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RESEARCH

Evaluating Grain Yield in Spring Wheat with Canopy Spectral Reflectance

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ABSTRACT

Worldwide, improving grain yield is the most important target for wheat (*Triticum aestivum* L.) breeders. Fast, cost-effective, and nondestructive phenotyping methods for important traits are needed to increase the efficiency of cultivar development. The present study tested canopy spectral reflectance (CSR) as a potential high-throughput method for assessing wheat grain yield in a diverse set of 540 spring-habit accessions from the USDA–ARS National Small Grains Collection. Plots were grown under irrigated (IR) and terminal drought (DR) treatments over two growing seasons, and CSR was measured at several growth stages in each year. The CSR indices related to canopy water and N status, biomass, and photosynthetic area were evaluated for their relation to grain yield. The CSR indices were significantly correlated with yield at every growth stage, with anthesis and grain filling being the most useful for predicting grain yield in IR and DR environments. Single CSR indices selected up to 57% of the highest 25% yielding lines in DR conditions and the grain yield of accessions selected using CSR was 20% greater than randomly selected genotypes. Canopy spectral reflectance also identified up to 86% of the highest 10% yielding accessions. Canopy spectral reflectance may be valuable as a high-throughput means of selecting for yield in large trials of genetically diverse wheat genotypes.

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Abbreviations: CSR, canopy spectral reflectance; CSR_{25}, 25% plots for each canopy spectral reflectance at each growth stage; DR, terminal drought; DZNI, dry *Zea* N index; H^2, broad-sense heritability; HY_{10}, 10% highest yielding accessions; HY_{25}, 25% highest yielding accessions; IR, irrigated; NDVI, normalized difference vegetation index; NSGC, National Small Grains Collection; NWI, normalized water index; ONI, *Oryza* N index; PRI, photochemical reflectance index; PSSRa, pigment specific simple ratio chlorophyll-a; REML, restricted maximum likelihood; RNDVI, red normalized difference vegetation index; SI, standard index; WI, water index.

Wheat yields have increased over the past few decades, in part, because of genetic improvements such as the incorporation of dwarfing genes, increased disease resistance and abiotic stress tolerance, and development of locally adapted cultivars (Reynolds et al., 2009, 2012). However, from 1959 to 2008 the estimated yield gain has been 1.1% annually, with most of the yield increase occurring before 1984 (Graybosch and Peterson, 2010; Pingali, 2012). Current estimates of annual yield increase for wheat over the past 20 yr are <1% (Fischer and Edmeades, 2010) and are insufficient to meet the projected 1.7 to 2.4% increase needed to keep pace with the growing world population

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(Reynolds et al., 2012; Ray et al., 2013). In addition to the concern of population growth, the threat of worldwide climate change and its effects on cropping systems will be an additional challenge for breeders to overcome in efforts to improve wheat grain yield (Fedoroff et al., 2010; Malcolm et al., 2012; Stamp and Visser, 2012; Arbuckle et al., 2013). The use of novel germplasm and improved phenotyping tools would aid breeders by increasing their efficiency and widening the genetic base of wheat.

Fast, cost-effective, and nondestructive high-throughput phenotyping platforms have gained interest in recent years for use by breeders to decrease the time and costs required to assess new genotypes (Cabrera-Bosquet et al., 2012). While genotyping technologies have improved significantly, in-field phenotyping tools have not kept pace (Araus and Cairns, 2014). A problem when attempting to accurately phenotype large numbers of plants is controlling the growing conditions. The use of growth chambers and greenhouses allows for the highest levels of environment control but does not reflect actual field conditions, whereas field experiments suffer from heterogeneous soil and environmental conditions (Araus and Cairns, 2014). Statistical adjustments of spatial variation found in field conditions are commonly used to reduce the effects of environmental influences on data analysis. Canopy spectral reflectance is one of the first high-throughput phenotyping platforms applied to field assessment of crops (Aparicio et al., 1999). While the accuracy of phenotypic data taken in the field will most always be affected by heterogeneous environmental conditions to some extent, CSR indices have been used to not only assess crop characteristics but also as a means of mapping and adjusting for field heterogeneity (Araus and Cairns, 2014).

Canopy spectral reflectance is based on the differential pattern of light reflectance on leaves at photosynthetically active wavelengths (400–700 nm) and infrared wavelengths (700–1000 nm). Canopy spectral reflectance indices can be used to estimate plant characteristics such as leaf N content (Wright and Ritchie, 2003; 2004; Wei et al., 2008; Zhu et al., 2008; Feng et al., 2011), photosynthetic active biomass (Aparicio et al., 1999), leaf chlorophyll content, and plant water status (Penuelas et al., 1997a,b; Aparicio et al., 1999; Araus, 2002; Babar et al., 2006a,c; Prasad et al., 2007a; Gutierrez et al., 2010b).

Direct measurements of plant biomass, water status, photosynthetic capacity, and leaf N status are associated with agronomic traits such as yield and grain protein, but these conventional methods all have disadvantages. Measuring plant biomass requires destruction of the entire plant and is impractical for screening large numbers of genotypes (Van Ginkel et al., 1998). Assessing plant water status through excised leaves is destructive, requires tedious measurements of small changes in leaf weight over time, and sufficient time to completely dry plant tissue (McCaig and Romagosa, 1989). Similarly, plant N status measurements are time consuming and require removal of leaf tissue and laboratory procedures to assess N content (Feng et al., 2011).

