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remote sensing

Article

Using NDVI to Differentiate Wheat Genotypes Productivity Under Dryland and Irrigated Conditions

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Abstract: Crop breeders are looking for tools to facilitate the screening of genotypes in field trials. Remote sensing-based indices such as normalized difference vegetative index (NDVI) are sensitive to biomass and nitrogen (N) variability in crop canopies. The objectives of this study were (i) to determine if proximal sensor-based NDVI readings can differentiate the yield of winter wheat (*Triticum aestivum* L.) genotypes and (ii) to determine if NDVI readings can be used to classify wheat genotypes into grain yield productivity classes. This study was conducted in northeastern Colorado in 2010 and 2011. The NDVI readings were acquired weekly from March to June, during 2010 and 2011. The correlation between NDVI and grain yield was determined using Pearson's product-moment correlation coefficient (r). The k-means clustering method was used to classify mean NDVI and mean grain yield into three classes. The overall accuracy between NDVI and yield classes was reported. The findings of this study show that, under dryland conditions, there is a reliable correlation between grain yield and NDVI at the early growing season, at the anthesis growth stage, and the mid-grain filling growth stage, as well as a poor association under irrigated conditions. Our results suggest that when the sensor is not saturated, i.e., NDVI < 0.9, NDVI could assess grain yield with fair accuracy. This study demonstrated the potential of using NDVI readings as a tool to differentiate and identify superior wheat genotypes.

Keywords: NDVI; proximal sensor; genotypes; k-means clustering; dryland; irrigated

1. Introduction

Conventional breeding methods rely heavily on grain yield as a trait to identify, select, and breed new crop varieties. Likewise, crop biomass, as measured by destructive sampling, is another trait often used in conventional breeding to identify total aboveground biomass and total N in crop tissue. Regan et al. [1] reported that destructive sampling is an efficient method to select superior genotypes of spring wheat (*Triticum aestivum* L.) for early vigor under dryland conditions. However, the destructive sampling method is practical only when small numbers of samples are involved [2]. The direct estimation of grain yield and biomass through destructive sampling is tedious, expensive, labor-intensive, and time-consuming [3,4]. Experience has demonstrated that sampling errors cause difficulty in detecting prominent differences among samples and destructive sampling reduces the plot area for estimating final biomass and grain yield. Proximal canopy sensing can be used to estimate crop N status and crop biomass without destructive sampling and thus has the potential to provide a fast, inexpensive, and accurate technique to estimate plant biomass production [5,6] and grain yield [7] of genotypes. The indestructive assessment of grain yield prior to harvest would be beneficial for crop breeders in a superior cultivar selection process [2,4,8,9]. Also, proximal canopy sensors can be used to detect subtle differences in sparse canopies which makes them suitable for estimating crop growth at early stages [10,11]. Reynolds et al. [12] proposed that a proximal canopy sensor can be used as a promising tool to differentiate wheat genotypes based on productivity. Similarly, Araus et al. [13] showed that a spectral vegetation index such as the normalized difference vegetation index (NDVI) is a promising tool for screening wheat genotypes at Mediterranean climatic conditions. Remote sensing has been used to monitor N status in crop canopies and offer rapid as well as nondestructive ways of assessing leaf area index (LAI), crop canopy biomass in rice in subtropical climate [14], grain yield, and wheat nitrogen (N) stress [15–17]. Proximal canopy sensors with their own source of energy (i.e., active sensors) were developed to overcome the limitations of passive sensing devices and to minimize the effects of ambient light conditions on reflectance readings [18]. These sensors are small enough to be handheld or mounted on a tractor [4,16,19]. Proximal canopy sensing tools such as GreenSeeker (Trimble Navigation Limited, Sunnyvale, California, USA) can measure reflected light from the crop canopy to record vegetation indices such as the simple ratio or the NDVI. The NDVI is calculated by normalizing the ratio of the difference between near-infrared (NIR: correlated to leaf structure) and red (correlated to chlorophyll content) wavelength bands to the sum between NIR and red wavelength as presented in Equation (1):

$$\mathrm{NDVI} = \frac{\mathrm{NIR} - \mathrm{Red}}{\mathrm{NIR} + \mathrm{Red}} \quad (1)$$

where NIR is light reflectance in the near-infrared wavelength ranging from 720 to 1300 nm and Red is the light reflectance in the red wavelength ranging from 600 to 720nm

The vegetation reflectance intensity in the visible light (VIS waveband; 400–720 nm) is negatively correlated to leaf N content while NIR reflectance is positively correlated to leaf N content and biomass [20]. Plant anatomical characteristics are influenced by many environmental factors such as soil moisture, soil salinity, nutrient status or leaf age, and changes in anatomical characteristics that can affect reflectance measurements [21]. A strong linear relationship exists between leaf N concentration and leaf chlorophyll concentration [22,23]. Greater leaf area and green plant biomass translate into higher NDVI values [19,20]. The N content of the plant is directly related to leaf area and green plant biomass, and a higher N content in plants translates into higher NDVI values [19]. Raun et al. [24] demonstrated that a significant relationship (R^2 = 0.5, $p < 0.0001$) exists between NDVI and estimated grain yield in winter wheat. Likewise, Inman et al. [25] found a linear relationship (R^2 = 0.65) between NDVI and grain yield in corn. Several other studies revealed a significant correlation between NDVI and grain yield from heading to grain filling in wheat [9,12,17,26,27]. The most appropriate stage for estimating yield was reported to be at the mid-grain filling stage [9,27]. Also, Aparicio et al. [15] observed a significant correlation between wheat durum grain yield and NDVI at the maturity growth stage and non-significant correlation at the booting and mid-grain filling growth stages under the irrigated condition. While under dryland conditions, a strong correlation between grain yield and NDVI was observed throughout the growth stages of wheat.

It was demonstrated in the early 1990s that NDVI and other spectral reflectance indices [28] have the potential to differentiate spring wheat genotypes from heading to grain filling stages for crop biomass and grain yield under irrigated conditions [3,15,29]. Ma et al. [21] reported that NDVI could be used to differentiate between high and low grain yield among soybean genotypes. They concluded that NDVI could be a reliable and fast index for screening soybean genotypes and estimating grain yield under irrigated conditions. However, a review of the literature indicates that very few studies have been conducted to identify and differentiate winter wheat genotypes for grain yield using a proximal canopy sensor based on NDVI. The primary reason for selecting a common commercial sensor and a common vegetative index (NDVI) was to make the results suitable for broader audience.

