THE POTENTIAL FOR USING CANOPY SPECTRAL REFLECTANCE AS AN INDIRECT SELECTION TOOL FOR YIELD IMPROVEMENT IN WINTER WHEAT

By

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CHAPTER I

Introduction

Wheat represents about 25 percent of the total cereal grains production in the world, and nearly 1.5 billion people obtain the majority of their calories from wheat (Reynolds et al., 1999). Global demand for wheat is increasing at a rate of nearly 2% per year, but the genetic gains in yield potential are not able to cope with the pace of increasing demand (Sayre et al., 1997).

Plant breeding strategies for developing improved genotypes of wheat are extensively based on approaches that involve generating a large number of crosses and selecting segregating progenies, which has been successful over the past several decades (Jackson et al., 1996). Wheat breeders have made extensive use of the classical empirical approach, evaluating grain yield *per se* as the main selection criteria for identifying higher yielding genotypes (Aparicio et al., 2000). However, genetic gains are different in different environments, particularly successful in water non-limiting environments (Aparicio et al., 2002), but limited in dry environments (Reynolds et al., 2001). Yield gains in wheat are largely due to increased harvest index, whereas, improvement in total dry matter production has not been significant (Cox et al., 1988). Richards (2000) suggested that future improvement in wheat yield can be achieved by increasing total biomass.

Yield in a particular environment is directly or indirectly influenced by different morphological, physiological, and environmental factors. Selection of breeding lines in advanced nurseries often needs multiple testing to judge the worth of a genotype (Ball and Konzak, 1993). Analytical breeding strategy is an alternate breeding approach that requires a better understanding of the factors responsible for development, growth, and yield (Richards, 1982). This strategy considers the use of morpho-physiological selection criteria that can make empirical selection more efficient (Reynolds et al., 2001). But, the limited application of this analytical approach is probably due to improper knowledge and estimation of the physiological parameters, and their genetic associations with grain yield (Richards, 1996). Commonly used physiological selection criteria include stomatal conductance, canopy temperature depression, C_3 isotope discrimination of grains, etc. (Reynolds et al., 1999). Spectral properties of the plant is a new area of research that can be a potential selection tool for improved grain yield and biomass (Araus et al., 2001; Aparicio et al., 2002; Osborne et al., 2002; Royo et al., 2003).

Spectral reflectance from the canopy is based on the principle that specific traits of a plant are associated with the absorption of specific wavelengths of the electromagnetic spectrum. Leaf pigments like chlorophyll, xanthophylls, and carotenoids strongly absorb light in the visible wavelengths (400-700 nm), but not in the near infrared (700-1300 nm). The reflectance patterns in the near infrared part of the spectrum are influenced by the structural components of the leaf tissue (Araus et al., 2001; Peñuelas and Filella, 1998). Canopy spectral reflectance is associated with overall leaf area and other photosynthetic components, pigment composition, and physiological factors of plants. Thus, the measurement of light reflected from plants provides information to estimate green biomass, photosynthetic size, amount of absorbed photosynthetically active radiation, and canopy photosynthetic potential (Reynolds et al., 2001). Other physiological parameters, such as relative water content, nutrient deficiencies, and environmental stresses have been assessed by spectral reflectance (Araus et al., 2001; Reynolds et al., 2001).

Identifying promising genotypes in a breeding program will be facilitated if potential yield can be predicted before the crop is harvested. This early prediction will also be very helpful if the top performing families can be identified from a large number of segregating populations in a breeding program (Royo et al., 2003). Reynolds et al. (1999) indicated that morphological characters, such as number of grains per unit area and harvest index can be used in visual selection of breeding lines, but those traits are difficult to measure in small plots or at lower plant populations per unit area in the early generations. Several authors have suggested that grain yield can be approximated using spectral reflectance during the growth cycle of the crop (Araus et al., 2001; Aparicio et al., 2002; Osborne et al., 2002).

Spectral reflectance indices (SRI) are a formula based on typically a sum, difference, or ratio of two or more wavelengths that are indicative of important functions of the plant (Araus et al., 2001). The most commonly used indices are simple ratio (SR) and normalized difference vegetative index (NDVI) (Araus et al., 2002). Green biomass, leaf area index, green leaf area index, and fraction of photosynthetically active radiation have been reported positively correlated with SRI (Wiegand and Richardson, 1990; Baret and Guyot, 1991; Price and Bausch, 1995). Peñuelas et al. (1997) showed that water index (WI) can assess the salinity effect on barley. Water index has been demonstrated to estimate relative water content, leaf water potential, stomatal conductance, and canopy temperature (Peñuelas, et al., 1993).

The use of spectral reflectance as a selection criterion has not been exploited for assessing winter wheat grain yield and biomass. Information is limited even within spring and durum wheat environments. Royo et al. (2003) studied the relationship between some SRI and grain yield in durum wheat at different growth stages from booting to maturity and found that the milk grain stage was the most appropriate stage for reflectance measurements. Adequate discrimination between high and low yielding genotypes of soybeans was reported by using the NDVI (Ma et al., 2001). Serrano et al. (2000) previously reported that SR can give reliable prediction of wheat yield under different nitrogen levels. They also reported that NDVI only functioned if the leaf area index was less than three. Babar et al. (2006) demonstrated the usefulness of near infrared indices for identifying higher yielding genotypes in irrigated spring wheat under northwestern Mexican environments. Raun et al. (2001) reported that 50% of the yield variability was explained by NDVI in winter wheat under diverse nitrogen treatments. Bellairs et al. (1996) established a strong relationship between NDVI and biomass in wheat when the NDVI was measured before leaf area index reached 1.5. Peñuelas et al. (1997) reported that NDVI can differentiate biomass of barley during the early growth stages when total biomass was low.

Most studies have been carried out with a small number of genotypes and very specific wavelengths of the spectrum. As a result, investigation on the usefulness of spectral reflectance indices in a breeding program as a potential indirect selection criterion has been restricted. The routinely used indices saturate at a level LAI>3, which is not desirable in a selection program for grain yield. The practical use of spectral reflectance as an indirect selection tool requires the identification of appropriate SRI and growth stage(s) to detect genotypic differences for grain yield and biomass.

From a breeding perspective, a secondary trait must fulfill specific requirements before it can be utilized in a breeding program as a selection criterion. The efficiency of an indirect selection criterion compared to direct selection depends on the heritability of both direct and indirect traits, along with the genetic correlation between them (Falconer, 1989). The selected trait(s) must exhibit enough genetic variability, a high genetic correlation with yield, and a higher heritability than yield itself in genetic populations (Jackson et al., 1996). Information about the spectral reflectance indices in terms of these features is not available to conclude the efficiency of using SRI as an indirect selection tool for improving grain yield in winter wheat. Brancourt-Hulmel et al. (2005) reported that the magnitude of the genetic correlations between two traits may vary greatly, and depends strongly on the traits and the genetic materials under study. Improving wheat yield by selecting for resistance to stripe rust disease in a disease predominant area was reported more effective than direct selection for grain yield (Hill et al., 1999). Most selection efficiency studies have involved creating two diverse environments and selecting a trait in one environment that could be equally effective in another environment. The correlation coefficients between indirect and direct traits have been reported mostly as phenotypic correlations, not assessed in a breeding population, which may be biased. The heritability of the direct and indirect traits and the genetic correlations between them should be measured in genetic populations and in environments that closely represent the area where the improvement is being targeted. So, a comprehensive study is required in the U.S. Great Plains winter wheat environments to verify the use of SRI as an indirect selection tool for grain yield improvement.

Therefore, the objectives of the study were to i) identify suitable SRI that can best predict the winter wheat genotypes for grain yield and biomass production potential under Great Plains conditions, ii) identify the appropriate plant growth stage or stages when the indices can best determine the genotype's potential for grain yield and biomass production, iii) derive genetic correlations of the SRI with grain yield and biomass, iv) estimate heritability of grain yield and the SRI, v) examine the selection response for grain yield and correlated response for grain yield through SRI, vi) estimate relative selection efficiency of the SRI for grain yield, and vii) determine the capability of the SRI to select the top yielding genotypes.

CHAPTER II

Potential Use of Spectral Reflectance Indices as a Selection Tool for Grain Yield in Winter Wheat under Great Plains Conditions

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Abbreviations

GNDVI, green normalized difference vegetation index

LAI, leaf area index

NIR, near infrared

NWI, normalized water indices

NWI-1, normalized water index-1

NWI-2, normalized water index-2

NWI-3, normalized water index-3

NWI-4, normalized water index-4

PAR, photosynthetically active radiation

RNDVI, red normalized difference vegetation index

RIL, recombinant inbred lines

SR, simple ratio

SRI, spectral reflectance indices

WI, water index

Abstract

Selection criteria that would facilitate increased genetic gain for yield potential would be considered advantageous in plant breeding programs. We evaluated the potential of spectral reflectance indices (SRI) for assessing grain yield variability in winter wheat genotypes grown under Great Plains conditions. Three experiments were conducted, each in two different years or locations at Oklahoma State University research farms. The first experiment was composed of 25 winter wheat cultivars from the Great Plains and the other two experiments contained two groups of 25 $F_{4:6}$ and $F_{4:7}$ recombinant inbred lines from two crosses. Six previously reported SRI (red and green normalized difference vegetation index, RNDVI, GNDVI; simple ratio, SR; water index, WI; normalized water indices 1 and 2 (NWI-1, NWI-2), and two new normalized water indices (NWI-3 and NWI-4) were calculated at three growth stages, namely booting, heading, and grainfilling. Significant genotypic variation was observed for all SRI and growth stages, though the booting stage was the least associated with grain yield. The relationships between the minor water absorption band based indices (WI and NWI) and grain yield were stronger than with the RNDVI, GNDVI, and SR. The WI and the NWI always performed better in identifying superior genotypes, either at any individual growth stage or in a combination of growth stages, and especially at the heading and the grainfilling stages. Our study clearly demonstrated the potential of using SRI as a selection tool for grain yield in a winter wheat breeding program. However, genetic variation among the genotypes and environmental conditions should be considered when using the indices as a selection tool for grain yield.

Introduction

Breeding strategies for developing higher yielding genotypes of wheat are extensively based on approaches that involve generating a large number of crosses, and subsequently selecting among and within segregating populations. To date, wheat breeding around the world has been based primarily on empirical selection criteria (yield *per se*) for yield improvement (Araus et al., 2002). Since yield is characterized by low heritability and a high genotype-environmental interaction, the empirical selection may not be sufficient for achieving genetic gain (Jackson et al., 1996).

Identifying promising genotypes in a breeding program will be facilitated if potential yield can be predicted before the crop is harvested. This early prediction would also be very helpful if the top performing families could be identified from a group of hundreds or thousands of segregating populations in a breeding program (Royo et al., 2003). Reynolds et al. (1999) indicated that morphological characters such as number of grains per unit area and harvest index can be used in the visual selection of breeding lines, but those traits are difficult to measure in small plots or at the lower plant population densities commonly used in the early generations.

Yield in a given environment is directly or indirectly influenced by different morphological, physiological, and environmental factors. Selection of breeding lines for grain yield in advanced nurseries often needs repetition to make a selection decision because commonly used statistical procedures sometimes fail to produce sufficiently accurate results for identifying superior genotypes (Ball and Konzak, 1993). An analytical breeding strategy is an alternative breeding approach that requires a better understanding of the factors responsible for development, growth, and yield (Richards, 1982). This strategy considers morpho-physiological selection criteria that have potential to make empirical selection more efficient (Reynolds et al., 2001). The limited application of this analytical approach is probably due to the lack of an appropriate understanding of the physiological parameters, their estimation, and their true associations with grain yield (Richards, 1996). Commonly used physiological selection criteria include stomatal conductance, canopy temperature depression, and C_3 isotope discrimination of grains (Reynolds et al., 1999). Spectral properties of the plant came into focus as a potential selection tool for grain yield in more recent years (Araus et al., 2001; Aparicio et al., 2002; Osborne et al., 2002; Royo et al., 2003; Babar et al., 2006).

The basic principle governing canopy spectral reflectance is that specific plant traits are associated with the absorption of specific wavelengths of the spectrum (Reynolds et al., 1999). Leaf pigments like chlorophyll, xanthophylls, and carotenoids strongly absorb light in the photosynthetically active radiation (PAR) portion of the spectrum, but not in the near infrared (NIR) region. These pigments reduce reflectance of PAR, but not NIR where the magnitude of reflectance is governed by the structural discontinuities in the leaf (Araus et al., 2001; Peñuelas and Filella, 1998). Spectral reflectance by a crop canopy is related to the overall area of leaves and other photosynthetic organs in the canopy, pigment concentration, and other physiological factors (Araus et al., 2001). Thus, the measurement of the spectrum reflected from plants provides information that can be used to estimate a large number of parameters (Araus et al., 2001), such as green biomass of the canopy, photosynthetic area, amount of PAR absorbed by the canopy, and photosynthetic potential (Reynolds et al., 2001). Other parameters related to the physiological status of the canopy at the time of measurement, such as relative water content, nutrient deficiencies, environmental stresses, pigment concentration, and photosynthetic radiation use efficiency have been assessed by spectral reflectance measurements (Araus et al., 2001; Reynolds et al., 2001). Several researchers have suggested that grain yield can be estimated using spectral reflectance during different crop growth stages (Araus et al., 2001; Aparicio et al., 2002; Osborne et al., 2002; Babar et al., 2006).

The most commonly used spectral reflectance indices (SRI) are simple ratio (SR) and normalized difference vegetative index (NDVI) (Araus et al., 2002). Green biomass, leaf area index (LAI), green area index, green leaf area index, and fraction of absorbed photosynthetically active radiation are positively correlated with SRI (Wiegand and Richardson, 1990a, 1990b; Baret and Guyot, 1991; Price and Bausch, 1995). Measuring SRI periodically during different crop growth stages allows the estimation of leaf area duration that serves as an indicator of stress tolerance and the total PAR absorbed by the canopy, which are the most important factors for predicting yield (Wiegand and Richardson, 1990a). Stress assessment in plants is one of the important physiological tools associated with certain spectral indices. Water index (WI) has been demonstrated to predict relative water content, leaf water potential, stomatal conductance, and canopy temperature with sufficient water stress (Peñuelas et al., 1993). Peñuelas et al. (1997) showed the usefulness of using WI to assess the salinity effect on barley.

Ma et al**.** (2001) reported NDVI as a fast, reliable, and repeatable indicator for screening soybean genotypes for yield, and the index explained 44-80% of the yield variation. Serrano et al. (2000) worked on remote sensing for the assessment of grain yield in wheat under different nitrogen levels and determined that under stress conditions, SR provided a reliable predictor for yield. They also reported that NDVI only functioned if the LAI was less than three. Raun et al. (2001) reported that 50% of the yield variability was explained by NDVI in experiments with winter wheat at nine locations over two successive years. Tucker et al. (1980) calculated NDVI from a winter wheat field using 21 readings during the growing season and reported that 64% of the within field grain yield variability was explained by the index, especially if the measurements were taken at the growth stages from stem elongation through anthesis. Aparicio et al. (2000) conducted experiments with durum wheat genotypes in irrigated and rainfed environments and reported positive correlations between NDVI and SR with grain yield under rainfed conditions, which explained 52 and 59% of the yield variability, respectively. The commonly used SRI usually saturate at a level of LAI>3.0. So, these SRI cannot be used as a selection tool in a wheat breeding program, where the genotypes are selected at the later reproductive growth stages.

The present study was undertaken to explore the spectrum for wavelengths or combinations of wavelengths that can be used to adequately predict winter wheat genotypes for grain yield variation. The specific objectives of this study were to test the correlation of widely used SRI with grain yield variability, to identify new spectral indices that can better correlate with grain yield variability among winter wheat genotypes across environments, and to determine the optimum growth stage(s) for measurement of SRI.

Materials and Methods

Experimental materials

Three experiments, each containing 25 winter wheat genotypes developed by different breeding programs in the southern and central Great Plains were conducted during the 2003-2004 and 2004-2005 wheat growing seasons. The experiments were planted at the OSU Agronomy Research Farm, at Stillwater (STW), OK, and at Lake Carl Blackwell (LCB), located 25 km west of Stillwater. The soil types at these sites are silty clay loam with an average pH of 6.2 to 6.5 and fine sandy loam with an average pH of 6.7 to 6.9, respectively. The weather patterns of the two sites are presented in Table 1.

The first experiment, designated as Exp-1, was composed of commercial winter wheat cultivars from the southern and central Great Plains and was planted at both sites in year 2003-2004. The second experiment, designated as Exp-2, was composed of 25 $F_{4:6}$ and $F_{4:7}$ recombinant inbred lines (RIL) from the cross TX 95V5905 /Jagger and was planted at the LCB site in years 2003-2004 (F_6) and 2004-2005 (F_7). These lines were developed by the winter wheat breeding program at Kansas State University. The third experiment, designated as Exp-3, was composed of 25 $F_{4:6}$ and $F_{4:7}$ RIL from the cross TX93V4927/G1878 and was planted at the STW site in years 2003-2004 (F_6) and 2004-2005 (F_7). This population was developed by AgriPro-Coker.

All experiments were conducted under rainfed conditions and planted at a seeding rate of 70 kg ha^{-1}. Individual plot size for each experiment was 3.04 m long × 1.24 m wide. Each individual experiment was a 5 × 5 alpha lattice design with two replications. At both experimental sites, 90 kg ha^{-1} pre-plant nitrogen was applied. Folicur (25 % tebuconazole) was applied twice (late tillering and booting) to control foliar diseases and

Cygon (dimethoate) was applied at booting to control aphids. Grain yield for each plot was determined by mechanically harvesting the whole plot and expressed as kg ha^{-1}.

Spectral reflectance measurements

Spectral reflectance measurements were accomplished using a portable field spectro-radiometer (FieldSpec UV/VNIR, Analytical Spectral Devices, Boulder, CO). Reflectance measurements were taken with a 25^{0} field of view optic at vertical position. The spectro-radiometer was capable of measuring radiance from 350-1050 nm wavelengths with a sampling interval of 1.4 nm of the spectrum. Thus, 512 continuous data points were obtained with each reading. Measurements were taken during the middle of the day on cloudless days. The optical sensor was placed approximately 50 cm above the plant canopy. The incident spectrum was taken from the light reflected from a white reference panel and reflectance was calculated from the ratio of reflected light from the crop canopy against the total radiance reflectance from the white surface. The spectro-radiometer was recalibrated every ten plots. Four spectral measurements were taken randomly from four different places per plot and the mean of the four readings was used to calculate the spectral reflectance indices. Reflectance data were taken at three growth stages, namely booting (Zadoks stage 45), heading (Zadoks stage 59), and grainfilling (Zadoks stage 75) for all three experiments in both years and locations (Zadoks et al., 1974). Six previously reported spectral reflectance indices and two newly developed indices were used in this study. Descriptions of the indices are presented in Table 2.

Data analysis

The Alpha lattice analysis was performed by SAS MIXED procedures (SAS Institute, 2001) for grain yield and spectral reflectance indices. Pearson’s correlation coefficients were used to determine the association between grain yield and spectral reflectance indices measured at individual growth stages and across different growth stages. Genetic correlations between grain yield and SRI were calculated by the formula (Falconer, 1989),

$r_g = (\mathrm{Cov}_{G1G2}) / \sqrt{(\mathrm{Var}_{G1} \times \mathrm{Var}_{G2})}$, where Cov and Var indicate the genetic covariance between the two variables and variance components of the variables, respectively, and were estimated across growth stages and environments.

Regression analysis was performed to establish the linear relationship between grain yield and the SRI, and was accomplished by the SAS software (SAS Institute, 2001).

Selection for the 25% highest yielding and 25% lowest yielding genotypes was done by ranking the genotypes according to grain yield, and then ranking the genotypes based on the different SRI. The genotypes were ranked for SRI based on the mean of the readings obtained by combining the readings of the different growth stages. We also determined the yield *per se* of the 25% highest yielding and 25% lowest yielding genotypes. The yield estimate based on individual SRI was calculated using the regression equation of that specific index with grain yield. Percent yield differences were then estimated between yield *per se* and yield estimates based on different SRI.

Results

Genotypic variation and growth stage

Significant genotypic differences ($p<0.05$) for grain yield were found in all three experiments (Table 3). Genotypes in different experiments showed significant differences ($p<0.05$) for the spectral behavior at different crop growth stages, namely booting, heading, and grainfilling with a few exceptions (Table 4). In all cases, the grainfilling stage was the most significant one, followed by the heading stage; booting was the least significant stage for the behavior of the indices. The progression of growth stage from booting to grainfilling resulted in changes in the SRI values. The values for RNDVI, GNDVI, and SR decreased from the booting to the grainfilling stage (Table 4). These indices are calculated based on different combinations of red, green, and near infrared wavebands. The primary reason for the decreasing trend of these indices is the reduced reflectance in the near infrared region due to lower total leaf area and lower green leaf area, resulting in increased reflectance in the visible region (Aparicio et al., 2000). On the other hand WI, NWI-1, NWI-2, NWI-3, and NWI-4 gave two different types of patterns (Fig. 1). In year 2003-2004, these indices decreased from booting to heading, and then increased at the grainfilling stage, but in 2004-2005, they showed a continuous increase from the booting to the grainfilling stage (Fig. 1). These indices (WI and NWI) are based on the minor water absorption band (970 nm) and they measure the water status of the canopy (Babar et al., 2006; Peñuelas et al., 1997). In 2003-2004, the experiments had good water status during heading (71.4-74.9 mm rainfall) and the indices were lower, whereas, in 2004-2005, heading occurred under water deficit stress conditions (9.9-10.7

mm rainfall) and the indices were higher, since there is a negative relationship between water status and indices values.

Interaction between genotypes, growth stages, and years/locations

The analysis of variance presented in Table 5 signifies the main factor effects as well as the interaction effects of different factors for Exp-3. Among the main effects, genotypes and growth stage were found significant. Among the two way interactions, growth stage by genotype interaction was significant for all the indices. The three way interaction was significant for all the NIR indices. The analyses of variance for the other two experiments were similar (data not presented). The correlation between the heading and grainfilling stages for each individual index value was found significant for all experiments across locations and years, except Exp-1 at STW 03-04 (Table 6). For the other two combinations (booting and heading, and booting and grainfilling), the correlation coefficients were non-significant in most of the cases, except Exp-2 at LCB in 2004-2005 and a few other exceptions (Table 6).

Correlation between spectral reflectance indices and grain yield

Among the tested indices, six have been previously studied (WI, RNDVI, GNDVI, SR, NWI-1, and NWI-2), and two new indices (NWI-3 and NWI-4) have been calculated. Most of the reported indices have been previously used to estimate different physiological parameters, except NWI-1 and NWI-2, which were used to study genetic variation for grain yield among different spring wheat genotypes (Babar et al., 2006). Three indices (RNDVI, GNDVI, and SR) gave positive associations with grain yield, whereas, the other five showed negative associations (Table 7). Almost all the indices gave low correlation with grain yield at the booting stage and high correlation at heading and grainfilling stages with grain yield. The indices can be classified into two groups. One group includes the visible and near infrared wavebands and another group includes only the near infrared wavebands, especially the minor water absorption band of 970 nm (Table 2). The indices based on the minor water absorption band tended to give higher correlations with grain yield compared to the widely used RNDVI, GNDVI, and SR. In each individual experiment, the near infrared based indices gave a consistent and significant negative association with grain yield across years and locations. The vegetation based indices (RNDVI, GNDVI, and SR) gave correlations that were inconsistent in different experiments across years and locations.

