

RESEARCH

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

B. Prasad, B. F. Carver, M. L. Stone, M. A. Babar, W. R. Raun, and A. R. Klatt*

ABSTRACT

Selection criteria that would facilitate increased genetic gain for grain yield would be considered advantageous in plant breeding programs. We evaluated the potential of spectral reflectance indices (SRI) for assessing grain yield variability in winter wheat (*Triticum aestivum* L.) genotypes under Great Plains conditions. One experiment was conducted at two locations on the Oklahoma State University research farms for 1 yr, and two experiments were conducted for 2 yr at a single location. The first experiment included 25 winter wheat cultivars from the Great Plains, and the other two experiments contained two groups of 25 F_{4:6} and F_{4:7} recombinant inbred lines. Six reported SRI (red and green normalized difference vegetation index, RNDVI, GNDVI; simple ratio, SR; water index, WI; normalized water indices 1 and 2, NWI-1 and NWI-2), and two new normalized water indices (NWI-3 and NWI-4) were calculated at booting, heading, and early grain-filling stages using a FieldSpec UV/VNIR spectroradiometer. Significant genotypic variation was observed for SRI and growth stages, though the booting stage was least associated with grain yield. The relationships of grain yield with WI and NWI were stronger than with the RNDVI and SR. The WI and the NWI performed better in identifying superior genotypes, either at any individual growth stage or in a combination of growth stages. Our study clearly demonstrated the potential of using SRI as a selection tool for grain yield in a winter wheat breeding program.

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Abbreviations: GNDVI, green normalized difference vegetation index; LAI, leaf area index; NDVI, normalized difference vegetation index; NIR, near infrared; NWI, normalized water indices; NWI-1, normalized water index 1; NWI-2, normalized water index 2; NWI-3, normalized water index 3; NWI-4, normalized water index 4; PAR, photosynthetically active radiation; RIL, recombinant inbred lines; RNDVI, red normalized difference vegetation index; SR, simple ratio; SRI, spectral reflectance indices; WI, water index.

BREEDING STRATEGIES for developing higher-yielding genotypes of wheat (*Triticum aestivum* L.) are extensively based on approaches that involve generating a large number of crosses and subsequently selecting among and within segregating populations. To date, wheat breeding around the world has been based primarily on empirical selection criteria (yield per se) for yield improvement (Araus et al., 2002). Since yield is characterized by low heritability and a high genotype–environment interaction, empirical selection may be insufficient for achieving genetic gain (Jackson et al., 1996).

Identifying promising genotypes in a breeding program will be facilitated if grain yield can be predicted before the crop is harvested. This early prediction would also be very helpful if the top-performing families could be identified from a group of hundreds

Published in *Crop Sci.* 47:1426–1440 (2007).

doi: 10.2135/cropsci2006.07.0492

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or thousands of segregating populations in a breeding program (Royo et al., 2003). Reynolds et al. (1999) indicated that morphological characters such as number of grains per unit area and harvest index can be used in the visual selection of breeding lines, but those traits are difficult to measure in a large number of small plots in early generations due to labor intensiveness and time.

Yield in a given environment is directly and indirectly influenced by morphological, physiological, and environmental factors. Selection of breeding lines for grain yield in advanced nurseries often needs repetition to make a selection decision because commonly used statistical procedures sometimes fail to produce sufficiently accurate results for identifying superior genotypes (Ball and Konzak, 1993). An analytical breeding strategy is an alternative breeding approach that requires a better understanding of the factors responsible for development, growth, and yield (Richards, 1982). This strategy considers morphophysiological selection criteria that have potential to make empirical selection more efficient (Reynolds et al., 2001). The limited application of this analytical approach is probably due to the lack of an appropriate understanding of the physiological parameters, their estimation, and their true associations with grain yield (Richards, 1996). Commonly used physiological selection criteria include stomatal conductance, canopy temperature depression, and C_{13} isotope discrimination of grains (Reynolds et al., 1999). Spectral properties of the plant came into focus as a potential selection tool for grain yield in more recent years (Aparicio et al., 2002; Royo et al., 2003; Babar et al., 2006).

The basic principle governing canopy spectral reflectance is that specific plant traits are associated with the absorption of specific wavelengths of the spectrum (Reynolds et al., 1999). For example, leaf pigments like chlorophyll, xanthophylls, and carotenoids strongly absorb light in the photosynthetically active radiation (PAR) portion of the spectrum, but not in the near infrared (NIR) region. These pigments reduce reflectance of PAR, but not NIR where the magnitude of reflectance is influenced by the scattering and diffusion of light by different leaf tissues (Knippling, 1970). Spectral reflectance by a crop canopy is related to the overall area of leaves and other photosynthetic organs in the canopy, pigment concentration, and other physiological factors (Araus et al., 2001). Thus, the measurement of the spectrum reflected from plants provides information that can be used to estimate a large number of parameters (Araus et al., 2001), such as green biomass of the canopy, photosynthetic area, amount of PAR absorbed by the canopy, and photosynthetic potential (Reynolds et al., 2001). Other parameters related to the physiological status of the canopy at the time of measurement, such as relative water content, nutrient deficiencies, environmental stresses, pigment concentration, and photosynthetic radiation use efficiency, have been assessed by spectral reflectance

measurements (Araus et al., 2001; Reynolds et al., 2001). Several researchers have suggested that grain yield can be estimated using spectral reflectance during different crop growth stages (Araus et al., 2001; Aparicio et al., 2002; Osborne et al., 2002; Babar et al., 2006).