Previous studies have shown CSR indices to be predictors of yield in barley (*Hordeum vulgare* L.) (Hansen, 2002), rice (*Oryza sativa* L.) (Inoue and Moran, 1998), corn (*Zea mays* L.) (Teal et al., 2006), durum wheat (*Triticum turgidum* L.) (Aparicio et al., 1999), winter wheat (Hansen, 2002; Prasad et al., 2007a), and bread wheat (Gutierrez et al., 2010b). Babar et al. (2006a) found indices related to photochemical, biomass, and canopy water content related indices to be highly correlated (>0.80) with yields to explain upward of 50% of the yield variation observed across multiple years in a group of 15 high-yielding CIMMYT bread wheat genotypes.

Canopy spectral reflectance has the potential to aid breeding programs in which large numbers of individuals must be screened in a fast and cost-effective manner. Canopy spectral reflectance could facilitate line development by identifying superior genotypes at or before anthesis, allowing for crosses to be made before grain yields have been evaluated. Previous studies on CSR relation to yields have used small groups ($n < 50$) of advanced breeding lines (Babar et al., 2006c; Gutierrez et al., 2010b), biparental populations (Babar et al., 2006b; Prasad et al., 2007a), or commercial cultivars (Babar et al., 2006b; Prasad et al., 2007a). For a technology to be useful to breeders, it must be applicable to a wide range of genotypes and growing conditions. While genotype selection by CSR has been successfully implemented in several wheat growing environments (Gutierrez et al., 2010b), screening of large, genetically diverse panels of wheat genotypes has not been reported previously.

In the present study, we used CSR as a high-throughput phenotyping tool to assess grain yield in a diverse collection of accessions from the USDA–ARS National Small Grains Collection (NSGC). Our goals were to assess CSR indices for predicting grain yield under irrigated and water-stressed conditions and to identify high-yielding germplasm in a large, diverse set of wheat lines from the NSGC using CSR.

MATERIALS AND METHODS

Plant Material

The plant material used in this study consisted of 540 spring wheat accessions from the NSGC common wheat core subset based on heading dates and uniformity during an initial screen in 2010. The NSGC is a component of the National Plant Germplasm System in the USDA–ARS. The 540 spring wheat accessions originated from six continents and 81 countries and included cultivars, breeding lines, landraces, and accessions of uncertain improvement status. Two wheat cultivars, Agawam (PI648027) and Alpowa (PI566596) and three breeding lines from the University of Idaho wheat breeding program, IDO599, IDO686, and IDO702, were used as checks in 2011. PI428506 and PI520108 were chosen to replace IDO702 and PI648027 as checks to better represent the 540 lines from NSGC in the 2012 trial (Supplemental Table S1). Additional information on the plant material used for this study can be found at the USDA's Germplasm Resources Information Network (www.ars-grin.gov) and characteristics of the five checks can be found in Li et al. (2011).

Field Design and Experimental Conditions

Trials were arranged in an augmented complete block design 20 plots wide and 30 plots deep, as previously described (Zhang et al., 2014). In total, 540 unique accessions and five checks were planted in each experiment. Plots were 1.83 m long by 1.5 m wide and planted in seven rows at a rate of 364,500 kernels per hectare. Plots were divided into early, medium, and late maturity groups, each containing 180 accessions and 20 check plots for a total of 600 plots per trial. Each maturity group was further divided into four subblocks that contained a single plot of each of the five checks. The check lines were distributed so that each row contained two different checks and each column contained three different checks. Trials were planted in adjacent water-level treatments at the University of Idaho Aberdeen Research and Extension Center in Aberdeen, Idaho (42°57′36″ N, 112°49′12″ W, and elevation 1342 m). One treatment was irrigated throughout the entire growing season and the other was subjected to water stress at reproductive growth stages (DR). A drip-tape system was employed for precise irrigation control and to allow for each plot within the treatment to receive the same amounts of water. Individual plots had three 1.83 m sections of drip tape spaced every two rows. All plots were irrigated for a single 12 h period each week until heading at a rate of 2.5 L h^{-1} per 30.5 m of drip tape. Plots in the IR were irrigated until physiological maturity. Water stress conditions were induced in the DR treatment when 95% of the plots had headed.

The climate in Aberdeen, ID, is conducive to DR research, with annual precipitation between 20.3 and 27.9 cm, and mean annual temperatures between 7.2 and 8.3°C (Li et al., 2011). The least precipitation and highest air temperatures were recorded during June and July each year and occurred during the heading and flowering growth stages consistent with the onset of DR conditions. The average temperatures and total precipitation from June to August were 27.2°C and 3.5 mm in 2011, and 28.7°C and 11.7 mm in 2012. Trials received 66.3 and 46.2 mm of precipitation between planting and physiological maturity (April–August) in 2011 and 2012, respectively. The field soils were Declo loam (coarse-loamy, mixed, superactive, mesic Xeric Haplocalcids) with 0 to 2% slopes and pH of 8.1. Historical information on climate conditions for Aberdeen, ID, is available through AgriMet (http://www.usbr.gov/pn/agrimet/webarcread.html).

Agronomic Traits

In all trials, individual plots were harvested after physiological maturity with a Wintersteiger Classic small-plot combine equipped with a Harvest Master system (Wintersteiger Inc.). Yields were calculated from raw grain weight and converted to kilograms per hectare. Days to heading were calculated from the planting date until 50% of the heads within a plot were emerged. Heights were measured from the middle rows of each plot at maturity from the soil surface to top of the spike.

Canopy Spectral Reflectance

Canopy spectral reflectance measurements were made with a portable Ocean Optics Jaz spectrometer (Ocean Optics). This device measures the radiation reflected directly from the plot canopies. Measurements were taken between 10:00 AM and 3:00 PM on cloud-free days to minimize atmospheric interference and ensure consistent sunlight and when a majority of the plots were at specific growth stages, heading, and anthesis in 2011 and at booting, heading, anthesis, and grain filling in 2012. The spectrometer was calibrated using a barium sulfate ($BaSO_4$)-coated board to account for changes in the solar radiation intensity due to the position of the sun. New calibrations were taken every 40 plots or approximately every 20 min.

