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Analysis of genotype-by-environment interaction in winter wheat growth in organic production system

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ABSTRACT

The goal of this study was to evaluate the performance of 36 wheat winter (*Triticum aestivum* L.) lines in organic systems in three locations in Nebraska, to compare the performance of the released cultivars with experimental lines to help in the process of selection, to study the magnitude and behavior of genotype-by-environment interaction for grain yield, anthesis date, plant height, protein content, grain volume weight and vegetation index, and to identify the more stable genotypes. Linear mixed models and site regression model was implemented for reaching the objectives of the present research. Genotypic and GE interaction are significant across the three locations for all traits except for anthesis date. Environment were significant for the six traits. Yield is negative correlated with protein content and plant height. In general the genetic correlation explained more of the genotype performance, although the GE interaction was significant. The best genotypes for grain yield across the three environments were genotypes NW03666, SD07165, NE07444 and Overland. For vegetation index the best lines were: Lyman and Buckskin. For grain volume weight the best lines were: Lyman, NW03681, Danby and Goodstreak. For anthesis date all genotypes were similar. For plant height, the best lines were Goodstreak, Buckskin and Clarkscream. For protein content, the best lines were Goodstreak, Karl92, Lyman, and Clarkscream. In general the average grain yield of the experimental lines was better than the released lines. For anthesis date, the performance was similar between experimental and released lines. However, for vegetation index, plant height, grain volume weight and protein content, the average performance of the experimental lines was lower than the released lines.

Keywords: *Triticum aestivum* L.; Yield, Protein; Genotype x environment interaction; Stability

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a major crop to feed the world's population. Out of the total world production, about 53% is used in developed countries, and around 85% usage is reported in the developing countries (Denčić et al., 2011). In 2010, 647,497 hectares (ha) of wheat were planted in Nebraska and 602,981 ha were harvested with an average yield of 2891.75 kg/ha for a total production of 1,743,985.4 ton. But only small proportion of this production was produced organically. However, the demand of organic products continues to increase because organic wheat is an indispensable ingredient in many processed organic products. However, there has to date been little attention paid by public wheat breeders to evaluate and develop cultivars

adapted specifically to organic production systems. Remedying this lack of information and lack of truly adapted lines is of paramount importance because the traits needed for organic production are not the same as those needed for conventional production (Baenziger et al., 2011). In general, winter wheat cultivars are being used by organic farmers under conventional high-input conditions, however, these cultivars may not reach their full genetic potential because organic soils are frequently limited in nutrients and fertilizers. Better nutrient uptake efficiency would be of great value for organic farms and conventional farms producing under low-input conditions (Hildermann et al., 2008). For this reason, winter wheat cultivars are being developed that will improve profitability and competitiveness of organic producers (Baenziger et al., 2011). However, it

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is important to evaluate lines under organic production conditions in different environments. Multienvironment trials are important in plant breeding for evaluating genotypes for their overall stability and adaptability in the presence of genotype by environment (GE) interaction. An understanding of GE interaction is important at all stages of plant breeding, including ideotype design, parent selection, selection based on traits, including grain yield (Yan et al., 1998). The GE interaction causes should be identified in order to establish breeding objectives, identify ideal testing locations and conditions, and come up with suggestions for areas of optimal cultivar adaptation. Many studies had been conducted to study the GE interaction in winter wheat. For example, Mengistu et al. (2010) reported hard winter wheat cultivar blends for the grain yield between blends and their component cultivars over several locations in Nebraska to compare blend grain yield stability and they found that when compared with the average of component cultivars in the blend, cultivar blends were more stable over different environments with little or no reduction in grain yield. Taghouti et al. (2010) studied GE interaction for quality traits in durum wheat (*Triticum durum* L.) cultivars adapted to different environments. They concluded that the genetic variation for sedimentation volume, yellow pigment index and grain volume weight was larger than the environmental variation, indicating the greater influence of genotypes of these traits. However, for vitreousness and protein content, the environmental effect was greater than the genetic effect. These studies show that wheat yield and other agronomic and quality traits vary considerably as a result of genotype, environment and their interaction (Allard et al., 1964; Basford et al., 1998; Trethowan et al., 2007; Denčić et al., 2011). For these reasons, to develop excellent winter wheat cultivars for organic production, it is extremely important to select and test lines in organic systems in different ecological regions. For this reason, the main contribution of this paper will be that it is the first one that evaluate GE interaction of winter wheat cultivar under organic production. To develop organic wheat cultivars suited to increase competitiveness and profits of organic producers in Nebraska and the Northern Great Plains. The objective of this study was to evaluate the performance of 36 wheat winter lines for organic systems in three locations in Nebraska (Clay, Dixon and Saunders counties) to: (1) To compare the performance of the released cultivars with experimental lines to help in the process of selection, (2) to study the magnitude and behavior of genotype-by-environment interaction for six agronomic traits (grain yield, anthesis date, plant height, protein content, grain volume weight and vegetation index) and (3) to identify the more stable genotypes for these traits.

