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Evaluation of agronomic traits and spectral reflectance in Pacific Northwest winter wheat under rain-fed and irrigated conditions

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ABSTRACT

The US Pacific Northwest (PNW) is characterized by high latitude and Mediterranean climate where wheat production is predominantly rain-fed and often subject to low soil moisture. As a result, selection for drought-adaptive traits in modern cultivars has been an integral component of the regional breeding programs. The goal of this research was to evaluate phenotypic associations of morpho-physiological traits and their response to soil moisture variation in winter wheat germplasm adapted to the PNW. A panel of 402 winter wheat accessions (87 hard and 315 soft) was evaluated for spectral reflectance indices (SRIs), canopy temperature (CT), plant stature, phenology, grain yield, and yield components under rain-fed and irrigated conditions in 2012–2014. Variation in soil moisture and temperature cumulatively explained 86% of total yield variation across years and locations. The phenotypic associations of yield with phenology, plant height, and CT were environment dependent. Various SRIs related to biomass, stay green, pigment composition, and hydration status showed consistent patterns of response to drought and strong correlations with yield ($p < 0.001$). The compensatory interaction of grain number and weight was indicated in the negative correlation between thousand kernel weight and grain number per spike across moisture regimes. Area under vegetation index curve (AUVIC) explained 53–88% of the total variation in stay green estimated from visual score of flag leaf senescence ($p < 0.001$). Principal component analysis revealed three major clusters that explained more than 76% of interrelations among traits. The market classes within the study population showed differentiation with respect to these traits. This study highlights the potential use of spectral radiometry in field screening of winter wheat for grain yield and drought adaptation in Mediterranean-like environments.

1. Introduction

Winter wheat production in the US Pacific Northwest (PNW) is characterized by high latitude, relatively cool temperature, and a Mediterranean-like climate with most annual precipitation occurring in the winter (Mote, 2003). Annual precipitation ranges from less than 200 mm to more than 500 mm (Schillinger and Papendick, 2008). In addition to soil moisture deficits in semiarid areas, seasonal precipitation fluctuation between April and June is a major constraint of wheat production in the entire region (Lopez et al., 2003). Soil depth ranges from less than 1 m to over 7 m, causing spatial heterogeneity in water holding capacity and stress severity in the region (Shillinger and Papendick, 2008). Direct selection for grain yield has been successfully practiced for more than a century to improve yield across the precipitation zones. However, the genetic gain from this approach is generally low in the driest farms because of high genotype-environment interaction and unaccounted spatial heterogeneity (Blum, 2006).

Higher genetic progress in yield can be achieved by selecting secondary traits other than yield *per se*. Grain yield is determined by number of spikes per area, number of kernels per spike and kernel weight which are interrelated to each other and influenced by morpho-physiological traits such as early vigor, plant stature, flowering time, and physiological maturity (Alexander et al., 1984; McNeal et al., 1978; El-Mohsen et al., 2012; Mohammadi et al., 2012; Wu et al., 2012). The influence of environment on these secondary traits is relatively low and predictable resulting in higher genetic progress compared to yield based selection (Fischer et al., 2012; Wu et al., 2012). Recent reports suggest that some of the component traits are genetically independent suggesting the possibility

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of combining multiple traits in modern wheat cultivars (Dhungana et al., 2007).

The compensatory interactions and relative contributions of grain weight, grain number per spike, and spike number per area towards overall yield are affected by developmental characteristics and environmental factors (Cutforth et al., 1988; Duguid and Brule-Babel, 1994; Santra et al., 2009). Semi-dwarf stature (shorter and stiffer than standard height), early growth vigor, and early maturity are adaptive features for environments with terminal heat and drought stresses (Bai et al., 2004; van Ginkel et al., 1998; Morgan, 1995; Álvaro et al., 2008; Kirkegaard et al., 2001). On the other hand, dwarf stature and long grain-fill duration have yield advantage in optimum conditions (Bai et al., 2004; Blum, 1996; Gomez et al., 2014). As a result, indirect selection is an analytic approach that involves understanding interrelationships among various attributes, their yield advantages, and responses to environmental variation (McNeal et al., 1978; El-Mohsen et al., 2012).

Some physiological attributes contribute to grain yield and yield components like kernel weight by maintaining higher rate and duration of grain filling (Duguid and Brule-Babel, 1994). These physiological attributes include radiation use and photosynthetic efficiency, transpiration efficiency, water availability and retention capacity, biomass capacity, and assimilate translocation to the grain (El-Mohsen et al., 2012; Reynolds et al., 2012). However, direct measurements of these physiological attributes are labor and resource-intensive which limits their application to characterize large sets of germplasm.

Spectral radiometry and other indirect sensing methods became high-throughput phenotyping alternatives that enable evaluation of large germplasm collections over multiple target environments (Fiorani and Schurr, 2013). Spectral reflectance indices (SRIs) are calculated in the visible (VIS) and near infrared (NIR) ranges ($\lambda = 400–700$ and $\lambda > 700$ nm respectively). Three main categories of traits can be estimated using these indices: (1) biomass, pigment abundance, and area of photosynthetic canopy (Wiegand and Richardson, 1990; Haboudane et al., 2004; Naumann et al., 2009; Reynolds et al., 2012); (2) water availability and plant hydration status (Tucker and Sellers, 1986; Zarate-Valdez et al., 2012); and (3) composition of photo- and thermo-protectant molecules (Peñuelas et al., 1995; Ollinger, 2011).

A combination of both visible and infrared spectra are used to derive the vegetation indices of simple ratio (SR), normalized difference vegetation index (NDVI), and green normalized vegetation index (GNDVI) to discern minute differences in vegetative greenness, rate of senescence, and stay green duration (Gitelson et al., 1996; Stenberg et al., 2004; Babar et al., 2006; Edae et al., 2014; Lopez and Reynolds, 2012; Liu et al., 2015). The anthocyanin reflectance index (ARI) is derived from wavelengths in both the VIS and NIR regions as a surrogate for anthocyanin composition (Gitelson et al., 2002). Photochemical reflectance index (PRI), normalized chlorophyll-pigment ratio index (NCPI), and Xanthophyll pigment epoxidation state (XES) are derived from reflectance at the VIS light range to estimate the composition and abundance of plant pigments (Peñuelas et al., 1993; Peñuelas et al., 1995; Ollinger, 2011), whereas normalized water index (NWI) is derived from reflectance at NIR range to estimate plant hydration status (Babar et al., 2006).

Characterizing the interrelations of SRIs, developmental traits, and yield with respect to target environments is crucial to facilitate an integrated use of remotely-sensed and agronomic information in adaptation breeding (Edmeades et al., 1997; Farshadfar et al., 2013). Rigorous investigations have been carried out to fully account for the responses of SRIs in irrigated, warm, and low latitude spring wheat environments. Effects of environment and growth stages on SRIs were reported to be significant (Aparicio et al., 2002; Babar et al., 2006; Lopez and Reynolds, 2012). Aparicio et al. (2000) reported that association of SRIs with grain yield, biomass capacity, and leaf area index (LAI) was higher in rain-fed than irrigated condition. These reports suggest the need to carefully determine which growth stage and selection environment is most informative.

However, little or no previous assessment has been done on the properties and potential use of canopy spectral reflectance in the environments with a high latitude, cool to cold winter season, and strong photoperiod requirement. The main goal of this research was to evaluate various spectral reflectance indices associated with grain yield under dry, moist-cool, and irrigated conditions in winter wheat genotypes adapted to the PNW environment. Specific objectives were: (i) to evaluate phenotypic associations of SRIs with grain yield and morpho-physiological traits; (ii) to determine the interaction of these traits with environmental variables and developmental traits such as ear emergence, and (iii) to examine the trends of phenotypic associations across multiple growth stages in the crop's life cycle. Understanding the interrelationship and drought-responses of these developmental traits, morpho-physiological components, and agronomic performance will help in combining the yield advantage of multiple attributes through focused identification and introgression of traits that have synergetic effects on yield.