Plant genotypes respond differently to climate, soil type, irrigation, and other management practices, thus we need as many studies as possible to complement the existing literature. As the field of high throughput plant phenotyping (HTPP) for plant breeding and selection, is still in its infancy [30–32], this study is a valuable addition to this rapidly growing field [16,33–36]. Use of several genotypes in this study adds breadth to the relationship between spectral measurements and yield in winter wheat. Moreover, relatively few studies [36,37] have been conducted in the semi-arid climate to assess the use of spectral measurements for wheat genotype productivity estimation and classification. This study also includes two irrigation conditions, which provides more useful information for a diverse group of scientists and producers. Additionally, this project used clustering algorithms to classify grain yields regardless of genotype and compared it with clustering of NDVI data, which is a fairly new practice [38]. The results from clustering analysis of NDVI and yield could be utilized for management and productivity zone-based precision agriculture.

The overall goal of this study was to evaluate the possibility of using NDVI measured by a proximal canopy sensor as a tool to differentiate and classify wheat genotypes productivity in terms of yield in a semi-arid climate under two irrigation conditions. The specific objectives were (i) to determine if winter wheat genotypes can be identified and differentiated on the basis of their productivity via NDVI measurements and (ii) to determine if multiple winter wheat genotypes can be classified into grain yield productivity classes on the basis of NDVI measurements.

2. Materials and Methods

2.1. Study Site

This research was conducted on two fields, over two winter wheat growing seasons (2009–2010 and 2010–2011) at the USDA-ARS Limited Irrigation Research Farm, near the town of Greeley, Colorado, USA, and are referred to as site years I and II respectively. The geo-coordinates of the wheat fields were latitude 40° 26′ 58.87′′ N, and longitude −104° 38′22.56′′ W. Soils for both the fields were classified as Otera sandy loam soil series (coarse–loamy, mixed superactive, calcareous, mesic Aridic Ustorthents) with 0–3% slope and the fields were equipped with drip irrigation system [39]. The soil is deep, well-drained, and was formed by eolian deposits and mixed outwash parent material; it includes loam and clay loam underlying material. In site year I (2009–2010), the total precipitation received during the crop growing season from October 1st, 2009 to July 31st, 2010 was 292.9 mm. Moreover, the average daily temperature was 5.97 °C and average relative humidity (fraction) was 0.62. For site year II (2010-2011), the total precipitation received from October 1st, 2010 to July 31st, 2011 was 209.3 mm [40]. In addition, the average daily temperature was 7.23 °C and average relative humidity (fraction) was 0.59. The total precipitation received during the two site years was higher than the ten years average precipitation of 170.6 mm for the same time periods.

2.2. Experimental Procedure

A global positioning system unit was used to map the field boundaries and to geo-reference soil samples (Trimble Ag 114 GPS, CA, USA). The layout of the experiment is shown in the Supplementary Materials section. Soil samples were collected using a systematic unaligned grid sampling design for the entire study area on both site-years. Soil samples were collected at two depths, 0–20 cm, and 20–61 cm, at 15 locations within the 0.2-hectare study area (i.e., a sampling density of 72 samples per ha). Several soil cores were composited at each location to obtain each of the 30 composite soil samples (15 locations and two depths). Soil samples were then dried and sent to a commercial laboratory (Ag Source Harris Lab., Lincoln, NE) for chemical and physical soil property analysis. Particle size analysis was determined by using the hydrometer method [41]. Soil pH was measured using a 1:1 water to soil slurry [42]. Organic matter (OM) was determined using the loss on ignition method. Soil NO_3-N was measured using the cadmium reduction method [43]. A summary of soil properties for both sampling depths across the two site years is presented in Table 1.

Table 1. Summary of soil properties for soil samples acquired at depths of 0–20 cm and 20–61 cm.

Site year	Sampling depths (cm)		pH	O.M %	N at early spring Mg g^{-1}	N after harvest Mg g^{-1}	Sand	Silt %	Clay	Soil texture
I	0-20	Min	7.9	1	22	5	64.8	13.6	9.6	Sandy Loam
		Mean	8	1.1	31	7.9	68.4	16.5	15.1	
		Max	8.1	1.3	47	14	72.8	21.6	17.6	
	20-61	Min	7.9	0.9	11	5	60.8	13.6	11.6	Sandy Loam
		Mean	8	1.1	22.3	12.5	67.7	16.9	15.3	
		Max	8.2	1.3	40	37	72.8	21.6	17.6	
Site year	**Sampling depths (cm)**		**pH**	**O.M %**	**N at early fall Mg g^{-1}**	**N after harvest Mg g^{-1}**	**Sand**	**Silt %**	**Clay**	**Soil texture**
II	0-20	Min	7.8	1	30	8	58.8	4.4	12.8	Sandy Loam
		Mean	8	1.2	38	15.4	64.9	16.7	18.4	
		Max	8.1	1.5	54	22	70.8	24.4	30.8	
	20-61	Min	8	0.8	16	4	53.2	3.6	15.2	Sandy Loam
		Mean	8.2	1	22.4	9.8	61.7	17.7	20.5	
		Max	8.4	1.3	44	22.0	67.2	27.6	29.2	

OM = Organic Matter, Soil NO_3-N contents were determined on samples collected on March 22nd (at early spring) and August 19th of 2010 (after harvest) for site year I and November 8th, 2010 (early fall) and August 22nd, 2011 (after harvest) for site year II.

This study was a part of a large ongoing multi-disciplinary project. The experimental design for this study was a split-plot with three replications. Twenty-four winter wheat genotypes (sub-plots) were planted under both irrigated and dryland conditions (main plots). The genotypes were: Above, Ankor, Arlin, Avalanche, Baca, BillBrown, BondCL, CO940610, Danby, Good streak, Hatcher, Jagalene, Jagger, Keota, NuDakota, Platte, PrairieRed, Prowers99, Ripper, RonL, Sandy, Snowmass, TAM112, and Yuma. Further information on characteristics of winter weat genotypes can be found at [44] Dimensions of individual experimental plots were 1.4 m in width and 3.7 m in length with crop row width of 0.23 m between each row with a total 6 rows in each plot. Site years I and II were planted on October 11th, 2009 and October 8th, 2010, respectively, at a rate of 19.76 seeds m^{-2}. Nitrogen and phosphorus fertilizers were applied prior to planting on September 29th, 2009 and October 7th, 2010 for site year I and II, respectively. Nitrogen and phosphorous rates were determined based on soil samples analysis for site year I and II according to Colorado State University (CSU) Guidelines [45] as follows.

$$\text{N rate (kg ha}^{-1}) = 1.12 \times \{35 + [1.2 \times \text{EY (bu/A)}] - [8 \times \text{average ppm NO}_3{}^{-}\text{N in the soil}] - [0.14 \times \text{EY (bu/A)} \times \%\ \text{OM}] - \text{other N credits (lb/A)}\}.$$

where 1.12 is used to convert N recommendations from lbs (pounds) N $acre^{-1}$ to kg N ha^{-1}, EY = expected yield, A = acre, % OM = percent organic matter, $NO_3{}^{-}N$ = ppm available of Nitrate-N in the soil, other N credits = the N from legumes, manure, other organic materials, and N from irrigation water.