Mean indices over the three growth stages always gave higher correlations with grain yield compared to any individual growth stage (Table 7), except for the RNDVI, GNDVI, and SR indices in certain cases. The indices based on the minor water absorption band always gave a higher association with grain yield compared to the RNDVI, GNDVI, and SR, when the mean of the three growth stages was used. These patterns were also evident when indices were combined for the heading and grainfilling stages, but the correlation coefficients were lower than the coefficients using three growth stage combinations (data not shown).

The water based near infrared indices (WI and NWI) always gave higher genetic correlation with grain yield compared to the widely used indices (RNDVI, GNDVI, and SR) (Fig 2). These patterns were also observed when individual growth stages and individual year/location yield were considered (data not shown). In most cases, the performance of the minor water band based indices (WI and NWI) were indistinguishable from each other, but considering the overall performance, NWI-3 and NWI-4 gave better correlations with grain yield in the different experiments over different years and locations.

Functional relationship between spectral reflectance indices and grain yield

The functional relationship between grain yield and the different SRI for the three growth stages individually and in combination were established through regression analysis. Figure 3 shows the functional relationship of NWI-3 with yield in three growth stages for two experiments. The relationship of three different indices (NWI-2, NWI-3, and NWI-4) with grain yield are presented in Fig 4 for three different experiments, based on the mean of the three different growth stages in an individual year or location. The relationships of the other indices with grain yield were also tested (data not shown), and in most instances, the relationship was linear. Indices tended to explain more of the grain yield variability when mean data were used, for example, combining growth stages, combining years/locations, or combining both. Normalized water index -3 explained more of the grain yield variability at the heading and grainfilling stages, as compared to the booting stage (Fig 3). Our analysis based on combining growth stages revealed the superiority of the NWI-3 over the other indices in predicting yield, explaining 74% of the grain yield variation in one location and 63% of the variation in the other location for Exp -1 (Fig 4). The relationship of measured grain yield and predicted grain yield based on NWI-3 is presented in Fig 5. This relationship was established on the basis of all three experiments using the mean values of the three growth stages across locations and years. Although some differences were observed between the different experiments for grain yield and spectral behavior, the relationships revealed the significant linear predictability of grain yield based on index NWI-3.

Selection of genotypes

The percentage of selected genotypes (based on grain yield *per* se) among the 25% highest yielding and 25% lowest yielding genotypes were estimated based on the mean indices over three growth stages for individual year/location for each individual experiment (Table 8). The water index and the NWI showed accuracy in identifying both the highest yielding and the lowest yielding genotypes compared to the RNDVI, GNDVI, and SR. Similar trends were also observed when individual growth stages were considered (data not shown), but the combination of growth stages always worked better. The indices that included the minor water absorption band identified 50-83% of both the 25% highest yielding genotypes and the 25% lowest yielding genotypes in all three experiments. Among the near infrared based indices, NWI-3 and NWI-4 consistently performed better. This was evident when the mean of the two locations or two years data were combined for individual experiments, and also when the three experiments were considered together (Table 8).

Yield *per se* and yield estimates of the 25% highest yielding and 25% lowest yielding genotypes for the three experiments based on the linear regression equation of the different SRI are presented in Table 9. The percent difference between the harvested and predicted yield in different experiments was as close as 0.9 % for the highest yielding genotypes using NWI-3 in Exp-1. The largest difference between actual and predicted yield was 21.1 % for the RNDVI in Exp-3. In most cases, the percent yield difference was 10 % or less, with the near infrared based indices commonly having differences of 1-5 %. The widely used indices (RNDVI, GNDVI, and SR) showed inconsistency across years and locations in identifying the highest yielding and lowest yielding genotypes, while the near infrared indices showed remarkable accuracy and consistency in predicting the relative performance of the genotypes for grain yield across years and locations.

Discussion

Genotypic variation and growth stage

Significant genotypic variation for grain yield and spectral behavior at different growth stages, namely booting, heading, and grainfilling, confirms the existence of sufficient variation among the genotypes. The first experiment (Exp-1) includes widely used winter wheat cultivars that are available throughout the Great Plains of the U.S. The other two experiments contain sufficient variation in the respective RIL populations. Similar variations were also observed by other researchers working with spectral reflectance in wheat, including Babar et al. (2006) working with irrigated spring wheat, with irrigated and non-irrigated spring wheat (Gutiérrez-Rodríguez et al., 2004), with durum wheat under irrigation and under rainfed conditions in the Mediterranean region (Aparicio et al., 2000, 2002), and with durum wheat under rainfed conditions (Royo et al., 2003). The least significant difference among the genotypes for spectral reflectance indices at the booting stage was due to the homogeneity of the LAI among the genotypes, which normally reach maximum at the booting stage (Aparicio et al., 2000). But, at the later growth stages, these indices are highly influenced by the reproductive structures such as spike morphology, as well as the decrease in the LAI (Hatfield, 1981; Aparicio et al., 2000).

Interaction effect of genotypes, growth stages, and years/locations

We observed significant interaction between different growth stages and the genotypes for different indices (Table 5). This means the specific growth stages are a very important consideration when measuring the spectral reflectance. Similar observations were made in the studies of Babar et al. (2006) with irrigated spring wheat and by Aparicio et al. (2002) with rainfed durum wheat. We also observed very low associations of any individual index between different growth stages, especially between the booting and the heading stages, and between the booting and the grainfilling stages, with a few exceptions. This indicates that the genotypes were ranked differently for indices value in different growth stages. Babar et al. (2006) and Royo et al. (2003) reported similar observations. Correlations between the heading and grainfilling stages of individual indices were found significant for all the cases except for the Exp-1 at Stillwater in 2003-2004. Since the heading and the grainfilling stages are the two most important growth stages for spectral reflectance measurements, we recommend measuring spectral reflectance at these two growth stages.

Correlation between spectral reflectance indices and grain yield

Different studies have reported the usefulness of the spectral reflectance indices for predicting grain yield under different environmental conditions. Most studies considered the diversity of the environments in differentiating the genotypes, including different water regimes in durum wheat (Aparicio et al., 2000), salinity effects in barley (Peñuelas et al., 1997), fertilizer and water treatments in corn (Osborne et al., 2002), and plant population density in soybean (Ma et al., 2001). These studies measured the environmental influence in increasing the variability in the experiments rather than the genetic variability for grain yield due to the genotypes. However, Babar et al. (2006) established a strong relationship between the near infrared based indices and grain yield with irrigated spring wheat genotypes. In contrast, there has been little research in using spectral reflectance indices in predicting winter wheat genotypes for grain yield under

Great Plains rainfed conditions. Raun et al. (2001) previously reported the use of NDVI in predicting the yield of a few winter wheat cultivars under diverse nitrogen levels.

We used six published SRI (RNDVI, GNDVI, SR, WI, NWI-1, and NWI-2) and two new indices (NWI-3 and NWI-4) have been calculated (Table 2). Most indices taken at the booting stage showed non-significant correlations with grain yield in the different experiments, with a few exceptions. The relationship between SRI and yield became stronger as the growth stage progressed toward the reproductive phase. Babar et al. (2006) also reported lower correlations between the different spectral reflectance indices and grain yield at the booting stage. Ma et al. (2001) with soybean and Royo et al. (2003) with durum wheat also reported that the SRI correlated better with yield at the reproductive growth stages rather than at early vegetative growth stages. Aparicio et al. (2000) found significant correlations between grain yield and SRI at maturity with durum wheat under rainfed conditions.

Water index and the NWI gave higher correlations with grain yield compared with the RNDVI, GNDVI, and SR at heading and grainfilling (Table 7). Peñuelas et al. (1993) reported significant relationships between WI and relative water content, leaf water potential, stomatal conductance, and canopy temperature. The high correlation coefficients of the near infrared based indices with grain yield are due to the fact that the higher yielding genotypes retained more water in their canopy at the later growth stages. Increased water content in the plant canopy decreased the reflectance of the water band and consequently, we obtained negative correlations of these indices with grain yield. Babar et al. (2006), Royo et al. (2003), and Peñuelas et al. (1997) reported similar observations.

Correlations of the mean estimates of the SRI over the three growth stages with yield were higher compared to any individual growth stage (Table 7). This was also reported by Babar et al. (2006) and Aparicio et al. (2000). The near infrared based indices always gave higher correlations than the RNDVI, GNDVI, and SR when reflectance measurements were combined over different growth stages. In most cases, the mean of the indices at the heading and the grainfilling stages also gave higher correlations with grain yield than any individual growth stage, but lower than the correlation coefficients obtained through combining three growth stages (data not shown). We believe the mean indices over growth stages are a measurement of overall plant health during a critical time in plant development. Repeated reflectance measurements of a genotype over different growth stages assess the overall fitness of the genotype over time. Therefore, the indices identify the genotypes that maintain a healthier condition throughout the growing season and as a result, give higher correlations with final grain yield. We observed higher genetic correlations between the water based near infrared based indices and grain yield compared to widely used vegetation based indices (Fig 2). This observation indicated that these water based indices have higher predictability at the genotypic level for grain yield variation compared to the vegetation based indices.

Functional relationship between the SRI and grain yield

The functional relationship between the SRI and grain yield was linear (Fig 3 and Fig 4). Babar et al. (2006) and Gutiérrez-Rodríguez et al. (2004) also reported linear relationships between the different SRI and grain yield. Our results indicate that these SRI can be used for predicting grain yield irrespective of the yield potential of the genotypes. The near infrared based indices always showed better predictability when more data were included in the model (different growth stages, different years/locations). These indices also predicted grain yield well when an individual growth stage such as heading or grainfilling stages were considered, thus making them suitable as a selection tool in a breeding program for yield improvement. Considering the overall performance, NWI-3 and NWI-4 showed better performance over the other near infrared based indices.

Selection of genotypes

The efficiency of selecting superior genotypes is a primary concern when using an indirect selection tool for a specific trait. Our results showed that the water based near infrared indices identified a major proportion of the higher yielding as well as the lower yielding genotypes in the different experiments. In addition, the close approximation of actual grain yield also bears significance about the power of the indices for accurate prediction of the genotypes yield potential. The near infrared based indices, especially NWI-3 and NWI-4, showed very promising effectiveness in selecting the desired genotypes for grain yield.

Conclusions

The potential of spectral reflectance indices for predicting winter wheat genotypes for grain yield variability is demonstrated in our study. The randomly derived recombinant inbred lines established the genetic basis of the true relationship between the spectral reflectance indices and grain yield. The indices were related to grain yield in a linear fashion confirming the effectiveness for identifying the higher yielding genotypes irrespective of their yield potential. Combining the reflectance measurements from three growth stages provided the best relationship between spectral reflectance indices and grain yield, but for practical consideration, we recommend taking spectral reflectance measurements at the heading and the grainfilling stages for predicting the genotypic grain yield potential. Indices based on the minor water absorption band consistently provided the best relationships with grain yield, and among them NWI-3 and NWI-4 showed better performance in identifying the higher yielding genotypes in different winter wheat genetic backgrounds in the Great Plains.

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Tables

Table 1. Mean, maximum, and minimum monthly temperatures (^{0}C) and monthly total rainfall (mm) for the two growing seasons at two locations.

Stillwater (2003-2004)									
		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Mean temperature (^{0}C)	Max.	23.9	15.0	11.7	9.4	10.0	18.3	21.7	28.3
	Min.	8.9	3.9	-0.6	-2.8	-2.2	5.6	8.9	15.6
	Ave.	16.3	9.7	5.5	3.2	3.7	12.4	15.6	22.0
Total rainfall (mm)		74.9	54.4	42.7	56.9	42.4	101.1	71.4	5.8
Stillwater (2004-2005)									
Mean temperature (^{0}C)	Max.	23.3	15.0	12.8	8.9	13.3	16.7	22.8	26.7
	Min.	11.1	5.0	-2.2	-2.2	1.1	3.3	8.9	14.4
	Ave.	17.1	9.8	4.9	2.6	6.9	10.3	15.8	20.4
Total rainfall (mm)		116.1	126.0	24.4	70.4	33.3	17.5	9.9	97.8
Lake Carl Blackwell (2003-2004)									
Mean temperature (^{0}C)	Max.	23.9	15.0	11.7	9.4	10.0	18.3	21.7	27.8
	Min.	10.0	4.4	0.0	-2.8	-2.2	6.1	10.0	15.0
	Ave.	16.6	9.6	5.5	3.1	3.7	12.4	15.5	21.6
Total rainfall (mm)		84.3	60.7	33.0	62.2	39.6	110.2	74.9	3.6
Lake Carl Blackwell (2004-2005)									
Mean temperature (^{0}C)	Max.	23.3	14.4	12.8	8.3	12.8	16.7	22.2	26.1
	Min.	11.1	5.0	-1.1	-2.8	1.7	3.9	9.4	14.4
	Ave.	17.1	9.7	5.2	2.6	7.2	10.2	15.7	19.9
Total rainfall (mm)		127.8	162.6	24.1	65.3	38.4	19.6	10.7	69.1

Table 2. Description of the spectral reflectance indices employed in this study.

Spectral reflectance indices	Estimation†	Function	References
Red normalized difference vegetation index (RNDVI)	$(R_{780}-R_{670})/(R_{780}+R_{670})$	Canopy photosynthetic area	Raun et al. (2001)
Green normalized difference vegetation index (GNDVI)	$(R_{780}-R_{550})/(R_{780}+R_{550})$	Canopy photosynthetic area	Aparicio et al. (2000)
Simple ratio (SR)	(R_{900}/R_{680})	Canopy photosynthetic area	Gitelson et al. (1996)
Water index (WI)	(R_{970}/R_{900})	Canopy water status	Peñuelas et al. (1993)
Normalized water index -1 (NWI-1)	$(R_{970}-R_{900})/(R_{970}+R_{900})$	Canopy water status	Babar et al. (2006)
Normalized water index -2 (NWI-2)	$(R_{970}-R_{850})/(R_{970}+R_{850})$	Canopy water status	Babar et al. (2006)
Normalized water index -3 (NWI-3)	$(R_{970}-R_{920})/(R_{970}+R_{920})$	Canopy water status	Newly developed
Normalized water index -4 (NWI-4)	$(R_{970}-R_{880})/(R_{970}+R_{880})$	Canopy water status	Newly developed

† R, reflectance at a specific wavelength of the light spectrum (in nm).

Table 3. Variability parameters for grain yield (kg ha^{-1}) in three experiments presented as individual year/location.†

Parameters	Exp-1		Exp-2		Exp-3	
	STW 03-04	LCB 03-04	LCB 03-04	LCB 04-05	STW 03-04	STW 04-05
Minimum	3920	4229	3566	2482	3294	3381
Maximum	6460	6620	6576	3512	5808	5498
Mean	4950	5383	4989	2980	4667	4271
SE	530	430	589	249	437	361
LSD (5%)	1125	912	1250	527	927	765
CV (%)	10.2	7.8	11.7	7.9	8.6	8.5
Significance level	*	**	*	*	**	**

*$p<0.05$, **$p<0.01$

† STW, Stillwater site; LCB, Lake Carl Blackwell site.

Table 4. Variability parameters of different spectral reflectance indices estimated at different growth stages, presented for three experiments across different years and locations.

Indices†	Parameters	Exp-1‡			Exp-2§			Exp-3§		
		Booting	Heading	Grainfilling	Booting	Heading	Grainfilling	Booting	Heading	Grainfilling
WI	Minimum	0.810	0.784	0.891	0.824	0.843	0.866	0.806	0.794	0.869
	Maximum	0.876	0.906	0.964	0.866	0.910	0.980	0.871	0.870	0.953
	Mean	0.842	0.849	0.935	0.847	0.865	0.915	0.835	0.833	0.903
	SE	0.012	0.013	0.015	0.015	0.013	0.012	0.008	0.013	0.015
	Significance level	**	**	**	*		**	**	**	**
RNDVI	Minimum	0.928	0.779	0.524	0.841	0.757	0.518	0.886	0.869	0.612
	Maximum	0.945	0.943	0.834	0.941	0.900	0.828	0.948	0.933	0.837
	Mean	0.870	0.887	0.651	0.900	0.851	0.680	0.931	0.904	0.724
	SE	0.016	0.028	0.046	0.018	0.028	0.036	0.009	0.016	0.051
	Significance level		*	**	*		**	**	**	**
GNDVI	Minimum	0.755	0.660	0.564	0.768	0.680	0.620	0.773	0.776	0.606
	Maximum	0.846	0.858	0.736	0.830	0.810	0.741	0.869	0.849	0.746
	Mean	0.808	0.780	0.633	0.799	0.757	0.668	0.835	0.811	0.672
	SE	0.018	0.023	0.026	0.016	0.020	0.021	0.01	0.015	0.027
	Significance level	**	**	**		*	*	**	**	**
SR	Minimum	18.2	8.5	3.15	11.45	6.54	2.83	17.07	14.65	4.15
	Maximum	34.9	33.1	11.22	32.35	21.82	9.49	38.59	28.04	11.36
	Mean	27.8	19.9	5.12	21.49	13.44	5.52	29.07	20.49	6.81
	SE	3.86	2.665	1.013	4.043	2.48	0.911	3.417	2.91	1.42
	Significance level	*	**	**			*	**	**	**
NWI-1	Minimum	-0.105	-0.121	-0.058	-0.096	-0.085	-0.072	-0.108	-0.115	-0.070
	Maximum	-0.067	-0.049	-0.019	-0.072	-0.047	-0.010	-0.069	-0.07	-0.024
	Mean	-0.086	-0.081	-0.034	-0.083	-0.072	-0.044	-0.09	-0.09	-0.051
	SE	0.007	0.007	0.008	0.009	0.008	0.007	0.005	0.008	0.008
	Significance level	**	**	**	*	**	**	**	**	**
NWI-2	Minimum	-0.107	-0.118	-0.040	-0.095	-0.079	-0.061	-0.105	-0.105	-0.056
	Maximum	-0.066	-0.027	0.015	-0.066	-0.022	0.035	-0.053	-0.056	0.007
	Mean	-0.084	-0.072	-0.005	-0.081	-0.063	-0.02	-0.087	-0.080	-0.032
	SE	0.008	0.009	0.011	0.010	0.01	0.009	0.006	0.009	0.011
	Significance level	**	**	**	*	*	*	**	**	**
NWI-3	Minimum	-0.102	-0.118	-0.061	-0.094	-0.084	-0.072	-0.105	-0.111	-0.071
	Maximum	-0.067	-0.054	-0.026	-0.071	-0.052	-0.022	-0.070	-0.070	-0.031
	Mean	-0.084	-0.082	-0.041	-0.082	-0.073	-0.049	-0.08	-0.089	-0.054
	SE	0.006	0.007	0.007	0.008	0.007	0.006	0.004	0.007	0.007
	Significance level	**	**	**	*		**	**	**	**
NWI-4	Minimum	-0.017	-0.125	-0.055	-0.101	-0.087	-0.073	-0.111	-0.113	-0.070
	Maximum	-0.068	-0.043	-0.010	-0.074	-0.041	0.004	-0.066	-0.067	-0.015
	Mean	-0.087	-0.082	-0.027	-0.086	-0.073	-0.040	-0.092	-0.090	-0.048
	SE	0.008	0.008	0.009	0.01	0.008	0.008	0.005	0.008	0.009
	Significance level	**	**	**	*	*	**	**	**	**

*p<0.05, **p<0.01

† GNDVI, green normalized difference vegetation index; NWI-1, normalized water index-1; NWI-2, normalized water index-2; NWI-3, normalized water index-3; NWI-4, normalized water index-4; RNDVI, red normalized difference vegetation index; SR, simple ratio; WI, water index.

‡ Estimates were based on two locations in year 2003-2004.

§ Estimates were based on two years (2003-2004 and 2004-2005).

Table 5. Combined analysis of variance (mean squares) for different spectral reflectance indices across different years and growth stages (GS) in Exp-3.

		Spectral reflectance indices†							
Source of variation	df	WI	RNDVI	GNDVI	SR	NWI-1	NWI-2	NWI-3	NWI-4
Year	1	0.00091	0.000744	0.015693	7.88	0.000319	0.000109	0.000247	0.000438
Rep (year)	2	0.00523**	0.02528**	0.00965**	395.84**	0.00175**	0.002919**	0.00149**	0.002424**
Block (year × rep)	16	0.00036	0.001430	0.000548	21.63**	0.000106	0.000187*	0.000092*	0.000154 *
Genotype	24	0.00175**	0.006872**	0.00355**	79.87**	0.00059**	0.000874**	0.00046**	0.000702**
GS	2	0.15693**	1.26649**	0.77409**	12608**	0.05152**	0.091245*	0.04003**	0.062720*
GS × genotype	48	0.00049*	0.00322**	0.00118**	19.47**	0.000167*	0.000248*	0.000135*	0.000199*
Year × genotype	24	0.00029	0.001126	0.000499	15.41*	0.000097	0.000140	0.000073	0.000110
Year × GS	2	0.00048	0.000281	0.00417**	9.99	0.000141	0.001964**	0.000055	0.00075**
Year × GS × genotype	48	0.00029**	0.000916	0.000375	9.34	0.000095**	0.000134**	0.000075**	0.00011**
Residual	132								
Total	299								

*p<0.05, **p<0.01

† GNDVI, green normalized difference vegetation index; NWI-1, normalized water index-1; NWI-2, normalized water index-2; NWI-3, normalized water index-3; NWI-4, normalized water index-4; RNDVI, red normalized difference vegetation index; SR, simple ratio; WI, water index.

Table 6. Correlation coefficients between the estimates of different spectral reflectance indices for three growth stages in three experiments for two locations/years.

