SPECTRAL REFLECTANCE INDICES FOR ESTIMATING YIELD AND WATER CONTENT IN SPRING WHEAT GENOTYPES UNDER WELL IRRIGATED, WATER STRESS, AND HIGH TEMPERATURE CONDITIONS

By

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CHAPTER I

Introduction

Wheat (*Triticum aestivum* L.) is one of the major world crops supplying calories to the human diet and represents 25 percent of total cereal grain production (Reynolds *et al.*, 1999). The world demand for wheat is increasing at a rate of 2% per year; however, the genetic gain through breeding programs has lower rates (Sayre *et al.*, 1997).

Wheat-breeding strategies for developing new genotypes are based on the generation of large numbers of crosses for deriving segregated populations (Jackson *et al.*, 1996). Wheat breeding has been based extensively on the classical empirical approach of grain yield *per se* as the main selection criterion for identifying higher yielding genotypes (Aparicio *et al.*, 2000). A high number of genotypes needs to be evaluated in order to select the best ones for specific environments compared to the commercial cultivars (Ball and Konzak, 1993).

An adequate and alternative breeding strategy is required for a better understanding of the factors responsible for plant development and growth because grain yield in a given environment is directly and indirectly influenced by genetic, morphological, physiological, and environmental factors (Richards, 1996). Even though the genetic basis for yield improvement in wheat is not well established, genetic improvement in yield has been particularly successful for spring wheat under irrigated conditions, and there has also been significant progress under drought and heat stressed environments in the developing world (Richards *et al.*, 2001; Lantican *et al.*, 2002; Trethowan *et al.*, 2002). The use of morpho-physiological parameters could make the yield empirical selection more efficient (Reynolds *et al.*, 2001). The limited application of analytical approaches is probably due to improper knowledge and estimation of the physiological parameters and their genetic associations with grain yield (Richards, 1996).

Some efforts have been made to develop physiological selection criteria such as stomatal conductance, canopy temperature depression, carbon isotope discrimination (CID) of grains, etc. (Reynolds *et al.*, 1999). However, these efforts have been limited to specific environments, while CID is an expensive method.

Canopy spectral plant properties is a new area of research that has great potential as an indirect tool for selecting genotypes for high grain yield and biomass (Araus *et al.*, 2001; Aparicio *et al.*, 2002; Osborne *et al.*, 2002; Royo *et al.*, 2003). Methods that integrate the whole canopy for the yield assessment of many genotypes in a short time are highly desirable because field evaluation of genotypes for several years across locations is expensive and time consuming (Reynolds *et al.*, 1999).

Spectral reflectance of the canopy is based on the principle that leaf pigments (*i.e.*, chlorophyll and carotenoids) absorb light in the visible region (400-700 nm) of the electromagnetic spectrum, while the light is highly reflected in the near infrared region (700-1300 nm), which is influenced by structural components of the leaf tissue (Araus *et al.*, 2001; Peñuelas and Filella, 1998). Spectral reflectance indices (SRI) have been used to estimate diverse physiological traits such as leaf area, photosynthetic capacity, chlorophyll content, and absorbed radiation on plants (Penuelas *et al.*, 1993, Penuelas, 1998; Araus *et al.*, 2001).

Because spectral reflectance indices respond to physiological variables involved in crop growth that determine final grain yield, it is possible to use the indices for yield prediction in wheat, corn, and other crops (Rudorff and Batista, 1990; Wiegand *et al.* 1991). With periodic measurements of reflectance during the growing cycle of a crop, the grain yield and biomass can be predicted (Wiegand *et al.*, 1991; Rudorff & Batista, 1990, Gitelson *et al.*, 1996).

Similarly, it is possible to estimate changes in crop water content in wheat genotypes under water deficit conditions, and use these changes for selecting genotypes that produce high yields under water stress conditions (Penuelas *et al.*, 1993). Plant water content provides information for making irrigation decisions to prevent water deficit stress to the crop and for assessing the crop growth (yield) under drought conditions (Tucker, 1980; Penuelas *et al.*, 1993). Several water indices based on canopy reflectance measurements have been established to assess grain yield using different wavelengths in well irrigated, water stress, and rainfed conditions (Babar *et al.*, 2006a, and Prasad *et al.*, 2007a). This has an important implication in breeding programs because the selection of high yielding lines can be identified easily in different environments.

However, canopy spectral reflectance can be influenced or altered by traits related to the leaf surface (*i.e.*, cutin and wax) (Ribeiro, 2006). Leaf thickness, trichome abundance, and wax composition have an influence on the spectral reflectance pattern in different species (Ribeiro, 2006). Other leaf components such as cellulose and cutin also have also shown some influence on the canopy spectral reflectance pattern (Ribeiro, 1996). The spike reflected more energy in the visible region because of its lower chlorophyll concentrations and distinctive surface properties compared to leaves (Guyot, 1990). The difference in the internal and external morphology of spikes compared to leaves causes variations in the reflectance signals (Riedell and Blackmer 1999).

In summary, spectral reflectance techniques have high potential in breeding programs to identify high yielding wheat lines in diverse environments. Their employment could help us understand physiological changes in plant growth and plant water status (transpiration rate, canopy temperature) that influence final yield under adverse growing conditions. Spectral reflectance can also serve as an indirect selection tool to identify high yielding genotypes more easily and quickly in breeding programs.

CHAPTER Ⅱ

Spectral water indices for assessing yield in elite bread wheat genotypes grown under well irrigated, water deficit stress, and high temperature conditions

Abbreviations

CIMMYT, International Maize and Wheat Improvement

CR, correlated response

CRX/RX, efficiency of indirect selection

ESWYT, 24th Semi-Arid Wheat Yield Trial

GNDVI, green normalized difference vegetation index

h^2, broad sense heritability

HTWYT, 11th High Temperature Wheat Yield Trial

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

r_g= genetic correlation

R, response to selection

SAWYT, 11th Elite Spring Wheat Yield Trial

SR, simple ratio

SRI, spectral reflectance indices

WI, water index

Abstract

Spectral canopy reflectance can be employed for evaluating yield among genotypes for identifying and selecting those lines with high yield potential in wheat. Spring wheat genotypes were evaluated in Northwest Mexico during three growing seasons to determinate the relationship between diverse spectral reflectance indices (SRI) and grain yield, and to evaluate the SRI as an indirect selection tool for breeding purposes based on their genetic correlation, heritability, and correlated response under well-irrigated, water deficit, and high temperature conditions. Diverse advanced lines were used which corresponded to three international trials of the International Maize and Wheat Improvement Centre (CIMMYT); 24^{th} Elite Spring Wheat Yield Trial (ESWYT), 11^{th} Semi-Arid Wheat Yield Trial (SAWYT), and 11^{th} High Temperature Wheat Yield Trial HTWYT). The SRI were determined at three growth stages (booting, heading, and grain filling) during cloud free days using a field portable spectrometer (Analytical Spectral Devices, Boulder, CO) and diverse SRI were estimated. Significant genotypic differences for grain yield and for the SRI were found in the three environments for all the trials. In the diverse environments, the water indices (NWI-1 and NWI-3; normalized water index 1 and 3, respectively) always provided higher correlation with grain yield when heading and grain filling were combined, except for the high temperature environment (HTWYT trial). The vegetative indices RNDVI, GNDVI, and SR; red normalized difference vegetative index, green NDVI, and simple ratio showed inconsistency in their relationship with grain yield in individual years and across years for the well irrigated and water stress environments, but they showed a good association with grain yield in the high temperature environment. The water indices gave higher genetic correlations with grain yield than the vegetative indices in the three trials in all the environments when heading was combined with grain filling across years. Heritability was higher for the vegetative indices than for the water indices in all the environments in spite of their low phenotypic and genetic correlations, but the correlated response was higher for the water indices, except in the water stress environment (SAWYT trial). The relationship between grain yield and canopy temperature determined at grain filling was strongest in the high temperature environment. The high temperature environment also showed the strongest associations between SRI and grain yield demonstrating the potential of

SRI for achieving genetic gains in breeding for warmer climates. In conclusion, the water indices can be used for breeding purposes in well-irrigated, water deficit stress, and high temperature environments for selecting high yielding advanced wheat lines, and canopy temperature could complement and support the selection of high yielding lines.

Introduction

Breeding strategies for wheat (*Triticum aestivum* L.) involve a large number of segregating genotypes that are compared and evaluated for selecting high yielding genotypes among and within segregating populations (Ball and Konzak, 1993). This process also involves a large number of crosses for deriving new genotypes that have to be contrasted with commercial cultivars in specific environments. Selection of breeding lines for grain yield in advanced nurseries often needs repetition to enhance success (Ball and Konzak, 1993). However, this methodology is expensive and a time consuming process because more than one field evaluation must be made during several years and locations.

Wheat breeding around the world for yield improvement has been based primarily on the empirical selection criteria of yield per se; however, yield has demonstrated low heritability and a high genotype-environment interaction (Slafer and Andrade, 1991; Jackson *et al.*, 1996; Trethowan *et al*., 2003). An adequate breeding strategy requires a better understanding of the factors responsible for development and growth because grain yield in a given environment is directly and indirectly influenced by genetic, morphological, physiological, and environmental elements (Richards, 1996). Genetic improvement in yield has been particularly successful for spring wheat in irrigated environments, which mainly has been attributed to better partitioning of photosynthetic products (Calderini *et al.*, 1997; Sayre *et al.*, 1997; Richards *et al.*, 2001; Trethowan *et al.*, 2002). However, there has been significant progress under drought and heat stressed environments in the developing world (Heisey *et al.*, 2002).

Royo *et al.* (2003) indicated that promising high yielding genotypes could be identified in breeding programs before the crop is harvested (yield prediction) and hundreds of high yielding genotypes could be identified in segregating populations. For reducing the laborious and time-consuming process of yield selection, an easy, rapid, and inexpensive selection tool is desirable for helping breeders to screen a large number of genotypes in a relatively short time (Reynolds *et al.*, 1999). In addition, this selection tool would need to have high heritability and a strong correlation with grain yield for detecting high yielding genotypes rapidly and efficiently from a large number of early-generation lines and for advanced genotypes. Breeders often need to identify the very best yielding genotypes from among a sample of already superior lines and a method that integrates the whole canopy is highly desirable to assess many genotypes in a short time (Reynolds *et al.*, 1999). Several authors have been employing some physiological traits to improve grain yield in diverse environments like canopy temperature, which has shown a high association with grain yield in spring wheat genotypes in irrigated high-radiation environments (Reynolds *et al*., 1994, 1999). Carbon isotope discrimination (CID) is another method used successfully improve grain yield potential in wheat under water deficit environments (Condon *et al.*, 2002; Condon *et al*., 2004). However, CID determinations resulted expensive and time consuming process. Spectral reflectance indices are a potential technique that could assess yield at the genotypic level without destructive sampling (Reynolds *et al.*, 1999).

Assessments based on remote sensing techniques (canopy spectral reflectance) measured in the visible [400-700 nm], near-infrared [700-1200 nm], and mid-infrared [>1200 nm] regions) are convenient because they are noninvasive, and easy to use (Field *et al.,* 1994; Reynolds *et al.*, 1999; Araus *et al.*, 2001). Canopy reflectance properties are based mainly on the absorption of light at specific wavelengths associated with plant characteristics (Araus *et al.*, 2002). In the visible region, reflectance is relatively low because the light is absorbed by leaf pigments (chlorophyll, carotenoid and anthocyanins). In contrast, the reflectance in the NIR wavelengths is high because the radiation is scattered by plant tissue structures in the canopy.

Several spectral reflectance indices (SRI) have been established for estimating physiological traits and for predicting yield by periodic measurements of reflectance during the plant development in diverse crops (Rudorff and Batista, 1990; Wiegand *et al.,* 1991). The most commonly known index for analyzing vegetation is the normalized difference vegetation index (NDVI; $[R_{900}-R_{680}]/[R_{900}+R_{680}]$) (Araus *et al.,* 2001) used as an indirect assessment of canopy biomass, leaf area index, light-absorption, and potential photosynthetic capacity (Peñuelas, 1998; Araus *et al.,* 2001). Reynolds *et al.* (1999) found an association between NDVI and yield and biomass (r^2=0.36-0.44) in bread wheat genotypes in an irrigated environment. The red NDVI (RNDVI, $[R_{780}-R_{670}]/[R_{780}+R_{670}]$) and the green NDVI (GNDVI, $[R_{780}-R_{550}]/[R_{780}+R_{550}]$) have been established for estimating canopy photosynthetic area for predicting grain yield and biomass in wheat and corn under water stressed environments (Gitelson *et al.*, 1996; Raun *et al.*, 2001; Shanahan *et al.,* 2001; Gutierrez-Rodriguez *et al.*, 2004; Osborne *et al.*, 2002). The simple ratio (SR, R_{900}/R_{680}) is also used as an indicator of canopy photosynthetic active area (Aparicio *et al.*, 2000). Other studies in durum wheat genotypes have demonstrated a strong association ($r^2>0.80$) between several SRI (*i.e.*, NDVI, SR) and grain yield and biomass under rainfed and irrigated conditions (Aparicio *et al.* 2002; Royo *et al.,* 2003).

Similarly, it is possible to estimate the canopy water content using SRI (Peñuelas *et al.*, 1993). The water index (WI, R_{970}/R_{900}) proposed by Peñuelas *et al.* (1993) is an indicator of the plant water status at the leaf and canopy level. It can assess the changes of relative water content, leaf water potential and stomatal conductance when water stress is considerable (Peñuelas *et al.*, 1993). Babar *et al.*, (2006a) proposed two normalized water indices (NWI-1=$[R_{970}-R_{900}]/[R_{970}+R_{900}]$ and NWI-2=$[R_{970}-R_{850}]/[R_{970}+R_{850}]$) based on the water index proposed by Peñuelas *et al.* (1993) for screening grain yield in spring wheat. Two other normalized water indices (NWI-3=$[R_{970}-R_{880}]/[R_{970}+R_{880}]$ and NWI-4=$[R_{970}-R_{920}]/[R_{970}+R_{920}]$) were proposed for screening grain yield in winter wheat (Prasad *et al.*, 2007a). These five water indices based on NIR wavelengths can be used for predicting yield because they have shown strong relationships with grain yield in spring and winter wheat genotypes (r=-0.40 to -0.88) over time under well irrigated, water deficit stress, and rainfed conditions (Babar *et al.*, 2006a, b; Prasad *et al.*, 2007b). Genetic variation for biomass production and canopy temperature in spring wheat can also be effectively estimated under irrigated conditions using the water indices (Babar *et al.*, 2006c). The water indices explained a large part of grain yield variability and they are an alternative breeding/selection tool for grain yield in different breeding lines (Babar *et al.*, 2006a; Prasad *et al.*, 2007a).

An alternative indirect selection for grain yield is appropriate if the genetic correlation between the selected and unselected traits is high and if heritability is higher for the selected trait than for the unselected trait (Falconer, 1989). Indirect selection is based on the fact that the primary trait (yield) and the secondary trait (SRI) are subjected to the same selection pressure in the same environment. Reynolds *et al.* (1998) found that canopy temperature explained the grain yield variation in diverse spring wheat genotypes and it was easier, cheaper and quicker to measure in the field than grain yield.

The wheat breeding program at the International Maize and Wheat Improvement Center (CIMMYT) releases advanced breeding lines every year for developing countries where spring wheat is grown (Trethowan and Crossa, 2007). The international yield trials distributed include the Elite Spring Wheat Yield Trial (ESWYT), Semi-Arid Wheat Yield Trial (SAWYT), High Temperature Wheat Yield Trial (HTWYT) and others (Trethowan and Crossa, 2007; Lage *et al.*, 2008). The ESWYT includes advanced breeding lines that are targeted to highly productive irrigated wheat production areas; the SAWYT includes advanced lines for the semi arid regions, and the HTWYT has advanced lines for heat-stressed areas (Lillemo *et al.*, 2004; Trethowan and Crossa, 2007). High yielding and well adapted lines have been derived for many areas where spring wheat is grown in developing countries (Trethowan *et. al.*, 2002).

The main goal of the present work is to determinate the relationship between diverse SRI, especially for the normalized water indices, and grain yield in advanced breeding lines that were included in the 24th Elite Spring Wheat Yield Trial (ESWYT), 11th Semi-Arid Wheat Yield Trial (SAWYT), and 11th High Temperature Wheat Yield Trial (HTWYT) determined at three growth stages (booting, heading, and grain filling). Secondly, to evaluate the potential of the SRI as an indirect selection tool based on their genetic correlation, heritability, and correlated response for breeding purposes under well-irrigated, water deficit, and high temperature conditions during three growing seasons.

Materials and Methods

Experimental materials

Bread wheat genotypes (*Triticum aestivum* L.) from CIMMYT (International Maize and Wheat Improvement Center) were used for this study. The genetic materials represented advanced breeding lines developed by CIMMYT that corresponded to three international trials; 24^{th} ESWYT (25 genotypes) represented advanced lines developed for irrigation conditions, 11^{th} SAWYT (40 genotypes) represented advanced lines for reduced irrigation, and 11^{th} HTWYT (18 genotypes) represented advanced lines for high temperature conditions (Elite Spring Wheat Yield Trial, Semi-Arid Wheat Yield Trial, and High Temperature Wheat Yield Trial, respectively). The ESWYT genotypes were planted under well irrigated conditions, SAWYT genotypes under well irrigated and water stress conditions, and HTWYT genotypes under water stress, high temperature and well irrigated conditions. The genetic materials were previously selected for desirable agronomic traits and grain yield potential for each environment.

Growing conditions

The genotypes were grown during the winter season at CIMMYT's experiment station in Cd. Obregon, Northwest Mexico (27.3^{o}N, 109.9^{o}W, 38 m above sea level). The weather is mostly sunny and dry during the winter cropping cycle and hot for the April-June months (Table 1). The soil type is coarse sandy clay, mixed montmorillonitic type caliciorthid, low in organic matter and slightly alkaline (pH 7.7) in nature (Sayre *et al.*, 1997).

The seeding rate for each experiment was 78 kg ha^{-1}. Nitrogen and phosphorous were applied to the plots at the rate of 150 kg ha^{-1} and 22 kg ha^{-1}, respectively. Field plots consisted of two raised beds 5 m long (80 cm width each) with 2 rows, 10 cm apart on each bed. An alpha lattice design with 2 repetitions was employed for all experiments.

For the well irrigated and water stress experiments, the planting dates were in November and plants reached booting and heading during February-March. For the experiments under high temperature conditions, the genotypes were planted in February and plants reached booting and heading in April-June (ambient temperature around 30-35^{o}C) (Table 1). There were three crop growing years for all experiments planted in the fall that are referred to as years; 2006 for the cycle 2005-2006, 2007 for the cycle 2006-2007 and 2008 for the cycle 2007-2008. The HTWYT trial under water stress conditions was grown only in 2007 and 2008. The HTWYT under late sowing for high temperature conditions was grown in all three growing cycles (2006, 2007 and 2008).

Flood irrigation was applied every 20-25 days for well-irrigated treatments. In trials subjected to drought stress conditions, one irrigation was applied before seeding (providing approximately 100 mm of available water), and two irrigations of 50-70 mm prior to the booting stage. For the trial of high temperature conditions, irrigations were also applied as needed to prevent drought stress.

Folicur (Tebuconazole) was applied at the booting and heading-grain filling stages at the rate of 0.5 L ha^{-1} to protect the experimental materials from leaf rust (caused by *Puccinia triticina*)

Spectral reflectance measurements

Canopy reflectance was measured in the 350 to 1100 nm range, collected at 1.5-nm intervals using a FieldSpec spectroradiometer (Analytical Spectral Devices, Boulder, CO). Data were collected during cloud-free days at solar noon between (10:30 and 14:00 hrs) and a previous calibration was carried out using a white plate of barium sulphate ($BaSO_4$) that provides maximum irradiance (Labsphere Inc., North Sutton, USA). Four measurements in each plot were taken at heights of 0.5 m above the canopy with a field of view of 25^o. Each reflectance measurement was the average of 10 scans from an area of 18.94 cm^2 of the plot. The sensor was mounted with the help of a pistol grip approximately 50 cm above the canopy facing the center of the plot. Canopy reflectance measurements were taken at random places in each plot during booting, heading and grain filling growth stages.

Eight SRI were calculated following the equations with wavelengths (nm) described by several authors. Three vegetative indices were estimated; red normalized difference vegetative index ($RNDVI=[R_{780}-R_{670}]/[R_{780}+R_{670}]$), the green NDVI ($GNDVI=[R_{780}-R_{550}]/[R_{780}+R_{550}]$) and simple ratio ($SR=R_{900}/R_{680}$) (Gitelson *et al.*, 1996; Aparicio *et al.*, 2000; Raun *et al.*, 2001). The water index proposed by Peñuelas *et al.* (1993) was estimated ($WI=R_{970}/R_{900}$) and four normalized water indices proposed by Babar *et al.* (2006a) and Prasad *et al.* (2007a) ($NWI\text{-}1=[R_{970}-R_{900}]/[R_{970}+R_{900}]$; $NWI\text{-}2=[R_{970}-R_{850}]/[R_{970}+R_{850}]$, $NWI\text{-}3=[R_{970}-R_{880}]/[R_{970}+R_{880}]$ and $NWI\text{-}4=[R_{970}-R_{920}]/[R_{970}+R_{920}]$) were also estimated.

Estimation of genetic correlations

Genetic correlations between traits were estimated using the SAS software with proc mixed, following the method described by Singh and Chaudhary (1977) (SAS Inst., 2001). The formula used to estimate genetic correlation was:

$r_g = (CovXY)/(\sqrt{VarX \bullet VarY})$

where Var and Cov, respectively, refer to the components of variance and covariance.

The genetic correlations between grain yield and the SRIs were estimated within each growing year (2006, 2007 and 2008) and across years in all the trials and environments for all SRI in each growth stage (booting, heading, grain filling stages) and by combining them.

Broad-sense heritability

To calculate broad-sense heritability, the variance components associated with genotype (σ^2_g), genotype x year interaction (σ^2_{gy}), and residual (σ^2_e) were estimated for all SRI. The broad-sense heritability within and across years was estimated by using the following formulae:

Heritability (within year), $h^2 = (\sigma^2_g)/(\sigma^2_g + \sigma^2_e)$

Heritability (across years), $h^2 = (\sigma^2_g)/([\sigma^2_g + \sigma^2_{gy}]/[y + \sigma^2_e / y \bullet r])$

where y and r are the number of years and replications, respectively.

Estimation of selection response, correlated response, and efficiency of indirect selection

Expected response to selection (*R*), correlated response to selection (*CR*), and efficiency of indirect selection (*CR*X/*R*X) were estimated according to Falconer (1989), and are described below:

$R = h^2_x \sigma_x$

where h^2_x and σ_x are the heritability and phenotypic standard deviation values for trait X, respectively:

$CR = h_x h_y r_g \sigma_y$

where h_x and h_y are the square root of the heritability of trait X and Y, respectively; r_g is the genetic correlation between trait X and Y; and σ_y is the phenotypic standard deviation for trait Y.

The efficiency of indirect selection using SRI was as follows:

$CRX/RX = h_y r_g / h_x$

Mean values of SRI combining heading and grain filling and grain yield were obtained across years for all the experiments for R, CR, and CR/R.

Selection of high and low yielding lines (25% range)

Selection for the 25% highest yielding and 25% lowest yielding genotypes was made according to Prasad *et al.* (2007b). The genotypes were ranked according to grain yield and SRI across two growth stages (heading and grain filling). Grain yield differences between the genotypes of the two selection groups were based on the 25% highest and the 25% lowest SRI values. Percent of yield differences were estimated between yield per se and yield estimates based on different SRI.

Grain yield

In all experiments grain yield was measured after physiological maturity by harvesting and threshing the four rows of the plot, excluding a 0.5-m border at each end. Prior to grain harvest, a random subsample of 100 spike-bearing culms was removed from the plot. The subsample was oven-dried, weighed, and threshed. The grain weight was recorded and individual kernel weight estimated using a subsample of 200 kernels.

Canopy temperature

During the grain filling stage a hand-held infrared thermometer (Mikron M90 Series, Mikron Infrared Instrument Co. Inc., Oakland, NJ) was used to measure canopy temperature depression. The difference in temperature between the canopy and air was estimated with a thermistor built into the infrared thermometer. The mean of four readings was obtained from the same side of each plot at an angle of approximately 30^o with respect to the horizontal angle in order to integrate many leaves without viewing the soil. The measurements were taken during the afternoon (13:00-14:00 h) when the crop was experiencing maximum transpiration rates.

Statistical analysis

The three experiments were analyzed according to the alpha lattice design by using proc mixed in the SAS program for each growth stage, growing year, and combining growth stages and years (SAS, 2001). Pearson correlation coefficients were used to estimate the phenotypic relationship of spectral reflectance indices and yield and other parameters. In addition, the genetic correlation between traits was also estimated using proc mixed following the method described by Singh and Chaudhary (1977). A multiple regression analysis was conducted using Proc Stepwise for the SRI and grain yield.

Data from the different SRI, canopy temperature and grain yield for each environment and trial were analyzed by principal component analysis (PCA) using SAS. The PCA was conducted using mean of heading and grain filling combined for the SRI, canopy temperature at grain filling, and grain yield averaging the three growing seasons (2006, 2007, and 2008), except for the HTWYT trial in water stress conditions (2007 and 2008). The PCA was conducted for ESWYT (well irrigated), SAWYT (well irrigated, water stress, and combining both growth conditions), and HTWYT (well irrigated, water stress, high temperature, and combining the three growth conditions).

Results

The SRI were classified into two groups; one group called vegetative indices included the visible and NIR wavebands (RNDVI, RNDVI and SR), and another group called water indices that only included NIR wavebands primarily based on the 970 nm water absorption band (WI, NWI-1, NWI-2, NWI-3, NWI-4). However, two normalized water indices (NWI-1, NWI-3) gave the best relationship with grain yield. These two water indices were better than the other three (WI, NWI-2 and NWI-4) in their relationship with grain yield, generally by 3 to 10% across years. As a consequence, we decided to discuss only these two water indices (NWI-1 and NWI-3) instead of all of the other water indices estimated. Because of the minimal differences between SRI in each group, the results will be discussed primarily on the basis of water indices versus vegetative indices. When significant differences occurred within the group, they will be indicated and discussed.

Other well known SRI indices were also analyzed in a multiple regression but their individual association was lower than the vegetative and water indices in explaining grain yield variations in the three environments in each international trial. These spectral indices were the ratio analysis of reflectance spectra for chlorophyll a (RARSa), for chlorophyll b (RARSb) and for carotenoids (RARSc), the structural independent pigment index (SIPI), the photochemical reflectance index (PRI), and the normalized phaeophytinization index (NPQI).

Genotypic variation for spectral reflectance indices and grain yield

Significant genotypic differences for grain yield ($p \leq 0.05$and 0.01) were found for the trials ESWYT, SAWYT and HTWYT under well irrigated, water stress and high temperature conditions (Table 2). The only exception was for the combined years in the HTWYT under water stress conditions. The lack of significant genetic differences across years was caused by the minimal differences in grain yield among genotypes for the year 2007 (0.98-1.80 t ha^{-1}), while the range was wider and higher for the year 2008 (2.89-4.31 t ha^{-1}). As a consequence, when both years were combined, the average yield did not give significant differences.

Also, genotypic differences were found for nearly all SRI at different crop growth stages (booting, heading and grain filling) for the ESWYT, SAWYT and HTWYT trials in the three environments (Table 3). The only exception was for the booting stage in the SAWYT under water stress conditions for the water indices. The vegetative indices were higher at booting and lower at the heading and grain filling stages. In contrast, the water indices were lower at booting and higher at the heading and grain filling stages.

Interaction between genotypes, growth stage, and years

The ANOVA revealed that genotypes and growth stage main effects were significant in the three environments for all SRI (data not shown). Also, the growth stage by genotype interaction was significantly different for the well irrigated and high temperature environment for all the SRI, but not for the water stress environment for the SAWYT and HTWYT trials where the water indices were mainly not significant. The main effect of years also showed significant differences in all the environments, as well as the interaction between years, genotypes, and growth stage.

Phenotypic correlation between spectral reflectance indices and grain yield

The water indices always exhibited a negative association with grain yield in each individual experiment in every year and across years in the three environments when the association was significant. On the other hand, vegetative indices always showed positive correlations with grain yield.

In the ESWYT trial under well irrigated conditions, the association between the water indices and grain yield showed a higher relationship with grain yield at heading and grain filling compared to booting in every year and across years (Table 4). In contrast, the vegetative indices showed low correlation coefficients, especially in the year 2006 and 2007, but if the SRI were combined over years they showed a significant association with grain yield. The relationship between all SRI and grain yield was low and not significant for the growing year 2006, but combining the SRI over the three years resulted in a significant relationship. When the growth stages were combined, the correlations coefficients were slightly higher or similar to the highest correlation coefficient of any individual growth stage. The weak correlation values obtained with the booting stage affected the association when booting, heading and grain filling were combined in each year and across years. The relationship between the water indices and grain yield was much stronger when the SRI at heading and grain filling were combined, and the water indices gave higher associations than the vegetative indices.

In the SAWYT trial under well irrigated and drought conditions, the relationship between the water indices and grain yield generally showed a higher association at heading, grain filling, and by combining the two growth stages in every year and across years compared to the booting stage (Table 4). The low correlation values at booting in most growing seasons negatively affected the association of average SRI with grain yield when the three growth stages were combined (booting, heading and grain filling) in both environments for the SAWYT trial. For the water stress environment, the combination of heading and grain filling showed a stronger association each year and across years, except for the year 2007. In comparison, the vegetative indices showed a low association with grain yield among years and across years under well irrigated conditions, with some exceptions in the year 2008. Under water stress conditions, the vegetative indices showed some significant correlation coefficients with grain yield in the three growing seasons; however, across years the vegetative indices did not show any strong relationship with grain yield. The vegetative indices were inconsistent in their relationship with grain yield in the different growing years and across years for the two environments, while the water indices showed a better and more consistent association at heading, grain filling and by combining heading and grain filling.

In the HTWYT trial, the association between the water indices and grain yield also showed a higher relationship at the heading and grain filling stages across years except for the year 2007 under well irrigated conditions (Table 5). Under water stress conditions, this association at the booting stage was lower compared to the heading and grain filling stages, but significant for the majority of years. When the two years were combined (2007 and 2008) for the water stress environment, the booting stage gave similar correlation coefficients than the correlation coefficients obtained by combining growth stages. For the high temperature environment, booting, heading and grain filling stages did not show big differences in each year or across years. The highest association (highly significant at $p \leq 0.01$) between the water indices and grain yield was obtained in this environment. In contrast, the vegetative indices generally showed a low relationship with grain yield under well irrigated conditions with some exceptions in the year 2007. The same pattern occurred under water stress conditions with some significant associations in the year 2008, but across years the association with grain yield was lower and not significant. Under high temperature conditions, the three vegetative indices gave a very strong association with grain yield, but always lower than the water indices. When growth stages were combined for the water indices, the three growth stages (booting, heading and grain filling) were generally lower than averaging two growth stages (heading and grain filling) in the irrigated environment, but the differences between three and two growth stages combined were minimal in the water stress and high temperature environment, especially across years.

The mean grain yield and mean index value of NWI-3 for three years combining heading and grain filling for the ESWYT, SAWYT and HTWYT trials under well irrigated, water stress and high temperature conditions are shown in Fig. 1. The two water indices; NWI-1 and NWI-3, showed minimum differences in their relationship with grain yield for the three environments in all the trials, but NWI-3 generally gave a slightly higher association across years when combining heading and grain filling. The relationship between NWI-3 and grain yield was described by a linear model, and the strongest relationship was obtained in the high temperature environment for the HTWYT trial.

A multivariate approach was conducted to compare the relationship of all SRI with grain yield (Fig. 2). The two water indices (NWI-1 and NWI-3) were spread in a negative direction while grain yield and the vegetation indices were spread in a positive direction in the three environments. Uncorrelated variables in a biplot are at 90^{o} while a bigger or smaller angle indicates a higher association. The principal component analysis revealed that the water indices had a stronger relationship with grain yield (negative correlation) compared to the vegetation indices in every environment (well irrigated, water stress and high temperature). When the SRI where analyzed combining environments for the SAWYT (well irrigated and water stress) and the HTWYT (well irrigated, water stress and high temperature), NWI-3 and canopy temperature maintained significant relationships with grain yield in both trials, while RNDVI had a lower relationship (Fig. 3).