2. Materials and methods

2.1. Study population

The study was conducted on two PNW winter wheat subpopulations: hard winter ($n = 87$), and soft winter ($n = 315$). The winter wheat germplasm in the region has been continuously subjected to selection for yield, yield stability, end-use qualities, farming preferences, and disease resistance (Barrett and Kidwell, 1998; Chen, 2005; Schillinger and Papendick, 2008). Donaldson (1996) indicated that wheat cultivars adapted to the region contain significant variations for emergence, early canopy establishment, root growth and development, winter survival, osmotic adjustment, optimum maturity, and plant architecture. Barret and Kidwell (1998) attributed the broad and stratified genetic basis for these agronomic traits to the breeding effort in region that has been in place for more than a century. Similarly, the study population is known to have a genetic stratification that align with market class and breeding history. In particular, population structure analysis differentiated hard winter genotypes from club winter genotypes with only a slight overlap (Naruoka et al., 2015).

Genotypes were selected from mapping populations, advanced breeding lines, and cultivars from PNW breeding programs targeted to Oregon, Washington, and Idaho. The hard red winter wheat cultivar 'Norwest 553' (PI 655030) and the soft white winter cultivar 'Madsen' (PI 511673) were included as local checks. Madsen is known for its wide adaptation and disease resistance and has been grown in the PNW for over 20 years, whereas Norwest 553 has high yield potential, good disease resistance, and was the most commonly grown hard red cultivar in the PNW when the trial was initiated. Because both accessions have semi-dwarf plant height and photoperiod sensitivity, the variation across years and locations is expected to have low effect on their performance making them ideally suited to account for spatial variations within each trial.

2.2. Experimental conditions and field design

The study population was grown in three moisture regimes at the following Washington State University agronomy research farms: Central Ferry (46° 4′ N; 117° 8′ W), Pullman (46° 4′ N; 117° 5′ W), and Othello (46° 5′ N; 119° 2′ W) (Table 1). Central Ferry has a

Table 1
Geographical location, soil type, precipitation, and growing degree days of the three experimental sites.

Variable	Central Ferry	Pullman	Othello
Geographic position (Lat, Long)	46.4; 117.8	46.4; 117.5	46.5; 119.2
Altitude (masl)	206	717	323
Precipitation (mm)[a]	317	533	214
Growing degree days (GDD, °C)[b]	3408	2138	2774
Soil type	Chard silt loam	Palouse silt loam	Shano silt loam
Years of experiment Rain-fed	2012–2014 (3)	2012–2014 (3)	2013 (1)
Irrigated	2013–2014 (2)		2013 (1)

[a] Cumulative precipitation in the growing seasons in millimeters.
[b] Calculated for growing seasons of the study periods using 0 °C as base temperature.

well-drained and moderately permeable Chard silt loam soil with water holding capacity ranging from 220–280 mm. Othello has a well-drained and moderately permeable Shano silt loam soil with 170–220 mm water holding capacity. The Palouse silt loam soil in Pullman is the most fertile and highly cultivated soil with deep profile, moderate permeability, and high water holding capacity. Planting of winter wheat in the study area is usually between late September and mid-October. The annual rainfall is highest in Pullman followed by Central Ferry and Othello (Table 1). In Central Ferry and Othello, the population was planted in two treatments, a rain-fed planting representing the drought condition and irrigated treatment representing the water optimum condition. In Pullman, the population was planted only in a rain-fed condition representing the moist-cool condition. The irrigated trials were conducted using solid-set sprinkler systems for 4–8 h, one or two times a week depending on the weather. Overhead sprinkler irrigation system is recommended in the region to minimize runoff. This system delivered approximately 600 mm of water over the growing season. Irrigation started on booting (Feekes 9), before the soil moisture depletes and any sign of stress was detected, and continued until the onset of physiological maturity.

In all trials, a modified augmented design (Federer and Raghavarao, 1975; Lin and Poushinsky, 1983) was used with 16–20% of the plots assigned to the replicated local checks, Norwest 553 and Madsen. Planting was done using a Wintersteiger plot seeder (Wintersteiger AG, Austria). The seeding rates were 5.4 g/m^2 for drought and 10.8 g/m^2 for both irrigated and moist-cool rain-fed treatments in 4.65 m^2 plots. Pre-plant seed treatment was done with Sedaxane + Difenoconazole + Mefenoxam + Thiamethoxam (Cruisermaxx Vibrance®, EPA Reg. No. 100-1383, at 0.06% by weight of the product; Syngenta Crop Protection, Greensboro NC) for healthy root growth. Fungicide (Quilt®, 140 g/ha) and herbicides (Huskie™—840 g/ha, Starene®—7m00 g/ha, and PowerFlex®—140 g/ha) were applied at Feekes stages 4–6 following label instructions. This was done to eliminate the confounding effects of stripe rust resistance and herbaceous weeds on yield potential.

2.3. Measurement and calculation of traits

Heading date was recorded as the number of days from sowing until full exposure of spikes in 50% of the plot. Plant height (PHT) was measured between the base of the plant stand and the tip of fully emerged spike excluding awns. Peduncle extrusion (PE) was measured as the portion of peduncle that emerged out of its sheath. Degree of flag leaf greenness was visually scored on a 1–10 scale (1 = fully senesced and 10 = fully green) three times between heading date and physiological maturity. Canopy reflectance was measured using a handheld CROPSCAN multispectral radiometer (CROPSCAN, Inc. Rochester, USA) installed with filters that selectively measure incident and reflected radiation at 16 different wavelengths between 430 and 970 nm. Three to five measurements were taken per plot at one to two week intervals between heading and late grain-fill. While the up-welling sensor measures the incoming radiation, the down-welling sensor positioned 40 cm above the canopy measure the reflection from plant surface. All reflectance measurement was done between 10 a.m. and 2 a.m. avoiding shadow, cloud, and strong wind. A handheld infrared thermometer (Sixth Sense LT300, Total Temperature Instrumentation, Inc., Burlington, VT) was used to measure canopy temperature (CT) during the grain fill stages (Feekes 10–11). Plots were harvested with a Wintersteiger NurseryMaster small plot combine (Wintersteiger AG, Austria) after ripening (Feekes 11). Grain yield (t/ha) was calculated from the grain weight per plot. Thousand kernel weight (g) and test weight (kg/hl) were processed from sample grain whereas grain number per spike (count) was obtained by hand-threshing and counting grain numbers in five spikes sampled from the plots. In 2014, spectral reflectance values were not collected on all plot entries, and thus these data only represent 2012 and 2013 measurements.

Genotypic stay green (SG) was calculated from multiple scores of flag leaf greenness as area under greenness curve by slightly modifying the method to estimate the area under SPAD decline curve (AUSDC) in Rosyara et al. (2007):

$$SG = \sum_{i=1}^{n-1} \left[\frac{G(i) + G(i+1)}{2} \right] * [D(i+1) - D(\mathrm{i})]$$

where G(i) and G(i + 1) are consecutive scores of flag leaf greenness, and D(i) and D(i + 1) are days after sowing for (Gi) and G(i + 1) measurements, respectively.

Reflectance values were used to derive seven vegetation indices that are presented in Table 2. Three different parameters derived from NDVI have been previously reported to be effective estimators of the degree of greenness (pigment abundance), rate of senescence, and stay green duration: NDVI at specific growth stages (Babar et al., 2006; Edae et al., 2014; Liu et al., 2015), slope of NDVI over growth stages (Lopes and Reynolds, 2012), and arithmetic mean of NDVI over growth stages (Babar et al., 2006). We tested these approaches along with two new parameters: area under vegetation index curve (AUVIC) modified from Rosyara et al. (2007) and weighted mean, which uses the population mean of each measurement as a weighting factor. We calculated these parameters for all studied vegetation indices and tested their phenotypic associations with grain yield. Stay green estimates based on flag leaf senescence were also compared with growth-specific reflectance indices and the derived parameters.

2.4. Data analysis

Trait values were adjusted for spatial variation within each field experiment (called trial hereafter) using the MIXED procedure (SAS, Cary, NC) which accounts for the fixed effect of un-replicated genotypes and random effect of blocks in a mixed linear model as follows:

$$Y_{ij} = \mu + \tau_{ij} + b_j + e_{ij}$$

Table 2
Vegetation indices evaluated as proxies for stay green, grain yield, and drought tolerance in Pacific Northwest winter wheat.