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

Experiments in multiple locations for predicting grain yield of wheat using SRI have shown that approximately 50 to 65% of yield variability can be explained by NDVI and SR (Tucker et al., 1980; Aparicio et al., 2000; Serrano et al., 2000; Raun et al., 2001). At LAI values above 3, the canopy absorbs all of the incident red light, leaving no more red light to be absorbed by higher vegetation fractions. Hence, the common SRI that utilize the red band are insensitive to variation in vegetation fraction or LAI values beyond 3 (Serrano et al., 2000). Therefore, these SRI cannot be used as a selection tool in wheat breeding programs where genotypes are selected at later reproductive growth stages.

This study explored the spectrum for wavelengths or combinations of wavelengths that could be used to adequately predict winter wheat genotypes for grain yield variation. Specific objectives were to test the correlation of widely used SRI with grain yield variability, to identify new spectral indices better correlated with grain yield variability among winter wheat genotypes across environments, and to determine the optimum growth stage(s) for measurement of SRI.

MATERIALS AND METHODS

Experimental Materials

Three experiments, each containing 25 winter wheat genotypes developed by different breeding programs in the southern and central Great Plains, were conducted during the 2003/2004 and 2004/2005 wheat growing seasons. The experiments were

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

Parameters		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Mean/sum [†]
Stillwater (2003/2004)										
Mean temp. (°C)	Max.	23.9	15.0	11.7	9.4	10.0	18.3	21.7	28.3	17.3
	Min.	8.9	3.9	-0.6	-2.8	-2.2	5.6	8.9	15.6	4.7
	Ave.	16.3	9.7	5.5	3.2	3.7	12.4	15.6	22.0	11.1
Total rainfall (mm)		74.9	54.4	42.7	56.9	42.4	101.1	71.4	5.8	449.6
Stillwater (2004/2005)										
Mean temp. (°C)	Max.	23.3	15.0	12.8	8.9	13.3	16.7	22.8	26.7	17.4
	Min.	11.1	5.0	-2.2	-2.2	1.1	3.3	8.9	14.4	4.9
	Ave.	17.1	9.8	4.9	2.6	6.9	10.3	15.8	20.4	11.0
Total rainfall (mm)		116.1	126.0	24.4	70.4	33.3	17.5	9.9	97.8	495.4
Lake Carl Blackwell (2003/2004)										
Mean temp. (°C)	Max.	23.9	15.0	11.7	9.4	10.0	18.3	21.7	27.8	17.2
	Min.	10.0	4.4	0.0	-2.8	-2.2	6.1	10.0	15.0	5.1
	Ave.	16.6	9.6	5.5	3.1	3.7	12.4	15.5	21.6	11.0
Total rainfall (mm)		84.3	60.7	33.0	62.2	39.6	110.2	74.9	3.6	468.5
Lake Carl Blackwell (2004/2005)										
Mean temp.(°C)	Max.	23.3	14.4	12.8	8.3	12.8	16.7	22.2	26.1	17.1
	Min.	11.1	5.0	-1.1	-2.8	1.7	3.9	9.4	14.4	5.2
	Ave.	17.1	9.7	5.2	2.6	7.2	10.2	15.7	19.9	11.0
Total rainfall (mm)		127.8	162.6	24.1	65.3	38.4	19.6	10.7	69.1	517.6
Long-term [‡] average temp. (°C)		16.0	8.2	2.5	1.2	3.6	5.9	14.3	19.7	8.9
Total rainfall (mm)		59.9	41.9	29.2	22.4	29.5	47.5	71.1	105.6	407.1

[†]Values represent mean temperature and total rainfall from Oct. to May.

[‡]Data represent both sites averaged over past 100 yr.

planted at the Oklahoma State University Agronomy Research Farm at Stillwater, Oklahoma (36°11' N, 97°05' W), and at Lake Carl Blackwell (36°16' N, 97°28' W), located 25 km west of Stillwater. The soil types at these sites are Kirkland silt loam (fine, mixed, thermic Udertic Paleustolls) with an average pH of 6.2 to 6.5, and Pulaski fine sandy loam (coarse-loamy, mixed, nonacid, thermic Typic Ustifluent) with an average pH of 6.7 to 6.9. The weather patterns of the two sites are presented in Table 1. All four site-years were above the long-term average temperature (8.9°C) and long-term total precipitation (407.1 mm), although March and April were drier in 2004/2005 at both sites compared to the long-term average.

The first experiment, designated as Exp-1, was composed of commercial winter wheat cultivars from the southern and central Great Plains and was planted at both sites in year 2003/2004. The second experiment, designated as Exp-2, was composed of 25 F_{4.6} and F_{4.7} recombinant inbred lines (RIL) from the cross TX 95V5905/Jagger and was planted at the Lake Carl Blackwell site in years 2003/2004 (F₆) and 2004/2005 (F₇). These lines were developed by the winter wheat breeding program at Kansas State University, Manhattan. The third experiment, designated as Exp-3, was composed of 25 F_{4.6} and F_{4.7} RIL from the cross TX93V4927/G1878 and was planted at the Stillwater site in years 2003/2004 (F₆) and 2004/2005 (F₇). This population was developed by AgriPro Coker (Vernon, TX).

All experiments were conducted under rain-fed conditions and planted at a seeding rate of 70 kg ha⁻¹. Individual plot size for each experiment was 3 m long × 1.2 m wide. Each individual experiment was a 5 × 5 alpha lattice design with two replica-

tions. At both experimental sites, 90 kg ha⁻¹ preplant nitrogen was applied. Folicur 3.6F (38.7% tebuconazole; Bayer Crop-Science, NC) was applied twice (late tillering, Zadoks stage 29 and booting, Zadoks stage 45 [Zadoks et al., 1974]) to control foliar diseases, and Cygon 2E (23% dimethoate; Southern Agricultural Insecticides, Inc., FL) was applied at booting to control aphids. Grain yield for each plot was determined by mechanically harvesting the whole plot and expressed as kg ha⁻¹.