MATERIALS AND METHODS

Cultivar selection and growth conditions

Thirty-six hard winter wheat lines were used in this study of which 20 lines were released. Clarkscream and Buckskin were released in the early 1970's. Lyman and McGill were released in 2009 and 2010 respectively and the other 16 were released before 2009. Sixteen lines are under consideration for release. The lines were grown in three Nebraska locations (Dixon, Clay and Saunders counties) that are in the western cornbelt ecoregion. The general attributes of each location are given in Table 1. Locations hereafter referred to by their respective counties.

Traits measured

Yield is measured in kilograms per ha (kg ha^{-1}). Grain volume weight (GVW) is the weight of a sample of grain in a small cup, converted to kilograms per hectoliter (kg/hl kg hl^{-1}). Anthesis date (Adate) is the date in May at which half of the tillers in a plot reach anthesis (indicated by visible emergence of anthers). The Normalized Difference Vegetation Index (NDVI) is the relative reflectance of plant material and soil at two wavelengths: Visible (red, 660 nm) and infra-red (NIR, 770 nm). $\text{NDVI} = (\pi\text{NIR} - \pi\text{VIS}) / (\pi\text{NIR} + \pi\text{VIS})$. NDVI was measured with a hand-held Greenseeker® at 0.6096 meters (m) above the canopy, and is the average of 40 to 60 measurements per plot. Measurements were taken at Feekes 7 or 8 stage. Height is the visual average distance from soil to top of the head excluding awns for mature plants for each plot and was measured in cms. Protein content was measured by near infra-red reflectance (NIR) as a grams of protein per kg (g kg^{-1}) of flour using a FOSS Tecator NIR.

Design and analysis

At each location in 2011, the 36 lines were grown in a randomized complete block design (RCBD) and we measured grain yield, anthesis date, plant height, protein content, GVW, and NDVI. In Clay all traits had 6 replication with exception of Adate which was only measured in the first two repetitions. In Dixon all the traits were measured on 4 replications, while in Saunders all the traits were measured on 5 replications with the exception of anthesis date which was measured using 4 replicates. The combined linear model over all locations for one trait, Y_l , is:

$$Y_{ijk l} = \mu_l + E_{jl} + R(E)_{jkl} + G_{il} + GE_{ijl} + \varepsilon_{ijk l} \quad (1)$$

where μ_l is the mean effect on trait l , E_{jl} is the effect of location j on trait l , $R(E)_{jkl}$ is the effect of block k within location j on trait l , G_{il} is the effect of genotype (or pedigree) i on trait l , GE_{ijl} is the effect of the

Table 1: Attributes of each location

Attributes	Location		
	Dixon	Clay county	Saunders
Longitude	96.57.31.7	98.08.40.4	92.29.40.6
Latitude	42.23.03.0	40.34.29.5	41.09.14.3
Previous crop	oats/clover	Soybeans	Soybeans
Tillage after previous crop	Disc	Rototill	None
Soil test	NA	NA	NA
Soil type	Nora Silty Clay Loam	Hastings Silt Loam	Tomek Silt Loam
Planting date	9/30/2011	10/8/2011	10/7/2011
Row spacing	7.5 inches	7.5 inches	7.5 inches
Planting depth	1.0 to 1.5 inches	1.5 inches	0.5 to 1.5 inches

interaction between genotype i and environment j on trait l , and $\epsilon_{ijk l}$ is the random experimental error effect associated with genotype i and block k within location j on trait l . All variance components need $R(E)_{jkl} \sim N(0, \sigma_{R(E)}^2)$, $G_{il} \sim N(0, \sigma_G^2)$, $GE_{ijl} \sim N(0, \sigma_{GE}^2)$ and $\epsilon_{ijk l} \sim N(0, \sigma^2)$, are assumed random, while locations, were considered fixed, because only three environments were studied.