The spectrometer used for this experiment had three channels with gratings #3, #4, and #14 (Ocean Optics). Across all gratings, continuous wavelengths from 339 to 1259 nm were recorded with an average interval of 0.324 nm. Grating #4 has an optimal range of 530 to 1100 nm and a full-width, half-maximum optical resolution of 1.17 nm, which encompassed all required wavelengths except for 520 nm. Grating #4 was used in the present study because 526 nm is just slightly outside its optimal range and all other required wavelengths can be optimally measured with it.

Only the centers of plots were measured, to avoid edge effects, using a constant-scanning method 50 cm above the canopy with a 25° field of view (386 cm^3 footprint). An average of 100 individual measurements was taken per wavelength recorded with a single scan of each plot used for measurements. The constant-scanning method entails scanning across the center region of each plot for the duration of the measurements. The same accessions were recorded in both IR and DR treatments within a single day and all plots were measured within a single week.

Index Calculations

The wavelengths needed for calculating CSR indices were generated by averaging the four reflectance intensities closest to the needed wavelength. In addition, while recording reflectance values the spectrometer implemented a three-step boxcar smoothing. Averaged values were used as a means of noise reduction. For example, the water index (WI) is calculated using the minor water absorption band at 970 nm and reference frequency 900 nm. A single value for 970 and 900 nm is calculated by averaging reflectance values at the frequencies shown in the equation below:

$$\text{Water index (WI)} = \frac{\text{Average (969.89, 970.21, 970.52, 970.85)}}{\text{Average (900.16, 900.49, 900.82, 901.16)}}$$

Three general categories of CSR indices were used: water-based, vegetation-based, and N-based. The water-based and vegetation-based indices were chosen because they are correlated with yields in wheat and are indicators of canopy water status and photosynthetic biomass. The two N-based indices were chosen because they are related to plant height, leaf area, dry matter accumulation, and chlorophyll content in corn and rice (Xue et al., 2004; Zhao et al., 2003).

The water-based indices used here are WI (WI = 970 nm/900 nm) (Penuelas et al., 1993), normalized water index 1 [NWI1 = (970 nm − 900 nm)/(970 nm + 900 nm)], NWI2 [(970 nm – 850 nm)/(970 nm + 850 nm)], NWI3 [(970 nm – 920 nm)/(970 nm + 920 nm)], and NWI4 [(970 nm – 880 nm)/(970 nm + 880 nm)] (Babar et al., 2006b; Prasad et al., 2007b). The vegetative based indices used here are simple ratio (SR = 900 nm/680 nm) (Tucker and Sellers, 1986), red normalized difference vegetation index [RNDVI = (780 nm − 670 nm)/(780 nm + 670 nm)] (Raun et al., 2001), normalized difference vegetation index [NDVI = (900 nm − 680 nm)/(900 nm + 680 nm)] (Tucker and Sellers, 1986), photochemical reflectance index [PRI = (531 nm – 570 nm)/(531 nm + 570 nm)] (Penuelas et al., 1997a), and pigment specific simple ratio chlorophyll-a (PSSRa = 800 nm/680 nm) (Blackburn, 1999). The index PSSRa specifically measures chlorophyll-a content but has been used for estimating vegetative biomass (Babar et al., 2006b). The indices used here to estimate canopy N status were the *Oryza* N index (ONI = 810 nm/560 nm) (Xue et al., 2004) and the dry *Zea* N index (DZNI = 575 nm/526 nm) (Zhao et al., 2003).

Statistical Analysis

Collected data was adjusted for maturity blocks as well as days to heading by restricted maximum likelihood (REML) models. Analysis of variance (ANOVA), Student *t*-test, and Pearson correlation coefficients were calculated using JMP version 11 statistical software (SAS Institute, 2013). Checks were used to estimate broad-sense heritability (H^2) by REML models with rows and columns as fixed effects and genotypes as random effects.

Canopy Spectral Reflectance Selection of High-Yielding Accessions

Subsets of accessions were selected based on yields and CSR values. A subset of the 25% highest yielding accessions (HY_{25}) and the 10% highest yielding accessions (HY_{10}) were selected in each treatment for both years. For each CSR index at each growth stage, 25% of the plots (CSR_{25}) were selected to represent accessions having values presumably associated with increased grain yield. Thus, accessions were chosen with the highest 25% index values for NDVI, RNDVI, PRI, PSSRa, SR, and ONI and the lowest 25% index values for WI, NWI1, NWI2, NWI3, NWI4, and DZNI. Average yield from a random selection of 135 (25%) accessions, repeated 1000 times, was calculated for comparison to the CSR_{25} yield values. Bootstrapping at a 95% confidence was used to determine significance of yield increase due to selection.

RESULTS

Analysis of Grain Yield

Terminal drought conditions had significant effects on yields in both 2011 and 2012 (Table 1). Lines in the IR treatments produced significantly higher yields than the DR treatment and the average of yields of all lines for both treatments was larger in 2011 than 2012 ($p < 0.05$) (Table 1). The average yields of all lines in IR were 5795.22 kg ha^{-1} in 2011 and 4503.95 kg ha^{-1} in 2012, while in DR they were 5376.78 and 3697.30 kg ha^{-1} in 2011 and 2012, respectively. The H^2 of yield was 0.64 and 0.54 in 2011, IR and DR, respectively, and 0.3 and 0.25 in 2012, IR and DR, respectively.