Exp-1						
Indices†	STW 03-04			LCB 03-04		
	Boot-Hd‡	Boot-Gf§	Hd-Gf¶	Boot-Hd‡	Boot-Gf§	Hd-Gf¶
WI	0.217	0.043	0.076	0.122	-0.183	0.699**
RNDVI	0.043	-0.317	0.373	0.273	-0.133	0.602**
GNDVI	0.310	-0.135	0.071	0.389	0.296	0.667**
SR	-0.003	-0.218	0.324	0.106	-0.035	0.792**
NWI-1	0.208	0.031	0.074	0.133	-0.185	0.694**
NWI-2	0.258	0.102	0.057	0.005	-0.272	0.679**
NWI-3	0.257	0.078	0.096	0.148	-0.118	0.725**
NWI-4	0.297	0.154	0.090	0.099	-0.202	0.690**
Exp-2						
Indices	LCB 03-04			LCB 04-05		
	Boot-Hd‡	Boot-Gf§	Hd-Gf¶	Boot-Hd‡	Boot-Gf§	Hd-Gf¶
WI	0.289	-0.041	0.664**	0.717**	0.367	0.656**
RNDVI	0.016	-0.104	0.804**	0.690**	0.185	0.482*
GNDVI	0.258	0.279	0.791**	0.748**	0.415*	0.604**
SR	-0.197	-0.320	0.842**	0.683**	0.048	0.363
NWI-1	0.295	-0.037	0.665**	0.716**	0.386	0.652**
NWI-2	0.130	-0.181	0.695**	0.646**	0.173	0.528**
NWI-3	0.353	-0.106	0.674**	0.691**	0.459*	0.705**
NWI-4	0.205	-0.168	0.666**	0.668**	0.286	0.593**
Exp-3						
Indices	STW 03-04			STW 04-05		
	Boot-Hd‡	Boot-Gf§	Hd-Gf¶	Boot-Hd‡	Boot-Gf§	Hd-Gf¶
WI	0.505**	0.105	0.614**	0.126	0.128	0.804**
RNDVI	0.196	-0.244	0.623**	0.746**	0.489*	0.771**
GNDVI	0.388	-0.119	0.589**	0.707**	0.430*	0.813**
SR	0.288	-0.137	0.648**	0.836**	0.522**	0.765**
NWI-1	0.485*	0.097	0.619**	0.127	0.140	0.802**
NWI-2	0.464*	0.081	0.609**	0.216	0.199	0.762**
NWI-3	0.506**	0.085	0.605**	0.110	0.140	0.798**
NWI-4	0.425*	0.107	0.616**	0.173	0.203	0.770**

*p<0.05, **p<0.01

† GNDVI, green normalized difference vegetation index; NWI-1, normalized water index-1; NWI-2, normalized water index-2; NWI-3, normalized water index-3; NWI-4, normalized water index-4; RNDVI, red normalized difference vegetation index; SR, simple ratio; WI, water index.

‡ Correlation between booting and heading stages.

§ Correlation between booting and grainfilling stages.

¶ Correlation between heading and grainfilling stages.

Table 7. Correlation coefficients between different spectral reflectance indices and grain yield at three growth stages (GS) for three experiments. †

Indices	GS	EXP-1		EXP-2		EXP-3	
WI		STW 03-04	LCB 03-04	LCB 03-04	LCB 04-05	STW 03-04	STW 04-05
	Boot	-0.374	-0.272	-0.105	-0.358	-0.588**	-0.139
	Hd	-0.674**	-0.733**	-0.643**	-0.512**	-0.686**	-0.569**
	GF	-0.516**	-0.672**	-0.657**	-0.752**	-0.571**	-0.685**
	Mean‡	-0.814**	-0.763**	-0.676**	-0.653**	-0.785**	-0.623**
RNDVI							
	Boot	0.081	0.266	0.029	0.372	0.319	0.402
	Hd	0.401*	0.481*	0.499*	0.400*	0.259	0.525**
	GF	0.129	0.474*	0.567**	0.522**	0.150	0.591**
	Mean‡	0.183	0.567**	0.570**	0.574**	0.232	0.605**
GNDVI							
	Boot	0.130	0.295	0.210	0.473*	0.397*	0.469*
	Hd	0.622**	0.557**	0.680**	0.475*	0.494*	0.509**
	GF	0.108	0.387	0.512**	0.478*	0.058	0.567**
	Mean‡	0.312	0.524**	0.624**	0.559**	0.341	0.571**
SR							
	Boot	0.107	0.312	-0.112	0.348	0.313	0.484*
	Hd	0.445*	0.554**	0.604**	0.464*	0.367	0.594**
	GF	0.052	0.535**	0.606**	0.533**	0.224	0.608**
	Mean‡	0.381	0.618**	0.471*	0.533**	0.435*	0.595**
NWI-1							
	Boot	-0.364	-0.268	-0.097	-0.366	-0.579**	-0.135
	Hd	-0.673**	-0.730**	-0.650**	-0.515**	-0.682**	-0.600**
	GF	-0.518**	-0.663**	-0.662**	-0.750**	-0.577**	-0.684**
	Mean‡	-0.819**	-0.758**	-0.671**	-0.653**	-0.788**	-0.618**
NWI-2							
	Boot	-0.429*	-0.278	-0.069	-0.334	-0.598**	-0.172
	Hd	-0.668**	-0.690**	-0.617**	-0.522**	-0.650**	-0.614**
	GF	-0.442*	-0.596**	-0.652**	-0.645**	-0.511**	-0.670**
	Mean‡	-0.759**	-0.702**	-0.690**	-0.657**	-0.741**	-0.622**
NWI-3							
	Boot	-0.423*	-0.260	-0.106	-0.348	-0.560**	-0.132
	Hd	-0.704**	-0.740**	-0.678**	-0.522**	-0.666**	-0.581**
	GF	-0.514**	-0.703**	-0.742**	-0.729**	-0.579**	-0.698**
	Mean‡	-0.865**	-0.798**	-0.776**	-0.666**	-0.777**	-0.668**
NWI-4							
	Boot	-0.499*	-0.281	-0.089	-0.335	-0.581**	-0.164
	Hd	-0.707**	-0.727**	-0.649**	-0.516**	-0.658**	-0.598**
	GF	-0.478*	-0.646**	-0.721**	-0.670**	-0.551**	-0.689**
	Mean‡	-0.845**	-0.810**	-0.753**	-0.673**	-0.761**	-0.684**

*p<0.05, **p<0.01

† Boot, booting stage; GF, grainfilling stage; GNDVI, green normalized difference vegetation index; Hd, heading stage; NWI-1, normalized water index-1; NWI-2, normalized water index-2; NWI-3, normalized water index-3; NWI-4, normalized water index-4; RNDVI, red normalized difference vegetation index; SR, simple ratio; WI, water index.

‡ Correlation between yield and mean spectral reflectance indices over three growth stages.

Table 8. The percentage of selected genotypes among the 25% highest yielding and 25% lowest yielding genotypes (values in parenthesis) selected on the basis of different spectral reflectance indices.

Experiments	Spectral reflectance indices†							
	WI	RNDVI	GNDVI	SR	NWI-1	NWI-2	NWI-3	NWI-4
EXP-1								
STW 03-04	83 (83)	17 (33)	50 (33)	50 (50)	83 (67)	67 (67)	83(83)	83 (67)
LCB 03-04	83 (50)	50 (50)	50 (50)	50 (67)	67 (50)	83 (50)	67 (83)	83 (67)
Mean‡	67 (50)	50 (33)	50 (50)	50 (33)	67 (50)	67 (50)	83 (83)	83 (83)
EXP-2								
LCB 03-04	67 (50)	67 (50)	67 (50)	67 (50)	67 (50)	67 (50)	83 (67)	83 (67)
LCB 04-05	67 (50)	67 (50)	50 (50)	50 (50)	50(50)	67 (50)	83 (67)	83 (67)
Mean‡	50 (50)	50(33)	33 (67)	50 (50)	50 (50)	67 (33)	67 (67)	67 (67)
EXP-3								
STW 03-04	50 (67)	33 (17)	33 (33)	50 (50)	50 (67)	67 (50)	67 (67)	67 (67)
STW 04-05	67 (50)	50 (50)	50 (50)	83 (50)	67 (50)	83 (50)	83 (50)	83 (67)
Mean‡	67 (50)	67 (17)	83 (33)	83 (33)	67 (67)	67 (67)	83 (83)	83 (83)
Overall mean§	61 (50)	56 (28)	55 (50)	61 (39)	61 (56)	67 (50)	78 (78)	78 (78)

† GNDVI, green normalized difference vegetation index; NWI-1, normalized water index-1; NWI-2, normalized water index-2; NWI-3, normalized water index-3; NWI-4, normalized water index-4; RNDVI, red normalized difference vegetation index; SR, simple ratio; WI, water index.

‡ Correlation between mean spectral reflectance indices over three growth stages and mean grain yield over locations/years for each individual experiment.

§ Correlation between mean spectral reflectance indices over three growth stages and mean grain yield over locations/years for all experiments.

Table 9. Yield *per se* (kg ha^{-1}) and yield estimates (kg ha^{-1}) based on the linear regression equations of different spectral reflectance indices with grain yield for the 25% highest yielding and 25% lowest yielding genotypes (values in parenthesis).

Experiments	Spectral reflectance indices†							
	WI	RNDVI	GNDVI	SR	NWI-1	NWI-2	NWI-3	NWI-4
EXP-1								
STW 03-04 Yield *per se* 5678 (4207)	5620 (4386)	5071 (4797)	5170 (4963)	5228 (4677)	5624 (4380)	5595 (4379)	5627 (4333)	5622 (4352)
% Difference	1.0 (4.2)	10.7 (14.0)	9.0 (11.6)	7.9 (11.1)	1.0 (4.1)	1.5 (4.1)	0.9 (3.0)	1.0 (3.4)
LCB 03-04 Yield *per se* 6248 (4758)	6006 (4837)	5852 (5029)	5764 (4983)	5852 (4994)	5993 (4845)	6001 (4865)	6000 (4857)	6005 (4843)
% Difference	3.9 (1.7)	6.3 (5.7)	7.7 (4.7)	6.3 (5.0)	4.1 (1.8)	4.0 (2.2)	4.0 (2.1)	3.9 (1.8)
EXP-2								
LCB 03-04 Yield *per se* 5995 (4157)	5621 (4337)	5504 (4455)	5573 (4383)	5567 (4439)	5628 (4327)	5585 (4358)	5662 (4280)	5631 (4302)
% Difference	6.2 (4.3)	8.2 (7.2)	7.0 (5.4)	7.1 (6.8)	6.1 (4.1)	6.8 (4.8)	5.6 (3.0)	6.1 (3.5)
LCB 04-05 Yield *per se* 3317 (2611)	3205 (2725)	3147 (2765)	3139 (2770)	3177 (2753)	3211 (2728)	3208 (2739)	3197 (2744)	3203 (2739)
% Difference	3.4 (4.4)	5.1 (5.9)	5.4 (6.1)	4.2 (5.4)	3.2 (4.5)	3.3 (4.9)	3.6 (5.1)	3.4 (4.9)
EXP-3								
STW 03-04 Yield *per se* 5642 (3511)	5407 (3809)	4931 (4450)	5042 (4324)	5157 (4272)	5410 (3804)	5388 (3873)	5402 (3791)	5396 (3853)
% Difference	4.2 (7.8)	12.6 (21.1)	10.6 (18.8)	8.6 (17.8)	4.1 (7.7)	4.5 (9.3)	4.2 (7.4)	4.3 (8.8)
STW 04-05 Yield *per se* 5008 (3640)	4728 (3800)	4686 (3857)	4653 (3844)	4758 (3864)	4728 (3802)	4742 (3799)	4723 (3807)	4747 (3802)
% Difference	5.6 (4.4)	6.4 (5.9)	7.1 (5.6)	5.0 (6.2)	5.6 (4.4)	5.3 (4.4)	5.7 (4.6)	5.2 (4.4)

† GNDVI, green normalized difference vegetation index; NWI-1, normalized water index-1; NWI-2, normalized water index-2; NWI-3, normalized water index-3; NWI-4, normalized water index-4; RNDVI, red normalized difference vegetation index; SR, simple ratio; WI, water index.

Figures

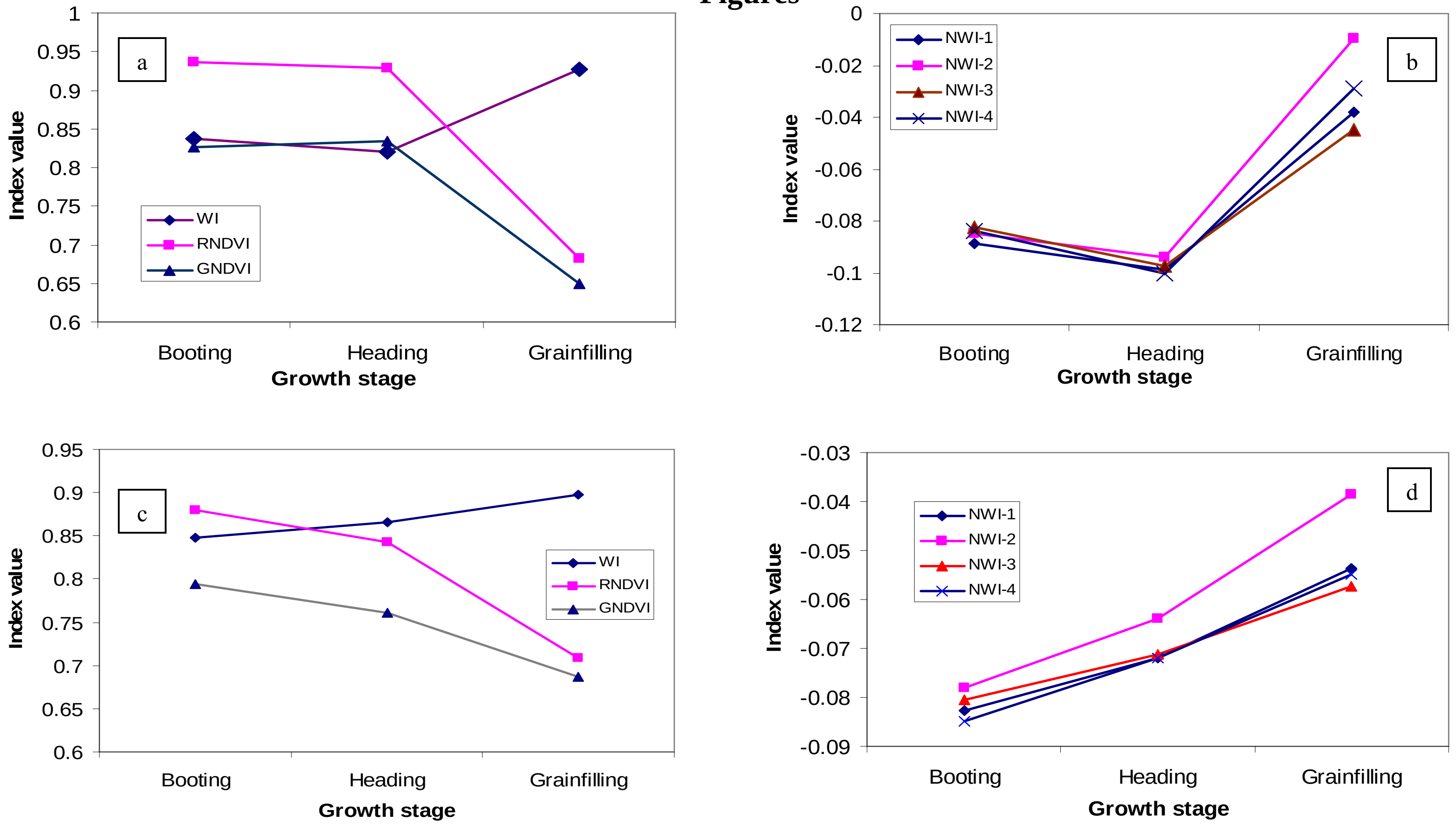


Fig 1. Changes in the pattern of different spectral reflectance indices with the advancement of growth stages. Fig a and Fig b represent Exp-1 in year 2003-2004 at Stillwater and Fig c and Fig d represent Exp-2 in 2004-2005 at Lake Carl Blackwell.

GNDVI, green normalized difference vegetation index; NWI-1, normalized water index-1; NWI-2, normalized water index-2; NWI-3, normalized water index-3; NWI-4, normalized water index-4; RNDVI, red normalized difference vegetation index; WI, water index.

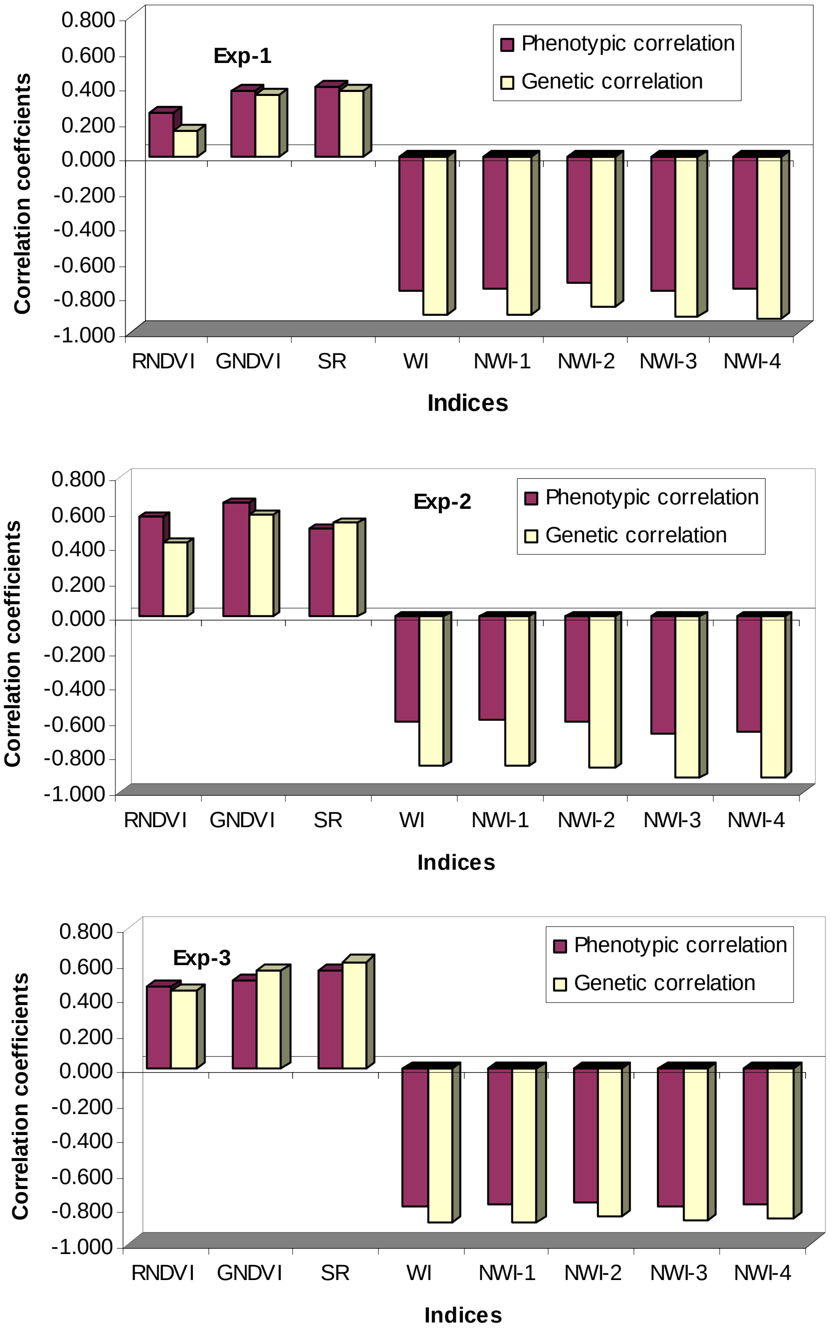
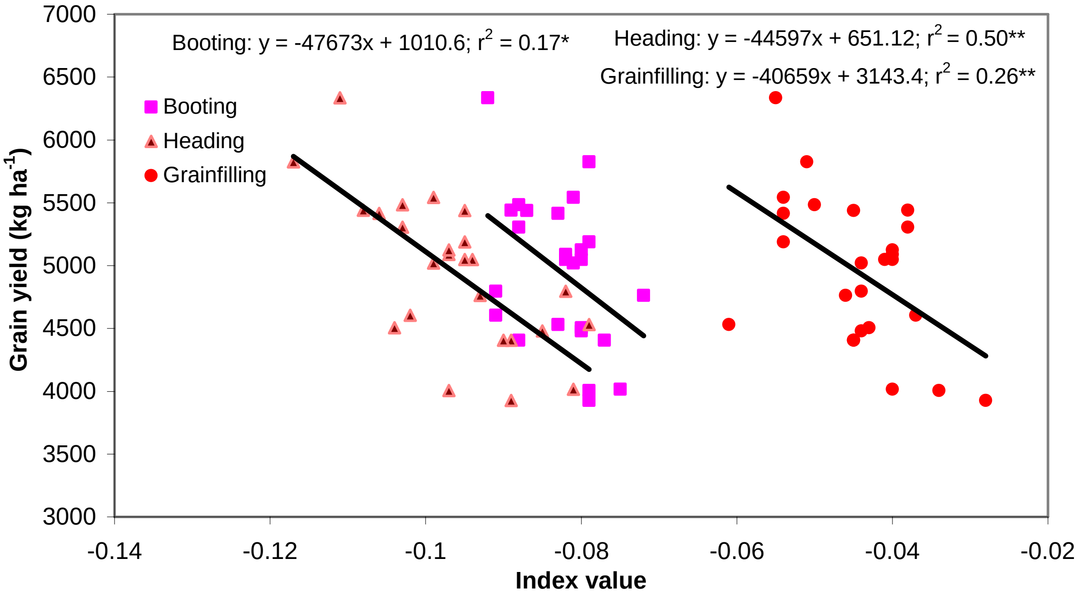


Fig 2. Phenotypic and genetic correlation coefficients between different spectral reflectance indices (mean of different growth stages across year/location) and grain yield (mean of two locations in Exp-1 and two years in Exp-2 and Exp-3).

GNDVI, green normalized difference vegetation index; NWI-1, normalized water index-1; NWI-2, normalized water index-2; NWI-3, normalized water index-3; NWI-4, normalized water index-4; RNDVI, red normalized difference vegetation index; SR, simple ratio; WI, water index.



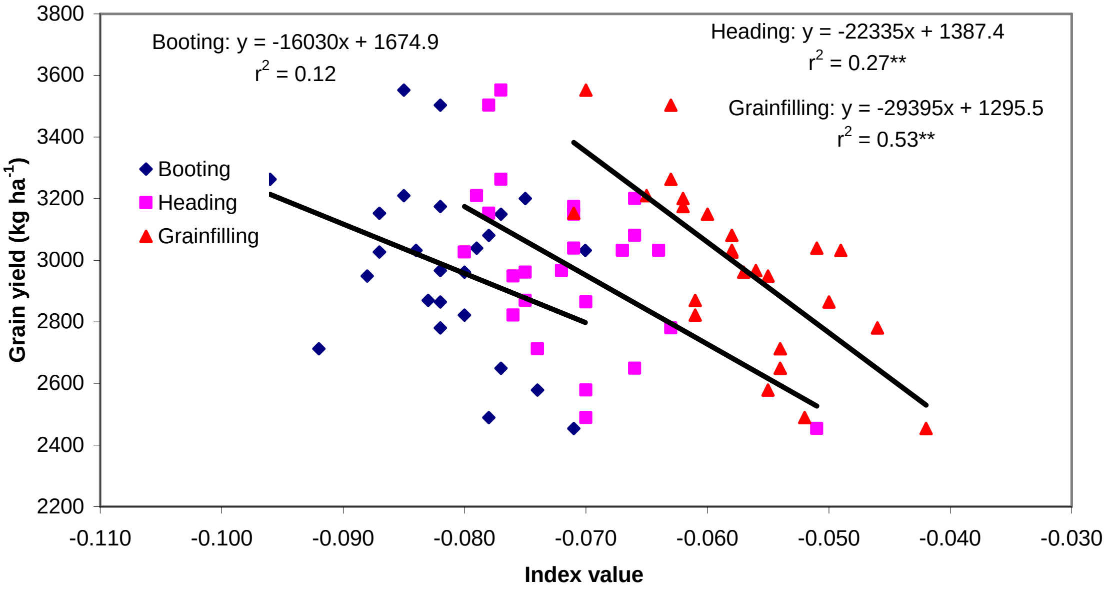


Fig 3. Functional relationship between grain yield and normalized water index-3 (NWI-3) at three growth stages for two experiments. Top figure represents the relationship for Exp-1 and the bottom figure represents Exp-2. $^*p<0.05$, $^{**}p<0.01$.