An ANOVA was performed for each location and for each response variable using a RCBD. A combined ANOVA over the three locations (Clay, Dixon and Saunders) was performed for each trait after checking the homogeneity of error variances. We estimated Heritability (h^2) according to (Cooper et al., 1994) from the analysis of variance. The broad sense heritability over all environments

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GE}^2 / r + \sigma_E^2 / rL}, \sigma_G^2$$

denotes the genotypic variance, σ_{GE}^2 denotes the genotype–environment interaction variance, σ_E^2 is the residual error variance, L is the number of locations and r is the number of replications. Genotypic and phenotypic correlations between traits were estimated using REML following (Holland, 2006). The combined linear models over all traits and locations is given by:

$$Y_{ijkl} = \mu + E(Trait)_{jl} + R(E)(Trait)_{jkl} + G(Trait)_{il} + GE(Trait)_{ijl} + \epsilon_{ijk l}(Trait) \quad (2)$$

Where μ the overall mean and is fixed term, $E(Trait)_{jl}$ is considered a fixed term because we have only three environments, $R(E)(Trait)_{jkl} \sim N(0, \sigma_{R(E)(Trait)}^2)$, $G(Trait)_{il} \sim N(0, \sigma_{G(Trait)}^2)$, $GE(Trait)_{ijl} \sim N(0, \sigma_{GE(Trait)}^2)$, and $\epsilon_{ijk l}(Trait) \sim N(0, \sigma^2)$. Using this model

(Eq. 2) we can estimate the genetic covariance, the GE covariance and the error covariance matrices. For estimating the phenotypic correlation matrix among locations in model (2) we exchange Trait by E and vice versa. All analyses were performed using SAS software (SAS, 2003).

Site regression model (SREG)

To characterize the genotype and GE covariance matrices, the SREG (Crossa et al., 2002; Crossa and Cornelius, 1997) model was used. The SREG model is equal to

$$\bar{y}_{ij} = \mu_j + \sum_{k=1}^t \lambda_k \alpha_{ik} \gamma_{jk} + \bar{\epsilon}_{ij} \quad (3)$$

Where \bar{y}_{ij} is the mean of a trait of the itb cultivar in the jtb environment for g genotypes and L locations ($i = 1, 2, \dots, g$ and $j = 1, 2, \dots, L$); $\bar{\epsilon}_{ij}$ is the site mean; λ_k ($\lambda_1 \geq \lambda_2 \geq \dots, \lambda_t$) are singular values scaling constants) that allow the imposition of orthonormality constraints on the singular vectors for genotypes, $\alpha_k = (\alpha_{1k}, \dots, \alpha_{gk})'$ and locations, $\gamma_k = (\gamma_{1k}, \dots, \gamma_{lk})'$, such that $\sum_i \alpha_{ik}^2 \sum_j \gamma_{jk}^2 = 1$ and $\sum_i \alpha_{ik} \alpha_{ik'} \sum_j \gamma_{jk} \gamma_{jk'} = 0$

for $k \neq k'$. α_{ik} and γ_{jk} for $k = 1, 2, \dots$, are called “primary,” “secondary,” “tertiary,” effects of the itb cultivar and the jtb location, respectively; $\bar{\epsilon}_{ij}$ is the residual error assumed to be normally and independent distributed with mean of zero and variance σ^2 / r (where σ^2 is the pooled error variance and r is the number of replicates). The number of bilinear terms is $t \leq \min(g, L)$. Estimates of the multiplicative parameters in the kth bilinear term are obtained as the kth component of the deviations from the additive part of the model. In the SREG model, only the main effects of cultivars plus the G x E interaction are absorbed into the bilinear terms (Crossa and Cornelius, 1997).