Similarly, Phosphorus rate was calculated as follows.

P rate (kg P_2O_5 ha^{-1}) = 1.12 × [48 − 5 × (Extractable-P)], where Extractable P = DB-DTPA extracted P in ppm.

Nitrogen dry fertilizer was applied at a rate of 84 and 112 kg N ha^{-1} as Urea (46-0-0) and phosphorous dry fertilizer was applied at a rate of 56 and 44.8 kg P_2O_5 ha^{-1} as Mono-Ammonium Phosphate (11-52-0) for site year I and II, respectively. Plots were irrigated as prescribed by Colorado State University Extension [46] to match the total daily evapotranspiration. The crop was harvested by hand on July 15th, 2010 for site year I and on July 25th, 2011 for site year II.

2.3. Proximal Canopy Sensing

A GreenSeeker Model 505 (Trimble Navigation Limited, Sunnyvale, California, USA) handheld active optical sensor (with its own source of light and not affected by ambient radiation) unit was used to obtain NDVI values from crop canopies [47]. Because active sensors such as GreenSeeker have their own source of light and are not affected by ambient radiation, thus they can be used at any time of day or night and in different areas and different ambient radiation conditions. Having its own source of light and a small distance between the sensor and canopy makes active canopy sensors suitable for various ambient light conditions. In-field reflectance from the wheat crop canopies were obtained by holding the GreenSeeker unit at the recommended height of 0.9m above the crop canopy and walking through the middle of each experimental plot. The measurements of 20 to 50 NDVI readings were collected in approximately two to five seconds duration of sensing in each experimental plot. The NDVI readings were obtained weekly on cloud-free days between 10:00 am and 2:00 pm local time. Readings were collected starting at the early spring (March 29th) growth stage to after the mid-grain filling growth stage (June 21st) for site year I and from March 22nd to June 17th for site year II (Figure 1).

Figure 1. A GreenSeeker handheld optical sensor unit was used to collect NDVI measurements from crop canopy. Highlighted white dashed lines are delineated to differentiate the boundaries of each plot.

2.4. Statistical Analysis

All statistical analysis was performed in statistical software R [48]. The linear relationship between NDVI and grain yield was determined by using Pearson's product-moment correlation coefficient (r) measuring the strength of the relationship between NDVI and grain yield. To determine if NDVI readings could classify multiple wheat genotypes into grain yield productivity classes, both yield and NDVI data were clustered. Two clustering methods were used to classify grain yield into productivity classes: (i) k-means clustering algorithm and (ii) stratification clustering. The mean grain yield was classified into three classes (e.g., low, medium, and high) for each clustering method. The NDVI data were also classified to create three classes (e.g., low, medium, and high) using the k-means clustering algorithm and stratification clustering. The NDVI classes were generated independently for each of the 11 or 14 dates for site year I or site year II respectively and likewise independently for both irrigation conditions. The k-means clustering method aims at dividing data into classes or clusters that minimize the within-cluster sum of squares and is described in detail by Hartigan et al. [49]. Stratification clustering was based on the "cumulative square root of the frequency" method and it is described in detail by Scheaffer et al. [50]. The NDVI or grain yield averaged across the three replications was used to classify the 24 wheat genotypes into three classes. In order to verify the performance of the clustering methods, the grain yield, and NDVI classes were compared creating contingency tables [51] for each clustering method (e.g., k-means on yield vs. stratification on NDVI and so on). The overall accuracy of agreement between grain yield and NDVI for twenty-four wheat genotypes was used to determine the best method among the four methods. The best combination of methods would then be used to measure the overall accuracy of NDVI classes against yield classes, which is the main objective of this section.

3. Results

3.1. Crop Growth Stages and Changes in NDVI

The temporal pattern of NDVI values portrayed a typical corn growth curve (Figure 2). Site year II had lower NDVI values during mid-season, which is reflective of the drought conditions, the crop was experiencing. Also, as logically expected, towards the end of the crop season (in June, anthesis to mid-grain filling stages), the NDVI values decreased as the crop approached maturity. The mean NDVI values for individual winter wheat genotype (CO940610) (shown as a representative genotype) in late season (16 June) were 0.34 under dryland conditions (Figure 2) and 0.58 under irrigated conditions (data not shown) for site year I. Similarly, for site year II, the mean NDVI values during late season (17 June) were 0.24 and under dryland (Figure 2) and 0.62 under irrigated conditions (data not shown). Again, consistently lower NDVI values under dryland conditions than under irrigated conditions at the end of the season were reflective of water stress and could have been translated to NDVI values. Table 2 shows the temporal variability of NDVI and yield across the growing season for all genotypes under study.