Genetic correlation between spectral reflectance indices and grain yield

The water indices gave significant genetic correlations with grain yield in the three trials (ESWYT, SAWYT and HTWYT) under different growing conditions (well irrigated, water stress and high temperature conditions) when heading was combined with grain filling in each year and across years (Table 6). The genetic correlation ranged from -0.31 to -0.95 for the water indices in the three environments, while the vegetative indices showed a few significant genetic correlations for the well irrigated and water stress conditions. However, they showed a highly significant relationship with grain yield in the high temperature environment, but the genetic correlation coefficients were lower than the water indices. The same behavioral relationship occurred each year and across years in the different trials and growth conditions and a similar pattern was obtained when individual growth stages were tested (data not shown). In all environments, the genetic correlation values were higher than the phenotypic correlations in every year and across years for both groups of SRI.

Heritability, selection response, correlated response, and relative selection efficiency

The water indices gave moderate to high heritability values in all environments, with a range of 0.41 to 0.96, and the vegetation indices showed heritability values that ranged from 0.48 to 0.96 (Table 7). Even though the vegetative indices gave low and moderate phenotypic and genetic correlations in some years under well irrigated and water stress conditions, they showed high heritability. The heritability was higher for the vegetative indices than for the water indices in all the environments. Grain yield heritability generally showed high values for every year and across years in the three environments for the three trials.

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 8 for heading and grain filling averaged across years. In general, the vegetative indices showed higher selection response compared to grain yield and the water indices in the three environments across years. However, the correlated response was higher for the water indices compared to the vegetative indices except for the water stress environment in the SAWYT trial where both SRI groups showed low values. The relative selection efficiency of vegetative indices was low due to low values of correlated response. In contrast, the water indices showed high correlated response values under well irrigated and high temperature conditions, but not for the water stress environment. The relative selection efficiency gave significant relationships for the water indices in the three environments, except for the HTWYT under water stress conditions.

Genotype selection using the water indices and grain yield

When the selection was based on the 25% highest and the 25% lowest using the two water indices (NWI-1 and NWI-3), the percentage of comparable lines selected by grain yield and by SRI were from moderate to high (Table 9). These two water indices performed better than the vegetative indices that gave low and inconsistent relationship with grain yield (data not shown). In addition, both water indices gave similar trends when individual growth stages were considered, but the combination of heading and grain filling always worked better for all the trials in the three environments. When the 25% highest yielding genotypes and the 25% lowest yielding genotypes from the ESWYT under well irrigated conditions were identified, the efficiency of selection ranged from 17-83% across years, for the SAWYT under well irrigated conditions it ranged from 10-80%, from 20-80% under water stress conditions, for the HTWYT under well irrigated conditions the efficiency was from 20-80%, from 20-80% under water stress conditions, and from 40-100% under high temperature conditions. Once again, the best results for selecting high yielding genotypes and/or for rejecting low yielding genotypes were obtained in the high temperature environment.

Phenotypic correlation between spectral reflectance indices and grain yield across environments

Because of a stronger relationship between the water indices and grain yield compared with the vegetative indices, the water indices measured in one environment for the same trial were correlated with grain yield of another environment. The water indices were averaged combining the heading and grain filling stages across years. An association between the water indices measured in one environment and the yield of the same genotype in another environment would mean that the water indices could be used to predict yield in diverse environments.

For the SAWYT trial, the water indices for irrigated conditions compared to the grain yield under water stress gave a low relationship (data not shown). Similar results were obtained by combining the opposite relationship. For the HTWYT trial, the water indices for irrigated conditions compared with its yield in the high temperature environment resulted in a significant relationship (Table 10). The opposite combination between the water indices under high temperature conditions and the yield in the irrigated environment resulted in lower correlation values (not significant). Other comparisons resulted in low relationships.

Interseason correlation for grain yield

The interseason correlation among years for grain yield resulted in a significant relationship for the ESWYT and SAWYT trials for the well irrigated environments (Table 11). For the same environment, the correlation only resulted significant between the years 2006 and 2008 in the HTWYT trial. In the water stress environment, the interseason correlation for grain yield gave the lowest correlation values for the SAWYT and HTWYT trials even though the correlations were significant for the SAWYT trial. Finally, in the high temperature environment, the interseason correlation resulted significant for the three years in the HTWYT trial.

Canopy temperature and grain yield

The association between grain yield and canopy temperature determined at grain filling resulted in some significant associations for the ESWYT and SAWYT trials under well irrigated conditions in every year and across years (Table 12). In the water stress environment, the relationship with grain yield was significant for two years and across years in the SAWYT trial and only for one year in the HTWYT trial. For the high temperature environment, the relationship between canopy temperature and grain yield was highly significant for every year and across years. This association showed a similar pattern to the relationship between water indices and grain yield because the strongest association was obtained in the high temperature environment (Fig. 2). When the diverse environments were combined in the SAWYT (well irrigated and water stress) and HTWYT (well irrigated, water stress and high temperature), canopy temperature showed a lower relationship with grain yield for the SAWYT trial than for the HTWYT trial (Fig. 3).

Discussion

Genotypic variation and growth stages

Significant genotypic variation for grain yield was found in this study for the three trials under well irrigated, water deficit stress and high temperature conditions (Table 2). Also, we observed a wide range of genetic variation for the different SRI in the three environments at different growth stages referred to as booting, heading, and grain filling stages confirming the existence of sufficient genetic variation in each trial for SRI and yield (Table 3). Similar variation has been reported in earlier studies under irrigated conditions in spring wheat (Babar *et al.*, 2006a), under water deficit stress conditions (Gutierrez-Rodriguez *et al.*, 2004; Babar *et al.*, 2006b), under rainfed conditions in durum wheat (Royo *et al.*, 2003), and under rainfed conditions in winter wheat (Prasad *et al.*, 2007a).

Interaction between genotypes, growth stage, and years

In this study, we observed significant interaction between growth stages and genotypes in regard to their SRI values. The interactions of growth stages by genotype suggests that the growth stage for predicting yield based on SRI needs to be identified with caution for accurately selecting high yielding genotypes in breeding programs (Babar *et al.,* 2006a,b; Prasad *et al.*, 2007a). Other studies have also reported a significant interaction between growth stages and spectral indices (NDVI's and SR and water indices) in spring, winter, and durum wheat (Aparicio *et al.*, 2002; Babar *et al.,* 2006a,b; Prasad *et al.*, 2007a).

Phenotypic correlation between spectral reflectance indices and grain yield

Our results showed that the vegetation indices (RNDVI, GNDVI, and SR) generally had positive correlation coefficients with grain yield (Tables 4, 5). Similar positive associations have been reported in spring wheat, durum winter and winter wheat (Royo *et al.*, 2003; Babar *et al.*, 2006a, b, c; Prasad *et al.*, 2007a). In contrast, the water indices (NWI-1 and NWI-3) always showed strong negative correlations with grain yield in all the three environments tested. The negative association between the water indices and grain yield has previously been reported under well irrigated and water stress conditions for spring wheat (Babar *et al.*, 2006a, b) and for winter wheat under rainfed conditions (Prasad *et al.*, 2007a). Peñuelas *et al.* (1993, 1997) reported an inverse relationship between the water indices and water potential, relative water content, and leaf temperature in wheat and other crops. A decrease in plant water content causes an increase in the amount of light reflected at 970nm, and lower water content in the canopy results in lower grain yield (Babar *et al.*, 2006a, b; Prasad *et al.*, 2007a). Moreover, the association between the water indices and grain yield indicates that canopy water content plays a vital role in yield among genotypes under diverse growth conditions (Babar *et al.*, 2006b; Prasad *et al.*, 2007a). One advantage of the water indices is that the NIR wavelengths penetrate deeper into the canopy for estimating the water status and for indicating a higher water content at heading (14 to 22%) than at the grain filling stage in spring wheat (Babar *et al.*, 2006c).

Phenotypic correlations between grain yield and the water indices in our study were stronger when heading and grain filling were combined for the well irrigated, water stress and high temperature environments. The water indices always provided a higher association with grain yield compared to the three vegetative indices that showed inconsistency in their relationship with grain yield in the well irrigated and water stressed environments (Table 4, 5). The association between the SRI combining heading and grain filling across years is clearly observed for each trial and environment (Fig. 2). Two first principal components explained more than 80% of the variance for the well irrigated and water stress environments (ESWYT, SAWYT, and HTWYT), and explained 95% of the variance in the high temperature environment (HTWYT). It is clear that the water indices showed a higher relationship than the vegetative indices in all the environments. This indicates that the water indices explained a large amount of the variation related to grain yield among genotypes that was not caused by or derived from environmental effects. The combination of SRI from three growth stages gave lower correlations for the well irrigated and water stress environments, while under high temperature conditions combining two or three growth stages gave similar results (Table 5). We are assuming that growth development under high temperature is accelerated resulting in major morphological differences among genotypes during booting, heading and grain filling in the HTWYT trial. Other studies have shown that combining SRI across two growth stages (heading and grain filling) gave a better relationship with grain yield than any individual growth stage in spring and winter wheat (Babar *et al.*, 2006a, b; Prasad *et al.*, 2007b). The genetic variability for grain yield can be estimated by determining canopy reflectance during the heading and grain filling stages, and by combining the SRI from both growth stages, and yield prediction can be further improved in diverse environments (Babar *et al.*, 2006a, b, c). Prasad *et al.* (2007a) postulated that the overall fitness of a genotype can be determined over time by estimating the water indices at anthesis and grain filling.

The five water indices gave similar correlation values with grain yield but the NWI-1 and NWI-3 showed a slightly higher association with grain yield (Fig. 1, 2). Babar *et al.* (2006a) reported that the normalization of the water index did not give better results for predicting yield in spring wheat under optimal or adverse growing conditions. However, in our study, the NWI-1 and NWI-3 gave better results (3-10%) in their association with grain yield compared to the water index proposed by Peñuelas *et al.* (1993).

For most SRI, genotypes cannot be distinguished from one another at the booting stage and therefore, gave a low association with grain yield, especially under irrigated and water stress conditions (Table 4, 5). This could be attributed to the morphological uniformity of leaves (no presence of reproductive organs) and large leaf area index (LAI), which over shadowed the differences among genotypes. Aparicio *et al.* (2000), found that diverse SRI (NDVI, SR, and WI) did not show significant differences among wheat genotypes at the booting stage due to large LAI, which normally reaches maximum values at this growth stage. The presence of spike and differences in its size increases morphological variation among genotypes at heading and during grain filling derived from a decrease of LAI. Several authors have reported that genotypic variability increased as the crop growth progressed because of spike size and/or its morphology (Asrar *et al.*, 1984; Ahlrichs and Bauer, 1983). In our study, the entries in the ESWYT trial showed a low relationship between the water indices and grain yield in the well irrigated environment for the year 2006. Probably, large LAI caused low morphological genotypic differences at booting, heading and grain filling because grain yield had the lowest range in the year 2006 compared to the other two years (2007 and 2008) (Table 2, 4). In contrast, the strongest relationship between the water indices and grain yield was obtained for the year 2008 when grain yield had the biggest range. When plants showed a wider range in grain yield, it suggests major differences in LAI compared with the year 2006.

Even though the vegetative indices (mainly NDVI's) have been reported to have significant correlations with grain yield in bread and durum wheat under well watered and water deficit stress conditions (Ball and Konzak, 1993; Raun *et al.*, 2001; Royo *et al.*, 2003), our results indicated that the vegetative indices performed inconsistently in these environments (Table 4, 5). The vegetative indices cannot be used for predicting yield under well-irrigated and water stress conditions for the advanced lines of the ESWYT and SAWYT trails that we tested. We don't have a clear explanation why the association with grain yield was generally low for both environments. However, they gave a similar association with grain yield compared to the water indices in the high temperature environment. In this environment, the association for all SRI resulted highly significant for the vegetative and water indices during the three seasons. Under high temperature conditions, plant growth is accelerated and we assume that the HTWYT genotypes had a major genotypic diversity for LAI compared to the well-irrigated and water stress conditions. Of course, the morphological differences are also associated with the size, erectness and wax content in spikes and leaves in every genotype in each trial. We believe that in the HTWYT trial the genotypes have a major morphological diversity for the traits mentioned; however, this hypothesis needs to be corroborated. There was a lower association between the water indices and grain yield in the water stress environment for the HTWYT trial, where we assumed high morphological diversity. However, the advanced lines in this trial were selected for high temperature conditions and not for water stress conditions. The resistant and high yielding genotypes for high temperature conditions are not the same kind of genotypes as those selected for the water stress conditions. We did not find any relationship between the water indices under water stress conditions and the yield of the genotypes under high temperature conditions, or for the opposite relationship (Table 10).

Our study demonstrates a high efficiency of the water indices to evaluate the yield performance of genotypes selected for the three environments; well-irrigated (ESWYT), water stress (SAWYT), and high temperature conditions (HTWYT trial) during three growing seasons. The genotypes of the HTWYT showed the strongest association under high temperature. In fact, this is the first study reporting the association between the water indices and grain yield under high temperatures conditions. The potential of using SRI as a tool in breeding programs for selecting genotypes for increased yield potential has been demonstrated in spring wheat and winter wheat genotypes (Babar *et al.*, 2006a; Prasad *et al.*, 2007a). The water indices showed stability over time and environment that is a major concern for breeders in evaluating genotypes for a particular environment. The water indices have higher predictability at the genotypic level for grain yield variation compared to the vegetation based indices for selecting superior genotypes for grain yield for the three environments tested. The water indices NWI-1 and NWI-3 gave the best results in selecting the top yielding genotypes for grain yield and for discarding low yielding genotypes. The identification of low yielding lines has important implications in breeding programs because these lines are not desirable for making new crosses. Similar results were reported by Prasad *et al.* (2007a) for the water indices where NWI-3 also showed the highest relationship with grain yield. The two water indices (NWI-1 and NWI-3) proved to be quite accurate in selecting the top 25% and the 25% lowest yielding (Table 9). Once again, the high temperature environment gave the best results for selecting high yielding genotypes and/or for rejecting low yielding genotypes.

When the environments were combined in the SAWYT and HTWYT trials, the relationship between NWI-3 and grain yield was maintained significant and resulted stronger for the HTWYT trial (Fig. 3). The two first components explained 89% of the variance for the HTWYT, while for the SAWYT, only explained 68% of the variance.

Genetic correlation between spectral reflectance indices and grain yield

The water indices showed a higher association at the genetic level than the vegetation indices suggesting that canopy water content is more powerful in predicting grain yield. The genetic coefficients calculated based on individual growth stages, combining three growth stages (booting, heading, and grain filling), and combining two growth stages (heading and grain filling) gave similar results. However, higher genetic correlations were obtained when heading and grain filling were combined for the well irrigated, water stress and high temperature conditions (Table 6). The genetic correlation was stronger than the phenotypic correlation in the diverse environments in our study. This strong correlation is also evidence of an improved association between SRI and grain yield over years and growth stages, which has not been reported before for high temperature conditions. Babar *et al.* (2007) and Prasad *et al.* (2007a) also reported strong genetic correlations for the water indices under well irrigated, water stress and rainfed conditions.

Heritability, selection response, correlated response, and relative selection efficiency

The heritability calculated in our study is the proportion of phenotypic variance derived from genetic effects and indicates repeatability of SRI at different times (Falconer, 1989). The water indices showed moderate to high heritability while the vegetative indices showed the highest heritability for the three environments. Even though the vegetative indices had high heritability, they cannot be used for predicting yield because of their low phenotypic and genetic correlations (Table 4, 5, 6, 7). The inconsistency of the vegetative indices is highly repeatable (highly heritable) for both environments. However, they can be used for predicting yield in the high temperature environment because they had similar phenotypic and genetic correlations and higher heritability than the water indices. Jackson (2001) indicated that an indirect selection trait should have higher heritability than the direct trait, and high genetic correlation with the direct trait. Regarding the water indices in our study, they generally showed strong phenotypic and genetic correlations, and reasonably high heritability for the three environments. A genetic gain in grain yield by selection with the water indices (indirect selection criteria) can be achieved in breeding programs.

Grain yield also had high heritability for every year and across years in the three environments (Table 7). The advanced lines selected for the three environments in each trial demonstrated high heritability. Selecting genotypes by grain yield (direct selection) could be achieved for the three environments evaluated, but this method consumes considerable time when a large number of genotypes are evaluated in the field compared to the use of the water indices.

The vegetative indices showed a higher response to selection (R) than the water indices, but they had low correlated response (CR) and low efficiency of indirect selection (CR/R) in the well irrigated and water stress environments (Table 8). In contrast, the water indices showed a higher CR and CR/R than the vegetative indices in both environments. For the high temperature environment, the vegetative indices and the water indices had similar R, CR, and CR/R values. Similar results have been reported for spring and winter wheat genotypes (Babar *et al.*, 2006a, b; Prasad *et al.*, 2007a). 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 strong phenotypic and genetic correlation, heritability, CR, and CR/R suggest that the use of the water indices has significant potential for achieving greater genetic gain in grain yield in the three environments.

Canopy temperature and grain yield

Canopy temperature gave some strong relationships with grain yield in the well irrigated environment (Table12). In the water stress environment, the association between canopy temperature and grain yield was also significant. However, the association between canopy temperature and grain yield was the highest in the high temperature environment. Canopy temperature followed the same pattern as the water indices, showing the best association in this environment. This means that the advanced lines in the HTWYT can be selected indirectly for high grain yield using either the water indices or the canopy temperature (Fig. 2, 3). Both methods offer great advantages because they are cheaper, easier and quicker to measure in the field, especially when a large number of genotypes are being screened for yield. Also, canopy temperature could complement and support the selection of high yielding lines in other environments because it showed additive effects with the water indices for explaining grain yield according to a multiple analysis when heading and grain filling were combined (data not shown), especially for the well irrigated environment in the three trials (4-19%). For the other environments, the canopy temperature showed low additive effects (1-4%).

Conclusions

The water indices (NWI-1 and NWI-3, as well as NWI-2 and NWI-4) demonstrated great potential to differentiate high and low yielding genotypes in advanced lines of spring wheat under well irrigated, water stress and high temperature conditions in the diverse trials. This is the first study reporting the association between the water indices and grain yield for the high temperature environment that resulted in the best association. The combined growth stages of heading and grain filling can be used to differentiate genotypes for grain yield. The relationship between the water indices and grain yield also demonstrated a genetic base (high genetic correlation and heritability). The water indices can be used for breeding purposes in a well-irrigated, water deficit, and high temperature environments for selecting high yielding advanced lines of spring wheat because yield can be predicted using SRI. Additionally, canopy temperature could be used for predicting grain yield, especially in the high temperature environment. In other environments, canopy temperature could support the selection of high yielding lines by its additive effects with the water indices in the well irrigated environment for the three trials.

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Tables

Table 1. Mean, maximum and minimum temperature (^oC) and monthly total rainfall (mm) for three growing seasons in Northwest, Mexico.

	Normal growing season								
				Late growing season					Mean/Sum
Cycle	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	
2005-06									
Min	12.3	7.6	5.7	8.4	8.7	10.8	15.0	22.5	11.4
Max	31.7	26.7	25.8	26.6	27.0	32.1	35.2	38.1	30.4
Average	22.0	17.2	15.7	17.5	17.8	21.4	25.1	30.3	20.9
Total rainfall	0.0	1.0	0.0	0.2	1.0	0.0	0.0	31.6	34.8
2006-07									
Min	13.0	7.7	6.2	7.3	8.3	10.8	13.6	22.0	11.1
Max	31.6	24.7	21.7	25.2	28.6	29.3	34.2	36.7	29.0
Average	22.3	16.2	14.0	16.3	18.5	20.1	23.9	29.4	20.1
Total rainfall	0.0	4.4	19.0	0.4	0.0	0.2	0.4	0.0	24.4
2007-08									
Min	14.1	7.9	7.1	6.9	7.3	10.3	14.0	22.3	11.2
Max	29.9	22.6	23.8	25.4	27.0	31.6	33.3	35.8	28.7
Average	22.0	15.3	15.4	16.1	17.2	21.0	23.7	29.0	20.0
Total rainfall	14.8	44.4	6.2	0	1.0	0.0	0.0	3.0	69.4

Table 2. Mean, maximum and minimum yield levels (t ha^{-1}) for pair wise genotypic comparisons and significance levels for Elite Spring Wheat Yield Trial (ESWYT), Semi-Arid Wheat Yield Trial (SAWYT) and High Temperature Wheat Yield Trial (HTWYT) during three growing years and across years.

Year	**ESWYT**	**SAWYT**		**HTWYT**		
	Irrigated	Irrigated	Water stress	Irrigated	Water stress	High temperature
2006						
Min	6.36	3.33	0.51	5.70		1.87
Max	8.38	8.35	2.49	8.29		3.80
Mean	7.35	6.91	1.53	7.33		3.16
LSD (5%)	0.57	0.73	0.69	0.65		0.41
Significance level	**	**	**	**		**
2007						
Min	5.07	4.86	0.36	4.61	0.98	1.11
Max	7.93	9.45	3.13	7.85	1.80	3.90
Mean	6.57	6.32	1.85	6.03	1.39	2.66
LSD (5%)	0.78	1.13	0.89	1.10	0.24	0.76
Significance level	**	*	*	*	*	*
2008						
Min	4.80	4.48	1.97	4.64	2.89	1.26
Max	7.21	7.24	4.58	7.49	4.31	3.46
Mean	5.95	6.12	3.23	6.01	3.45	2.28
LSD (5%)	0.68	0.51	0.80	0.76	0.31	0.70
Significance level	*	**	**	**	*	*
Combined						
Min	4.80	3.33	0.36	4.61	0.98	1.11
Max	8.38	9.45	4.58	8.29	4.31	3.90
Mean	6.62	6.45	2.20	6.46	2.42	2.70
LSD (5%)	0.86	0.72	1.04	0.97	1.72	0.77
Significance level	**	**	**	**		**

*,**Significant at the 0.05 and 0.01 probability level, respectively.

Table 3. Mean (±SE) of spectral reflectance indices at three growth stages for Elite Spring Wheat Yield Trial (ESWYT), Semi-Arid Wheat Yield Trial (SAWYT) and High Temperature Wheat Yield Trial (HTWYT) grown in three different growing conditions. Estimates were based on combined years.

Growth stage	Vegetative indices†			Water indices‡	
	RNDVI	GNDVI	SR	NWI-1	NWI-3
			ESWYT-Well irrigated		
Booting	0.917±0.004**	0.808±0.004**	24.4±0.9**	-0.076±0.002**	-0.075±0.002**
Heading	0.886±0.006**	0.785±0.006**	18.2±0.8**	-0.094±0.002**	-0.094±0.003**
Grain filling	0.859±0.008**	0.767±0.008**	15.1±0.7**	-0.092±0.003**	-0.092±0.003**
			SAWYT-Well irrigated		
Booting	0.910±0.004**	0.793±0.003**	22.9±0.9**	-0.076±0.002**	-0.076±0.002**
Heading	0.865±0.003**	0.767±0.004**	15.1±0.4**	-0.095±0.002**	-0.096±0.002**
Grain filling	0.830±0.004**	0.729±0.005**	11.9±0.4**	-0.092±0.002**	-0.092±0.002**
			SAWYT-Water stress		
Booting	0.858±0.008**	0.755±0.008**	16.2±1.0**	-0.063±0.003	-0.059±0.003
Heading	0.791±0.011**	0.712±0.007**	10.5±0.6**	-0.048±0.002*	-0.045±0.003*
Grain filling	0.592±0.018**	0.598±0.011**	4.8±0.3**	-0.027±0.002*	-0.023±0.002*
			HTWYT-Well irrigated		
Booting	0.919±0.004**	0.806±0.006**	25.0±1.3**	-0.070±0.001**	-0.071±0.001**
Heading	0.880±0.006**	0.781±0.007**	17.1±0.9**	-0.092±0.003**	-0.094±0.003**
Grain filling	0.832±0.011**	0.737±0.014**	12.7±0.9**	-0.090±0.004**	-0.090±0.003*
			HTWYT-Water stress		
Booting	0.873±0.005**	0.778±0.007**	16.8±0.8**	-0.063±0.002*	-0.062±0.002*
Heading	0.749±0.030**	0.696±0.021**	10.5±1.3**	-0.046±0.005**	-0.041±0.005*
Grain filling	0.650±0.039**	0.647±0.024**	7.2±1.0**	-0.035±0.004**	-0.030±0.004*
			HTWYT-High temperature		
Booting	0.752±0.024**	0.664±0.014**	8.8±0.8**	-0.042±0.004**	-0.042±0.004**
Heading	0.746±0.020**	0.675±0.012**	8.4±0.6**	-0.045±0.004**	-0.042±0.004**
Grain filling	0.617±0.030**	0.602±0.018**	5.2±0.5**	-0.034±0.004**	-0.030±0.004**

*,**Significant at the 0.05 and 0.01 probability level, respectively.

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

‡NWI-1, normalized water index 1; NWI-3, normalized water index 3.

Table 4. Phenotypic correlations between spectral reflectance indices and grain yield for Elite Spring Wheat Yield Trial (ESWYT), and Semi-Arid Wheat Yield Trial (SAWYT) grown under well irrigated and water stress conditions during three years and across years.

Spectral index	Growth stage	ESWYT-Irrigated				SAWYT-Irrigated				SAWYT-Water stress			
		2006	2007	2008	Combined	2006	2007	2008	Combined	2006	2007	2008	Combined
Vegetative indices[†]													
RNDVI	Booting	0.23	0.21	0.68**	0.45*	0.07	0.25	0.25	0.20	0.06	-0.06	0.31*	-0.16
	Heading	-0.03	0.33	0.70**	0.42	0.18	-0.02	0.41**	0.17	0.51**	0.39*	0.45**	0.37*
	Grain filling	0.06	0.30	0.73**	0.48*	0.12	0.10	0.41**	0.19	0.30	0.28	0.44**	0.13
	Boot-Head-GF[‡]	0.08	0.30	0.73**	0.47*	0.15	0.11	0.40**	0.20	0.39*	0.33*	0.44**	0.17
	Head-GF[§]	-0.05	0.33	0.73**	0.46*	0.15	0.05	0.42**	0.18	0.44**	0.34*	0.46**	0.22
GNDVI	Booting	0.44*	0.28	0.65**	0.51**	0.12	0.15	0.17	0.18	0.07	0.05	0.08	-0.30*
	Heading	0.19	0.38	0.70**	0.49*	0.03	-0.10	0.34*	0.04	0.39*	0.50**	0.30	0.29
	Grain filling	0.26	0.37	0.66**	0.50*	0.02	0.04	0.29	0.02	0.20	0.17	0.17	-0.06
	Boot-Head-GF[‡]	0.34	0.38	0.70**	0.53**	0.06	0.05	0.30	0.07	0.27	0.33*	0.21	-0.01
	Head-GF[§]	0.17	0.39	0.69**	0.51**	0.03	-0.01	0.32*	0.03	0.33*	0.36*	0.24	0.08
SR	Booting	0.26	0.54**	0.66**	0.44*	0.12	0.24	0.25	0.27	0.07	-0.09	0.29	-0.30
	Heading	0.01	0.36	0.68**	0.43*	0.14	-0.01	0.39*	0.17	0.48**	0.09	0.48**	0.14
	Grain filling	0.09	0.41*	0.67**	0.41*	0.01	0.15	0.37*	0.15	0.30	0.32*	0.41**	0.11
	Boot-Head-GF[‡]	0.17	0.32	0.69**	0.45*	0.06	0.21	0.36*	0.24	0.34*	0.03	0.41**	-0.06
	Head-GF[§]	-0.02	0.34	0.69**	0.43*	0.09	0.08	0.39*	0.16	0.47**	0.15	0.47**	0.14
Water indices[‡]													
NWI-1	Booting	-0.18	-0.09	-0.45*	-0.19	-0.36*	-0.18	-0.33*	-0.05	-0.38*	-0.49**	-0.19	-0.07
	Heading	-0.26	-0.43*	-0.77**	-0.56**	-0.50**	-0.41**	-0.62**	-0.63**	-0.63**	-0.59**	-0.40**	-0.46**
	Grain filling	-0.28	-0.52**	-0.84**	-0.64**	-0.45**	-0.45**	-0.63**	-0.57**	-0.53**	-0.46**	-0.59**	-0.40**
	Boot-Head-GF[‡]	-0.28	-0.40*	-0.76**	-0.51**	-0.34*	-0.46**	-0.60**	-0.55**	-0.63**	-0.62**	-0.42**	-0.40**
	Head-GF[§]	-0.29	-0.52**	-0.82**	-0.62**	-0.51**	-0.44**	-0.64**	-0.61**	-0.65**	-0.60**	-0.52**	-0.47**
NWI-3	Booting	-0.21	-0.06	-0.41*	-0.17	-0.41**	-0.17	-0.35*	-0.01	-0.35*	-0.49**	-0.25	-0.16
	Heading	-0.25	-0.41*	-0.74**	-0.54**	-0.52**	-0.41**	-0.66**	-0.66**	-0.67**	-0.57**	-0.44**	-0.53**
	Grain filling	-0.28	-0.48*	-0.84**	-0.63**	-0.42**	-0.46**	-0.63**	-0.56**	-0.54**	-0.41**	-0.64**	-0.42**
	Boot-Head-GF[‡]	-0.29	-0.36	-0.74**	-0.49*	-0.30	-0.47**	-0.62**	-0.55**	-0.65**	-0.62**	-0.49**	-0.47**
	Head-GF[§]	-0.27	-0.49*	-0.81**	-0.61**	-0.51**	-0.46**	-0.67**	-0.63**	-0.67**	-0.57**	-0.59**	-0.52**

*,**Significant at the 0.05 and 0.01 probability level, respectively.
[†]RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio.
[‡]NWI-1, normalized water index 1; NWI-3, normalized water index 3.
[§]Boot-Head-GF**,** average of the booting, heading and grain filling stages.
[¶]Head-GF, average of the heading and grain filling stages.

Table 5. Phenotypic correlations between spectral reflectance indices and grain yield for High Temperature Wheat Yield Trial (HTWYT) grown under well irrigated, water stress, and high temperature conditions during three years and across years.

