Using Spectral Reflectance for Genome Wide Association Study and Germplasm Selection for Yield in Pacific Northwest Winter Wheat Breeding Programs

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Introduction: Winter wheat germplasm in the Pacific Northwest (PNW) has been selected for yield, yield stability, end-use quality, farming preferences, and disease resistance for over 100 years. Wheat cultivars adapted to the region vary for emergence, early canopy establishment, root growth and development, winter survival, osmotic adjustment, optimum maturity, and plant architecture. Aside from these agronomic characteristics, adaptation to different climatic conditions is needed as well. Not only do climate conditions change about every 20 km in any direction in the PNW, year to year variations can be extreme as well. For example, in 2011 and 2012, the PNW saw very cool, wet spring conditions with very dry summer months. In 2015, conditions were hot and dry in both the spring and summer months, whereas 2016 showed very mild temperatures and increased precipitation during the summer. These variations make it difficult to select breeding lines and predict their performance across climactic regions and years. Recent advancements in genomics and high-throughput phenotyping (HTP) have led many plant breeders to ask if selection on these parameters can be used to predict performance of breeding lines across locations and years. Recent research using large diversity panels and genome-wide association mapping has indicated that genomics and HTP can be used as indirect selection parameters for yield potential under different production regions. Few publications are found validating that data generated from these populations can be transferred to a breeding program, where breeding lines can be selected for and advanced based on models derived from other populations. The objective of this research was to validate if 1) genomic regions and 2) canopy spectral reflectance indices associated with grain yield potential can predict performance across locations and years.

Materials and Methods: Two populations were used for this research. Panel #1 was derived from a soft white winter wheat diversity panel of 480 lines from regional breeding programs in the PNW. Panel #2 consisted of 319 lines derived primarily from the WSU and USDA soft winter wheat breeding programs. Panels were grown from 2012 to 2016. Panel #1 was grown in Pullman, WA for three years and in Lind, WA for one year. Panel #2, in order to validate the results from Panel #1, was grown in Central Ferry and Othello, WA, under irrigated and rainfed conditions for two years and in Pullman, WA for three years under rainfed conditions. Plots were planted using a double disc planter in 4.65 m² plots. Grain yield was collected using a Zurn 150 small plot combine (Zurn Harvesting; Germany). Data were collected on grain yield (kg/ha), thousand kernel weight (TKW, g), test weight (TW, kg/hl), and grain number per spike (GNS). Canopy reflectance was measured at multiple growth stages using the CROPSCAN multispectral radiometer (CROPSCAN, Inc.; Rochester, USA), and used to derive several spectral reflectance indices as described by Gizaw (2016). Both panels were genotyped using the Illumina Infinium iSelect SNP marker array as described by Cavanagh et al. (2013).

Canopy spectral reflectance indices were calculated for Panel #1 from Pullman in year one. For each index, the top 25% and bottom 25% of lines were grouped separately. One-way ANOVAs were used to compare the grain yield means between the two groups at each of the four locations where yield data was collected (Pullman Years 1-3 and Lind Year 1). Those indices which showed significant p-values ($P < 0.05$) across all four locations were validated in Panel #2 in a similar manner. In Panel #2, the selected indices calculated from canopy spectral reflectance data collected across three years in nine environments were used to separate the top 25% and bottom 25% of lines into groups in the same way as was done in Panel #1. A one-way ANOVA test was used to compare the mean yield of the two groups at each location based on the groupings done using canopy spectral reflectance data at that same location.

Data for grain yield, canopy spectral reflectance indices, and 3653 SNP markers were used to perform a genome-wide association study on Panel #1 as described in Gizaw (2015). In order to validate results from Panel #1 GWAS, all QTL markers found were run on Panel #2. Yield data were then compared between lines with differing numbers of favorable alleles to estimate the effect of selection on genomic regions associated with grain yield potential.
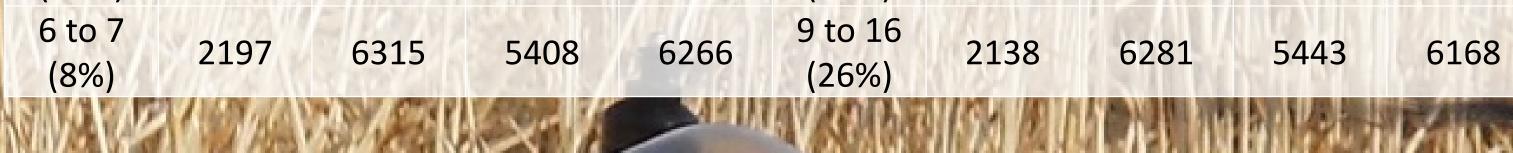
Works Cited:
Cavanagh et al. (2013) PNAS 100:8057-8062
Gizaw et al. (2016) Field Crops Research 196:168-179
Gizaw (2015) PhD Dissertation, Washington State University

Table 1 Grain yield potential of Panel #2 when selected for different numbers of accumulated favorable alleles for grain yield and reflectance indices based on GWAS markers from Panel #1.