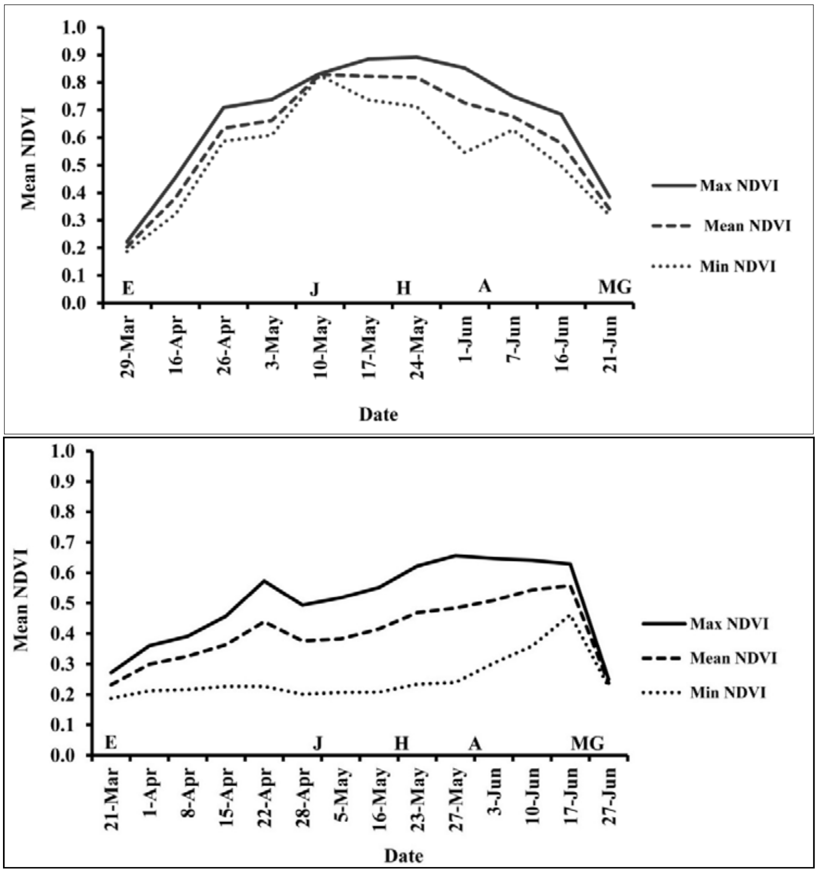

Figure 2. Individual winter wheat genotype (CO940610) selected across the growing season shows the characterization of the generalized wheat crop growth-curve on the basis of the time-series of maximum, mean, and minimum of NDVI readings for the site I (top) (NDVI readings obtained for 11 dates) and for site II (NDVI readings for 14 dates) (bottom). Critical growth stages are indicated as A = anthesis (60-69 Zadoks Scale for growth stage) growth stage, E = early spring (26-29 Zadoks Scale for growth stage) growth stage, J = jointing (30-39 Zadoks Scale for growth stage) growth stage, H = heading (50-59 Zadoks Scale for growth stage) growth stage, and MG = mid-grain filling (70-77 Zadoks Scale for growth stage) growth stage.

3.2. *In-field Spatial Variability of Winter Wheat*

In-field spatial variability in NDVI across wheat genotypes was observed in both site years and under both dryland and irrigated conditions (Figure 3). Visual assessment of the data indicates that a higher level of spatial variability existed under dryland conditions for site year II across the 24 wheat genotypes. This may potentially be explained by different water stress responses across genotypes which could differently affect the chlorophyll content and green biomass from one genotype to the other [15]. The spatial variability of NDVI measurements was also observed by other studies [52,53].

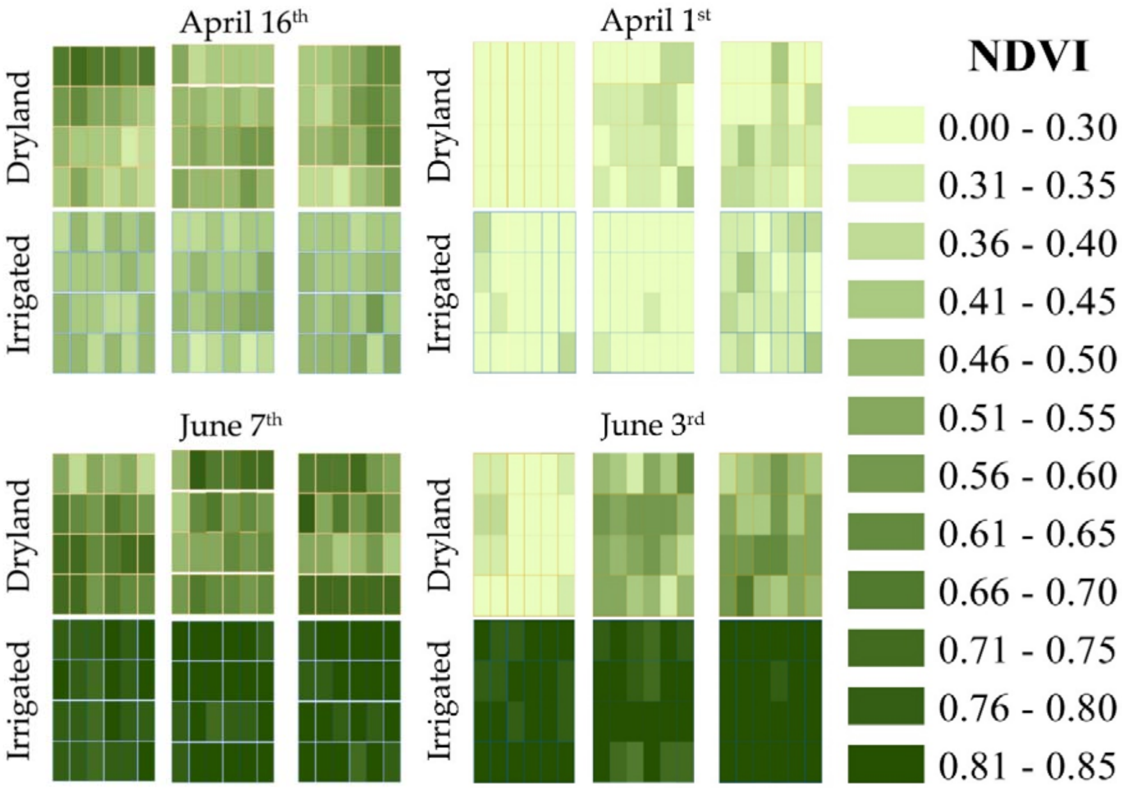

Figure 3. Map of average NDVI values for each genotype of each replication for both dryland and irrigated plots on April 16th and June 7th in site-year I (left panel), and April 1st and June 3rd in site-year II (right panel). Actual location of the replications relative to each other was modified to accommodate this figure.

Table 2. Mean grain yield of 24 winter wheat genotypes and mean NDVI values at four growth stages under two irrigation conditions across two site years.