Vegetation Index	Formula[b]	References
Normalized difference vegetation index (NDVI)	$(R_{800} - R_{680})/(R_{800} + R_{680})$	Rouse et al., 1973
Simple ratio (SR)	R_{800}/R_{680}	Stenberg et al., 2004
Green-NDVI (GNDVI)	$(R_{780} - R_{550})/(R_{780} + R_{550})$	Gitelson et al., 1996
Photochemical reflectance index (PRI)	$(R_{530} - R_{570})/(R_{530} + R_{570})$	Peñuelas et al., 1993
Normalized chlorophyll pigment ratio index (NCPI)	$(R_{680} - R_{430})/(R_{680} + R_{430})$	Peñuelas et al., 1993
Anthocyanin reflectance index (ARI)	$R_{800}(1/R_{550} - 1/R_{700})$	Gitelson et al., 2002
Normalized water band index (NWI)[a]	$(R_{900} - R_{970})/(R_{900} + R_{970})$	Babar et al., 2006
Xanthophyll pigment epoxidation state (XES)	R531	Peñuelas et al., 1995

[a] The formula for NWI is slightly modified so that it has similar dimension to hydration status.

[b] The letter "R" followed by three digit number stands for wavelength of respective reflectance.

where μ is the overall trait mean, τ_{ij} is the calculated estimator used to adjust mean of genotype i within the block j, b_j is the effect of block j, and e_{ij} is model residual calculated from replicated checks. Adjusted trait value is obtained by adding μ and τ_{ij}.

Analysis of variance was performed using the following equation to determine if there was significant effect of genotype, environment (years, locations and moisture regimes), and genotype × environment interaction on the traits:

$$Y_{ijkl} = \mu + E_i + Bji + G_k + GE_{ik} + e_{ijkl}$$

where Y_{ijkl} is the measurement of genotype k on plot l in block j, and trial i; μ is the overall mean of all plots in all trials; E_i is the effect of trial i; B_{ji} is the effect of block j within trial i using replicated check; G_k is the effect of genotype k; GE_{ik} is the interaction of genotype i with trial k; e_{ijkl} is the plot residual.

Stress intensity of each moisture regime was calculated according to Fischer and Maurer (1978):

$$SI = 1 - \left(\frac{\bar{Y}i}{\bar{Y}p}\right)$$

where $\bar{Y}_i$ is mean yield under stress and $\bar{Y}_p$ is mean yield under optimum condition. Cumulated growing degree days (GDD) was calculated from the maximum day and night temperature using 0 °C as baseline in a wheat thermal model (modified from Saiyed et al., 2009).

The phenotypic correlation coefficients (r) between the LS-means of spectral reflectance and grain yield were calculated overall population and within narrow phenology groups (lower 25 percentile—early; 25–75 percentile—intermediate; and top 25 percentile—late ear emergence) using multivariate CORR function (SAS, Cary, NC). Principal component analysis was performed among traits using genotypic trait values in order to infer population differentiation, covariance among traits, and reduce the number of yield predictive variables.

3. Results

3.1. Environmental conditions and germplasm responses

Moisture deficit in drought treatment started at booting stage (Feekes 9) and progressively increased through maturity. Pullman received the highest precipitation and lowest temperature whereas Othello received lowest precipitation and intermediate temperature and Central Ferry received intermediate precipitation and highest temperature (Fig. 1). The irrigated trials in Central Ferry and Othello differed in their maximum temperature as well as respective accumulated growing degree days. The moisture and temperature gradient observed in this study represent distinctive growing conditions with considerable yield differences. The presence of population stratification had significant effect on all traits ($p < 0.05$), the highest effect being on grain number per spike (38%).

3.2. Response in grain yield and yield components

The highest yield was recorded in moist-cool rain-fed condition followed by irrigated and drought conditions, respectively (Table 3). Stress intensity (SI) in the drought condition, calculated as reduction of grain yield in reference to moist-cool treatment, was 52% for hard winter and 55% for soft winter wheat. This result suggests that drought intensity and yield response of winter wheat germplasm was affected by the combination of high temperature and soil moisture deficit (Fig. 1). The two classes of wheat (soft and hard) responded similarly to the water treatments. The irrigated condition showed 36 and 30% SI in hard and soft winter subpopulations, respectively. The yield variation across years and locations was significant in all treatments ($p < 0.001$, Fig. 2a). The yield increase in response to irrigation was 15% in Central Ferry and 45% in Othello. A linear regression model indicated that differences in water regime and temperature explained 77% ($p < 0.001$) of the variation in yield with about 10% more yield variation attributed to the interaction of available moisture and temperature (Fig. 2b).

Like grain yield, thousand kernel weight was lowest in drought, intermediate in irrigated, and highest in moist-cool rain-fed condition. The moist-cool rain-fed condition showed highest test weight and lowest grain number per spike. Irrigated and drought conditions didn't show consistent and statistically significant difference in grain number and test weight. The soft winter wheat subgroup had higher grain number per spike compare to hard winter in all environmental conditions. Thousand kernel weight and test weight were significantly higher in hard winter wheat under drought condition, but didn't show significant difference between the subgroups under moist-cool rain-fed and irrigated conditions.

3.3. Response in phenology: heading date and stay green

Heading was late in moist-cool rain-fed condition, intermediate in irrigated, and early in drought conditions (Table 3). In reference to moist-cool condition, early ear emergence was observed under drought (4.7–6.3%) and irrigation (3.0–4.2%). Heading date and stay green showed a direct relationship, the highest degree of stay green being obtained in late heading genotypes followed by medium and early genotypes (Fig. 3). Difference in phenology poses a confounding effect on hydration status and subsequently on the relationship between yield and component traits. To minimize this confounding effect, the study population was divided into early, intermediate, and late phenology subgroups. Genotypes with heading date lower than the 25 percentile cut off were grouped as early phenology groups. Genotypes with heading date between 25 and 75 percentile were grouped as intermediate phenology; and geneotypes with heading date above the 25 percentile were grouped as late phenology groups.

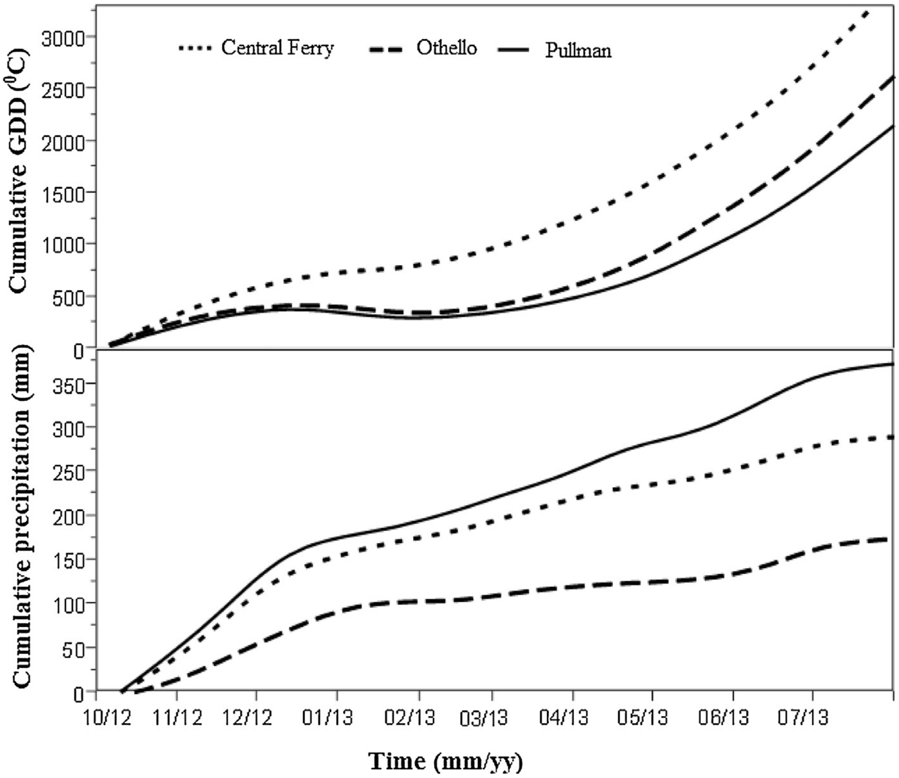

Fig. 1. Cumulative precipitation and temperature of the experimental sites in 2012–2013 growing season: Lowest temperature and highest precipitation was obtained in Pullman; intermediate temperature and lowest precipitation in Othello; and highest temperature and intermediate precipitation in Central Ferry.