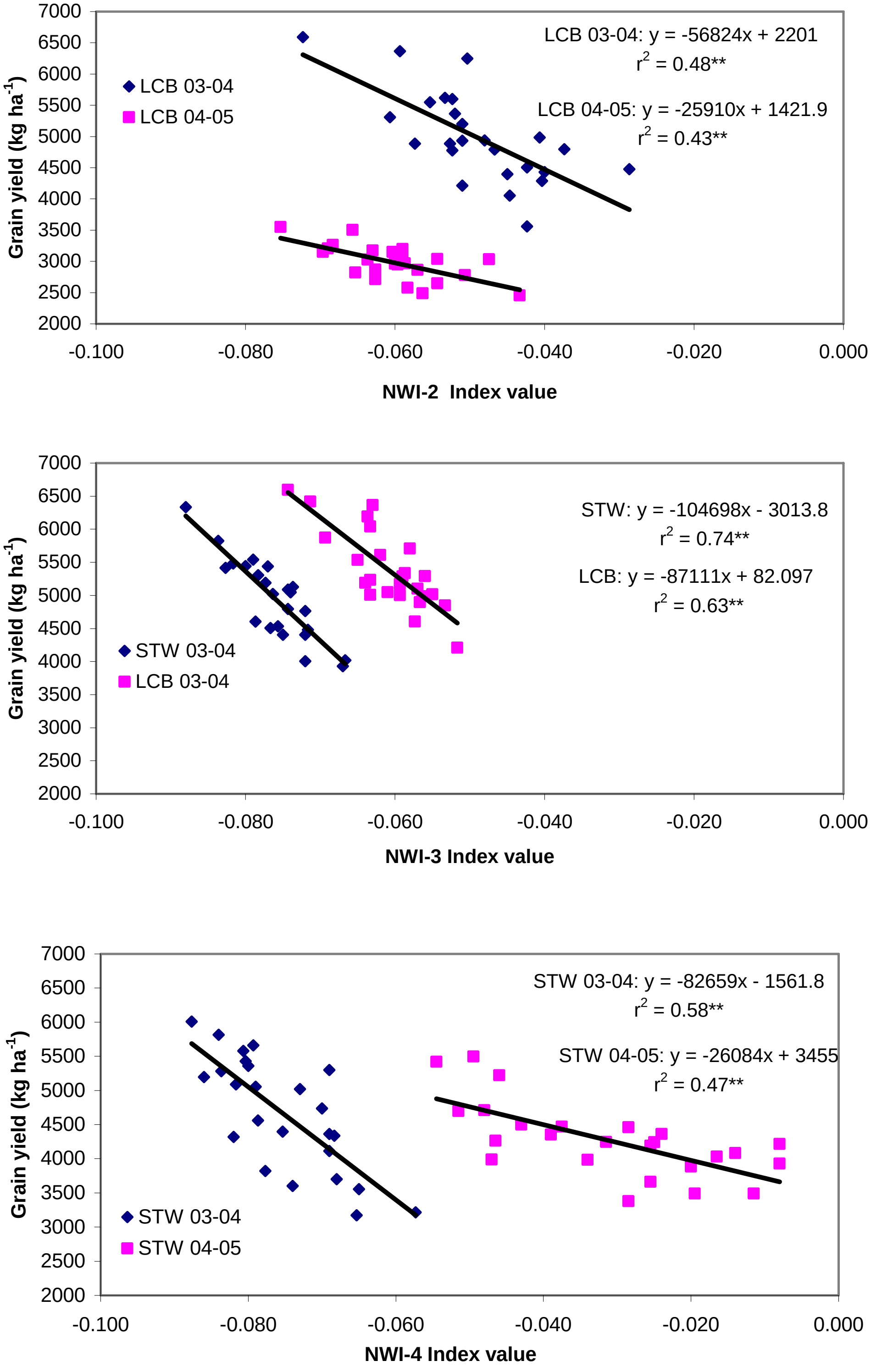


Fig 4. Functional relationship between grain yield and normalized water index-2 (NWI-2), normalized water index-3 (NWI-3), and normalized water index-4 (NWI-4) (estimated as the mean of the three growth stages). Top figure represents Exp-2, middle figure represents Exp-1, and bottom figure represents Exp-3. STW and LCB denote Stillwater and Lake Carl Blackwell sites, respectively. *p<0.05, **p<0.01.

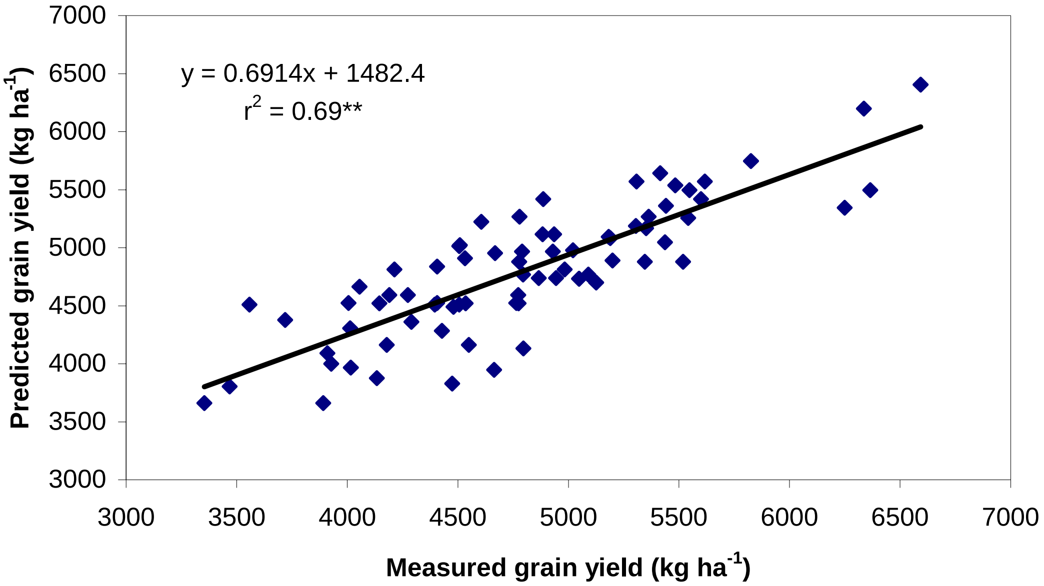


Fig 5. Relationship between measured grain yield and predicted grain yield based on the linear equation using normalized water index-3 (NWI-3) as the predictor, estimated using the mean values of three growth stages for all three experiments across two locations/years. $^{**}p<0.01$.

CHAPTER III

Spectral Reflectance Indices as a Selection Tool for Improving Winter Wheat Biomass

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Abbreviations

GNDVI, green normalized difference vegetation index

NWI, normalized water indices

NWI-1, normalized water index-1

NWI-2, normalized water index-2

NWI-3, normalized water index-3

NWI-4, normalized water index-4

RARSa, ratio analysis of reflectance spectra - chlorophyll a

RARSb, ratio analysis of reflectance spectra - chlorophyll b

RARSc, ratio analysis of reflectance spectra - carotenoids

RNDVI, red normalized difference vegetation index

SR, simple ratio

SRI, spectral reflectance indices

WI, water index

Abstract

Increased dry matter production is one of the important criteria for future grain yield improvement in wheat. The potential of using spectral reflectance indices (SRI) has been evaluated to assess dry matter production in different winter wheat genetic backgrounds. Three experiments were conducted for two years at Oklahoma State University research farms. The first experiment was composed of 25 winter wheat cultivars developed by different breeding programs in the U.S. Great Plains. The other experiments contained two sets of 25 $F_{4:6}$ and $F_{4:7}$ recombinant inbred lines from two crosses. Three groups of SRI (vegetation based, pigment based, and water based) were tested for their discriminating capacity for biomass production at three distinct growth stages (booting, heading, and grainfilling). Among the vegetation based indices, simple ratio gave the best consistency for predicting biomass. Pigment based indices lacked predictability for biomass production among the genotypes. Water index and the normalized water indices gave the highest genetic correlations and linear predictability for biomass production, and these relationships were stronger at the reproductive growth stages. Our study clearly demonstrated the potential of using SRI, especially the water based indices, as a breeding tool for identifying genotypes with enhanced biomass production in a winter wheat breeding program.

Introduction

Higher biomass production has been recognized as one of the important characteristics for higher grain yield production of wheat. So far, significant improvement of wheat yield has been achieved by conventional breeding approaches. One of the main reasons for yield improvement has been attributed to the higher partitioning of biomass towards the grain, a term commonly known as harvest index (Austin et al., 1980; Cox et al., 1998). The increase in harvest index has been achieved without a substantial change in total biomass production in wheat (Siddique et al., 1989; Slafer and Andrade, 1989). It has been reported that harvest index in wheat has a theoretical upper limit that has already been achieved (Austin et al., 1980). Several authors have indicated that further yield improvement in wheat can only be accomplished through increased biomass production while maintaining the harvest index (Reynolds et al., 1999; Slafer and Andrade, 1991; Richards, 2000). A positive association between higher biomass production and higher yield in wheat has been reported in several studies (Sayre et al., 1997; Reynolds et al., 2005). As a consequence, selection of genotypes with higher biomass is an important objective in wheat breeding programs concerned with yield improvement.

Biomass assessment on a field basis is not only monotonous work, but it is also considered as a slow and inaccurate procedure that often produces errors in detecting genotypic differences (Whan et al., 1991). Moreover, by using destructive sampling, there is a loss of plot area for other important observations necessary for the evaluation of the genotypes. Such sampling techniques cannot be utilized in a breeding program having a large number of test entries that are being evaluated for a number of traits. Several researchers have found canopy spectral reflectance as a good determinant for biomass production potential (Tucker, 1979; Smith et al., 1993; Peñuelas et al., 1997; Babar et al., 2006).

Spectral reflectance is based on the principle that specific traits of a plant are associated with the absorption of specific wavebands of the spectrum, which are commonly associated with the overall area of the leaves and other photosynthetic organs, pigment composition, and physiological properties of the plants (Reynolds et al., 1999). Chlorophylls, xanthophylls, and carotenoids absorb light in the visible part of the spectrum, but they are insensitive to the near infrared parts of the light spectrum (Araus, 1996). The magnitude of the near infrared reflectance is governed by the scattering of light by plant tissue at different level in the crop canopy (Knipling, 1970). Therefore, measuring the spectral reflectance from a plant canopy can provide useful information about a large number of parameters such as green biomass, amount of photosynthetic area, amount of light absorption by the canopy at the photosynthetic active radiation level, and photosynthetic capacity of the plant canopy (Reynolds et al., 2001).

Spectral reflectance indices (SRI) are developed by combining several useful wavebands of the spectrum into a simple ratio form or as differences (Araus, 1996). The first group of SRI was developed on the basis of a simple ratio between near infrared and visible wavebands. The first of these is widely known as simple ratio (SR), and another one is commonly known as normalized difference vegetation index (NDVI), which uses the difference of the near infrared and visible bands in a normalized fashion. The association of these two indices with crop variables has been studied by different researchers and it has been reported that these indices can quantify total dry matter, leaf area index, green area index, pigment composition, and other useful physiological parameters related to the crop canopy (Tucker, 1979; Weigand et al., 1991; Elliot and Regan, 1993; Bellairs et al., 1996). Studies with these SRI have been done on a small scale basis with only a few genotypes, or at early growth stages for differentiating genotypes for biomass production. Babar et al. (2006) reported that the NDVI and SR were not predictable for biomass production at the later growth stages while working with a large number of irrigated spring wheat genotypes under northwestern Mexican conditions.

Several pigment based indices have been developed by Chappelle et al. (1992) for the assessment of chlorophylls and carotenoids and these are commonly known as ratio analysis of reflectance spectra (RARS). They developed RARSa, RARSb, and RARSc for the estimation of chlorophyll-a, chlorophyll-b, and carotenoids at the crop canopy level, respectively. Usefulness of these pigment based indices has never been tested at the field level for differentiating winter wheat genotypes for biomass production. However, Babar et al. (2006) reported that these pigment based indices were capable of predicting spring wheat genotypes for biomass production under irrigated conditions.

Another very important region of the spectrum is related to the water content of the canopy. The water index (WI) was developed using the minor water absorption band 970 nm and the near infrared waveband 900 nm in a ratio form. Water index has been used to assess the relative water content, leaf water potential, stomatal conductance, and canopy temperature (Peñuelas et al., 1993), as well as in assessing different stress effects, such as salinity resistance in barley (Peñuelas et al., 1997). Very recently, Babar et al. (2006) reported the usefulness of water band indices for predicting spring wheat genotypes for biomass production under irrigated conditions. Until now, no information is available about differentiating winter wheat genotypes for biomass production under Great Plains rainfed environments by using SRI.

There is a considerable amount of information available about the different SRI for predicting different growth parameters of wheat. Bellairs et al. (1996) emphasized the influence of the crop growth stage or canopy structure on the relationship between SRI and crop growth parameters. However, a comprehensive study is required to establish the usefulness of SRI in assessing winter wheat biomass production potential with different genetic backgrounds, in different crop growth stages, and across different environments. Studies are needed to establish the usefulness of SRI in estimating total biomass production in winter wheat genotypes. Therefore, the objectives of the study were to (i) test the predictability of the SRI for biomass production potential of diverse winter wheat cultivars and advanced lines; (ii) develop new SRI that can be more predictable for biomass production in winter wheat compared to the commonly used indices; and (iii) identify the optimum growth stage(s) for the measurement of the SRI.

Materials and Methods

The experiments were conducted during the 2003-2004 and 2004-2005 wheat growing seasons at Oklahoma State University research farms at Stillwater, OK and at Lake Carl Blackwell, 25 km west of Stillwater. The soil types at these sites are silty clay loam with an average pH of 6.2-6.5 and fine sandy loam with an average pH of 6.7- 6.9, respectively. The experiments were conducted under rainfed conditions and planted at a seeding rate of 70 kg ha^{-1}. Individual plot size for each experiment was 3.04 m long × 1.24 m wide. At both the sites, 90 kg ha^{-1} pre-plant nitrogen was applied. Folicur (25 % tebuconazole) was applied at the late tillering and booting stages to control foliar diseases and Cygon (dimethoate) was applied at the booting stage to control aphids.

Experimental materials

Three experiments, each containing 25 winter wheat genotypes developed by different breeding programs of the southern and central Great Plains were used.

Experiment 1: This experiment contained commercial winter wheat cultivars from the southern and central Great Plains and was planted at the Lake Carl Blackwell site in 2003-2004 and at Stillwater in 2004-2005. The cultivars were planted in a 5 × 5 alpha lattice design with two replications.

Experiment 2: This experiment contained 25 $F_{4:6}$ and $F_{4:7}$ lines from the cross TX95V5905/Jagger and was planted at Lake Carl Blackwell in 2003-2004 (F_6) and at Stillwater in 2004-2005 (F_7). These experimental lines were developed by the winter wheat breeding program of Kansas State University. This experiment was also planted in a 5 × 5 alpha lattice design with two replications.

Experiment 3: This experiment was composed of 25 $F_{4:6}$ and $F_{4:7}$ lines from the cross TX93V4927/G1878 and was planted at the Stillwater site in 2003-2004 (F_6) and 2004-2005 (F_7). These experimental lines were developed by the winter wheat breeding program of AgriPro-Coker. This experiment was established in a 5 × 5 alpha lattice design with two replications.

Canopy spectral reflectance measurements

A portable field spectro-radiometer (FieldSpec UV/VNIR, Analytical Spectral Device, Boulder, CO) was used to measure the spectral reflectance from the wheat canopy. The field of view of the instrument was set at 25^0. The spectro-radiometer has the capability of collecting the reflectance from 350-1050 nm of the spectrum, thus covering the entire visible and part of the near infrared portions of the light spectrum. The machine gave reflectance measurements at 1.4 nm intervals and 512 continuous data points from each reading. Reflectance measurements were taken during the middle of the day under sunny conditions. The optic fiber of the sensor was placed 50 cm above the plant canopy. The incident reflectance was calibrated against the reflectance from a white reflectance plate coated with $BaSO_4$. Four readings were collected from each plot from four randomly chosen places and the mean of the four readings was used for the estimation of reflectance indices. Reflectance measurements were taken at booting (Zadoks stage 45), heading (Zadoks stage 59), and grainfilling (Zadoks stage 75) in all three experiments in both years (Zadoks et al., 1974).

Several previously reported indices and two newly developed indices were used in the study. The indices are grouped into three categories: vegetation based indices, pigment based indices, and water based indices. The formula for the indices calculation used in the study are provided in Table 1.

Biomass estimation

From each plot, all plants from an area 0.5 m long × 0.96 m wide (total 0.48 m^2) were clipped with scissors from the soil level. The outer two rows were excluded from each plot to avoid border effects. Biomass harvesting coincided with the reflectance measurements from the plots. Biomass was harvested at booting (Zadoks stage 45), heading (Zadoks stage 59), and grainfilling (Zadoks stage 75) for all three experiments in both years. After clipping, total fresh biomass weight was measured from each plot and a representative sample of the fresh biomass was dried in the oven at 65^0C for 72 hours. The oven-dried samples were weighed and converted into total dry weight of each plot and finally calculated as gm m^{-2}.

Statistical analysis

The alpha lattice analysis for biomass and the SRI were carried out by the SAS MIXED procedure (SAS Institute, 2001). Phenotypic and genetic correlation coefficients were estimated to reveal the association between the SRI and biomass. The genetic correlation coefficients between SRI and dry biomass were calculated by the formula (Falconer, 1989),

$r_g = (\text{Cov}_{X1X2}) / \sqrt{(\text{Var}_{X1} \times \text{Var}_{X2})}$, where Cov_{X1X2} is the genetic covariance of the two variables and Var_{X1} and Var_{X2} were the genetic variances of the variables, respectively, and were estimated across growth stages. The correlation coefficients were estimated with SAS software (SAS Institute, 2001). Regression analysis was performed between the SRI and dry matter production with SAS software (SAS Institute, 2001).

Results

Genotype performance

Variability parameters (mean ± SE) for biomass production at three growth stages, namely booting, heading, and grainfilling, for three experiments in two different years are presented in Table 2. In general, genotypic variation for fresh and dry biomass production for the genotypes in the different experiments were significant with a few exceptions.

The means (± SE) of the different vegetation and pigment based indices, and means (± SE) of water based indices for different experiments at three growth stages in two years are presented in Table 3 and Table 4, respectively. In most cases, genotypic variations for all three groups of indices were significant with some exceptions.

Growth stages affect

Mean dry matter production increased with growth stage from booting to grainfilling (Table 2). The differences in dry matter production were comparatively higher from booting to heading compared with heading to grainfilling (Table 2). The vegetation based indices (RNDVI, GNDVI, and SR) showed a gradual decrease in the index value from the booting to the grainfilling stage (Table 3, Fig 1). Among the pigment based indices, RARSa gradually increased from the booting to the grainfilling stage, whereas RARSb and RARSc decreased as the growth stage progressed (Table 3, Fig 1). The water based indices (WI and NWI) decreased from the booting to the heading stage, and then increased at the grainfilling stage (Table 4, Fig 1). In Exp-1 and Exp-2, there were some positive index estimates for NWI-2 for some genotypes, and hence, a very high index value was obtained when all the genotypic values were averaged for each experiment (Table 4). Dry matter production in Exp-1 at the different growth stages along with estimates of the SRI in 2004-2005 are plotted in Fig 1. This figure indicates the increase in biomass production with advancement in growth stage. However, the vegetation based indices gradually decreased with advanced growth stage, while the water based indices had the lowest values at heading, but increased significantly at grainfilling. Chlorophyll-a index (RARSa) gradually increased over time, and RARSb and RARSc followed patterns similar to the vegetation based indices.

Association of vegetation and pigment based indices with dry matter production

Significant phenotypic correlations between dry biomass and the vegetation and pigment indices were observed at the heading and grainfilling stages, with some exceptions at the booting stage (Table 5). All indices were associated positively with dry matter production except for RARSa, which gave negative correlations with dry matter (Table 5). Genetic correlation coefficients between the SRI and dry matter were significant in all instances, except for RARSb (Exp-2 and Exp-3 in 2003-2004). The associations between the SRI and dry matter were more consistent at the heading and grainfilling stages than at the booting stage (Table 5). Coefficients of determination (r^2) between SRI and dry matter production are presented in Fig 2. Simple ratio (SR) was more consistent and predictive over different experiments compared to the other vegetation and pigment based indices (Fig 2). In general, the vegetation indices were more predictive for biomass than the pigment based indices (Fig 2).

Water based indices and biomass

Significant phenotypic and genetic correlation coefficients were observed between the water status based indices and dry matter for all three experiments at three growth stages in both years (Table 6). The coefficients were negative and consistent irrespective of growth stage. The genetic correlations between the indices and dry matter were highly significant (Table 6). The coefficients of determination (r^2) between the indices and dry matter production are presented in Fig 2. The indices were less predictive for Exp-2 compared to the other two experiments (Fig 2). The trends of the coefficients were similar for the different water status based indices, thus making them essentially indistinguishable from each other (Fig 2). The water based indices were more predictable for biomass production among the genotypes at both the phenotypic and genetic levels compared to the vegetation and pigment based indices (Table 5, Table 6, and Fig 2).

Functional relationship between SRI and biomass

The functional relationships of the pigment index (RARSc), the vegetation index (RNDVI), and the water based index (NWI-4) with dry matter production at individual growth stages in different experiments were established through regression analysis (Fig 3). The relationship was tested for all other indices (data not shown). All the relationships were linear. The water based index showed stronger relationship compared to the vegetation and pigment based indices. The relationship between the predicted biomass and actual biomass using the linear regression equation based on NWI-4 for the three experiments individually and in combination are shown in Fig 4.The relationship revealed a highly significant predictability of NWI-4 for biomass production (Fig 4).