Table 1. Grain yield, plant height, and days to heading recorded in 2011 and 2012 irrigation (IR) and terminal drought (DR) treatments, as well as the broad-sense heritability (H^2).

Trait	Treatment	2001 Mean	H^2 2011	2012 Mean	H^2 2012
Yield (kg ha^{-1})	IR	5795.22a†	0.64	4503.95a	0.30
	DR	5376.78b	0.54	3697.30b	0.25
Height (cm)	IR	107.24a	0.67	105.79a	0.63
	DR	109.60b	0.69	110.00b	0.73
Days to heading (d)	IR	69.66a	0.88	70.96a	0.32
	DR	69.33b	0.78	70.03b	0.20

† Values not connected by letters indicate significant differences $p < 0.05$ within columns.

Canopy Spectral Reflectance Heritability and Changes by Growth Stage

Heritability of CSR indices ranged from 0.0 at heading in 2012 DR to 0.68 at heading in 2011 DR. Heritabilities of RNDVI at heading in 2011DR and at booting in 2012 IR and DR were higher than that of yield in both years. Heritabilities of DZNI at most growth stages over 2 yr were higher than those of yield in 2012 (Table 1, 2). Heritabilities of WI and NWI1–NWI4 varied with growth stages, treatments, and years (Table 2). All water-based CSR indices recorded in 2011 DR at HD had higher H^2 than yield and NWI3 had H^2 values equal to yield H^2 in 2012 IR at anthesis. No other water-based CSR H^2 was larger than their respective yield H^2. In 2011, NDVI and RNDVI were the only vegetative indices to have H^2 higher than yield H^2 in DR at HD. All vegetative biomass- and N-status-related indices had higher H^2 than yield H^2 in 2012 IR at booting, but not at other growth stages (Table 1, 2). The indices PRI, NDVI, RNDVI, and DZNI had higher H^2 than yield in 2012 DR booting and anthesis growth stages but not heading or grain filling. Overall, NDVI and RNDVI were found to have H^2 values in three of the four trials in at least one growth stage. The water-based NWI3 had H^2 values greater than yield H^2 in two trials, as did the vegetative biomass index PRI and N-status-based DZNI.

The CSR indices of all lines showed significant differences between IR and DR treatments in all growth stages in both years except for DZNI in 2011 at anthesis and ONI and PRI in 2011 at heading (Table 3). At most growth stages measured each year, accessions had significantly higher values in DR vs. IR for WI, NWI1, NWI2, NWI3, NWI4, and DZNI and lower values in DR than IR for PRI, NDVI, RNDVI, PSSRa, SR, and ONI. At heading in 2011, the WI values were significantly higher in the IR treatment than DR and the vegetation index PRI showed no difference between IR and DR treatments. In 2012, the highest values for the water-related CSR indices

Table 2. Heritability of single canopy spectral reflectance indices at each growth stage measured in 2011 and 2012 irrigated (IR) and terminal drought (DR) treatments. Growth stages: Bt, booting; Hd, heading; Ant, anthesis; and GF, grain filling.

	2011 IR		2011 DR		2012 IR				2012 DR			
Index	Hd	Ant	Hd	Ant	Bt	Hd	Ant	GF	Bt	Hd	Ant	GF
WI	0.28	0.44	0.57	0.27	0.27	0.20	0.27	0.26	0.21	0.05	0.17	0.14
NWI1	0.29	0.42	0.54	0.25	0.22	0.20	0.27	0.25	0.21	0.04	0.15	0.13
NWI2	0.30	0.43	0.61	0.23	0.28	0.17	0.19	0.21	0.22	0.03	0.13	0.09
NWI3	0.30	0.41	0.54	0.26	0.20	0.22	0.30	0.28	0.21	0.04	0.16	0.15
NWI4	0.32	0.44	0.59	0.26	0.26	0.19	0.23	0.24	0.21	0.03	0.14	0.11
SR	0.45	0.29	0.33	0.12	0.63	0.18	0.21	0.17	0.17	0.00	0.14	0.15
PRI	0.47	0.29	0.31	0.23	0.46	0.14	0.20	0.25	0.26	0.05	0.36	0.23
NDVI	0.25	0.44	0.65	0.22	0.51	0.24	0.21	0.22	0.61	0.07	0.28	0.11
RNDVI	0.33	0.41	0.68	0.21	0.58	0.22	0.17	0.20	0.57	0.05	0.28	0.09
PSSRa	0.45	0.31	0.34	0.11	0.60	0.17	0.19	0.15	0.15	0.00	0.13	0.18
ONI	0.22	0.31	0.27	0.19	0.66	0.20	0.29	0.24	0.16	0.04	0.20	0.19
DZNI	0.46	0.50	0.36	0.27	0.49	0.15	0.22	0.28	0.33	0.06	0.37	0.23

(WI, NWI1, NWI2, NWI3, and NWI4) were recorded at booting and the lowest at heading, followed by an increase from heading to grain filling. The two sets of readings taken in 2011 follow this trend with an increase in CSR values from heading to anthesis. Photochemical (PSSRa and PRI) and vegetative biomass related indices (NDVI, RNDVI, and SR) generally decreased throughout the growing season in both years. The ONI index increased during the early season then decreased after heading. The DZNI index, in contrast, decreased during the early season then increased after heading (Table 3).

