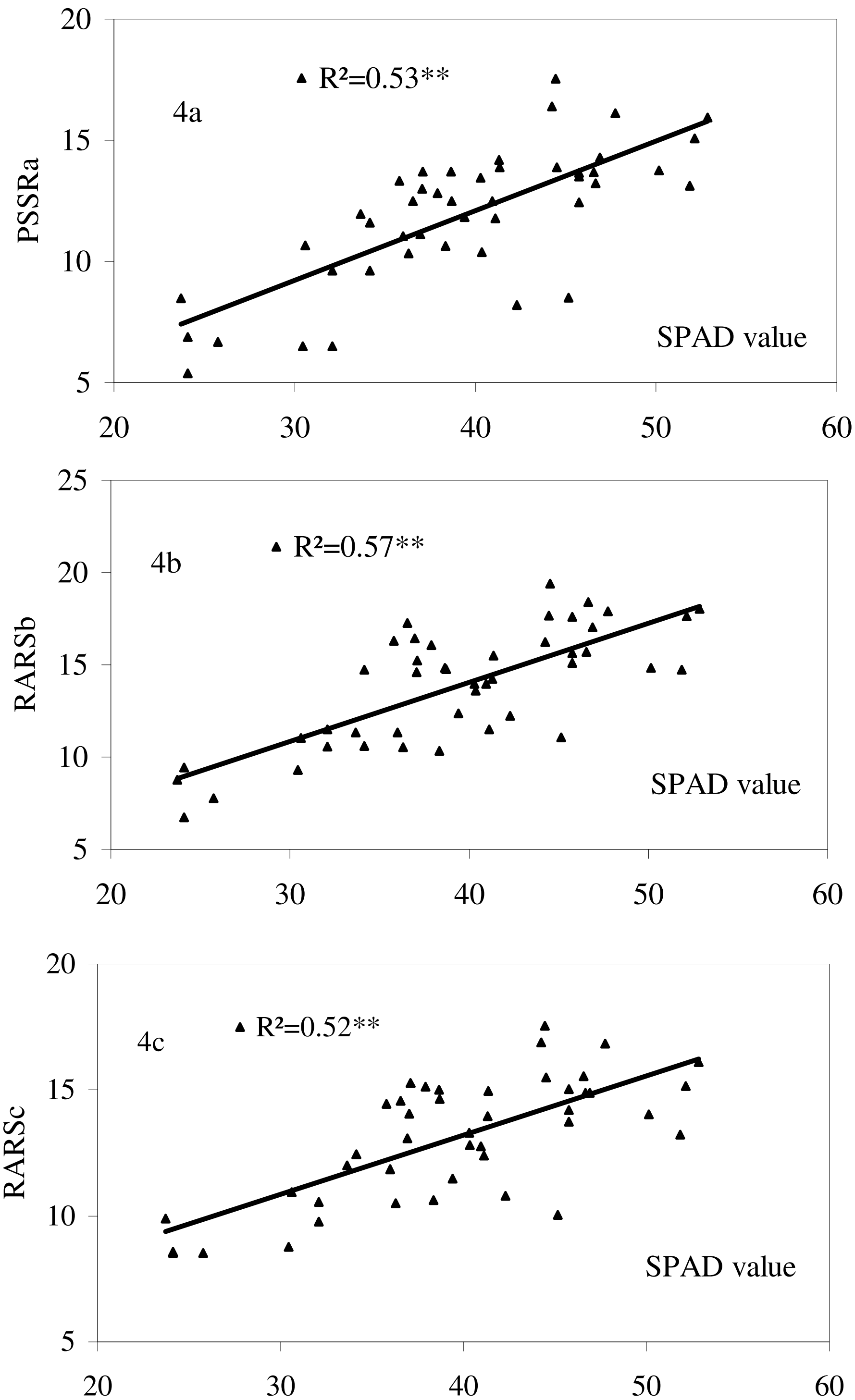


Fig 4. Relationship between chlorophyll content (SPAD values) and pigment specific simple ratio-chlorophyll a (PSSRa), ratio analysis of reflectance spectra-chlorophyll b (RARSb), and ratio analysis of reflectance spectra-carotenoids (RARSc) across three years in experiment GHIST at grainfilling stage.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

CHAPTER IV

Association between Grain Yield and Spectral Reflectance Indices and Their Heritability in Wheat Under Water Limiting Environments

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Abbreviations

CR, Correlated response

NDVI, Normalized Difference Vegetation Index

NIR, Near Infrared Radiation

NWI-1, Normalized Water Index-1

NWI-2, Normalized Water Index-2

PDR, Proportion of Direct Response to Selection

R, Direct response to the selection

r_g Genetic correlation coefficients

r_p Phenotypic correlation coefficients

SR, Simple Ratio

SRIs, Spectral Reflectance Indices

VIS, Visible wavelengths

WI, Water Index

$\sigma^2_{e,}$ Residual Variance

σ^2_g, Genotype Variance

σ^2_{gm}, Genotype-Moisture Environment Interaction Variance

Abstract

The objectives of this research were to study the association between spectral reflectance indices (SRIs) and grain yield, estimate their heritability, and the correlated response to selection (*CR*) for grain yield estimated from SRIs under water limiting environments in bread wheat. Reflectance was measured at three different growth stages (booting, heading and grainfilling) and five SRIs were calculated, namely normalized difference vegetation index (NDVI), simple ratio (SR), water index (WI), normalized water index-1 (NWI-1), and normalized water index-2 (NWI-2). Three field experiments were conducted (each with 30 advanced lines) in three different years. Two moisture environments were created, namely a one-irrigation level (pre-plant), and a two-irrigation level (pre-plant and booting stage), both representing levels of drought. Genotypic variations for all SRIs were significant. Three NIR (near infrared radiation) based indices (WI, NWI-1, and NWI-2) gave the highest level of association (both phenotypic and genotypic) with grain yield under both reduced moisture conditions. Using the mean SRI values averaged over growth stages or the progressive integration of SRIs from booting to grainfilling increased the capacity to explain variation among genotypes for yield. A higher level of broad-sense heritability was found with the two-irrigations regime (0.80) than with one-irrigation (0.63). Overall, 50% to 75% of the top 12.5 percent highest yielding genotypes, and 50% to 87% of the top 25 percent highest yielding genotypes were selected when the NWI-2 index was applied as an indirect selection tool. Strong genetic correlations, moderate to high heritability, correlated response for grain yield close to direct selection for grain yield, and a very high efficiency of selecting superior genotypes indicate the potential of using these three SRIs in breeding programs for selecting for increased genetic gains in grain yield under water limiting environments.

Introduction

Wheat breeders in last several decades have made successful yield improvement using grain yield as a selection criteria, which involves the trial and error method of selecting for yield *per se* (Slafer and Andrade 1991; Loss and Siddique 1994; Trethowan et al., 2001, 2002, 2003; Lillemo et al., 2004). However, the rate of genetic improvement in yield has been different in different environments (Pingali and Rajaram, 1999). Genetic improvement in yield has been particularly successful under irrigated conditions, but the progress is less under water-limiting environments (Richards et al., 2001; Trethowan, 2002). Water-limiting environments are characterized by either unpredictable and highly variable rainfall patterns or uneven moisture distribution patterns in the soils. Thus, yield is highly variable, and shows high genotype by environment interaction and low heritability (Richards et al., 2001). As a consequence, the genetic gain in the yield under water-limiting conditions has been limited.

In classical breeding programs, a large number of advanced breeding genotypes are evaluated with the best commercial cultivars in order to select suitable genotypes for the target environments. Although, the large-scale field evaluation process is expensive in terms of time and financial resources, such field evaluations are still necessary to effectively identify winning genotypes in a real-life setting. Often, even additional evaluations are necessary in successive years and in different locations. Proper statistical procedures are needed to cope with the undesirable genotype-environment interactions, thereby reducing the chance of discarding a good line in the trials (Ball and Konzak, 1993). To avoid or at least help reduce this laborious, time consuming, and cumbersome process, an easy, rapid and inexpensive selection tool is needed to help breeders reliably screen large numbers of genotypes in a relatively short time on small plots before expensive yield trials are carried out (Reynolds et al., 1999). It would be very advantageous if such a selection tool could detect high yielding genotypes rapidly and efficiently from a large number of genotypes.

Using morphological and physiological selection criteria to differentiate grain yield is an indirect breeding approach. Richards et al. (2001; 2002) have emphasized that future improvement in grain yield of wheat under water-limiting environments will be achieved by using different physiological traits. The use of these physiological tools in breeding programs is limited because of the limited understanding of their relationship with yield and frequently due to the proposed evaluation procedure being complex (Loss and Siddique, 1994; Richards 1996). The understanding of physiological traits that limit yield under drought conditions has improved in recent years (Richards et al., 2001). This has created a new opportunity for plant breeders to use those tools as selection criteria for grain yield improvement. In recent years, different authors have investigated the use of various physiological traits to improve grain yield in diverse environments. Canopy temperature, which can be sensed remotely using IR thermometry, has been shown to be well associated with yield of wheat cultivars (Reynolds et al., 1994; Fischer et al., 1998), as well as that of recombinant inbred lines and advanced breeding materials (Reynolds et al., 1998; 1999) in irrigated, high radiation environments. Condon and Richards (1992) and Condon et al (2004) proved that the use of carbon isotope discrimination was a useful trait to improve grain yield potential in water-limiting environment.