	Site Year I										Site Year II									
	Dryland					Irrigated					Dryland					Irrigated				
	NDVI					NDVI					NDVI					NDVI				
Wheat Genotype	**E[+]**	**J⁎**	**A***	**MG†**	**Grain Yield ± SD ‡ (Mg ha^{-1})**	**E**	**J**	**A**	**MG**	**Grain Yield ± SDyacohi (Mg ha^{-1})**	**E**	**J**	**A**	**MG**	**Grain Yieldyacohi ± SD (Mg ha^{-1})**	**E**	**J**	**A**	**MG**	**Grain Yield ± SD (Mg ha^{-1})**
Above	0.20	0.69	0.56	0.42	4.00 ± 1.5	0.20	0.77	0.81	0.76	7.23 ± 0.5	0.25	0.37	0.50	0.21	3.68 ± 0.8	0.22	0.67	0.83	0.55	8.18 ± 0.6
Ankor	0.20	0.81	0.69	0.64	4.63 ± 0.4	0.18	0.78	0.78	0.77	6.25 ± 0.3	0.20	0.30	0.39	0.22	3.66 ± 1.6	0.19	0.54	0.81	0.71	7.57 ± 1.0
Arlin	0.18	0.69	0.46	0.37	3.65 ± 1.4	0.20	0.83	0.77	0.73	7.84 ± 0.9	0.22	0.35	0.42	0.20	3.31 ± 1.2	0.19	0.55	0.76	0.59	7.50 ± 1.9
Avalanche	0.21	0.84	0.71	0.59	5.31 ± 1.1	0.20	0.87	0.81	0.79	7.00 ± 0.7	0.22	0.33	0.41	0.20	3.09 ± 1.2	0.24	0.60	0.79	0.58	7.49 ± 1.6
Baca	0.17	0.77	0.59	0.47	3.31 ± 1.8	0.20	0.88	0.81	0.81	6.63 ± 0.9	0.23	0.37	0.50	0.24	3.29 ± 1.0	0.24	0.70	0.84	0.64	6.62 ± 1.2
Bill Brown	0.19	0.70	0.66	0.59	4.91 ± 1.0	0.21	0.87	0.81	0.82	7.48 ± 1.1	0.22	0.36	0.46	0.22	3.92 ± 1.7	0.21	0.59	0.83	0.71	8.09 ± 1.1
Bond CL	0.18	0.84	0.51	0.33	2.90 ± 0.9	0.19	0.80	0.79	0.75	7.14 ± 0.7	0.20	0.30	0.41	0.20	3.81 ± 1.1	0.19	0.56	0.82	0.65	8.15 ± 0.9
CO940610	0.20	0.83	0.68	0.58	4.76 ± 1.1	0.20	0.77	0.79	0.77	7.63 ± 0.1	0.23	0.38	0.51	0.24	3.80 ± 1.1	0.21	0.58	0.78	0.62	7.73 ± 0.4
Danby	0.17	0.83	0.63	0.51	3.69 ± 0.6	0.20	0.82	0.80	0.79	8.10 ± 0.9	0.24	0.36	0.48	0.26	4.04 ± 0.5	0.19	0.56	0.84	0.73	7.73 ± 1.0
Good Streak	0.17	0.73	0.64	0.58	3.23 ± 1.1	0.19	0.85	0.80	0.80	5.99 ± 0.7	0.21	0.26	0.40	0.27	3.45 ± 0.9	0.20	0.55	0.83	0.66	7.38 ± 1.1
Hatcher	0.19	0.84	0.64	0.50	3.87 ± 0.4	0.19	0.81	0.79	0.76	6.93 ± 2.0	0.21	0.31	0.48	0.26	3.42 ± 0.5	0.18	0.58	0.84	0.73	8.14 ± 0.9
Jagalene	0.18	0.82	0.64	0.50	3.57 ± 1.4	0.20	0.87	0.79	0.78	6.58 ± 1.0	0.23	0.35	0.39	0.22	3.42 ± 0.5	0.21	0.59	0.81	0.69	7.76 ± 0.6
Jagger	0.18	0.75	0.64	0.52	3.92 ± 2.0	0.19	0.80	0.82	0.80	7.00 ± 1.4	0.22	0.30	0.40	0.20	3.02 ± 1.4	0.20	0.52	0.77	0.70	7.96 ± 1.5
Keota	0.16	0.68	0.48	0.40	2.64 ± 1.0	0.21	0.77	0.77	0.76	7.87 ± 0.6	0.21	0.30	0.40	0.23	3.36 ± 1.0	0.21	0.62	0.78	0.65	8.22 ± 1.3
NuDakota	0.16	0.80	0.55	0.45	3.04 ± 1.2	0.20	0.74	0.81	0.77	7.37 ± 0.3	0.21	0.28	0.43	0.23	3.91 ± 0.8	0.21	0.64	0.81	0.65	8.92 ± 0.2
Platte	0.18	0.69	0.55	0.43	2.99 ± 1.2	0.19	0.81	0.81	0.73	7.01 ± 0.9	0.22	0.33	0.43	0.20	3.28 ± 1.1	0.19	0.52	0.81	0.68	7.66 ± 0.8
Prairie Red	0.18	0.77	0.62	0.52	4.29 ± 0.3	0.19	0.82	0.78	0.74	7.35 ± 0.7	0.22	0.28	0.39	0.20	3.13 ± 0.9	0.20	0.62	0.79	0.70	8.30 ± 0.9
Prowers 99	0.20	0.77	0.73	0.62	4.50 ± 1.3	0.22	0.83	0.81	0.83	6.86 ± 1.3	0.21	0.28	0.33	0.24	2.89 ± 0.9	0.22	0.55	0.84	0.64	6.78 ± 1.2
Ripper	0.22	0.79	0.66	0.56	5.46 ± 0.8	0.20	0.86	0.81	0.76	7.02 ± 1.0	0.26	0.37	0.45	0.21	3.52 ± 0.8	0.24	0.69	0.82	0.60	9.21 ± 1.7
RonL	0.18	0.83	0.60	0.49	3.64 ± 2.4	0.20	0.83	0.82	0.79	7.51 ± 1.7	0.21	0.26	0.33	0.22	3.06 ± 0.7	0.22	0.61	0.79	0.68	8.50 ± 1.2
Sandy	0.18	0.84	0.66	0.61	4.03 ± 0.8	0.19	0.88	0.82	0.78	7.12 ± 0.3	0.22	0.33	0.46	0.24	3.60 ± 0.9	0.20	0.62	0.84	0.65	7.32 ± 1.3
Snowmass	0.18	0.74	0.63	0.61	4.54 ± 1.5	0.19	0.88	0.79	0.81	7.75 ± 0.4	0.22	0.31	0.44	0.21	3.42 ± 0.8	0.21	0.67	0.82	0.59	7.54 ± 1.0
TAM 112	0.19	0.82	0.59	0.54	4.20 ± 0.6	0.20	0.86	0.79	0.75	7.02 ± 1.5	0.23	0.35	0.45	0.26	4.27 ± 0.9	0.23	0.69	0.80	0.68	9.12 ± 0.9
Yuma	0.19	0.72	0.64	0.52	4.39 ± 0.8	0.20	0.76	0.77	0.76	6.08 ± 0.6	0.21	0.35	0.43	0.20	3.85 ± 1.4	0.20	0.66	0.83	0.68	8.63 ± 1.6

[+] E = early spring growth stage,⁎J = jointing growth stage, * A = anthesis growth stage, † MG = mid-grain filling growth stage, ‡ SD = Standard Deviation.

3.3. Relationship between NDVI and Grain Yield

For site year I, under dryland conditions, the correlation coefficient (r) between NDVI and grain yield with 72 observations (24 wheat genotypes times 3 replications) was significant (p-value < 0.05) for all growth stages. The correlation was greater at early growth stages and after anthesis than in the middle of the season (Figure 4). Detailed correlation plots and coefficients (r) between NDVI measurements for 11 dates and grain yield with 72 observations (24 wheat genotypes times three replications) under dryland conditions at site year I is provided in the Supplementary Materials section (Figure S1).

Under irrigated conditions, the correlation coefficient between NDVI and grain yield was significant (p-value < 0.05) throughout the season with the exception of jointing and mid-grain filling stages (Figure 4). The highest correlation coefficient was observed in the early spring stage on March 29th (r = 0.47), under irrigated conditions. Supplementary material shows correlation plots and coefficients (r) between NDVI measurements for 11 dates and grain yield with 72 observations (24 wheat genotypes times 3 replications) under irrigated conditions at site year I (Figure S2). In general, the correlation coefficient was lower in irrigation conditions than in dryland conditions.