Correlation between Canopy Spectral Reflectance Indices and Yield

Pearson correlation coefficients between yield and CSR indices were significant ($P < 0.001$) at all growth stages in IR and DR each year (Table 4). The indices WI, NWI1, NWI2, NWI3, NWI4, and DZNI were consistently negatively correlated with yield, and PRI, RNDVI, NDVI, PSSRa, SR, and ONI were positively correlated with yield. No single index had consistently higher associations with yield than other indices. Water indices (WI, NWI1, NWI2, NWI3, and NWI4) showed similar correlation coefficients ranging from −0.20 at heading in 2011 IR to −0.66 at anthesis in 2012 DR. Vegetative and photochemical indices (SR, NDVI, RNDVI, PRI, PSSRa) also showed similar correlation coefficients ranging from 0.16 at heading in 2011 IR to 0.71 at grain filling in 2012 IR (Table 4).

In general, correlation increased during the growing season with the most significant correlations occurring at anthesis followed by grain filling in 2012. The grain filling growth stage had the highest correlations for RNDVI in 2012 IR and DR, for NDVI in 2012 IR, and for DZNI in 2012 DR. Water-based indices were more highly correlated with yields in the IR treatments than DR at all readings except at booting in 2012 (Table 4).

Canopy Spectral Reflectance-Based Selections

The CSR_{25} selections encompassed 32 to 55% of HY_{25} and 37 to 86% of HY_{10}. The proportion of genotypes selected by both HY_{25} and CSR_{25} in DR treatments was higher than in IR treatments at both growth stages in 2011 except for PRI and ONI at anthesis. The HY_{25} selections in 2012 found both DR and IR treatments to select similar percentages by CSR_{25}. For HY_{10}, the DR treatment had much higher selection rates than the IR treatment at anthesis in 2011 and at all growth stages in 2012. The CSR_{25} selections made after heading identified a larger proportion of HY_{25} than earlier growth stages for most indices. The index NWI2 at anthesis had the highest HY_{10} selection rates at 86%, and PRI at grain filling had the highest HY_{10} selection rates at 85%, both in the DR treatment in 2012 (Table 5).

Accessions identified by CSR had average yields significantly higher than accessions selected at random. Accessions selected using CSR measured at anthesis consistently had the largest gain in yield compared with the random selections. In 2011, the yield increase of CSR_{25} at anthesis was 9.1 and 10.2% above randomly selected accessions in IR and DR, respectively. In 2012 the average yield increase for accessions selected by CSR at anthesis was 20.3% in IR and 20.8% in DR. Accessions selected based on the PRI showed the greatest yield increases in 2011: 10.7% for IR and 12.9% for DR. In 2012, accessions selected with PSSRa had 20.9% greater yield than randomly selected accessions in the IR, and those selected with NWI2 had showed a 24.4% increase over the random sample.

DISCUSSION

Genetic Variation of Canopy Spectral Reflectance Indices

Evaluation and selection of high-yielding wheat genotypes using CSR indices have been successfully applied in earlier studies (Aparicio et al., 1999; Babar et al., 2006a,c; Prasad et al., 2007a; Gutierrez et al., 2010b). Previous studies

Table 3. Average canopy spectral reflectance index value and standard deviations of measurements taken in 2011 and 2012 irrigated (IR) and terminal drought (DR) treatments. Growth stages: Bt, booting; Hd, heading; Ant, anthesis; and GF, grain filling.