Table 3
Descriptive summary of grain yield, yield components, heading date, stay-green, and spectral reflectance indices in PNW winter wheat: Means and standard errors (SE) were calculated from multiple trials within treatments.

Trait[a]	Hard[b]						Soft					
	Drought		Irrigated		Moist cool RF[c]		Drought		Irrigated		Moist cool RF	
	Mean	SE[d]	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
DTH	222.26^{c}	0.208	226.52^{b}	0.201	234.06^{a}	0.323	225.46^{c}	0.076	229.53^{b}	0.118	236.04^{a}	0.182
SG	0.87^{c}	0.023	1.01^{b}	0.028	1.10^{a}	0.027	1.02^{c}	0.015	1.16^{a}	0.013	1.09^{b}	0.017
Yield	3.61^{c}	0.052	4.91^{b}	0.100	7.42^{b}	0.097	3.60^{c}	0.028	5.68^{b}	0.051	8.04^{a}	0.047
GNS	36.32^{b}	0.727	41.05^{a}	0.675	31.29^{c}	0.602	46.10^{a}	0.444	44.74^{b}	0.446	36.49^{c}	0.341
TKW	32.83^{c}	0.306	34.68^{b}	0.334	35.73^{a}	0.363	31.06^{c}	0.176	34.63^{b}	0.175	36.52^{a}	0.240
TW	75.15^{b}	0.166	73.51^{c}	0.181	76.93^{a}	0.141	73.48^{b}	0.089	73.06^{b}	0.090	76.09^{a}	0.062
PHT	93.54^{c}	0.975	112.33^{a}	1.176	108.73^{b}	1.242	81.66^{c}	0.391	103.39^{a}	0.399	95.13^{b}	0.470
PE	13.24^{b}	0.387	20.96^{a}	0.576	21.20^{a}	0.483	9.44^{c}	0.163	18.74^{a}	0.191	14.93^{b}	0.173
CT	26.32^{a}	0.115	23.11^{b}	0.086	23.07^{b}	0.088	24.58^{b}	0.076	22.27^{c}	0.129	28.33^{a}	0.207
NDVI	0.66^{c}	0.006	0.76^{a}	0.012	0.75^{b}	0.006	0.68^{c}	0.003	0.81^{a}	0.005	0.77^{b}	0.003
SR	5.69^{c}	0.075	7.72^{a}	0.239	6.36^{b}	0.085	6.19^{c}	0.050	8.76^{a}	0.117	6.81^{b}	0.051
GNDVI	0.66^{c}	0.004	0.70^{a}	0.009	0.69^{b}	0.004	0.67^{c}	0.002	0.74^{a}	0.004	0.71^{b}	0.002
NWI	0.04^{c}	0.001	0.08^{a}	0.002	0.08^{a}	0.001	0.05^{c}	0.001	0.09^{a}	0.001	0.08^{b}	0.001
PRI	−0.11^{a}	0.001	−0.11^{a}	0.001	−0.12^{b}	0.001	−0.10^{a}	0.000	−0.10^{a}	0.001	−0.11^{b}	0.001
NCPI	0.46^{a}	0.006	0.34^{c}	0.015	0.39^{b}	0.006	0.40^{a}	0.003	0.31^{c}	0.007	0.36^{b}	0.002
ARI	0.89^{a}	0.035	−0.33^{c}	0.103	0.40^{b}	0.038	0.62^{a}	0.016	−0.58^{c}	0.055	0.21^{b}	0.018
XES	5.01^{a}	0.023	4.94^{a,b}	0.026	4.78^{b}	0.028	4.94^{a}	0.026	4.38^{b}	0.028	4.38^{b}	0.023

Different superscript across moisture regimes within the same market class shows statistically significant difference (Alpha = 0.05).

[a] *DTH* days to heading (measured in days after sowing), *NWI* normalized water index, *PRI* photochemical reflectance index, *NCPI* normalized chlorophyll-pigment ratio index, *ARI* anthocyanin reflectance index *SG* stay green, *NDVI* normalized difference vegetation index, *SR* simple ratio, *XES* xanthophyll pigment epoxidation state, *Yield* measured in (t/ha), *GNS* grain number per spike, *TKW* thousand kernel weight (g), *TW* test weight (kg/hl), *PHT* plant height (cm), *PE* Peduncle extrusion (cm), *CT* canopy temperature (°C).

[b] Hard and soft groups represent the two market classes that delineate the two subpopulations.

[c] Moist-cool rain-fed environment was the high precipitation condition in Pullman, WA.

[d] SE was calculated from multiple trials within treatments.

3.4. Responses in plant height and peduncle extrusion

Plant height was lowest in drought, intermediate in high precipitation, and highest in irrigated environments. The soft winter subgroup had significantly lower mean plant height than the hard winter subgroup. Peduncle extrusion showed the second highest response to drought (40–50% reduction) next to yield reduction.

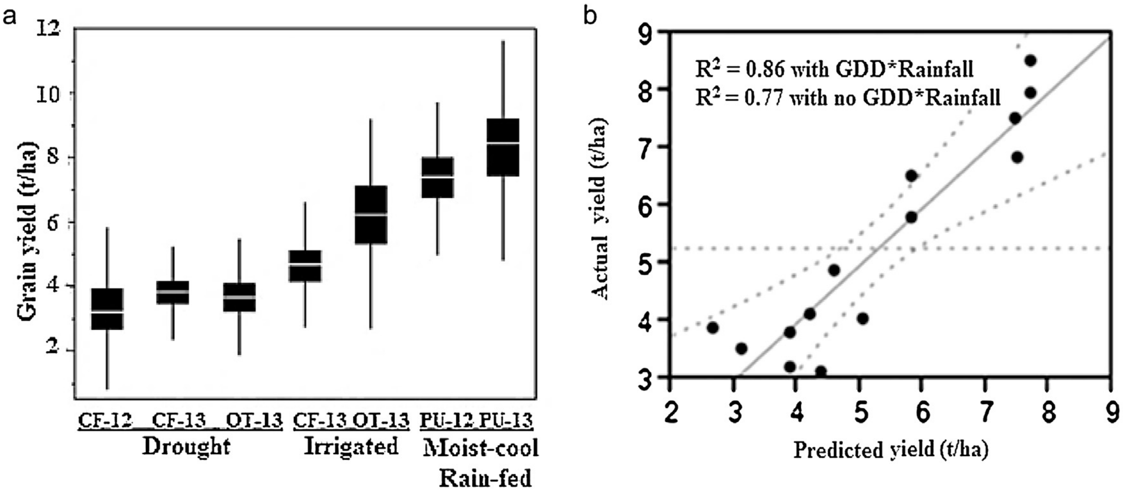

Fig. 2. (a) Mean grain yield over trials (CF, OT and PU stands for Central Ferry, Othello and Pullman experimental sites, respectively; and the two digits stands for year of experiment 2012 and 2013) and (b) model explaining grain yield (t/ha) by environmental variables (precipitation and thermal time).

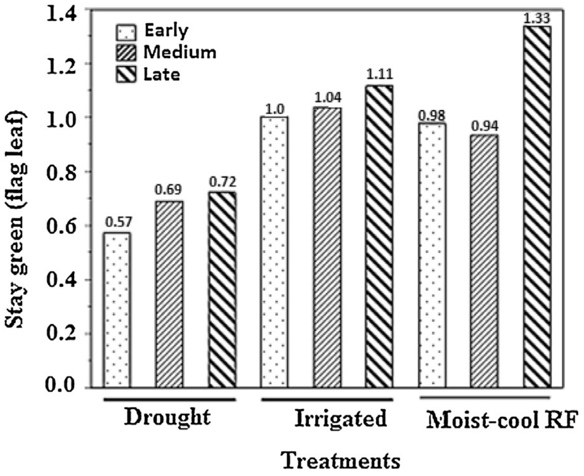

Fig. 3. Mean stay green estimate based on flag leaf senescence score in early, medium, and late heading genotypes of hard winter subgroup in drought, irrigated, and high precipitation conditions.