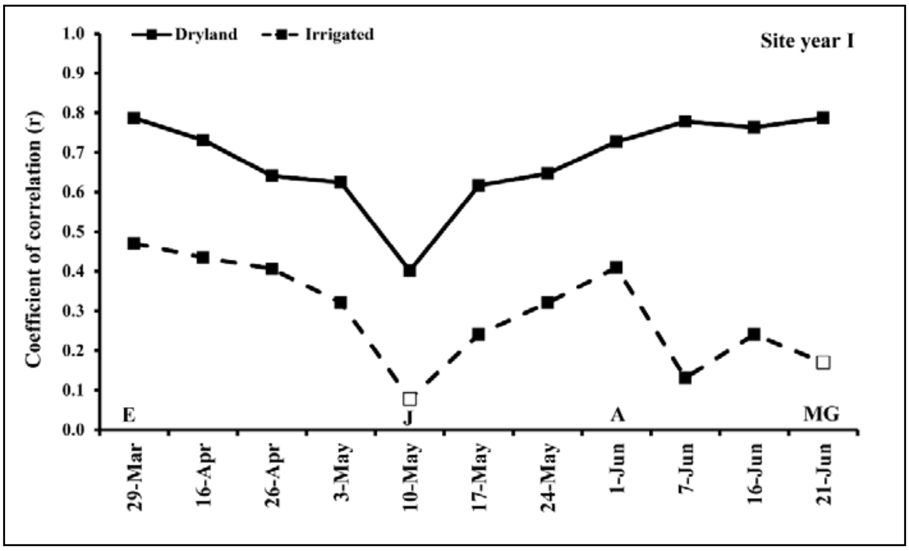

Figure 4. Correlation coefficient (r) between NDVI and grain yield across 24 winter wheat genotypes for site year I under dryland (solid line) and irrigated (dashed line) conditions across crop growth stages. Critical growth stages are indicated as A = anthesis growth stage, E = early spring growth stage, J = jointing growth stage, and MG = mid-grain filling growth stage. Solid symbols show a significant correlation between NDVI and yield (p-value < 0.05).

For site year II, under dryland conditions, the correlation coefficients between NDVI and grain yield were significant for all wheat growth stages (p-value < 0.05), increasing gradually from early growth stages to late growth stages. The highest correlation coefficient (r = 0.91) was observed between anthesis and mid-grain filling stages (Figure 5). Supplementary material shows the correlation coefficients (r) between NDVI measurements for 14 dates and grain yield with 72 observations (24 wheat genotypes times three replications) under dryland conditions at site year II (Figure S3).

While the correlation coefficient between NDVI and grain yield was significant for all growth stages (p-value < 0.05) under irrigated conditions, the highest correlation coefficient was observed during early spring (r = 0.53) and after mid-grain filling (r = 0.54) stages. Lower correlation coefficients were observed around anthesis stages as illustrated in Figure 5. Supplementary material shows correlation coefficient (r) between NDVI measurements for 14 dates and grain yield with 72 observations (24 wheat genotypes times three replications) under irrigated conditions at site year II (Figure S4).

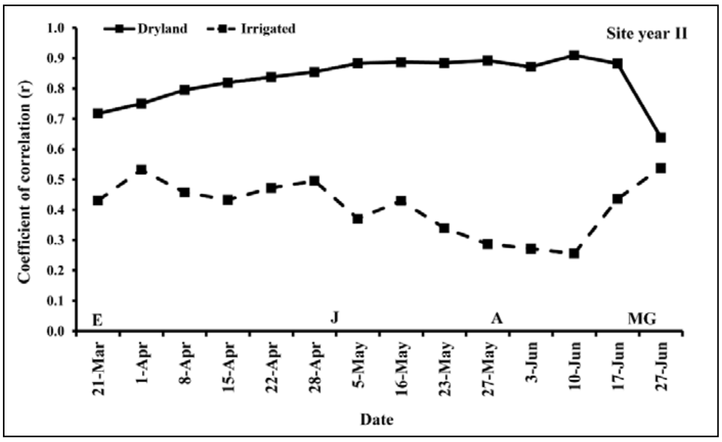

Figure 5. Correlation coefficient (r) between NDVI and grain yield across 24 winter wheat genotypes for site year II under dryland (solid line) and irrigated (dashed line) conditions across crop growth stages. Critical growth stages are indicated as A = anthesis growth stage, E = early spring growth stage, J = jointing growth stage, and MG = mid-grain filling growth stage. Solid symbols show a significant correlation between NDVI and yield (p-value < 0.05).

3.4. Comparing NDVI and Yield Classification

The two clustering methods (k-means and stratification clustering) employed in grain yield data classification produced almost the same results under both dryland and irrigated conditions (Figures 6 and 7).

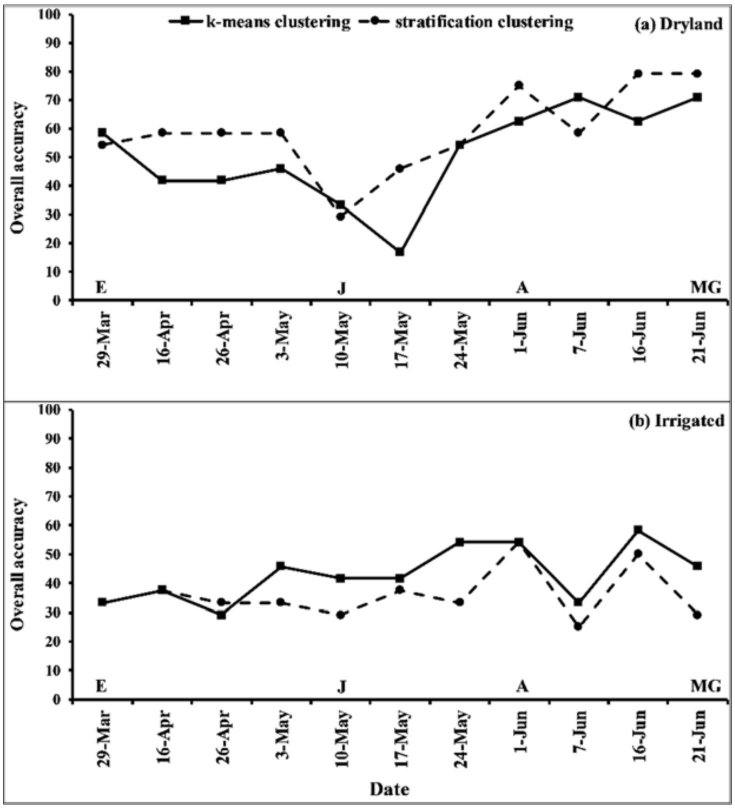

Figure 6. Overall accuracy results from contingency table comparing mean grain yield classes to mean NDVI classes using k-means clustering (solid line with squares) and stratification clustering (dashed line with circles). The overall accuracy is presented across 11 dates for site year I under (**a**) dryland conditions and (**b**) irrigated conditions. Critical growth stages are indicated as A = anthesis growth stage, E = early spring growth stage, J = jointing growth stage, and MG = mid-grain filling growth stage.