Spectral Reflectance Measurements

Spectral reflectance measurements were accomplished using a portable field spectroradiometer (FieldSpec UV/VNIR, Analytical Spectral Devices, Boulder, CO). Reflectance measurements were taken with a 25° field of view optic at vertical position. The spectroradiometer was capable of measuring radiance from 350- to 1050-nm wavelengths with a sampling interval of 1.4 nm of the spectrum. Thus, 512 continuous data points were obtained with each reading. Measurements were taken during the middle of the day on cloudless days. The optical sensor was placed approximately 50 cm above the plant canopy in nadir position. The incident spectrum was taken from the light reflected from a white reference panel, and reflectance was calculated from the ratio of reflected light from the crop canopy against the total radiance reflectance from the white surface. The spectroradiometer was recalibrated every 10 plots. Four spectral measurements were taken randomly from four different places per plot, and the mean of the four readings was used to calculate the spectral reflectance indices. Reflectance data were taken at three growth stages, namely, booting, heading (Zadoks

stage 59), and early grain-filling (Zadoks stage 75) for all three experiments in both years and locations. There was a difference of 3 to 7 d among the genotypes in reaching booting, heading, and early grain-filling stages. The reflectance measurements were taken at the middle of this range to ensure minimal influence on the readings. We calculated about 200 new indices using different combinations of visible and NIR wavebands in ratio and normalized forms and chose two new indices based on the overall performance across experiments and compared with six previously reported indices. Descriptions of the indices are presented in Table 2.

Data Analysis

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

$$r_g = (\text{Cov}_{G1G2}) / \sqrt{(\text{Var}_{G1} \times \text{Var}_{G2})}$$

where Cov indicates components of covariance between grain yield (G1) and SRI (G2), and Var indicates components of variance of grain yield and SRI.

Relationship between SRI and grain yield was established by regression analysis and was accomplished by the SAS software (SAS Institute, 2001). Data were fitted into different models based on the least-square method of parameter estimation. Residuals were checked for model fitting. Coefficients of

determination were compared for different models. Root mean square error was calculated based on work by Kobayashi and Salam (2000).

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

RESULTS

Genotypic Variation and Growth Stage

Significant genotypic differences ($p < 0.05$) for grain yield were found in all three experiments (Table 3). Differences ($p < 0.05$) were observed for spectral behavior at different crop growth stages, namely booting, heading, and early grain-filling (Table 4). The values for RNDVI, GNDVI, and SR decreased from the booting to the grain-filling stage (Table 4, Fig. 1). On the other hand WI, NWI-1, NWI-2, NWI-3, and NWI-4 provided two different types of patterns (Fig. 1). In year 2003/2004, these indices

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

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

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

Table 3. Statistical parameters for grain yield in three experiments presented as individual year/location.

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

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

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

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

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

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

[†]GNDVI, green normalized difference vegetation index; NWI-1, normalized water index 1; NWI-2, normalized water index 2; NWI-3, normalized water index 3; NWI-4, normalized water index 4; RNDVI, red normalized difference vegetation index; SR, simple ratio; WI, water index.[‡]Estimates based on two locations in year 2003/2004.[§]Estimates based on 2 yr (2003/2004 and 2004/2005).

decreased from booting to heading, and then increased at the grain-filling stage (Fig. 1a, b), but in 2004/2005, they showed a continuous increase from the booting to the grain-filling stage (Fig. 1c, d). In all instances, the values of the water-based indices were higher in the grain-filling stage compared to booting stage (Table 4, Fig. 1).

Interaction between Genotypes, Growth Stages, and Years/Locations

The ANOVA for Exp-3 (Table 5) revealed that genotypes and growth stage main effects were significant, as well as the growth stage by genotype interaction. The three-way interactions were significant for all the water-based NIR