Discussion

Genotype performance

Significant genotypic variation for biomass production and spectral behavior at different growth stages (booting, heading, and the grainfilling) confirms the existence of sufficient genetic variation for these traits among the genotypes in this study. Previously, significant genetic variation for biomass production at different growth stages had been reported in irrigated spring wheat (Babar et al., 2006) and in durum wheat (Aparicio et al., 2002). Until now, no comprehensive studies involving winter wheat genotypes or environments have been reported that assess the genetic variability among genotypes using canopy spectral reflectance. Genetic variation for SRI was reported in irrigated spring wheat (Babar et al., 2006), in irrigated and rainfed spring wheat (Gutiérrez-Rodríguez et al., 2004), and in rainfed and irrigated durum wheat (Aparicio et al., 2000; Royo et al., 2003). In our study, we tested three groups of materials, of which the first group contained winter wheat cultivars from the U.S. Great Plains and two other groups contained recombinant inbred populations from two different crosses. In Exp-2, there were some instances of non-significant variations for SRI. We believe this was caused by the heterogeneity of some plots due to uneven germination and varying plant populations. Due to increased soil exposure in some plots affected by irregular germination, higher estimates for NWI-2 were obtained, and as a result, the average index value for NWI-2 over the genotypes in Exp-1 and Exp-2 was high. Elliot and Regan (1993) and Babar et al. (2006) previously reported that incomplete canopy coverage can influence the reflectance of the near infrared wavelengths. In general, sufficient genetic variations for

SRI and biomass signify the usefulness of SRI in differentiating winter wheat genotypes for total biomass production.

Growth stages affect

We observed a continuous increase in dry matter accumulation from booting to grainfilling in all three experiments in both years. Our studies were conducted under rainfed conditions, although total water received by the experiments throughout the crop growth cycle (October – May) was high (450 and 517 mm, respectively in the two years). As a consequence, dry matter production continued to increase. Several researchers reported similar increases in dry matter production when water was non-limiting (Babar et al., 2006; Aparicio et al., 2000, 2002).

The highest values for the vegetation based indices (RNDVI, GNDVI, and SR) were observed at the booting stage in all three experiments in both years, and this can be attributed to the occurrence of the highest green leaf area and leaf area index at this growth stage. The gradual decrease in the value of these indices from booting to grainfilling stages was due to a loss of photosynthetic area as the older leaf tissues started losing their greenness and becoming chlorotic (Aparicio et al., 2000; Babar et al., 2006). These changes resulted in an increase of visible reflectance and a decrease in near infrared reflectance, the two regions of the light spectrum used to calculate these vegetation indices.

The increase in RARSa with the progression of the growth cycle from booting to grainfilling was due to the gradual decrease in chlorophyll content as the plants approached the later growth stages (heading and grainfilling). The increased reflectance of the chlorophyll-a band (675 nm) increased the RARSa value from the booting to the grainfilling stage. Reduced values for the other two pigment indices (RARSb and RARSc) were due to the variation in leaf pigment concentration at later growth stages compared to the early vegetative growth stage (Calderini et al., 1997; Babar et al., 2006).

The third group of indices (WI and NWI) included the minor water absorption band (970 nm), and other near infrared bands as reference bands (850, 880, 900, and 920 nm). Bull (1991) reported that sensitivity of the water absorption band is a combination of two factors; the extent of light penetration into canopy and the canopy water content. Royo et al. (2003) reported the influence of crop biomass on the behavior of WI. The values for these water based indices tended to be lowest at the heading stage, which indicates the highest amount of water content in the canopy at heading. It has been reported that reflectance at the near infrared wavebands is higher for vertical canopy compared to a horizontal one (Jackson and Erza, 1985). The heading stage represents changes in the canopy architecture from horizontal to more vertical due to the extended flag leaves. Penetration of the near infrared wavebands is higher during this time period and that causes lower reflectance of the near infrared wavebands. We have observed that the canopy contained the highest amount of water at heading (data not shown). Higher light penetration and higher canopy water content at the heading stage resulted in the lower values for the water based indices at the heading stage.

Association of vegetation based and pigment based indices with dry matter

The vegetation based indices gave positive correlations with dry matter at all three growth stages. Dry matter production at heading and grainfilling was significantly correlated with the vegetation indices in the different experiments. The biomass at booting was poorly correlated with these indices. These observations can be attributed to the relative uniformity of total crop vegetation at booting for the different genotypes. On the other hand, combining the index values for the growth stages and mean dry matter over the growth stages gave significant phenotypic and genetic correlations for all the vegetation based indices, although the increase in the correlation coefficients compared to any individual growth stage was not observed. A higher association of the RNDVI, GNDVI, and SR at the heading and grainfilling stages with dry biomass was previously reported in irrigated durum wheat (Aparicio et al., 2000, 2002), in irrigated spring wheat (Babar et al., 2006), and in durum wheat for grain yield (Royo et al., 2003).

The chlorophyll-a based index (RARSa) gave significant negative correlations at the grainfilling stage with dry matter. The mean indices and mean dry matter production over the booting, heading, and the grainfilling stages also gave significant phenotypic and genetic correlations. Elliot and Regan (1983) reported a strong negative association between the visible wavebands and dry matter production in wheat. This higher association at grainfilling is due to the variation of the genotypes for their green pigment content as they approach the reproductive phase. The carotenoids index (RARSc) gave significant correlations at the phenotypic and genetic levels, whereas, the RARSb was poorly associated with dry matter production. The higher association of the vegetation based indices over the pigment based indices (RARSa and RARSb) for dry matter production was due to the incorporation of the near infrared bands for the estimation of the vegetation indices (see Table 1 for index calculation). As heading and grainfilling stages were more predictive, we recommend measuring SRI at these two growth stages for prediction of the genotypes biomass production potential.

Association of water based indices and dry matter

Significant phenotypic and genetic correlations between the water based indices (WI and NWI) and dry matter production at the different growth stages indicate that these indices offer an alternative method to classify genotypes for biomass production. The lower association between the water based indices and biomass production in Exp-2 compared to the other experiments is believed to be the effect of varying plant populations due to uneven germination in some plots. The higher phenotypic correlations at the grainfilling stage in a number of occasions signify the better predictability of these indices for assessing biomass production at grainfilling. On the other hand, mean values of the indices and biomass over booting, heading, and the grainfilling stages gave higher correlations at both the phenotypic and genetic levels, thus confirming a strong genetic basis for the relationship. The work of Babar et al. (2006) in irrigated spring wheat fully supports our findings. Greater water retention by the canopy translates to lower values of these indices; hence, we obtained negative associations between these indices and biomass production. The association of these indices with biomass production was high at the later growth stages; therefore, we suggest measuring canopy spectral reflectance at the heading and grainfilling growth stages for assessment of biomass. Since, total biomass at the reproductive growth stage is usually indicative of grain yield potential for a given genotype, these indices may also serve as an effective and efficient selection tool for higher grain yield in a wheat breeding program.

Functional relationship between biomass and SRI

Significant linear relationships between different groups of indices with dry matter accumulation in different experiments revealed that the indices maintained their predictability at higher levels of biomass. This observation has a significant implication in a crop breeding program. Breeders can use this tool as a selection criterion regardless of the level of biomass production of the breeding materials. Similar linear relationships were also reported in irrigated spring wheat (Babar et al., 2006). The water based indices showed better predictability for biomass production over the vegetation and pigment based indices, and among the water based indices, NWI-4 appeared to be the most promising. The closer estimation of biomass production using the linear regression equation of NWI-4 with biomass provides ample evidence of the power of this index in assessing dry matter production and its suitability as a selection tool in a wheat breeding program.

Conclusions

The ability to predict winter wheat genotypes for biomass production using SRI in different genetic backgrounds has been shown in this study. The different genetic populations signify the strong genetic basis for the relationship between the SRI and biomass. The indices were related to biomass linearly, which bears a significant implication in a selection program. The water based indices (WI and NWI) had the strongest relationship with biomass. The relationship between the water based indices and biomass were equally significant at the different growth stages, with more consistency and predictability at the generative growth stages (heading and grainfilling). As the total dry matter at the reproductive growth stage appears to be one of the most important determining factors for increased grain yield, we recommend estimating the water based indices at the reproductive growth stages. Our study clearly demonstrated the usefulness of water based indices to incorporate in a winter wheat breeding program for predicting biomass production potential among the genotypes.

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Tables

Table 1. Description of the spectral reflectance indices (SRI) used in these studies.

SRI	Formula†	References
Vegetation based indices		
Red normalized difference vegetation index (RNDVI)	$(R_{780}-R_{670})/(R_{780}+R_{670})$	Raun et al., 2001
Green normalized difference vegetation index (GNDVI)	$(R_{780}-R_{550})/(R_{780}+R_{550})$	Aparicio et al., 2000
Simple Ratio (SR)	(R_{900}/R_{680})	Gitelson et al., 1996
Pigment based indices		
Ratio analysis of reflectance spectra (RARSa)	R_{675}/R_{700}	Chappelle et al., 1992
Ratio analysis of reflectance spectra (RARSb)	$R_{675}/(R_{650}R_{700})$	Chappelle et al., 1992
Ratio analysis of reflectance spectra (RARSc)	R_{760}/R_{500}	Chappelle et al., 1992
Water based indices		
Water index (WI)	(R_{970}/R_{900})	Peñuelas et al., 1993
Normalized water index (NWI-1)	$(R_{970}-R_{900})/(R_{970}+R_{900})$	Babar et al., 2006
Normalized water index (NWI-2)	$(R_{970}-R_{850})/(R_{970}+R_{850})$	Babar et al., 2006
Normalized water index (NWI-3)	$(R_{970}-R_{920})/(R_{970}+R_{920})$	Newly developed
Normalized water index (NWI-4)	$(R_{970}-R_{880})/(R_{970}+R_{880})$	Newly developed

† R and the subscript indicate the light reflectance at the specific wavebands (in nm).

Table 2. Variability parameters (mean ± SE) related to biomass production at three distinct growth stages for the three experiments in two years.

Experiment	Year	Growth stages	Fresh weight (g m^{-2})	Dry weight (g m^{-2})
Exp-1	2003-2004	Booting	3293 ± 332*	627 ± 51*
		Heading	3647 ± 338*	1409 ± 116**
		Grainfilling	3443 ± 357**	1586 ± 122**
	2004-2005	Booting	3648 ± 441	806 ± 94
		Heading	3707 ± 412*	1069 ± 102*
		Grainfilling	3327 ± 459*	1509 ± 175*
Exp-2	2003-2004	Booting	3027 ± 356*	562 ± 44**
		Heading	4046 ± 364**	1207 ± 86**
		Grainfilling	3877 ± 290**	1690 ± 97**
	2004-2005	Booting	3822 ± 429*	822 ± 98
		Heading	4196 ± 403	1213 ± 88*
		Grainfilling	3704 ± 472	1682 ± 125*
Exp-3	2003-2004	Booting	4677 ± 345**	750 ± 58**
		Heading	6180 ± 518**	1720 ± 133**
		Grainfilling	4755 ± 610*	2066 ± 184*
	2004-2005	Booting	3328 ± 375**	764 ± 86*
		Heading	3774 ± 481*	1191 ± 154*
		Grainfilling	3324 ± 379	1484 ± 99*

*$p<0.05$, **$p<0.01$

Table 3. Mean estimates (± SE) of vegetation and pigment based spectral reflectance indices at three growth stages (GS) for three experiments in two years.

Experiment	Year	GS	Vegetation based indices†			Pigment based indices‡		
			RNDVI	GNDVI	SR	RARSa	RARSb	RARSc
Exp-1	2003-2004	Booting	0.919 ± 0.014	0.790 ± 0.013**	25.18 ± 1.80**	0.337 ± 0.016**	13.92 ± 0.754**	21.97 ± 1.41**
		Heading	0.845 ± 0.034**	0.726 ± 0.028**	13.07 ± 3.14*	0.402 ± 0.03*	12.52 ± 0.986**	16.53 ± 2.48
		Grainfilling	0.620 ± 0.047**	0.615 ± 0.023**	4.57 ± 0.95**	0.588 ± 0.032	8.82 ± 0.882	8.64 ± 1.23**
	2004-2005	Booting	0.942 ± 0.006**	0.851 ± 0.009**	33.87 ± 3.06**	0.339 ± 0.009**	22.04 ± 2.04**	30.49 ± 2.14**
		Heading	0.926 ± 0.011*	0.842 ± 0.012**	26.53 ± 3.08**	0.391 ± 0.018**	20.05 ± 1.97**	26.09 ± 2.12**
		Grainfilling	0.822 ± 0.043	0.740 ± 0.032*	11.31 ± 2.04**	0.519 ± 0.032	11.82 ± 1.42**	13.64 ± 1.85**
Exp-2	2003-2004	Booting	0.920 ± 0.015	0.803 ± 0.016	26.26 ± 3.92	0.342 ± 0.023	14.43 ± 1.42	23.5± 2.38
		Heading	0.859 ± 0.030	0.752 ± 0.023*	14.64 ± 3.06	0.402 ± 0.032	13.53 ± 1.33*	18.48 ± 2.56
		Grainfilling	0.651 ± 0.039**	0.649 ± 0.022	4.89 ± 0.768**	0.578 ± 0.026**	10.04 ± 0.715**	9.58 ± 1.15*
	2004-2005	Booting	0.952 ± 0.005**	0.873 ± 0.006**	36.64 ± 3.62**	0.338 ± 0.014*	27.81 ± 1.49**	37.18 ± 2.40**
		Heading	0.930 ± 0.011	0.850 ± 0.012	28.13 ± 3.70	0.388 ± 0.020*	20.51 ± 1.51*	27.80 ± 2.48*
		Grainfilling	0.722 ± 0.051	0.670 ± 0.027	6.56 ± 1.33	0.541 ± 0.041	8.71 ± 0.763	10.31 ± 1.43
Exp-3	2003-2004	Booting	0.933 ± 0.003**	0.820 ± 0.007**	29.11 ± 1.47**	0.352 ± 0.005**	13.50 ± 0.944**	23.51 ± 0.998**
		Heading	0.904 ± 0.010**	0.805 ± 0.013**	19.97 ± 1.92**	0.404 ± 0.012**	14.28 ± 1.18*	21.42 ± 1.40**
		Grainfilling	0.728 ± 0.044**	0.670 ± 0.026**	6.82 ± 1.23**	0.559 ± 0.032**	10.27 ± 1.01**	10.18 ± 1.22
	2004-2005	Booting	0.949 ± 0.005**	0.855 ± 0.007**	38.19 ± 3.72**	0.313 ± 0.012**	21.30 ± 1.76**	33.17 ± 2.09**
		Heading	0.905 ± 0.020	0.819 ± 0.017*	21.23 ± 3.41*	0.432 ± 0.030*	20.60 ± 2.08	21.03 ± 2.34*
		Grainfilling	0.721 ± 0.053*	0.673 ± 0.026**	6.80 ± 1.53*	0.563 ± 0.044**	9.50 ± 0.968*	10.08 ± 1.40**

*p<0.05, **p<0.01

† RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio.

‡ RARSa, ratio analysis of reflectance spectra - chlorophyll a; RARSb, ratio analysis of reflectance spectra - chlorophyll b; RARSc, ratio analysis of reflectance spectra - carotenoids.

Table 4. Mean estimates (± SE) of water based spectral reflectance indices at three growth stages (GS) for three experiments in two years.

Experiment	Year	GS	Water based indices†				
			WI	NWI-1	NWI-2	NWI-3	NWI-4
Exp-1	2003-2004	Booting	0.847 ± 0.007**	-0.083 ± 0.004**	-0.083 ± 0.004**	-0.081 ± 0.004**	-0.085 ± 0.005**
		Heading	0.878 ± 0.016*	-0.065 ± 0.009	-0.051 ± 0.011*	-0.065 ± 0.008*	-0.062 ± 0.01*
		Grainfilling	0.943 ± 0.008**	-0.030 ± 0.006**	-0.001 ± 0.008**	-0.036 ± 0.005**	-0.023 ± 0.007**
	2004-2005	Booting	0.846 ± 0.008**	-0.083 ± 0.004**	-0.079 ± 0.006**	-0.081 ± 0.004**	-0.083 ±0.006**
		Heading	0.832 ± 0.011**	-0.092 ± 0.006**	-0.082 ± 0.008**	-0.085 ± 0.006**	-0.085 ± 0.007**
		Grainfilling	0.873 ± 0.012	-0.068 ± 0.007**	-0.057 ± 0.009**	-0.068 ± 0.006**	-0.068 ± 0.008**
Exp-2	2003-2004	Booting	0.846 ± 0.012	-0.084 ± 0.007	-0.085 ± 0.008	-0.082 ± 0.007	-0.086 ± 0.008
		Heading	0.865 ± 0.014	-0.073 ± 0.008	-0.061 ± 0.011*	-0.073 ± 0.007	-0.072 ± 0.009
		Grainfilling	0.932 ± 0.011**	-0.035 ± 0.006**	-0.001 ± 0.007**	-0.042 ± 0.005**	-0.024 ± 0.007**
	2004-2005	Booting	0.816 ± 0.007**	-0.101 ± 0.004**	-0.097 ± 0.004**	-0.098 ± 0.004**	-0.103 ± 0.004**
		Heading	0.808 ± 0.013	-0.106 ± 0.008*	-0.099 ± 0.009	-0.100 ± 0.007*	-0.102 ± 0.008*
		Grainfilling	0.887 ± 0.020	-0.060 ± 0.011	-0.043 ± 0.014	-0.062 ± 0.010	-0.058 ± 0.012
Exp-3	2003-2004	Booting	0.836 ± 0.004**	-0.089 ± 0.003**	-0.090 ± 0.003**	-0.088 ± 0.002**	-0.093 ± 0.003**
		Heading	0.833 ± 0.007**	-0.090 ± 0.004**	-0.081 ± 0.005**	-0.088 ± 0.004**	-0.087 ± 0.005**
		Grainfilling	0.907 ± 0.013**	-0.049 ± 0.007**	-0.026 ± 0.009**	-0.052 ± 0.006**	-0.043 ± 0.008**
	2004-2005	Booting	0.848 ± 0.008**	-0.082 ± 0.005**	-0.077 ± 0.005**	-0.077 ± 0.004**	-0.079 ± 0.005**
		Heading	0.833 ± 0.015**	-0.091 ± 0.009*	-0.080 ± 0.010*	-0.087 ± 0.008*	-0.085 ± 0.010*
		Grainfilling	0.898 ± 0.014*	-0.054 ± 0.008*	-0.037 ± 0.011*	-0.055 ± 0.007*	-0.052 ± 0.009*

*$p<0.05$, **$p<0.01$

† WI, water index; NWI-1, normalized water index-1; NWI-2, normalized water index-2; NWI-3, normalized water index-3; NWI-4, normalized water index-4.

Table 5. Phenotypic (r_p) and genetic (r_g) correlation coefficients between dry biomass production and vegetation and pigment based indices at three growth stages (GS) for three experiments in two years.

Indices†	Correlations	GS	Exp-1		Exp-2		Exp-3	
Vegetation indices			2003-2004	2004-2005	2003-2004	2004-2005	2003-2004	2004-2005
RNDVI	r_p	Booting	0.619**	0.695**	0.439*	0.613**	0.350	0.580**
		Heading	0.567**	0.581**	0.540**	0.355	0.649**	0.632**
		Grainfilling	0.548**	0.515**	0.603**	0.613**	0.447*	0.613**
		Mean‡	0.539**	0.739**	0.561**	0.617**	0.535**	0.562**
	r_g	Mean§	0.545**	0.601**	0.588**	0.628**	0.635**	0.451*
GNDVI	r_p	Booting	0.498*	0.656**	0.315	0.523**	0.379	0.479*
		Heading	0.555**	0.584**	0.544**	0.205	0.598**	0.638**
		Grainfilling	0.598**	0.485*	0.404*	0.459*	0.286	0.611**
		Mean‡	0.567**	0.737**	0.540**	0.678**	0.455*	0.629**
	r_g	Mean§	0.604**	0.525**	0.555**	0.623**	0.531**	0.647**
SR	r_p	Booting	0.645**	0.683**	0.457*	0.588**	0.311	0.600**
		Heading	0.615**	0.527**	0.642**	0.329	0.661**	0.595**
		Grainfilling	0.654**	0.503*	0.649**	0.601**	0.485*	0.566**
		Mean‡	0.728**	0.782**	0.705**	0.650**	0.521**	0.633**
	r_g	Mean§	0.638**	0.518**	0.715**	0.635**	0.603**	0.714**
Pigment indices								
RARSa	r_p	Booting	-0.596**	-0.403*	-0.405*	-0.379	0.058	-0.647**
		Heading	-0.531**	-0.114	-0.587**	-0.337	-0.395	-0.498*
		Grainfilling	-0.461*	-0.429*	-0.605**	-0.596**	-0.679**	-0.562**
		Mean‡	-0.523**	-0.458*	-0.542**	-0.516**	-0.484*	-0.531**
	r_g	Mean§	-0.502*	-0.590**	-0.636**	-0.616**	-0.533**	-0.741**
RARSb	r_p	Booting	0.361	0.407*	0.103	0.455*	0.209	0.472*
		Heading	0.279	0.478*	0.333	0.081	0.071	0.441*
		Grainfilling	0.371	0.357	-0.018	0.579**	-0.221	0.138
		Mean‡	0.409*	0.567**	0.310	0.483*	-0.076	0.384
	r_g	Mean§	0.616**	0.730**	0.115	0.616**	-0.042	0.441*
RARSc	r_p	Booting	0.649**	0.569**	0.383	0.530**	0.285	0.559**
		Heading	0.598**	0.369	0.677**	0.214	0.605**	0.551**
		Grainfilling	0.613**	0.366	0.582**	0.557**	0.442*	0.587**
		Mean‡	0.661**	0.636**	0.633**	0.586**	0.462*	0.574**
	r_g	Mean§	0.566**	0.782**	0.468*	0.658**	0.537**	0.658**

*$p<0.05$, **$p<0.01$

† RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio; RARSa, ratio analysis of reflectance spectra - chlorophyll a; RARSb, ratio analysis of reflectance spectra - chlorophyll b; RARSc, ratio analysis of reflectance spectra - carotenoids.

‡ Phenotypic correlation coefficients based on mean of three growth stages in individual year.

§ Genetic correlation coefficients based on mean of three growth stages in individual year.

Table 6. Phenotypic (r_p) and genetic (r_g) correlation coefficients between different water based indices and dry biomass production at three distinct growth stages (GS) for three experiments in two years.