Some recent studies suggest that spectral reflectance is another promising remote sensing technique for screening genotypes for grain yield (Araus et al., 2001). Canopy light reflectance properties mainly based on the absorption of light at specific wavelengths are associated with specific plant characteristics. The spectral reflectance in the visible (VIS) wavelengths (400-700nm) depends on the absorption of light by leaf chlorophyll and associated pigments such as carotenoid and anthocyanins. The reflectance in the VIS range is low because of the high absorption of light energy by these pigments. The reflectance in the near infrared (NIR) wavelengths (700-1300nm) is high because of the multiple scattering of light by different leaf tissues (Knipling, 1970). Spectral reflectance indices (SRIs) were developed on the basis of simple mathematical formulae such as ratios or differences between the reflectance at given wavelengths (Araus et al., 2001). The Simple Ratio (SR=NIR/VIS) index and the Normalized Difference Vegetation Index [NDVI=(NIR-VIS)/(NIR+VIS)] were the first SRIs to be described, combining 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 (Tucker and Seller, 1986). SRIs have been developed based only on VIS, such as the Photochemical Reflectance Index [PRI=(R_{531}nm-R_{570}nm)/(R_{531}nm+R_{570}nm)] to assess radiation use efficiency by plants (Peñuelas et al., 1995), and also only on NIR, such as the Water Index (WI=R_{970}nm/R_{900}nm) to assess the water status of the canopy (Peñuelas et al., 1993). SRIs have been widely reported by different authors to assess different physiological conditions of the canopy such as the estimation of total dry matter, leaf area index, photosynthetic capacity (Sellers, 1987), as well as green leaf area index and fractional photosynthetically active radiation absorption (Wiegand and Richardson, 1990; Baret and Guyot, 1991). SRIs have also proven to be useful in the assessment of early biomass and vigor of different wheat genotypes (Elliot and Regan, 1993), water status in gerbera and barley (Peñuelas et al., 1993; Peñuelas et al., 1997), and different pigment concentrations in the leaves of soybean (Chappelle et al., 1992). The potential for using NDVI to predict in-season grain yield has also been reported in wheat under water stressed environments (Raun et al., 2001).

Attempts have been made to evaluate the potential use of SRIs in wheat breeding to differentiate genotypes for yield under well-watered conditions in bread wheat (Ball and Kazak, 1993; Babar et al., 2005), under moisture stressed conditions in durum wheat (Aparicio et al., 2000; Royo et al., 2003), and in soybean (Ma et al., 2001). Babar et al. (2005) made an extensive study of spectral reflectance indices (SRIs) under irrigated conditions at the same location where the present studies were conducted (Obregon, North-West Mexico). The study showed that three NIR based indices, water index (WI), normalized water index-1 (NWI-1), and normalized water index-2 (NWI-2) had strong associations with grain yield (both phenotypic and genotypic) of wheat cultivars and random sister lines (F_5, F_6, and F_7 generations). This study sought to verify the potential use of these three NIR based indices under water limiting conditions.

In this study, two water-limiting environments were created, a one-irrigation (low yield potential environment, 2-2.5 t/ha) and a two-irrigation (moderate yield potential environment, 5-5.5 t/ha) to test the validity of using the three above mentioned NIR based indices to differentiate genotypes for grain yield. The yield potential of the experimental location is >7.0 t/ha under fully irrigated conditions (Sayre et al., 1997). The two-irrigation condition was used in all three years of the study (year 2001-02, 2002-03, and 2003-04), whereas the one-irrigation condition was used only in year 2003-04. Unpredictable rainfall added to these two moisture environments, and the total amount of moisture received from rain ranged from 18.3 to 191.0 mm. Early heat stress in the second and third week of March in year 2003-04 created an additional unpredictable stressed condition. These unusual situations created a useful opportunity to test the potentiality of the three NIR based indices under different environmental conditions.

Thus, the objectives of the study were i) to evaluate the three NIR based indices as a potential screening tool under water-limiting environments, ii) compare their effectiveness with the most widely used indices, NDVI and SR, iii) determine suitable growth stage or stages when these tools can be applied, iv) determine the efficiency of the SRIs in selecting the top yielding genotypes, and v) estimate broad-sense heritability and correlated selection response for grain yield estimated from SRIs.

Materials and Methods

Three experiments, each with 30 elite advanced genotypes developed by the wheat breeding program of the International Maize and Wheat Improvement Center (CIMMYT), were conducted during three years (year 2001-02, year 2002-03, and year 2003-04). Experiment-1 (designated as Expt-1) was conducted with two irrigations in the year 2001-02 and year 2003-04. Experiment-2 (Expt-2) was conducted with two irrigations in the year 2002-03 and year 2003-04, and with just one irrigation in year 2003-04. Experiment-3 (Expt-3) was grown under one and two irrigations in the 2003-04 crop cycle. All experiments were conducted at the CIMMYT experimental station near Ciudad Obregon in Sonora, Mexico (lat. 27.33^{o} N, long 109.09^{o} 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 (January-May). The weather pattern of the location is described in Table 1. The experiments were planted on raised beds and each 3-meter plot consisted of two beds. The width of the beds was 80 cm. Each plot area was 4.8 m^2 (1.6 m x 3 m). In the first and third years, three rows were planted on each bed with 15 cm distance between rows. In the second year, two rows were planted on the beds with 20 cm row spacing. The experiments were laid out in a 6x5 alpha-lattice design with two replications.

The seeding rate was 73 kg/ha in the first two years, and 63 kg/ha under both irrigation conditions in the third year. In the first two years with two irrigations, a total of 200 kg/ha nitrogen was supplied split into two equal proportions: the first half was applied at land preparation shortly prior to planting and the other half at the booting stage during the supplemental irrigation. Fifty kg/ha $P_2O_5$was also supplied during land preparation with two irrigations. Aphix (1 L/ha) was applied to control aphids. In the third year, 100 kg/ha nitrogen was supplied during land preparation in both the one and two-irrigation regimes, and 75 kg/ha nitrogen was top-dressed at the booting stage during the supplemental irrigation under the two-irrigations regime.

In the one-irrigation regime, 80 mm of water was supplied shortly before planting of the experiments. The two irrigations regime consisted of one pre-plant irrigation (80 mm) and one supplemental irrigation (80 mm) at the booting stage. In the two irrigations regime, the supplemental irrigation was applied at 65 days after planting in the first two years. However, in the third year, the supplemental irrigation was applied after 85 days because 45 mm of rainfall was received during the fourth week of February and first week of March. In the third year of the study, both the one and two-irrigation regimes received 190 mm rainfall during the growing season, which reduced drought stress. The rainfall in all three years is presented in Table 1.

Radiometric measurements

The spectral reflectance measurements were taken using a portable narrow-bandwidth Spectroradiometer (Model FieldSpec UV/VNIR, Analytical Spectral Devices, Boulder, Colorado) with 25^o field of view. This instrument can detect reflected light from the canopy ranging from 350nm to 1100nm. Therefore, it covers the visible and part of the near infrared (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. With the help of a pistol grip the sensor was held 40-50 cm above the canopy facing the center of the bed. The Spectroradiometer was recalibrated against a white reference plate ($BaSO_4$) every 10 plots. The reflectance measurements were taken between 10:30 am to 1:30 pm under sunny conditions, and from four different places randomly within each plot. The mean of the four readings was used to calculate spectral indices.

The spectral reflectance measurements were taken at the booting stage (designated as 'Boot', Zadoks stage 39-47), heading (designated as 'Hd', Zadoks stage 55-69), and grainfilling (designated at 'GF', Zadoks stage 75-83) in the three different experiments (Zadoks et al., 1974). In the two-irrigations regime, spectral reflectance measurements were taken at three growth stages in all three experiments in year 2003-04, two growth stages (Hd and GF) in Expt-2 in year 2002-03, and during one growth stage in Expt-1 in year 2001-02. For the one-irrigation regime, the reflectance measurements were taken at two growth stages (Hd and GF) in Expt-2 and Expt-3 in year 2003-04.

Calculation of indices

Based on our prior study under irrigated conditions at the same location, the results of two indices (based on NIR) are presented in this paper. The two indices combined information from 850 nm, 900 nm, 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 nm 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).

The different spectral reflectance indices calculated were:

Water index, WI = R_{970}/R_{900} (Peñuelas et al., 1993), which indicates canopy water status.

Normalized difference vegetation index, NDVI = $(R_{780}-R_{670})/(R_{780}+R_{670})$ (Raun et al., 2001), which indicates canopy photosynthetic area.

$SR=R_{900}/R_{680}$ (Aparicio et al., 2000), which is also an indicator of canopy photosynthetic active areas.

The above mentioned normalized water indices were calculated as follows:

Normalized water index-1, NWI-1 = $(R_{970}-R_{900})/(R_{970}+R_{900})$, and

Normalized water index-2, NWI-2 = $(R_{970}-R_{850})/(R_{970}+R_{850})$.

'R' and the sub-index in all of the above formulae indicate the reflectance of light at that specific wavelength (in nm).

Data analysis

Alpha-lattice analyses for different SRIs and grain yield under each individual moisture environment and across moisture environments were carried out using the MIXED procedure of the SAS software (SAS Inst., 2001). Phenotypic and genotypic correlations were used to estimate the relationship between SRIs and grain yield at the different growth stages. Phenotypic correlations were taken as simple Pearson's correlations, which were also estimated using the SAS software (SAS Inst., 2001).

Estimation of genetic correlations, broad-sense heritability, expected response to the selection (*R*) and correlated selection response (*CR*)

The genotypic correlations were estimated using SAS software (SAS Inst., 2001) by following Falconer (1990):

$r_g=(Cov_{XY})/(\sqrt{Var_X*Var_Y})$, where 'Var' and 'Cov' respectively refer to the components of variance and covariance.

To calculate heritability, we estimated the mean square values (MS) from the analysis of variance of genotype (σ^2_g), genotype-moisture environment interaction (σ^2_{gm}), and residual (σ^2_e) variance, using the mean values (average of heading and grainfilling) of the SRIs. The broad-sense heritability within and between moisture environments was estimated on a genotypic mean basis by using the following formulae:

Heritability (within environment) = $(\sigma^2_g)/(\sigma^2_g + \sigma^2_e)$

Heritability (between environment) = $(\sigma^2_g)/(\sigma^2_g + \sigma^2_{gm}/e + \sigma^2_e/m.r)$

Where 'm' and 'r' are the number of moisture environments and replications, respectively. The average values of two growth stages (heading and grainfilling) were used to estimate heritability. Expected response to selection (*R*) and correlated response to selection (CR) were calculated followed by Falconer (1990), which is described below

$R = h_x \sigma_x$, where h_x and σ_x is the square root of heritability and genotypic standard deviation for trait X, respectively.

$CR = h_x r_g \sigma_{gy}$, where h_x is the square root of heritability of trait X, r_g is the genetic correlation between trait X and Y, and σ_{gy} is the genotypic standard deviation for trait Y.

Selection of superior genotypes for grain yield

We identified the highest yielding genotypes (top 12.5% and top 25 %) in each experiment. First, we ranked the genotypes according to their grain yield and selected the top 12.5% and 25% highest yielding genotypes. Afterwards we ranked the genotypes according to the NIR based indices. We selected genotypes with the lowest indices values (12.5% and 25%), because the NIR based indices are negatively correlated with grain yield. The genotypes were ranked for each growth stage and for the average of the growth stages.