3.5. Response in reflectance indices and canopy temperature

Treatments showed significant differences in growth-specific SRI measurements, their means, and rate of decline over growth stages (Table 3). NDVI, SR, GNDVI, and NWI were highest in irrigated condition, intermediate in moist cool rain-fed, and lowest in drought condition (Table 3). Conversely, NCPI and ARI were lowest in irrigated condition, intermediate in moist-cool rain-fed, and highest in drought condition. There was no significant difference in mean PRI between treatments. The overall rate of NDVI decline was highest in drought, intermediate in moist-cool rain-fed, and lowest in irrigated condition. NDVI decline between heading and early grain fill was lowest in moist-cool rain-fed followed by irrigated and drought (Fig. 4). Canopy temperature in rain-fed condition was higher than irrigated conditions. The CT measurement under moist-cool rain-fed condition in soft winter wheat was excluded from analysis for its inconsistency.

3.6. Phenotypic association between grain yield and various traits

Grain yield showed positive correlation with grain number per spike in hard winter wheat and with thousand kernel weight in soft winter consistently across moisture regimes (Table 4). In soft winter wheat, grain number per spike and thousand kernel weight were negatively correlated under all environments whereas in hard winter, the negative correlation was statistically significant only in the irrigated condition.

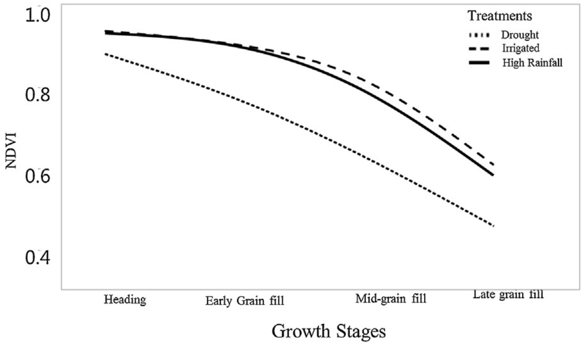

Fig. 4. Trend of NDVI decline over growth stages in PNW winter wheat population under drought, irrigated, and moist-cool rain-fed conditions.

The correlation between grain yield and days to heading under drought condition was not statistically significant in both subpopulations. In the hard winter subgroup, grain yield and days to heading were positively correlated under irrigated and moist-cool rain-fed conditions, whereas in soft winter subgroup slightly negative correlation was obtained under irrigated condition (Table 4). In soft winter wheat, thousand kernel weight was negatively correlated with days to heading in both subpopulations and across moisture regimes even though the association in hard winter wheat was weak and statistically insignificant under drought condition ($r = 0.13$; $p = 0.26$).

Negative correlation between grain yield and plant height was obtained in hard winter wheat under all moisture conditions whereas in soft winter wheat, these two traits showed positive correlation under drought, negative under irrigation, and statistically insignificant under moist-cool rain-fed condition. Peduncle extrusion and grain yield were positively correlated under drought and moist cool rain-fed conditions in soft winter whereas the two traits didn't show significant association in hard winter wheat.

Yield was positively correlated with NDVI, SR, GNDVI, and PRI, and negatively correlated with NCPI, ARI, and XES ($p < 0.001$) (Table 5). Generally, reflectance indices taken at mid-grain-fill stage showed stronger correlation with grain yield as compared to other

Table 4
Phenotypic correlations of developmental traits and yield components in Pacific Northwest winter wheat under drought, irrigated, and moist-cool rain-fed environments (association in soft winter is presented in upper diagonal and the association in hard winter wheat is presented in lower diagonal).

Variable[a]	TRT[b]	GY		GNS		TKW		TW		DTH		PHT		PE		CT	
		R[c]	*Sig.*[d]	r	*Sig.*	r	*Sig.*	r	*Sig.*	r	*Sig.*	r	*Sig.*	r	*Sig.*	*r*	*Sig.*
GY	I			0.10	0.08	0.20	***	0.24	****	−0.08	ns	0.34	****	0.33	****	−0.15	*
	II			−0.03	ns	0.21	****	0.11	0.07	−0.14		−0.19	**	−0.02	ns	−0.09	ns
	III			0.08	ns	0.26	***	−0.12	*	0.09	ns	0.08	ns	0.21	***	−0.24	****
GNS	I	0.28	*			−0.38	****	−0.39	****	0.16	**	−0.10	ns	−0.19	***	0.16	**
	II	0.36	**			−0.19	**	−0.36	****	0.18	**	0.00	ns	−0.17	**	−0.05	ns
	III	0.23	0.06			−0.22	***	−0.25	****	0.08	ns	−0.12	*	−0.21	***	0.22	***
TKW	I	0.16	ns	0.07	ns			0.34	****	−0.23	****	0.17	**	0.15	*	−0.21	***
	II	0.02	ns	−0.31	**			0.14	*	−0.28	****	−0.08	ns	−0.04	ns	0.05	ns
	III	0.02	ns	−0.03	ns			0.25	****	−0.15	**	0.03	ns	0.09	ns	0.01	ns
TW	I	−0.02	ns	0.15	ns	−0.07	ns			−0.07	ns	0.33	****	0.24	****	−0.11	0.06
	II	−0.01	ns	−0.26	*	0.04	***			−0.26	****	0.14	*	0.05	ns	−0.05	ns
	III	−0.14	ns	−0.09	ns	0.11	***			−0.20	***	0.19	**	0.11	0.07	−0.13	*
DTH	I	−0.05	ns	−0.08	ns	−0.13	***	−0.41	***			0.14	*	−0.10	0.07	0.08	ns
	II	0.23	8	0.18	ns	−0.23	*	−0.20	0.07			0.34	****	0.01	ns	−0.10	ns
	III	0.35	**	0.15	ns	−0.36	**	−0.43	***			0.31	****	0.22	***	−0.04	ns
PHT	I	−0.21	0.07	−0.04	ns	−0.04	ns	0.02	ns	0.02	ns			0.32	****	−0.20	***
	II	−0.28	**	0.07	ns	−0.20	0.07	0.15	ns	−0.04	ns			0.33	****	−0.18	**
	III	−0.32	**	0.17	ns	−0.19	ns	−0.16	ns	0.07	ns			0.47	****	−0.38	****
PE	I	0.09	ns	0.03	ns	−0.05	ns	0.08	ns	−0.11	ns	0.47	****			−0.19	**
	II	0.08	ns	0.01	ns	−0.12	ns	0.22	*	0.07	ns	0.11	ns			−0.06	ns
	III	−0.15	ns	0.29	*	−0.25	*	−0.19	ns	0.23	0.06	0.71	****			−0.21	***
CT	I	−0.05	ns	0.01	ns	−0.35	**	−0.03	ns	0.03	ns	−0.35	**	−0.24	*	−0.05	
	II	−0.22	*	−0.04	ns	−0.08	ns	−0.03	ns	−0.03	ns	0.17	ns	−0.08	ns	−0.22	
	III	−0.03	ns	−0.14	ns	−0.06	ns	0.07	ns	−0.06	ns	0.09	ns	−0.13	ns	−0.03	

[a] *GY* Grain yield, *GNS* Grain number per spike, *TKW* thousand kernel weight, *TW* test weight, *DTH* days to heading, *PHT* plant height, *PE* peduncle extrusion, *CT* canopy temperature.

[b] Treatments representing drought (I) irrigated (II), and moist cool rain-fed (III) conditions.

[c] r Correlation coefficient.

[d] Statistical significance as indicated by *p*-value *ns* nonsignificant: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$. Non-significant associations close to the 0.05 thresholds are indicated as actual *p* value.

Table 5
Phenotypic correlation of grain yield with mean vegetation indices derived from multiple measurements across growth stages in Pacific Northwest winter wheat.