Spectral index	**Growth stage**	**Irrigated**				**Water stress**			**High temperature**			
		2006	2007	2008	Combined	2007	2008	Combined	2006	2007	2008	Combined
Vegetative indices†												
RNDVI	Booting	0.20	0.38	0.24	0.22	-0.09	0.46*	-0.05	0.69**	0.75**	0.83**	0.86**
	Heading	0.42	0.40	0.36	0.38	0.22	0.47*	0.19	0.75**	0.83**	0.83**	0.85**
	Grain filling	0.43	0.20	0.43	0.35	-0.06	0.54*	-0.21	0.77**	0.64**	0.80**	0.88**
	Boot-Head-GF§	0.40	0.36	0.37	0.34	0.11	0.51*	-0.18	0.76**	0.79**	0.82**	0.88**
	Head-GF¶	0.44	0.33	0.41	0.37	0.14	0.51*	-0.21	0.75**	0.77**	0.81**	0.88**
GNDVI	Booting	0.27	0.42	0.12	0.23	-0.01	0.48*	-0.19	0.70**	0.77**	0.83**	0.84**
	Heading	0.44	0.34	0.24	0.30	0.16	0.34	-0.10	0.77**	0.81**	0.81**	0.82**
	Grain filling	0.41	0.15	0.21	0.26	-0.07	0.33	-0.07	0.80**	0.63**	0.78**	0.83**
	Boot-Head-GF§	0.41	0.34	0.21	0.28	-0.03	0.39	-0.12	0.80**	0.88**	0.81**	0.85**
	Head-GF¶	0.43	0.25	0.26	0.28	-0.04	0.33	-0.09	0.77**	0.76**	0.81**	0.84**
SR	Booting	0.05	0.49*	0.27	0.22	-0.01	0.41	-0.09	0.68**	0.56*	0.82**	0.80**
	Heading	0.31	0.48*	0.36	0.38	0.22	0.42	-0.15	0.71**	0.71**	0.83**	0.81**
	Grain filling	0.32	0.22	0.41	0.30	-0.03	0.49*	-0.19	0.72**	0.52*	0.81**	0.84**
	Boot-Head-GF§	0.22	0.53*	0.34	0.29	0.06	0.46*	-0.13	0.72**	0.65**	0.83**	0.83**
	Head-GF¶	0.32	0.46*	0.38	0.35	0.16	0.45	-0.17	0.71**	0.68**	0.83**	0.84**
Water indices‡												
NWI-1	Booting	-0.23	-0.69**	-0.51*	-0.41	-0.43	-0.65**	-0.54*	-0.77**	-0.86**	-0.93**	-0.92**
	Heading	-0.30	-0.66**	-0.66**	-0.55*	-0.64**	-0.66**	-0.52*	-0.78**	-0.88**	-0.94**	-0.87**
	Grain filling	-0.46*	-0.69**	-0.77**	-0.61**	-0.62**	-0.69**	-0.53*	-0.77**	-0.79**	-0.81**	-0.88**
	Boot-Head-GF§	-0.36	-0.79**	-0.72**	-0.58**	-0.60**	-0.71**	-0.57*	-0.76**	-0.89**	-0.92**	-0.92**
	Head-GF¶	-0.39	-0.74**	-0.73**	-0.59**	-0.63**	-0.69**	-0.54*	-0.78**	-0.87**	-0.90**	-0.90**
NWI-3	Booting	-0.22	-0.64**	-0.59**	-0.45	-0.43	-0.68**	-0.56*	-0.75**	-0.88**	-0.93**	-0.92**
	Heading	-0.33	-0.62**	-0.69**	-0.57*	-0.66**	-0.68**	-0.48	-0.75**	-0.89**	-0.94**	-0.88**
	Grain filling	-0.43	-0.69**	-0.79**	-0.67**	-0.69**	-0.68**	-0.54*	-0.80**	-0.73**	-0.89**	-0.92**
	Boot-Head-GF§	-0.38	-0.79**	-0.79**	-0.64**	-0.64**	-0.72**	-0.55*	-0.71**	-0.90**	-0.93**	-0.93**
	Head-GF¶	-0.42	-0.74**	-0.78**	-0.63**	-0.69**	-0.70**	-0.53*	-0.75**	-0.87**	-0.92**	-0.92**

*,**Significant at the 0.05 and 0.01 probability level, respectively.
†RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio.
‡NWI-1, normalized water index 1; NWI-3, normalized water index 3.
§Boot-Head-GF**,** average of the booting, heading and grain filling stages.
¶Head-GF, average of the heading and grain filling stages.

Table 6. Genetic correlations between spectral reflectance indices and grain yield for Elite Spring Wheat Yield Trial (ESWYT), Semi-Arid Wheat Yield Trial (SAWYT), and High Temperature Wheat Yield Trial (HTWYT) grown under different growth conditions. Average of combined growth stages (heading and grain filling stages) during three years and across years.

Year	**Vegetative indices†**			**Water indices‡**	
	RNDVI	GNDVI	SR	NWI-1	NWI-3
		ESWYT-Well irrigated			
2006	0.03	0.22	0.08	-0.49*	-0.45*
2007	0.20	0.25	0.22	-0.50*	-0.45*
2008	0.79**	0.72**	0.72**	-0.84**	-0.83**
Combined	0.46*	0.52**	0.45*	-0.63**	-0.62**
		SAWYT-Well irrigated			
2006	0.16	0.01	0.08	-0.63**	-0.63**
2007	0.01	-0.11	0.04	-0.58**	-0.62**
2008	0.47**	0.36*	0.43**	-0.75**	-0.77**
Combined	0.18	0.01	0.18	-0.74**	-0.77**
		SAWYT-Water stress			
2006	0.46**	-0.36*	0.59**	-0.76**	-0.89**
2007	0.10	0.01	-0.33*	-0.31*	-0.33*
2008	0.56**	0.28	0.62**	-0.55**	-0.53**
Combined	-0.04	-0.25	-0.26	-0.38*	-0.46**
		HTWYT-Well irrigated			
2006	0.46*	0.46*	0.35	-0.40	-0.46*
2007	0.38	0.29	0.52*	-0.98**	-0.98**
2008	0.31	0.25	0.30	-0.73**	-0.68**
Combined	0.42	0.32	0.39	-0.63**	-0.71**
		HTWYT-Water stress			
2007	0.12	-0.01	0.14	-0.70**	-0.76**
2008	0.65**	0.37	0.50*	-0.72**	-0.71**
Combined	-0.16	-0.35	-0.30	-0.58**	-0.62**
		HTWYT-High temperature			
2006	0.75**	0.74**	0.71**	-0.85**	-0.84**
2007	0.84**	0.86**	0.64**	-0.89**	-0.89**
2008	0.83**	0.81**	0.84**	-0.92**	-0.95**
Combined	0.92**	0.86**	0.85**	-0.97**	-0.97**

*,**Significant at the 0.05 and 0.01 probability level, respectively.

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

‡NWI-1, normalized water index 1; NWI-3, normalized water index 3.

£PRI, photochemical reflectance index.

Table 7. Broad-sense heritability for spectral reflectance indices and grain yield for Elite Spring Wheat Yield Trial (ESWYT), Semi-Arid Wheat Yield Trial (SAWYT), and High Temperature Wheat Yield Trial (HTWYT) grown under different growing conditions. Average of combined growth stages (heading and grain filling) during three years and across years.

Year	Grain yield	Vegetative indices†			Water indices‡	
		RNDVI	GNDVI	SR	NWI-1	NWI-3
		ESWYT-Well irrigated				
2006	0.92	0.92	0.93	0.93	0.80	0.77
2007	0.96	0.96	0.97	0.95	0.87	0.86
2008	0.48	0.84	0.91	0.87	0.69	0.69
Combined	0.81	0.89	0.94	0.92	0.80	0.79
		SAWYT-Well irrigated				
2006	0.96	0.87	0.89	0.83	0.83	0.83
2007	0.50	0.65	0.65	0.67	0.77	0.72
2008	0.90	0.83	0.84	0.87	0.79	0.81
Combined	0.77	0.86	0.89	0.85	0.83	0.83
		SAWYT-Water stress				
2006	0.64	0.44	0.53	0.29	0.39	0.42
2007	0.69	0.77	0.67	0.70	0.51	0.52
2008	0.69	0.88	0.89	0.87	0.81	0.79
Combined	0.62	0.49	0.56	0.44	0.37	0.41
		HTWYT-Well irrigated				
2006	0.86	0.94	0.98	0.93	0.93	0.91
2007	0.73	0.92	0.93	0.85	0.69	0.70
2008	0.77	0.82	0.91	0.87	0.67	0.62
Combined	0.72	0.95	0.96	0.89	0.75	0.71
		HTWYT-Water stress				
2007	0.79	0.93	0.95	0.95	0.91	0.85
2008	0.86	0.72	0.88	0.85	0.74	0.70
Combined	0.74	0.96	0.97	0.94	0.87	0.87
		HTWYT-High temperature				
2006	0.84	0.97	0.97	0.97	0.96	0.96
2007	0.85	0.96	0.97	0.96	0.94	0.95
2008	0.93	0.97	0.97	0.97	0.91	0.95
Combined	0.78	0.90	0.92	0.87	0.83	0.84

†RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio.
‡NWI-1, normalized water index 1; NWI-3, normalized water index 3.
£PRI, photochemical reflectance index.

Table 8. Selection response (R) for the spectral reflectance indices and grain yield, correlated response (CR) for grain yield using SRI, and relative selection efficiency (CR/R) for grain yield for Elite Spring Wheat Yield Trial (ESWYT), Semi-Arid Wheat Yield Trial (SAWYT) and High Temperature Wheat Yield Trial (HTWYT) grown under different growing conditions. Average of heading and grain filling stages across years.

	Selection parameter	**Grain yield**	**Vegetative indices**[†]			**Water indices**[‡]	
			RNDVI	GNDVI	SR	NWI-1	NWI-3
					ESWYT-Well irrigated		
Combined years	R	0.39	0.47*	0.64**	0.53*	0.30	0.29
	CR		0.19	0.22	0.19	-0.25	-0.24
	CR/R		0.40*	0.34	0.36	-0.81**	-0.84*
					SAWYT-Well irrigated		
Combined years	R	0.53**	0.64**	0.67**	0.61**	0.45**	0.38*
	CR		0.10	-0.01	0.10	-0.41**	-0.43**
	CR/R		0.16	-0.01	0.16	-0.91**	-1.12**
					SAWYT-Water stress		
Combined years	R	0.20	0.20	0.18	0.15	0.12	0.14
	CR		-0.01	-0.05	-0.04	-0.06	-0.08
	CR/R		-0.03	-0.28	-0.29	-0.51**	-0.57**
					HTWYT-Well irrigated		
Combined years	R	0.36	0.80**	0.82**	0.76**	0.25	0.22
	CR		0.17	0.13	0.16	-0.23	-0.26
	CR/R		0.22	0.16	0.21	-0.94**	-1.18**
					HTWYT-Water stress		
Combined years	R	0.65**	0.97**	0.99**	0.93**	0.82**	0.73**
	CR		-0.08	-0.18	-0.15	-0.29	-0.30
	CR/R		-0.09	-0.18	-0.17	-0.35	-0.41
					HTWYT-High temperature		
Combined years	R	0.39	0.56*	0.58**	0.60**	0.36	0.38
	CR		0.38	0.36	0.35	-0.39	-0.40
	CR/R		0.69**	0.63**	0.58**	-1.10**	-1.06**

*,**Significant at the 0.05 and 0.01 probability level, respectively.
[†]RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio.
[‡]NWI-1, normalized water index 1; NWI-3, normalized water index 3.
[£]PRI, photochemical reflectance index.

Table 9. Percentage of the 25% highest and lowest yielding genotypes selected by the two water indices compared with direct selection by grain yield in Elite Spring Wheat Yield Trial (ESWYT), Semi-Arid Wheat Yield Trial (SAWYT) and High Temperature Wheat Yield Trial (HTWYT) grown under different growth conditions during three years and across years.

Trial	**NWI-1**[†]		**NWI-3**[†]	
	Lowest (%)	Highest (%)	Lowest (%)	Highest (%)
ESWYT-Well irrigated				
2006	33	17	33	17
2007	17	17	17	17
2008	83	50	83	50
Mean[‡]	83	33	83	33
SAWYT-Well irrigated				
2006	50	10	50	10
2007	80	40	80	50
2008	70	60	70	60
Mean[‡]	60	30	60	30
SAWYT-Water stress				
2006	60	40	60	40
2007	50	60	60	50
2008	60	70	60	80
Mean[‡]	40	20	40	30
HTWYT-Well irrigated				
2006	60	20	60	20
2007	40	60	40	60
2008	80	80	80	80
Mean[‡]	60	40	80	40
HTWYT-Water stress				
2007	40	80	60	80
2008	60	60	60	60
Mean[‡]	60	20	80	40
HTWYT-High temperature				
2006	80	40	100	40
2007	80	60	80	60
2008	100	80	80	80
Mean[‡]	80	60	100	60

[†]NWI-1, normalized water index 1; NWI-3, normalized water index 3.
[‡]Average of the heading and grain filling stages.

Table 10. Inter-environmental correlations between spectral reflectance indices and grain yield for the trial High Temperature Wheat Yield Trial (HTWYT) combining growth conditions (well irrigated, water stress and high temperature). Average of combined years.

Spectral Indices[†]	**Grain yield** Boot-Head-GF[‡]	Head-GF[§]
Well irrigated	**High temperature**	
NWI-1	-0.55*	-0.58*
NWI-3	-0.54*	-0.57*
High temperature	**Well irrigated**	
NWI-1	-0.37	-0.38
NWI-3	-0.36	-0.43

*,**Significant at the 0.05 and 0.01 probability level, respectively.
[†]NWI-1, normalized water index 1; NWI-3, normalized water index 3.
[‡] Boot-Head-GF**,** average of the booting, heading and grain filling stages.
[§]Head-GF, average of the heading and grain filling stages.

Table 11. Interseason correlations for grain yield during diverse growing seasons for Elite Spring Wheat Yield Trial (ESWYT), Semi-Arid Wheat Yield Trial (SAWYT) and High Temperature Wheat Yield Trial (HTWYT) grown under different growth conditions.

	Well irrigated					
	ESWYT		**SAWYT**		**HTWYT**	
	2006	2007	2006	2007	2006	2007
2007	0.71**		0.45**		0.39	
2008	0.52**	0.48*	0.66**	0.55**	0.65**	0.34
	Water stress				**High temperature**	
	SAWYT		**HTWYT**		**HTWYT-**	
	2006	2007	2006	2007	2006	2007
2007	0.34*				0.49*	
2008	0.37*	0.39*		0.23	0.58**	0.64**

*,**Significant at the 0.05 and 0.01 probability level, respectively.

Table 12. Phenotypic correlations between grain yield and canopy temperature determined at grain filling stage for Elite Spring Wheat Yield Trial (ESWYT), Semi-Arid Wheat Yield Trial (SAWYT) and High Temperature Wheat Yield Trial (HTWYT) grown under different growth conditions during three years and across years.

Trial	2006	2007	2008	Combined
Well irrigated				
ESWYT	-0.14	-0.15	-0.50*	-0.38
SAWYT	-0.26	-0.19	-0.27	-0.34*
HTWYT	-0.14	-0.58**	-0.59**	-0.37
Water stress				
SAWYT	-0.13	-0.45**	-0.33*	-0.40**
HTWYT		-0.22	-0.48*	-0.12
High temperature				
HTWYT	-0.74**	-0.50*	-0.86**	-0.82**

*,**Significant at the 0.05 and 0.01 probability level, respectively.

Figures

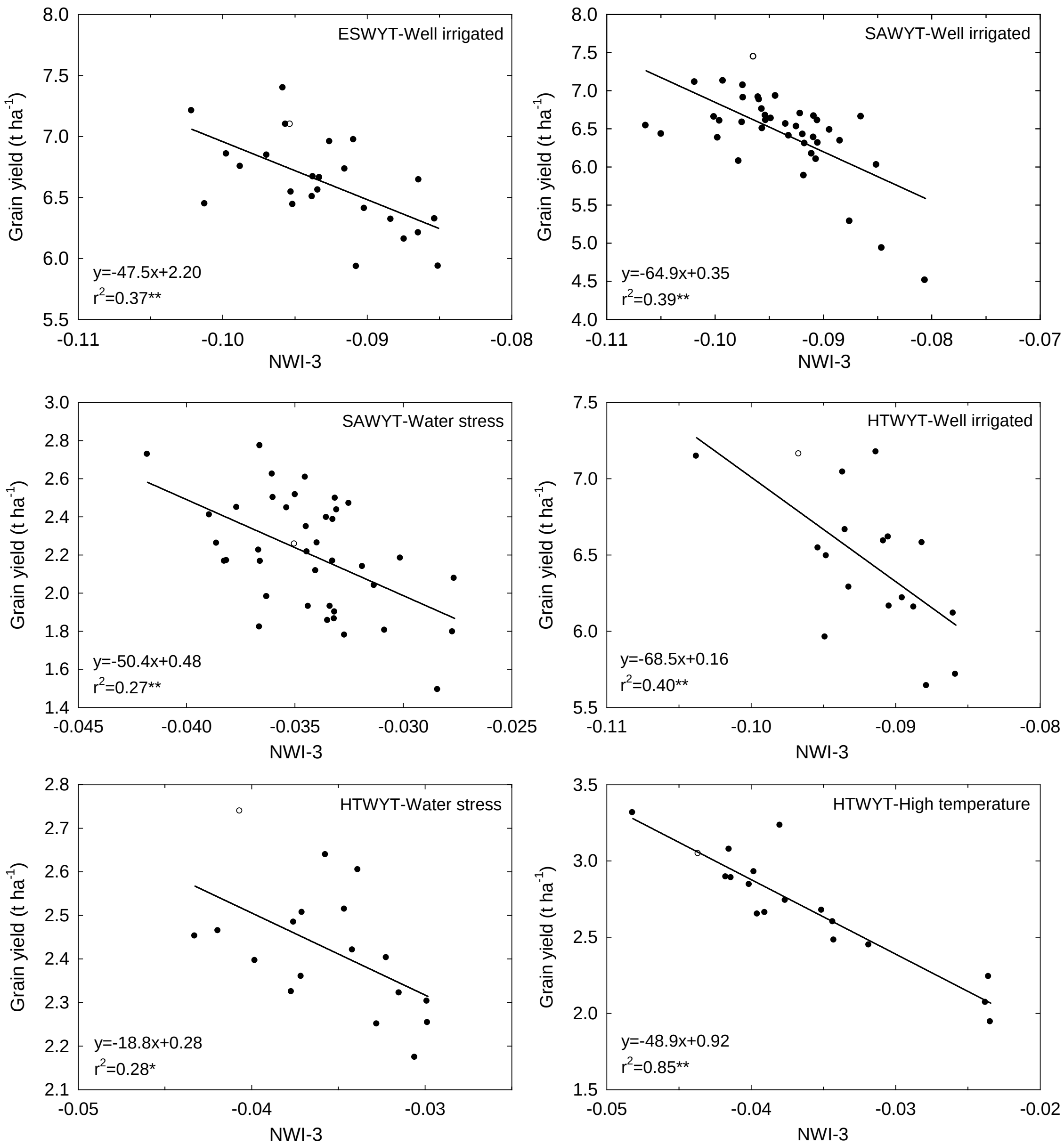

Figure 1. Linear relationship between grain yield and normalized water index 3 (NWI-3) combining heading and grain filling stages for Elite Spring Wheat Yield Trial (ESWYT), Semi-Arid Wheat Yield Trial (SAWYT) and High Temperature Wheat Yield Trial (HTWYT). Average of combined years.

*,**Significant at the 0.05 and 0.01 probability level, respectively.

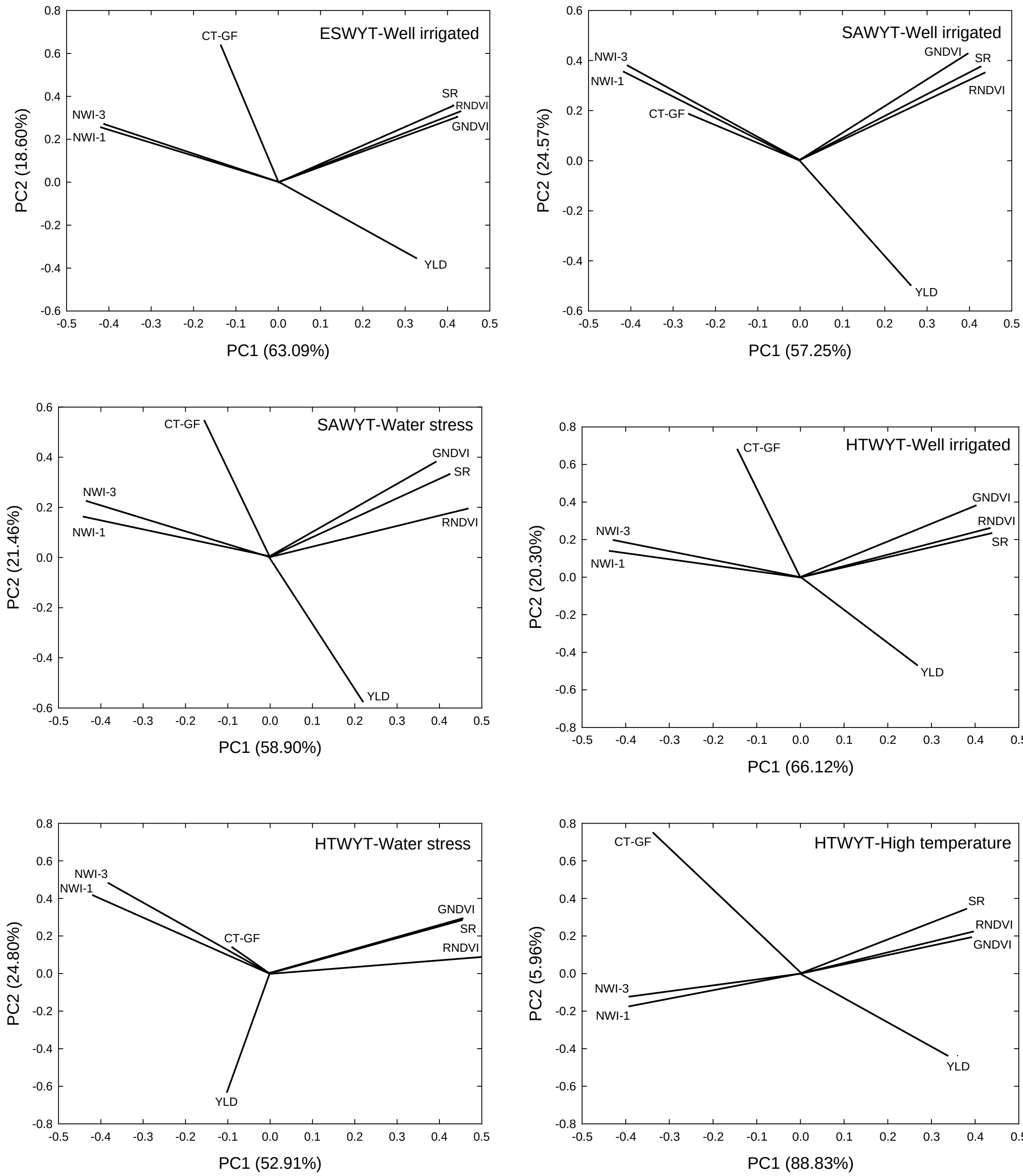

Figure 2. Two-dimensional distributions of coefficients of the first two principal components (PC) obtained by a multivariate analysis of different spectral reflectance indices and grain yield for the Elite Spring Wheat Yield Trial (ESWYT), Semi-Arid Wheat Yield Trial (SAWYT) and High Temperature Wheat Yield Trial (HTWYT) grown in three environments. Average of heading and grain filling stages across years.

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-3, normalized water index 3; PRI, photochemical reflectance index; CT-GF, canopy temperature at grain filling; YLD, yield

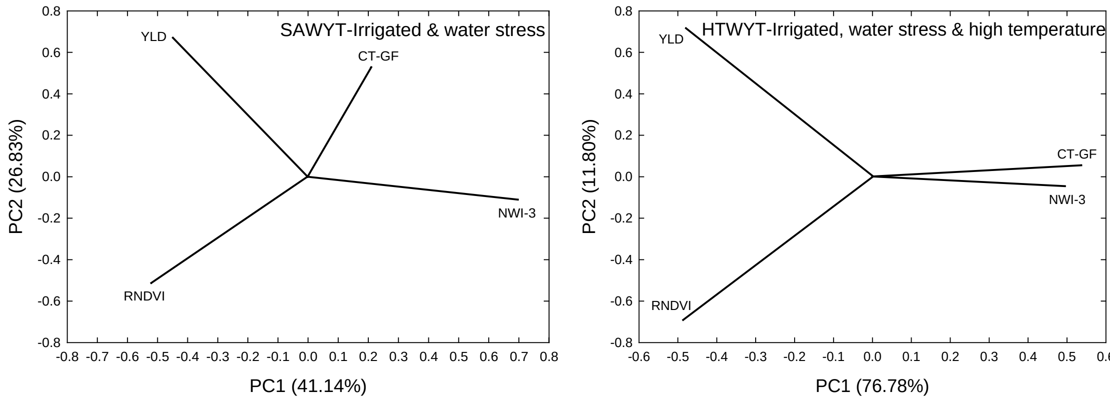

Figure 3. Two-dimensional distributions of coefficients of the first two principal components (PC) obtained by a multivariate analysis of NWI-3, RNDVI, grain yield and canopy temperature at grain filling combining environments in Semi-Arid Wheat Yield Trial (SAWYT) and High Temperature Wheat Yield Trial (HTWYT). Average of heading and grain filling stages across years.

RNDVI, red normalized difference vegetation index; NWI-3, normalized water index 3; CT-GF, canopy temperature at grain filling; YLD, yield.

CHAPTER III

Indirect selection for grain yield in diverse nurseries worldwide using parameters locally determined in NW Mexico in spring bread wheat

Abbreviations

CIMMYT, International Maize and Wheat Improvement

ESWYT, Semi-Arid Wheat Yield Trial

HTWYT, High Temperature Wheat Yield Trial

NWI-1, normalized water index-1

NWI-2, normalized water index-2

NWI-3, normalized water index-3

NWI-4, normalized water index-4

SAWYT, Elite Spring Wheat Yield Trial

WI, water index

Abstract

A strong relationship has been previously reported between the spectral reflectance parameter normalized water index three (NWI-3) and grain yield in NW Mexico at the principal wheat breeding station of The International Maize and Wheat Improvement Centre (CIMMYT). This study determined the relationship between NWI-3 and canopy temperature with the grain yield of multi-location yield trials of advanced spring wheat lines included in the 24th ESWYT (elite spring wheat yield trial), 11th SAWYT (semi-arid wheat yield trial), and 11th HTWYT (high temperature wheat yield trial) planted at international locations in 2003. The NWI-3, canopy temperature, and grain yield were determined in NW Mexico in distinct environments for each international trial: 24th ESWYT (well irrigated), 11th SAWYT (well irrigated and water stress) and 11th HTWYT (well irrigated, water stress, and high temperature) during three growing seasons (2006, 2007, and 2008). The database from CIMMYT for the 24th ESWYT, 11th SAWYT, and 11th HTWYT was used to obtain grain yield data for each international trial in diverse worldwide nurseries for the year 2003. All trials were planted in an alpha lattice design with two replications at every location. The analysis encompassed data from fifty yield testing sites of the 24th ESWYT, twenty nine sites for the 11th SAWYT, and twenty two sites for the 11th HTWYT. The mean grain yield of each nursery site showed great diversity during the year 2003, ranging from 0.75 to 9.0 t ha^{-1} for the 24th ESWYT entries, from 0.62 to 8.17 t ha^{-1} for the 11th SAWYT entries, and from 0.41 to 6.98 t ha^{-1} for the 11th HTWYT entries. The overall mean was 4.47 t ha^{-1} for the 24th ESWYT entries, 3.48 t ha^{-1} for the 11th SAWYT, and 3.73 t ha^{-1} for the 11th HTWYT. The NWI-3, canopy temperature, and grain yield obtained from NW Mexico in distinct environments (well irrigated, water stress, and high temperature) showed significant associations with the grain yield of genotypes in several nurseries located in different regions worldwide for the three international trials (24th ESWYT, 11th SAWYT and 11th HTWYT). Depending on the environment in which NWI-3 and CT were measured, they showed significant relationships with specific locations distributed in worldwide sites. However, when the top 25% yielding lines for each international trial and environment (6 lines for 24th ESWYT, 10 lines for 11th SAWYT, and 5 lines for 11th HTWYT) were selected according to NWI-3, canopy temperature and grain yield in NW Mexico, the number of significant

associations increased dramatically. For the 24th ESWYT, significant correlations were obtained with nurseries located mainly in Central Asia, North Africa, Southern Europe, and North America; for the 11th SAWYT, using parameters determined in the irrigated and water stress environments gave significant correlations with the grain yield of nurseries from Central Asia, North Africa, Southern Europe, and South and North America; and for the 11th HTWYT, several significant correlations were obtained using the NWI-3 and canopy temperature measurements from the irrigated, water stress, and high temperature environments, especially for nursery locations in Central Asia. NWI-3 gave higher number of significant correlations than canopy temperature for predicting yield performance of the advanced breeding lines in diverse regions worldwide.

Introduction

A typical wheat breeding program must evaluate a large number of advanced lines for high yield potential, and the methodology used normally involves field evaluation during several years and locations (Ball and Konzak, 1993). An early estimate of grain yield is particularly important for breeding purposes to detect, identify and select high yielding genotypes (Marti *et al.*, 2007). Indirect selection criterion might offer better knowledge of factors involved in growth and grain yield (Richards, 1982; Shorter *et al.,* 1991). Yield prediction based on models derived from remotely sensed information can be used for this purpose (Bouman, 1995). A technique for assessing yield of diverse genotypes in a fast, cheap and accurate way could reduce work and time for breeders because high yielding genotypes could be detected among thousands of lines in different environments (Royo *et al.*, 2003). The development of a new selection index must integrate several traits, trait interrelations, and repeatability for predicting yield into breeding programs (Baker, 1986).

Spectral reflectance indices (SRI) are a potential tool for assessing yield among genotypes (Reynolds *et al.*, 1999). The most widely used SRI is the normalized difference vegetation index (NDVI) that has been used to predict grain yield in wheat and corn under well watered and stressed environments (Osborne *et al.*, 2002). The red NDVI (RNDVI) has shown to be a good predictor of grain yield and biomass in winter wheat (Raun *et al.*, 2001; Moges *et al.*, 2004). The green NDVI (GNDVI) has also been associated with yield in corn and wheat genotypes (Shanahan *et al,.* 2001; Gutierrez-Rodriguez *et al.*, 2004). Five water indices based on near infrared wavelengths; one water index and four normalized water indices (WI and NWIs, respectively), have been used for predicting yield and they have shown a strong relationship with grain yield in spring and winter wheat genotypes over time (three growing seasons) under well irrigated, water deficit stress, and rainfed conditions (Babar *et al.*, 2006; Prasad *et al.*, 2007). Our results have also demonstrated strong associations between the water indices and grain yield in advanced lines of spring wheat in irrigated, water stress, and high temperature environments (Gutierrez *et al.*, 2008). As a result, the water indices are an alternative breeding/selection tool for predicting grain yield in different environments in wheat.

The wheat breeding program at the International Maize and Wheat Improvement Center (CIMMYT) develops advanced breeding lines every year for developing countries where spring wheat is grown (Trethowan and Crossa, 2007). Diverse countries collaborate in the testing of the breeding lines and share their own germplasm for new crosses at CIMMYT. In addition, every collaborator sends yield data to CIMMYT, which are collected and analyzed across sites (Trethowan and Crossa, 2007). Advanced lines have been distributed around the world through yield trials by CIMMYT since 1964 (Trethowan and Crossa, 2007). The yield trials are called; Elite Spring Wheat Yield Trial (ESWYT), Semi- Arid Wheat Yield Trial (SAWYT), High Temperature Wheat Yield Trial (HTWYT), and others (Trethowan and Crossa, 2007; Lage *et al.*, 2008). High yielding and well adapted lines have been derived through this exchange program for many regions where spring wheat is grown in developing countries (Trethowan *et. al.*, 2002). The ESWYT includes advanced breeding lines that are targeted to highly productive irrigated wheat areas, the SAWYT includes advanced lines for the semi arid regions, and the HTWYT has advanced lines for heat-stressed areas; these trials are distributed annually to international cooperators (Lillemo *et al.*, 2004; Lillemo *et al.*, 2005; Trethowan and Crossa, 2007). In addition to providing approximately 1,000 new genotypes annually to national wheat programs worldwide as a public good, the international yield trials represent an important information source of feedback on how effective the targeting of germplasm is, and information on how the physiological traits expressed in the selection environments relate to international performance could complement this data base.

Several studies of international trials have been reported using yield data of nurseries from CIMMYT's database (Peterson and Pfeiffer, 1989; DeLacy, *et al.,* 1994; Trethowan *et al.,* 2001, 2003; Lillemo *et al.,* 2004, 2005). Trethowan *et al.* (2001) evaluated and examined the grain yield data for the advanced lines included in the SAWYT at 122 locations representing diverse environments over a six year period. The impact of CIMMYT wheat germplasm in highly productive environments in developing countries has increased significantly, but drought reduces and may even eliminate yield performance advantages in some semi arid environments (Trethowan *et al.*, 2001). The yield testing of advanced lines in diverse environments or regions is important for identifying environmental factors that affect crop yield performance (Lillemo *et al.*, 2005). For example, the yield performance of advanced lines in diverse nurseries was analyzed to the amount of annual rainfall received in each testing site for explaining yield progress across years for the ESWYT (Trethowan *et al.,* 2001).