Index	Treatment	Bt	Hd	Ant	GF
WI	2011 IR	–	0.165 ± 0.014a†	0.181 ± 0.023a	–
	2011 DR	–	0.163 ± 0.018b	0.199 ± 0.027b	–
	2012 IR	0.71 ± 0.109a	0.162 ± 0.014a	0.182 ± 0.026a	0.222 ± 0.032a
	2012 DR	0.787 ± 0.058b	0.191 ± 0.025b	0.201 ± 0.027b	0.278 ± 0.038b
NWI1	2011 IR	–	−0.717 ± 0.021a	−0.695 ± 0.032a	–
	2011 DR	–	−0.721 ± 0.026b	−0.669 ± 0.039b	–
	2012 IR	−0.174 ± 0.078a	−0.722 ± 0.021a	−0.693 ± 0.037a	−0.638 ± 0.043a
	2012 DR	−0.12 ± 0.037b	−0.68 ± 0.035b	−0.666 ± 0.037b	−0.566 ± 0.047b
NWI2	2011 IR	–	−0.719 ± 0.021a	−0.694 ± 0.033a	–
	2011 DR	–	−0.722 ± 0.026b	−0.667 ± 0.042b	–
	2012 IR	−0.17 ± 0.081a	−0.717 ± 0.024a	−0.687 ± 0.042a	−0.623 ± 0.05a
	2012 DR	−0.119 ± 0.039b	−0.675 ± 0.037b	−0.657 ± 0.04b	−0.545 ± 0.049b
NWI3	2011 IR	–	−0.71 ± 0.021a	−0.687 ± 0.032a	–
	2011 DR	–	−0.713 ± 0.026b	−0.664 ± 0.038b	–
	2012 IR	−0.169 ± 0.075a	−0.716 ± 0.02a	−0.687 ± 0.035a	−0.635 ± 0.041a
	2012 DR	−0.117 ± 0.034b	−0.675 ± 0.035b	−0.661 ± 0.036b	−0.568 ± 0.045b
NWI4	2011 IR	–	−0.72 ± 0.021a	−0.697 ± 0.032a	–
	2011 DR	–	−0.724 ± 0.026b	−0.672 ± 0.04b	–
	2012 IR	−0.172 ± 0.077a	−0.722 ± 0.022a	−0.695 ± 0.038a	−0.635 ± 0.045a
	2012 DR	−0.121 ± 0.037b	−0.681 ± 0.036b	−0.666 ± 0.038b	−0.562 ± 0.047b
SR	2011 IR	–	20.579 ± 6.829a	12.633 ± 5.856a	–
	2011 DR	–	19.284 ± 5.105b	10.059 ± 4.139b	–
	2012 IR	11.119 ± 4.001a	12.02 ± 3.984a	6.997 ± 3.365a	3.992 ± 2.239a
	2012 DR	8.077 ± 2.782b	7.858 ± 3.486b	4.91 ± 2.191b	1.816 ± 0.865b
PRI	2011 IR	–	−0.023 ± 0.014a	−0.062 ± 0.026a	–
	2011 DR	–	−0.023 ± 0.016a	−0.065 ± 0.026b	–
	2012 IR	−0.145 ± 0.021a	−0.144 ± 0.021a	−0.165 ± 0.036a	−0.198 ± 0.036a
	2012 DR	−0.154 ± 0.017b	−0.162 ± 0.024b	−0.185 ± 0.028b	−0.22 ± 0.017b
NDVI	2011 IR	–	0.897 ± 0.032a	0.82 ± 0.086a	–
	2011 DR	–	0.892 ± 0.035b	0.785 ± 0.092b	–
	2012 IR	0.812 ± 0.082a	0.829 ± 0.063a	0.695 ± 0.143a	0.516 ± 0.199a
	2012 DR	0.744 ± 0.129b	0.732 ± 0.119b	0.613 ± 0.144b	0.243 ± 0.163b
RNDVI	2011 IR	–	0.861 ± 0.059a	0.808 ± 0.093a	–
	2011 DR	–	0.887 ± 0.04b	0.766 ± 0.104b	–
	2012 IR	0.817 ± 0.071a	0.809 ± 0.078a	0.653 ± 0.172a	0.444 ± 0.237a
	2012 DR	0.748 ± 0.115b	0.702 ± 0.133b	0.56 ± 0.161b	0.144 ± 0.17b
PSSRa	2011 IR	–	19.909 ± 6.568a	12.19 ± 5.659a	–
	2011 DR	–	18.813 ± 5b	9.544 ± 4.053b	–
	2012 IR	11.162 ± 3.845a	11.442 ± 4.054a	6.61 ± 3.373a	3.64 ± 2.207a
	2012 DR	8.122 ± 2.696b	7.413 ± 3.384b	4.486 ± 2.129b	1.569 ± 0.834b
ONI	2011 IR	–	9.027 ± 2.468a	6.365 ± 2.215a	–
	2011 DR	–	8.934 ± 2.028a	5.719 ± 1.706b	–
	2012 IR	5.585 ± 1.552a	6.515 ± 1.479a	4.603 ± 1.324a	3.075 ± 0.834a
	2012 DR	4.554 ± 1.164b	4.911 ± 1.412b	3.821 ± 0.933b	2.122 ± 0.458b
DZNI	2011 IR	–	1.098 ± 0.039a	1.199 ± 0.08a	–
	2011 DR	–	1.09 ± 0.043b	1.203 ± 0.078a	–
	2012 IR	1.52 ± 0.109a	1.479 ± 0.08a	1.578 ± 0.141a	1.701 ± 0.146a
	2012 DR	1.531 ± 0.086b	1.529 ± 0.084b	1.632 ± 0.116b	1.778 ± 0.076b

† Values not connected by the same letter are significantly different, $p < 0.05$.

evaluated groups of fewer than 50 genotypes consisting either of advanced breeding lines, elite cultivars, or biparental populations. In the present study, we found that CSR indices were able to distinguish high-yielding genotypes from a large and diverse collection of wheat accessions that included cultivars, breeding lines, and landraces.

Water-based indices (WI, NWI1, NWI2, NWI3, and NWI4) responded as expected in each treatment and were negatively correlated with yields at all growth stages. Water indices use the 970-nm wavelength minor-water absorption band and are indicators of plant water status. Increases in the water-based index values indicate

Table 4. Pearson correlation coefficient of yield and canopy spectral reflectance indices at different growth stages in two irrigation regimes (IR, irrigation; DR, terminal drought) over two growing seasons. Growth stages: Bt, booting; Hd, heading; Ant, anthesis; and GF, grain filling. All values significant at $p < 0.001$.

Index	2011 IR (Hd)	2011 IR (Ant)	2011 DR (Hd)	2011 DR (Ant)	2012 IR (Bt)	2012 IR (Hd)	2012 IR (Ant)	2012 IR (GF)	2012 DR (Bt)	2012 DR (Hd)	2012 DR (Ant)	2012 DR (GF)
WI	−0.21	−0.41	−0.25	−0.39	−0.32	−0.48	−0.63	−0.58	−0.43	−0.41	−0.55	−0.45
NWI1	−0.21	−0.41	−0.25	−0.38	−0.31	−0.48	−0.63	−0.58	−0.44	−0.41	−0.56	−0.46
NWI2	−0.21	−0.42	−0.29	−0.37	−0.37	−0.52	−0.66	−0.62	−0.44	−0.44	−0.59	−0.50
NWI3	−0.20	−0.40	−0.24	−0.35	−0.30	−0.46	−0.62	−0.56	−0.42	−0.40	−0.54	−0.43
NWI4	−0.20	−0.41	−0.27	−0.35	−0.36	−0.51	−0.64	−0.60	−0.44	−0.42	−0.57	−0.47
SR	0.22	0.37	0.27	0.43	0.54	0.54	0.64	0.61	0.26	0.37	0.53	0.53
PRI	0.25	0.48	0.39	0.44	0.53	0.58	0.69	0.67	0.29	0.41	0.54	0.51
NDVI	0.25	0.46	0.28	0.39	0.57	0.56	0.69	0.70	0.26	0.39	0.49	0.45
RNDVI	0.16	0.46	0.30	0.41	0.57	0.57	0.70	0.71	0.25	0.41	0.52	0.53
PSSRa	0.22	0.38	0.28	0.44	0.56	0.55	0.65	0.62	0.26	0.39	0.56	0.55
ONI	0.26	0.40	0.30	0.34	0.56	0.51	0.59	0.50	0.22	0.32	0.40	0.28
DZNI	−0.24	−0.48	−0.38	−0.41	−0.50	−0.58	−0.70	−0.66	−0.30	−0.42	−0.40	−0.47