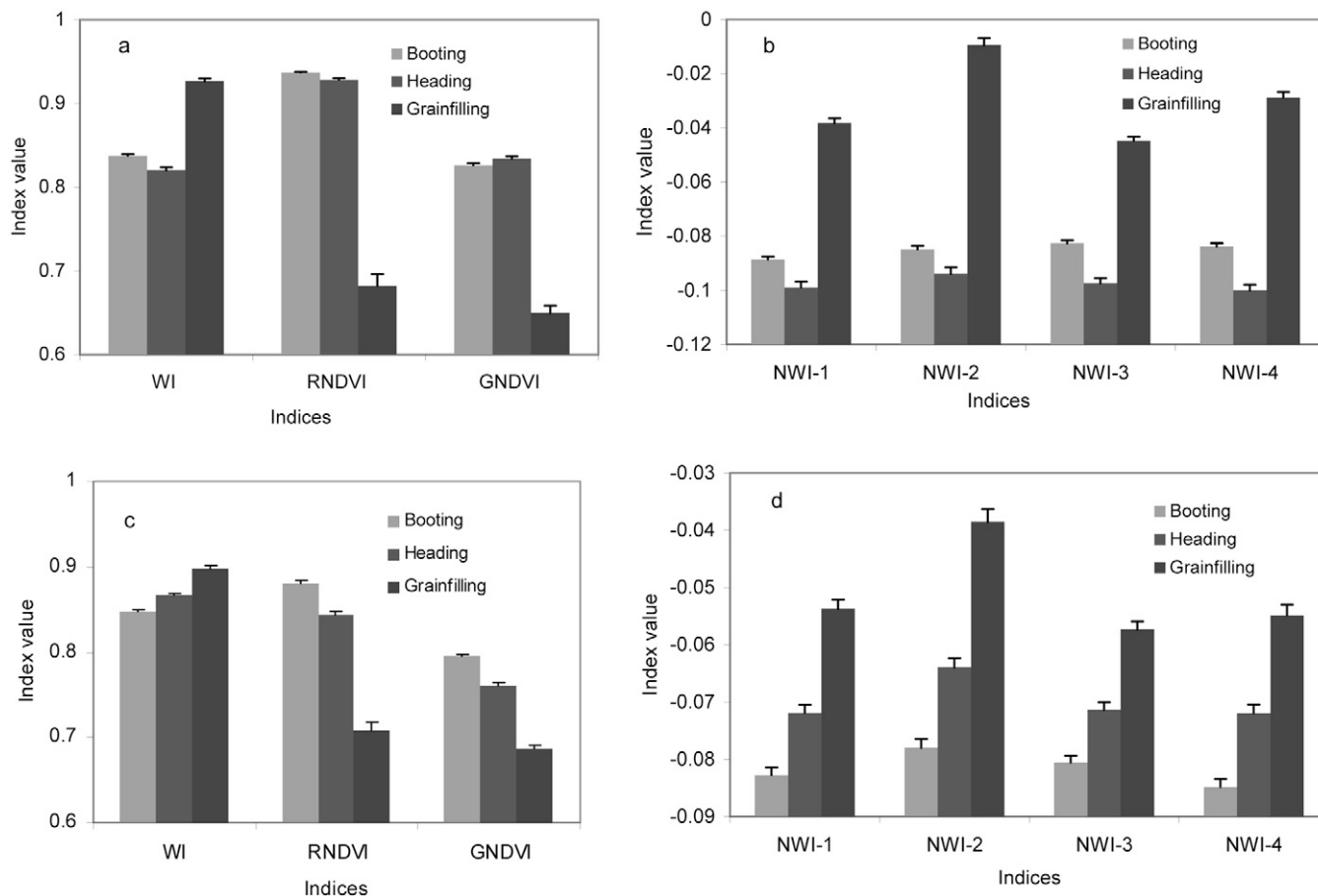


Figure 1. Changes in the pattern of different spectral reflectance indices with the advancement of growth stages: (a) and (b) represent Exp-1 in year 2003/2004 at Stillwater, and (c) and (d) represent Exp-2 in 2004/2005 at Lake Carl Blackwell. GNDVI, green normalized difference vegetation index; NWI-1, normalized water index 1; NWI-2, normalized water index 2; NWI-3, normalized water index 3; NWI-4, normalized water index 4; RNDVI, red normalized difference vegetation index; WI, water index. The bars indicate standard errors.

indices. The analyses of variance for the other two experiments were similar (data not presented). The correlation coefficients between the heading and grain-filling stages for each individual index value were significant for most experiments across locations and years (Table 6). On the other hand, the correlation coefficients of the index value between booting and heading stages, as well as between the booting and grain-filling stages, were nonsignificant in most cases (Table 6).

Correlation between Spectral Reflectance Indices and Grain Yield

Among the tested indices, six have been previously studied (WI, RNDVI, GNDVI, SR, NWI-1, and NWI-2), and two new indices (NWI-3 and NWI-4) have been calculated. The reported indices have been previously used to estimate different physiological parameters and yield, mostly with durum wheat (*Triticum turgidum* L. subsp. *durum*) under Mediterranean rain-fed conditions or spring wheat (*Triticum aestivum* L.) under irrigation. Three indices (RNDVI, GNDVI, and SR) provided positive associations with grain yield, whereas, the other five showed negative associations (Table 7). Almost all the indices showed low correlation

with grain yield at the booting stage and high correlations at heading and grain-filling stages with grain yield. The indices can be classified into two groups. One group includes the visible and NIR wavebands, and another group includes only the NIR wavebands, especially the minor water absorption band of 970 nm (Table 2). The indices based on the minor water absorption band tended to provide higher correlations with grain yield compared to the widely used RNDVI, GNDVI, and SR. In each individual experiment, the NIR-based indices exhibited a consistent negative association with grain yield across years and locations. The vegetation-based indices (RNDVI, GNDVI, and SR) gave correlations that were inconsistent in different experiments across years and locations.

Mean indices over the three growth stages always provided higher correlations with grain yield compared to any individual growth stage (Table 7), except for the RNDVI, GNDVI, and SR indices in certain cases. The indices based on the minor water absorption band always provided a higher association with grain yield compared to the RNDVI, GNDVI, and SR when the mean of the three growth stages was used. These patterns were also evident when indices were combined for the heading and grain-filling stages, but

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

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

[†]Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

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

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

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

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

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

[‡]Correlation between booting and heading stages.

[§]Correlation between booting and grain-filling stages.

[¶]Correlation between heading and grain-filling stages.

the correlation coefficients were lower than the coefficients using three growth stage combinations (data not shown).

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

Functional Relationship between Spectral Reflectance Indices and Grain Yield

The functional relationship between grain yield and the different SRI for the three growth stages individually and in combination were established through regression analysis. Figure 3 shows the functional relationship of NWI-3 with yield in three growth stages for two experiments. The NWI-3 explained more of the grain yield variability at heading and grain-filling stages compared to the booting stage (Fig. 3). The relationship of three different indices (NWI-2, NWI-3, and NWI-4) with grain yield are presented in Fig. 4 for three different experiments, based on the mean of the three different growth stages in an

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

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

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

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

[‡]Correlation between yield and mean spectral reflectance indices across three growth stages.