Northwest Mexico (Yaqui Valley) has been reported as a good site for developing advanced lines for diverse environments (irrigated, drought, and high temperature) around the world based on yield per se, especially for developing countries (Lillemo *et al.*, 2005). The main goal of the present work was to compare the expression of yield and two remotely, sensed selection criteria -spectral indices and canopy temperature, when measured in the selection environment of NW Mexico with expression of yield across a range of international target locations. Specific objectives were (i) to determine the level of association between SRI (vegetative and water indices) determined in Northwest, Mexico (Yaqui Valley) during 3 growing seasons at two growth stages (heading and grain filling), and average grain yield of the advanced breeding lines that were included in the 24th ESWYT, 11th SAWYT and 11th HTWYT nurseries for the year 2003, and (ii) to evaluate the potential of the SRI for predicting average yield performance of the respective advanced lines at the international testing sites.

Materials and Methods

International trials

Advanced breeding lines from CIMMYT (International Maize and Wheat Improvement Center) were used for this study. The genetic material corresponded to three international trials; Elite Spring Wheat Yield Trial (24^{th} ESWYT) (25 genotypes) comprised of advanced lines developed for irrigation conditions, Semi-Arid Wheat Yield Trial (11^{th} SAWYT) (40 genotypes) composed of advanced lines developed for reduced irrigation or semi arid conditions, and the High Temperature Wheat Yield Trial (11^{th} HTWYT) (18 genotypes) containing advanced lines for high temperature regions.

Growing conditions for Northwest Mexico (Yaqui Valley)

The 24^{th} ESWYT genotypes were planted under well irrigated conditions, the 11^{th} SAWYT genotypes under well irrigated and water stress conditions, and the 11^{th} HTWYT genotypes under well irrigated, water stress, and high temperature conditions. The genotypes were grown during the winter season at CIMMYT's experimental station in Cd. Obregon, NW Mexico (27.3^{o}N, 109.9^{o}W, 38 m above sea level). The seeding rate for each experiment was 78 kg ha^{-1}. Nitrogen and phosphorous were applied at the rate of 150 kg ha^{-1} and 22 kg ha^{-1}, respectively. Field plots consisted of two raised beds 5 m long (80 cm width each) with 2 rows, 10 cm apart on each bed. An alpha lattice design with 2 replications was employed for all experiments.

Planting was accomplished in November and plants reached booting and heading during February-March for the well irrigated and water stress conditions. For the experiments under high temperature conditions, the genotypes were planted in February to reach booting and heading in April-May (ambient temperature around 35-40^{o}C). There were three crop growing seasons for all experiments referred to as years; 2006, 2007 and 2008. The 11^{th} HTWYT trial under water stress conditions was grown only in 2007 and 2008.

Flood irrigation was applied every 20-25 days for well-irrigated treatments. In trials submitted to drought stress conditions, one irrigation was applied before seeding providing approximately 100 mm of available water, and two irrigations of 50-70 mm prior to the booting stage. For the high temperature trial, irrigations were also applied as needed to prevent drought stress.

Grain yield was determined at maturity by harvesting the complete plot, but excluding a 0.5-m border at each end.

Spectral reflectance measurements in Northwest Mexico

Canopy reflectance was measured in the 350 to 1100 nm range using a FieldSpec spectroradiometer (Analytical Spectral Devices, Boulder, CO). Data were collected during cloud-free days at solar noon between (10:30 and 14:00 hrs) with a previous calibration using a white plate of barium sulphate ($BaSO_4$) that provides maximum irradiance (Labsphere Inc., North Sutton, USA). Four measurements in each plot were taken at heights of 0.5 m above the canopy with a field of view of 25^o during the heading and grain filling growth stages.

Five water indices ($WI=R_{970}/R_{900}$, $NWI\text{-}1=[R_{970}-R_{900}]/[R_{970}+R_{900}]$, $NWI\text{-}2=[R_{970}-R90_{850}]/[R_{970}+R_{850}]$, $NWI\text{-}3=[R_{970}-R_{880}]/[R_{970}+R_{880}]$, and $NWI\text{-}4=[R_{970}-R_{920}]/[R_{970}+R_{920}]$) and other spectral reflectance indices were determined at booting, heading, and grain filling in advanced lines of the 24th ESWYT (well irrigated), 11th SAWYT (well irrigated and water stress) and 11th HTWYT (well irrigated, water stress, and high temperature) in NW Mexico (Penuelas *et al.*, 1993; Babar *et al.*, 2006; Prasad *et al.*, 2007). The combination of heading and grain filling for the NWI-3 was employed in the present study, which gave the best association with grain yield in all environments in NW Mexico for predicting yield in the 24th ESWYT, 11th SAWYT, and 11th HTWYT (Gutierrez *et al.*, 2008).

Canopy temperature

Canopy temperature during grain filling was determined in diverse advanced lines of the 24th ESWYT (well irrigated), 11th SAWYT (well irrigated and water stress) and 11th HTWYT (well irrigated, water stress, and high temperature) in NW Mexico, and was employed for the present study (Gutierrez *et al.*, 2008). A hand-held infrared thermometer (Mikron M90 Series, Mikron Infrared Instrument Co. Inc., Oakland, NJ) was used to measure canopy temperature depression.

Grain yield data for international nurseries

The database from CIMMYT for the 24th ESWYT, 11th SAWYT, and 11th HTWYT for the year 2003 was used to obtain grain yield data. This database contains yield data from every location for the diverse array of collaborators. Some information such as latitude, longitude, soil type, and soil pH were reported by some cooperators (Table 1). However, the information provided by many collaborators was incomplete.

The advanced breeding genotypes of the 24th ESWYT, 11th SAWYT and 11th HTWYT were planted in an alpha lattice trial with two replications. All trials were packaged and randomized at CIMMYT, Mexico and each nursery was sown under local agronomic practices.

The advanced breeding lines in the 2003 nurseries corresponded to the same lines planted in NW Mexico (Cd. Obregon) during the years 2006, 2007 and 2008. For other years, the breeding lines in each international nursery are different because CIMMYT sends new advanced breeding material to its collaborators each year.

Statistical analysis

Grain yield data for the diverse genotypes in each trial location were analyzed by SAS (SAS Institute, 2001) using proc mixed and the adjusted means were obtained according to the alpha lattice design. The SRI determined at heading, grain filling, and by combining both growth stages were averaged for the three seasons (2006, 2007, and 2008) in NW Mexico for each trial and environment. Pearson correlation coefficients were used to estimate the phenotypic relationship between the NWI-3 and canopy temperature (diverse environments in NW Mexico) and the grain yield of international nurseries.

Data from the different SRI were analyzed by principal component analysis (PCA) using SAS. PCA was conducted using NWI-3 from heading-grain filling and canopy temperature measured from grain filling, and grain yield of NW Mexico averaging three growing seasons

(2006, 2007, and 2008), while the grain yield of nurseries was averaged using yield data for the year 2003. The PCA was conducted for the 24^{th} ESWYT, 11^{th} SAWYT, and 11^{th} HTWYT trials with their respective nurseries (Table 1).

Results

As previously reported, the combination of measurements at heading and grain filling for the normalized water index three (NWI-3) gave the most significant associations with grain yield across environments for the three international trials; 24th ESWYT (well irrigated), 11th SAWYT (well irrigated and water stress), and 11th HTWYT (well irrigated, water stress, and high temperature) (Gutierrez *et al.*, 2008). The vegetative indices (*i.e.*, RNDVI and GNDVI) determined in the same environments in NW Mexico showed lower relationships with grain yield. In this study, the NWI-3 (at heading and grain filling) and canopy temperature (mean of grain filling) were compared for their association with grain yield of the genotypes in the three international nurseries at diverse locations.

Grain yield diversity of nurseries and their association with NW Mexico parameters

There was high average grain yield diversity among nursery sites where the advanced lines of the 24th ESWYT, 11th SAWYT, and 11th HTWYT were evaluated (Table 2). The average grain yield of nurseries ranged from 0.75 to 9.0 t ha^{-1} for the 24th ESWYT entries, from 0.62 to 8.17 t ha^{-1} for the 11th SAWYT entries, and from 0.41 to 6.98 t ha^{-1} for the 11th HTWYT entries. There were fifty five international nursery sites for the 24th ESWYT, twenty nine sites for the 11th SAWYT, and twenty two sites for the 11th HTWYT (not including the environments of NW Mexico). The overall mean was higher for the 24th ESWYT (4.47 t ha^{-1}), than for the 11th SAWYT (3.48 t ha^{-1}) and for the 11th HTWYT (3.73 t ha^{-1}). The analyses of variance using nursery yields showed significant differences for environment and genotype as main effects, as well as for the genotype by environment interactions for the three international trials (data not shown). There was a large amount of yield variability among the genotypes at the different sites (Fig. 1, 2, 3). The interaction between the parameters determined in NW Mexico (NWI-3, canopy temperature, and grain yield from the irrigated, water stressed, and high temperature environments) and the grain yield of genotypes at different nursery sites were analyzed by the multivariate approach of principal component analysis (PCA) for showing their distribution for each international trial (Fig. 1, 2, 3).

The wide yield diversity of advanced lines among international nursery sites in every international trial (24th ESWYT, 11th SAWYT, and 11th HTWYT) is clearly observed in the PCA biplots (Fig. 1-5). The parameters determined in NW Mexico (NWI-3, canopy temperature and grain yield) in distinct environments (irrigated, water stress, and high temperature) revealed that some international nursery sites are closely associated (significant relationships) with them and yield performance can be successfully predicted in those nurseries.

Diverse nurseries showed significant relationships ($p \leq 0.05$ and 0.01) with the parameters determined in the irrigated environment in NW Mexico for the 24th ESWYT trial (Fig. 1). NWI-3 correlated well with eleven nurseries, which were located in Central Asia (Afghanistan, India [five], and Nepal), West Asia (Turkey), Central Africa (Zambia), Southern Europe (Italy), and South America (Argentina) (Table 3; Fig. 1). Canopy temperature showed significant correlations with nurseries in Central Asia (India [two] and Pakistan), South Africa (Angola, South Africa, and Zimbabwe), Southern Europe (Spain), and South and North America (Argentina and Canada [two], respectively). Grain yield from NW Mexico had significant relationships with nurseries sites in Central Asia (Afghanistan [two], India [two], Iran, and Nepal).

For the 11th SAWYT trial, parameters from NW Mexico were determined for two environments (irrigated and water stress) (Fig. 2). NWI-3 from the irrigated environment showed significant relationships with the nurseries from North Africa (Morocco) and Southern Europe (Spain), and canopy temperature for Central Asia (Afghanistan and Pakistan) and Southern Europe (Serbia Montenegro). Grain yield from NW Mexico was correlated with nurseries sites in Central and West Asia (India and Turkey), North Africa (Morocco [two]), Southern Europe (Spain), South America (Argentina), and North America (Mexico-Ciano). For the parameters determined in the water stress environment, NWI-3 showed significant relationships with nurseries from Central Asia (India [two] and Pakistan) and North Africa (Morocco). Canopy temperature showed significant relationships with nurseries from Central Asia (Pakistan), North Africa (Morocco), Southern Europe (Spain) and South America (Argentina), while grain yield from NW Mexico was correlated with nurseries from Central Asia (India [two]) and Central Africa (Kenya). When the parameters from the two environments were averaged and correlated with the grain yield of international nurseries (Fig. 4), the number of significant correlations decreased drastically for the NWI-3 because only one nursery gave a significant relationship (Morocco in North Africa). Canopy temperature showed a few significant correlations with nurseries from Central Asia (Pakistan), North Africa (Morocco) and South America (Argentina), while grain yield in NW Mexico increased, and included nurseries in Central Asia (India and Pakistan), North Africa (Morocco [two]), Southern Europe (Spain), and South and North America (Argentina and Mexico-Ciano, respectively) (Table 3).

Three environments for the 11th HTWYT were managed in NW Mexico, for well irrigated, water stress, and high temperature (Fig. 3). The NWI-3 determined in the well irrigated environment showed only one significant relationship with a nursery in Central Europe (Hungary), and there were no other significant associations for the water stress and high temperature environments. Canopy temperature measured in the irrigated and water stress environments did not show any significant relationship, but when determined in the high temperature environment, there was one significant correlation with a nursery from Pakistan (Central Asia). Grain yield measured in the irrigated environment in NW Mexico showed significant associations with nurseries from Central Asia (India [two]) and North Africa (Morocco), and when determined in the water stress environment showed significant associations with nurseries from North Africa (Morocco) and North America (Canada). Grain yield from the high temperature environment gave significant associations with nurseries from Central Asia (India and Pakistan). If the three environments from NW Mexico were combined (Fig. 5), the mean NWI-3 did not show any significant association with an international nursery site, canopy temperature only showed two associations for nurseries from Central Asia (India and Pakistan), and grain yield also gave two significant correlations with nurseries from Central Asia (India) and North Africa (Morocco) (Table 3).

Selection of the top 25% yielding lines from NW Mexico environments

When the parameters from the 25% top yielding lines from each trial in every environment from NW Mexico (24th ESWYT [well irrigated], 11th SAWYT [well irrigated and water stress], and 11th HTWYT [well irrigated, water stress, and high temperature]) were considered, the number of significant correlations with international nursery sites increased (Table 3). The 25% top yielding lines were selected using each parameter determined in NW Mexico (NWI-3, canopy temperature, and grain yield in each international trial and environment (6 lines for the 24th ESWYT, 10 lines for the 11th SAWYT, and 5 lines for the 11th HTWYT).

For the 24th ESWYT, using the NWI-3, canopy temperature, and grain yield from NW Mexico (irrigated environment), there were nurseries of diverse worldwide regions showing significant correlations, especially for the Central Asia region (Table 3). Generally, NWI-3 and grain yield from NW Mexico had more significant correlations with international sites from his region than canopy temperature. However, other regions (West Asia, North, Central and South Africa, Southern and Central Europe, and South and North America) showed few differences in number of significant correlations (less than three significant correlations) among international nursery sites and NWI-3, canopy temperature and grain yield from NW Mexico.

For the 11th SAWYT, NWI-3 from the irrigated environment showed more significant correlations with nurseries from Central Asia (eleven) than canopy temperature (seven) or grain yield (seven) of NW Mexico (Table 3). NWI-3, canopy temperature, and grain yield of NW Mexico measured in the water stressed environment did not show any differences in the number of significant associations with nurseries from Central Asia. There were a few significant correlations (no more than three) in nursery sites from West Asia, North, Central and South Africa, Southern Europe, and South and North America employing parameters determined in the irrigated and water stressed environments.

For the 11th HTWYT, NWI-3 determined in the irrigated, water stress, and high temperature showed more significant correlations with international nursery sites than canopy temperature, and grain yield from NW Mexico (six, ten, and eight significant correlations for NWI-3; four, five, and six for canopy temperature, and three, five, and seven for grain yield in the irrigated, water stress and high temperature environment, respectively) (Table 3). For international nurseries from West Asia, North Africa, Southern and Central Europe, and North America there were two or less significant correlations in each region for the NWI-3, canopy temperature and grain yield determined in the irrigated, water stressed and high temperature environments (Table 3).

Discussion

The diverse associations between the NWI-3 and canopy temperature determined in NW Mexico and grain yield at international sites was the result of wide yield differences among the international trials (24th ESWYT, 11th SAWYT, and 11th HTWYT), as demonstrated by their distribution in the PCA biplots (Table 2, Fig. 1, 2, 3). The yield diversity of the advanced breeding lines among nurseries suggests that they were influenced by local agronomic practices and international factors. In fact, we found significant genotype by environment interactions for the three international trials (data not shown). The nursery grouping in our study was based only on the grain yield for the year 2003, and the lack of information prevented us from associating the nursery yields with other factors such as environmental conditions (temperature, precipitation, etc), amount of fertilizer, local tillage practices, soil type, etc.

Even though there was a wide range in grain yield among nurseries, the NWI-3, canopy temperature, and grain yield measured in the three environments in NW Mexico showed that yield performance can be predicted in certain nurseries (Fig. 1, 2, 3). Even though NWI-3 and canopy temperature were determined in a different environment in Mexico, these indirect selection parameters successfully predicted genotype performance in some nurseries, mainly those in Central Asia, North Africa, Southern Europe, and South and North America. The mean NWI-3, canopy temperature, and grain yield of NW Mexico obtained by combining two environments (irrigated and water stress) in the 24th SAWYT and three environments (irrigated, water stress, and high temperature) in the 11th HTWYT did not improve yield prediction for the international nursery sites (Table 3; Fig. 4, 5). There were very few differences when the environments in NW Mexico were combined in both trials compared with individual environments.

Several nursery sites of the 24th ESWYT were significantly associated with the NWI-3 and canopy temperature measured in the irrigated environment in NW Mexico (Table 3). Even though the advanced lines were developed by CIMMYT for high yield in irrigated environments, it is evident that other factors affected the genotype yield performance (yield ranged from 0.75 to 9.0 t ha^{-1}) in diverse nurseries (Table 2). It seems that drought was a determining factor affecting the yield performance in many nurseries of the 24th ESWYT, either because of low rainfall and/or limited irrigations (data not shown). Trethowan *et al.* (2001) reported that drought reduces and may even eliminate yield performance advantages in some semi arid environments in diverse worldwide regions. However, when the top 25% yielding lines were selected for their grain yield in NW Mexico, the number of nurseries that showed significant associations with the NWI-3 and canopy temperature increased, especially for the Central Asia region. There were thirty different nurseries in Central Asia that were associated with the parameters from NW Mexico, while in other regions, the number of nurseries was lower (less than two significant associations) in West Asia, North, Central and South Africa, Southern Europe, South and North America.

In the 11th SAWYT, the parameters determined in the irrigated and water stress environments of NW Mexico, as well as their combination, generally a showed similar pattern to the associations with yield per se in Central Asia and North Africa (Table 3; Fig. 2, 4). Averaging the parameters of NW Mexico across both environments did not increase or improve the yield prediction of the advanced lines in other worldwide regions. Even though there were some significant associations of NWI-3 and canopy temperature from NW Mexico for the 11th SAWYT (advanced lines selected for semiarid regions), the NWI-3 did not predict yield performance in well known nurseries that suffer continuous drought from Central Asia, North Africa and Southern Europe (Mediterranean region). The lack of information for rainfall, amount of irrigation and other environmental factors for many nurseries (not reported) did not permit us to postulate why these results occurred. However, if parameters from the 25% top yielding lines were used, the number of associated nurseries increased using the NWI-3 and canopy temperature determined in NW Mexico, especially for the Central Asia region.

The 11th HTWYT represented advanced lines selected for high yield in hot environments (Lillemo *et al.*, 2005). The NWI-3 and canopy temperature obtained from this environment in NW Mexico only predicted the yield performance of few nurseries in Central Asia. The parameters of NW Mexico determined in the irrigated and water stress environments, and their combination showed similar patterns (low number of nurseries associated) (Fig. 3, 5). When the 25% top yielding lines were selected based on the NW Mexico parameters, the number of significantly associated nurseries increased dramatically in Central Asia, North Africa, Southern and Central

Europe, and North America regions. It means that certain advanced lines (same lines for all locations) showed a higher adaptation than others in diverse nursery sites, and that these lines with high yield performance can be detected using the NWI-3 and canopy temperature for predicting the yield in international nursery sites. In a previous study (Gutierrez *et al.*, 2008), the direct selection of lines for grain yield and the indirect selection using the NWI-3 resulted in the similar selection (same genotypes) of the top yielding lines (25%) in the three international trials and for every environment in NW Mexico (well irrigated, water stress, and high temperature). Similarly to the 24th ESWYT, not all the advanced lines selected in NW Mexico for high yields in hot environments of the 11th HTWYT were well adapted in many worldwide locations. Every nursery site represents a particular environment with distinctive traits such as altitude, latitude, soil type, rainfall, temperature, and other factors. In addition, the genotype by environment interactions indicated that many genotypes were not well adapted to all the environments (locations) where the advanced lines were tested. Trethowan *et al.* (2002) reported that grain yield of individual sites is inaccurate in estimating yield progress over time and used the five highest yielding genotypes from the ESWYT and SAWYT at each location over time (20 years) to determine the relationship between locations. In our study, significant correlations were obtained between the parameters determined in the three environments from NW Mexico and the grain yield of diverse sites when the 25% top yielding lines were used in the three international trials (Fig. 1-5).

Lage *et al.* (2008) grouped individual sites into clusters using a shifted multiplicative model based on environmental data and grain yield (35 years averaged) and found that 18 sites were similar and 23 were contrasting. The ESWYT genotypes were clustered in 29 nurseries, SAWYT in 20, and HTWYT in 15, and this established that the grain yield of NW Mexico (Yaqui Valley) was similar to six nurseries in Western and Central Asia; two regions each in Turkey (Southwest), Pakistan (Northeast and Northwest), and Syria. Several authors have proposed that other sites around the world could be used for testing the advanced breeding genotypes from CIMMYT for diverse environments (Trethowan *et al.,* 2006; Lage *et al.*, 2008). In this study, the grain yield of locations mainly in Central Asia gave significant correlations with the NWI-3, canopy temperature, and grain yield from NW Mexico for the 24th ESWYT, 11th SAWYT, and 11th HTWYT. The nurseries from Central Asia presented the major number of significant associations for the parameters measured in NW Mexico for the three international trials, especially with the top yielding advanced lines. Trethowan *et al.* (2003) found that some nurseries from Egypt and Pakistan were also associated with NW Mexico. Trethowan and Crossa (2007) reported that other nurseries located in North Africa, Western Asia and South America (Argentina) were also similar to NW Mexico. In our study, the nurseries from Morocco in North Africa showed high associations with the parameters from NW Mexico for the three international trials, especially for the 24th ESWYT. Trethowan and Crossa (2007) identified five environments for the HTWYT employing environmental factors: continuous heat stress; terminal heat stress; temperate non-heat stressed; dry heat; and humid heat. The authors found that late planting in NW Mexico (February) was a good predictor of grain yield in sites with high temperatures such as Tandojam, Pakistan and Indore, India. In our study, the NWI-3 and canopy temperature determined in the high temperature environment in NW Mexico did not predict the yield performance using the complete set of advanced lines of the 11th HTWYT at many worldwide nurseries, but when employing the top 25% high yielding lines, the parameters from NW Mexico can predict yield in diverse worldwide regions, especially for Central Asia (Table 3).

The evaluation of diverse genotypes, individual traits, trait interrelationships, and their predictive repeatability are considered by breeders for selecting potential high yielding lines (Baker, 1986). The potential of developing new selection indices needs to be based on the fact that an index could integrate several traits for predicting yield into breeding programs. Optimum selection indices must incorporate a genetic base, interaction of several traits, and the relative economic value for selecting or evaluating genotypes (Baker, 1986). When selection is based on accurate parameter estimates, the potential index could provide the best solution to maximize genetic improvement for the selection goal (Milligan *et al.*, 2003). Using the NWI-3 and other water indices determined at heading and grain filling in NW Mexico, high genetic gains (high genetic correlation and heritability) can be obtained in diverse environments (irrigated, water stress, and high temperature) (Gutierrez *et al.*, 2008). In our study, we used a selection index based on canopy spectral reflectance (NWI-3) and canopy temperature for integrating the whole plant canopy for predicting yield. NWI-3 from NW Mexico was more predictive of yield at more international nursery sites than canopy temperature. Depending on the environment in which the parameters from NW Mexico were determined, they can be used to predict grain yield in many worldwide locations (nurseries), especially in Central Asia, North Africa, Southern Europe and North America (Table 3).

Conclusions

The NWI-3, canopy temperature and grain yield obtained from NW Mexico in three environments (well irrigated, water stress, and high temperature) showed significant associations with the grain yield of nurseries located in diverse worldwide regions for the three international trials (24^{th} ESWYT, 11^{th} SAWYT and 11^{th} HTWYT). Depending on the environment where the NWI-3 and canopy temperature where determined, these parameters demonstrated significant associations with certain nurseries. Many significant associations were obtained when the 25% top yielding lines were used for the relationship between the NW Mexico parameters and the grain yield of genotypes at international nursery sites. This means that the best yielding lines in NW Mexico are frequently the same high yielding lines in other regions of the world. Locations from Central Asia, North Africa, Southern Europe, and North America showed the stronger associations with NWI-3 and canopy temperature measurements from NW Mexico in diverse environments, and NWI-3 showed a greater number of significant associations than canopy temperature, especially when the 25% top yielding lines were selected. The NWI-3 and canopy temperature successfully predicted yield performance of advanced breeding lines in the three international trials (24^{th} ESWYT, 11^{th} SAWYT, and 11^{th} HTWYT), especially for selected locations in Central Asia, North Africa, Southern Europe and North America regions. Our results indicate that these two indirect selection parameters have the potential to identify genotypes with high yield and broad adaptation, but further studies are required to confirm these observations.

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Tables

Table 1. List of international locations where advanced lines of the 24th Elite Spring Wheat Yield Trial (ESWYT), 11th Semi-Arid Wheat Yield Trial (SAWYT), and 11th High Temperature Wheat Yield Trial (HTWYT) were planted in 2003 under diverse environmental conditions.

Country	Site	Latitude	Longitude	Altitude (m)	Soil	pH	International trial		
Northwest Mexico (2006, 2007 and 2008)									
Mexico	Cd. Obregon	27°24'N	109°56W	38	Caliciorthid	7.7	ESWYT	SAWYT	HTWYT
International nurseries (2003)									
1. Afghanistan	Behsud	34°26'N	70°02'E	570					HTWYT
2. Afghanistan	Coll. of Agriculture						ESWYT		
3. Afghanistan	Darul	34°28'N	69°03'E	1841		5.6-7	ESWYT		
4. Afghanistan	Dehdadi	36°65'N	66°96'E	477		7.1-8	ESWYT	SAWYT	
5. Afghanistan	Khoja	34°04'N	32°01'E	1198				SAWYT	
6. Afghanistan	Kunduz R. Station	36°43'N	68°51'E	403			ESWYT		
7. Afghanistan	Shesham Bagh	34°42'N	70°74'E	552		5.6-7	ESWYT		
8. Afghanistan	Urdokhan	34°01'N	62°01'E	1096			ESWYT		
9. Algeria	El Khroub				Vertisol	7.1-8	ESWYT	SAWYT	
10. Angola	Humpata						ESWYT		
11. Argentina	Marcos J.	32°42'S	62°07'W	110	Arguidol	5.6-7	ESWYT	SAWYT	
12. Argentina	Pergamino				Pergamino	5.6-7	ESWYT	SAWYT	
13. Argentina	Tucuman-Obispo						ESWYT		
14. Canada	Aafc Glenlea F. St.				Black Orthic	7.1-8	ESWYT		
15. Canada	Kernen Res. F.				Sutherland		ESWYT		HTWYT
16. Canada	Swift Current						ESWYT	SAWYT	HTWYT
17. Egypt	Sids						ESWYT		
18. Hungary	Szeged					5.6-7			HTWYT
19. India	Azad University	26°28'N	80°24'E	406		7.1-8			HTWYT
20. India	Banaras H. U. V.	25°16'N	82°57'E			7.1-8			HTWYT
21. India	Bari					7.6-8	ESWYT	SAWYT	HTWYT
22. India	Bihar Agric. Coll. F.								HTWYT
23. India	D. Plant Breeding					5.6-7	ESWYT		
24. India	Durgapura					7.1-8	ESWYT		
25. India	Dwr-Karnal	15°42'N	76°07'E	638		7.1-8	ESWYT	SAWYT	HTWYT
26. India	Gwalior				Alluence	7.1-8	ESWYT		
27. India	Iari Genetics Div.						ESWYT	SAWYT	HTWYT
28. India	Indore	22°37'N	75°05'E	600	Black cotton	5.6-7	ESWYT		
29. India	Livestock Farm	23°00'N	79°58'E	412	Medium black	5.6-7	ESWYT		HTWYT
30. India	Nepz, Ubkv				Flurauents	5.6-7	ESWYT		
31. India	Niphad	20°06'N	74°06'E	549	Medium black	>8			HTWYT
32. India	Pantnagar	29°00'N	79°30'E	243		7.1-8	ESWYT		
33. India	Powarkheda				Vertisol	7.1-8		SAWYT	
34. India	Pusa-Iari							SAWYT	
35. India	Vijapur	23°35'N	75°45'E	126		>8	ESWYT		HTWYT
36. Iran	Ahwaz	31°17'N	48°40'E	20	Terriorthents	7.1-8			HTWYT
37. Iran	Araghee Mohaleh	36°54'N	54°25'E	132	Xerochrepts	7.1-8	ESWYT		
38. Iran	Fars				Entisols	7.1-8	ESWYT		HTWYT
39. Iran	Moghan	39°49'N	47°50'E	60		7.1-8	ESWYT		
40. Iran	Safiabad A. Res.				Calarious	7.1-8	ESWYT		
41. Iran	Zargan	29°46'N	52°43'E	1603	Calcixerollix	7.1-8	ESWYT		
42. Italy	Montelibretti	47°07'N	12°42'E	80			ESWYT		
43. Kenya	Npbrc-Njoro						ESWYT	SAWYT	
44. Mexico	CIANO	27°24'N	109°56W	38	Caliciorthid	7.1-8		SAWYT	
45. Morocco	Marchouch						ESWYT	SAWYT	HTWYT
46. Morocco	Tassaout						ESWYT	SAWYT	HTWYT
47. Nepal	Nwrp- Bhairahwa	27°30'N	83°27'E	105	Hablaquets	7.1-8	ESWYT		
48. Pakistan	Bannu	32°05'N	70°05'E	285		>8		SAWYT	
49. Pakistan	Barani	32°05'N	72°05'E	490				SAWYT	
50. Pakistan	Dera	31°50'N	70°54'E	171	Aridosol	7.1		SAWYT	HTWYT
51. Pakistan	Jarm Res. S.	33°05'N	71°05'E	500		7.1-8		SAWYT	
52. Pakistan	Narc Islamabad	33°05'N	73°00'E	683			ESWYT	SAWYT	
53. Pakistan	Pirsabak	35°05'N	71°05'E	340			ESWYT	SAWYT	
54. Pakistan	Quetta Ari Sariab					>8		SAWYT	
55. Pakistan	Regional Agric. R.					>8	ESWYT		
56. Pakistan	Sakrand	26°31'N	68°03'E	31		7.1-8	ESWYT		
57. Pakistan	Sariab					7.1-8	ESWYT		
58. Pakistan	Wheat Res. I.					7.1-8	ESWYT	SAWYT	HTWYT
59. Poland	Danko-Choryn					5.6-7	ESWYT		
60. Poland	Radzikow P. Breed					5.6-7	ESWYT		
61. Portugal	P. Alentejo				Alluvial	7.1-8	ESWYT	SAWYT	HTWYT
62. Saudi Arabia	Tabuk Ars					7.1-8	ESWYT		
63. Serbia Montenegro	Kragujev	44°02'N	20°56'E	182	Vertisol	5.6-7	ESWYT	SAWYT	
64. South Africa	Pannar					5.6-7	ESWYT	SAWYT	
65. Spain	Alameda O.				F. xerochrept	>8	ESWYT		
66. Spain	Gimenells	41°35'N	0°32'E	290		>8		SAWYT	
67. Spain	Tomejil	27°24'N	5°35'W	72	Vertisol	7.1-8	ESWYT		
68. Turkey	Aegean	38°04'N	27°00'E	10		7.1-8	ESWYT		
69. Turkey	SE Anatolian					7.1-8		SAWYT	HTWYT
70. Turkey	Univ. of Cukurova	35°01'N	37°01'E	90			ESWYT		HTWYT
71. Turkey	Ziraat	38°42'N	28°45'E	10	Xerofluvient	7.1-8	ESWYT		
72. Zambia	Golden Valley					7.1-8	ESWYT		
73. Zimbabwe	Rattray				Salisbury SE	5.6-8	ESWYT		

Table 2. Minimum, maximum, and mean grain yield (t ha^{-1}) of advanced lines from the 24th Elite Spring Wheat Yield Trial (ESWYT), 11th Semi-Arid Wheat Yield Trial (SAWYT), and 11th High Temperature Wheat Yield Trial (HTWYT) planted in NW Mexico and diverse worldwide sites.