Results

Effects of genotype, growth stages and moisture environments on SRIs

The mean values (±SE) of the five spectral reflectance indices (SRIs) at different growth stages of the wheat crop in different years, and grown under two moisture environments (one and two irrigations) are presented in Table 2. The genotypic variations for the SRIs were significant in all growth stages in all three experiments. Considering the main effect of growth stages, the values for NDVI and SR (developed based on NIR and visible wavelengths) were similar in the booting and heading stages with two irrigations (year 2003-04; Table 2). The values of these indices decreased in similar fashion from heading to grainfilling under both irrigation conditions (Table 2). With two-irrigations, the NIR based indices (WI, NW-1, and NWI-2) generally showed a decreasing trend from booting to heading in 2003-04. The values of these three indices increased from heading to grainfilling with two irrigations in years 2002-03 and 2003-04, and with one irrigation in year 2003-04 (Table 2).

The values of NDVI and SR were higher with two irrigations compared to their values after one irrigation, while the NIR-based indices were lower with two irrigations than one irrigation (Table 2).

The interaction effects between genotypes, growth stages, and moisture environments

The interaction values between genotypes and growth stages were significant for all five SRIs in both moisture regimes (data not presented). The F-test values of interaction effects (Expt-2) were much smaller for the three NIR based indices (2.84** to 3.91** with two irrigations and 2.03** to 2.33** with one irrigation) than with their corresponding genotypic main effect (10.2** to 12.1** with two irrigations and 4.78** to 5.09** with one irrigation). The interaction effects of the two components for NDVI and SR (F-values were 6.49** to 8.70** and 4.26** to 7.04** with two and one irrigation, respectively) were either equal to or smaller than the genotypic main effect for those two SRIs (F-values were 6.50** to 6.99** with two irrigations and 10.26** to 15.8** with one irrigation). A similar tend also was observed in Expt-3 (data not shown).

The phenotypic correlations (r_p) between the SRIs measured at different growth stages for Expt-2 are provided in Table 3. In general, the NIR-based indices gave higher correlations than NDVI and SR. Under the two-irrigations regime, the NIR-based indices gave higher correlations between booting and heading, and between heading and grainfilling than the correlations between booting and grainfilling, while NDVI and SR showed strong correlations only between booting and heading. With one irrigation, all indices gave similar correlations between heading and grain filling. A similar trend for correlations was observed in Expt-3 also (data not shown).

The combined analysis across two moisture environments using the mean values for heading and grainfilling revealed that the interaction between genotypes and moisture environments was significant for all five SRIs in both Expt-2 and Expt-3 in the 2003-04. In general, the F-test values for these interactions for all SRIs were smaller than the corresponding genotypic main effects except for NDVI in Expt-2. The F-test values of interaction effects for three NIR based indices were 2.96** to 3.67** (Expt-2) and 5.90** to 7.71** (Expt-3), while their corresponding genotypic main effects were 7.30** to 9.61** (Expt-2) and 13.9** to 17.5** (Expt-3). The F-test values for interaction and genotypic main effects for NDVI were 8.10** to 19.2** and 7.30** to 19.55**, respectively, while those values for SR were 4.82** to 10.8** (interaction) and 6.58** to 17.4** (genotypic effect).

The correlations between the values of the same indices measured in the two moisture environments were highly significant for SR and the three NIR-based indices, but not for NDVI.

The correlation values ranged from 0.538** to 0.655**for the three NIR based indices, from 0.481** to 0.516** for SR, and from 0.194 to 0.242 for NDVI.

The genotype, moisture environments and their interaction effects on grain yield

The minimum, maximum, and means values for grain yield across years and experiments were 3.53 t ha^{-1}, 6.50 t ha^{-1}, and 4.97 t ha^{-1} under two irrigations conditions (data not shown). In the year 03-04 when experiments were conducted under both moisture regimes, the minimum, maximum and mean values for grain yield were 3.53 t ha^{-1}, 5.50 t ha^{-1}, and 4.56 t ha^{-1}, respectively under two irrigations, while those values under one irrigation were 3.16 t ha^{-1}, 5.46 t ha^{-1}, and 4.25 t ha^{-1}, respectively (data not shown). Under two-irrigations grain yield was much higher in the earlier two years than in 2003-04. The genotypes varied significantly for grain yield in all three years and under both moisture conditions. The main effect of moisture environments on grain yield was not significant for both experiments (data not shown). The genotypic effects across the environments and the interaction were highly significant. The F-test values for the interaction (2.24*, and 3.66** in Expt-2 and Expt-3, respectively) were much lower than the genotypic effects (8.72** and 10.07** for Expt-2 and Expt-3, respectively) in both experiments. The combined analysis across years revealed significant effect for year, genotype and genotype by year interaction in both Expt-1 and Expt-2 (data not shown).

Correlations between SRIs and grain yield

The phenotypic (r_p) and genetic (r_g) correlations between the SRIs and grain yield in different years and for the two moisture regimes are presented in Tables 4 and 5. The NIR-based indices (WI, NWI-1, and NWI-2) gave significant negative phenotypic correlations with grain yield in both moisture environments, in all growth stages, and in all three years. In general with two irrigations, the highest phenotypic correlations between grain yield and the NIR-based indices were noted either in the heading or grainfilling stages with two irrigations (Table 4). With one-irrigation, the indices gave higher phenotypic correlations in the heading stage compared to the grainfilling stage (Table 5). The mean values of indices (average of booting, heading, and grainfilling within a year) gave higher phenotypic correlations with grain yield compared to the correlation between SRIs and grain yield at any individual growth stage for the two-irrigation regime. Using mean values of indices taken at two growth stages (heading and grainfilling) did not give higher phenotypic correlations with grain yield than the correlations between SRIs and grain yield at any individual growth stage with either moisture regime (Table 4 and 5).

Strong genotypic correlations were observed between grain yield and the NIR-based indices when calculated between the mean values of indices (averaged over different growth stages within a year) and grain yield (Tables 4 and 5). The three NIR based indices showed a similar level of association (both phenotypic and genotypic) with grain yield and none of the three showed any definite superiority in the prediction of grain yield variability. Therefore, in order to simplify the description of the results, NWI-2 has been selected for further presentation in the simple linear models, which will in general reflect the predictive capacity of all three NIR-based indices in explaining grain yield variability. More than fifty percent of the variation in the mean grain yield (average of two moisture environments) was explained by mean NWI-2 (average of two moisture environments) in both Expt-2 and Expt-3 (Fig 1).

In general with two irrigations, NDVI and SR showed significant positive phenotypic correlations with grain yield at the grainfilling stage, except in Expt-1 (Table 4). While under the one-irrigation regime these two indices produced significant positive phenotypic correlations with grain yield at heading and grainfilling (Table 5). Correlation values for these two indices with yield increased from the vegetative to the reproductive growth stages under two irrigations (Table 4). Lower genotypic correlations between these indices and grain yield were also observed. The NIR-based indices consistently showed higher correlations with grain yield than either NDVI or SR.

Table 6 presents the percentage of variation in grain yield that is explained by combining NWI-2, NDVI and SR values from the different growth stages. Combining information from the three growth stages (booting, heading, and grainfilling) increased the predictive capacity to explain grain yield variability considerably compared to any individual growth stage, while combining values from just two growth stages (heading and grainfilling) did not increase the percentage variability in grain yield explained relative to the individual scores. The other NIR-based indices gave similar results, but NDVI and SR gave low predictability with the multiple regression model.

The mean grain yields (average of two moisture regimes) of the genotypes were plotted against NWI-2 from two individual moisture environments (one and two irrigations) in two experiments (Fig 2a, Expt-2, and Fig. 2b, Expt-3) in 2003-04. NWI-2 with two irrigations explained 36-58% variations in mean grain yield, and NWI-1 with one-irrigation regime explained 29-54% of the variability in the mean grain yield in the two experiments. The mean values of NWI-2 (average of two moisture environments) were plotted against the yield of genotypes from two individual moisture environments in the two different experiments (3a, Expt-2, and 3b, Expt-3) in 2003-04. The mean values of NWI-2 explained 40-53% of the grain yields variation with two irrigations in the two experiments, and 30-54% of the grain yield variations were explained with the one-irrigation regime in the two experiments. NWI-2 showed a marginal superiority in explaining grain yield variability with two irrigations compared to the one-irrigation regime. Similar results were demonstrated by the other NIR-based indices (data not shown).

Selection of superior genotypes for grain yield

The selection of the 12.5% and 25% highest yielding genotypes based on NWI-2 is presented in Table 7. Overall, 50% to 75% of the top 12.5% highest yielding genotypes, and 50% to 87% of the top 25 percent highest yielding genotypes were selected based on combining selection on individual growth stages using NWI-2 or based on the mean NWI-2 values (averaged over different growth stages), with a single exception. The selection of 12.5% superior genotypes based on combining information from individual growth stages was basically equivalent to the selection based on the mean values (average of different growth stages). The selection based on individual growth stages performed slightly better over selection based on mean values (average of different growth stages) when the top 25 percent highest yielding genotypes were selected.

The mean values for the highest and lowest yielding 25% genotypes based on yield *per se* versus the corresponding 25% genotypes selected by NWI-2 revealed a very close association (Table 8). The differences for grain yield of the selected genotypes using both methods showed less than a 3% difference (except for one case) for the top yielding genotypes and less than a 4% percent difference for the bottom yielding genotypes. The differences between mean NWI-2 values for the 25% highest and lowest yielding genotypes selected by these two methods differed by less than 7%, with one exception (Table 8).

Heritability of SRIs and grain yield, and correlated response

The broad-sense heritability values of SRIs and grain yield estimated on the genotypic mean basis, for within and between moisture environments, and the correlated selection response

(*CR*) for grain yield estimated from SRIs and the direct selection response (*R*) for grain yield in two different experiments and under two moisture regimes are presented in Table 9. In individual environments, heritability of NDVI and SR ranged from 0.55 to 0.92 while NIR based indices ranged from 0.57 to 0.87, and grain yield itself from 0.54 to 0.57. NIR based indices gave higher heritability with two irrigations than with one, while grain yield and the other two indices (NDVI and SR) did not show any clear pattern. The heritability between moisture environments was in general lower for all the SRIs than the heritability of SRIs within individual moisture environment. The heritability ranged from 0.34 to 0.68 for NDVI and SR, from 0.57 to 0.71 for the three NIR based indices (WI, NWI-1 and NWI-2), and from 0.54 to 0.65 for grain yield.