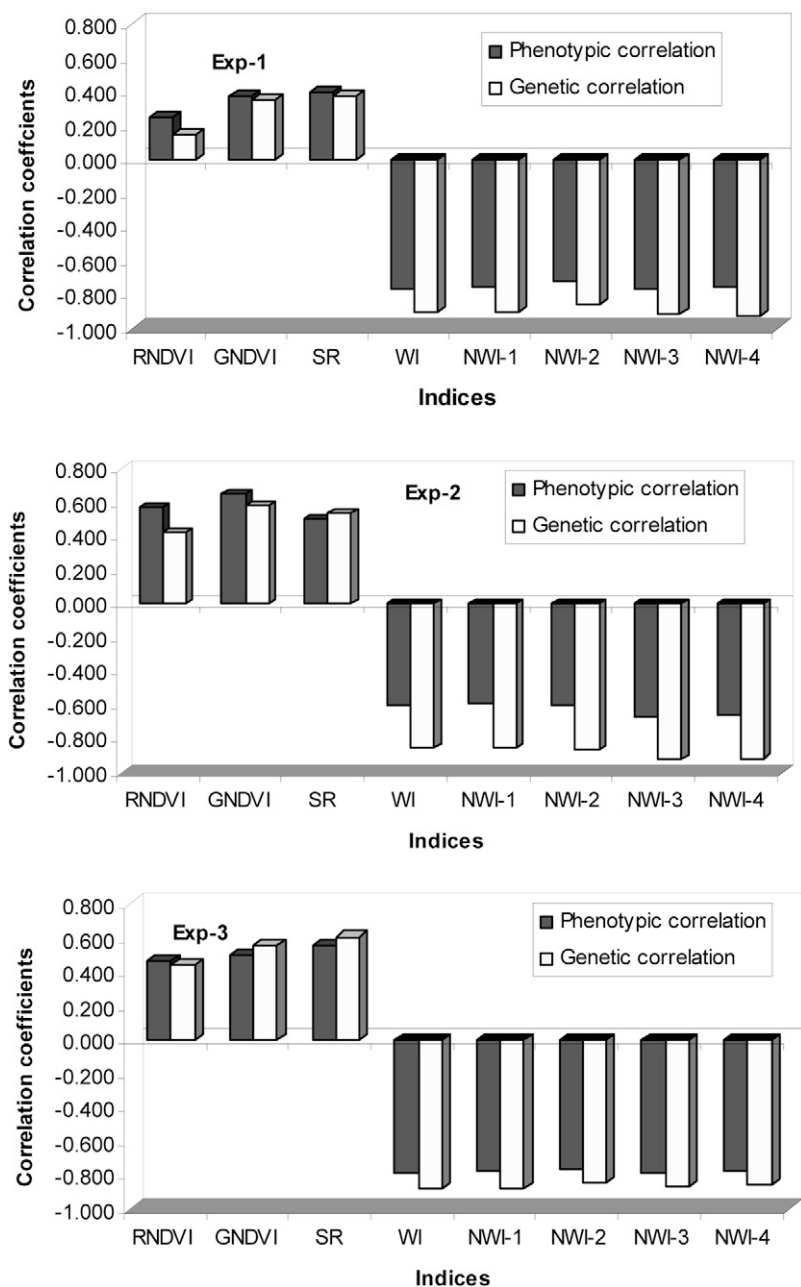


Figure 2. Phenotypic and genetic correlation coefficients between different spectral reflectance indices (mean of different growth stages across year/location) and grain yield (mean of two locations in Exp-1 and 2 yr in Exp-2 and Exp-3). GNDVI, green normalized difference vegetation index; NWI-1, normalized water index 1; NWI-2, normalized water index 2; NWI-3, normalized water index 3; NWI-4, normalized water index 4; RNDVI, red normalized difference vegetation index; SR, simple ratio; WI, water index.

individual year or location. The relationships of the other indices with grain yield were also tested (data not shown), and in all instances the model fit better with a linear relationship having the higher coefficient of determination compared to other models. Indices tended to explain more of the grain yield variability when mean data were used, for example, combining growth stages, combining years/locations, or combining both. Our analysis based on combining growth stages revealed the superiority of NWI-3

over the other indices in predicting yield, explaining 74% of the grain yield variation in one location and 63% of the variation in the other location for Exp-1 (Fig. 4). The relationship of measured grain yield and predicted grain yield based on NWI-3 is presented in Fig. 5. This relationship was established on the basis of all three experiments using the mean values of the three growth stages across locations and years. Although some differences were observed between the different experiments for grain yield and spectral behavior, the relationships revealed the significant linear predictability of grain yield based on NWI-3.

Selection of Genotypes

Significant difference between genotypic grain yields of the two selection groups based on the 25% highest and the 25% lowest SRI values was observed for all the water-based SRI (data not shown). The water index and the NWI showed greater accuracy in identifying both the highest-yielding and the lowest-yielding genotypes compared to the RNDVI, GNDVI, and SR (Table 8). Similar trends were also observed when individual growth stages were considered (data not shown), but the combination of growth stages always worked better. The indices that included the minor water absorption band identified 50 to 83% of both the 25% highest-yielding genotypes and the 25% lowest-yielding genotypes in all three experiments. Among the NIR-based indices, NWI-3 and NWI-4 consistently performed better. This was also evident when the mean of the two locations or 2-yr data were combined for individual experiments, and also when the three experiments were considered together (Table 8).