The SREG biplots plot the primary and secondary effects of genotypes and locations. Useful conclusions can be drawn from the biplot about relationships among environments, genotypes and GE interaction. For example, environments located in the same direction of the biplot equally discriminate genotypes, whereas locations in the opposite direction ranked the genotypes differently. (Crossa et al., 2002) pointed out that if the primary effects of locations were all of the same sign, the first component in biplots of SREG would be related to non-crossover genotype plus GE interaction variability, whereas the second component accounted for crossover genotype plus GE interaction variability, such that the ideal test environment or the genotype should have a large first primary effect and a near-zero secondary effect (Yan et al., 2001; Yan et al., 2007).

RESULTS

Means on individual locations indicated as average grain yield for all lines ranged from 3042.27 $kg\ ha^{-1}$ at Dixon (Table 2) to 3515.88 $kg\ ha^{-1}$ at Saunders (Table 3). Average Adate for all lines ranged from 26.3 days at Saunders (Table 3) to 41.3 days at Dixon (Table 2). For plant height, the average for all lines ranged from 79 cms at Dixon (Table 2) to 96.28 cms at Saunders (Table 3). For protein content the average ranged from 124.21 at Dixon (Table 2) to 126.04 grams per kg of flour at Saunders (Table 3). For GVW the average for all lines is between 69.94 $kg\ hl^{-1}$ at Saunders (Table 3) to 72.94 $kg\ hl^{-1}$ at Clay (Table 3). Finally, average NDVI for all lines ranged from 0.4044 at Dixon (Table 2) to 0.7175 at Saunders (Table 3). The lowest heritability on individual locations was for Adate (for all the genotypes) ranged from 0.2 at Cay (Table 8) to 0.79 at Saunders (Table 3). While the largest heritability was for protein content ranged from 0.90 at Dixon (Table 2) to 0.97 at Saunders (Table 3).

The combined analysis of variance for the six traits (grain yield, Adate, height, protein content, GVW and NDVI) is presented in Table 7. Genotypic effect was highly significant ($P < 0.01$) across the three locations for all the six traits except for Adate that was not significant ($P > 0.1$). While the effects of location were highly significant ($P < 0.01$) for the six traits (Table 7). Also the GxE interaction was highly significant ($P < 0.01$) for five traits except for plant height that was marginally significant ($P < 0.1$) (Table 7). This means that there are differential responses of the lines relative to each other across the three locations of Nebraska, which implies that the selection of lines should be focused on either genotypes with excellent adaptation to specific locations or stable genotypes that perform well in the three across environments (Mengistu et al., 2010).

For each trait the variation among genotypes was partitioned to compare released cultivars to experimental lines. McGill compared with the average of the experimental lines was highly significant ($P < 0.01$) for plant height, GVW and protein content, but was not significant for grain yield, NDVI, and Adate (Table 7). Lyman was highly significant ($P < 0.01$) for four traits (NDVI, plant height, GVW and protein content) but not significant for grain yield and Adate when compared with the average of the experimental lines (Table 7). The average of Lyman plus McGill, compared with the average of experimental lines were highly significant ($P < 0.01$) for four traits (NDVI, plant height, GVW and protein) but not significant for yield and Adate. While the average of Buckskin plus Clarkscream vs the average of experimental lines (Table 7) were highly significant ($P < 0.01$) for four traits grain yield, plant height, GVW and protein. However, the average grain yield of the experimental lines was higher by 135.45 $kg\ ha^{-1}$ over the average yield of the released cultivars, and was higher by 683.20 $kg\ ha^{-1}$ over the average of the older cultivars (Buckskin plus Clarkscream). The average of the experimental cultivars for NDVI was 0.06428 lower than the average of Lyman, was 0.03472 lower than the average of Lyman plus McGill, was 0.01936 lower than the average of Buckskin plus Clarkscream and was 0.01575 lower than the released cultivars. For plant height, the average of the experimental lines was lower by 3.54, 5.48, 5.51, 20.84, 2.66 cms compared with the McGill, Lyman, Lyman plus McGill, Buckskin plus Clarkscream and released lines, respectively. For GVW the average of the experimental lines was lower by 0.84, 1.68, 1.26, 0.90, 0.53 kg/hl compared with the McGill, Lyman, Lyman plus McGill, Buckskin plus Clarkscream and released lines, respectively. Finally, in protein content the average of the experimental lines was higher by 2.85 g/kg over the average of McGill. However, the average of the experimental lines was lower by 12.77, 4.96, 11.72, 3.70 $g\ kg^{-1}$ compared to Lyman, Lyman plus McGill, Buckskin plus Clarkscream and released lines, respectively. When comparing Karl 92 vs experimental lines only there was a highly significant ($P < 0.01$) difference for grain yield plant height and protein content. Expedition vs experimental lines only differ significantly ($P < 0.01$) for grain volume weight. While Camelot vs experimental lines only differed significantly ($P < 0.01$) for protein content. The average of McGill plus Camelot only differed from the average of experimental lines for grain volume weight ($P < 0.01$) and for plant height ($P < 0.05$). When comparing the average of Lyman plus Expedition vs the experimental lines only there were no significant differences for grain yield and for Adate ($P > 0.05$).