Subgroup	Trait[a]	Drought			Irrigated			Moist-cool rain-fed		
		Early[b]	Medium	Late	Early	Medium	Late	Early	Medium	Late
Hard winter wheat	NDVI	0.51[c]	0.62	0.45	0.70	0.48	0.62	0.73	0.67	0.66
	SR	0.60	0.61	0.47	0.82	0.62	0.70	0.84	0.77	0.77
	GNDVI	0.50	0.55	0.40	0.81	0.65	0.73	0.77	0.69	0.71
	NWI	0.69	0.78	0.66	0.72	0.53	0.63	0.82	0.76	0.69
	PRI	0.38	0.45	0.27	0.74	0.52	0.48	0.66	0.63	0.52
	NCPI	−0.52	−0.73	−0.61	−0.71	−0.46	−0.53	−0.75	−0.70	−0.68
	ARI	−0.59	−0.70	−0.69	−0.71	−0.46	−0.51	−0.82	−0.79	−0.74
	XES	−0.59	−0.13	−0.25	−0.67	−0.41	−0.75	−0.61	−0.46	−0.66
Soft winter wheat	NDVI	0.65	0.68	0.68	0.49	0.51	0.35	0.54	0.37	0.41
	SR	0.64	0.60	0.65	0.51	0.54	0.31	0.66	0.51	0.50
	GNDVI	0.65	0.68	0.68	0.50	0.62	0.21	0.56	0.41	0.50
	NWI	0.76	0.75	0.70	0.63	0.67	0.46	0.68	0.59	0.39
	PRI	0.24	0.20	0.43	0.33	0.21	0.23	0.27	0.30	0.26
	NCPI	−0.59	−0.55	−0.60	−0.50	−0.38	−0.33	−0.54	−0.46	−0.38
	ARI	−0.65	−0.57	−0.66	−0.48	−0.44	−0.31	−0.68	−0.59	−0.51
	XES	−0.35	−0.41	−0.40	−0.33	−0.33	−0.26	−0.47	−0.35	−0.29

[a] *NDVI* normalized difference vegetation index, *NWI* normalized water index, *PRI* photochemical reflectance index, *NCPI* normalized chlorophyll-pigment ratio index, *ARI* anthocyanin reflectance index, *SG* stay green, *SR* simple ratio, *XES* xanthophyll pigment epoxidation state.

[b] Early, medium, and late represents phenology groups based on the respective heading date.

[c] All correlations were significant at $p = 0.01$.

growth stages (Table 6). However, the growth stage specific measurements and their slope over growth stage were less consistent among trials (Table 6).

Grain yield showed consistently positive correlation with stay green across moisture categories (S. Fig. 1). The correlations of grain number per spike and thousand kernel weight with NWI were statistically significant and consistent across moisture regimes ($p < 0.05$) whereas the associations with other indices were either population or environment dependent (data not shown). Canopy temperature showed negative correlations with grain yield and thousand kernel weight that were statistically significant at least in one moisture regime ($p < 0.05$). Canopy temperature was also significantly associated with plant height (Table 4) and various veg-

Table 6
Phenotypic correlation of grain yield with growth stage-specific NDVI measurements and parameters derived from multiple measurements.

Subgroup[a]	Parameter[b]	Trials[c]						
		I	II	III	IV	V	VI	VII
Hard	AUVIC	0.65	0.42	0.48	0.50	0.69	0.50	0.57
	Slope	0.06	0.48	0.43	0.38	0.66	0.49	0.25
	Mean	0.66	0.50	0.50	0.45	0.67	0.70	0.55
	Heading	0.55	0.39	0.13	0.22	0.11	0.47	0.30
	Early GF	0.67	0.44	0.54	0.52	0.09	0.69	0.46
	Mid GF	0.60	0.52	0.47	0.50	0.73	0.70	0.58
	Late GF	0.41	0.47	0.40	0.35	0.62	0.68	0.37
Soft	AUVIC	0.71	0.64	0.36	0.28	0.64	0.65	0.50
	Slope	0.31	0.47	0.25	0.10	0.53	0.66	0.30
	Mean	0.72	0.50	0.37	0.28	0.64	0.67	0.50
	Heading	0.56	0.02	0.10	0.33	0.56	0.41	0.29
	Early GF	0.58	0.49	0.42	0.32	0.39	0.64	0.47
	Mid GF	0.71	0.54	0.43	0.29	0.63	0.64	0.45
	Late GF	0.55	0.42	0.11	0.06	0.56	0.67	0.43

[a] Hard and soft market classes were considered as population subgroups.

[b] AUVIC area under vegetation index curve, slope and mean of NDVI calculated from growth.

[c] I Central Ferry 2012 (rain-fed), II Central Ferry 2013 (rain-fed), III Central Ferry 2013 (irrigated), IV Pullman 2012 (rain-fed), V Pullman 2013 (rain-fed), VI Othello 2013 (rain-fed), VII Othello 2013 (irrigated).

etation indices at least in drought and irrigated conditions (data not shown).

Overall, higher yield was associated with higher NDVI at grain-fill stages (Feekes 10–Feekes 11) (S. Fig. 3) and a low rate of NDVI decline over growth stages (Fig. 5). Correlation coefficients were strong and consistent across environments and subgroups when the average of SRI was used. Genotypes that have low yield in rain-fed conditions exhibited the lowest mean NDVI and sharpest decline over growth stages. The moist cool rain-fed condition had lower mean NDVI than that of irrigated condition at all stages except at the early grain-fill (Fig. 5). Despite this lower NDVI, highest yield was scored in the moist cool rain-fed condition due to the longer vegetative period (pre-heading) and delayed onset of physiological maturity.

Similar to the stay green character, growth stage-specific reflectance indices and their derived parameters were strongly correlated with heading date. In general, ARI, and NCPI were higher in early genotypes followed by intermediate and late. GNDVI, NDVI, NWI, PRI, and SR were generally higher in late heading genotypes followed by medium and early heading groups with the exception that NWI under drought and PRI under irrigation didn't show significant differences among early, intermediate, and late heading genotypes.

The stay green estimate calculated from flag leaf senescence was moderately correlated with canopy level stay green. The correlation of stay green with AUVIC ranged from 0.41 to 0.73 in the overall population and from 0.26 to 0.76 within phenology-based subgroups. Among the growth stage-specific NDVI measurements, the highest correlation was obtained either in the mid or late grain fill stage ($r = 0.58$ to 0.78; $p < 0.001$). The association was statistically significant and independent to the time of transitioning between vegetative and reproductive phases (S. Fig. 3).

3.7. Principal component analysis of traits

The principal component analysis based on covariance of agronomic and remotely sensed traits revealed a remarkable differentiation of the Pacific Northwest winter wheat that align with the soft and hard winter wheat market classes (Fig. 6). The soft white and club white subgroups showed high degree of overlap in this analysis. The first three principal components explained 76% of trait covariance across trials, two of which accounted for the 53%. The three components cumulatively explained 75%, 89%, and 84% of covariance among traits under drought, irrigated, and moist cool rain-fed conditions, respectively. In all conditions, ARI, NCPI, and CT were positioned in close proximity with each other and distant from the other traits including yield (Fig. 6), which is in agreement with the negative association obtained through correlation analysis (Table 5). The traits that were differentiated across the first dimension of PCA are presumed to be informative for comparative assessment of genotypes across the subgroups that were slightly differentiated in the second dimension. However, this has to be further investigated to determine the relevant dimensions that can be translated to indication of agronomic performance.

Likewise, the positions of GNDVI, NDVI, NDVI, NWI, PRI, and SR were mostly within a 90° radius relative to yield indicating the positive phenotypic association. Similar evaluation of the relative distance among indices suggests that ARI, NCPI, PRI, and NDVI have moderate co-linearity among one another and show better representation of the principal components. On the other hand, GNDVI and SR were positioned in close proximity with NDVI and NWI, respectively, indicating stronger co-linearity and possible redundancy of information.

4. Discussion

The highest yield recorded in moist cool rain-fed condition is consistent with the historical yield average of the site and is mainly attributed to its cool-moist growing condition. In contrast, the drought stress observed in this study (Fig. 1) is a typical pattern in PNW semiarid areas that starts with low soil water budget during planting and progressively worsens through grain fill (Donaldson, 1996). Othello and Central Ferry experience warmer summers that exacerbate the drought stress. Even when optimum soil moisture was maintained through irrigation, the heat stress was sufficient to trigger early senescence in most of the PNW winter wheat. The model that accounts for both temperature and soil moisture clearly showed that the drought stress was a combined effect of moisture deficit and high temperature which is a typical drought scenario in the region (Miles et al., 2010).