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

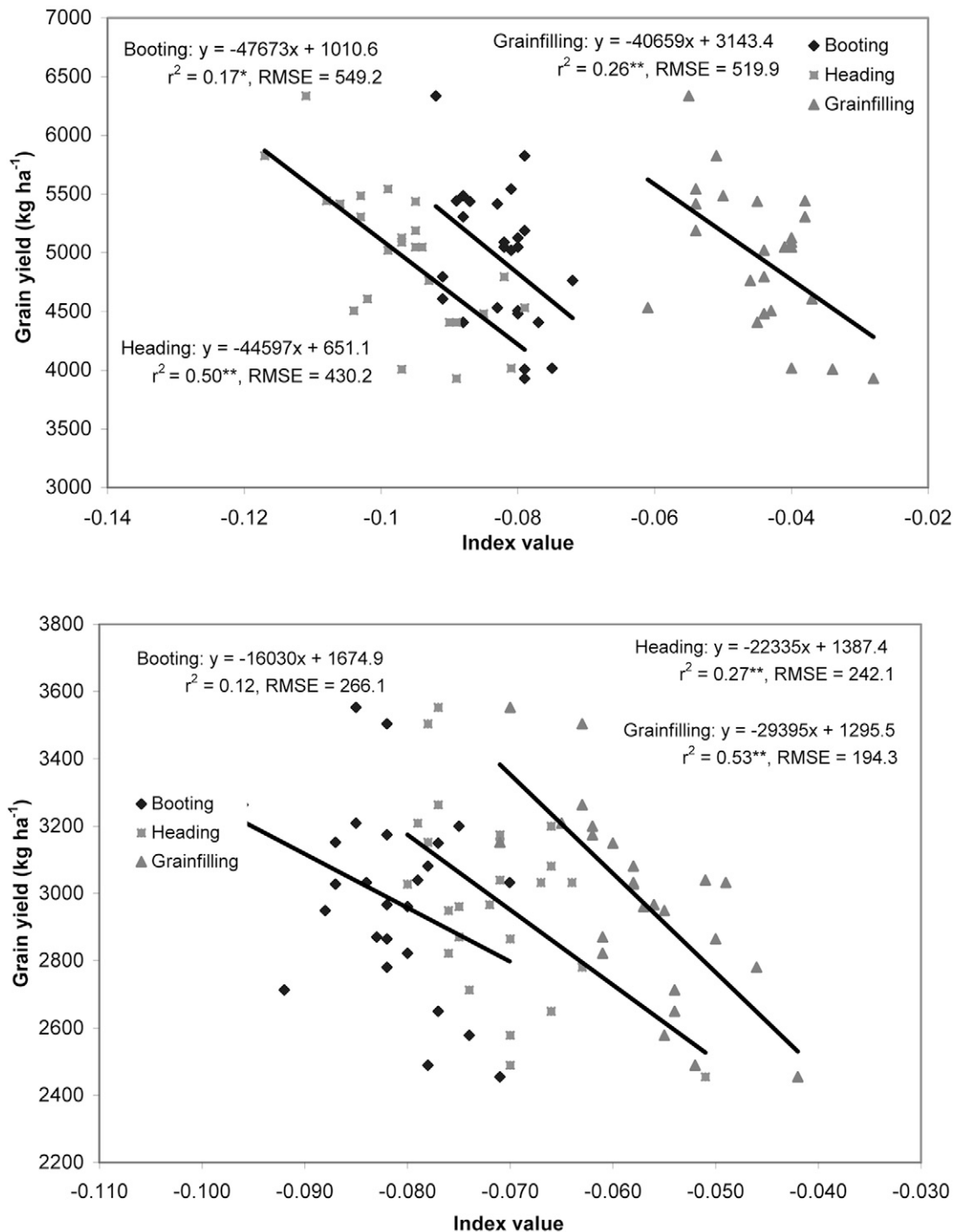


Figure 3. Functional relationship between grain yield and normalized water index 3 (NWI-3) at three growth stages for two experiments. Top: Exp-1; bottom: Exp-2. *Significant at the 0.05 probability level; **significant at the 0.01 probability level. RMSE, root mean square error.

DISCUSSION

Genotypic Variation and Growth Stage

Significant genotypic variation for grain yield and spectral behavior at different growth stages, namely booting, heading, and grain-filling, confirms the existence of sufficient variation among the genotypes. The first experiment (Exp-1) includes widely used winter wheat cultivars available throughout the Great Plains of the USA. The other two experiments contain sufficient variation in the respective RIL populations. Similar variations were

also observed by other researchers working with spectral reflectance in wheat, including Babar et al. (2006) working with irrigated spring wheat, with irrigated and nonirrigated spring wheat (Gutiérrez-Rodríguez et al., 2004), with durum wheat in the Mediterranean region (Aparicio et al., 2000, 2002), and with durum wheat under rain-fed conditions (Royo et al., 2003). The primary reason for the decreasing trend of RNDVI, GNDVI, and SR from booting to grain-filling is the reduced reflectance in the NIR region due to loss of green tissue as growth