The correlated selection response (*CR*) for grain yield estimated from three NIR based indices was much higher than NDVI and SR under both moisture regimes (Table 9). The *CR* values for grain yield estimated from these three NIR based indices were higher than the direct selection response (*R)* based on grain yield itself in Expt-2 under 2-irrigations conditions, but were lower in other experiments. The average *CR* values for grain yield estimated from the three NIR based indices ranged from 88% to 93% of *R* of grain yield under two-irrigation conditions, while average *CR* values ranged from 82% to 87% of R values under one irrigation level (deduced from Table 9). The average *R*-values for grain yield were similar under both irrigation conditions, but average *CR* values were higher under two irrigation conditions.

Discussion

Effect of genotype, growth stages, and their interactions on SRIs

The significant genotypic variations for the different SRIs have been reported by different authors in durum wheat under rainfed conditions, and in bread wheat under irrigated conditions. Babar et al. (2005) and Gutierrez-Rodriguez (2004) have found significant genotypic variations for SRIs in bread wheat under irrigated conditions at the same location where the research reported here was conducted. Babar et al. (2005) observed the increased genotypic variations for the SRIs as the growth stage progressed from vegetative to reproductive under irrigated conditions, while Aparicio et al. (2000) reported significant genotypic variations from the early growth stages to later growth stages under rainfed conditions in durum wheat. In the present study, the genotypic variations were significant at all growth stages in all three years, and in both moisture-stressed environments. This clear genotypic variation among these mostly unrelated genotypes demonstrates the potential of using these indices to differentiate genotypes with different genetic backgrounds. The indices were able to differentiate the genotypes for grain yield variability. The genotypic variations in all three years and in both moisture-stressed environments signify the relative strength of using these indices under different environmental conditions.

The use of SRIs to follow physiological changes in different growth stages has been documented in durum wheat under rainfed conditions (Aparicio et al., 2000; 2002), and in bread wheat under irrigated conditions (Leamer et al., 1980; Hatfield, 1981). At the early growth stages, there is more absorption of light in the visible wavelengths because of the higher amount of photosynthetically active plant tissues, but plants reflect more light at the NIR wavelengths. As growth progresses, active plant tissues mature, which results in an increased reflectance of the visible wavelengths and a decrease in the reflectance of NIR. NDVI and SR were developed based on a combination of visible and NIR wavelengths, and give the highest values at the early growth stages, and decreased values as growth stages progress because of the death (senescence) of the lower plant tissues. With two irrigations, the values for NDVI and SR were the same at booting and flowering, because the plant maintained green leaf area, and after that the values decreased at the grainfilling stage. With one irrigation, values of these indices decreased from heading to grainfilling, which was as expected and as had previously been reported by other authors (Aparicio et al., 2000; 2002) in durum wheat.

The three NIR based indices (WI, NWI-1 and NWI-2) were developed by combining information from the reflectance at 850nm, 900nm, and 970nm wavelengths. 970nm is a water absorption band, and the sensitivity of this band depends on the total water content at the canopy level (Bull, 1991). In the year 2003-04, the values of these indices increased from heading to grainfilling with the one-irrigation regime, which indicates that water stress increased from heading to grainfilling. With two irrigations, the values of the three indices decreased from booting to heading, and then increased at grainfilling, which observed in the same year. The decreasing trend of the values from booting to heading indicates that the canopy contained more water at the heading stage, which resulted from a 45mm rainfall prior to the heading stage (experiments received 37.1 mm and 8.4 mm rainfall on February 25 and March 3, respectively, while spectral reflectance measurements at heading were taken on March 1 and March 5 in different experiments) Babar et al. (2005) demonstrated a similar trend under fully irrigated conditions and found that the canopy contained more total water (fresh weight minus dry weight) at the heading stage. Due to higher water content, more energy was absorbed from the radiation that penetrated the canopy. The values of the three indices increased from heading to grainfilling, which indicates increased water stress at that stage with two irrigations, and the same trend was found in both years of the experiment (year 2002-03 and 2003-04).

A significant but low genotype by growth stage interaction was found in the present study and is also reported by others (Aparicio et al., 2002; Babar et al., 2005). A low level of interaction between growth stages and genotypes indicates the SRIs can be used in different growth stages to discriminate genotypes for grain yield in the breeding trails. However, in general, the NIR based indices expressed more consistency across growth stages and between varying moisture stresses compared to NDVI and SR.

Effects of moisture environments and their interactions with genotypes

Aparicio et al.(2000) demonstrated a significant effect of moisture level on NDVI and SR, and found significantly higher values for those two indices in irrigated conditions than in rainfed conditions at all growth stages. Under water limiting conditions, the plants showed a decrease in reflectance in the NIR wavelengths and an increased reflectance of the visible wavelengths as they loose their green areas faster than under irrigated conditions. Such changes caused NDVI and SR to have higher values with the two irrigations than with the one-irrigation regime. On the other hand, all three NIR based indices showed lower values with two irrigations than with one. Water index is used to assess the canopy water status and is used to track changes of relative water content, canopy temperature, leaf water potential, and stomatal conductance (Peñuelas et al., 1993; Peñuelas et al., 1997). The higher values of a water index are an indicator of low water status in the canopy. So the higher and lower values of the three NIR based indices are indicative of higher and lower levels of water stress, which is demonstrated by our experiments. Between the two moisture regimes, the difference in total water supply was only 80-90 mm, and both moisture regimes received the same rainfall. Therefore, the significant differences between the two moisture environments found in this study in year 03-04, was somewhat surprising. The effect may have resulted due to the 45 mm of rainfall that fell just prior to the spectral reflectance data being taken at the heading stage under the two irrigation regime in that year. Because of the rainfall, the water and turgor status of the canopy was higher at that stage, and this difference was detected by the sensor, thereby leading to a significant effect of moisture levels.

A low but significant level of interaction effects for genotype by moisture environment were found for the SRIs, and the interaction effects were higher for NDVI and SR than for the three NIR based indices. The correlation of the three NIR based indices, measured in two different moisture conditions in year 03-04, was highly significant (r=0.60**), but did not explain most of the variability among the environments. These results indicate that care must be taken to identify suitable moisture levels that effectively differentiate genotypes for their grain yield.

The effects of genotypes, moisture environments and their interaction effects on grain yield

The genotypic variation for grain yield was significant in all experiments, in all three years and under both moisture regimes, as expected. The year effects were very high and the genotypes produced much higher grain yields in 2001-02 and 2002-03 than in 2003-04 with two irrigations. The experiments received more water in 2003-04 compared to other two years due to unusual rains, but early heat during the second and third weeks of March caused floral abortion, thus resulting in a reduction in grain yield for that year compared to fully irrigated conditions. The interaction between the genotypes and moisture environments was significant, but much lower than the main effect of genotypes on grain yield. Although the correlation between the two moisture environments for grain yield was highly significant (r=0.60** for Expt-2 and 0.55** for Expt-3), this did not explain a high amount of the total variation.

Correlations between SRIs and grain yield

Various authors have suggested the potential use of different SRIs to differentiate advanced breeding lines in durum wheat under rainfed conditions (Aparicio et al., 2000; Royo et al., 2003), elite bread wheat lines under irrigation conditions (Ball and Konzak, 1993) as well as different early generation bread wheat materials, such as F5, F6, or F7 (Babar et al., 2005), and in soybean under well watered conditions (Ma et al., 2001). Babar et al. (2005) in an earlier study reported the use of three NIR based indices (WI, NWI-1, and NWI-2) as very promising selection criteria to differentiate genotypes for grain yield in bread wheat at the same location as this study. Two water-limiting environments were created by reducing the normal number of irrigations to achieve optimal yield levels from five to just one and two. Both environments received a total of 191 mm rainfall. The unusual rainfall patterns in 2003-04 created unpredictable environmental conditions. Our experiments tested the potential of these SRIs to continue to provide fairly accurate estimates of yield even under such unpredictable environmental conditions. The two earlier crop cycles (2001-02 and 2002-03) experienced relatively usual environmental conditions for that location (Table1).

The NIR based indices gave significant negative correlations with grain yield at all growth stages in all three years under both moisture-stressed environments. These results demonstrated the relative strength of these indices to differentiate genotypes under different environmental conditions. With two irrigations, genotypes were differentiated at all three-growth stages. The mean values of the indices (average of three growth stages) demonstrated even higher phenotypic correlations with grain yield than any single growth stage. The three NIR based indices indicate the water status at the canopy level, and NDVI and SR measure the greenness of the canopy. Both types of measurements are indicative of healthy plant conditions in the field. The repeated measurements on the same genotypes at different growth stages basically accumulated information on the respective health of the genotypes over a period of time. We hypothesize that the mean values of the indices for the different growth stages represent cumulative information on the health of the canopy, which translates into a higher correlation with final grain yield. This is also an indication that the underlying genotypic correlation is stronger than the phenotypic one, which was evident in our study by higher genotypic correlations between mean SRIs (average of different growth stages) and grain yield than the phenotypic correlations between SRIs and grain yield in individual growth stages.

As the time required to take the SRI data in the field is just 30-40 seconds/plot, it would be desirable to take the readings more than once. At a minimum, one reading at booting and another one after heading might effectively differentiate genotypes for grain yield. The multiple regression models also demonstrated the usefulness of taking data at more than one growth stages. In the one-irrigation scenario, no data were taken at the booting stage, but data at the reproductive stage showed strong correlations with grain yield. Aparicio et al. (2000) and Ma et al. (2001) demonstrated an increased trend in the relationship of NDVI and SR with grain yield with the advancement of growth stages.

Strong genotypic correlations existed between grain yield and the SRIs, which indicated that the association could be mainly attributed to the genotype, and less to environmental factors. The significant interaction between the genotypes and the moisture environments led us to investigate which of the two moisture-stressed environments might be more suitable for taking the SRIs. None of these two environments showed any definite superiority, but indices taken on the two-irrigations regime performed slightly better than with the one-irrigation regime (Fig.2 and 3). It was evident that the NIR based indices performed much better under both moisture environments than either NDVI or SR.

However, the performance among the three NIR-based indices (WI, NWI-1 and NWI-2) was indistinguishable. 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. However, in our study normalizing the water index (NWI-1, and NWI-2) did not seem to improve the relationship dramatically.