Site regression model (SREG)

For each response variable (grain yield, NDVI, Adate, plant height, GVW and protein content) we created SREG biplot

Table 2: Mean performance (Blups and blues) and heritability for each trait in location Dixon for the 36 lines studied

Entry	Blup		Blue					
	Pedigree	Yield	Yield	Adate	Height	Protein	GVW	NDVI
1	Hatcher	2567.67	2397.46	42.50	71.12	123.50	71.94	0.3370
2	Camelot	3155.46	3196.06	44.00	78.11	125.50	74.47	0.3688
3	Ne03490	3045.33	3046.43	42.75	70.49	118.50	72.06	0.3868
4	Wahoo	3459.88	3609.64	40.25	85.09	122.25	72.03	0.4713
5	Goodstreak	3254.46	3330.56	43.75	95.25	128.75	75.22	0.4183
6	Pronghorn	3070.08	3080.05	39.75	86.36	126.50	74.38	0.3965
7	Buckskin	3016.87	3007.76	41.50	96.52	125.50	74.19	0.4828
8	Clarkscream	2339.98	2088.11	39.50	100.33	140.25	73.63	0.3328
9	Danby	2744.63	2637.88	44.25	74.93	116.25	73.06	0.3815
10	Alice	2846.10	2775.74	40.75	68.58	125.00	72.78	0.4055
11	Karl92	2599.84	2441.18	40.75	66.68	130.25	72.94	0.3925
12	Darrell	3248.27	3322.15	39.50	83.19	123.25	73.75	0.4223
13	NE99495	3166.60	3211.19	42.25	77.47	125.25	72.53	0.4085
14	Wesley	2907.97	2859.81	42.75	72.39	129.25	71.66	0.3780
15	Alliance	2846.10	2775.74	43.25	79.38	115.25	73.38	0.4528
16	Millennium	3127.00	3157.39	41.75	82.55	126.25	73.69	0.3693
17	Overland	3419.04	3554.16	41.25	78.74	121.75	74.28	0.4505
18	Expedition	3140.61	3175.88	42.00	78.11	120.50	73.66	0.4250
19	McGill	2982.22	2960.68	38.75	80.65	120.25	73.09	0.4000
20	NW03666	3290.34	3379.31	44.75	77.47	123.25	71.25	0.4118
21	NW07505	3045.33	3046.43	43.50	79.38	120.00	72.00	0.4170
22	NW03681	2990.88	2972.45	43.00	76.20	129.75	74.09	0.4430
23	NE04424	2886.93	2831.23	42.00	74.30	124.00	73.09	0.3630
24	NE05496	3165.36	3209.51	41.50	77.47	123.25	72.69	0.4190
25	NE05548	3129.48	3160.75	38.50	86.36	128.75	72.75	0.4468
26	NE08457	3056.47	3061.56	40.25	71.76	128.00	73.47	0.3915
27	NIO8708	2993.36	2975.81	41.25	71.76	121.00	70.31	0.4023
28	NE02558	2895.60	2842.99	39.00	75.57	123.00	72.22	0.3493
29	NE06545	3223.52	3288.53	39.25	75.57	118.50	72.06	0.3608
30	NE07444	3036.67	3034.66	40.25	78.11	124.75	73.38	0.3888
31	NE05425	2974.79	2950.59	39.50	72.39	126.00	72.78	0.4075
32	NE05430	3334.89	3439.84	41.50	87.00	122.00	73.41	0.4153
33	SD07165	3223.52	3288.53	40.00	76.20	118.75	72.59	0.4788
34	Hallam	3369.54	3486.91	42.00	81.92	120.25	69.94	0.4060
35	Lyman	3223.52	3288.53	42.50	83.19	136.50	75.38	0.4655
36	NX05M41806	2743.39	2636.20	38.25	73.66	119.75	69.06	0.3135
	Var_Entry		80437.03	2.15	54.71	23.89	1.70	0.0011
	Var_Resid		115389.50	3.52	9.50	10.23	0.50	0.0023
	Gmean		3042.27	41.34	79.00	124.21	72.87	0.4044
	LSD		476.27	2.63	4.32	4.48	0.99	0.0676
	CV		7.90	3.21	2.76	1.82	0.69	8.4281
	Heritability		0.74	0.71	0.96	0.90	0.93	0.6519
	Nreps		4	4	4	4	4	4