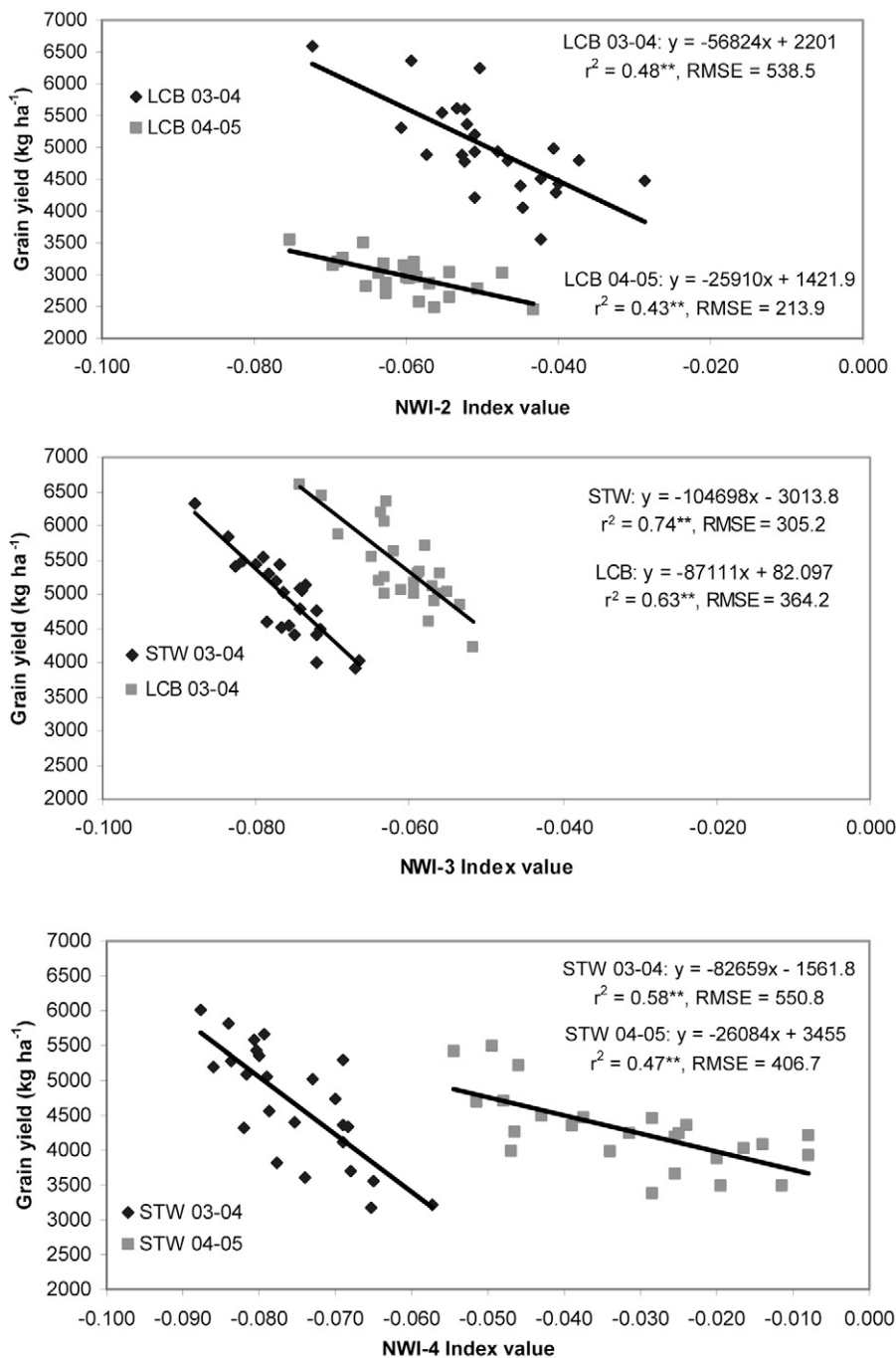


Figure 4. Functional relationship between grain yield and normalized water index 2 (NWI-2), normalized water index 3 (NWI-3), and normalized water index 4 (NWI-4) (estimated as the mean of the three growth stages). Top: Exp-2; middle: Exp-1; bottom: Exp-3. STW and LCB denote Stillwater and Lake Carl Blackwell sites, respectively. **Significant at the 0.01 probability level. RMSE, root mean square error.

stage progressed from booting to grain-filling, as well as increased reflectance in the visible region (Aparicio et al., 2000). The NIR indices (WI and NWI) are based on the minor water absorption band (970 nm), and they measure the water status of the canopy (Peñuelas et al., 1997; Babar et al., 2006). In 2003/2004, the experiments had good water status during heading (71.4–74.9 mm rainfall) and the indices were lower than at booting stage, whereas, in 2004/2005, heading occurred under water deficit stress

conditions (9.9–10.7 mm rainfall) and the indices were higher at heading compared to booting stage (Fig. 1), since there is a negative relationship between water status and indices values. The nonsignificant difference among the genotypes for the SRI in some instances at the booting stage was due to the homogeneity of the LAI among the genotypes, which normally reach maximum at the booting stage (Aparicio et al., 2000). But, at the later growth stages, these indices are highly influenced by the reproductive structures such as spike morphology, as well as the decrease in the LAI, and indices detected the significant variation among the genotypes (Hatfield, 1981; Aparicio et al., 2000). The commonly used indices (NDVI and SR) usually saturate at LAI > 3, which is due to the lack of sensitivity of red light at the higher vegetation fractions. Whereas, the NIR bands of the water-based indices (WI and NWI) can penetrate into the higher vegetation fraction of the canopy and assess the water status of the canopy as well as the amount of green biomass (Babar et al., 2006).

Interaction Effect of Genotypes, Growth Stages, and Years/Locations

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

Correlation between Spectral Reflectance Indices and Grain Yield

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

We used six published SRI (RNDVI, GNDVI, SR, WI, NWI-1, and NWI-2), and two new indices (NWI-3 and NWI-4) were calculated (Table 2). Most indices taken at the booting stage showed nonsignificant correlations with grain yield in the different experiments, with a few exceptions. The relationship between SRI and yield became stronger as the growth stage progressed toward the reproductive phase. Babar et al. (2006) also reported lower correlations between the different spectral reflectance indices and grain yield at the booting stage. Ma et al. (2001) with soybean and Royo et al. (2003) with durum wheat also reported that the SRI correlated better with yield at the reproductive growth stages rather than at early vegetative growth stages. Aparicio et al. (2000) found significant correlations between grain yield and SRI at hard dough stage (Zadoks stage 87) with durum wheat under rain-fed conditions.

Water index and the NWI gave higher correlations with grain yield compared with the RNDVI, GNDVI, and SR at heading and grain-filling (Table 7). Peñuelas et al. (1993) reported significant

relationships between WI and relative water content, leaf water potential, stomatal conductance, and canopy temperature. The minor water absorption band (970 nm) and other NIR bands used in the calculation of WI and NWI make these indices more sensitive to higher fractions of green vegetation at the heading and grain-filling growth stages compared to NDVI and SR (Babar et al., 2006). The higher correlation coefficients of the NIR-based indices with genotypic grain yield compared to NDVI and SR are

Table 8. The percentage of 25% highest-yielding and 25% lowest-yielding (values in parentheses) genotypes selected by different spectral reflectance indices.