Selection of superior genotypes for grain yield

We tested the accuracy of the NIR based indices as a tool for selecting superior genotypes for grain yield. In most cases we were able to identify the higher yielding genotypes in all moisture regimes via this indirect approach. Ball and Konzak (1993) selected eight and five of the higher yielding genotypes out of a total of 35 genotypes based on the NDVI, when data were taken at two occasions during the grainfilling stage under well-watered conditions. In our study, NDVI did not give such high accuracy. Our study did demonstrate a very high accuracy of NIR based indices to select superior genotypes for grain yield.

Heritability of SRIs and grain yield

Broad-sense heritability indicates the proportion of phenotypic variance attributable to genotypic differences. In most cases, heritability for all SRIs was higher than grain yield under both moisture environments. The heritability for the NIR-based SRIs was higher with two irrigations than one. The heritability across the two moisture environments was lower, but higher than grain yield. These results indicate that the NIR based indices would be more reliable under moderate yield potential environments than under very low yield potential environments. High heritability of these SRIs indicates that the genetic gain can be achieved by selecting in favor of these SRIs.

Correlated response to selection reflects improvements in one character, namely grain yield, based on the selection for another trait, such as SRIs. Yield improvement will be more by selecting in favor of NIR based indices, rather than NDVI and SR, and a higher yield improvement could be achieved under two-irrigation conditions than one-irrigation conditions. Using an indirect selection tool is mainly suggested if the genetic correlation between the selected and unselected traits is very high, the heritability is much higher for the selected trait than for the unselected trait, and the correlated response in the unselected trait based on the selected trait is higher than the direct response to the selection in the unselected trait (Falconer, 1990; Kearsey and Pooni 1996; Jackson, 2001). In practice these situations are rarely obtained (Kearsey and Pooni 1996; Jackson, 2001). Nonetheless, an indirect selection criterion could be used if it is less time consuming, cost effective, and the desirable trait is difficult to measure (Kearsey and Pooni 1996; Jackson, 2001). In our study, we have found very strong genetic correlations between the three NIR based indices and grain yield, high broad-sense heritability for the NIR based indices, and the average correlated response to selection for grain yield estimated from NIR based SRIs were close to the direct response to the selection of grain yield itself under the two irrigation condition. A very high efficiency for selecting superior phenotypes under both moisture conditions was also demonstrated in our study. The time requirement for taking measurements in the field is very minimal (average 40 seconds/plot for four reading) with the machine used in the present study. A research project is currently underway to develop a new spectral sensor to measure these NIR based indices in the field. The successful completion of the project will facilitate faster measurements in the field and will bring down the current cost of the equipment to a minimum level. These facilities will help breeders to work with these tools in the field to discriminate genotypes for grain yield in the breeding trails.

Conclusions

The potential to differentiate genotypes for grain yield by using SRIs has been demonstrated in this study with different types of breeding lines of spring wheat under water-limiting environments. The SRIs showed genotypic variations at different growth stages and under different moisture conditions. Using mean values of SRIs (average of growth stages), or a progressive addition of SRIs from different growth stages increased the grain yield variations compared to the variations explained by a single growth stage. Thus, the best results could be achieved by combining data from both vegetative and reproductive growth stages. When comparing various indices, the indices based on NIR (WI, NWI-1, and NWI-2) demonstrated consistently higher levels of association, explained a higher proportion of the variability for grain yield, and allowed the identification of a higher percentage of the top-yielding lines, compared with the other spectral indices (NDVI and SR). A moderate to high level of broad-sense heritability was observed for these three SRIs. Strong genotypic correlations between grain yield and NIR-based SRIs, higher heritability values than grain yield, high accuracy in selecting superior genotypes, and *CR* for grain yield based on these NIR indices close to *R* values based on grain yield itself suggest that the use of SRIs would achieve effective genetic gain for grain yield under moisture limited conditions. Our study indicate that the genetic gain for yield improvement will be more successful under moderate grain yield environments rather than low yield potential environments because of the higher values of the above mentioned genetic parameters under two-irrigation level rather than one irrigation.

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Tables

Table 1. Maximum and minimum temperatures, total monthly rainfall, solar radiation from January to May for three cropping cycles, and long term mean (LT).

Year		Jan.	Feb.	Mar.	April.	May
		Mean temperature (^oC)				
2002	Maximum	25.5	26.2	27.4	32.4	37.1
	Minimum	5.8	8.5	7.8	13.0	13.6
2003	Maximum	28.7	25.5	28.5	32.2	36.1
	Minimum	8.5	11.3	8.6	11.5	14.8
2004	Maximum	23.1	24.1	30.1	29.5	34.8
	Minimum	8.3	6.5	10.3	11.8	15.2
LT	Maximum	23.3	24.9	27.1	31.3	33.7
	Minimum	6.9	6.8	8.2	10.2	16.3
		Total rainfall (mm)				
2002		0.0	17.0	0.0	0.0	0.0
2003		0.0	19.7	0.0	8.8	0.0
2004		146.8	37.8	8.9	4.6	0.3
LT		12.0	7.9	2.7	1.4	1.3
		Solar radiation ($MJ/(m^2d^1)$)				
2002		14.6	18.1	22.7	24.4	29.1
2003		15.9	17.0	24.0	27.5	30.8
2004		14.8	19.1	24.4	26.4	31.1

Table 2. Mean values (± SE) of five different spectral reflectance indices at three different growth stages booting (Boot), heading (Hd) and grainfilling (GF)) in three sets of advanced lines under two moisture regimes (1 irrigation or 2 irrigations) during three years.

Years	Experiment	Environment	Growth stage	NDVI	SR	WI	NWI-1	NWI-2
2001-02	Expt-1	2-Irrig.	GF	0.689±0.024**	5.59±1.14**	0.887±0.007**	-0.062±0.003**	-0.050±0.005**
2002-03	Expt-2	2-Irrig.	Hd	0.883±0.017**	16.3±2.59**	0.838±0.006**	-0.070±0.003**	-0.068±0.004**
			GF	0.831±0.041**	10.9±0.713**	0.871±0.007**	-0.065±0.004**	-0.063±0.006**
2003-04	Expt-1	2-Irrig.	Boot	0.918±0.009**	23.3±2.47**	0.871±0.009**	-0.069±0.004**	-0.065±0.005**
			Hd	0.920±0.029**	24.6±2.91**	0.836±0.012**	-0.089±0.007**	-0.086±0.007**
			GF	0.586±0.037**	13.3±0.652**	0.915±0.008**	-0.072±0.004**	-0.069±0.005**
2003-04	Expt-2	2-Irrig.	Boot	0.918±0.010**	23.73±2.44**	0.874±0.009**	-0.067±0.005**	-0.062±0.006**
			Hd	0.923±0.007**	25.22±2.22**	0.801±0.009**	-0.089±0.006**	-0.084±0.006**
			GF	0.876±0.027**	17.49±1.86**	0.837±0.011**	-0.073±0.007**	-0.066±0.008**
		1-Irrig.	Hd	0.833±0.006**	17.5±1.72**	0.854±0.008**	-0.070±0.005**	-0.065±0.005**
			GF	0.761±0.007**	11.22±1.34**	0.942±0.006**	-0.066±0.003**	-0.060±0.004**
2003-04	Expt-3	2-Irrig.	Boot	0.917±0.006**	23.02±1.72**	0.878±0.008**	-0.065±0.005**	-0.060±0.005**
			Hd	0.923±0.004**	24.99±1.38**	0.802±0.007**	-0.092±0.004**	-0.089±0.004**
			GF	0.837±0.026**	14.87±1.01**	0.848±0.008**	-0.082±0.005**	-0.074±0.005**
		1-Irrig.	Hd	0.905±0.010**	20.49±1.79**	0.864±0.009**	-0.073±0.006**	-0.066±0.006**
			GF	0.784±0.014**	10.36±1.35**	0.872±0.008**	-0.066±0.005**	-0.060±0.005**

** Genotypic variations are significant at 0.01 probability.

NDVI, Normalized difference vegetation index; SR, Simple ratio; WI, water index; NWI-1, Normalized water index-1; NWI-2, Normalized water index-2.

Table 3. The phenotypic correlations (r_p) between five different SRIs, taken at different growth stages in Expt-2, under two moisture regimes (1 irrigation or 2 irrigations).

	NDVI		SR		WI		NWI-1		NWI-2	
	Heading	Grain filling	Heading	Grain filling	Heading	Grain filling	Heading	Grain filling	Heading	Grain filling
Year 02-03										
Heading (2-Irrig.)		0.782**		0.782**		0.876**		0.867**		0.879**
Year 03-04										
Booting (2-Irrig.)	0.737**	395*	0.693**	0.354	0.702**	0.479**	0.695**	0.473*	0.687**	0.481**
Heading (2-Irrig.)		0.121		0.114		0.681**		0.682**		0.676**
Heading (1-Irrig.)		0.696**		0.673**		0.719**		0.702**		0.700**

* Significant at 0.05 probability.

** Significant at 0.01 probability.

NDVI, Normalized difference vegetation index; SR, Simple ratio; WI, water index; NWI-1, Normalized water index-1; NWI-2, Normalized water index-2.

Table 4. The phenotypic (r_p) and genetic (r_g) correlation coefficients between grain yield and five different spectral indices at different growth stages in different years under 2-irrigations for three experiments.

	Y01-02	Y02-03				Y03-04				
	r_p	r_p			r_g	r_p				r_g
Indices	Grainfilling	Heading	Grainfilling	Mean††	Mean‡‡	Booting	Heading	Grainfilling	Mean††	Mean‡‡
					Expt-1					
NDVI	0.263					0.014	0.332	0.156	0.373	0.482**
SR	0.297					0.039	0.303	0.173	0.392*	0.476 **
WI	-0.632**					-0.497**	-0.670**	-0.456*	-0.769**	-0.796**
NWI-1	-0.617**					-0.492**	-0.680**	-0.461*	-0.775**	-0.807**
NWI-2	-0.662**					-0.518**	-0.668**	-0.472*	-0.774**	-0.782**
					Expt-2					
NDVI		0.597**	0.613**	0.624**	0.676**	0.248	0.003	0.492**	0.452*	0.379*
SR		0.594**	0.611**	0.639**	0.742**	0.298	0.011	0.562**	0.235	0.062
WI		-0.687**	-0.726**	-0.736**	-0.779**	-0.692**	-0.511**	-0.628**	-0.704**	-0.795**
NWI-1		-0.688**	-0.728**	-0.740**	-0.800**	-0.679**	-0.516**	-0.693**	-0.703**	-0.798**
NWI-2		-0.666**	-0.744**	-0.738**	-0.767**	-0.683**	-0.544**	-0.670**	-0.737**	-0.856**
					Expt-3					
NDVI						-0.284	-0.183	0.590**	0.541**	0.507**
SR						-0.241	-0.144	0.476**	0.219	0.115
WI						-0.498**	-0.401*	-0.598**	-0.644**	-0.643**
NWI-1						-0.495**	-0.387*	-0.595**	-0.639**	-0.648**
NWI-2						-0.530**	-0.522**	-0.591**	-0.678**	-0.640**

* Significant at 0.05 probability.