Indices†	Correlations	GS	Exp-1		Exp-2		Exp-3	
			2003-2004	2004-2005	2003-2004	2004-2005	2003-2004	2004-2005
WI	r_p	Booting	-0.806**	-0.703**	-0.673**	-0.645**	-0.685**	-0.635**
		Heading	-0.710**	-0.660**	-0.650**	-0.599**	-0.652**	-0.682**
		Grainfilling	-0.583**	-0.653**	-0.689**	-0.734**	-0.769**	-0.654**
		Mean‡	-0.792**	-0.850**	-0.659**	-0.783**	-0.851**	-0.649**
	r_g	Mean§	-0.612**	-0.749**	-0.704**	-0.777**	-0.884**	-0.851**
NWI-1	r_p	Booting	-0.797**	-0.703**	-0.673**	-0.648**	-0.680**	-0.635**
		Heading	-0.719**	-0.657**	-0.661**	-0.590**	-0.652**	-0.676**
		Grainfilling	-0.588**	-0.643**	-0.687**	-0.739**	-0.765**	-0.653**
		Mean‡	-0.801**	-0.846**	-0.665**	-0.776**	-0.847**	-0.647**
	r_g	Mean§	-0.619**	-0.756**	-0.731**	-0.789**	-0.826**	-0.780**
NWI-2	r_p	Booting	-0.810**	-0.713**	-0.692**	-0.658**	-0.713**	-0.647**
		Heading	-0.646**	-0.669**	-0.581**	-0.656**	-0.716**	-0.682**
		Grainfilling	-0.473*	-0.646**	-0.699**	-0.714**	-0.737**	-0.633**
		Mean‡	-0.710**	-0.840**	-0.636**	-0.789**	-0.884**	-0.661**
	r_g	Mean§	-0.863**	-0.756**	-0.754**	-0.752**	-0.826**	-0.780**
NWI-3	r_p	Booting	-0.816**	-0.693**	-0.643**	-0.652**	-0.679**	-0.655**
		Heading	-0.740**	-0.672**	-0.672**	-0.627**	-0.626**	-0.688**
		Grainfilling	-0.645**	-0.646**	-0.694**	-0.733**	-0.768**	-0.658**
		Mean‡	-0.824**	-0.846 **	-0.665 **	-0.772 **	-0.828 **	-0.668 **
	r_g	Mean§	-0.921**	-0.741 **	-0.769 **	-0.822 **	-0.918 **	-0.891 **
NWI-4	r_p	Booting	-0.823**	-0.713 **	-0.683 **	-0.650 **	-0.710 **	-0.671 **
		Heading	-0.702**	-0.682 **	-0.637 **	-0.687 **	-0.691 **	-0.682 **
		Grainfilling	-0.537**	-0.657 **	-0.702 **	-0.720 **	-0.769 **	-0.638 **
		Mean‡	-0.765**	-0.856 **	-0.670 **	-0.793 **	-0.873 **	-0.680 **
	r_g	Mean§	-0.898**	-0.759 **	-0.819 **	-0.859 **	-0.959 **	-0.851 **

*p<0.05, **p<0.01

† WI, water index; NWI-1, normalized water index-1; NWI-2, normalized water index-2; NWI-3, normalized water index-3; NWI-4, normalized water index-4.

‡ Phenotypic correlation coefficients based on mean of three growth stages in individual year.

§ Genetic correlation coefficients based on mean of three growth stages in individual year.

Figures

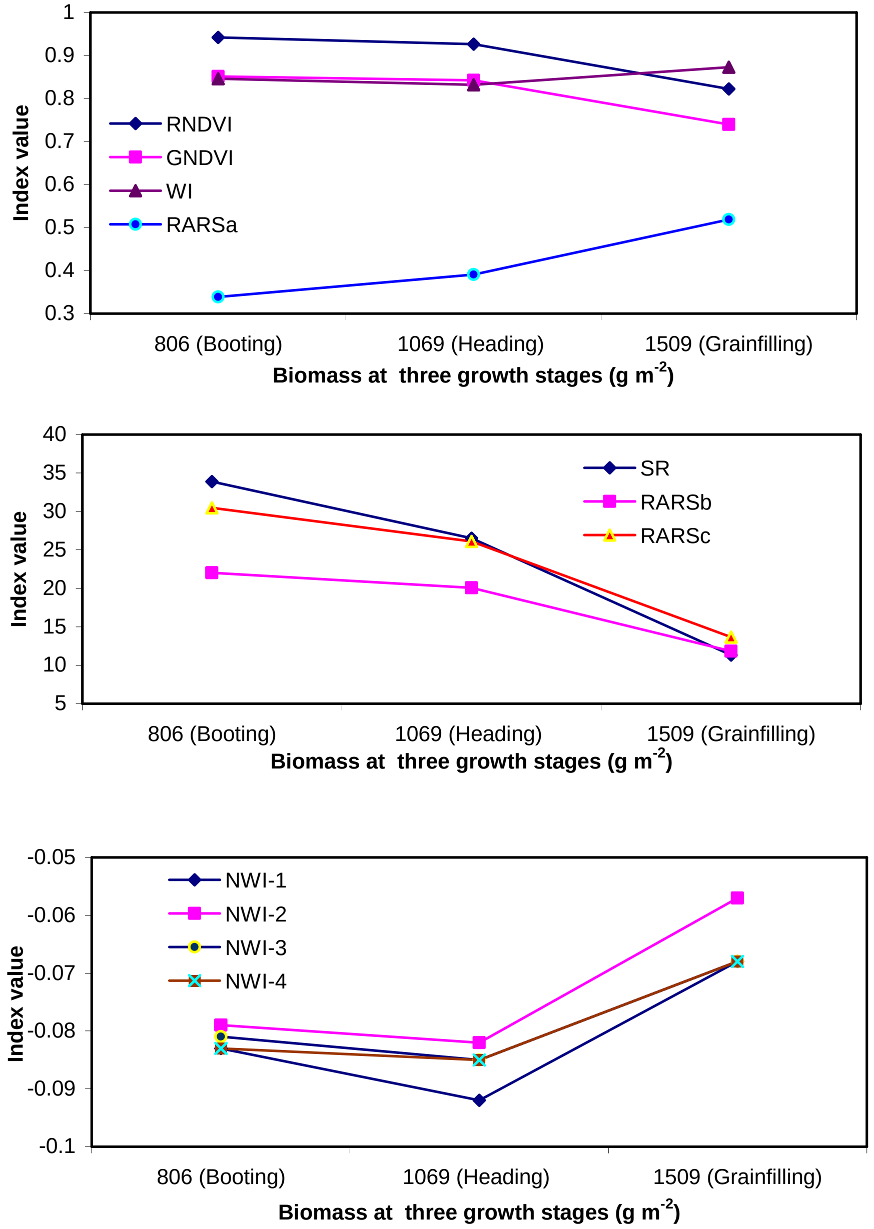

Fig 1. Patterns of change of the spectral reflectance indices and biomass production during different growth stages for Exp-1 in year 2004-2005. RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; WI, water index; RARSa, ratio analysis of reflectance spectra - chlorophyll a; SR, simple ratio; RARSb, ratio analysis of reflectance spectra - chlorophyll b; RARSc, ratio analysis of reflectance spectra - carotenoids; NWI-1, normalized water index-1; NWI-2, normalized water index-2; NWI-3, normalized water index-3; NWI-4, normalized water index-4.

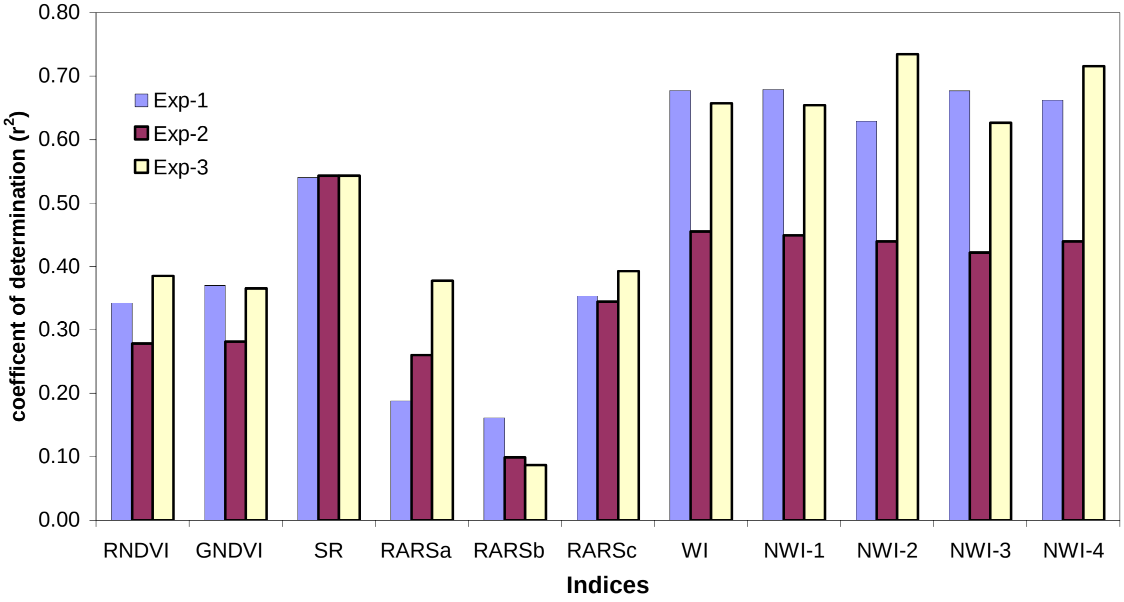

Fig 2: Coefficients of determination (r^2) for biomass production in three experiments as explained by the different spectral reflectance indices based on the mean indices and biomass values of three growth stages over two years. RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio; RARSa, ratio analysis of reflectance spectra - chlorophyll a; RARSb, ratio analysis of reflectance spectra - chlorophyll b; RARSc, ratio analysis of reflectance spectra - carotenoids; WI, water index; NWI-1, normalized water index-1; NWI-2, normalized water index-2; NWI-3, normalized water index-3; NWI-4, normalized water index-4.

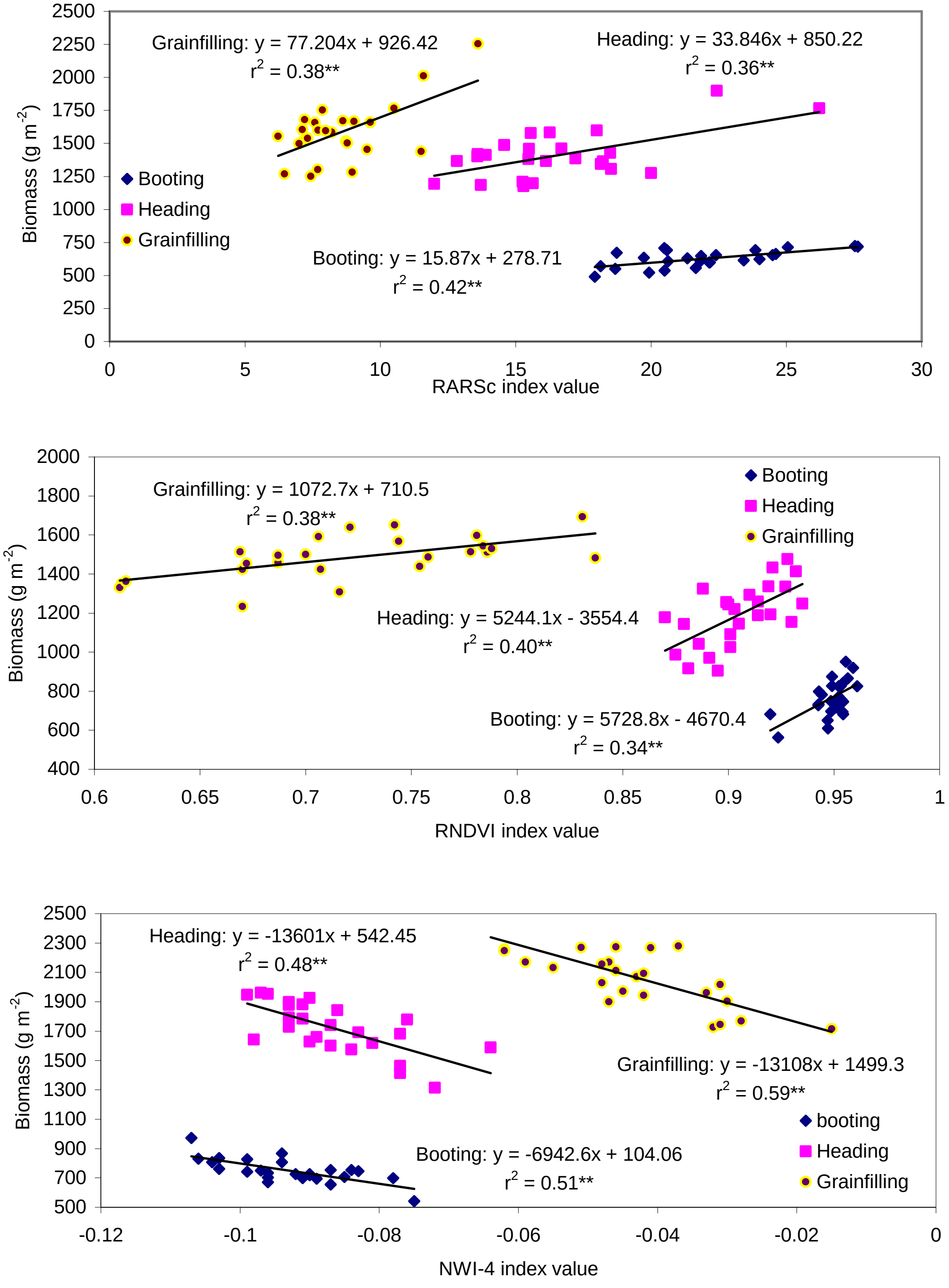

Fig 3. Functional relationship between biomass at three growth stages and ratio analysis of reflectance spectra - carotenoids (RARSc) (Exp-1, year 2003-2004), red normalized difference vegetation index (RNDVI) (Exp-3, year 2004-2005), and normalized water index-4 (NWI-4) (Exp-3, year 2004-2005). $^{**}p<0.01$.

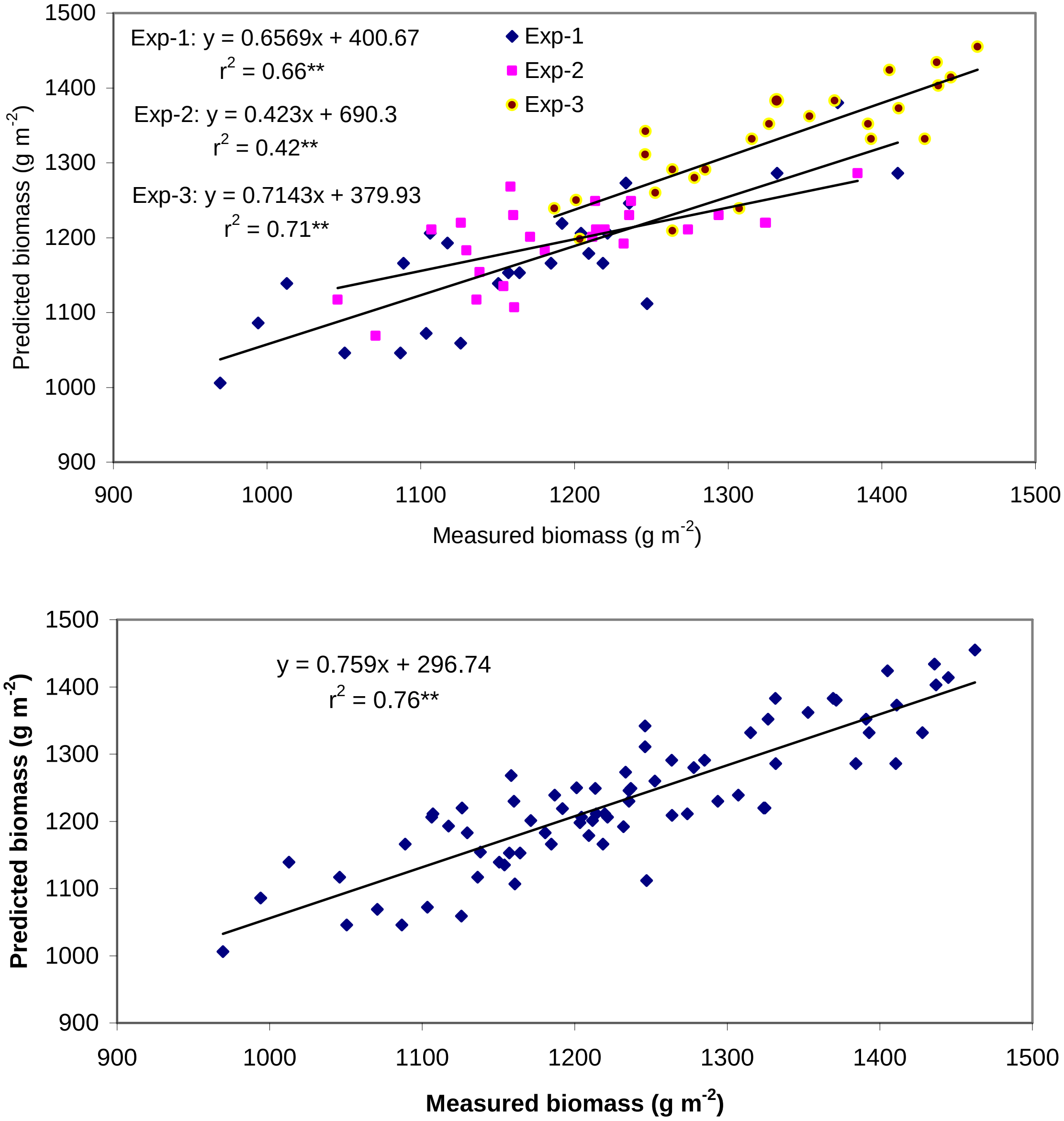

Fig 4. Relationship between actual biomass production and predicted biomass production based on a linear relationship using normalized water index-4 (NWI-4) in all three experiments individually (top figure) and in combination (bottom figure) using the mean values of the index and biomass production over three growth stages and two years. **p<0.01.

CHAPTER IV

Genetic Analysis and Indirect Selection for Winter Wheat Grain Yield Using Spectral Reflectance Indices

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Abbreviations

GNDVI, green normalized difference vegetation index

NWI, normalized water indices

NWI-1, normalized water index-1

NWI-2, normalized water index-2

NWI-3, normalized water index-3

NWI-4, normalized water index-4

RNDVI, red normalized difference vegetation index

SR, simple ratio

SRI, spectral reflectance indices

WI, water index

Abstract

Indirect selection for grain yield in winter wheat using spectral reflectance indices (SRI) has been assessed in this study. The genetic correlations, heritability, and response to selection for grain yield and SRI, correlated response of grain yield, and relative selection efficiency of the SRI for grain yield were analyzed in different winter wheat genetic backgrounds. Three experiments were conducted in each of two years. The first experiment had 25 winter wheat cultivars. The other two experiments contained two different populations, each having 25 recombinant inbred lines. Six previously reported SRI (RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio; WI, water index; NWI-1, normalized water index-1 and NWI-2, normalized water index-2) were calculated and two new indices were developed (NWI-3, normalized water index-3 and NWI-4, normalized water index-4). The indices were estimated at three distinct growth stages (booting, heading, and grainfilling). The water based indices (WI and NWI) gave higher genetic correlations with grain yield together with higher heritability compared to the other indices and grain yield. The combination of growth stages gave better correlations compared to any individual growth stage. Up to 83% of the top 25% highest yielding genotypes were selected by NWI-3 and NWI-4. Overall, strong genetic correlations, higher heritability and selection response, correlated response equal to or higher than direct response, and relative selection efficiency greater than one for NWI-3 and NWI-4 indicate the strong genetic basis for indirect selection with these indices for winter wheat grain yield.

Introduction

Selection of advanced breeding materials for grain yield is a labor intensive procedure, and sometimes produces inaccurate results due to the genetic behavior of yield, the most complex trait for every crop species (Ball and Konzak, 1993). Grain yield is influenced directly or indirectly by a number of factors, such as morphology, physiology, and especially environmental influences. Grain yield in wheat is characterized by low heritability and possesses a high genotype by environment interaction, and hence, selection becomes more difficult in a given environment (Jackson et al., 1996). Selection for grain yield by measuring yield itself is a classical approach, whereas selection for grain yield by considering different indirect traits is an analytical approach (Richards, 1982). A good understanding of the factors responsible for growth and development is required to identify an indirect selection tool for improving grain yield in wheat (Richards, 1982).

Reynolds et al. (2001) emphasized the potential of using different morpho-physiological selection criteria to complement empirical selection for grain yield, which eventually can make the selection process more efficient. So far, the use of this strategy is not well established in a large scale breeding program due to the lack of good knowledge about the indirect traits (Richards, 1996). Moreover, no comprehensive studies have been reported from the breeding and genetics point of view about the usefulness of secondary traits for obtaining greater genetic gain for grain yield. However, there are reports that stomatal conductance, C_3 isotope discrimination of grains, and canopy temperature depression can complement empirical selection for grain yield in different wheat growing areas (Reynolds et al., 1999). Canopy spectral reflectance has recently been reported to be useful for predicting grain yield in wheat (Araus et al., 2001; Aparicio et al., 2000; Royo et al., 2003; Babar et al., 2006a).

The concept behind canopy spectral reflectance is that specific plant traits are associated with absorption of specific wavebands of the light spectrum and thus, give a characteristic reflectance pattern at a certain waveband of the light spectrum (Reynolds et al., 1999). For example, chlorophylls, xanthophylls, and carotenoids strongly absorb light in the visible wavebands, and thus, reflect only a smaller proportion of the visible wavelengths. The near infrared portion of the light spectrum is not absorbed by these pigments, and hence, reflect a significant portion of the near infrared light from the canopy. The reflection pattern of the near infrared wavebands is governed by the structural behavior of the plant tissues (Peñuelas and Filella, 1998). Measurements of canopy spectral reflectance can provide useful information about green biomass, photosynthesis, relative water content, nutrient deficiencies, and environmental stresses (Araus et al., 2001; Reynolds et al., 2001).

Estimation of different morpho-physiological properties of crop plants can be done by using spectral reflectance indices (SRI), which are based on mathematical equations between different wavelengths, such as sums, ratios, or differences (Araus et al., 2001). Among the indices, normalized difference vegetation index and simple ratio are the two most widely used SRI that have been reported to assess green biomass, leaf area index, and fraction of absorbed photosynthetically active radiation in different crop species (Wiegand and Richardson, 1990; Baret and Guyot, 1991; Price and Busch, 1995). The estimation of leaf area duration, an estimator of stress tolerance and absorbed photosynthetically active radiation, has been used to predict grain yield with periodic measurements of canopy spectral reflectance (Wiegand and Richardson, 1990). Prediction of early biomass in wheat was also reported by Elliot and Regan (1993). The water index (WI), developed by using the minor water absorption band at 970 nm, has been demonstrated to approximate relative water content and other traits related to water content of the canopy (Peñuelas et al., 1993, 1997). Normalized difference vegetation index has been reported to predict grain yield in soybean (Ma et al., 2001), in winter wheat (Raun et al., 2001), and in durum wheat (Aparicio et al., 2000). Serrano et al. (2000) reported that simple ratio can reliably predict winter wheat grain yield under different nitrogen stresses. Babar et al. (2006a) reported the efficiency of the near infrared based indices in estimating spring wheat grain yield under northwestern Mexican conditions. The published literature is not decisive about the value of the SRI as an indirect selection criterion in winter wheat environments.