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

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

[‡]Selection based on mean spectral reflectance indices across three growth stages and mean grain yield across locations/years for each individual experiment.

[§]Selection based on mean spectral reflectance indices across three growth stages and mean grain yield across locations/years for all three experiments.

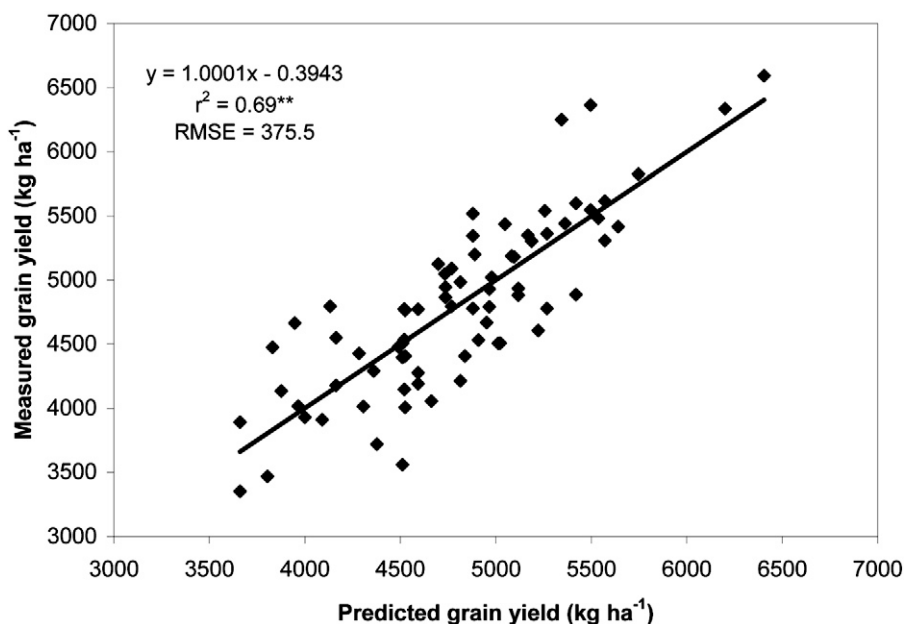


Figure 5. Relationship between measured grain yield and predicted grain yield based on the linear equation using normalized water index 3 (NWI-3) as the predictor, estimated using the mean values of three growth stages for all three experiments across two locations/years. **Significant at the 0.01 probability level. RMSE, root mean square error.

Table 9. Comparison between measured grain yield (kg ha⁻¹) and predicted grain yield (kg ha⁻¹) of the 25% highest-yielding and 25% lowest-yielding (values in parentheses) genotypes in each experiment using spectral reflectance indices.

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

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

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

[§]Difference between measured grain yield and predicted grain yield, expressed as percentage.

due to better differentiation of the genotypes at heading and grain-filling. It has been also reported that WI can predict relative water content, stomatal conductance, and leaf water potential (Peñuelas et al., 1993). The correlation coefficients of different water-based indices with grain yield were different but negligible, which was due to the use of different NIR wavebands to normalize the 970-nm water band in different NIR indices. Increased water content in the plant canopy decreased the reflectance of the water band, and consequently, we obtained negative correlations of these indices with grain yield. Babar et al. (2006) reported similar observations.

Correlations of the mean estimates of the SRI across three growth stages with yield were higher compared to any individual growth stage (Table 7). This was also reported by Babar et al. (2006) and Aparicio et al. (2000). The NIR-based indices always gave higher correlations than the RNDVI, GNDVI, and SR when reflectance measurements were combined across different growth stages. In most cases, the mean of the indices at the heading and the grain-filling stages also gave higher correlations with grain yield than any individual growth stage, but lower than the correlation coefficients obtained through combining three growth stages (data not shown). We believe the mean indices across growth stages are a measurement of overall plant health during a critical time in

plant development. Repeated reflectance measurements of a genotype from different growth stages assess the overall fitness of the genotype with time. Therefore, the indices identify the genotypes that maintain a healthier condition throughout the growing season and, as a result, give higher correlations with final grain yield. We observed higher genetic correlations between the water-based NIR indices and grain yield compared to the widely used vegetation based indices (Fig. 2). This observation indicated that these water-based indices have higher predictability at the genotypic level for grain yield variation compared to the vegetation based indices under rain-fed conditions.

Functional Relationship between the SRI and Grain Yield

The functional relationship between the SRI and grain yield was linear (Figs. 3 and 4). Babar et al. (2006) and Gutiérrez-Rodríguez et al. (2004) also reported linear relationships between the different SRI and grain yield. Our results indicate that these SRI can be used for predicting grain yield irrespective of the yield potential of the genotypes. The NIR-based indices always showed better predictability when more data were included in the model (different growth stages, different years/locations). These indices also predicted grain yield well when an individual growth stage such as heading or grain-filling stages was

considered, thus making them suitable as a selection tool in a breeding program for yield improvement. Considering the overall performance, NWI-3 and NWI-4 showed better performance over the other NIR-based indices.

Selection of Genotypes

The efficiency of selecting superior genotypes is a primary concern when using an indirect selection tool for a specific trait. Significant yield differences between the two groups of genotypes based on the highest and lowest index values for the water-based indices indicate that these indices can properly discriminate the genotypes for grain yield. Our results showed that the water-based NIR indices identified a major proportion of the higher-yielding as well as the lower-yielding genotypes in the different experiments. In addition, the close approximation of actual grain yield reveals the power of the indices for prediction of the genotypes grain yield. Use of the NIR-based indices, especially NWI-3 and NWI-4, shows promise in selecting desired genotypes for higher grain yield.

CONCLUSIONS

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

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