Country	Mean	Min.	Max.
Northwest Mexico†	**24th ESWYT**		
Well Irrigated	6.63**	5.93	7.21
International sites			
2. Afghanistan, C. Agric.	5.45**	4.15	6.24
3. Afghanistan, Darul	1.22**	0.46	2.38
4. Afghanistan, Dehdadi	4.64*	3.93	5.29
6. Afghanistan, Kunduz	4.69*	3.97	5.24
7. Afghanistan, Shesham	4.02	3.29	4.69
8. Afghanistan, Urdokhan	3.03**	1.98	3.74
9. Algeria, El Khroub	5.27**	1.49	7.78
10. Angola, Humpata	3.12*	1.87	4.45
11. Argentina, Marcos J.	4.20**	2.39	5.09
12. Argentina, Pergamino	3.90**	3.14	4.54
13. Argentina, Tucuman	1.50**	1.14	2.11
14. Canada, Aafc	3.53**	2.88	4.35
15. Canada, Kernen	2.64**	1.90	3.19
16. Canada, Swift	1.57**	0.71	2.41
17. Egypt, Sids	7.15	5.31	9.95
21. India, Bari	3.07*	2.15	3.95
23. India, D. Plant B.	7.13**	4.57	9.37
24. India, Durgapura	1.38	1.13	1.65
25. India, Dwr-Karnal	3.74**	2.79	5.08
26. India, Gwalior	4.86**	3.86	5.71
27. India, Iari	4.36*	3.79	5.24
28. India, Indore	7.87**	7.20	8.64
29. India, Livestock	3.02*	1.84	3.68
30. India, Nepz	3.58**	2.70	4.32
32. India, Pantnagar	4.16**	3.35	5.00
35. India, Vijapur	3.87**	2.73	4.91
37. Iran, Araghee	4.08**	1.23	5.64
38. Iran, Fars	5.36	4.00	6.68
39. Iran, Moghan	5.09**	2.39	7.10
40. Iran, Safiabad	5.61**	4.44	6.70
41. Iran, Zargan	6.05**	5.08	7.25
42. Italy, Montelibretti	6.44**	4.61	7.49
43. Kenya, Npbrc	0.75**	0.38	1.24
45. Morocco, Marchouch	5.85**	4.68	7.72
46. Morocco, Tassaout	6.24	5.06	7.15
47. Nepal, Nwrp	2.50**	2.03	2.92
52. Pakistan, Narc	3.09	2.16	3.62
53. Pakistan, Pirsabak	4.43	3.06	5.12
55. Pakistan, Reg. Agric.	3.17**	2.20	4.16
56. Pakistan, Sakrand	2.66	1.50	3.59
57. Pakistan, Sariab	2.72*	2.27	4.22
58. Pakistan, Wheat R. I.	3.74**	3.13	4.26
59. Poland, Danko	6.84**	5.78	8.13
60. Poland, Radzikow	2.94**	2.32	3.47
61. Portugal, P. Alentejo	4.13	3.11	5.03
62. Saudi Arabia, Tabuk	8.52**	4.96	10.83
63. Serbia Mont., Kragujev	4.60	2.63	5.63
64. South Africa, Pannar	9.00*	7.29	10.02
65. Spain, Alameda	5.58**	3.41	7.32
67. Spain, Tomejil	4.29**	2.64	5.72
68. Turkey, Aegean	7.28*	4.97	8.70
70. Turkey, U. Cukurova	7.39	5.74	9.24
71. Turkey, Ziraat	2.51	1.61	3.17
72. Zambia, Golden V.	6.49	3.68	8.44
73. Zimbabwe, Rattray	7.22**	3.03	8.99
Overall mean	4.47		

Country	Mean	Min.	Max.
Northwest Mexico†	**11th SAWYT**		
Well Irrigated	6.42**	4.51	7.12
Water stress	2.20**	1.49	2.77
International sites			
4. Afghanistan, Dehdadi	2.05*	1.46	3.35
5. Afghanistan, Khoja	0.70**	0.30	0.97
9. Algeria, El Khroub	5.21**	1.10	7.12
11. Argentina, Marcos J.	3.46**	1.48	4.77
12. Argentina, Pergamino	2.61**	1.46	3.45
16. Canada, Swift	1.59**	0.83	1.97
21. India, Bari	3.37**	2.35	4.25
25. India, Dwr-Karnal	4.03**	2.91	5.54
27. India, Iari	1.30	0.67	1.81
33. India, Powarkheda	5.90**	4.84	7.26
34. India, Pusa	2.84**	0.81	4.15
43. Kenya, Npbrc	0.64**	0.22	1.33
44A. Mexico, CIANO	5.52**	3.31	6.63
44B. Mexico, CIANO	5.43**	3.44	6.66
45. Morocco, Marchouch	5.61**	3.38	7.25
46. Morocco, Tassaout	5.70**	3.32	7.50
48. Pakistan, Bannu	1.79**	0.50	2.47
49. Pakistan, Barani	2.21**	1.50	3.00
50. Pakistan, Dera	1.36**	0.84	2.04
51. Pakistan, Jarm	3.36	1.70	5.30
52. Pakistan, Narc	3.43*	2.56	4.37
53. Pakistan, Pirsabak	3.25*	2.29	4.17
54. Pakistan, Quetta	0.62	0.37	0.95
58. Pakistan, Wheat R. I.	3.02**	1.97	4.14
61. Portugal, P. Alentejo	4.26**	3.05	5.45
63. Serbia Mont., Kragujev	8.26**	5.69	9.80
64. South Africa, Pannar	1.25**	0.22	2.04
66. Spain, Gimenells	8.17*	6.05	9.39
69. Turkey, SE Anatolian	3.79**	2.89	4.49
Overall mean	3.48		

Country	Mean	Min.	Max.
Northwest Mexico†	**11th HTWYT**		
Well Irrigated	6.42**	5.64	7.17
Water stress	2.40	2.17	2.64
High Temperature	2.69**	1.94	3.31
International sites			
1. Afghanistan, Behsud	4.69	3.51	5.48
15. Canada, Kernen	2.76**	2.08	3.19
16. Canada, Swift	1.57**	1.01	2.13
18. Hungary, Szeged	4.27**	2.99	5.11
19. India, Azad	4.14**	3.34	5.52
20. India, Banaras	3.40**	2.77	3.91
21. India, Bari	3.54**	2.64	4.04
22. India, Bihar	2.71	2.00	3.30
25. India, Dwr-Karnal	3.48	2.27	4.32
27. India, Iari	2.78**	2.14	3.24
29. India, Livestock	3.67*	2.83	4.54
31. India, Niphad	1.58	1.08	2.26
35. India, Vijapur	3.16**	2.07	4.19
36. Iran, Ahwaz	4.84	3.55	5.73
38. Iran, Fars	6.97	5.42	8.13
45. Morocco, Marchouch	5.66	4.73	6.75
46. Morocco, Tassaout	5.66*	4.24	7.28
50. Pakistan, Dera	0.41**	0.26	0.71
58. Pakistan, Wheat R. I.	2.65**	1.91	3.31
61. Portugal, P. Alentejo	3.39*	2.81	4.15
69. Turkey, SE Anatolian	3.86**	2.99	4.55
70. Turkey, U. Cukurova	6.98*	4.32	9.20
Overall mean	3.73		

†Average of three years (2006, 2007 and 2008)

*, ** Significant at the 0.05 and 0.01 probability level, respectively.

Table 3. Nursery distribution by region that showed significant correlations between the parameters measured in NW Mexico and grain yield of nurseries for the 24th Elite Spring Wheat Yield Trial (ESWYT), 11th Semi-Arid Wheat Yield Trial (SAWYT), and 11th High Temperature Wheat Yield Trial (HTWYT).

Trial	Parameter NW Mexico[†]	Nurseries by region			
		Asia	Africa	Europe	America
			24th ESWYT		
Irrigated	NWI-3	Central (7)[‡] & West (1)	Central (1)	Southern (1)	South (1)
	CT	Central (3)	Central (1) & South (1)	Southern (1)	South (1) and North (2)
	Yield	Central (6)	-	-	-
			11th SAWYT		
Irrigated	NWI-3		North (1)	Southern (1)	-
	CT	Central (2)	-	Southern (1)	-
	Yield	Central (1) &West (1)	North(2)	Southern (1)	South (1) & North (1)
Water stress	NWI-3	Central (3)	North (1)	-	-
	CT	Central (2)	North (1)	Southern (1)	-
	Yield	Central (3)	Central (1)	-	-
			11th HTWYT		
Irrigated	NWI-3	-	-	Central (1)	-
	CT	-	-	-	-
	Yield	Central (2)	North (1)	-	-
Water stress	NWI-3	-	-	-	-
	CT	-	-	-	North (1)
	Yield	-	North (1)	-	North (1)
High temperature	NWI-3	-	-	-	-
	CT	Central (1)	-	-	-
	Yield	Central (2)	-	-	-
			Mixed environments from NW Mexico		
			11th SAWYT (irrigated and water stress)		
Combined	NWI-3	-	North (1)	-	-
	CT	Central (1)	North (1)	-	South (1)
	Yield	Central (2)	North(2)	Southern (1)	South (1) & North (1)
			11th HTWYT (irrigated, water stress, and high temperature)		
Combined	NWI-3	-	-	-	-
	CT	Central (1)	North (1)	-	-
	Yield	Central (2)	-	-	-
			Selecting the top 25% yielding lines from NW Mexico		
			24th ESWYT		
Irrigated	NWI-3	Central (23) & West (4)	North (3), Central (2) & South (2)	Southern (3) & Central (1)	South (1) & North (3)
	CT	Central (18) & West (1)	North (3), Central (1) & South (2)	Southern (3) & Central (2)	South (2) & North (2)
	Yield	Central (22) & West (4)	North (2), Central (2) & South (2)	Southern (2) & Central (2)	South (3) & North (1)
			11th SAWYT		
Irrigated	NWI-3	Central (11)	North (2)	Southern (1)	South (1) & North (1)
	CT	Central (7) & West (1)	North (1), Central (1) & South (1)	Southern (1)	South (2) & North (1)
	Yield	Central (7) &West (1)	North(3) & South (1)	Southern (1)	South (2) & North (2)
Water stress	NWI-3	Central (7 & West (1))	North (1)	Southern (1)	South (1) & North (3)
	CT	Central (7) & West (1)	North (2), Central (1) &South (1)	Southern (2)	South (1) & North (1)
	Yield	Central (7) & West (1)	North (1) & South (1)	Southern (2)	North (2)
			11th HTWYT		
Irrigated	NWI-3	Central (6) & West (2)	North (1)	Southern (1) & Central (1)	North (1)
	CT	Central (4) & West (2)	North (1)	Central (1)	North (1)
	Yield	Central (3) & West (2)	North (1)	Central (1)	North (1)
Water stress	NWI-3	Central (10) & West (1)	North (1)	Southern (1) & Central (1)	North (2)
	CT	Central (5) &West (1)	North (1)	Central (1)	-
	Yield	Central (5) & West (1)	North (2)	-	-
High temperature	NWI-3	Central (8) & West (2)	North (1)	Southern (1) & Central (1)	North (2)
	CT	Central (6) & West (2)	North (1)	Central (1)	-
	Yield	Central (7)	North (1)	Southern (1) & Central (1)	North (2)

[†]NWI-3, normalized water index three; CT, canopy temperature.
[‡]parenthesis number indicates the total of nurseries in each region.

Figures

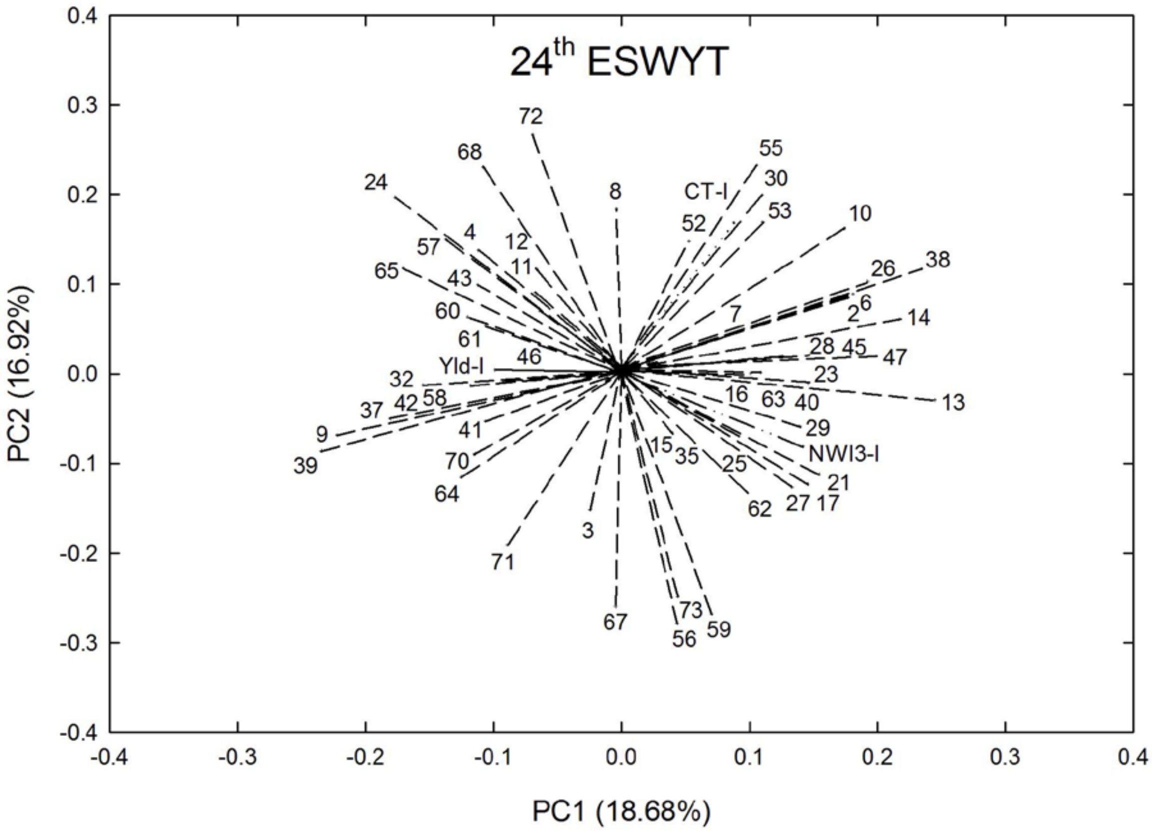

Figure 1. Two-dimensional distributions of coefficients of the first two principal components (PC) obtained by a multivariate analysis of NWI-3, canopy temperature, and grain yield from NW Mexico and grain yield of diverse nurseries for the Elite Spring Wheat Yield Trial (ESWYT). Estimates were based on three combined years (2006, 2007, and 2008) for NW Mexico and one year (2003) for the international nursery sites.

Yld-I, grain yield-irrigated; NWI3-I, normalized water index three-irrigated; CT-I, canopy temperature-irrigated; Numbers indicates sites (see Table 1).

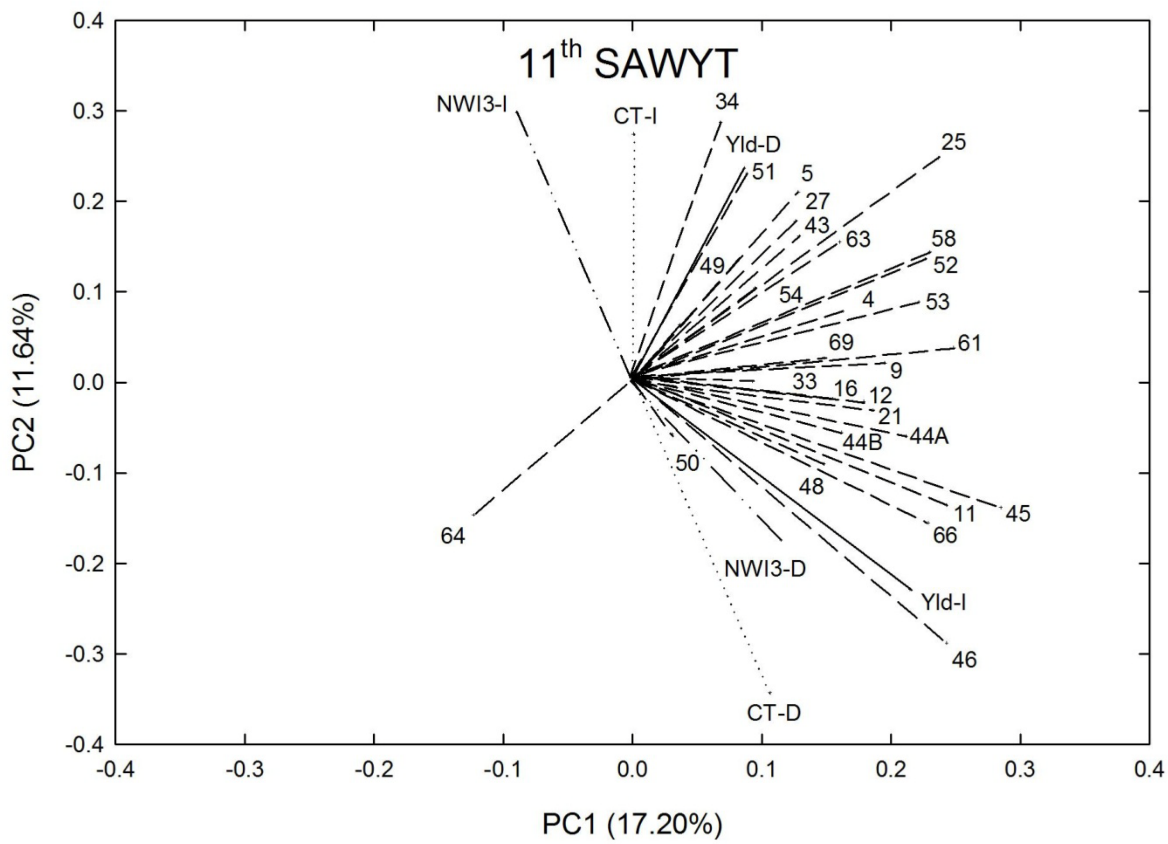

Figure 2. Two-dimensional distributions of coefficients of the first two principal components (PC) obtained by a multivariate analysis of NWI-3, canopy temperature, and grain yield from NW Mexico and grain yield of diverse nurseries of the Semi-Arid Wheat Yield Trial (SAWYT). Estimates were based on three combined years (2006, 2007, and 2008) for NW Mexico and one year (2003) for the international nursery sites.

Yld-I, grain yield-irrigated; Yld-D, grain yield-drought; NWI3-I, normalized water index three-irrigated; NWI3-D, normalized water index three-drought; CT-I, canopy temperature-irrigated; CT-D, canopy temperature-drought; Numbers indicates sites (see Table 1).

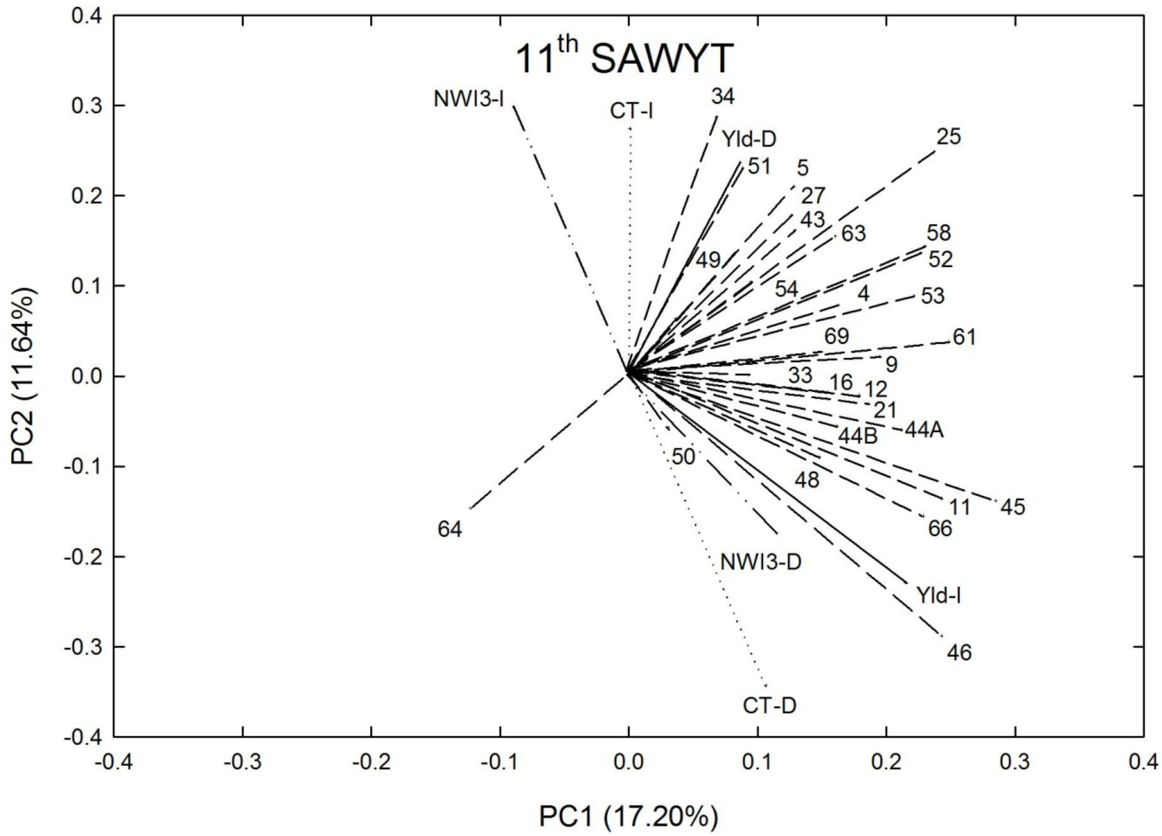

Figure 3. Two-dimensional distributions of coefficients of the first two principal components (PC) obtained by a multivariate analysis of NWI-3, canopy temperature, and grain yield from NW Mexico and grain yield of diverse nurseries of the 11th High Temperature Wheat Yield Trial (HTWYT). Estimates were based on three combined years (2006, 2007, and 2008) for NW Mexico and one year (2003) for the international nursery sites.

Yld-I, grain yield-irrigated; Yld-D, grain yield-drought; Yld-H, grain yield-heat; NWI3-I, normalized water index three-irrigated; NWI3-D, normalized water index three-drought; NWI3-H, normalized water index three-heat; CT-I, canopy temperature-irrigated; CT-D, canopy temperature-drought; CT-H, canopy temperature-heat; Numbers indicates sites (see Table 1).

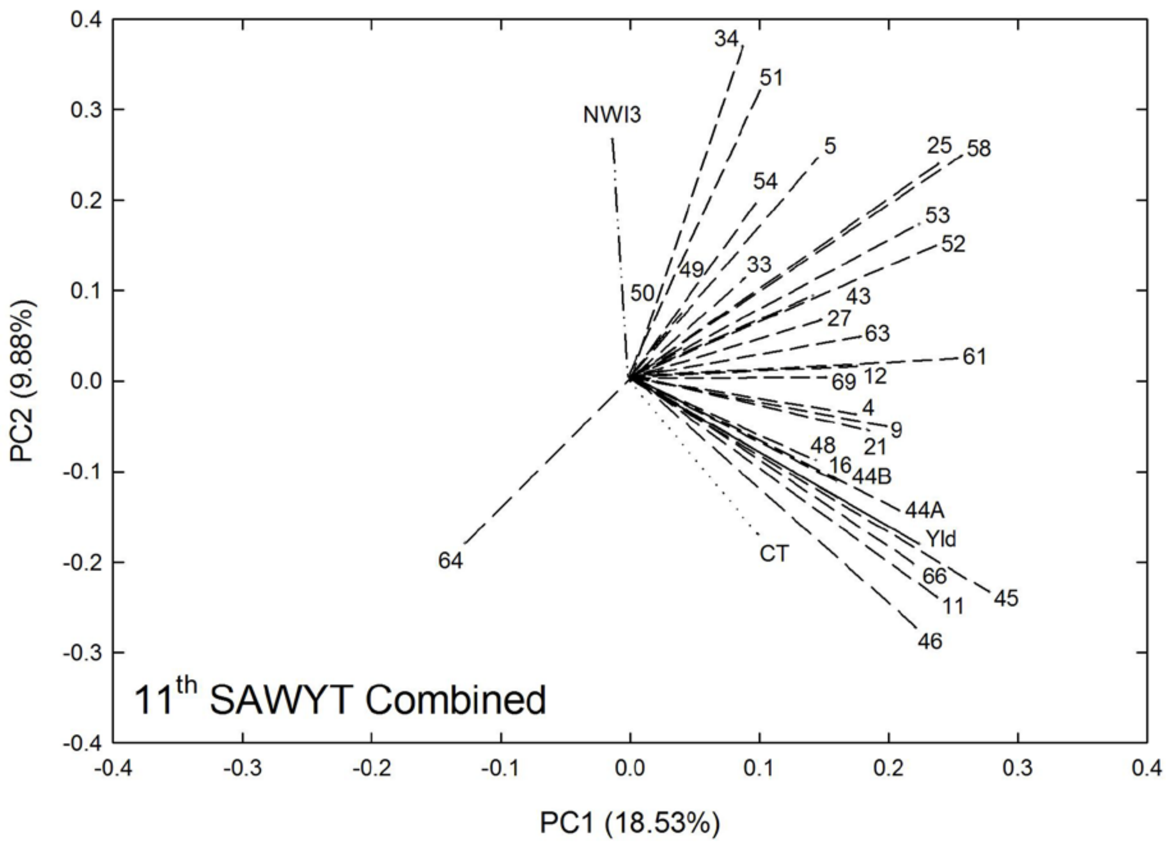

Figure 4. Two-dimensional distributions of coefficients of the first two principal components (PC) obtained by a multivariate analysis of NWI-3, canopy temperature, and grain yield from NW Mexico and grain yield of diverse nurseries for the 24th Elite Spring Wheat Yield Trial (ESWYT). Average of heading and grain filling stages across years (2006, 2007, and 2008) and environments (well irrigated and water stress).

Yld, grain yield; NWI3-I, normalized water index three; CT, canopy temperature; Numbers indicates sites (see Table 1).

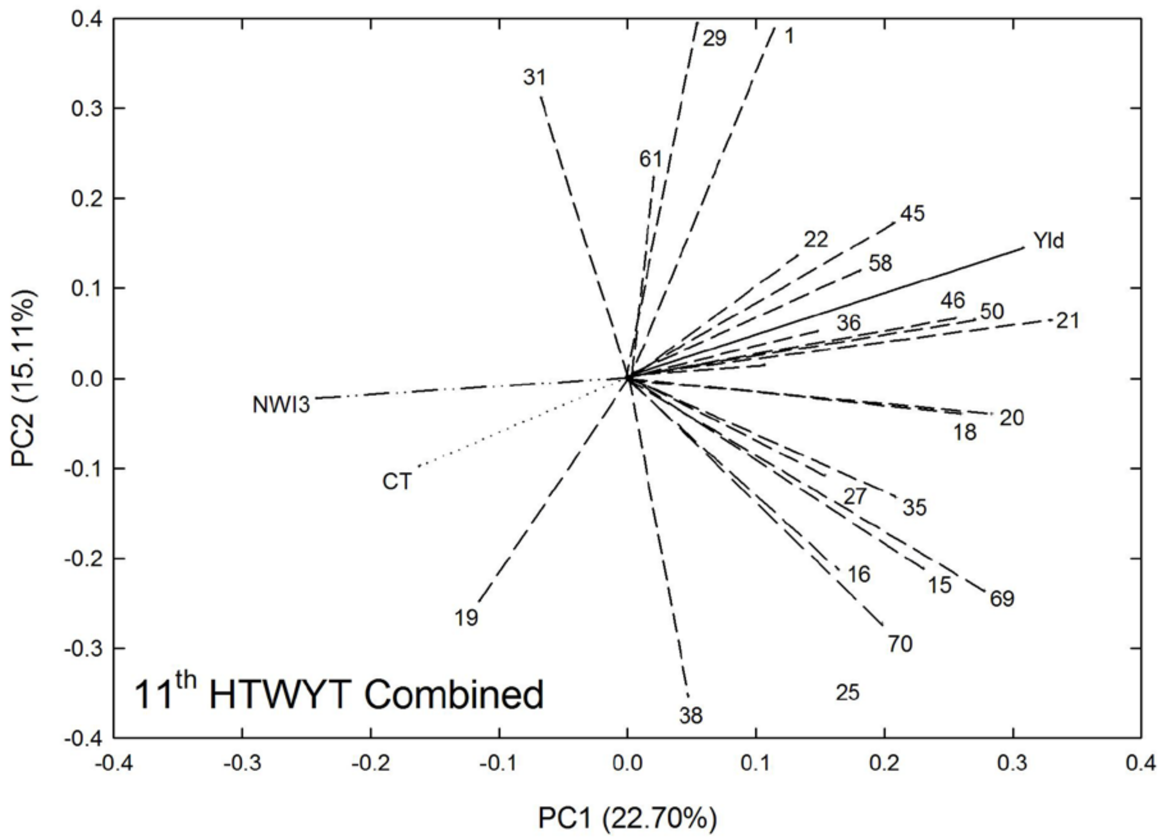

Figure 5. Two-dimensional distributions of coefficients of the first two principal components (PC) obtained by a multivariate analysis of NWI-3, canopy temperature, and grain yield from NW Mexico and grain yield of diverse nurseries for the 24th Elite Spring Wheat Yield Trial (ESWYT). Average of heading and grain filling stages across years (2006, 2007, and 2008) and environments (well irrigated, water stress, and high temperature).

Yld, grain yield; NWI3-I, normalized water index three; CT, canopy temperature; Numbers indicates sites (see Table 1).

CHAPTER IV

Association between water spectral indices and plant water status in spring wheat under water stress conditions

Abbreviations

CIMMYT, International Maize and Wheat Improvement

SBS-I, subset of advanced sister lines in the year 2006 and 2007

SBS-II, subset of sister lines in the year 2008

WUE-I, advanced lines selected for high water use efficiency in the year 2006

WUE-II, advanced lines selected for high water use efficiency in the year 2007

SYNDER, advanced synthetic derivative lines selected for high grain yield under drought

SRI, spectral reflectance indices

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

RWC, relative water content

Abstract

The use of spectral reflectance indices for estimating the plant water status in adverse growth conditions (*i.e.*, water stress) offer great advantages in wheat. Several water indices were determined to establish their relationship with water potential, relative water content (RWC), canopy temperature, soil moisture, and root weight in spring wheat lines under water stress field conditions. Diverse advanced breeding lines from the International Maize and Wheat Improvement Centre (CIMMYT) were employed that corresponded to five trials: a subset of sister lines including parents (SBS-I and SBS-II), lines selected for high water use efficiency (WUE-I and WUE-II), and a group of synthetic derivatives (SYNDER) selected for high grain yield under drought conditions. All genotypes were planted at CIMMYT's experiment station in Northwest, Mexico during three growing seasons (2006, 2007, and 2008). Five water indices (WI and four NWIs; water index and normalized water indices, respectively) were determined at diverse growth stages (booting, heading, and grain filling) using a field portable spectrometer (Analytical Spectral Devices, Boulder, CO). The relationships between the normalized water index three (NWI-3) and the water potential were significant for the WUE-I, WUE-II, and SBS-II trials when both parameters were correlated in individual growth stages. However, when growth stages were combined (booting, anthesis and grain filling), the relationships between the NWI-3 and water potential were stronger for the SBS-II (r^2=0.85) and SYNDER (r^2=0.76) trials in the year 2008, and explain a larger proportion of the water potential variations. Similarly, canopy temperature showed a strong association with the water indices (r^2=0.81 and 0.78 for SBS-II and SYNDER, respectively) as well as with water potential (r^2=0.61 and 0.72 for SBS-II and SYNDER, respectively) combining midday determinations. The SBS-II and SYNDER genotypes showed stronger relationships between NWI-3 and water potential, canopy temperature, grain yield, and biomass. In addition, there were good relationships between the NWI-3 and soil moisture. Apparently, resistant genotypes with high water content, high grain yield, and low canopy temperature access deeper soil layers for water uptake. The use of the water indices, especially NWI-3, offers great advantages in wheat breeding programs because they can be determined in

an easy and quick manner integrating the complete canopy at low economic cost, and large genotype numbers can be evaluated for estimating additional parameters (*i.e.*, water potential)

Introduction

Water availability is an important factor that limits plant growth in semiarid areas and reduces crop yield and causes economic losses in diverse regions worldwide (Hanks, 1988, Araus *et al.*, 2002). Water status refers to the amount of water in a plant, crop, or soil, and it is influenced by environmental conditions, agronomic practices, and soil properties (Hanks, 1988). The plant water status provides information that can be used to prevent crop water deficit stress through irrigation and to assess crop growth under drought conditions (Tucker, 1980; Peñuelas *et al.*, 1993).