The efficiency of an indirect selection criterion compared to direct selection depends on the heritability of both the direct and indirect traits along with the genetic correlation between them, considering that equal selection intensities for both traits were practiced (Falconer, 1989). Information from the spectral reflectance indices in terms of these two features is not available to ascertain the efficiency of using SRI as an indirect selection tool for improving grain yield in winter wheat. Brancourt-Hulmel et al. (2005) reported that the magnitude of genetic correlations between two traits may greatly vary, and depends largely on the traits, and the genetic materials under study. Indirect selection has been shown to be more efficient, less efficient, or equally efficient compared to direct selection when different input levels were considered and selection was practiced to improve a trait in one environment by selecting the trait in another environment (Atlin and Frey, 1989; Calhoun et al., 1994; Sinebo et al., 2002). Improving wheat yield by selecting resistance to stripe rust in a disease predominant area was more effective than direct selection for grain yield (Hill et al., 1999). Most of the selection efficiency studies involved creating two diverse environments and selecting a trait in one environment that could be equally effective in another environment. The selection based on some combination of morphological traits has been reported to be equally effective as selection for dry matter directly in maize (Gallais, 1984). The correlation coefficients between indirect and direct traits have been reported mostly as phenotypic correlations, not assessed in a breeding population, which may be biased upwards or downwards due to error effects or the correlation between environments. One of the important considerations for an indirect trait relevant to a breeding program is that the heritability of the direct and indirect traits and the genetic correlations between them should be measured in genetic populations closely representing the area where the improvement is being targeted. So, a comprehensive study is required in the U.S. Great Plains to determine the usefulness of the SRI for improvement of grain yield by using genetic materials developed in this region. It is also important to consider the relationship between expected grain yield and predicted grain yield using SRI to compare the effectiveness.

Therefore, the objectives of the study were to i) estimate genetic correlations between the SRI and grain yield, ii) estimate the heritability for grain yield and SRI, iii) estimate the efficiency of direct selection for grain yield and indirect selection for grain yield through SRI, and iv) estimate relative selection efficiency of the SRI for grain yield.

Materials and Methods

The experiments were conducted at Oklahoma State University research farms at Stillwater and at Lake Carl Blackwell, 25 km west of Stillwater, OK during the wheat growing season of 2003-2004 and 2004-2005.These sites have soil types of silty clay loam to fine sandy loam with an average pH of 6-7. The experiments were conducted under rainfed condition with a seeding rate of 70 kg ha^{-1}. At both sites, 90 kg ha^{-1} nitrogen was applied before planting. Individual plot size was 3.04 m long × 1.24 m wide. To control foliar diseases, Folicur (25% tebuconazole) was applied twice (late tillering and booting stage) and Cygon (dimethoate) was applied once at the booting stage to control aphids.

Plant materials

The plant materials consisted of three groups of winter wheat genotypes developed by different wheat breeding programs of the southern and central Great Plains. The first experiment (Exp-1) was composed of 25 commercial winter wheat cultivars from the Great Plains of U.S. and was planted at Stillwater and Lake Carl Blackwell in the 2003-2004 and 2004-2005 cropping seasons, respectively. The second experiment (Exp-2), designated as population 1, contained 25 $F_{4:6}$ and $F_{4:7}$ recombinant inbred lines from the cross KS920709-B-5-2-2/Stanof, and was planted at Stillwater in 2003-2004 (F_6) and at Lake Carl Blackwell (F_7) in the 2004-2005. The third experiment (Exp-3), designated as population 2, consisted of 25 $F_{4:6}$ and $F_{4:7}$ recombinant inbred lines from the cross Longhorn/98IWS26, and was planted at Lake Carl Blackwell in both years (F_6 in 2003-2004 and F_7 in 2004-2005). All three experiments were established in a 5 × 5 alpha lattice design with two replications. Grain yield from each plot was determined by mechanical harvesting of the whole plot and expressed as kg ha^{-1}.

Reflectance measurements

Canopy spectral reflectance measurements were carried out with a field spectro-radiometer (FieldSpec UV/VNIR, Analytical Spectral Device, Boulder, CO). The radiometer has the capability of collecting the reflectance from 350-1050 nm of the light spectrum. Five hundred and twelve continuous data points from each reading were obtained with an interval of 1.4 nm between each reading. Cloud free days were chosen to measure the reflectance and data were taken in the middle of the day. The optical fiber of the sensor was placed 50 cm above the canopy with a field of view of 25^0. The incoming reflectance was calibrated against the reflectance from a white reflectance plate coated with $BaSO_4$. Reflectance measurements were taken from four random places from each plot and the mean of the four readings was used to estimate reflectance indices. Reflectance measurements were taken at booting (Zadoks stage 45), heading (Zadoks stage 59), and grainfilling (Zadoks stage 75) stages in all three experiments in both years (Zadoks et al., 1974).

Spectral reflectance indices were calculated as follows:

Vegetation based indices that are related to canopy photosynthetic area are:

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

Green normalized difference vegetation index (GNDVI) = $(R_{780}-R_{550})/(R_{780}+R_{550})$, Aparicio et al. (2000).

Simple Ratio (SR) = (R_{900}/R_{680}), Gitelson et al. (1996).

Water based indices that indicate the canopy water status are:

Water index (WI) = (R_{970}/R_{900}), Peñuelas et al. (1993).

Normalized water index-1 (NWI-1) = (R_{970}-R_{900})/(R_{970}+R_{900}), Babar et al. (2006a).

Normalized water index-2 (NWI-2) = (R_{970}-R_{850})/(R_{970}+R_{850}), Babar et al. (2006a).

Two new normalized water indices were calculated as follows:

Normalized water index-3 (NWI-3) = (R_{970}-R_{920})/(R_{970}+R_{920}).

Normalized water index-4 (NWI-4) = (R_{970}-R_{880})/(R_{970}+R_{880}).

'R' and the subscript indicate the light reflectance at the specific wavebands (in nm).

Statistical analysis

SAS MIXED procedure (SAS Institute, 2001) was used for alpha lattice analysis for grain yield and spectral reflectance indices. Phenotypic and genetic correlation coefficients were estimated to reveal the association between the indices and grain yield. The genetic correlation coefficients between the SRI and grain yield were calculated by the formula (Falconer, 1989),

$r_g = (\mathrm{Cov}_{X1X2}) / \sqrt{(\mathrm{Var}_{X1} \times \mathrm{Var}_{X2})}$, where Cov_{X1X2} is the genetic covariance between the two variables, and Var_{X1} and Var_{X2} were the genetic variances of the variables, respectively, and were estimated across growth stages. The coefficients of the phenotypic and genetic correlations were estimated with SAS software (SAS Institute, 2001). Broad sense heritabilities (h^2) were calculated on a plot mean basis as defined by Falconer (1989). Broad sense heritability is the proportion of the phenotypic variance to the total genetic variance, and estimated as: $h^2 = \sigma^2g/(\sigma^2g + \sigma^2e)$, where, σ^2g is the genotypic variance and σ^2e is the error variance. Response to selection (R) of the SRI and grain yield and correlated response (CR) for grain yield by using SRI were calculated according to Falconer (1989) as: $R = h_x \sigma_x$, where h_x is the square root of heritability and σ_x is the genotypic standard deviation.

$CR = h_x r_g \sigma_y$, where h_x is the square root of heritability of trait x (SRI), r_g is the genetic correlation between the trait x (SRI) and y (yield), and σ_y is the genotypic standard deviation of trait y (yield), assuming the selection intensity is equal in different generations and for the traits. The relative efficiency of selection was calculated as the ratio of CR of grain yield for a specific SRI and R of grain yield (Falconer, 1989).

Data from the different SRI were analyzed to obtain principal components by principal component analysis to reduce the redundant information of the indices. Principal component analysis provides a better way to classify the contribution of different indices rather than each index individually (Filella et al., 1995). The principal component analysis was done for each experiment with mean grain yield over two years and mean index values over different growth stages and years.

Selection of genotypes

For selection of genotypes, we first ranked the genotypes based on grain yield *per se* and selected the top 25% of the highest yielding genotypes. Then, we ranked the genotypes based on the SRI values and selected the top 25 % of the genotypes. The ranking of the genotypes was done based on mean index values over three growth stages. Mean yield *per se* and mean predicted yield of the top 25% highest yielding genotypes using linear regression equation between grain yield and SRI were compared, and percent yield differences were calculated to know the effectiveness of the SRI in selecting for higher yielding genotypes.

Results and Discussion

Genotypes and growth stages

Genotype grain yield was significantly different ($p<0.05$) in all three experiments in both years and across years. Mean grain yield of the cultivars in Exp-1 was 5026 kg ha^{-1} in year 2003-2004, 3571 kg ha^{-1} in year 2004-2005, and 4299 kg ha^{-1}, when data were combined for two years. In Exp-2 (Population 1), mean grain yield was 4608 kg ha^{-1}, 3177 kg ha^{-1}, and 3899 kg ha^{-1} for year 2003-2004, 2004-2005, and across years. Mean grain yield in Exp-3 (Population 2) was 4301 kg ha^{-1}, 2521 kg ha^{-1}, and 3411 kg ha^{-1} in year 2003-2004, 2004-2005, and across years, respectively. Significant differences among the genotypes for grain yield in all three sets of materials suggested the presence of considerable genetic variation among the genotypes for grain yield. Genetic variations for grain yield were also reported in irrigated spring wheat (Babar et al., 2006a), in rainfed durum wheat (Aparicio et al., 2000; Royo et al., 2003), and in rainfed winter wheat (Prasad et al., 2006).

The mean (± SE) of the SRI at three growth stages (booting, heading, and grainfilling) based on two years data for three experiments are presented in Table 1. All indices showed significant variation ($p<0.05$) among genotypes in the different experiments at the heading and grainfilling stages, with some non-significant variations at the booting stage. This result suggests that the heading and grainfilling stages are the best growth stages for measuring the SRI. Similar observations were reported in irrigated spring wheat (Babar et al., 2006a), in durum wheat (Aparicio et al., 2000; Royo et al., 2003), and in winter wheat (Prasad et al., 2006). The estimates of the vegetation based indices (RNDVI, GNDVI, and SR) showed a decreasing trend from the booting to grainfilling stage (Table 1). The highest estimates of these indices at the booting stage are due to the highest amount of green leaf area and the highest leaf area index of the genotypes occurring at this stage. The decrease in green tissues with the progression of growth stages resulted in a decrease in the index values in subsequent growth stages. Since these three indices were developed by using the visible and near infrared wavebands, they follow the changes in the genotypic signature for the loss of green mass. It has been reported that reflectance in the visible wavebands increases as the green tissues start to collapse and near infrared reflectance decreases (Knipling et al., 1970). The near infrared based indices (WI and NWI) always had the highest value at the grainfilling stages, and in most cases, they showed the lowest values at the heading stage with a few exceptions (Table 1). Theses indices contain the minor water absorption band 970 nm (see materials and methods for index calculation). The most important property of this 970 nm waveband has been reported to approximate the water content of the canopy (Peñuelas et al., 1997). The highest values at the grainfilling stage indicate the lowest amount of canopy water content at grainfilling. Conversely, the lowest values for the water based indices at the heading stage indicate the highest water content in the canopy. This behavior of the water based indices was also reported in irrigated spring wheat (Babar et al., 2006a) and in winter wheat (Prasad et al., 2006). Overall, the clear genotypic variations for the SRI among different cultivars and populations of recombinant inbred lines demonstrate the potential of using SRI to classify winter wheat genotypes for grain yield.

Correlation between SRI and grain yield

Significant phenotypic correlations between the SRI and grain yield were observed in both years and across years, with a few exceptions (Table 2). The vegetation based indices (RNDVI, GNDVI, and SR) always gave positive correlation coefficients. Similar positive correlations have been reported in rainfed durum wheat environments (Aparicio et al., 2000; Royo et al., 2003). In the year 2004-2005, this experiment (Exp-1) was affected by uneven germination, and thus showed some inconsistent correlations between the vegetation based indices and grain yield. In most cases, RNDVI, GNDVI, and SR gave higher phenotypic correlations when data were combined from the different growth stages, especially combining the three growth stages (Table 2).

The indices based on the minor water absorption band (WI and NWI) always gave significant and higher phenotypic correlation coefficients compared to the vegetation based indices (Table 2). The sign of the coefficients were always negative. The correlation became stronger when the relationship was assessed by combining the three growth stages (Table 2). Combining two growth stages (heading and grainfilling) also produced similar trends, but they were lower than the three growth stage combinations. The better association between the SRI and grain yield based on the combined growth stage information can be hypothesized as being a better picture of overall plant health during the crop growing period assessed by the SRI. The water based indices measure water content of the canopy, and the higher associations between these indices with grain yield indicate that canopy water content plays a vital role in the productivity of a given genotype under rainfed conditions. Using reflectance information from different growth stages for predicting grain yield was previously reported in irrigated spring wheat (Babar et al., 2006a) and in winter wheat (Prasad et al., 2006). Although three readings always gave higher associations compared to one or two readings, from a practical point of view, we recommend taking reflectance measurements at heading and at grainfilling and combining the information from the two readings.

It has been suggested that phenotypic correlation does not always reflect the true association between two traits (Jackson, 2001). The correlation can be biased either upward or downward due to the influence of error or correlated environmental effects. Genetic correlation between two traits can reveal the true relationship between them (Jackson, 2001). With this in mind, we estimated the genetic correlation between the SRI and grain yield in two populations (Exp-2 and Exp-3) of recombinant inbred lines (Table 3). All indices showed significant genetic correlation with grain yield in both years and across years for both populations. The sign of the coefficients were positive for the vegetation based indices (RNDVI, GNDVI, and SR) and negative for the water based indices (WI and NWI). The water based indices gave a higher association at the genetic level than the vegetation based indices, suggesting that canopy water content is more powerful in predicting genotypes yield potential. Higher genetic correlations were also observed between the same water based indices measured in two years compared to the vegetation based indices (data not shown). These observations suggest that the water based indices were less affected by the environment, and showed lower genotype by environment interactions compared to the commonly used vegetation indices. Higher genetic correlations between the water based indices and grain yield in randomly chosen recombinant lines established the genetic basis for the relationship.

The percent of the variation for grain yield that is explained by the SRI in the two populations (Exp-2 and Exp-3) is presented in Table 4. The coefficients were calculated based on individual growth stages, and two combinations of growth stages (combining booting, heading, and grainfilling, and combining heading and grainfilling). The water based indices explained more of the grain yield variability than the vegetation based indices (Table 4). The combination of growth stages, especially combining three growth stages gave higher predictive capability than the individual growth stages in most cases with some exceptions. We suggest measuring SRI multiple times, at least one at heading and again at grainfilling and utilizing mean of the two readings.

The mean grain yield for two years and mean index value of NWI-3 for the three growth stages and across years for population 1 are plotted in Fig 1. The relationship was linear. The index is capable of predicting the genotypes yield potential at any yield level. It did not saturate at the higher yield levels, which means breeders can effectively use this index for discriminating genotypes in different breeding populations for grain yield at any yield level.

The water based indices were basically indistinguishable from each other. It was reported earlier that normalizing can improve the relationship over a ratio index (Tucker, 1979). Babar et al. (2006a, 2006b) reported that the normalized index did not produce any significantly better results over the ratio index for predicting yield and biomass in spring wheat. This is also evident in our studies. We used a multivariate approach to look at all the indices together, instead of each index separately. Filella et al. (1995) suggested that the multivariate approach can classify the variables according to their relationship with each other, and can identify unique variables that are a true representation of the function of the variables. We obtained principal components for three experiments individually by using the mean index values over three growth stages and across years and grain yield for each experiment over two years. Axis 1 measures the normalized water indices in a positive direction. Yield and the vegetation based indices (RNDVI, GNDVI, and SR) measure in a negative direction in all three experiments (Fig 2). The relationship between the different indices can be easily visualized from the plots. All the normalized water indices grouped together with a very strong negative correlation with grain yield (Fig 2). We conclude that all the normalized water indices are measuring the same parameters within the crop canopy. Similarly, RNDVI, GNDVI, and SR are measuring the same parameters within the crop canopy, as they were grouped together. The principal component analysis also revealed that the water based indices have a stronger relationship with grain yield compared to the vegetation based indices (Fig 2).

Heritability

Broad sense heritabilities for grain yield ranged from 0.444 to 0.627 in two populations in two different years and across years. Grain yield heritabilities were slightly different in the two years and across years in the two populations (Table 6). The heritabilities of the vegetation indices (RNDVI, GNDVI, and SR) ranged from 0.459 to 0.739 in the different populations in the two years and across years. The water based indices showed higher heritability compared to the vegetation indices. The heritability calculated in this study is the proportion of phenotypic variance that is due to the genetic effect. These heritabilities indicate the repeatability of the indices at different times. These heritabilities may be biased to some extend by the selection that occurred in the populations in the early generations. The recombinant lines of these populations were selected in the F_2, F_3, and F_4 for traits like disease resistance, and other important agronomic traits, such as maturity and height. This selection pressure might reduce genetic variances and heritability, but, the reduction in genetic variance of the populations may not be limited since they were never selected for grain yield. On the other hand, the reduction in the genetic variance may be compensated by a reduction in the environmental variance because of the selection of the more highly heritable traits in the earlier generations. We believe the bias in heritability estimates is minimal. Grain yield heritability was lower compared to the reflectance indices, indicating that grain yield is more environmentally influenced than the SRI. On the other hand, the water based indices showed a higher heritability than the vegetation based indices, indicating that the water based indices are more repeatable than the vegetation based indices. High heritability of the water based indices is indicative of the scope for higher genetic gain in grain yield through selection.

Selection response, correlated response, and relative selection efficiency

The response to selection (R) for SRI and grain yield, correlated response (CR) for grain yield using SRI, and relative selection efficiency of the SRI for grain yield are presented in Table 6. The response to selection for grain yield ranged from 0.403 to 0.597 in the different populations in two years and across years, whereas, the SRI showed higher selection response compared to grain yield (Table 6). Among the SRI, the water based indices had a higher direct response compared to the vegetation based indices. The correlated response was higher for the water based indices compared to the direct response for grain yield (Table 6). The ratio between correlated response for a primary trait via a secondary trait and the response to selection for the primary trait is a measure of the relative selection efficiency (Falconer, 1989). The relative selection efficiency of the SRI for grain yield revealed that indirect selection can be equally effective via SRI for grain yield selection (Table 6), especially with the water based indices. The value of the relative selection efficiency depends on the genetic correlation between the direct and indirect traits and heritability of the traits (Falconer, 1989). We observed the relative selection efficiency more than one for some of the water based indices. Among the indices, NW1-3 and NWI-4 consistently gave relative selection efficiencies more than one in the two populations over different years and across years. Jackson (2001) indicated that an indirect selection trait should have higher heritability than the direct trait and high genetic correlation with the direct trait. In addition, the indirect trait should have convenience in measurement along with low costs of measurement. In the context of our study, greater genetic gain can be obtained through the indirect traits (NWI-3 and NWI-4) than selecting for the direct trait (yield) itself. Strong genetic correlation, moderate to high heritability, higher correlated response that the direct response, and relative selection efficiency equal to or more than one for the water based indices provided ample evidence for the potential of the water based indices to incorporate them into a breeding program for obtaining greater genetic gain in grain yield.

Selection of higher yielding genotypes

The percent of selected genotypes among the 25% highest yielding genotypes along with the percent yield difference between the harvested yield and predicted yield based on the linear regression equation between the SRI and yield are presented in Table 7. The data presented in this table are based on the average values for each index over three individual growth stages. Similar observations were also observed when the mean of two growth stages (heading and grainfilling) were used to calculate the indices (data not shown). The water based indices showed a clear cut advantage over the vegetation based indices. Ball and Kozak (1993) demonstrated the efficiency of normalized difference vegetation index in selecting higher yielding genotypes under water non-limiting conditions using two readings at the grainfilling stage, while, Babar et al. (2006b) reported the higher efficiency of the near infrared based indices for grain yield selection under partially irrigated spring wheat environments. We have shown that the water based indices are efficient in selecting higher yielding genotypes in winter wheat rainfed environments. Up to 83 % of the top 25% highest yielding genotypes can be detected with the normalized water indices (Table 7). From the percent yield difference data between actual yield and predicted yield, it is evident that water based indices gave a close approximation of actual grain yield. These data indicate that the water based indices, especially NWI-3 and NWI -4, can serve as an efficient indirect selection tool for selection of genotypes with higher yield potential in a winter wheat breeding program.

Conclusions

The genetic basis for the indirect selection of winter wheat grain yield using SRI has been demonstrated in this study. The water based indices (WI and NWI) showed higher genetic correlations with grain yield compared to RNDVI, GNDVI, and SR. The heritability estimates were also higher for the water based indices than for grain yield *per se*. Response to selection for the two newly developed indices (NWI-3 and NWI-4) was higher than the direct response for grain yield. The correlated response of these indices for grain yield was equal to or higher than the direct response for grain yield, and the relative selection efficiency was always more than one. These newly developed indices detected a significant proportion of the highest yielding genotypes. Based on these genetic parameters, greater genetic gains can be obtained through the utilization of the NWI-3 and NWI-4 as an indirect selection tool for grain yield in winter wheat.

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Tables

Table 1. Mean (± SE) of different spectral reflectance indices at three growth stages for three experiments, estimates based on combined years.

Experiments	Growth stages	Spectral reflectance indices†							
		RNDVI	GNDVI	SR	WI	NWI-1	NWI-2	NWI-3	NWI-4
Exp-1	Booting	0.899 ± 0.021	0.807 ± 0.018**	22.2 ± 2.58**	0.846 ± 0.013**	-0.083 ± 0.008**	-0.08 ± 0.009**	-0.082 ± 0.007**	-0.089 ± 0.009**
	Heading	0.862 ± 0.018**	0.764 ± 0.025**	15.5 ± 1.38**	0.864 ± 0.01**	-0.073 ± 0.006**	-0.064 ± 0.006 **	-0.073 ± 0.005 **	-0.073 ± 0.006**
	Grainfilling	0.756 ± 0.044*	0.692 ± 0.033**	8.51 ± 1.60**	0.892 ± 0.013**	-0.057 ± 0.007**	-0.045 ± 0.009**	-0.059 ± 0.006 **	-0.056 ± 0.008**
Exp-2	Booting	0.912 ± 0.014	0.823 ± 0.012	27.6 ± 2.69*	0.836 ± 0.009	-0.090 ± 0.005	-0.087 ±0.007	-0.088 ± 0.005*	-0.091 ± 0.006
	Heading	0.908 ± 0.012*	0.807 ± 0.013**	23.2 ± 2.29**	0.834 ± 0.012*	-0.091 ± 0.007*	-0.085 ±0.009	-0.090 ± 0.007*	-0.094 ± 0.008*
	Grainfilling	0.607 ± 0.033**	0.602 ± 0.020**	5.13 ± 0.720**	0.934 ± 0.011**	-0.034 ± 0.006**	-0.013 ± 0.008	-0.039 ± 0.005**	-0.031 ± 0.007**
Exp-3	Booting	0.878 ± 0.024	0.746 ± 0.016**	17.2 ± 2.88*	0.886 ± 0.015**	-0.061 ± 0.008**	-0.058 ± 0.009**	-0.061 ± 0.007**	-0.062 ± 0.009**
	Heading	0.857 ± 0.022**	0.743 ± 0.019**	14.1 ± 1.92**	0.861 ± 0.012**	-0.075 ± 0.007**	-0.067 ± 0.007**	-0.074 ± 0.006**	-0.075 ± 0.007**
	Grainfilling	0.743 ± 0.038**	0.677 ± 0.024**	7.29 ± 1.26**	0.892 ± 0.014**	-0.057 ± 0.008**	-0.043 ± 0.01**	-0.060 ± 0.007	-0.057 ± 0.009**

*p<0.05, **p<0.01

† RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio; WI, water index; NWI-1, normalized water index 1; NWI-2, normalized water index 2; NWI-3, normalized water index 3; NWI-4, normalized water index 4.