Table 5. Percentage of the 25% highest-yielding accessions (HY_{25}) genotypes selected by 25% of the plots for each canopy spectral reflectance at each growth stage (CSR_{25}) and percentage yield gain compared with the mean yield of a random selection of 25% ($n = 135$) of all genotypes ($Mean_{Rand25\%}$). Values within parentheses are the percentage of the top 10% yielding (HY_{10}) genotypes selected by CSR_{25}. Bootstrapped yields at a 95% confidence interval ($p < 0.0001$). Growth stages: Bt, booting; Hd, heading; Ant, anthesis; and GF, grain filling. IR, irrigation; DR, terminal drought.

Experiment	2011 IR				2011 DR				2012 IR				2012 DR			
$Mean_{Rand25\%}$	5779				5374				4508				3700			
Growth stage	Hd	Gain	Ant	Gain	Hd	Gain	Ant	Gain	Ant	Gain	GF	Gain	Ant	Gain	GF	Gain
kg ha^{-1}									%							
$WI_{25\%}$	33 (55)	6.2	40 (51)	8.8	39 (39)	5.8	45 (56)	9.9	48 (57)	20.4	48 (54)	18.8	53 (84)	22.5	48 (78)	17.6
$NWI1_{25\%}$	33 (55)	6.2	40 (51)	8.8	39 (39)	5.7	45 (56)	9.8	48 (57)	20.4	48 (54)	18.8	53 (84)	22.5	48 (78)	17.6
$NWI2_{25\%}$	32 (49)	5.3	38 (49)	8.5	41 (44)	6.6	42 (54)	9.3	48 (55)	20.1	51 (58)	19.9	57 (86)	24.4	49 (78)	19.2
$NWI3_{25\%}$	33 (55)	6.4	38 (49)	8.8	38 (37)	5.6	43 (54)	9.3	46 (51)	19.4	48 (52)	18.5	53 (82)	21.5	45 (71)	15.6
$NWI4_{25\%}$	32 (51)	5.9	39 (49)	8.5	41 (44)	6.8	42 (54)	8.9	49 (57)	20.2	51 (58)	19.3	55 (84)	23.2	47 (78)	17.6
$SR_{25\%}$	34 (49)	8.1	38 (51)	8.1	40 (49)	5.7	44 (59)	10.9		20.7	48 (54)	18.1	50 (84)	19.6	51 (83)	1537
$PRI_{25\%}$	32 (45)	5.1	46 (58)	10.7	46 (60)	10.5	44 (63)	12.9		19.8	47 (52)	17.8	42 (76)	21.4	50 (80)	19.2
$NDVI_{25\%}$	32 (51)	7.8	38 (51)	8.7	41 (50)	6.2	44 (57)	10.4		20.7	48 (54)	18.1	50 (84)	19.6	53 (83)	15.7
$RNDVI_{25\%}$	33 (43)	5.5	46 (60)	8.8	48 (56)	6.8	49 (70)	10.4		20.6	46 (52)	18.2	50 (82)	21.1	53 (85)	17.8
$PSSRa_{25\%}$	35 (49)	7.6	38 (53)	8.7	39 (50)	7	44 (57)	10.6		20.9	48 (56)	18.2	48 (43)	21.1	50 (83)	17.1
$ONI_{25\%}$	38 (51)	8.3	44 (60)	10.1	43 (55)	7.3	41 (52)	9		20.1	50 (56)	19.5	45 (70)	16.1	39 (68)	10.5
$DZNI_{25\%}$	35 (51)	4.8	38 (51)	10.5	37 (45)	10.1	37 (45)	11		19.9	48 (56)	18.2	48 (82)	16.4	50 (83)	19.9
Average gain		6.4		9.1		7		10.2		20.3		18.6		20.8		17

a decrease in the amount of water within the canopy, while decreases in water-based index values indicate increased water status. The trend of increasing water-based index values from heading to grain filling follows the expected decrease in canopy water as the growing season progressed, which has been reported in previous studies (Aparicio et al., 1999; Babar et al., 2006a,c; Prasad et al., 2007b; Gutierrez et al., 2010b). The indices RNDVI, NDVI, SR, PRI, and PSSRa were expected to behave similarly since they are used as indicators of biomass (Aparicio et al., 1999; Babar et al., 2006a,c; Prasad et al., 2007b; Gutierrez et al., 2010b). These indices estimate vegetative biomass or photosynthesis-related chemical content through measurements of chlorophylls (RNDVI, NDVI, and SR) or xanthophyll (PRI), which absorb at 670- to 680- and 531-nm wavelengths, respectively. Near infrared wavelengths (700–1300 nm) are used in each of vegetative indices, except PRI, because such wavelengths are not absorbed by plant material and have a higher level of reflectance. The vegetative biomass indices assume that the leaf tissue area is related to photosynthetic tissue and, consequently, decreases as the plants mature, leaves senesce, and photochemicals are recycled.