Measurements of relative water content (RWC) and water potential are the standard parameters for determining the plant water content under water deficit stress conditions (Slatyer, 1967; Nobel, 1983). The RWC provides information about the plant water content by measuring the amount of water that the plant requires to reach maximum turgor, and it is expressed as a percentage in specific growth stages (Slatyer, 1967). Nobel (1983) established that the leaf water potential is the most accurate indicator of the plant/crop water status. Because of value differences in the soil, plant, and atmosphere, the water potential is considered the main driving force in transpiration, providing information about the water content of the plant and soil as an integrated system (Kozlowski *et al.*, 1991). The pressure chamber technique developed by Scholander *et al.* (1964) is the most common method for determining leaf water potential where the pressure applied to the leaf is approximately equal to the plant water potential (Kramer and Boyer, 1995). Water potential and RWC have been employed for assessing plant water content in diverse wheat genotypes under water stressed environments, and their combination with canopy temperature, and grain yield are used to distinguish drought resistant from drought susceptible genotypes in wheat (Munjal and Dhanda, 2005).

The stomatal conductance and transpiration indicate stomata opening level and are also used to screen water status in diverse crops (Lu *et al.*, 1998). Both parameters depend highly on the plant water content in adverse or optimal growth conditions (Condon *et al.*, 2004; Lu *et al.*, 1998). Modern cultivars of Pima cotton and bread wheat with superior high yield potential showed enhanced stomatal conductance compared to old cultivars under irrigated and high temperature conditions (Lu *et al.*, 1998). Breeding for high yield potential in Pima cotton and bread wheat generated better stomatal conductance rates than photosynthetic rates (Lu *et al.*, 1998). Similarly, spring wheat cultivars delivered in diverse years (1962-1988) in northwest Mexico had increased stomatal conductance (63%), higher photosynthetic rate (23%), and reduced canopy temperature (0.6^{o}C cooler), while grain yield was only improved 27% (Fisher *et al.*, 1998). Elevated stomatal conductance is associated with enhanced leaf cooling at flowering and boll filling in Pima cotton and at anthesis and grain filling in bread wheat, especially for high demanding environments (Fisher *et al.*, 1998; Lu *et al.*, 1998). Stomatal conductance can be used in breeding programs as a selection criterion for high grain yield in wheat in irrigated, drought and hot environments (Lu *et al.*, 1998; Condon *et al.,* 2004).

Another method for detecting crop water status is canopy temperature, which is based on the assumption that a plant transpires water through its leaves, thereby reducing their temperature (cooling system) (Reynolds *et al.*, 1994). Plant canopy temperature indicates how transpiration cools leaves, and indicates the cooling efficiency under high demanding environments (Araus *et al.*, 2008). High transpiration rates and stomatal conductance mean better cooling in leaves for optimizing the photosynthesis process (Araus *et al.*, 2008). Lower canopy temperature in particular genotypes also indicates their capacity for taking water from the soil to maintain satisfactory plant water status (Araus *et al.*, 2008). Selecting lines with high transpiration rates is an alternative manner for selecting for high yield potential (Reynolds *et al*., 1999). The maintenance of favorable plant water status during a water stress (high water potential) implies reduced water loss through stomata and maximizes water uptake through the root system (Barnabás, 2008). When the soil water availability decreased, leaf water potential, canopy temperature, transpiration rate, stomatal conductance and photosynthesis rate are reduced (Sharma and Pannu, 2008). Screening for canopy temperature has been conducted in vegetative (non complete ground cover) and in reproductive growth stages (full groundcover) under drought stressed and irrigated crops (Royo *et al*., 2005). Thermal imaging is an innovative phenotyping method for the spatial examination of canopy temperature patterns associated with transpiration at the canopy or leaf level (Chaerle *et al.*, 2007, Grant *et al*., 2007). Thermal imaging is a promising phenotyping technique for screening canopy temperature in a large number of genotypes with high efficiency in a short time (Araus *et al.*, 2008; Chapman 2008).

Another promising potential trait for determining crop water status is the carbon isotope discrimination (CID) (discrimination for the stable isotope ^{13}C). CID has been proposed as a predictive selection criterion for high grain yield in wheat under water stressed environments (Araus *et al.*, 1998; 2001; Condon *et al.*, 2002). In fact, Australia has delivered two commercial wheat cultivars for rainfed conditions by selecting genotypes with low CID (high transpiration) at tillering (Richards, 2006). Similarly, CID measured in mature grains is also positively correlated with grain yield in wheat in Mediterranean regions (Araus *et al*., 1998; Condon *et al*., 2004). CID has been correlated with stomatal conductance and grain yield in Pima cotton and bread wheat cultivars, and is also associated with yield progress in wheat cultivars (Lu *et al.*, 1998, Fischer *et al.*, 1998). The positive relationship between CID and grain yield in genotypes with low CID indicates a better water status (Araus *et al*., 2002; Condon *et al*., 2004). Even though CID offers various advantages, this technique has had low acceptance due to the high cost of processing samples (Araus *et al.*, 2008).

The term water use efficiency (WUE) refers to the efficiency of water consumed by a crop for producing biomass or grain yield by carbon assimilation, and is an important parameter in semiarid regions (Tambussi *et al.*, 2007). WUE is defined as the biomass produced per mm of water extracted from the soil and transpirated by plant. Genotypes with high WUE indicate high biomass capacity per mm of water, and CID can be used for this purpose (Condon *et al.*, 2002). Wheat genotypes with high biomass production were more efficient for extracting soil water (11%) compared with genotypes with low biomass production (Reynolds and Trethowan, 2007). The WUE also offers great potential for breeding purposes to increase grain yield in wheat (Condon *et al.*, 2004).

Several of the methods and techniques described previously offer great advantages for determining the crop water content and grain yield in wheat, but many of these physiological approaches have limitations due to high costs of processing samples (CID), time for determining a specific parameter (stomatal conductance, water potential, RWC), and determination of additional parameters (WUE). Spectral reflectance is an alternative that offer great advantages, such as easy and quick determinations, complete canopy integration, and additional parameters estimation (*i.e.*, photosynthetic capacity, leaf area index, intercepted radiation, and chlorophyll content) (Araus *et al.*, 2001). Moreover, the canopy reflectance is also considered as an efficient phenotyping technique for screening many field plots (Chapman, 2008). Plant water status can be assessed by remote sensing systems by using canopy spectral reflectance indices associated with the changes in crop water content (Peñuelas *et al.*, 1997; Ustin *et al.*, 1998; Stimson *et al.*, 2005). Several spectral water indices have been proposed using different wavelengths for detecting changes in plant water status in diverse crops (Peñuelas *et al.*, 1993; Gao, 1996; Peñuelas *et al.*, 1997; Serrano *et al.*, 2000; Stimson *et al.*, 2005). Energy is strongly absorbed by water in specific wavelengths and diverse indices have been proposed (simple ratios) for predicting crop water content. Accurate estimations can be obtained using wavelengths which penetrate far into canopies (Sims and Gamon, 2003). Diverse wavelengths in the near infrared (700-1300 nm) and short infrared (1300-2500 nm) have been employed for monitoring plant water status and several water bands have been proposed in the electromagnetic spectrum at 970, 1240, 1400, and 2700 nm (Tucker, 1980; Peñuelas *et al.*, 1993; Gao, 1996; Zarco-Tejada and Ustin, 2001; Anderson *et al.*, 2004; Stimson *et al.,* 2005). Gao (1996) developed the normalized difference water index (NDWI; $[R_{860}-R_{1240}]/[R_{860}+R_{1240}]$) to determine canopy water content in soybean and corn (Anderson *et al.*, 2004). Stimson *et al.* (2005) found that the NDWI and the normalized difference vegetation index (NDVI; $[R_{900}-R_{680}]/[R_{900}+R_{680}]$) showed significant correlation with water potential (r^2=0.68, and 0.71, respectively) in *Pinus*. Zarco-Tejada and Ustin (2001) proposed the simple ratio water index (SRWI, R_{860}/R_{1240}) to measure the water status in forest canopies.

The water index (WI, R_{970}/R_{900}) proposed by Peñuelas *et al.* (1993) was used to estimate water status in *Phaseolus vulgaris*, *Capsicum annuum* and *Gerbera jamesonii*, and was related with the RWC under reduced water conditions. In broccoli plants, the WI explained the plant water content variations and total biomass under diverse water treatments (El-Shikha *et al.*, 2007). Babar *et al.*, (2006) proposed two normalized water indices (NWI-1=$[R_{970}-R_{900}]/[R_{970}+R_{900}]$

and NWI-2=[R_{970}-R_{850}]/[R_{970}+R_{850}]) based on the water index proposed by Peñuelas *et al.* (1993) for screening grain yield in spring wheat genotypes under well irrigated and water deficit stress conditions. Two additional normalized water indices (NWI-3=[R_{970}-R_{880}]/[R_{970}+R_{880}] and NWI-4=[R_{970}-R_{920}]/[R_{970}+R_{920}]) were proposed for screening grain yield of advanced lines of winter wheat under rainfed conditions (Prasad *et al.*, 2007). These five water indices (WI and four NWIs) explained a large proportion of grain yield variability and are alternative approaches for selecting high yielding lines in diverse environments (Babar *et al.*, 2006; Prasad *et al.*, 2007). The normalized water indices (NWIs) are based on the hypothesis that the NIR wavelengths (970 nm) penetrate deeper into the canopy and accurately estimate water content at heading and grain filling (Babar *et al.*, 2006; Prasad *et al.*, 2007; Gutierrez *et al.*, 2008). The association between the water indices and grain yield indicates that canopy water content plays a vital role in yield among wheat genotypes under optimal and adverse growth conditions (Babar *et al.*, 2006; Prasad *et al.*, 2007). However, a large number of studies have been reported using several formulas and diverse wavelengths based on theoretical perspectives, but there is relatively little validation with field data (Serrano *et al.*, 2000; Sims & Gamon, 2003). The objective of the present study is to establish the relationship between water indices (WI and NWIs) and water potential, RWC, canopy temperature, soil moisture, and root weight in wheat genotypes during booting, anthesis (heading), and grain filling under water stress field conditions in Northwest, Mexico.

Materials and Methods

Experimental materials

Spring bread wheat and advanced synthetic derivatives from the International Maize and Wheat Improvement Center (CIMMYT) were used for this study. Fourteen sister lines from the cross Seri-M82/Babax plus the two parents were used in a trial called subset of sister lines (SBS-I) in the year 2006 and 2007. The number of sister lines was reduced to six plus the two parents in the year 2008 (SBS-II). The sister lines used in 2006 and 2007, and subsequently in 2008 were selected on the basis of grain yield and physiological performance (*i.e.*, canopy temperature) under water stress conditions. Another trial included 16 advanced lines selected for high water use efficiency (WUE-I) in the year 2006. In the year 2007, the WUE-II trial included four advanced lines from WUE-I and twelve new advanced lines selected for high grain yield and high water use efficiency under water stress conditions. Finally, ten advanced synthetic derivative lines (SYNDER) selected for high grain yield under water stress conditions were tested in the year 2008.

Growing conditions

The genotypes were grown during the winter season at CIMMYT's experiment station in Cd. Obregon, Northwest Mexico (27.3^{o}N, 109.9^{o}W, 38 m above sea level). The weather is mostly sunny and dry during the winter cropping cycle (see Gutierrez *et al.,* 2008 for environmental conditions in the years 2006, 2007, and 2008). The soil type is coarse sandy clay, mixed montmorillonitic type caliciorthid, low in organic matter and slightly alkaline (pH 7.7) in nature (Sayre *et al.*, 1997).

The seeding rate for each experiment was 78 kg ha^{-1}. Nitrogen and phosphorous were applied to the plots at the rate of 150 kg ha^{-1} and 22 kg ha^{-1}, respectively. Field plots consisted of two raised beds, 5 m long (80 cm wide each) with 2 rows, 10 cm apart on each bed. An alpha lattice design with 2 repetitions was employed for all experiments.

The planting dates were in November and plants reached booting and heading during February-March and were harvested in May. The crop growing seasons for all experiments are referred to as years: 2006 for the cycle 2005-2006, 2007 for the cycle 2006-2007 and 2008 for the cycle 2007-2008. The SBS-I, WUE-I, and WUE-II trials were planted under water stress conditions in the 2006 and 2007 growing seasons. The SBS-II and SYNDER trials were also planted under water stress conditions during the 2008 growing season.

The drought stress conditions were achieved by applying one irrigation before seeding that provided approximately 100 mm of available water, followed two irrigations of about 50-70 mm each applied prior to the booting stage.

Folicur was applied at the booting, heading, and grain filling stages at the rate of 0.5 L ha^{-1} to protect the experimental materials from leaf rust caused by *Puccinia triticina*.

Spectral reflectance measurements

Canopy reflectance was measured in the 350 to 1100 nm range and collected at 1.5-nm intervals using a FieldSpec spectroradiometer (Analytical Spectral Devices, Boulder, CO). Data were determined during cloud-free days at midday between (10:30 and 14:00 hrs) after a calibration using a white plate of barium sulphate ($BaSO_4$) that provides maximum irradiance (Labsphere Inc., North Sutton, USA). Four measurements in each plot were taken at heights of 0.5 m above the canopy with a field of view of 25^o. Each reflectance measurement was the average of 10 scans from an area of 18.94 cm^2 of the plot. Canopy reflectance measurements were taken at random places in each plot during booting (SBS-II and SYNDER), anthesis (SBS-II, and SYNDER), and grain filling (all trials) under water stress conditions.

The water index proposed by Peñuelas *et al.* (1993) was estimated ($WI=R_{970}/R_{900}$) and four normalized water indices proposed by Babar *et al.* (2006) and Prasad *et al.* (2007), $NWI\text{-}1=[R_{970}-R_{900}]/[R_{970}+R_{900}]$, $NWI\text{-}2=[R_{970}-R_{850}]/[R_{970}+R_{850}]$, $NWI\text{-}3=[R_{970}-R_{880}]/[R_{970}+R_{880}]$, and $NWI\text{-}4=[R_{970}-R_{920}]/[R_{970}+R_{920}]$, were also estimated.

Water potential and relative water content (RWC)

The water potential and RWC were estimated using flag leaves during booting (SBS-II and SYNDER), anthesis (SBS-II, and SYNDER), and grain filling (all trials) one day before or one day after the spectral reflectance measurements. Four flag leaves in each plot were used to determine water potential using a pressurized pump (Scholander's pump) in the morning close to sunrise (6:00-8:30 h), at midday (13:00-15:00 h), and at night (22:00-24:30 h).

The RWC was taken almost synchronously with the spectral measurements, and fresh samples of four flag leaves per plot (7-10 cm^2) were collected and immediately weighed (fresh weight, FW). Intact leaves were transferred to sealed tubes, rehydrated in de-ionised water (around 8-12 h until fully turgid at 25^{o}C), and weighed again (turgid weight, TW). Finally, the leaf samples were oven dried at 78^{o}C for 24 h and weighed (dry weight, DW). The RWC was calculated by the following formula: RWC (%) = (FW - DW)/(TW - DW)100.

Canopy temperature

A hand-held infrared thermometer (Mikron M90 Series, Mikron Infrared Instrument Co. Inc., Oakland, NJ) was used to measure canopy temperature depression during grain filling in all the experiments. The mean of four readings was obtained from the same side of each plot at an angle of approximately 30^o with respect to the horizontal angle to integrate as many leaves as possible without viewing the soil. The measurements were taken in the afternoon (13:00-14:00 h) when the crop experienced maximum transpiration rates.

Dry root weight and soil moisture

For estimating the soil moisture content, a hydraulic probe (tube of 2.5 inches in diameter and 2 m length) connected to a tractor was used for collecting soil samples at different depths (0-30, 30-60, 60-90 90-120 cm) during booting, anthesis, and maturity in the SBS-II and SYNDER experiments. For the SBS-I, WUE-I and WUE-II the soil moisture was determined only at maturity (2006 and 2007). After the biomass harvesting in each stage, the probe was placed where shoots had been cut and four soil samples were collected (soil plus roots) at different depths in each plot. The soil samples were kept in a plastic bag in a cooler to avoid moisture loss. The soil from each depth was mixed in the plastic bag and a subsample of 100-150 g was taken for recording fresh weight. Later, the soil samples were oven dried at 78^{o}C for 48 h to register the soil dry weight.

The moisture content was determined by dividing the fresh weight by the dry weight and multiplying by the apparent soil density (1.3 g cm^{-3}) and expressed in mm units.

Another soil subsample (100-150 g) was dissolved in water for 8-12 h for extracting the roots at each soil depth. The roots were collected using a mesh, washed with water, and placed in sealed bottles. All roots were put in a Petri dish with a dark bottom to facilitate the root collection, and then placed in sealed tubes and oven dried at 78ºC for 48 h for recording root dry weight.

Grain yield and biomass

In all experiments grain yield was determined after physiological maturity by harvesting and threshing the entire plot, excluding a 0.5 m border at each end. Prior to grain harvest, a random subsample of 100 spike-bearing culms was removed from the plots. The subsample was oven-dried, weighed, and threshed. The grain weight was recorded and individual kernel weight estimated using a subsample of 200 kernels.

For the biomass harvesting, all the plants in a 0.5 m long area were cut at soil level in one of the two beds of each plot. The area harvested for biomass was 0.4 m^2 (0.5 by 0.8 m). The SRI data were taken randomly before biomass harvesting. The biomass was collected randomly in the middle of the 5 m plot. After the biomass harvesting, the total fresh weight was taken and a representative sample was oven dried at 78^{o}C for 48 h. The dry weight of the biomass was recorded for estimating biomass by area (g m^{-2}). The biomass was sampled at booting, anthesis, and maturity in the SBS-II and SYNDER experiments for the year 2008. During the previous years (2006 and 2007), the biomass was just determined at physiological maturity in the SBS-I, WUE-I and WUE-II trials.

Statistical analysis

All the experiments were analyzed according to the alpha lattice design by using Proc Mixed in the SAS program for each growth stage and year (SAS, 2001). Pearson correlation coefficients were used to estimate the phenotypic relationship of the water indices to water potential, RWC, grain yield, soil moisture and root weight.

Results

According to previous studies (Gutierrez *et al.*, 2008) as well as in this study, two normalized water indices: NWI-1 and NWI-3, have given the strongest relationship between water potential and grain yield, but NWI-3 was slightly better (non-significant) than the other water indices (WI, NWI-1, NWI-2, and NWI-4) during three growing seasons under water stress conditions. As a result, we will discuss only this water index as an exemplification of the other three normalized water indices. Even though other SRI were estimated (RNDVI, GNDVI, and SR; red normalized vegetative difference index, green NDVI, and simple ratio, respectively) they showed lower relationships with water potential, canopy temperature, relative water content, soil moisture, and root weight (data not shown) in all the experiments.

In order to know the value range and significance in certain parameters determined in our study, we simplified their examination by combining growth stages for the normalized water index three (NWI-3), water potential, and biomass for SBS-II and SYNDER trials during 2008 (Table 1). In all the trials and years, canopy temperature and grain yield were analyzed at grain filling and maturity, respectively. However, the SBS-II and SYNDER trials were analyzed in each individual growth stage and time of determination (water indices and water potential) during the year 2008. The NWI-3 did not give significant differences for the SBS-I, WUE-I and WUE-II trials in the years 2006 and 2007, but it gave highly significant results in the year 2008 for SBS-II and SYNDER. Water potential did not show significant difference for the SBS-I and WUE-I trials during 2006, but it was significant for the SBS-I and WUE-II trials during 2007 as well as for the SBS-II and SYNDER trials during 2008. Canopy temperature was only significant for the SBS-II and SYNDER trials in the year 2008, but not for previous years (2006 and 2007). Grain yield showed significant differences for all the trials, except for the SBS-I during 2007. Finally, biomass was significant for the SBS-II and SYNDER trials averaging booting, anthesis, and grain filling during 2008.

Association of the water indices with water potential and relative water content

Different normalized water indices (NWIs) always gave negative relationships with water potential during booting, anthesis, and grain filling in all the trials under the water stress conditions (Tables 2, 3). The relationship between NWI-3 and water potential showed moderate correlations in the SBS-I during 2006 and 2007, while this relationship was significant for the same years in the WUE-I and WUE-II trials (Table 2). There were few differences in the water potential determinations taken in the morning or at the night in both years in the three trials. However, when the genotype number was reduced from sixteen (SBS-I) to eight (SBS-II), the correlations between the water potential and the NWI-3 showed stronger relationships (highly significant) for the anthesis stage in the year 2008 (Table 3). There were minimal differences for the NWI-3 determined at midday (11:00 h, 13:00 h, and 15:00 h) and their corresponding relationships with water potential, but correlations generally were slightly lower at 15:00 h. When water potential was determined at night for the year 2008, the relationships were lower than when determined at midday for the SBS-II trial (Table 3). For the SYNDER trial, the relationships between the NWI-3 and water potential ranged from low to moderate (non significant).

When the NWI-3 determinations taken at midday were averaged (11:00 h, 13:00 h, and 15:00 h) and related to the water potential determinations at midday at booting, anthesis, and grain filling (not averaged), the relationship was highly significant ($p \leq 0.01$) for the SBS-II (r^2=0.85) and for the SYNDER (r^2=0.76) trials (Fig. 1). Similar results were obtained using other normalized water indices (NWI-1, NWI-2 and NWI-4) (data not shown). The relationship was reduced using night determinations of water potential, but remained significant for both trials (r^2=0.59 for the SBS-II and r^2=0.64 for the SYNDER).

On the other hand, the relationships between the RWC and NWI-3 (same for other NWIs) did not show a clear association in diverse experiments during the three growing seasons for all the experiments. The association between NWI-3 and RWC was low and negative for two years (2006 and 2007) in the SBS-I, WUE-I, and WUE-II trials, while some positive and negative correlations were obtained during the year 2008 in the SBS-II and SYNDER trials (Tables 2, 3).

Relationship between water indices and canopy temperature

The correlations between the water indices and canopy temperature were positive and ranged from low during 2006 to moderate during 2007 with one significant correlation at grain filling in the SBS-I trial (Table 2). The same relationship was highly significant at the anthesis and grain filling stages for the SBS-II trial during 2008 (Table 3). However, for SYNDER, the correlations were lower and only one correlation was significant at booting.

The mean value of the NWI-3 at midday (averaging determinations at 11:00 h, 13:00 h, and 15:00 h) for each growth stage (booting, anthesis, and grain filling) showed a highly significant relationship ($p \leq 0.01$) with canopy temperature using determinations at booting and at grain filling (Fig. 2). The NWI-3 showed a highly significant association with canopy temperature for the SBS-II (r^2=0.81) and for the SYNDER (r^2=0.78) trials. In the same way, canopy temperature also showed a significant relationship ($p \leq 0.01$) with water potential in the same trials during 2008 (r^2=0.61 for SBS-II and r^2=0.72 for SYNDER) (Fig.3).

Association of the water indices with soil moisture and root weight

Soil moisture gave significant correlations with the NWI-3 at 30-60 cm during 2006, 0-30 cm and 90-120 cm during 2007 for the SBS-I, while for the WUE-II, a significant correlation was found at the 30-60 cm soil depth (Table 2). For the year 2008, the significant correlations were found at grain filling at 0-30 cm, 30-60 cm, and 90-120 cm soil depths for the SBS-II, while for the SYNDER trial, the significant correlations were found at 60-90 cm soil depth at grain filling (Table 3). When the mean value of NWI-3 at midday (averaging determinations at 11:00 h, 13:00 h, and 15:00 h) for booting, anthesis, and grain filling were combined and correlated with the soil moisture, the relationship between both parameters was significant for the SBS-II (r^2=0.13-0.74) and for SYNDER (r^2=0.42-0.72) (Fig. 4). The relationship was stronger at superficial soil layers (0-30 cm) than at deeper layers (90-120 cm).

Root weight at 60-90 cm soil depth showed highly significant correlations with the NWI-3 in SBS-II at anthesis and grain filling, and the SYNDER trial showed one significant correlation at 0-30 cm for the booting stage and at 30-60 cm soil depth for the booting and grain filling stages.

Association of the water indices and canopy temperature with grain yield and biomass

The relationships between grain yield and the NWI-3 gave low correlation values (non significant) for the WUE-I and WUE-II trials during 2006 and 2007, while in the SBS-I trial, the relationship between both parameters was significant during 2007 (Table 4). The correlations between grain yield and the NWI-3 were highly significant for the anthesis and grain filling stages and when the two growth stages were combined in the SBS-II for the year 2008. For SYNDER, the correlations with grain yield were significant for the grain filling stages.

The NWI-3 showed highly significant correlations with biomass at anthesis, grain filling, and when both growth stages were combined in the SBS-II trial during 2008 (Table 4). For the SYNDER trial, the significant correlations were found at grain filling, and when anthesis and grain filling were combined.

Canopy temperature showed strong relationships with grain yield and biomass in the SBS-II and SYNDER trials for the year 2008, but not for previous years (Table 4).

Discussion

There were few significant differences for the parameters determined during 2006 and 2007, but major differences were obtained for the water parameters (NWI-3, water potential, and canopy temperature), grain yield, and biomass in the SBS-II and SYNDER trials during 2008.

Association of the water indices with water potential and relative water content

The relationship between NWI-3 (same for other NWIs) and water potential were significant for the WUE-I and WUE-II trials grown during 2006 and 2007 at grain filling, and was highly significant at anthesis in the SBS-II during 2008 (Table 2, 3). NWI-3 and water potential showed low and moderate associations in individual growth stages; however, the best relationships ($p \leq 0.01$) were obtained when the three growth stages (booting, anthesis and grain filling) were combined (not averaged), and when the NWI-3 midday determinations were averaged (11:00 h, 13:00 h, and 15:00 h) in the SBS-II and SYNDER trials during 2008 (Fig. 1). The water potential variations were explained by NWI-3 (Fig 1) and the other water indices (data not shown). Leaf water potential is considered the most accurate indicator of plant water status, and some authors have used it for evaluating plant water content and drought resistance in diverse wheat genotypes in water stressed environments (Nobel, 1983; Munjal and Dhanda, 2005). These relationships were similar for SBS-II and for SYNDER using midday (r^2=0.85 and r^2=0.76, respectively) and night (r^2=0.59 and r^2=0.64, respectively) determinations of water potential (Fig. 1). At midday (high sunshine), plants express the maximum response to drought resistance with higher temperatures and the most resistant genotypes maintain higher water content than the sensitive plants. The enhanced water content in certain genotypes allows the plants to maintain growth in reduced soil water conditions, which results in higher yields. Our results indicate that NWI-3 can be used for identifying genotypes with better canopy water content that also leads to high stomatal conductance, transpiration, and lower leaf temperature.

Rapid and easy determination, complete canopy integration, low technique cost, screening of large genotype numbers in a short time, estimation of additional physiological parameters (water potential), and a strong correlation with grain yield are some advantages that the water indices offer to wheat breeding (Babar *et al.*, 2006; Prasad *et al.*, 2007; Gutierrez *et al.*, 2008). It is much easier to determine the water indices for assessing canopy water content instead of the time consuming method of measuring water potential with Scholander's pressure pump. In fact, estimation of the crop water stress by remote sensing is an important goal for irrigation scheduling because the plant water status provides information to prevent crop water stress (Jackson, 1986). If crop water stress is detected (low crop water content) in certain critical growth stages (*i.e.*, anthesis and grain filling) using a water index, yield losses could be prevented by applying irrigations. Koksal (2008) evaluated several water spectral indices to develop a water deficit index for irrigation purposes based on the crop water content during the crop growing season.

Diverse studies have been using spectral reflectance indices for estimating plant water status and water stress in several crops (Luquet *et al.*, 2003; Penuelas *et al.*, 1993, Anderson *et al.*, 2004; Stimson *et al,* 2005; Zarco-Tejada and Ustin, 2001). In our study, the four normalized water indices (NWIs) explained the water potential variations and they could be used for predicting plant/crop water content (Fig. 1). Other studies have found that the relationship between spectral indices and plant water status decreased with low and moderate levels of water stress (Peñuelas *et al.*, 1997; Stimson *et al.*, 2005). Our results demonstrated that the correlations between the water indices and water potential were stronger under high water stress field conditions in diverse trials (Table 2, 3; Fig 1). The plant water potential ranged from –0.59 to –2.70 MPa for the year 2006, from –0.72 to –1.54 MPa for the year 2007, and from -1.01 to –3.99 MPa for the year 2008. The selection of specific wavelengths with strong absorption by water is essential to increase the sensitivity of selected spectral indices for explaining changes in plant water status (Eitel *et al.*, 2006). The 970 nm wavelength employed in the four normalized water indices resulted sensitive for detecting water content differences among wheat genotypes growing under water deficit conditions.

Under reduced soil water content, plants close stomata on leaves to conserve water in order to maintain adequate water content (Serrano *et al.*, 2000). Our hypothesis that the water indices are associated with the plant water content is confirmed because the water indices consistently detected changes in water potential and are also associated with canopy temperature (high stomatal conductance, transpiration, and low leaf temperature). An increase in plant water content causes a decrease in the amount of light energy that is reflected at the 970 nm, and lower water content in the canopy results in lower grain yield and biomass (Babar *et al.*, 2006; Prasad *et al.*, 2007; Gutierrez *et al.*, 2008). The association between the water indices and grain yield indicates that canopy water content plays a vital role in yield among genotypes under diverse environments (well irrigated, water stress, rainfed, and high temperature conditions) in spring and winter wheat (Babar *et al.*, 2006; Prasad *et al.*, 2007; Gutierrez *et al.*, 2008). The water indices explained a large part of grain yield variability and they can be used as an alternative breeding tool for selecting high yielding wheat lines (indirect selection) (Babar *et al.*, 2006; Prasad *et al.*, 2007, Gutierrez *et al.*, 2008).

Even though RWC has been reported to estimate plant water content (Slatyer, 1967; Chaves *et al.*, 2002); in our study, we did not find any relationship between NWI-3 and RWC. The RWC determinations did not show any pattern of association with the water indices. In another study, the normalized difference water index (NDWI) showed a high correlation with RWC at the leaf (r^2 = 0.94) and canopy levels (r^2 = 0.60), but the plant water content variations over time were not explained by the RWC (Eitel *et al.*, 2006). Kozlowski *et al.* (1991) reported that RWC has limitations when the full saturation of leaves cannot be determined appropriately, thereby reducing its accuracy (Bradford and Hsiao, 1982).

Association between the water indices and canopy temperature

The relationship between the water indices and canopy temperature was stronger when growth stages were combined (booting, anthesis, and grain filling) for the SBS-II and SYNDER trials (Fig. 3). Plant canopy temperature indicates that transpiration cools leaves, and indicates the cooling efficiency under demanding environments (Araus *et al.*, 2008). If the plant water content decreases, the transpiration rate is reduced, thereby losing the cooling efficiency of the leaves. The positive relationship between the water indices and canopy temperature found in our study (Fig. 2), means that plants with lower canopy temperature maintain adequate water content for growing in adverse growth conditions (drought). Genotypes with better drought resistance (high yielding) in SBS-II and SYNDER could be identified using the water indices and/or canopy temperature because the most drought resistant genotypes maintained better water content (lower water potentials) under water stress conditions (Fig. 3). In consequence, the plant water status can be estimated using the water indices and/or canopy temperature because both parameters showed a significant relationship with water potential in the SBS-II and the SYNDER trials (Fig. 2, 3). The NWI-3 and canopy temperature are easy to measure in the field for evaluating large number of genotypes and both could be used for predicting plant water content in genotypes with high yield potential (high water content). There are few advantages of using the NWI-3 instead of canopy temperature because other spectral reflectance indices can be used for estimating additional physiological traits (*i.e.*, leaf area index and intercepted radiation). However, the parameters could be used together to confirm the selection the best yielding lines.