** Significant at 0.01 probability.

†† Phenotypic correlation between yield and mean spectral indices averaged over different growth stages within the same year.

‡‡ Genetic correlation between yield and mean spectral indices averaged over different growth stages within the same year.

Table 5. The phenotypic (r_p) and genetic (r_g) correlation coefficients between grain yield and five different SRIs at four different growth stages under the one-irrigation scenario.

	Expt 2				Expt 3			
		r_p		r_g		r_p		r_g
Indices	Heading	Grain filling	Mean††	Mean‡‡	Heading	Grain filling	Mean††	Mean‡‡
NDVI	0.376*	0.373*	0.416*	0.549**	0.416*	0.134	0.207	0.168
SR	0.431*	0.428*	0.485**	0.512**	0.414*	0.167	0.241	0.241
WI	-0.640**	-0.499**	-0.694**	-0.818**	-0.763**	-0.639**	-0.759**	-0.812**
NWI-1	-0.646**	-0.501**	-0.697**	-0.783**	-0.773**	-0.641**	-0.770**	-0.801**
NWI-2	-0.667**	-0.486**	-0.691**	-0.785**	-0.801**	-0.610**	-0.771**	-0.834**

* Significant at 0.05 probability.

** Significant at 0.01 probability.

†† Phenotypic correlation between yield and mean spectral indices averaged over different growth stages within the same year.

‡‡ Genetic correlation between yield and mean spectral indices averaged over different growth stages within the same year.

NDVI, Normalized difference vegetation index; SR, Simple ratio; WI, water index; NWI-1, Normalized water index-1; NWI-2, Normalized water index-2.

Table 6. The percentage of variation (R^2) in grain yield that is explained by integrating different SRIs values from different growth stages under two moisture conditions (1 irrigation or 2 irrigations) in three experiments.

Years	Experiment	Environments	Growth stages	SRIs	R^2
2002-03	Expt-2	2-Irrig.	Heading+Grainfilling	NWI-2	0.56**
				NDVI	0.41**
				SR	0.41**
2003-04	Expt-1	2-Irrig.	Booting+Heading+Grainfilling	NWI-2	0.65**
				NDVI	0.22*
				SR	0.20*
	Expt-2	2-Irrig.	Booting+Heading+Grainfilling	NWI-2	0.63**
				NDVI	0.27**
				SR	0.33**
		1-Irrig	Heading+Grainfilling	NWI-2	0.50**
				NDVI	0.18
				SR	0.24*
	Expt-3	2-Irrig.	Booting+Heading+Grainfilling	NWI-2	0.51**
				NDVI	0.45**
				SR	0.33**
		1-Irrig.	Heading+Grainfilling	NWI-2	0.65**
				NDVI	0.22*
				SR	0.20*

** Significant at 0.01 probability.

NDVI, Normalized difference vegetation index; SR, Simple ratio; NWI-2, Normalized water index-2.

Table 7. The number of selected genotypes among the top 12.5% and 25% highest yielding genotypes based on normalized water index-2 (NWI-2) and the number of highest 12.5% and 25% genotypes (in parenthesis) in three experiments in different years under two moisture regimes (1 irrigation or 2 irrigations) are presented.

Years	Experiment	Environments	Selected genotypes (12.5 %)		Selected genotypes (25%)	
			1*	2*	1*	2*
2001-02	Expt-1	2-Irrig.	2 (3)	-	5 (7)	-
2002-03	Expt-2	2-Irrig.	2 (4)	2 (4)	6 (8)	7 (8)
2003-04	Expt-1	2-Irrig.	2(3)	2 (3)	5 (6)	6 (6)
	Expt-2	2-Irrig.	2 (4)	2 (4)	4 (8)	4 (8)
		1-Irrig.	2 (4)	3 (4)	5 (8)	7 (8)
	Expt-3	2-Irrig.	1 (4)	2 (4)	3 (8)	5 (8)
		1-Irrig.	2 (4)	2 (4)	5 (8)	6 (8)

Codes 1 and 2 indicate genotypes were selected based on the average values over different growth stages and using individual growth stages, respectively.

Table 8. Average yield of highest and lowest (25%) genotypes based on yield *per se* as compared to the yield of the highest and lowest (25%) genotypes selected based on mean normalized water index-2 (NWI-2) values (average of different growth stages), in two experiments under two moisture regimes (1 irrigation or 2 irrigations) in the year 2003-04.

		Expt-2		Expt-3	
		2-Irrig (t ha^{-1})	1-Irrig (t ha^{-1})	2-Irrig (t ha^{-1})	1-Irrig (t ha^{-1})
Highest 25%	Based on yield *per s* (t ha^{-1})	5.19 (-0.085)	4.75 (-0.067)	4.80 (-0.089)	4.71 (-0.069)
	Based on NWI-2 (t ha^{-1})	5.06 (-0.089)	4.65 (-0.069)	4.41 (-0.095)	4.57 (-0.072)
	Difference (%).	2.7 (6.3)	2.2 (2.98)	8.8 (6.7)	3.1 (3.75)
Lowest 25%	Based on yield *per se* (t ha^{-1})	4.37 (-0.063)	4.0 (-0.059)	3.75 (-0.071)	3.59 (-0.066)
	Based on NWI-2 (t ha^{-1})	4.41 (-0.059)	4.13 (-0.057)	3.85(-0.061)	3.67 (-0.054)
	Difference (%).	0.9 (4.7)	3.25 (3.5)	2.7 (16.4)	2.2 (4.8)
	Overall mean difference (%)	1.8 (5.5)	2.7 (3.24)	5.8 (11.55)	2.6 (4.275)

Values in the parenthesis represent the mean NWI-2 scores.

Table 9. Broad sense heritability values of five different SRIs and of grain yield within and across two moisture regimes (1 irrigation or 2 irrigations), and correlated selection response (*CR*) for grain yield estimated from SRIs, and expected selection response (*R*) for grain yield within individual environments in two experiments in year 2003-04.

			NDVI	SR	WI	NWI-1	NWI-2	Yield
Expt-2	2-Irrig	Heritability	0.79	0.79	0.80	0.80	0.85	0.54
		CR	0.21	0.03	-0.43	-0.43	-0.48	0.40 (*R*)
	1-Irrig	Heritability	0.83	0.75	0.66	0.66	0.68	0.59
		CR	0.34	0.30	-0.45	-0.43	-0.44	0.52 (*R*)
	Across 2 and 1-Irrig	Heritability	0.41	0.68	0.69	0.69	0.71	0.65
Expt-3	2-Irrig	Heritability	0.93	0.88	0.89	0.89	0.91	0.65
		CR	0.40	0.09	-0.49	-0.49	-0.50	0.65 (*R*)
	1-Irrig	Heritability	0.61	0.72	0.59	0.57	0.64	0.57
		CR	0.09	0.14	-0.43	-0.42	-0.46	0.52 (*R*)
	Across 2 and 1-Irrig	Heritability	0.34	0.54	0.57	0.57	0.60	0.54

NDVI, Normalized difference vegetation index; SR, Simple ratio; WI, water index; NWI-1, Normalized water index-1; NWI-2, Normalized water index-2.

Figures

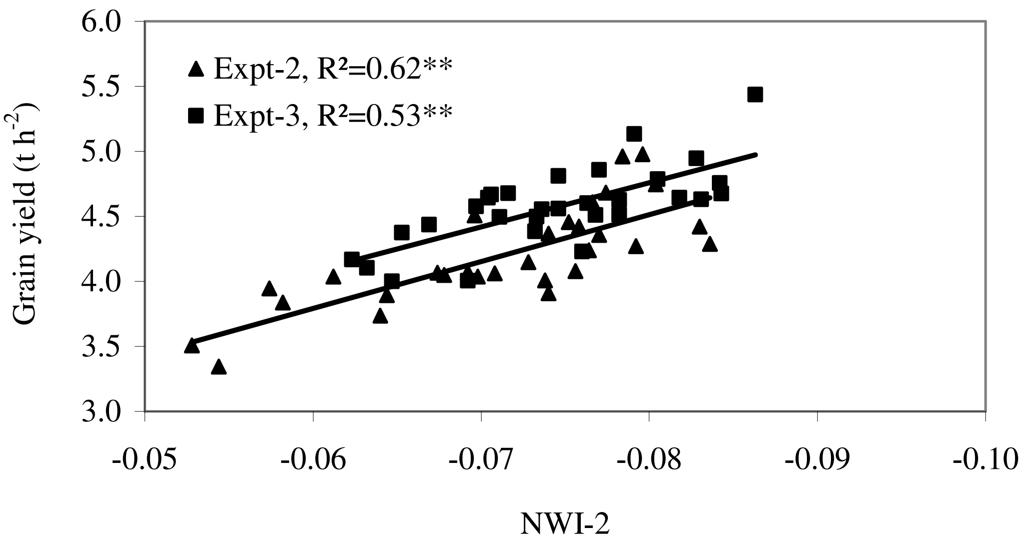


Fig 1. Variation (R^2) in mean grain yield (average of two moisture environments) explained by the mean normalized water index-2 (NWI-2, average of two moisture environments) in two experiments in year 2003/04. ** Significant at P=0.01.