The difference between IR and DR index values for CSR was greater in 2012 than in 2011. This result could be due to the higher precipitation in 2011, which would reduce the impact of DR. In a study by Gutierrez et al. (2010b), the change in CSR index values as the season progressed was higher in water-stressed treatments than well-irrigated treatments, as found in the present study. Over the growing season, the more rapid and larger change in CSR indices seen in the DR treatment of this experiment indicates the water-stress response. Water-stressed plants need to rely almost entirely on nutrient and water reserves stored in stems and leaves, while nonstressed plants are still able to take up available water and nutrients during the reproductive growth stage. Thus, stored water and nutrients are depleted more rapidly in the DR than IR treatments. The cumulative effects of lower levels of biomass, photosynthetic chemicals, and canopy are reflected in total yields (Foulkes et al., 2011).

A common finding in several past studies is the inconsistency between experiments of correlation and regression analysis of vegetative and photochemical related indices (Babar et al., 2006b; Prasad et al., 2007b; Gutierrez et al., 2010b). Gutierrez et al. (2010b) reported water-based index values to be more consistent in both irrigated and water-stressed treatments, but suggested the use of vegetative indices in high-temperature treatments. Babar et al. (2006a) also found that CSR indices were more associated with final yields when recorded after heading, and CSR measurements from multiple growth stages were more highly correlated with yields than each growth stage taken singly.

Here, we found CSR indices to be more highly correlated with yields in the later stages of anthesis and grain filling, but we did not find vegetative indices to be more highly associated with yields in the DR treatments than the IR except for 2011 anthesis and 2012 booting. It is possible that the DR conditions used in this study were not adequate to have this effect.

Indirect Selection of Yield using Canopy Spectral Reflectance

Estimates of H^2 in the present study were lower than those reported previously (Babar et al., 2006a; Prasad et al., 2007a,b). Prasad et al. (2007a) found CSR H^2 values of RNDVI, SR, WI, NWI1, NWI2, NWI3, and NWI4 to range from 0.48 to 0.78, with a majority of the H^2 values exceeding 0.5 across multiple growth stages, years, and environments. Similarly, Babar et al. (2006a) found H^2 of WI values as well as WI and NDVI of 0.6 in a majority of their trials. However, the low H^2 values in the present study should be expected when evaluating large numbers of diverse genotypes in unreplicated plots, as unreplicated trials are inherently imprecise (Federer and Raghavarao, 1975). Yet, even with low H^2 values, indirect selection tools can still be valuable to breeders if the measurement being used for indirect selection has a higher H^2 than the trait of interest (Babar et al., 2006a; Prasad et al., 2007a,b). Here we found that CSR indices NDVI and RNDVI taken at or before heading and at anthesis in DR treatment in 2012 had higher H^2 values than yield in three of our four trials and are possibly suitable for indirect selection.

When considering correlation of yields with CSR indices, selections at anthesis or grain filling would seem to be the most accurate, as these CSR readings were more highly correlated with yield in both IR and DR each year. However, in the present study, CSR selections in DR were better than in IR at identifying the highest yielding genotypes. In optimal water conditions, plants are able to remain green for the maximum amount of time. Yield potential has been shown to be related to water status and biomass, but in IR conditions these traits could be masked by the longevity of green tissue survival. In DR, genotypes that remain green longer will have lower water-based index values and higher biomass or photochemical index values at later growth stages. These genotypes will likely have the highest biomass- or photochemical-related yield potential. Therefore, selection using CSR indices under DR allows for more efficient selection of genotypes that remain green longer and have a higher chance of increased yields than genotypes that lose green tissue quickly in DR.

In comparison with previous studies that evaluated yield selection by CSR indices, the selections made here match closest to the high-temperature, water-stressed treatment used by Gutierrez et al. (2010a) with ambient daily temperature at anthesis of 30 to 35°C. They reported average selection efficiencies of 80 and 100% using NWI1 and NWI3, respectively. Here we found the CSR indices in DR able to identify 82% of HY_{10} at anthesis. We observed lower selection rates of HY_{25} genotypes than previously reported (Babar et al., 2006c; Prasad et al., 2007b; Gutierrez et al., 2010b) but comparable selection rates of HY_{10} genotypes with water-, vegetative-, and photochemical-based indices. In this study, CSR indices were found to be suitable for indirect selection in DR environments because of their high correlation with yield, ability to identify a large proportion of the highest yielding plots at the anthesis growth stage, and significantly increased average yields of selected accessions over random selection.

CONCLUSIONS

Developing improved cultivars by introducing superior traits or combinations of traits that would benefit wheat growers is the most important job for breeders worldwide. Currently, the genetic gain achieved by breeders is not adequate to keep pace with the growing world population. Some researchers have speculated that the reduction in the rate of yield gain in wheat is because the crop is approaching its theoretical yield limit (Foulkes et al., 2011; Reynolds et al., 2012; Cavanagh et al., 2013). It was shown in an analysis of multiple wheat genomes that during the domestication and subsequent development of modern cultivars the diversity of alleles was greatly reduced, creating a genetic bottleneck for breeders (Rostoks et al., 2006; Brenchley et al., 2012; Cavanagh et al., 2013). Achieving sufficient genetic gains will require introduction of new alleles to broaden the genetic pool available to breeders. Germplasm repositories, such as the NSGC, are an underutilized resource that could be a source of new alleles (Feuillet et al., 2008; Fischer and Edmeades, 2010; Fischer, 2011).

Identifying alleles that could increase the rate of genetic gain in wheat would require screening large numbers of wheat genotypes. Canopy spectral reflectance is a tool that could greatly decrease the time needed to screen new genotypes. Reducing the number of genotypes early in the breeding process would significantly reduce the costs of cultivar development. Based on correlations with yield and selections made using CSR, the anthesis and grain filling growth stages are most suitable for indirect selections. Selections made here by CSR indicate that the best results would be obtained in drought conditions. This study also suggests that additional replicated trails using a smaller number of accessions may improve our results and are needed to validate the above findings.

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