Association of water indices with soil moisture and root growth

There are no reports in the literature to explain the relationship between canopy spectral indices and soil water content in different soil layers. Spectral soil reflectance has only been used to estimate moisture on surface layers, type of texture, and organic matter content (Hummel *et al.*, 2001). The SBS-II genotypes always showed highly significant correlations between the water indices and plant water content parameters (water potential and canopy temperature), and they also showed an association with soil moisture (Table 2, 3; Fig. 4). This seems to verify that these eight genotypes, which were selected on the basis of grain yield under water stress conditions, have good resistance to drought (Table 3, 4). The relationship between the NWI-3 and soil moisture was significant for diverse soil depths in SBS-II and SYNDER (Fig. 4). It indicates that canopy water content estimated by the water indices (especially NWI-3) gives a relationship with soil moisture content at different soil depths. In other words, the most drought resistant genotypes (high water content, transpiration, grain yield, and low canopy temperature) develop a root system into deeper soil layers. In drought and hot-irrigated environments, deeper root growth permits better access to soil water to maintain high transpiration rates (better cooling) (Reynolds, and Trethowan, 2007). According to the root weight results in this study, the SBS-II genotypes showed a significant relationship with the water indices at the 60-90 cm soil depth, while the SYNDER genotypes showed some significant correlations at the surface layers (at 0-30 cm and 30-60 cm depths) (Table 3). If the water indices indicate higher water content in high yielding genotypes, then one would expect to find a relationship between root weight and the water indices. The significant correlations at the 60-90 cm soil depth could be related to this assumption in the SBS-II genotypes, but this assumption needs further investigation.

Water indices and yield

The lower relationship of the NWI-3 with water potential and canopy temperature during 2006 and 2007 in the SBS-I, WUE-I and WUE-II trials was the result of a weaker relationship between NWI-3 and grain yield (Table 4). However, for the SBS-II and SYNDER trials in 2008, the significant association of the water indices with water potential and canopy temperature paralleled the relationship between NWI-3 and grain yield and biomass, especially for the SBS-II trial (Table 4). The eight advanced lines in the SBS-II trial gave the strongest relationship between NWI-3 and grain yield. The canopy temperature also showed a significant relationship with grain yield and biomass in both trials during 2008, while the water potential gave a relationship with grain yield and biomass in the three years (data not shown).

Conclusions

The relationship between the water indices and leaf water potential was highly significant for the SBS-II and SYNDER genotypes under water stress conditions during 2008. The strongest association between the water indices and water potential were obtained using midday determinations. The NWI-3 explained a large proportion of the water potential variations when booting, anthesis, and grain filling were combined. Similarly, the canopy temperature showed a strong association with NWI-3 and with water potential if growth stages were combined. The use of NWI-3 and other water indices offer great advantages as an indirect selection tool in wheat breeding, for example, determinations are quick and easy, low economic cost, complete canopy integration, additional parameter estimation (water potential and canopy temperature), and easy in evaluation of large genotype numbers. The water indices and canopy temperature showed strong correlations with water potential and grain yield and they could be used for detecting plant water content and for predicting high yield potential under water stressed environments. The hypothesis that plant water content is an important factor in high yielding genotypes was corroborated. The time consuming methods for estimating water potential is greatly reduced by using the water indices for selecting genotypes with high water content. The significant association between the water indices and soil moisture in the SBS-II and SYNDER genotypes could suggest that resistant genotypes with better water content access water in deeper soil layers. Better water content among high yielding genotypes can be detected by the water indices, which indicates high stomatal conductance, transpiration, and low leaf temperature.

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Tables

Table 1. Minimum, maximum, mean, and least significant difference (LSD) for the normalized water index three (NWI-3), water potential, canopy temperature, grain yield, and biomass in a subset of sister lines (SBS-I and SBS-II), lines selected for high water use efficiency (WUE-I and WUE-II), and synthetic derivatives (SYNDER) grown under water stress conditions.

Trial	Year	Minimum	Maximum	LSD	Mean	Significance
				NWI-3		
SBS-I	2006	-0.039	-0.004	0.020	-0.020	NS
WUE-I	2006	-0.026	-0.007	0.020	-0.010	NS
SBS-I	2007	-0.042	-0.001	0.022	-0.016	NS
WUE-II	2007	-0.034	-0.001	0.015	-0.015	NS
SBS-II†	2008	-0.062	-0.011	0.007	-0.038	**
SYNDER†	2008	-0.069	-0.011	0.010	-0.036	**
				Water potential‡ (MPa)		
SBS-I	2006	-3.99	-2.70	0.59	-3.42	NS
WUE-I	2006	-3.99	-2.01	1.33	-2.78	NS
SBS-I	2007	-3.15	-1.15	0.72	-1.90	**
WUE-II	2007	-3.98	-0.78	1.54	-2.19	*
SBS-II†	2008	-3.73	-2.29	0.35	-3.04	*
SYNDER†	2008	-3.99	-2.98	0.20	-3.59	**
				Canopy temperature (^{o}C)		
SBS-I	2006	27.4	29.1	1.14	28.1	NS
WUE-I	2006	28.6	33.6	2.53	31.3	NS
SBS-I	2007	26.0	28.1	0.90	27.3	NS
WUE-II	2007	31.7	34.5	1.12	33.3	NS
SBS-II†	2008	28.9	31.0	0.23	29.6	**
SYNDER†	2008	26.2	31.2	1.23	29.0	**
				Grain yield (Kg ha^{-1})		
SBS-I	2006	0.38	3.48	0.60	1.30	**
WUE-I	2006	0.28	2.36	0.74	1.13	*
SBS-I	2007	0.64	0.76	0.53	0.71	NS
WUE-II	2007	0.83	2.12	0.39	1.44	**
SBS-II†	2008	1.69	4.28	0.16	3.25	**
SYNDER†	2008	2.13	3.96	0.18	2.98	**
				Biomass (Kg ha^{-1})		
SBS-II†	2008	4.71	8.11	0.71	6.34	**
SYNDER†	2008	2.02	9.41	1.88	4.78	**

*,**Significant at the 0.05 and 0.01 probability level, respectively. NS; non significant differences.
†Booting, anthesis and grain filling were averaged.
‡The water potential determinations were averaged for the determinations at 11:00, 13:00 and 15:00 h during 2008

Table 2. Relationship between the normalized water index three (NWI-3) and diverse water content parameters in a subset of sister lines (SBS-I) and lines selected for high water use efficiency (WUE-I and WUE-II) grown under water deficit stress conditions during 2006 and 2007.

Water indices†	**Growth stage**	**Water status**		**Canopy temp.**	**Soil moisture**‡			
		Water potential†	RWC	Grain filling	0-30 cm	30-60 cm	60-90 cm	90-120 cm
SBS-I (n=16)								
2006		Morning						
NWI-3	Grain filling	-0.48	-0.21	0.38	-0.31	-0.69**	-0.33	-0.20
2007		Night						
NWI-3	Grain filling	-0.47	-0.17	0.58*	-0.61*	-0.26	0.39	0.58*
WUE-I (n=16)								
2006		Morning						
NWI-3	Grain filling	-0.53*	-0.12	0.30	-0.05	-0.34	-0.13	-0.33
WUE-II (n=16)								
2007		Night						
NWI-3	Grain filling	-0.57*	-0.32	0.49	-0.12	0.50*	0.37	0.47

*,**Significant at the 0.05 and 0.01 probability level, respectively.
†Water potential was determined in the morning (6:00-8:30 h) and at night (22:00-24:30 h).
‡Soil moisture was determined at maturity.

Table 3. Relationship between the normalized water index three (NWI-3) and diverse parameters for a subset of sister lines (SBS-II) and synthetic derivatives (SYNDER) grown under water deficit stress conditions during 2008.

Water indices	**Growth stage**	Time for the NWIs	**Water status**			**Canopy temperature**	**Dry root weight[§]**				**Soil moisture**			
			Water potential[†]		RWC	Grain filling	0-30 cm	30-60 cm	60-90 cm	90-120 cm	0-30 cm	30-60 cm	60-90 cm	90-120 cm
SBS-II (n=8)			Midday	Night										
NWI-3	Booting	13:00	-0.66	-0.24	0.14	0.70	0.44	0.21	-0.69**	0.04	-0.36	-0.70	0.29	-0.14
	Anthesis	11:00	-0.90**	-0.84**	0.47	0.91**	0.10	0.23	-0.94**	-0.55	-0.36	0.67	0.53	0.65
		13:00	-0.90**	-0.86**	0.31	0.87**	0.12	0.32	-0.91**	-0.57	-0.21	0.66	0.65	0.74*
		15:00	-0.96**	-0.75*	0.25	0.89**	0.10	0.12	-0.95**	-0.58	-0.35	0.53	0.56	0.65
	Grain filling[‡]	11:00	-0.40	-0.12	-0.21	0.94**	-0.06	-0.09	-0.91**	-0.59	-0.86**	-0.83**	-0.17	0.69
		13:00	-0.31	-0.11	-0.31	0.95**	0.09	-0.04	-0.96**	-0.57	-0.87**	-0.79*	-0.17	0.73*
		15:00	-0.20	-0.02	-0.37	0.95**	0.13	0.03	-0.96**	-0.59	-0.86**	-0.72*	-0.14	0.74*
SYNDER (n=10)			Midday	Night										
NWI-3	Booting	13:00	-0.49	0.10	-0.11	0.61*	-0.80**	0.28	0.15	-0.38	-0.39	-0.42	0.15	0.04
	Anthesis	11:00	-0.45	-0.33	-0.57	-0.36	0.21	-0.23	-0.02	0.25	0.27	-0.10	0.11	-0.07
		13:00	-0.57	-0.31	-0.53	-0.35	0.16	-0.06	0.12	0.30	0.29	-0.22	0.02	-0.24
		15:00	-0.49	-0.44	-0.55	-0.43	0.35	-0.18	-0.02	0.30	0.25	-0.04	-0.03	-0.16
	Grain filling[‡]	11:00	-0.56	-0.59	0.28	0.34	-0.35	-0.60	-0.39	-0.37	0.24	-0.01	-0.57	-0.48
		13:00	-0.49	-0.46	0.34	0.41	-0.28	-0.68*	-0.29	-0.37	0.28	0.25	-0.61*	-0.43
		15:00	-0.44	-0.58	0.31	0.43	-0.28	-0.77**	-0.45	-0.43	0.30	0.21	-0.61*	-0.39

*,**Significant at the 0.05 and 0.01 probability level, respectively.
[†]Water potential was determined at midday (13:00-15:00 h) and at night (22:00-24:30 h).
[‡]The NWI-3 determined at grain filling was associated with soil moisture determined at maturity.
[§]Root weight was determined at anthesis.

Table 4. Relationship of grain yield and biomass to the normalized water index three (NWI-3) and canopy temperature in a subset of sister lines (SBS-I and SBS-II), lines selected for high water use efficiency (WUE-I and WUE-II), and synthetic derivatives lines (SYNDER) grown under water stress conditions during three years.

		WUE-I	**WUE-II**	**SBS-I**		**SBS-II**		**SYNDER**	
		Grain yield		Grain yield					
Parameter	**Growth stage**	2006	2007	2006	2007	Grain yield 2008	Biomass§ 2008	Grain yield 2008	Biomass§ 2008
NWI-3†	Anthesis					-0.91**	-0.71*	-0.33	-0.15
	Grain filling	-0.33	-0.38	-0.38	-0.56*	-0.96**	-0.95**	-0.68*	-0.79**
	Anth-GF‡					-0.95**	-0.96**	-0.43	-0.64*
Canopy temp.	Grain filling	-0.10	-0.06	-0.27	-0.21	-0.95**	-0.94**	-0.68*	-0.76**

*,**Significant at the 0.05 and 0.01 probability level, respectively.
†Midday determinations of NWI-3 (11:00, 13:00 and 15:00 h) were averaged for the year 2008.
‡Anth-GF, average of anthesis and grain filling.
§Biomass determined at physiological maturity.

Figures

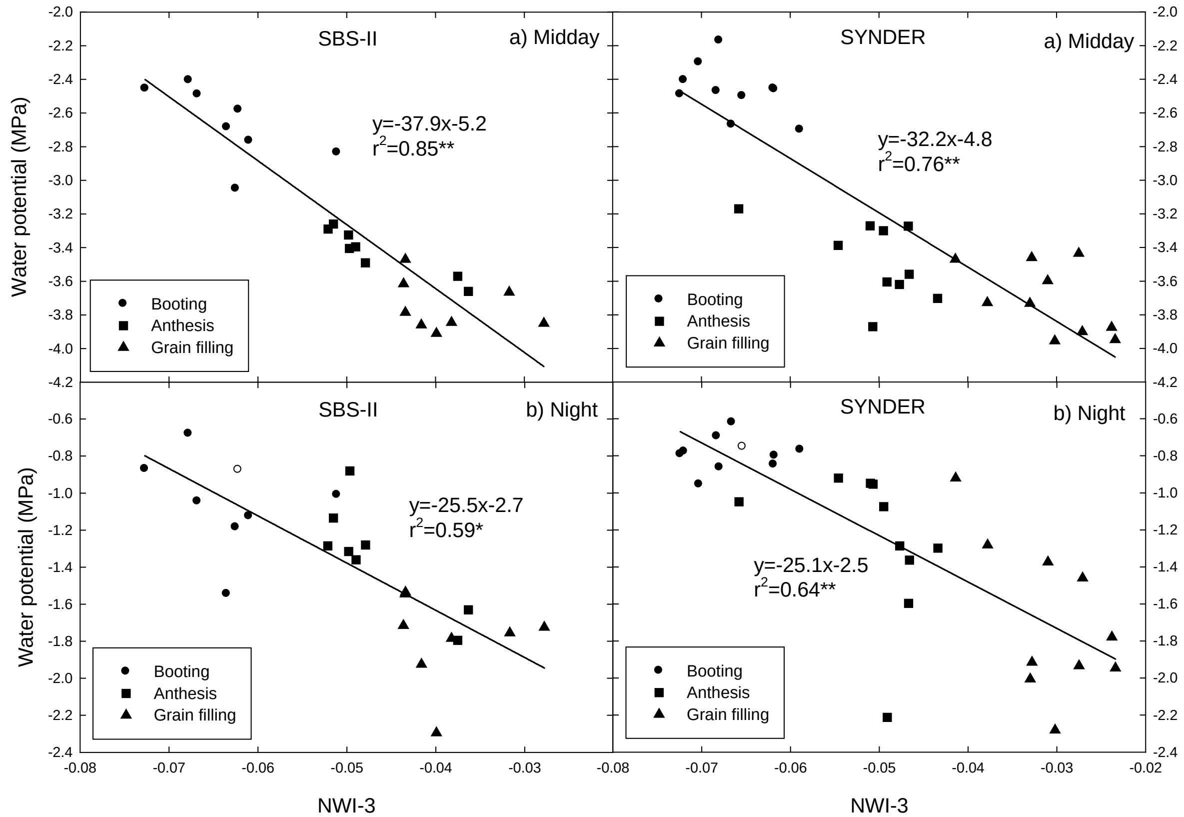

Figure 1. Relationship between the normalized water index three (NWI-3) and water potential determined at midday (13:00-15:00 h) and at night (22:00-24:30 h) in a subset of sister lines (SBS-II) and synthetic derivatives lines (SYNDER) under water stress conditions during 2008.

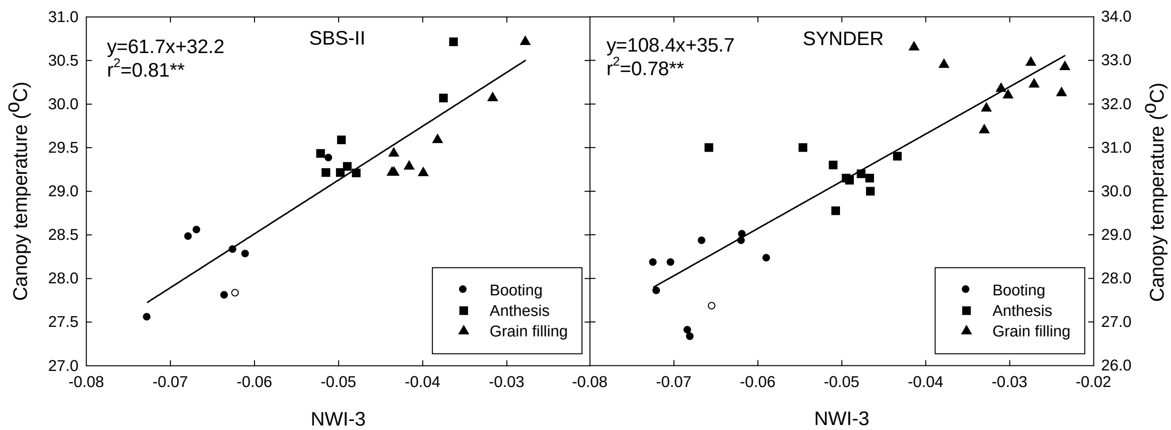

Figure 2. Relationship between the normalized water index three (NWI-3) and canopy temperature in a subset of sister lines (SBS-II) and synthetic derivatives lines (SYNDER) under water stress conditions during 2008.

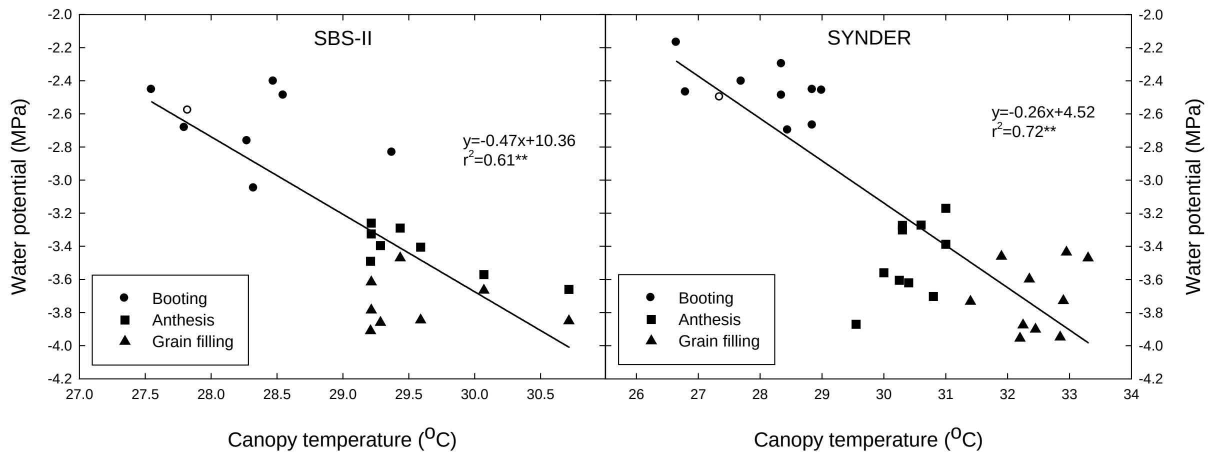

Figure 3. Relationship between canopy temperature and water potential in a subset of sister lines (SBS-II) and synthetic derivatives lines (SYNDER) under water stress conditions during 2008.

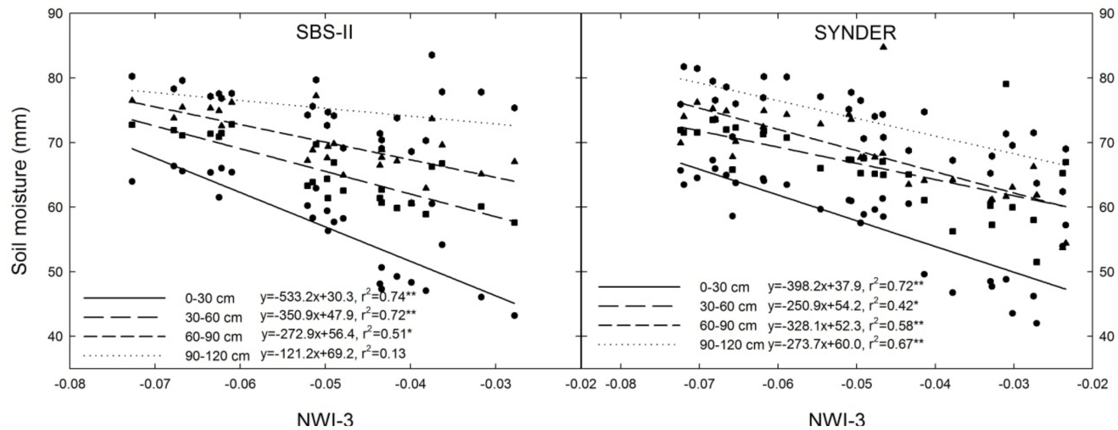

Figure 4. Relationship between the normalized water index three (NWI-3) and soil moisture at diverse depths in a subset of sister lines (SBS-II) and synthetic derivatives lines (SYNDER) under water stress conditions during 2008.

CHAPTER V

Effect of morphological traits over spectral reflectance indices in spring wheat

Abbreviations

CIMMYT, International Maize and Wheat Improvement

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

SRI, spectral reflectance indices

WI, water index

Abstract

Spectral reflectance indices are directly influenced by plant architecture, especially leaf distribution, but other reproductive organs have considerable influence. Diverse morphological traits (leaf and spike wax content, leaf and spike orientation, and awns on spikes) were studied in spring wheat genotypes to determine their influence on different spectral reflectance indices (SRI) and on the relationship between the respective SRI and grain yield under well irrigated conditions. Twenty advanced lines with contrasting morphological differences on leaves and spikes developed by the International Maize and Wheat Improvement Center (CIMMYT) were used. Ten bread wheat advanced lines, eight sister lines, and two double haploid lines were selected. All genotypes were planted at CIMMYT's experiment station in NW Mexico during two growing seasons (2007 and 2008). Three vegetative indices (red normalized difference vegetative index, green NDVI, and simple ratio; RNDVI, GNDVI and SR, respectively) and two water indices (normalized water indices one and three; NWI-1, and NWI-3) were determined at heading and grain filling during two growing seasons using a field portable spectrometer (Analytical Spectral Devices, Boulder, CO). A multiple regression model demonstrated that leaf and spike wax content and leaf orientation were the traits that showed major influences on the vegetative indices (14-30%) at heading, grain filling, and by combining both growth stages. The water indices were affected mainly by spike orientation and by the presence of awns on spikes (14-24%), and the same character affected grain yield per se (6-17%). Each morphological trait was used as as covariable for obtaining adjusted means and for estimating the relationship between the SRI and grain yield. The vegetative indices were more sensitive to the leaf morphological traits (orientation and wax content), and the water indices to the spikes morphological traits (orientation and awns). The association between the vegetative indices and yield was improved by adjusting for leaf orientation, but the relationship between both groups of SRI and grain yield was decreased by adjusting for spike orientation and awns on spikes.

Introduction

Assessments based on canopy spectral reflectance are convenient for identifying promising high yielding lines in breeding programs before the crop is harvested (yield prediction) (Royo *et al.*, 2003). Several spectral reflectance indices (SRI) have been established to estimate physiological traits and grain yield in diverse crops (Rudorff and Batista, 1990; Wiegand *et al.,* 1991; Araus *et al.*, 2001). The normalized difference vegetation index (NDVI, $[R_{900}-R_{680}]/[R_{900}+R_{680}]$) is the SRI most widely used to predict grain yield in wheat and corn under well watered and stressed environments (Raun *et al.*, 2001; Osborne *et al.*, 2002). The red NDVI (RNDVI, $[R_{780}-R_{670}]/[R_{780}+R_{670}]$) has been a good predictor (r^2=0.82) of grain yield and biomass in winter wheat (r^2=0.76) (Moges *et al.*, 2004). The green-NDVI (GNDVI, $[R_{780}-R_{550}]/[R_{780}+R_{550}]$) has also been associated with yield in corn and wheat genotypes (Shanahan *et al,.* 2001; Gutierrez-Rodriguez *et al.*, 2004). The water index (WI, R_{970}/R_{900}) and four normalized water indices (NWI-1=$[R_{970}-R_{900}]/[R_{970}+R_{900}]$, NWI-2=$[R_{970}-R_{850}]/[R_{970}+R_{850}]$, NWI-3=$[R_{970}-R_{880}]/[R_{970}+R_{880}]$, and NWI-4=$[R_{970}-R_{920}]/[R_{970}+R_{920}]$) have been used to screen grain yield in spring and winter wheat genotypes (Babar *et al.*, 2006; Prasad *et al.*, 2007). These five water indices have explained a large proportion of grain yield variability and represent an alternative method for selecting high yielding lines in diverse environments (well irrigated, water stress, and rainfed conditions) for breeding purposes (Babar *et al.*, 2006; Prasad *et al.*, 2007). Our results have also demonstrated strong associations between the water indices and grain yield in advanced lines of spring wheat in high temperature environments (Gutierrez *et al.*, 2008).

Energy reflected from plant surfaces (canopy) is related to the geometric form of objects and is an important consideration in remote sensing systems (Lillesand *et al.*, 2004). Plant architecture is a consequence of stem and leaf arrangement (shape, angle, distribution of layers), making canopies highly heterogeneous (Darvishzadeh *et al.*, 2008; Serrano, 2008). Canopy reflectance is affected not only by plant architecture, but also by internal and external factors of leaf structure (*i.e.*, trichomes, epidermis and mesophyll thickness) (Datt, 1998). These scattering properties of leaves cause additive effects over the SRI due to differences in leaf morphology, and the applicability of SRI is reduced over a wide range of species (Darvishzadeh *et al.*, 2008;

Serrano, 2008). For instance, canopy reflectance for estimating chlorophyll content is highly influenced by anatomical leaf characteristics (Datt, 1999). The effects on canopy reflectance of leaf properties such as leaf arrangement, leaf number, and leaf area have been studied in seven deciduous trees and Mediterranean scrubs. Thicker leaves showed lower values for the vegetative indices compared to thinner leaves with similar chlorophyll content (Serrano, 2008). Some vegetative indices were highly affected and others were less affected by external noise caused by soil, leaf angle, and leaf distribution (Serrano, 2008). Datt (1998) developed a reflectance index that corrected for leaf surface differences in 21 Eucalyptus species. Similarly, Sims and Gamon (2002) studied the effects on SRI of structural variations of leaves for estimating pigment content in more than 50 different species, proposing a new spectral index (mSR705) that corrects for leaf scattering.

Canopy spectral reflectance is also modified by factors associated with leaf constituents such as cutin, wax content, leaf thickness, trichome abundance, and wax composition, producing alterations on the SRI in diverse deciduous tree species (Ribeiro, 2006). Thicker wax and high trichome amounts affected spectral canopy reflectance by causing a pronounced attenuation at the 1031 nm wavelength in *Acer rubrum*. When wax was relatively thin on leaves, spectra reflectance was strongly influenced by inner tissue layers (*i.e.*, cellulose, cutin) (Ribeiro, 2006). Holmes and Keiller (2002) established that epicuticular wax is an effective reflector of UV radiation, and leaf hairs reduce the amount of PAR (photosynthetic active radiation, 400-700 nm) arriving at the leaf surface.

When the relationship between the SRI (vegetative and water indices) and grain yield is assessed for breeding purposes in diverse wheat genotypes in a particular environment, the SRI can be used to detect, identify, and select high yielding genotypes (Babar *et al.*, 2006, Prasad *et al.*, 2007, Gutierrez *et al.*, 2008). Genotypes represent genetic diversity for grain yield, but also for other features like morphological traits on leaves and spikes due to the specific genes of each genotype. Some differences in spike and leaf orientation, wax content, and other traits are evident in the canopy reflectance of every genotype. The effect of awns on spikes and other morphological traits on the SRI has not been considered in wheat. The main goal of the present work is to determine how morphological differences of spikes and leaves influence the relationship between the SRI (vegetative indices and water indices) and grain yield. For this purpose, twenty advanced lines with contrasting differences in leaf and spike orientation, presence or absence of awns on spikes, and wax content on leaves and spikes were used.

Materials and Methods

Experimental materials

Spring wheat genotypes from the International Maize and Wheat Improvement Center (CIMMYT) were used for this study. The genetic material represented twenty advanced lines developed by CIMMYT in diverse breeding trials. These lines were selected for contrasting morphological traits of leaves and spikes (Table 1). The trial was composed of ten bread wheat advanced lines (four lines from a 'Babax' cross, three lines from a 'Rialto' cross, one line from a 'Koel' cross, plus two other advanced lines called 'Cunningham' and 'Pastor') with differences for leaf orientation (curved and erect leaves) and leaf-spike wax content. Four pairs of bread sister lines with awned and anwless spikes (each pair was contrasting). Two double haploid lines (61DHB and 126DHB) were selected due to high wax content on spike and leaves. The two double haploid lines were obtained from the cross between the cv. Rialto and an advanced line (L14) which has large spikes. The twenty lines were also selected for similarity in time to anthesis (around 90 days) and maturity (around 125 days).

Growing conditions

The genotypes were grown during the winter season at CIMMYT's experiment station in Cd. Obregon, Northwest Mexico (27.3^{o}N, 109.9^{o}W, 38 m above sea level). The weather is mostly sunny and dry during the winter cropping cycle (see Gutierrez *et al.*, 2008). The soil type is coarse, sandy clay, mixed montmorillonitic type caliciorthid, low in organic matter and slightly alkaline (pH 7.7) in nature (Sayre *et al.*, 1997).

The seeding rate for each experiment was 78 kg ha^{-1}. Nitrogen and phosphorous were applied to the plots at the rate of 150 kg ha^{-1} and 22 kg ha^{-1}, respectively. Field plots consisted of two raised beds 80 cm apart and 5m long, each with 2 rows 10 cm apart on each bed. An alpha lattice design with 2 repetitions was employed for all experiments.

The planting dates were in November and plants reached booting and heading during February-March and were harvested in May. The crop growing seasons for all experiments are referred to as years: 2007 for the cycle 2006-2007 and 2008 for the cycle 2007-2008. In both years the genotypes were planted under well irrigated conditions. Flood irrigation was applied to the plots every 20-25 days, providing approximately 100 mm of water. Folicur was applied at the booting, heading, and grain filling stages at the rate of 0.5 L ha^{-1} to protect the experimental materials from leaf rust caused by *Puccinia triticina*.

Spectral reflectance measurements

Canopy reflectance was measured in the 350 to 1100 nm range, collected at 1.5-nm intervals using a FieldSpec spectroradiometer (Analytical Spectral Devices, Boulder, CO). Data were collected during cloud-free days at midday between (10:30 and 14:00 hrs) after calibration with a white plate of barium sulphate ($BaSO_4$) that provides maximum irradiance (Labsphere Inc., North Sutton, USA). Four measurements in each plot were taken at heights of 0.5 m above the canopy with a field of view of 25^o. Each reflectance measurement was the average of 10 scans from an area of 18.94 cm^2 of the plot. The sensor was mounted with the help of a pistol grip approximately 50 cm above the canopy facing the center of the plot. Canopy reflectance measurements were taken at random places in each plot during anthesis (heading) and grain filling growth stages.

Eight SRI were calculated following the equations with wavelengths (nm) described by several authors. Three vegetative indices were estimated; the red normalized difference vegetative index ($RNDVI=[R_{780}-R_{670}]/[R_{780}+R_{670}]$), the green NDVI ($GNDVI=[R_{780}-R_{550}]/[R_{780}+R_{550}]$) and the simple ratio ($SR=R_{900}/R_{680}$) (Gitelson *et al.*, 1996; Aparicio *et al.*, 2000; Raun *et al.*, 2001). The water index proposed by Peñuelas *et al.* (1993) was estimated ($WI=R_{970}/R_{900}$) and two normalized water indices proposed by Babar *et al.* (2006) and Prasad *et al.* (2007) ($NWI\text{-}1=[R_{970}-R_{900}]/[R_{970}+R_{900}]$ and $NWI\text{-}3=[R_{970}-R_{880}]/[R_{970}+R_{880}]$) were also estimated.