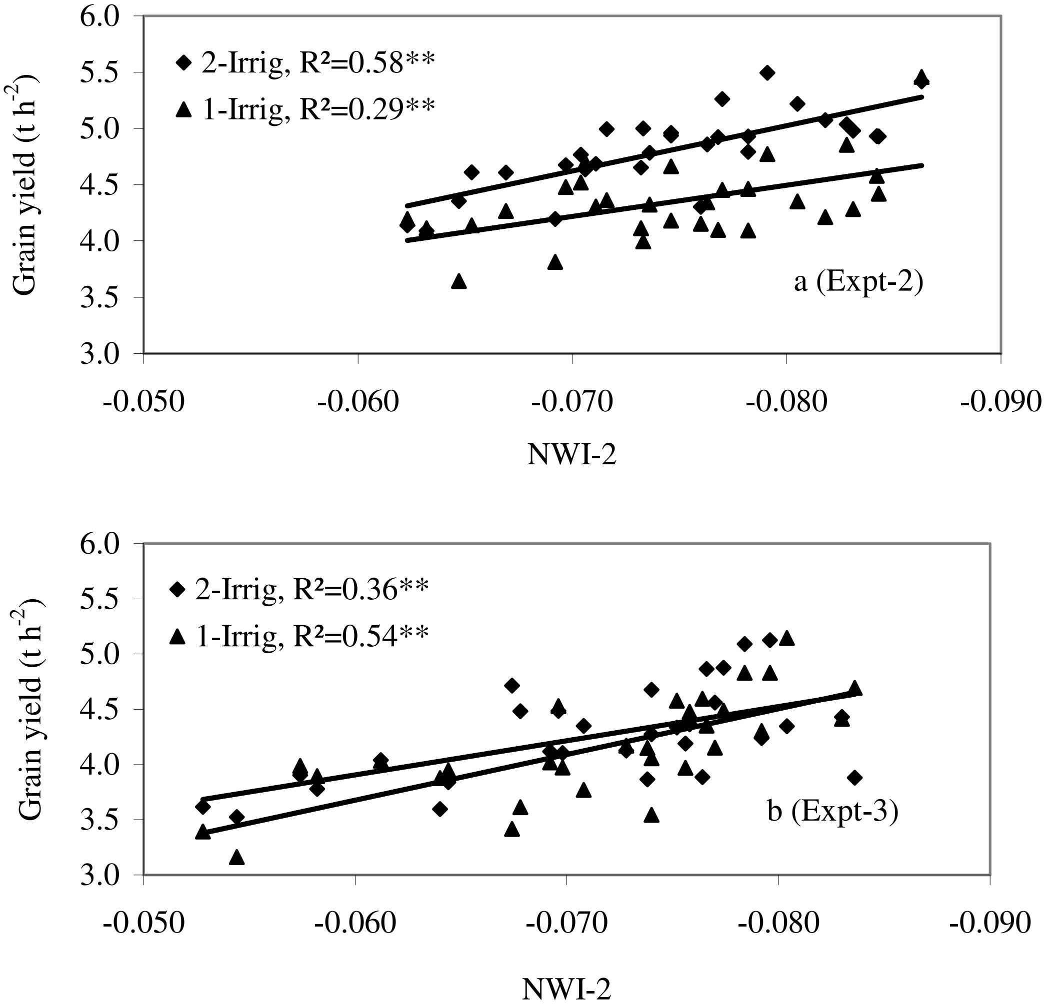
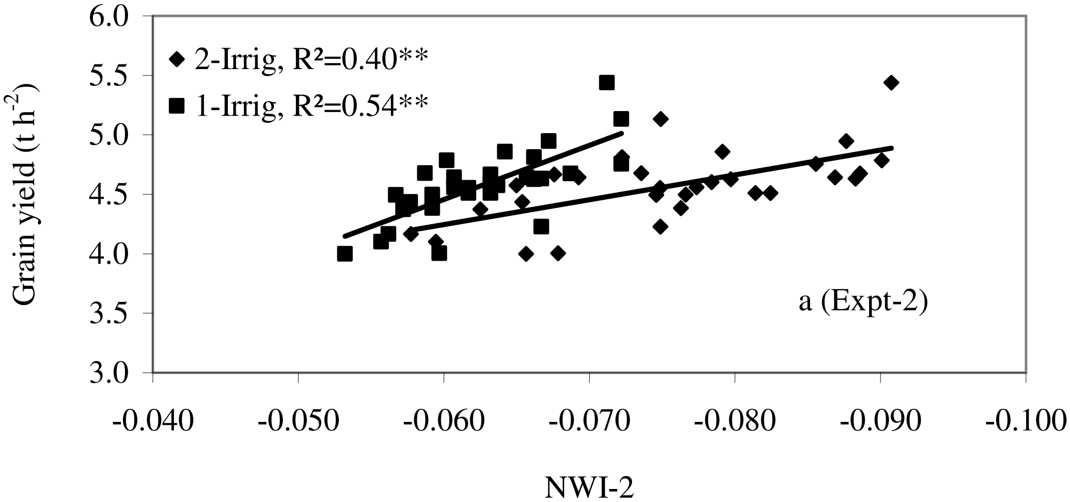


Fig 2. Variability (R^2) in mean grain yield (average of two moisture environments) as explained by normalized water index-2 (NWI-2) from each of the two moisture environments in two different experiments (Figs. a and b) in year 2003/04. ** Significant at P=0.01. '2-Irrig' and '1-Irrig' indicate experiments that were conducted under two and one-irrigation levels, respectively.



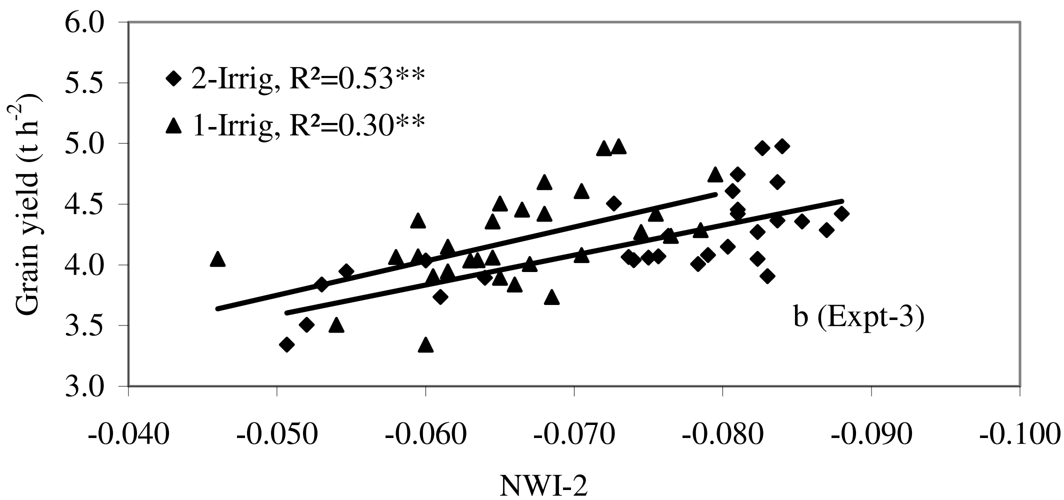


Fig 3. The variation (R^2) in grain yield in each of the two moisture environments as explained by normalized water index-2 (NWI-2, average of two moisture environments) in two experiments (Figs. a and b) in year 2003-04. **Significant at P=0.01. '2-Irrig' and '1-Irrig' indicate experiments that were conducted under two and one-irrigation levels, respectively.

CHAPTER V

Conclusions

Spectral reflectance indices potentially can differentiate genotypes of spring wheat for grain yield and biomass production under well watered and water limiting environments. Significant genotypic variations for different spectral reflectance indices were found in different individual growth stages within years, across growth stages, and across different years in different genetic background. Spectral reflectance indices based on NIR (WI, NWI-1, and NWI-2) consistently demonstrated higher levels of association and explained a higher proportion of the variability for grain yield and biomass production compared with the other spectral indices (RNDVI, GNDVI, SR, and PRI) under irrigated conditions. The best growth stages to apply the indices to differentiate genotypes for grain yield and biomass production were heading and grainfilling.

Under irrigated conditions, the three NIR based indices (WI, NWI-1 and NWI-2) showed strong genetic correlations with grain yield within environments and with grain yield averaged over a period of time (Appendix A and Appendix B). The level of genetic correlation between grain yield and the three NIR based indices was much higher than with the other indices (Appendix A and Appendix B). In general, all indices showed higher broad-sense and realized heritability than grain yield (Appendix C and Appendix D). The spectral reflectance indices based on visible and near infrared radiation (RNDVI, GNDVI, and SR) and based on only visible wavelength (PRI) showed higher heritability than the NIR based indices (Appendix C), while the realized heritability was higher for the NIR based indices than for the others with a few exceptions (Appendix D). The expected selection response for all the indices was higher than grain yield, and the NIR based indices showed lower selection response than the other indices (Appendix E). In contrast, the efficiency of correlated selection response for grain yield estimated from the three NIR based indices was much higher than with the other indices (Appendix F). The ratio of correlated response for grain yield estimated from the indices compared to the direct response of selection for grain yield revealed a much higher ratio in favor of the three NIR based indices than for the other indices (Appendix E). The ratio values for the three NIR-based indices were very close to one (Appendix E). The efficiency of the indices to select superior genotypes was tested and all indices demonstrated a high efficiency in selecting superior genotypes, but in general, the efficiency of the three NIR-based indices was consistently higher than the other indices (Appendix F). The strong genetic correlations, higher heritability and response to direct selection than grain yield, and correlated response to selection for grain yield was close to the direct selection response based on grain yield itself in two different populations, confirm a genetic basis or link between yield and the NIR-based spectral reflectance indices, and demonstrates the potential use for these indices as an indirect selection tool under irrigated conditions.

The potential to differentiate different types of spring wheat genotypes for grain yield by using SRIs under water-limited environments has also been demonstrated in this study. Using the mean values of SRIs (average of three different growth stages), or a progressive addition of SRIs from different growth stages increased the correlations with grain yield compared to the correlations with a single growth stage. This indicates that the underlying genotypic correlation is higher than the phenotypic correlation. Thus, the best results could be achieved by combining data from both vegetative and reproductive growth stages. Indices based on NIR (WI, NWI-1, and NWI-2) also demonstrated consistently higher levels of association and explained a higher proportion of the variability for grain yield compared with the other spectral indices (NDVI and SR) in the water limiting conditions. A moderate to high level of broad-sense heritability was also observed for these three SRIs. Strong genotypic correlations between grain yield and the NIR based indices, higher heritability than grain yield *per se*, higher expected selection response than grain yield, and very close values for correlated response to selection for grain yield estimated from the NIR based indices and the expected selection response based on the grain yield itself were observed under water limiting conditions. These results indicate that the use of SRIs would achieve effective genetic gain for grain yield under water-limiting environments, as well as with irrigated conditions with spring wheat, and would be efficient in terms of time and cost.