of the 36 genotypes, which are depicted in Fig. 1. The three locations (environments) are located to the right side of the biplot for all the traits measured with the exception of Adate. For grain yield and NDVI the first SREG component explained 68.22% and 55.93% respectively of the genotype plus GE interaction, whereas the second component accounted for 21.22% and 32.72% of the variability respectively (Fig. 1A and 1B). This means that the grain yield and NDVI are substantially variable from location to location.

With regard to the grain yield in Fig. 1A genotypes NW03666, SD07165, NE07444 and Overland have a positive response in terms of genotype and GE interaction for the three locations because they are in the same direction. This mean that these 4 genotypes produce the best yield which is in agreement with the combined analysis given in Table 4, where we can see that the yield for these four genotypes are 3444.39, 3521.25, 3299.00 and 3546.46 $kg\ ha^{-1}$ respectively. On the other hand, genotypes

Table 3: Mean performance (blups and blues) and heritability for each trait in location Sanders for the 36 lines studied

Entry	Blup		Blue					
	Pedigree	Yield	Yield	Adate	Height	Protein	GVW	NDVI
1	Hatcher	3397.71	3382.68	26.25	87.88	126.20	68.35	0.7264
2	Camelot	3692.02	3713.55	26.25	95.50	126.80	68.50	0.7658
3	Ne03490	3823.99	3871.84	26.25	88.39	116.60	70.08	0.6638
4	Wahoo	3473.08	3467.41	27.25	99.06	125.60	69.63	0.7338
5	Goodstreak	3596.98	3609.56	26.75	110.74	141.20	72.98	0.7818
6	Pronghorn	2974.21	2906.55	26.00	102.62	131.40	71.08	0.7354
7	Buckskin	2986.17	2920.00	26.50	115.82	128.60	68.58	0.7572
8	Clarkscream	2622.48	2511.12	26.75	123.44	145.60	70.58	0.7040
9	Danby	3638.18	3653.02	26.25	89.41	120.00	72.40	0.6672
10	Alice	3177.59	3135.20	25.50	83.82	126.80	69.88	0.7458
11	Karl92	3357.04	3336.95	25.25	83.82	138.80	69.18	0.7424
12	Darrell	3232.62	3197.07	27.25	98.04	127.80	71.15	0.6572
13	NE99495	3564.01	3569.63	26.00	93.98	129.00	69.55	0.7822
14	WESLEY	3723.12	3748.52	26.75	82.80	128.40	70.48	0.7152
15	Alliance	3477.87	3472.79	25.75	98.55	118.40	69.83	0.6728
16	Millennium	3477.87	3472.79	27.75	98.04	125.40	71.23	0.6850
17	Overland	3799.69	3834.60	27.00	97.03	122.40	70.18	0.6466
18	Expedition	3778.15	3810.39	26.25	91.95	124.00	70.63	0.6884
19	McGill	3611.86	3623.43	26.25	96.52	121.20	70.58	0.6820
20	NW03666	3616.65	3628.81	26.25	97.03	121.00	68.78	0.6910
21	NW07505	3396.52	3381.33	26.00	97.03	123.60	69.73	0.7688
22	NW03681	3379.77	3362.50	27.25	93.47	135.20	72.00	0.7072