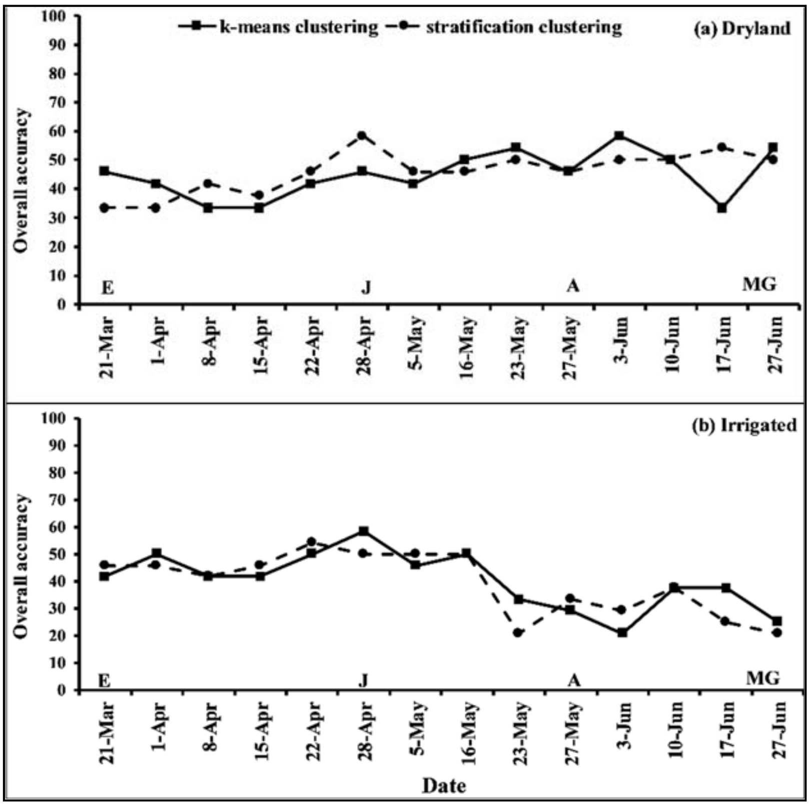

Figure 7. Overall accuracy results from contingency table comparing mean grain yield classes to mean NDVI classes using k-means clustering (solid line with squares) and stratification clustering (dashed line with circles). The overall accuracy is presented across 14 dates for site year II under (**a**) dryland conditions and (**b**) irrigated conditions. Critical growth stages are indicated as A = anthesis growth stage, E = early spring growth stage, J = jointing growth stage, and MG = mid-grain filling growth stage.

Table 3 shows the comparison of NDVI classes to grain yield classes for all dates and both site years in this study. The diagonal sum of the contingency table gives the agreement of the overall accuracy between NDVI classes and grain yield classes. The overall accuracy measured over the whole dataset of this study was 44.5%. For site year I, the overall accuracy between NDVI (all 11 dates together) and grain yield was 50.8% and 43.2% under dryland and irrigated conditions, respectively. For site year II, the overall accuracy between NDVI (all 14 dates together) and grain yield was 44.9% under dryland conditions and 40.2% under irrigated conditions. A detailed summary of cluster averages of NDVI (high, medium, and low), for each measurement period in both site years across two irrigation conditions, is provided in Supplementary Materials section (Table S1).

Table 3. Contingency table comparing grain yield classes to NDVI classes (k-means clustering) for all dates, site years and two irrigation conditions in this study merged together.

	———NDVI Class———			
Yield Class	**Low**	**Medium**	**High**	**% Accuracy**
Low	203	168	100	43.1
Medium	137	204	139	42.5
High	28	94	127	51
% accuracy	55.2	43.8	34.7	44.5 †

The overall accuracy is presented in the lower right end of the table. † Overall accuracy = (Sum of diagonal values/Sum of the whole table) × 100.

4. Discussion

4.1. Crop Growth Stages and Changes in NDVI

The results from this study show an accurate characterization of the generalized wheat crop growth-curve on the basis of NDVI measurements (Figure 2). A consistent increase in NDVI values from early season crop growth stages was observed until the growth-curve reached a plateau when the ground surface was completely covered by crop canopy. The plateau in NDVI values seems to correspond to the saturation of the sensor that loses sensitivity to changes in vegetation amount when LAI is higher than 3 [15,54,55]. The decrease in NDVI at the end of the season was attributed to physiological maturity, change in crop color, and leaf senescence which increased red band reflectance and decreased NIR band reflectance. This phenomenon was observed by previous studies [21,24] on vegetation with a lower amount of green biomass due to either water stress or to normal senescence happening from anthesis to mid-grain filling stage. As expected, increasing green biomass was observed in early spring and mid-season and decreasing green biomass in the late season, which was reflected in NDVI values. Other reflectance-based indices such as green NDVI, and simple ratio (SR) can also detect seasonal variations in green biomass [26,29].

4.2. Relationship between NDVI and Grain Yield

The lower correlation coefficient in site year I than in site year II and the lower correlation coefficient in the irrigated plot than in dryland plot were possibly due to differences in crop growth conditions and timing, rate, and distribution of precipitation during the growing season. Site year I received approximately 84 mm more precipitation than site year II during the growing season and irrigated plots received more water than dryland plots. Receiving more water enabled the wheat crop to reach a higher LAI which causes the NDVI sensor to saturate at about 0.9 NDVI values. NDVI saturation at a higher LAI values between 2-4 was also noted by [54,56]. Carlson et al. [56] stated that NDVI increases almost linearly with LAI, increasing until LAI values reach at 3–4, then NDVI is rapidly limited with dense vegetation. They reported that NDVI limit depends on vegetation type, age, and leaf water content. For this reason, a correlation was higher during the early and late growth stages, but also when the crop suffered from drought, preventing it from reaching high LAI values and saturating the sensor. These results are consistent with Aparicio et al. [15], who noted a positive relationship (R^2 = 0.52) between grain yield and NDVI values recorded under dryland conditions with durum wheat growth stages. Late season (i.e. anthesis and mid-grain filling stage) positive correlation between NDVI and grain yield was also observed in past studies [13,17,24,57]. It appears that the optimal period to assess wheat grain yield may be during the early and late crop growth stages, while mid-season should be avoided for the reasons observed in this study. However, these results in contrast with [34] who observed better correlations to predict grain yield of 23 wheat genotypes in irrigated condition than rainfed condition during grain filling growth stages using handheld sensors and UAV platforms measurements, which performed similarly in predicting yield.