The higher yield response to irrigation in Othello than in Central Ferry was due to the relatively high temperature that sped up the onset of senescence and shortened the grain-fill duration in the Central Ferry. The extended pre-flowering growth and biomass accumulation should be balanced with post-flowering assimilate use to maximize yield gain from irrigation (Reynolds et al., 2001). The phenological events between planting and physiological maturity are highly modulated by genes controlling vernalization

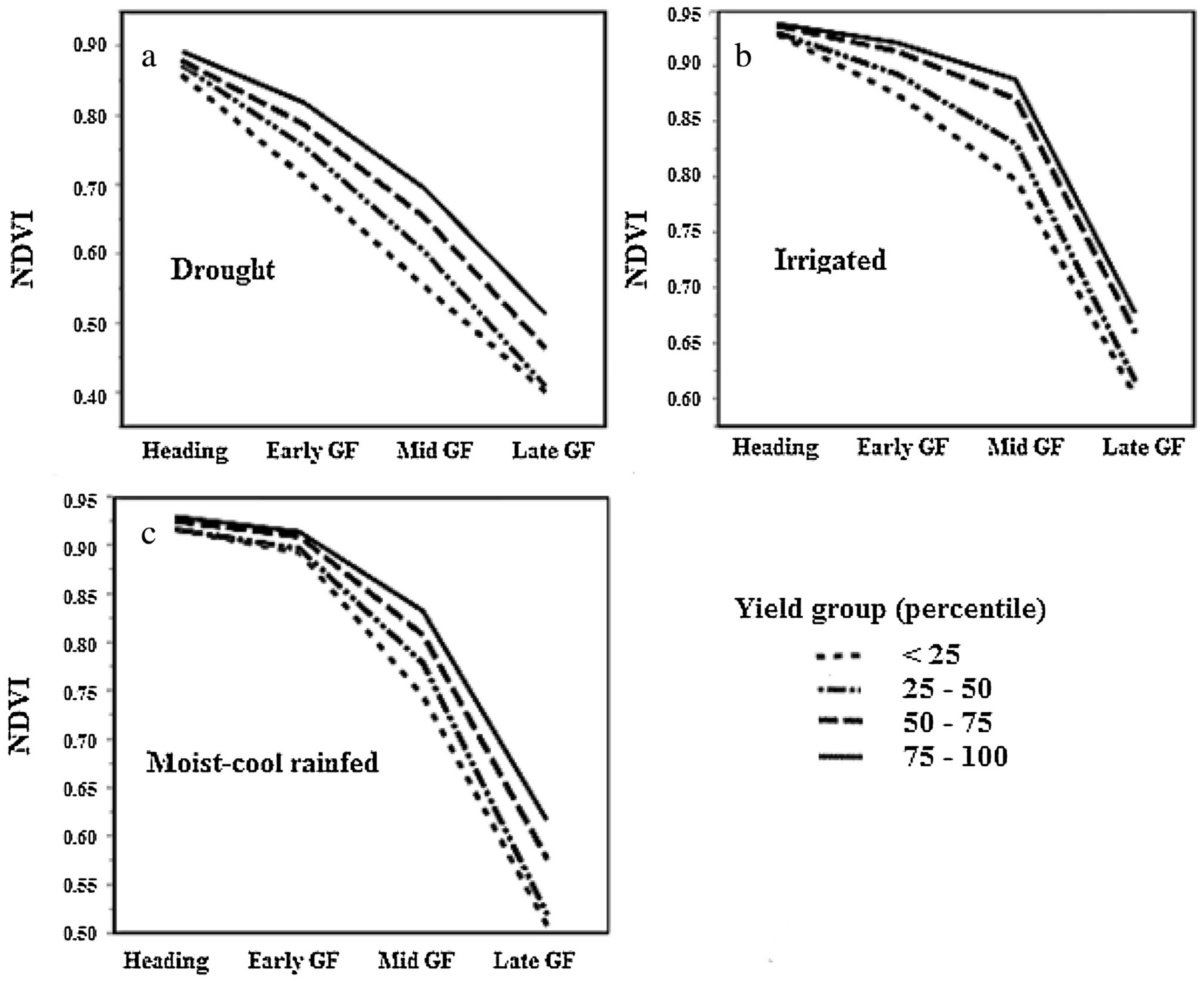

Fig. 5. Growth stage-specific measurements of NDVI and its decline curve over growth stages. The Y-axis shows the mean NDVI value and the X-axis shows the growth stage for each NDVI measurement. The trend of each line shows the change in mean NDVI across growth stages.

requirement (*Vrn*) and photoperiod sensitivity (*Ppd*) which determine the stem elongation, flowering, and onset of senescence (Chen et al., 2010). Prior information indicates that PNW winter wheat is predominantly photoperiod sensitive, meaning the accessions do not transition to reproductive phase before fulfilling the photoperiod requirement (Santra et al., 2009). As a result, only a few lines of the PNW accessions were observed to exhibit early transition to reproductive phase and physiological maturity. On the contrary, lines that have genetic relatedness to the Midwest breeding lines such as Jagger (PI 593688) and Karl (PI 564245) showed substantial degree of earliness. Some of the allelic variations in vernalization and photoperiod responsiveness genes reportedly have stress adaptive applications. Such variants have been used to develop early maturing winter wheat varieties that are designed to escape severe terminal drought and heat stress. We observed a strong correlation between the time to heading and physiological maturity. As a result, most of the early varieties had low biomass accumulation compared to the late genotypes as inferred from NDVI measurements (Fig. 4).

In our study, the variation in ear emergence between treatments was mainly in response to temperature differences. Early ear emergence as a result of moisture deficit was less than 2% when compared to the irrigated conditions in the same temperature condition. Such minimal drought response in phenology is expected when water deficit occurs after the onset of the reproductive stage. In support to this claim, the water stress in this experiment started at booting stage and progressively increased afterwards. Additionally, the soil water budget may have delayed the onset of drought response until after heading. This finding also suggests that grain yield is highly determined by those traits that are expressed during the grain fill stage.

4.1. Phenotypic association among agronomic traits

Early heading and completion of lifecycle before the onset of terminal heat and drought stress is perceived as a yield-positive adaptive mechanism. Such adaptive mechanisms are commonly used in Midwest through cultivars like Jagger and Karl to avoid terminal heat stress. However, we didn't observe a yield advantage of early heading and early physiological maturity in our study. Early maturity doesn't have overall yield advantage in environments where soil moisture deficit occur post-flowering and annual rain fall fluctuates between years (Foulkes et al., 2007). Early transitioning to reproductive stage followed by early onset of senescence has apparent yield advantage in years of sever terminal drought. This positive association was evident in 2013 and 2014 growing seasons when drought and high temperature started post flowering and progressed thereafter. However, early transitioning to repro-