21 favorable alleles possible	Yield markers				37 favorable alleles possible	Reflectance Index markers			
	Lind	Pullman				Lind	Pullman		
	Year 1	Year 1	Year 2	Year 3		Year 1	Year 1	Year 2	Year 3
12 to 15 (22%)	2123	6284	5425	6049	21 to 25 (18%)	2122	6453	5447	6178
10 to 11 (38%)	2159	6378	5342	6107	19 to 20 (27%)	2136	6255	5300	6057
8 to 9 (32%)	2121	6297	5418	6192	17 to 18 (29%)	2162	6354	5393	6148
6 to 7 (8%)	2197	6315	5408	6266	9 to 16 (26%)	2138	6281	5443	6168

Table 2 One-way ANOVA indicating if canopy spectral reflectance measurements taken at three different growth stages were able to predict grain yield potential across four location/years in Panel #1.

Index	Growth Stage	Lind	Pullman		
		Year 1	Year 1	Year 2	Year 3
NDVI	Heading	0.179	3.11E-02	3.86E-06	3.72E-06
NDVI	**Anthesis**	**8.02E-06**	**2.18E-03**	**1.76E-09**	**1.06E-02**
NDVI	Milk Stage	3.53E-07	4.59E-05	1.93E-09	0.509
SR	Heading	0.191	2.88E-02	2.54E-06	4.45E-06
SR	**Anthesis**	**8.02E-06**	**2.18E-03**	**1.76E-09**	**1.06E-02**
SR	Milk Stage	4.78E-07	3.48E-05	2.05E-09	0.49
GNDVI	**Heading**	**3.84E-02**	**3.29E-04**	**2.66E-08**	**7.28E-07**
GNDVI	**Anthesis**	**1.35E-07**	**1.11E-04**	**2.82E-09**	**4.82E-02**
GNDVI	Milk Stage	2.27E-08	4.98E-06	2.18E-11	0.343
PRI	Heading	1.88E-08	1.59E-13	9.53E-02	0.424
PRI	Anthesis	1.65E-08	7.57E-08	1.19E-04	0.771
PRI	Milk Stage	6.46E-07	0.143	2.54E-10	0.216
NCPI	Heading	5.22E-02	7.09E-01	9.42E-06	3.19E-03
NCPI	**Anthesis**	**3.91E-05**	**2.04E-02**	**1.03E-13**	**7.38E-03**
NCPI	Milk Stage	4.91E-07	0.127	9.34E-13	0.688
ARI	**Heading**	**7.98E-05**	**2.55E-04**	**3.89E-05**	**2.23E-04**
ARI	Anthesis	4.34E-09	1.30E-06	8.09E-09	0.29
ARI	Milk Stage	1.18E-01	8.82E-01	1.06E-11	0.283
NWI	Heading	4.53E-01	2.19E-01	1.22E-15	3.33E-07
NWI	Anthesis	8.14E-07	9.06E-02	1.29E-03	0.114
NWI	Milk Stage	2.87E-11	7.42E-12	7.65E-05	0.296
XES	Heading	1.98E-02	5.77E-02	2.98E-05	4.60E-04
XES	Anthesis	1.53E-06	9.52E-03	1.52E-03	9.86E-02

Results:

- 21 markers showed favorable association with grain yield components in panel #1 (Supp. Table)
- 37 markers showed favorable association with reflectance indices predictive of grain yield in panel #1 (Supp. Table)
- Selecting lines in panel #2 with more accumulated favorable alleles for either yield component or reflectance indices did not increase grain yield potential (Table 1)
- In panel #1, only three lines were in the top 10% of highest yielding lines, and in only three of the four location/years
- Six indices were predictive of grain yield potential in all four location/years in panel #1 (Table 2)
- Using these indices as selection criteria in panel #2, only two, GNDVI and ARI taken at heading, were predictive of grain yield potential across all locations and years tested (Table 3)

Table 3 One-way ANOVA indicating the ability of significant canopy spectral reflectance indices from Panel #1 to predict grain yield potential of the top and bottom 25% yielding lines in Panel #2 over years, locations, and cropping system management.