4.3. Comparing NDVI and Yield Classification

The results show that overall accuracy between yield classes and NDVI classes under dryland conditions was higher than overall accuracy under irrigated conditions for both site years. The results from correlation and clustering analysis were contrasted to evaluate the agreement between these two methods. For dryland conditions, clustering the genotypes as low, medium or high classes did not improve the overall accuracy between yield and NDVI (Figures 6 and 7), as compared to the correlation coefficients observed between NDVI and grain yield with 72 observations (Figures 4 and 5) across two site years. In site year I, under irrigated conditions, clustering of genotypes into three classes improved the overall accuracy (Figure 6) in general, when compared to the correlation coefficients over 72 observations (Figure 4). For site year II, there was a stronger relationship in mid-season using the clustering of genotypes (Figure 7) as compared to the correlation coefficients between NDVI and grain yield with 72 observations (Figure 5). It was thus concluded that clustering the genotypes into classes rather than comparing them independently could be useful when there is canopy closure or high LAI. Under these circumstances, the sensitivity of NDVI diminishes due to saturation and only substantial variations in yield can be detected using the GreenSeeker. The overall classification accuracy of clustering algorithm was 44.5% regardless of genotype, irrigation conditions, time of NDVI measurement, and season (site year I and II). Hence, this method could be useful for yield assessment rather than direct relationship between NDVI and yield.

For dryland conditions, based on overall accuracy results comparing grain yield classes to NDVI classes, anthesis to mid-grain filling stages would be the most appropriate stages to classify wheat genotypes. As opposed to dryland conditions, the results obtained from wheat genotypes grown under irrigated conditions did not respond the same way for both site years. In site year I, the overall accuracy was higher later in the season whereas in site year II, the overall accuracy was higher early in the season. In general, the decrease in the correlation and overall accuracy results between NDVI and grain yield across 24 wheat genotypes coincided with mid-season when NDVI saturated. The NDVI normally reaches a plateau with an LAI of three or more [10,54]. When NDVI reaches the plateau (or saturates), it loses its sensitivity to variations in biomass, LAI or chlorophyll content. It was reported that, with an LAI above 3, canopy closure is reached and red reflectance decreases to minimum values (around 3–4% of incident light) because 70–90 % of incident light is absorbed by chlorophyll in the upper leaves and the rest is transmitted [10]. In contrast, the NIR reflectance increases with canopy closure because chlorophyll reflects NIR more than bare soil [10]. Therefore, NDVI saturates because of the low red reflection in crop canopy and not because of GreenSeeker sensitivity. It is possible that using different wavelengths to calculate vegetation index may result in different outputs. The GreenSeeker uses the red waveband (around 656 nm), where reflectance approaches zero. This has the advantage of making the sensor very responsive to chlorophyll in its field-of-view under sparse canopy, but it has the disadvantage of saturating under canopy closure.

The results support the hypothesis of this study that it is possible with fair accuracy to use NDVI based on a proximal canopy sensor as a useful tool to differentiate wheat genotypes and classify it based on their productivity potential. In general, the results showed that NDVI differentiates and classifies better after anthesis and in mid-grain filling stages. In addition, NDVI differentiates and classifies better under dryland than irrigated conditions. These results indicate that wheat breeders who work on drought-resistant traits and grow wheat in dryland conditions may benefit from using NDVI as a screening index more than those who breed wheat for irrigated conditions. Hassan et al. [6] observed that the potential of NDVI for screening and ranking wheat genotypes based on their grain yield and it has also been demonstrated on soybean genotypes [21,58]. It is thus suggested that the use of NDVI as a tool to identify high yielding genotypes has potential with crops other than wheat.

5. Conclusions

NDVI from proximal canopy sensing could be a valuable tool for wheat genotype yield assessment. Under dryland conditions, a strong relationship was observed between grain yield and NDVI values across 24 genotypes of winter wheat. The results from this study suggest that NDVI could assess grain yield under dryland conditions, but show limitations under irrigated conditions. The overall accuracy between NDVI and grain yield classes across growth stages indicated that the most appropriate stage to differentiate and classify wheat genotypes were from anthesis growth stage to late-season at mid-grain filling growth stages. Our findings also show that winter wheat genotypes were successfully classified into grain yield classes based on NDVI readings collected by GreenSeeker under irrigated and dryland conditions. The outcomes of this study indicate the potential of differentiating winter wheat genotypes based on their productivity using NDVI measurements as a useful and promising tool. Moreover, the results from the clustering analysis could also be useful for management zone-based precision agriculture. More research is needed to clearly understand the relationship between NDVI and genotypic characteristics to accurately identify the most critical crop growth stages of wheat development which could aid breeders in recognizing the best genotypes.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-4292/12/5/824/s1, Figure S1: Correlation coefficient (r) between NDVI measurements for 11 dates and grain yield with 72 observations (24 wheat genotypes times 3 replications) under dryland conditions *across* site year I, Figure S2: Correlation coefficient (r) between NDVI measurements for 11 dates and grain yield with 72 observations (24 wheat genotypes times 3 replications) under irrigated conditions across site year I, Figure S3: Correlation coefficient (r) between NDVI measurements for 14 dates and grain yield with 72 observations (24 wheat genotypes times 3 replications) under dryland conditions *across* site year II, Figure S4: Correlation coefficient (r) between NDVI measurements for 14 dates and grain yield with 72 observations (24 wheat genotypes times 3 replications) under irrigated conditions across site year II, Figure S5: Experimental setup and genotype arrangement for site year I and site year II, in dryland and irrigation conditions. Outline: Yellow = Dryland; Blue = Irrigated, Filling: Red = replication 1; Yellow = replication 2; Green = replication 3, Letters: A = Above; B = Ankor; C = Arlin; D = Avalanche; E = Baca; F = BillBrown; G = BondCL; H = CO940610; I = Danby; J = Goodstreak; K = Hatcher; L = Jagalene; M = Jagger; N = Keota; O = NuDakota; P = Platte; Q = PrairieRed; R = Prowers99; S = Ripper; T = RonL; U = Sandy; V = Snowmass; W = TAM112; X = Yuma; Y = CO03W054-2. Actual location of the replications relative to each other was modified to accommodate this figure, Table S1: Cluster averages of NDVI showing three classes (low, medium, and high) for each measurement date for site year I and II across irrigation conditions.

Author Contributions: R.K. was the first author´s advisor, designed the study, provided supervision for research, and input for the paper. L.L. was responsible to design the study, provide training, and outline the methodology, statistical analysis, and laboratory analyses. M.A.N. performed statistical analyses and wrote the paper. S.D. contributed by reviewing, editing, analysis reviewing, and additions to the text. All authors have read and agreed to the published version of the manuscript.

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