Table 2. Phenotypic correlation between grain yield and different spectral reflectance indices in two years and across years in Exp-1.

Year	Growth stages	Spectral reflectance indices†							
		RNDVI	GNDVI	SR	WI	NWI-1	NWI-2	NWI-3	NWI-4
2003-2004	Booting	0.548**	0.588**	0.579**	-0.550**	-0.556**	-0.528**	-0.539**	-0.536**
	Heading	0.619**	0.588**	0.558**	-0.686**	-0.685**	-0.715**	-0.664**	-0.708**
	Grainfilling	0.672**	0.635**	0.629**	-0.735**	-0.737**	-0.701**	-0.751**	-0.717**
	Average‡	0.712**	0.685**	0.705**	-0.758**	-0.760**	-0.734**	-0.763**	-0.753**
	Average§	0.695**	0.651**	0.629**	-0.734**	-0.734**	-0.727**	-0.730**	-0.732**
2004-2005									
	Booting	0.685**	0.517**	0.635**	-0.810**	-0.807**	-0.774**	-0.798**	-0.779**
	Heading	0.432*	0.132	0.431*	-0.660**	-0.658**	-0.696**	-0.630**	-0.688**
	Grainfilling	0.295	-0.002	0.293	-0.693**	-0.690**	-0.583**	-0.723**	-0.651**
	Average‡	0.519**	0.205	0.588**	-0.834**	-0.833**	-0.819**	-0.832**	-0.840**
	Average§	0.375	0.059	0.407*	-0.748**	-0.750**	-0.730**	-0.753**	-0.772**
Across years									
	Booting	0.684**	0.580**	0.640**	-0.664**	-0.659**	-0.602**	-0.653**	-0.622**
	Heading	0.578**	0.357	0.543**	-0.661**	-0.657**	-0.681**	-0.630**	-0.678**
	Grainfilling	0.555**	0.353	0.497*	-0.706**	-0.704**	-0.649**	-0.721**	-0.677**
	Average‡	0.689**	0.466**	0.679**	-0.780**	-0.777**	-0.740**	-0.783**	-0.767**
	Average§	0.611**	0.376	0.558**	-0.723**	-0.720**	-0.704**	-0.713**	-0.719**

*p<0.05, **p<0.01

† RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio; WI, water index; NWI-1, normalized water index 1; NWI-2, normalized water index 2; NWI-3, normalized water index 3; NWI-4, normalized water index 4.

‡ Correlation coefficients were estimated based on average index value from booting, heading, and grainfilling stages.

§ Correlation coefficients were estimated based on average index value from heading and grainfilling stages.

Table 3. Genetic correlation between spectral reflectance indices (average of growth stages) and grain yield in two recombinant inbred populations for two years.

Experiments	Spectral reflectance indices†							
	RNDVI	GNDVI	SR	WI	NWI-1	NWI-2	NWI-3	NWI-4
Population 1								
Year 2003-2004	0.678**	0.663**	0.709**	-0.831**	-0.847**	-0.814**	-0.821**	-0.883**
Year 2004-2005	0.767**	0.767**	0.749**	-0.912**	-0.917**	-0.870**	-0.914**	-0.924**
Combined year	0.705**	0.745**	0.677**	-0.925**	-0.928**	-0.867**	-0.936**	-0.924**
Population 2								
Year 2003-2004	0.562**	0.656**	0.617**	-0.850**	-0.828**	-0.773**	-0.953**	-0.908**
Year 2004-2005	0.654**	0.678**	0.657**	-0.768**	-0.764**	-0.903**	-0.908**	-0.955**
Combined year	0.546**	0.611**	0.580**	-0.802**	-0.817**	-0.744**	-0.931**	-0.869**

**p<0.01

† RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio; WI, water index; NWI-1, normalized water index 1; NWI-2, normalized water index 2; NWI-3, normalized water index 3; NWI-4, normalized water index 4.

Table 4. Coefficient of determination (r^2) of different spectral reflectance indices for explaining grain yield variability in two recombinant inbred populations for two years.

Experiment	Growth stages	Spectral reflectance indices†							
		RNDVI	GNDVI	SR	WI	NWI-1	NWI-2	NWI-3	NWI-4
Population1									
Year 2003-2004	Booting	0.18*	0.24*	0.20*	0.41**	0.40**	0.43**	0.40**	0.40**
	Heading	0.30**	0.36**	0.27**	0.41**	0.41**	0.35**	0.39**	0.35**
	Grainfilling	0.20*	0.20*	0.28**	0.47**	0.46**	0.42**	0.47**	0.43**
	Average‡	0.24*	0.34**	0.40**	0.62**	0.62**	0.56**	0.65**	0.58**
	Average§	0.22*	0.28**	0.32**	0.52**	0.52**	0.46**	0.53**	0.48**
Year 2004-2005	Booting	0.13	0.37**	0.12	0.16*	0.15	0.16*	0.17*	0.18*
	Heading	0.57**	0.63**	0.53**	0.66**	0.67**	0.74**	0.62**	0.68**
	Grainfilling	0.57**	0.64**	0.56**	0.79**	0.79**	0.72**	0.80**	0.76**
	Average‡	0.61**	0.71**	0.43**	0.73**	0.72**	0.74**	0.71**	0.73**
	Average§	0.64**	0.73**	0.61**	0.77**	0.77**	0.77**	0.75**	0.77**
Population2									
Year 2003-2004	Booting	0.45**	0.19*	0.47**	0.46**	0.46**	0.49**	0.43**	0.47**
	Heading	0.34**	0.39**	0.22*	0.51**	0.50**	0.40**	0.54**	0.45**
	Grainfilling	0.13	0.14	0.13	0.32**	0.31**	0.17*	0.30**	0.19*
	Average‡	0.37**	0.35**	0.45**	0.63**	0.63**	0.53**	0.64**	0.58**
	Average§	0.23*	0.28**	0.20*	0.44**	0.43**	0.31**	0.44**	0.35**
Year 2004-2005	Booting	0.39**	0.20*	0.37**	0.40**	0.40**	0.38**	0.38**	0.38**
	Heading	0.39**	0.23*	0.34**	0.58**	0.59**	0.59**	0.59**	0.60**
	Grainfilling	0.28**	0.24*	0.27**	0.39**	0.40**	0.29**	0.39**	0.30**
	Average‡	0.40**	0.27**	0.40**	0.57**	0.58**	0.51**	0.58**	0.52**
	Average§	0.34**	0.26**	0.34**	0.52**	0.53**	0.43**	0.53**	0.45**

*$p<0.05$, **$p<0.01$

† RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio; WI, water index; NWI-1, normalized water index 1; NWI-2, normalized water index 2; NWI-3, normalized water index 3; NWI-4, normalized water index 4.

‡ Correlation coefficients were estimated based on average index value from booting, heading, and grainfilling stages.

§ Correlation coefficients were estimated based on average index value from heading and grainfilling stages.

Table 5. Broad sense heritability for grain yield and spectral reflectance indices (average of booting, heading, and grainfilling stages) in two recombinant inbred populations for two years and across years.

Experiments	Grain yield	Spectral reflectance indices†							
		RNDVI	GNDVI	SR	WI	NWI-1	NWI-2	NWI-3	NWI-4
Population 1									
Year 2003-2004	0.498	0.662	0.636	0.618	0.622	0.620	0.694	0.633	0.656
Year 2004-2005	0.627	0.739	0.715	0.580	0.783	0.780	0.685	0.779	0.799
Combined year	0.577	0.714	0.711	0.585	0.765	0.753	0.780	0.755	0.762
Population 2									
Year 2003-2004	0.565	0.501	0.459	0.491	0.681	0.641	0.730	0.692	0.759
Year 2004-2005	0.540	0.649	0.586	0.560	0.568	0.488	0.554	0.695	0.669
Combined year	0.444	0.683	0.622	0.628	0.713	0.587	0.687	0.682	0.665

† RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio; WI, water index; NWI-1, normalized water index 1; NWI-2, normalized water index 2; NWI-3, normalized water index 3; NWI-4, normalized water index 4.

Table 6. Selection response (R) for the spectral reflectance indices (SRI) and grain yield, correlated response (CR) for grain yield using SRI, and relative selection efficiency (CR/R) of the SRI for grain yield in two recombinant inbred populations.

Experiments	Selection parameter†	Grain yield	Spectral reflectance indices ‡							
			RNDVI	GNDVI	SR	WI	NWI-1	NWI-2	NW1-3	NWI-4
Population 1										
2003-2004	R	0.494	0.664	0.633	0.612	0.561	0.556	0.674	0.615	0.639
	CR		0.386	0.397	0.441	-0.459	-0.467	-0.548	-0.538	-0.542
	CR/R		0.782	0.803	0.892	-0.928	-0.945	-1.108	-1.088	-1.092
2004-2005	R	0.597	0.686	0.704	0.555	0.736	0.734	0.651	0.734	0.761
	CR		0.552	0.595	0.504	-0.617	-0.610	-0.587	-0.605	-0.623
	CR/R		0.925	0.996	0.844	-1.033	-1.022	-0.983	-1.013	-1.043
Across years	R	0.536	0.682	0.704	0.583	0.737	0.728	0.754	0.729	0.734
	CR		0.461	0.521	0.366	-0.571	-0.569	-0.541	-0.574	-0.561
	CR/R		0.860	0.971	0.681	-1.065	-1.060	-1.006	-1.071	-1.047
Population 2										
2003-2004	R	0.531	0.466	0.426	0.461	0.634	0.560	0.579	0.635	0.639
	CR		0.281	0.314	0.305	-0.495	-0.468	-0.467	-0.560	-0.539
	CR/R		0.529	0.591	0.575	-0.933	-0.882	-0.879	-1.054	-1.052
2004-2005	R	0.517	0.603	0.589	0.576	0.573	0.484	0.566	0.693	0.675
	CR		0.371	0.366	0.346	-0.476	-0.458	-0.474	-0.533	-0.550
	CR/R		0.717	0.707	0.669	-0.920	-0.885	-0.915	-1.031	-1.063
Across years	R	0.403	0.547	0.615	0.626	0.613	0.430	0.670	0.589	0.594
	CR		0.273	0.292	0.278	-0.410	-0.412	-0.373	-0.466	-0.428
	CR/R		0.678	0.723	0.690	-1.017	-1.023	-0.925	-1.154	-1.061

† Selection parameters were estimated based on the mean values of the SRI from heading and grainfilling stages.

‡ RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio; WI, water index; NWI-1, normalized water index 1; NWI-2, normalized water index 2; NWI-3, normalized water index 3; NWI-4, normalized water index 4.

Table 7. The percentage of selected genotypes among the 25% highest yielding genotypes based on the spectral reflectance indices (SRI), and percent yield difference (values in parenthesis) between yield *per se* and yield estimates based on SRI in two recombinant inbred populations.

Populations	Spectral reflectance indices†							
Population 1	RNDVI	GNDVI	SR	WI	NWI-1	NWI-2	NW1-3	NWI-4
Year 2003-2004	67 (7.0)	67 (5.6)	67 (4.6)	67 (3.6)	67 (3.6)	67 (4.4)	67 (3.5)	67 (4.1)
Year 2004-2005	67 (2.2)	50 (1.9)	50 (5.8)	67 (1.3)	67 (1.4)	67 (1.1)	67 (1.7)	67 (1.4)
Combined year	67 (3.4)	67 (2.4)	50 (5.4)	83 (2.2)	67 (2.3)	83 (2.6)	83 (2.2)	83 (2.7)
Population 2								
Year 2003-2004	33 (7.5)	50 (6.2)	33 (6.0)	50 (4.2)	50 (4.3)	33 (4.7)	67 (4.2)	67 (4.5)
Year 2004-2005	50 (7.3)	33 (7.9)	50 (6.7)	50 (6.2)	50 (6.1)	50 (6.1)	67 (6.2)	67 (6.3)
Combined year	50 (4.9)	33 (5.6)	50 (4.2)	67 (2.3)	67 (2.3)	67 (2.7)	83 (2.3)	83 (2.7)

† RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio; WI, water index; NWI-1, normalized water index 1; NWI-2, normalized water index 2; NWI-3, normalized water index 3; NWI-4, normalized water index 4.

Figures

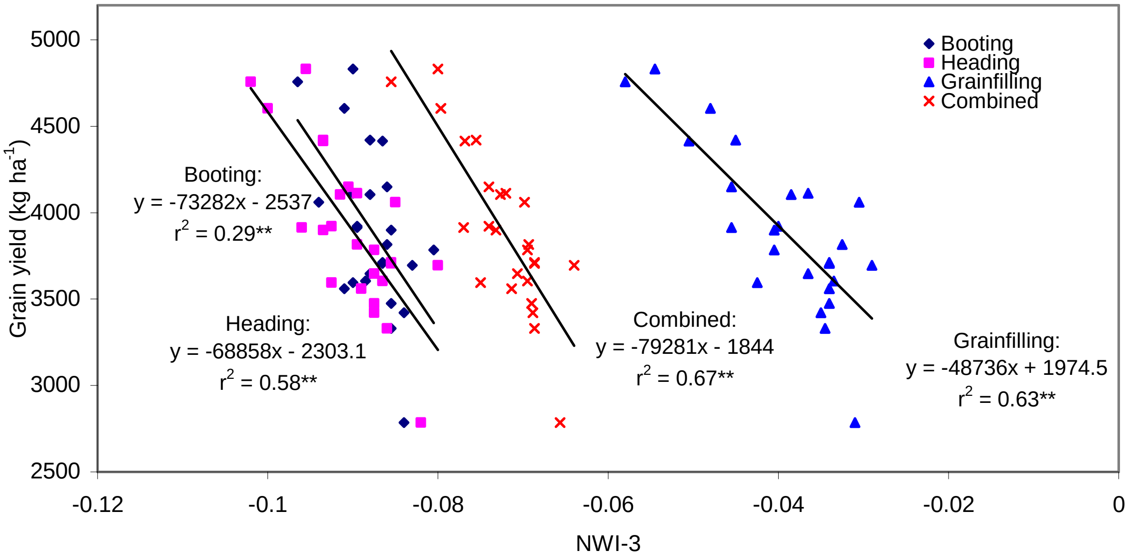

Fig 1: Functional relationship between grain yield and normalized water index-3 (NWI-3) in three growth stages and averaged over growth stages for population 1. **p<0.01.

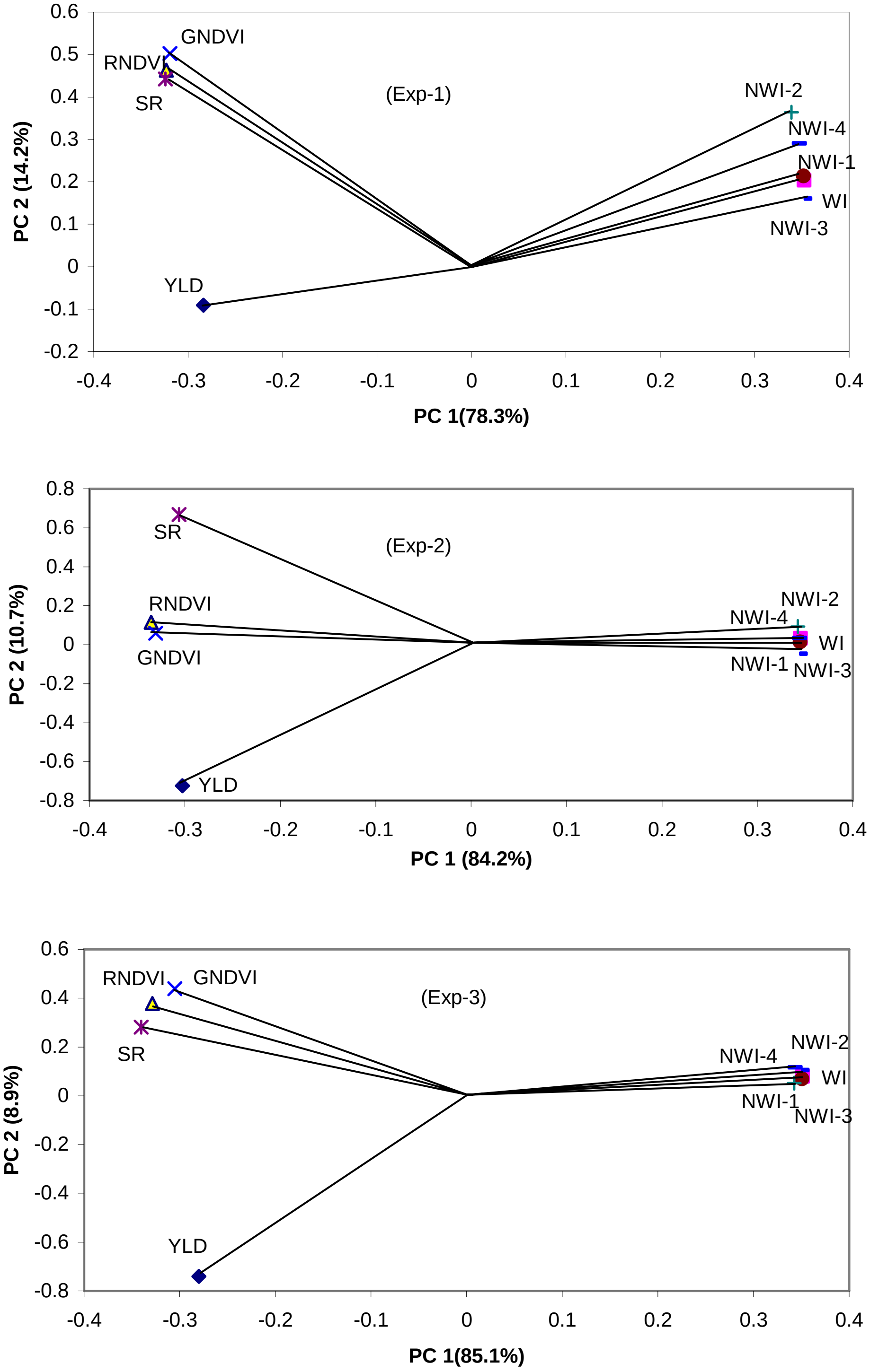

Fig 2. Two dimensional distributions of coefficients of the first two principal components obtained by a multivariate analysis of different spectral reflectance indices (see materials and methods for definitions of the variables) and grain yield for three experiments.

CHAPTER V

Conclusions

The potential of using spectral reflectance indices for differentiating winter wheat genotypes for grain yield and biomass production under U.S. Great Plains rainfed selection environments were demonstrated in our study. The randomly selected recombinant inbred lines developed in the Great Plains established the genetic basis of the true relationship between the SRI and grain yield and biomass production. The water based near infrared indices (WI and NWI) were more effective and efficient in predicting grain yield and biomass than the commonly reported indices (RNDVI, GNDVI, and SR). The indices were related to grain yield and biomass in a linear fashion, confirming their effectiveness for identifying the higher yielding genotypes irrespective of the production potential of the environment. For grain yield, combining reflectance information from three growth stages (booting, heading, and grainfilling) provided better associations compared to any individual growth stage. Whereas, the relationships between the water based indices and biomass were equally effective at all three growth stages, but were more predictable at the reproductive growth stages. We recommend taking at least two spectral reflectance measurements, one at heading and the other at grainfilling, and using the mean of the information from both growth stages for better identification of the genotypes with higher grain yield and biomass production potential. Indices based on the minor water absorption band consistently provided the best relationships with grain yield, and among them, NWI-3 and NWI-4 showed better performance over the other water based SRI. Since total dry matter at the reproductive growth stage is one of the most important determining factors for increased grain yield, the newly developed water based indices can be a good estimator of genotypic potential for higher grain yield via higher dry matter production.

The genetic basis of indirect selection for winter wheat grain yield using SRI was also demonstrated in this study. The water based indices (WI and NWI) showed higher genetic correlations with grain yield compared to the RNDVI, GNDVI, and SR. The water based indices also showed higher heritability than grain yield *per se*, and higher heritability than the widely used vegetation indices. The two newly developed indices (NWI-3 and NWI-4) gave higher direct response and equal or higher correlated responses for grain yield. The relative selection efficiency for grain yield of these two indices was always more than one, which signifies that at least equal or higher genetic gains can be obtained if selection is based on these indices. These newly developed indices also detected a significant proportion of the highest yielding genotypes. Based on the overall genetic analyses of the SRI, greater genetic gains for grain yield can be obtained through the use of the water based indices, especially the two newly developed indices (NWI-3 and NWI-4), and quite certainly these indices can enhance the breeding effort for grain yield in rainfed winter wheat environments in the Great Plains.

VITA

BISHWAJIT PRASAD

Candidate for the Degree of

Doctor of Philosophy

Thesis: **THE POTENTIAL FOR USING CANOPY SPECTRAL REFLECTANCE AS AN INDIRECT SELECTION TOOL FOR YIELD IMPROVEMENT IN WINTER WHEAT**

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Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title: **THE POTENTIAL FOR USING CANOPY SPECTRAL REFLECTANCE AS AN INDIRECT SELECTION TOOL FOR YIELD IMPROVEMENT IN WINTER WHEAT**

Pages in Study: 118 Candidate for the Degree of Doctor of Philosophy

Major: Crop Science

Scope and Methods of Study: Complementing breeding effort by deploying alternative methods of identifying higher yielding genotypes in a wheat breeding program is important for obtaining greater genetic gains. Spectral reflectance indices (SRI) are one of the many indirect selection tools that have been reported to be associated with different physiological process of wheat. A total of five experiments (a set of 25 released cultivars from winter wheat breeding programs of the U.S. Great Plains and four populations of randomly derived recombinant inbred lines having 25 entries in each population) were conducted in two years under Great Plains winter wheat rainfed environments at Oklahoma State University research farms. Grain yield was measured in each experiment and biomass was measured in three experiments at three growth stages (booting, heading, and grainfilling). Canopy spectral reflectance was measured at three growth stages and eleven SRI were calculated. Correlation (phenotypic and genetic) between grain yield and SRI, biomass and SRI, heritability (broad sense) of the SRI and yield, response to selection and correlated response, relative selection efficiency of the SRI, and efficiency in selecting the higher yielding genotypes by the SRI were assessed.

Findings and Conclusions: The genetic correlation coefficients revealed that the water based near infrared indices (WI and NWI) were strongly associated with grain yield and biomass production. The regression analysis detected a linear relationship between the water based indices with grain yield and biomass. The two newly developed indices (NWI-3 and NWI-4) gave higher broad sense heritability than grain yield, higher direct response to selection compared to grain yield, correlated response equal to or higher than direct response for grain yield, relative selection efficiency greater than one, and higher efficiency in selecting higher yielding genotypes. Based on the overall genetic analysis required to establish any trait as an efficient indirect selection tool, the water based SRI (especially NWI-3 and NWI-4) have the potential to complement the classical breeding effort for selecting genotypes with higher yield potential in a winter wheat breeding program.

ADVISOR'S APPROVAL: Arthur R. Klatt