APPENDIX

Appendix A. Genotypic correlations between different spectral reflectance indices (averaged over growth stages) and grain yield, presented separately for each of the two years in two different spring wheat random populations under irrigated conditions.

	RLs-1		RLs-2	
Indices	Year 03-04	Year 02-03	Year 03-04	Year 02-03
PRI	0.584**	-0.127	0.347*	0.586**
RNDVI	0.671**	0.682**	0.597**	0.381*
GNDVI	0.569**	0.784**	0.594**	0.601**
SR	0.534**	0.513**	0.691**	0.364*
WI	-0.739**	-0.715**	-0.860**	-0.803**
NWI-1	-0.728**	-0.822**	-0.859**	-0.778**
NWI-2	-0.768**	-0.726**	-0.849**	-0.788**

* Significant at 0.05 probability.

** Significant at 0.01 probability.

PRI, Photochemical reflectance index; WI, water index; RNDVI, Red normalized difference vegetation index; GNDVI, Green normalized difference vegetation index; SR, Simple ratio; NWI-1, Normalized water index-1; NWI-2, Normalized water index-2.

Appendix B. Genotypic correlations between different spectral reflectance indices (averaged over growth stages within each individual year) and grain yield (mean of both years) in two different spring wheat random populations under irrigated conditions, presented by year and over both years jointly.

	RLs1			RLs2		
Indices	Year 03-04	Year 02-03	Overall Mean†	Year 03-04	Year 02-03	Overall Mean†
PRI	0.320	0.103	0.257	0.446*	0.201	0.536**
RNDVI	0.640**	0.377*	0.654**	0.406*	0.131	0.519**
GNDVI	0.761**	0.436*	0.615**	0.528**	0.247	0.668**
SR	0.617**	0.546**	0.616**	0.445*	0.404*	0.584**
WI	-0.865**	-0.757**	-0.871**	-0.679**	-0.706**	-0.892**
NWI-1	-0.872**	-0.849**	-0.889**	-0.680**	-0.739**	-0.889**
NWI-2	-0.866**	-0.890**	-0.890**	-0.664**	-0.753**	-0.896**

* Significant at 0.05 probability.

** Significant at 0.01 probability.

† Genetic correlations between mean grain yield (over two years) and overall mean of spectral reflectance indices (average over growth stages and years).

PRI, Photochemical reflectance index; WI, water index; RNDVI, Red normalized difference vegetation index; GNDVI, Green normalized difference vegetation index; SR, Simple ratio; NWI-1, Normalized water index-1; NWI-2, Normalized water index-2.

Appendix C. Broad-sense heritability values of the different spectral reflectance indices and of grain yield within and between two years in two different spring wheat random populations under irrigated conditions, and the estimated heritability based on mean values averaged over the two years and growth stages.

	RLs1				RLs2			
Indices	Year 03-04	Year 02-03	Between Years	Mean	Year 03-04	Year 02-03	Between Years	Mean
PRI	0.937	0.519	0.718	0.874	0.889	0.759	0.882	0.878
RNDVI	0.810	0.618	0.386	0.833	0.717	0.696	0.821	0.723
GNDVI	0.736	0.694	0.604	0.816	0.712	0.630	0.782	0.701
SR	0.763	0.269	0.579	0.636	0.792	0.708	0.769	0.801
WI	0.788	0.572	0.676	0.754	0.693	0.721	0.788	0.732
NWI-1	0.781	0.588	0.657	0.751	0.682	0.721	0.777	0.728
NWI-2	0.801	0.654	0.699	0.795	0.706	0.748	0.796	0.742
Yield	0.741	0.487	0.546	0.673	0.462	0.541	0.726	0.639

PRI, Photochemical reflectance index; WI, water index; RNDVI, Red normalized difference vegetation index; GNDVI, Green normalized difference vegetation index; SR, Simple ratio; NWI-1, Normalized water index-1; NWI-2, Normalized water index-2.

Appendix D. Realized heritability values of different spectral reflectance indices and of grain yield in two different spring wheat random populations under irrigated conditions.

Indices	RLs1	RLs2
PRI	0.916	0.675
RNDVI	0.227	0.594
GNDVI	0.366	0.386
SR	0.380	0.923
WI	0.715	0.770
NWI-1	0.616	0.775
NWI-2	0.686	0.779
YIELD	0.217	0.441

PRI, Photochemical reflectance index; WI, water index; RNDVI, Red normalized difference vegetation index; GNDVI, Green normalized difference vegetation index; SR, Simple ratio; NWI-1, Normalized water index-1; NWI-2, Normalized water index-2.

Appendix E. Expected selection response (*R*) for spectral reflectance indices (SRIs) and for grain yield, efficiency of correlated selection response (*CR*) for grain yield estimated from SRIs in two spring wheat random populations under irrigated conditions, where *R* and *CR* were estimated based on mean of grain yield (over years) and mean SRIs (over growth stages and years).

	RLs1			RLs2		
Indices	*R*	*CR*	*CR/R*	*R*	*CR*	*CR/R*
PRI	0.854	0.197	0.287	0.874	0.371	0.629
RNDVI	0.822	0.489	0.712	0.706	0.326	0.553
GNDVI	0.803	0.456	0.663	0.696	0.413	0.701
SR	0.625	0.403	0.586	0.834	0.386	0.655
WI	0.696	-0.620	-0.903	0.708	-0.564	-0.956
NWI-1	0.691	-0.632	-0.920	0.705	-0.561	-0.950
NWI-2	0.735	-0.651	-0.947	0.683	-0.570	-0.967
Yield	0.688	-	-	0.591	-	-`

PRI, Photochemical reflectance index; WI, water index; RNDVI, Red normalized difference vegetation index; GNDVI, Green normalized difference vegetation index; SR, Simple ratio; NWI-1, Normalized water index-1; NWI-2, Normalized water index-2.

Appendix F. Average yield (t ha^{-1}) of highest 25% genotypes based on yield *per se* as compared to the highest 25% genotypes selected by different spectral reflectance indices (SRIs) values (average over growth stages) in two spring wheat random populations under irrigated conditions in two different years.

			PRI	RNDVI	GNDVI	SR	WI	NWI-1	NWI-2
RLs1	Y03-04	Yield *per se* (t ha^{-1})	5.08	5.08	5.08	5.08	5.08	5.08	5.08
		Based on SRIs (t ha^{-1})	4.91	4.95	4.83	4.89	4.92	4.92	4.93
		Difference (%)	3.5	2.6	5.2	3.9	3.3	3.3	3.0
	Y02-03	Yield *per se* (t ha^{-1})	6.22	6.22	6.22	6.22	6.22	6.22	6.22
		Based on SRIs (t ha^{-1})	5.76	5.86	6.04	5.86	6.02	6.02	6.04
		Difference (%)	8.0	6.1	3.0	6.1	3.3	3.3	3.0
RLs2	Y03-04	Yield *per se* (t ha^{-1})	5.81	5.81	5.81	5.81	5.81	5.81	5.81
		Based on SRIs (t ha^{-1})	5.58	5.62	5.53	5.60	5.65	5.68	5.68
		Difference (%)	4.1	3.4	5.1	3.8	2.8	2.3	2.3
	Y02-03	Yield *per se* (t ha^{-1})	6.87	6.87	6.87	6.87	6.87	6.87	6.87
		Based on SRIs (t ha^{-1})	6.59	6.41	6.60	6.47	6.64	6.64	6.76
		Difference (%)	4.3	7.2	4.1	6.2	3.5	3.5	1.6
		Overall mean difference (%)	5.6	4.8	4.3	5.6	3.2	3.1	2.5

PRI, Photochemical reflectance index; WI, water index; RNDVI, Red normalized difference vegetation index; GNDVI, Green normalized difference vegetation index; SR, Simple ratio; NWI-1, Normalized water index-1; NWI-2, Normalized water index-2.

VITA

Md. Ali Babar

Candidate for the Degree of

Doctor of Philosophy

Thesis: **SPECTRAL REFLECTANCE INDICES AS A SELECTION CRITERION FOR YIELD IMPROVEMENT IN WHEAT**

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Name: Md. Ali Babar Date of Degree: July, 2005

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: SPECTRAL REFLECTANCE INDICES AS A SELECTION CRITERION FOR YIELD IMPROVEMENT IN WHEAT

Pages in study: 148 Candidate for the Degree of Doctor of Philosophy

Major: Crop Science

Scope and Methods of Study: Yield in wheat (*Triticum aestivum* L.) is a complex trait and influenced by many environmental factors, and yield improvement is a daunting task for wheat breeders. Spectral reflectance indices (SRIs) have been used to study different physiological traits in wheat. SRIs have the potential to differentiate genotypes for grain yield. SRIs strongly associated with grain yield can be used to achieve effective genetic gain in wheat under different environments. Three experiments (15 adapted genotypes, 25 and 36 random sister lines derived from two different crosses) under irrigated conditions, and three experiments (each with 30 advanced genotypes) under water-limited conditions were conducted in three successive years in Northwest Mexico at the CIMMYT (International Maize and wheat Improvement Center) experimental station. SRIs and different agronomic data were collected for three years, and biomass was harvested for two years. Phenotypic and genetic correlations between SRIs and grain yield, between SRIs and biomass, realized and broad sense heritability, direct and correlated selection responses for grain yield, and SRIs were calculated.

Findings and Conclusion: Seven SRIs were calculated, and three near infrared based indices (WI, NWI-1 and NWI-2) showed higher level of genetic and phenotypic correlations with grain yield, yield components and biomass than other SRIs (PRI, RNDVI, GNDVI, and SR) under both irrigated and water limiting environments. Moderate to high realized and broad sense heritability, and selection response were demonstrated by the three NIR based indices. High efficiency of correlated response for yield estimation was demonstrated by the three NIR based indices. The ratio between the correlated response to grain yield based on the three NIR based indices and direct selection response for grain yield was very close to one. The NIR based indices showed very high accuracy in selecting superior genotypes for grain yield under both well-watered and water-limited conditions. These results demonstrated that effective genetic gain in grain yield improvement can be achieved by making selections with the three NIR based indices.

ADVISOR'S APPROVAL: ARTHUR R. KLATT