Index	Growth Stage	Central Ferry				Othello		Pullman	
		Irrigated		Rainfed		Irrigated	Rainfed	Rainfed	
		Year 3	Year 4	Year 5	Year 3	Year 4	Year 4	Year 3	Year 4
NDVI	Anthesis	0.165	0.935	2.00E-16	2.00E-16	0.722	5.45E-16	2.00E-16	5.06E-07
SR	Anthesis	0.169	0.935	2.00E-16	2.00E-16	0.722	5.45E-16	2.00E-16	5.06E-07
GNDVI	Heading	--	7.15E-08	2.00E-16	5.96E-06	2.97E-05	3.02E-12	2.00E-16	2.00E-16
GNDVI	Anthesis	0.241	0.996	2.00E-16	0.39	2.00E-16	0.42	1.14E-08
NCPI	Anthesis	0.892	0.707	2.47E-08	2.00E-16	0.903	9.00E-09	2.00E-16	1.48E-09
ARI	**Heading**	--	**4.58E-02**	**2.00E-16**	**1.03E-06**	**1.44E-07**	**8.67E-11**	**2.00E-16**	**7.34E-02**	**2.00E-16**

Discussion:

- Grain yield potential was variable across years and locations, with high genotype by environment interactions
- Selecting on favorable alleles for complex traits like grain yield potential was not useful under limited associated loci
- Identification of lines with higher accumulation of favorable alleles may show significant differences (15 favorable alleles out of 21 was the most identified for yield components)
- Selection using an increased number of alleles (genomic selection) should be more useful
- GNDVI and ARI, taken at heading, appear to be useful in predicting grain yield potential
- High-throughput phenotyping can be useful in selecting breeding lines with high yield potential across locations and years

Supplementary Table. List of markers significantly associated with grain yield components and spectral reflectance indices across four location/years in Washington using GWAS analysis from Panel #1.

Grain Yield Components								Reflectance Indices			
Tag SNP (9K index)	Chrom.	Position (cM)	Trait Associated with QTL	Tag SNP (9K index)	Chrom.	Position (cM)	Trait Associated with QTL GNDVI & NDVI	Tag SNP (9K index)	Chrom.	Position (cM)	Trait Associated with QTL GNDVI & SR
IWA3766	1A	61.63	GNS	IWA3740	1A	71.21		IWA2743	5A	122.84	
IWA2849	1A	63.98	GNS	IWA919	1B	121.86	PRI	IWA1	5A	123.21	XES
IWA4579	1A	68.51	PNC	IWA4587	1D	89.85	NCPI	IWA3391	5A	186.29	NCPI
IWA3883	1A	72.74	TKW	IWA2007	2A	84.73	PRI	IWA3479	5B	152.84	SR
IWA2818	1A	150.06	PNC	IWA2971	2A	117.57	XES	IWA3226	5B	153.10	GNDVI
IWA8135	1A	150.06	PNC	IWA2977	2B	114.69	GNDVI	IWA3226	5B	153.10	NDVI
IWA5483	1A	179.03	GY	IWA2253	2B	160.44	NCPI	IWA3226	5B	153.10	NCPI
IWA8084	1B	29.50	PNC	IWA7840	2D	168.49	NDVI	IWA7452	6B	50.76	NWI
IWA1496	2A	117.57	TKW	IWA6229	3A	64.84	NDVI	IWA2300	6B	72.44	PRI
IWA4095	2B	200.47	GY	IWA7476	3A	81.14	SR	IWA971	6B	83.04	GNDVI
IWA4097	2B	200.47	GNS	IWA4851	3A	116.24	PRI	IWA7111	6B	91.66	GNDVI & NDVI
IWA7353	3B	87.46	PNC	IWA4851	3A	116.24	NCPI	IWA4173	7A	174.73	XES
IWA4320	4A	35.30	PNC	IWA5980	3A	127.51	XES	IWA4175	7A	175.68	NDVI
IWA6196	5A	54.19	GY	IWA5982	3A	127.51	XES	IWA1437	7B	19.76	GNDVI
IWA6521	5B	47.01	PNC	IWA5212	3A	127.51	SR	IWA7830	7B	42.82	GNDVI
IWA6429	5B	52.14	GNS	IWA5982	3A	127.51	SR	IWA6667	7B	57.38	SR
IWA4757	5B	63.66	PNC	IWA3333	3B	85.13	NDVI				
IWA7776	5B	115.34	PNC	IWA1992	3A	43.98	NCPI				
IWA1997	6A	114.73	GY	IWA5831	4A	43.98	NCPI				
IWA3913	6D	117.60	TKW	IWA3489	4A	67.02	PRI				
IWA4173	7A	174.98	PNC	IWA1007	4B	68.33	XES				