Canopy spectral reflectance curves in the visible (400-700 nm) and in the infrared region (700-1100 nm) were compared for each morphological trait by selecting a certain group of genotypes with the same morphological trait (Fig. 1). For spike orientation, there were six genotypes with curved spikes and fifteen with erect spikes. There were nine genotypes with curved leaves and eleven genotypes with erect leaves (leaf orientation), six genotypes with awnless spikes and fourteen with awned spikes (awns on spikes), six genotypes with intermediate waxy content and fourteen with waxy spikes (spike wax content), eight genotypes with intermediate wax content and twelve with waxy leaves (wax content on leaves).

Morphological traits on leaves and spikes

The genetic diversity for morphological traits of leaves and spikes in the twenty genotypes is shown in Table 1. Every trait was estimated on a scale from 1 to 10 at heading (close to the flowering stage) during the year 2007 and confirmed during 2008. The number 10 represented high erectness and a high waxy content of leaves and spikes. For awns of spikes, the number 10 represented large awns, a number below 5 represented short awns and the number 1 a total absence of awns (awnless spikes).

Grain yield

In all experiments grain yield was determined after physiological maturity by harvesting and threshing the four rows of every plot, excluding a 0.5 m border at each end. Prior to grain harvest, a random subsample of 100 spike-bearing culms was removed from the plots. The subsample was oven-dried, weighed, and threshed. The grain weight was recorded and individual kernel weight estimated using a subsample of 200 kernels.

Statistical analysis

All the experiments were analyzed according to the alpha lattice design by using Proc Mixed in the SAS program for each growth stage and year (SAS, 2001). Pearson correlation coefficients were used to estimate the phenotypic relationship of the vegetation and water indices to grain yield. A multiple regression analysis was conducted using Proc Stepwise for all the SRI and morphological traits.

Additionally, every morphological trait (leaf and spike orientation, leaf and spike wax content, and awns on spikes) was used as covariables in a covariance analysis for obtaining adjusted means for each spectral index and grain yield. These adjusted means were used for estimating the relationship between SRI and grain yield using Pearson correlations.

Results

The two normalized water indices, NWI-1 and NWI-3, gave a stronger relationship with grain yield than the three vegetative indices (RNDVI, GNDVI, and SR) during the two growing seasons (2007 and 2008). Significant genotypic differences ($p \leq 0.01$) for the spectral reflectance indices (SRI) and grain yield were found for both years and across years (Table 2).

Individual effects of morphological traits over the spectral reflectance indices and yield

The effects of diverse morphological traits over the SRI and grain yield were detected through a multiple regression analysis (traits significant at $p \leq 0.05$) combining two years (2007 and 2008) for the twenty lines (Table 3). The wax content on leaves and spikes, and the leaf orientation were the morphological traits that influenced the vegetative indices at heading, grain filling, and when heading and grain filling were combined. Spike wax content showed the major more effect over the vegetative indices (7-15%) than the other two morphological traits (4-7%). In contrast, the water indices were influenced more by spike orientation (5-9%) and by awns on spikes (5-13%). Finally, grain yield was highly influenced by the awns on spikes (17%), and also spike orientation showed a significant influence (6%).

There were minor differences in the spectral reflectance curves in the visible region (400-700 nm) when a certain group of genotypes with the same morphological traits were compared (Fig. 1). Genotypes with erect spikes (n=15) showed a decreased of reflectance in the infrared region (700-1100 nm) compared to genotypes with curved spikes (n=5). The same happened when genotypes with waxy spikes (n=14) were compared with intermediate wax amount (n=6). In contrast, the presence of awns (n=14) increased the amount of reflectance in the infrared region compared with those genotypes that had awnless spikes (n=6). Similarly, genotypes with erect leaves (n=11) had increased canopy reflectance (n=9) compared with genotypes with curved leaves.

Association between the spectral reflectance indices and yield without considering morphological traits

The relationship between the SRI and grain yield was tested without considering the influence of any morphological trait (non adjusted means) (Table 4). The water indices always exhibited negative associations with grain yield, while the vegetative indices showed positive correlations. The correlation values between the water indices and grain yield were higher at both heading and grain filling than for the vegetative indices and grain yield during two years and across years. The vegetative indices showed lower correlation coefficients during 2008 with no significant relationships. The weaker correlation values for the vegetative indices in 2008 affected the correlation values across years when heading and grain filling were combined. The correlation coefficients were slightly higher or similar to the highest correlation coefficient of any individual growth stage for both groups of SRI.

Effects of morphological traits over the relationship between the spectral reflectance indices and yield

It was difficult to examine the effects of individual morphological traits over the relationship between the SRI and grain yield because every genotype presented two or more morphological traits (Table 1). However, every morphological trait was used as a covariable for obtaining adjusted means and estimating the relationship between the SRI and grain yield (Table 5). The correlation values obtained by using adjusted means for each morphological trait were compared to the correlations obtained without any adjustment (Table 5). Correlation values adjusted for leaf wax content did not show any effect on the relationship between the SRI (vegetative and water indices) and grain yield. Slight decreases in the correlation values occurred for the vegetative indices when adjusted for the amount of wax on spikes, while water indices were not affected by the amount of wax on the spikes. However, the correlations were improved (higher values) for the vegetative indices when means were adjusted for leaf orientation, whereas the water indices presented weaker correlations in their relationship with grain yield. Means adjusted for spike orientation decreased the relationship between both groups of SRI and grain yield because the correlations were lower, but remaining significant (except the vegetative indices at grain filling). The highest decrease in the correlation values between SRI and grain yield were caused by adjusting means for awns on spikes. The water indices remained significant, while the vegetative indices were not significant at grain filling.

RNDVI and NWI-3 were chosen to represent the vegetative indices and the water indices, respectively, to demonstrate how the different morphological traits affected the relationship between the SRI and grain yield (Fig. 2). Mean adjustments for awn on spikes showed that this trait had the most effect on the relationship between RNDVI and grain yield, while the correlations adjusted for leaf orientation improved this relationship. For NWI-3, none of the adjustments for morphological traits improved its relationship with grain yield, while adjusting for leaf orientation made the relationship non significant. For both spectral indices (RNDVI and NWI-3), correlations adjusted for awns on spikes and spike orientation reduced the relationship with grain yield, but it still remained significant.

Discussion

The combination of heading and grain filling showed a stronger relationship between the SRI and grain yield as has previously been reported by other authors (Babar *et al.*, 2006; Prasad *et al.*, 2007; Gutierrez *et al.*, 2008). The two normalized water indices (NWI-1 and NWI-3) gave more significant associations with grain yield than the vegetative indices (RNDVI, GNDVI, and SR) in the twenty lines tested. Similar results have been found in spring wheat for diverse environments (well irrigated, water stress, and high temperature) in NW Mexico (Gutierrez *et al.*, 2008).

Individual effects of morphological traits over the spectral reflectance indices and yield

There was a clear influence of the leaf and spike morphological traits over the canopy spectral reflectance and consequently, over the SRI (Table 4; Fig. 1). The vegetative indices were mainly affected by leaf and spike wax content and leaf orientation, while the water indices were mainly affected by spike orientation and awns on spikes. Moreover, a considerable amount of the grain yield variation was explained by the influence of awns on spikes.

The canopy spectral reflectance showed small changes in the visible region (400-700 nm) indicating few differences in the amount of chlorophyll and other leaf pigments among genotypes (Fig 1). However, considerable changes occurred in the infrared region (700-1000 nm) that is more related to the plant structure, especially of leaves (Lillesand *et al.*, 2004). Canopy reflectance of spikes and leaves followed a similar pattern in the amount of reflectance in both regions. Qifa and Jihua (2003) found that the spectral reflectance of rice spikes gave similar reflectance signals as leaves, but spikes reflected more energy in the visible region because of a lower chlorophyll concentration and distinctive surface properties. Guyot (1990) determined that the difference between the maximum and minimum reflectance peaks in the visible region was greater for spikes than for leaves. The difference in internal and external morphology of the spikes compared to leaves causes variations in the reflectance signal due to large spaces among grains, rachis branches, and high intercellular spaces among cells (Riedell and Blackmer 1999).

In our study, we determined reflectance in the entire plant canopy (spikes and leaves) for detecting major effects of the morphological traits.

Erect spikes and waxy spikes decreased the reflectance in the infrared region, while curved spikes and intermediate waxy spikes increased it. Particular genotypes with erect spikes could absorb more radiation (less reflectance) compared with those presenting curved spikes because there is less interference for radiation to reach leaves. Gaju *et al.*, (2009) found that two genotypes with large spikes (LPS1 and LPS2) showed lower radiation interception compared with a cultivar with shorter spikes (*cv.* Bacanora), thereby having less biomass. The lower radiation interception in genotypes with curved spikes (bigger size) can be associated with a major amount of energy reflected compared to genotypes with erect spikes found in our study (Fig. 1). Gausman *et al.* (1970) reported that the spectral reflectance of the spike is primarily influenced by chlorophyll and carotenoid in the visible region, while its internal structure affected the reflectance in the infrared region. In relation to the waxy spikes, the lower amount of reflectance detected could be caused by radiation being transmitted in different directions by the waxy reflection. However, this hypothesis needs further investigation.

The presence of awns on spikes in certain genotypes increased the reflectance in the infrared region, while the awnless genotypes decreased it. No studies have reported the effect of awns on canopy spectral reflectance in wheat and other crops, but the presence of awns might reflect a higher amount of radiation, especially in genotypes with curved spikes. If awns act as reflectors of the radiation, in curved spikes the amount of reflected radiation could be increased in a dense canopy, thereby reducing also the amount of radiation in lower leaf layers.

Genotypes with erect leaves reflected more radiation than genotypes with curved leaves in the infrared region (Fig. 1). We also expected to find differences in the visible region, but no differences were obtained. However, the effect of other traits (waxy leaves, curve spikes, and awned spikes) in genotypes with erect or curved leaves makes it difficult to establish a concrete tendency. In fact, the spectral reflectance curves plotted in Figure 1 were not corrected or adjusted by any particular morphological traits.

Morphological traits and the relationship between spectral reflectance indices and grain yield

The combined effects of morphological traits over the relationship between the SRI and grain yield followed a similar pattern as their individual effects over the SRI and grain yield (Table 5). The vegetative and water indices were little affected in their relationship with grain yield when the correlation values were adjusted for leaf and spike wax content.

An adjustment for leaf orientation improved the correlation values of the vegetative indices with grain yield, but the correlations for the water indices were decreased. Darvishzadeh *et al.* (2008) established that erectophile canopies reduced the efficiency of the SRI for assessing the leaf area index, while planophile canopies were less influenced. In our study, the effects of erect and curved leaves were considered together in their relationship with grain yield for the vegetative and water indices in the twenty lines (Fig. 2).

The spike orientation (adjusted means) was one of the morphological traits with the major effect over the vegetative and water indices (curved spikes), decreasing their relationship with grain yield (Table 5). A similar pattern was found for the effects of awns over the relationship of the vegetative and water indices with grain yield. It is evident that these two morphological traits negatively affect the relationship between the SRI and grain yield.

Even though genotypes had different combinations of morphological traits, our results demonstrated that leaf and spike orientation and awns on spikes influenced the SRI and their relationship with grain yield. The SRI described in the present study were adjusted to each morphological trait to show their influence over the SRI. In other studies, the SRI were corrected by chlorophyll content differences for estimating leaf area index in diverse plant species (perennial) (Nagler *et al.*, 2004; Wang *et al.*, 2005). If RNDVI and NWI-3 are adjusted for each morphological trait, the correlation values can be improved or decreased (Fig. 2). For example, the correlation values with RNDVI were improved by adjusting for leaf orientation, but NWI-3 and RNDVI correlation values decreased when both were adjusted for spike orientation and awns on spikes.

Conclusions

Morphological traits of leaves and spikes influenced SRI, affecting their relationship with grain yield. The vegetative indices were affected by leaf and spike wax content and leaf orientation, while the water indices and grain yield were affected by spike orientation and awns (individual effects). The relationship between SRI and grain yield (combined effects) was improved by adjusting for leaf orientation, but affected negatively by adjusting for spike orientation and awns. The relationship between water indices and grain yield was reduced by spike orientation (curved spikes) and awns on spikes. The vegetative indices were more sensitive to leaf morphological traits, and the water indices to the spikes morphological traits (same for grain yield). The association between the vegetative indices and yield was improved by adjusting for leaf orientation, but the relationship between both groups of SRI and grain yield decreased due to spike orientation and awns on spikes.

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Tables

Table 1. Diversity in morphological traits for leaves and spikes in twenty wheat genotypes.

		Orientation		Awns on	Wax content	
Lines	**Source/identification**	**Spikes**	**Leaves**	**spikes**	**Leaves**	**Spikes**
Bread wheat lines						
1	Babax cross	Erect	Curved	Awned	Intermediate waxy	Intermediate waxy
2	Babax cross	Erect	Curved	Awned	Intermediate waxy	Waxy
3	Babax cross	Erect	Erect	Awned	Waxy	Waxy
4	Babax cross	Erect	Erect	Awned	Intermediate waxy	Intermediate waxy
5	Rialto cross	Erect	Erect	Awned	Intermediate waxy	Intermediate waxy
6	Rialto cross	Erect	Curved	Awned	Waxy	Waxy
7	Rialto cross	Erect	Erect	Awned	Waxy	Waxy
8	Koel cross	Curved	Curved	Awned	Waxy	Waxy
9	Cunningham	Erect	Curved	Awned	Waxy	Intermediate waxy
10	Pastor	Erect	Curved	Awned	Intermediate waxy	Intermediate waxy
Sister lines						
11	FA2+	Erect	Curved	Awned	Waxy	Waxy
12	FA2-	Erect	Curved	Awnless	Waxy	Waxy
13	JA1+	Erect	Curved	Awned	Intermediate waxy	Waxy
14	JA1-	Erect	Erect	Awnless	Intermediate waxy	Waxy
15	WA2+	Curved	Erect	Awned	Intermediate waxy	Intermediate waxy
16	WA2-	Curved	Erect	Awnless	Waxy	Waxy
17	WA5+	Curved	Erect	Awned	Waxy	Waxy
18	WA5-	Erect	Erect	Awnless	Waxy	Waxy
Double haploid lines						
19	61DHB	Erect	Erect	Awnless	Waxy	Waxy
20	126DHB	Curved	Erect	Awnless	Waxy	Waxy

Table 2. Minimum, maximum, mean, and least significant difference (LSD) for the spectral reflectance indices (heading-grain filling) and grain yield in twenty lines grown under well irrigated conditions.

Year	**Grain yield**	**Vegetative indices**†			**Water indices**‡	
		GNDVI	RNDVI	SR	NWI-1	NWI-3
2007						
Min	4.40	0.633	0.584	4.78	-0.117	-0.120
Max	7.19	0.936	0.843	30.24	-0.052	-0.050
Mean	6.07	0.822	0.724	12.71	-0.086	-0.084
LSD (5%)	0.32	0.024	0.026	2.81	0.009	0.009
Significance level	**	**	**	**	**	**
2008						
Min	3.82	0.734	0.694	7.22	-0.097	-0.099
Max	8.69	0.901	0.827	20.69	-0.056	-0.050
Mean	6.79	0.847	0.761	13.48	-0.082	-0.082
LSD (5%)	**	0.012	0.014	1.20	0.005	0.008
Significance level	0.45	**	**	**	**	**
Combined						
Min	3.82	0.633	0.584	4.72	-0.117	-0.121
Max	8.69	0.936	0.843	30.24	-0.052	-0.050
Mean	6.43	0.834	0.743	13.10	-0.084	-0.085
LSD (5%)	0.27	0.013	0.014	1.45	0.005	0.006
Significance level	**	**	**	**	**	**

*,**Significant at the 0.05 and 0.01 probability level, respectively.
†RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio.
‡NWI-1, normalized water index 1; NWI-3, normalized water index 3.

Table 3. Correlation coefficients obtained from a stepwise multiple regression for explaining the influence of diverse morphological traits on the spectral reflectance indices and grain yield in twenty lines grown under well irrigated conditions. Estimates were based on combined years; variables in the model were significant at the 0.05 significance level.

Morphological Trait	**Grain yield**	**Vegetative indices**†			**Water indices**‡	
		RNDVI	GNDVI	SR	NWI-1	NWI-3
			Heading			
Leaf wax content	-	0.051	0.054	0.044	-	-
Spike wax content	-	0.150	0.088	0.153	-	-
Leaf orientation	-	0.046	-	0.050	-	-
Spike orientation	0.056	-	-	-	0.052	0.060
Awns on spikes	0.168	-	-	-	0.096	0.119
Total variation	0.224	0.247	0.143	0.247	0.148	0.179
			Grain filling			
Leaf wax content		0.065	-	0.075	0.052	-
Spike wax content		0.121	-	0.104	0.080	-
Leaf orientation		0.098	-	0.099	-	-
Spike orientation		-	-	-	0.105	0.129
Awns on spikes		-	-	-	-	0.093
Total variation		0.283	-	0.278	0.237	0.222
			Heading-Grain filling			
Leaf wax content		0.068	0.068	0.070	-	-
Spike wax content		0.134	0.072	0.153	-	-
Leaf orientation		0.082	0.060	0.079	-	-
Spike orientation		-	-	-	0.076	0.094
Awns on spikes		-	-	-	0.105	0.131
Total variation		0.283	0.200	0.302	0.181	0.225

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

‡NWI-1, normalized water index 1; NWI-3, normalized water index 3.

Table 4. Correlation coefficients between spectral reflectance indices and grain yield without considering the effect of morphological traits in twenty lines grown under well irrigated conditions during two years and across years.

Spectral index	Growth stage	2007	2008	Combined
Vegetative indices[†]				
RNDVI	Heading	0.71**	0.28	0.69**
	Grain filling	0.50*	0.49*	0.53*
	Heading-Grain filling	0.67**	0.43	0.65**
GNDVI	Heading	0.69**	0.13	0.62**
	Grain filling	0.39	0.38	0.40
	Heading-Grain filling	0.62**	0.29	0.55*
SR	Heading	0.69**	0.22	0.60**
	Grain filling	0.42	0.42	0.43
	Heading-Grain filling	0.65**	0.31	0.58**
Water indices[‡]				
NWI-1	Heading	-0.73**	-0.72**	-0.82**
	Grain filling	-0.75**	-0.75**	-0.85**
	Heading-Grain filling	-0.77**	-0.74**	-0.85**
NWI-3	Heading	-0.72**	-0.70**	-0.82**
	Grain filling	-0.75**	-0.78**	-0.86**
	Heading-Grain filling	-0.77**	-0.76**	-0.86**

*,**Significant at the 0.05 and 0.01 probability level, respectively.
[†]RNDVI, red normalized difference vegetation index; GNDVI, green normalized difference vegetation index; SR, simple ratio.
[‡]NWI-1, normalized water index 1; NWI-3, normalized water index 3.

Table 5. Correlations coefficient estimated using means adjusted by a covariance analysis for each morphological trait for the spectral reflectance indices and grain yield in twenty lines grown under well irrigated conditions. Estimates were based on combined years.

Growth stage	**Vegetative indices**†			**Water indices**‡	
	RNDVI	GNDVI	SR	NWI-1	NWI-3
		Non adjusted			
Heading	0.69**	0.62**	0.60**	-0.82**	-0.82**
Grain filling	0.53*	0.40	0.43	-0.85**	-0.86**
Heading-Grain filling	0.65**	0.55*	0.58**	-0.85**	-0.86**
		Leaf wax content			
Heading	0.67**	0.60**	0.54*	-0.82**	-0.80**
Grain filling	0.53*	0.40	0.44	-0.84**	-0.85**
Heading-Grain filling	0.62**	0.54*	0.57**	-0.85**	-0.85**
		Spike wax content			
Heading	0.70**	0.62**	0.62**	-0.81**	-0.81**
Grain filling	0.42	0.28	0.34	-0.82**	-0.86**
Heading-Grain filling	0.59**	0.46*	0.59**	-0.83**	-0.86**
		Leaf orientation			
Heading	0.78**	0.74**	0.74**	-0.82**	-0.72**
Grain filling	0.50*	0.46*	0.55*	-0.42	-0.27
Heading-Grain filling	0.74**	0.72**	0.74**	-0.73**	-0.52*
		Spike orientation			
Heading	0.58**	0.57**	0.60**	-0.79**	-0.79**
Grain filling	0.31	0.17	0.27	-0.77**	-0.63**
Heading-Grain filling	0.48*	0.38	0.52*	-0.77**	-0.73**
		Awns on spikes			
Heading	0.63**	0.55*	0.55*	-0.65**	-0.63**
Grain filling	0.35	0.32	0.34	-0.57**	-0.48*
Heading-Grain filling	0.55*	0.48*	0.54*	-0.61**	-0.56**

*,**Significant at the 0.05 and 0.01 probability level, respectively.

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

‡NWI-1, normalized water index 1; NWI-3, normalized water index 3.

Figures

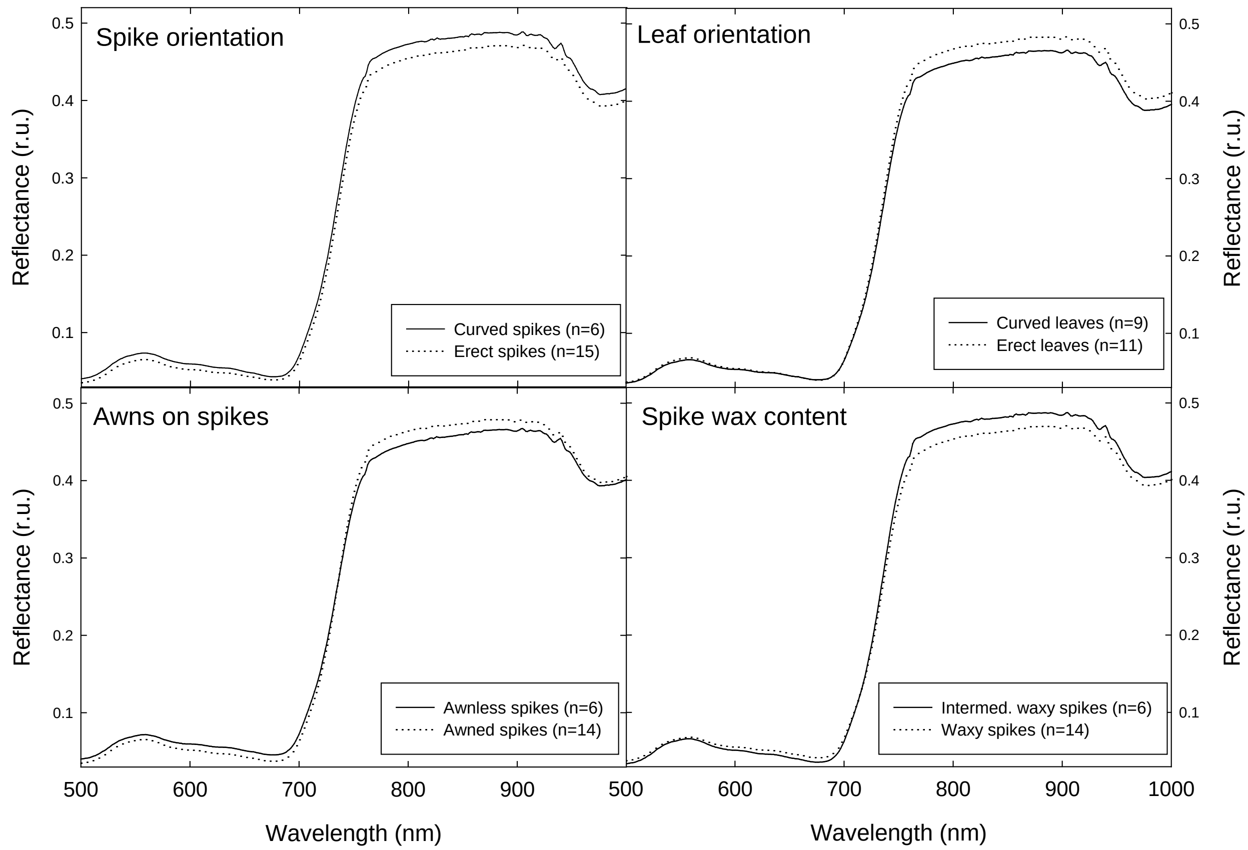

Figure 1. Canopy spectral reflectance response for wheat lines with differences in morphological traits on leaves and spikes grown under well irrigated conditions. Estimates were based on combined years.

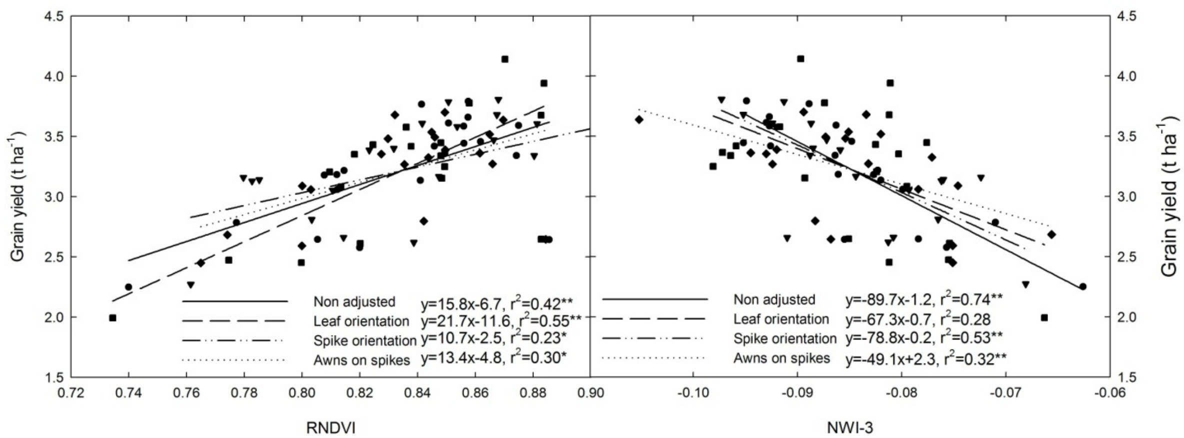

Figure 2. Relationship between spectral reflectance indices (RNDVI and NWI-3, red normalized difference vegetation index and normalized water index three, respectively) and grain yield without and with adjusting means for estimating their relationships. Estimates were based on combined years and growth stages (heading and grain filling).

CHAPTER VI

Conclusions

The potential of using spectral reflectance indices for differentiating high yielding lines in advanced spring wheat lines under well irrigated, water stress, and high temperature conditions was achieved using the water indices (WI and four NWIs). The water indices were more effective in predicting grain yield than the commonly reported indices (RNDVI, GNDVI, and SR), because they were strongly correlated to grain yield, thus demonstrating their effectiveness for detecting, identifying, and selecting high yielding advanced lines of the 24th ESWYT, 11th SAWYT, and 11th HTWYT during three individual years and across years in the three environments. The highest relationships were obtained under high temperature conditions for the 11th HTWYT, which is a new environment reported for this relationship. Combining canopy spectral reflectance from heading and grain filling, resulted in better relationships between the water indices and grain yield compared to individual growth stages. Two water indices (NWI-1 and NWI-3) demonstrated better relationships with grain yield in all the trials in the three environments. The water indices gave high genetic correlations and heritability (broad sense) with grain yield, demonstrating high potential for achieving genetic gains in all the environments. In addition, they also showed high response to selection and correlated response, relative selection efficiency, and efficiency in selecting the higher yielding genotypes. The water indices and canopy temperature determined in NW Mexico also can be used for predicting the yield in other nurseries located around the world where the advanced lines of the 24th ESWYT, 11th SAWYT, and 11th HTWYT where tested. Depending on the environment where NWI-3 and canopy temperature were measured, they can estimate and predict yield performance in certain nurseries, especially for the Central Asia region.

The water indices were related with parameters commonly employed for assessing the crop water status (*i.e.*, water potential). The relationships between water potential and canopy temperature to the water indices were highly significant in diverse advanced lines when booting, anthesis and grain filling were combined under water stress conditions. The majority of the water potential variability was explained by the water indices and canopy temperature confirming our hypothesis that the water indices are associated with the plant water content under adverse growth conditions (drought). In fact, the water indices can predict crop water deficit stress during the growing season and make irrigation decisions to avoid yield losses.

Our results also demonstrated that some changes in the relationship between the water indices and grain yield were influenced by morphological traits associated with leaves and spikes. The relationship between the water indices and grain yield was affected mainly by erect leaves and spike orientation. Erect spikes and awned spikes slightly affected the water indices.

The potential of employing the water indices for selecting high yielding lines represents a significant advantage in breeding programs because the top yielding lines can be selected among a group of advanced lines with high yield potential and low yielding lines can be discarded in an accurate, inexpensive, and easy manner. The water indices also can be employed for assessing crop water status under water stress conditions for avoiding yield losses.

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Candidate for the Degree of

Doctor of Philosophy

Dissertation: **SPECTRAL REFLECTANCE INDICES FOR ESTIMATING YIELD AND WATER CONTENT IN SPRING WHEAT GENOTYPES UNDER WELL IRRIGATED, WATER STRESS, AND HIGH TEMPERATURE CONDITIONS**

Major Field: Crop Science

Biographical:

Personal Data: Born in Mexico City, Mexico in September 12, 1966.

Education: Received Bachelor of Science (Biology) from National University of Mexico, Mexico in July, 1990, and Master of Science (Botany) from the Postgraduate College in Agricultural Sciences, Texcoco, Mexico in December, 2002. Completed requirements for the Doctor of Philosophy (Crop Science) at Oklahoma State University in July 2009.

Experience: Worked as an Assistant Researcher at the Postgraduate College in Agricultural Sciences, from March, 1991 to December, 2000 and as an Associate Researcher in the same Institution, from January, 2003 to July, 2005. Graduate Research Assistant, wheat breeding, Department of Plant and Soil Sciences, Oklahoma State University from October, 2005 to May, 2009.

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Pages in Study: 146 Candidate for the Degree of Doctor of Philosophy

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Scope and Method of Study: Alternative methods for selecting, detecting, and identifying higher yielding genotypes in wheat breeding programs are important for obtaining major genetic gains. The water indices can be used as an indirect selection tool because of their strong association with different physiological and yield components. Diverse spring wheat advanced lines were used, which corresponded to three international trials developed by the International Maize and Wheat Improvement Center (CIMMYT); 24th Elite Spring Wheat Yield Trial (ESWYT) with 25 lines, 11th Semi-Arid Wheat Yield Trial (SAWYT) with 40 lines, and 11th High Temperature Wheat Yield Trial (HTWYT) with 18 lines. Two other experiments also employed advanced lines for testing the relationship between water indices and water content parameters (10-16 lines) and for evaluating the influence of morphological traits (20 lines) over the water indices. Several water indices and other reflectance indices were estimated at three growth stages (booting, heading, and grain filling) using a field portable spectrometer (Analytical Spectral Devices, Boulder, CO). Field plots were planted in Northwest Mexico during three growing seasons (2006, 2007, and 2007). Grain yield, biomass, and some water status parameters were determined in diverse experiments.

Findings and Conclusions: There were high correlations (phenotypic and genetic) between grain yield and the water indices showing high heritability, response to selection and correlated response, relative selection efficiency, and efficiency in selecting the higher yielding genotypes. Two water indices showed the strongest relationships (NWI-1 and NWI-3) for all the parameters determined in the well irrigated, water stress, and high temperature environments. In addition, the water indices were related with parameters commonly employed for assessing the crop water status (*i.e.*, water potential) during booting, anthesis and grain filling under water stress conditions. Finally, our results demonstrated that the relationship between the water indices and grain yield was affected mainly by erect leaves and spike orientation. The potential for employing the water indices for selecting high yielding lines represents a significant advantage in breeding programs because the top yielding lines can be selected in an accurate, inexpensive, and easy manner. In addition, the best high yielding lines maintained high canopy water content under water stress conditions.

ADVISER'S APPROVAL: Arthur R. Klatt