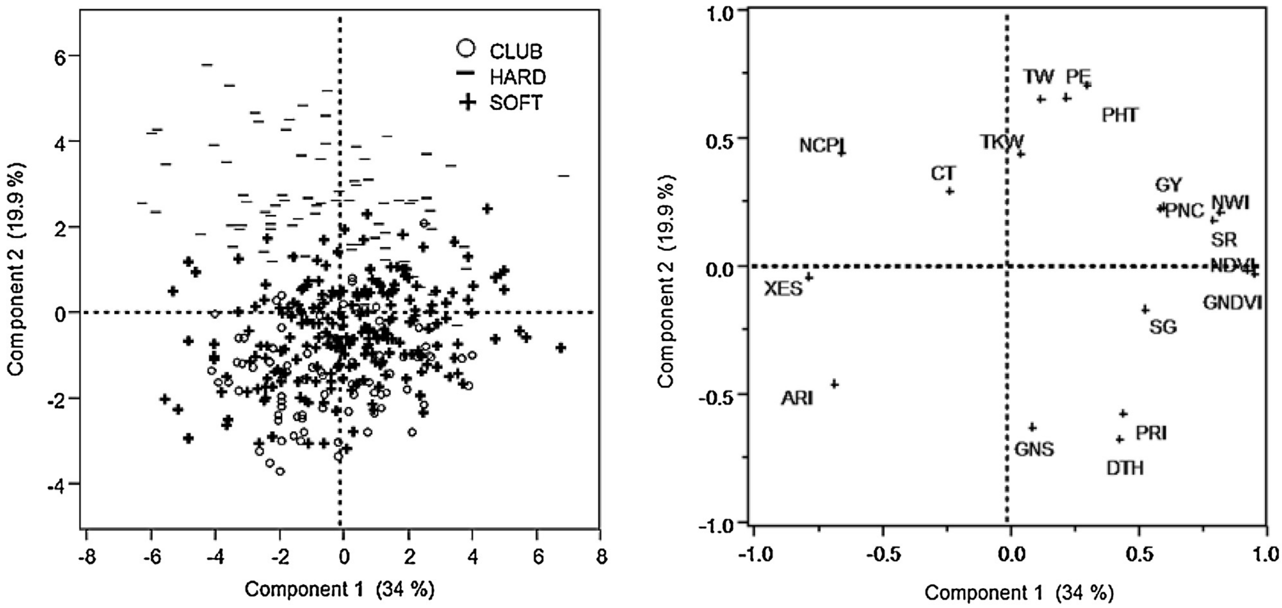

Fig. 6. The first two principal components of genetic covariance among major market classes (left) and agronomic and physiological traits (right) in Pacific Northwest winter wheat population: *DTH* days to heading, *SG* stay green, *PHT* plant height, *PE* peduncle extrusion, *GY* grain yield, *TW* test weight, *TKW* thousand kernel weight, *CT* canopy temperature, *NDVI* Normalized difference vegetation index, *SR* simple ratio, *NWI* Normalized water index, *PRI* Photochemical reflectance index, *NCPI* Normalized chlorophyll-pigment ratio index, *ARI* Anthocyanin reflectance index, *XES* Xanthophyll pigment epoxidation state.

ductive stage in response to combined effect of high temperature and water deficit would not have yield advantage when remnant soil moisture is left unused as evident in 2012 trial. These contrasting effects might have compensated with each other resulting a neutral effect of earliness during the study period.

The long vegetative period (late heading) observed under irrigation didn't increase yield (Table 4), suggesting that the accumulated assimilates during long pre-flowering period were not completely utilized during the short grain fill duration (Understande and Christiansen, 1986; Slafer and Rawson, 1994; Wheeler et al., 1996). Additionally, lack of standard plant stature in some accessions led to continuous increases in height and very late transition to reproductive phase, reducing overall efficiency of assimilate partitioning (Marri et al., 2005). On the other hand, the positive association between heading date and grain yield observed in moist cool rainfed condition resulted from the lower temperature and optimum soil moisture which extended the physiologically active period for assimilate synthesis, reserve, and relocation for normal grain development (Lopes and Reynolds, 2012). As a result, genetic variation in this trait could be utilized to improve yield in environments that have moderate thermal time and remnant soil moisture as the plants are close to maturity.

The traits that showed consistent association with grain yield across environments may be the most desired traits to stabilize yield in the changing environment. Thousand kernel weight, grain number per spike, and stay green fall in this category and will allow further yield improvement in wide range of environment. The compensatory interaction of grain number and grain weight was highlighted by the negative correlation of these traits in all moisture regimes. This relationship is attributed to insufficient rate and duration of grain fill as discussed in Duguid and Brule-Babel (1994). Due to this compensation effect, breeders often improve grain yield either through improving grain weight or grain number per plant. Such differential selection history may have caused the remarkable differentiation between soft and hard market classes of PNW winter wheat in the degree of phenotypic association between thousand kernel weight, grain number per spike, and grain yield. On the other hand, the environment specific associations of grain yield with canopy temperature, plant height, and days to heading indicate the possibility of improving local productivity by selecting alternative variants of these traits.

Positive association between yield and the stay green character was found in all studied conditions. Unlike phenology and plant stature, stay green is the genotypic ability to maintain hydration status and system integrity and to sustain photosynthesis and assimilate relocation (Thomas and Smart, 1993; Christopher et al., 2008). This result suggests that variation can be utilized to co-improve both yield potential and drought tolerance in PNW winter wheat. Genotypic stay green characteristics could be attributed to various morpho-physiological traits such as root access to deep soil moisture, efficient transpiration system, and plant architecture that were introgressed in due course of the regional breeding effort. Genotypes that have higher stay green in this study can be further evaluated if they possess distinctive composition of these alternative traits and explore the possibility of accumulating them to further increase agronomic performance.

4.2. Relationship between reflectance indices and grain yield

In general, SRIs measured at mid-grain-fill were more strongly associated with yield than at other growth stages. Previous reports have suggested that this stage is more informative because genotypic variations in leaf area index and subsequently in SRIs are low during heading and early vegetative stages (Babar et al., 2006). In addition, early measurements are also confounded by saturation problems of some indices (unable to discern genotypes for subtle differences) near their peak value (Jiang and Huete, 2010). The correlations across environments and subgroups were less consistent for growth stage specific measurements as compared to the parameters derived from multiple growth stages (Table 6).

The correlation coefficients of grain yield with NDVI measured at heading, early grain fill, and late grain fill were not significant in at least one trial. On the other hand, the correlation of grain yield with AUVIC, weighted mean, and arithmetic mean were comparable to the highest correlations of growth stage specific measurements. As a result, selection of high yielding genotypes at the grain fill stage seems more efficient. However, the environmental variation in multi-location and multi-year phenotyping may cause higher genotype-by-environment interaction on single compared to multiple measurements (Babar et al., 2006). In such cases, multiple measurements across growth stages may be preferred to capture the response of various physiological attributes that cumulatively affect yield.

4.3. Relationship between stay green and vegetation indices

The relationship between spectral reflectance and stay green can be further utilized to develop a throughput phenotyping alternative for the stay green trait. Stay green in wheat is not just a delay in leaf senescence, but also a slowly progressing loss of greenness in the peduncle and spike (Reynolds et al., 2009). As a result, the canopy level spectral reflectance provides a more inclusive estimate of stay green.

4.4. Multi co-linearity of traits and dimension reduction

As a major limitation at least from a statistical point of view, spectral reflectance indices exhibit substantial multi co-linearity among themselves. This is because most indices are derived from either infrared, visible, or both light spectra which plants exhibit similar absorption and reflectance behavior. The clustering of NDVI, SR, GNDVI, and PRI in one dimension of component analysis was of their physiological connection to vegetative growth, biomass, and pigment composition. As demonstrated in this study, analysis of genotypic covariance for different indices can be effectively used to reduce information redundancy by identifying distinctive indices that represent different physiological attributes. This analysis will help in developing a reduced-dimension predictive model for grain yield. The observation that some indices were found to be distinctive from each other validates previous reports that these indices represent different physiological attributes of yield and yield stability: NDVI with biomass, pigment abundance, and stay green; NCPI with photosynthetic efficiency in stressed condition, composition of stress related pigments; NWI for and plant hydration status (Babar et al., 2006; Reynolds et al., 2012).

5. Conclusions

Drought intensity in eastern Washington and yield response of the locally adapted winter wheat germplasm was affected by the combination of higher growing degree days and soil moisture deficit. Therefore, it is important to consider both growing degree days and available moisture to characterize yield and yield stability. The study also highlighted the importance of characterizing drought responses and phenotypic association of yield determining traits to fully understand the mechanisms of drought tolerance. The variation in phenology among treatments was mainly in response to temperature variation between the sites. Because the effect of earliness *per se* on yield can be neutral, positive, or negative depending on the timing and intensity of the stress, breeders usually standardize their genetic materials with desired phenology that maximizes the cultivar's performance in target environments. The moderate to high statistical power of SRI-based models to predict yield in all conditions shows an impending solution to overcome phenotyping bottleneck and characterize larger germplasm collections. Positive associations between yield and SRI-based estimates of stay green were found in all conditions making this trait one of the promising physiological traits that can be utilized to co-improve both yield potential and drought tolerance. In general, the proxy measurements will be useful to further improve yield potential and yield stability by facilitating selection for yield positive traits such as stay green, above ground biomass, and plant hydration status.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fcr.2016.06.018.

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