23	NE04424	3547.26	3550.80	26.50	93.98	123.60	71.70	0.6946
24	NE05496	3626.22	3639.57	26.25	92.46	124.40	70.25	0.6908
25	NE05548	3379.77	3362.50	27.75	108.20	132.80	70.70	0.6906
26	NE08457	3787.72	3821.15	26.25	90.42	132.20	70.53	0.7234
27	NIO8708	4031.78	4095.53	25.75	93.98	122.00	68.98	0.7764
28	NE02558	3555.63	3560.22	25.00	96.01	118.00	70.13	0.6708
29	NE06545	3934.87	3986.58	25.50	88.90	116.20	69.73	0.7504
30	NE07444	3476.67	3471.45	26.00	98.04	122.20	69.78	0.7250
31	NE05425	3638.18	3653.02	25.50	90.93	124.40	68.15	0.7280
32	NE05430	3430.01	3418.99	26.50	107.19	122.20	69.30	0.7124
33	SD07165	3796.10	3830.56	25.75	95.00	122.80	70.45	0.7234
34	Hallam	3580.75	3588.46	26.25	97.03	124.00	66.85	0.6938
35	Lyman	3595.11	3604.60	27.00	101.60	135.00	71.03	0.7990
36	NX05M41806	3394.12	3378.64	26.25	87.38	113.80	65.13	0.7296
	Var_Entry		86268.09	0.33	70.24	47.39	2.04	0.0009
	Var_Resid		53595.45	0.34	21.86	8.14	0.84	0.0034
	Gmean		3515.88	26.33	96.28	126.04	69.94	0.7175
	LSD		291.50	0.82	5.85	3.58	1.15	0.0725
	CV		4.19	1.58	3.07	1.44	0.83	5.1091
	Heritability		0.89	0.79	0.94	0.97	0.92	0.5604
	Nreps		5	4	5	5	5	5

Hatcher, NX05M41806 and Clarkscream are located on the opposite side of the biplot, this mean that they have a negative response in all sites. The grain yield of these genotypes are 2956.63, 2899.07 and 2563.12 $kg\ ha^{-1}$ respectively (Table 4 for yield). For NDVI genotypes Lyman and Buckskin have a positive response in terms of genotype and GE for the three sites because they are in the same direction and their corresponding means are 0.6535 and 0.6558 respectively. While genotype NX05M41806 have a negative response in all

sites (in opposite side of the biplot) with a mean of 0.5259 (See table 4 for NDVI).

While, for GVW, Adate, protein content and height the contribution of the first and second component was 81.47% and 12.44% (Fig. 1E); 74.67% and 16.27 (Fig. 1C); 93.97 and 4.78% (Fig. 1F) and, 95% and 3.19% (Fig. 1D), respectively. Recalling that a stable location demonstrates a large first primary effect (non-crossover GE variability) and near zero second effect (Crossover GE variability) in

Table 4: Mean performance (blups and blues) and heritability for each trait in the 3 locations (combined analysis) for the 36 lines studied

Entry	Blup		Blue					
	Pedigree	Yield	Yield	Adate	Height	Protein	GVW	NDVI
1	Hatcher	2870.06	2956.63	33.21	80.40	125.05	70.85	0.5751
2	Camelot	3366.80	3339.72	33.34	86.71	126.45	72.01	0.6050
3	NE03490	3432.33	3389.37	33.03	80.88	117.85	71.73	0.5667
4	Wahoo	3369.40	3341.70	32.34	90.37	123.80	71.27	0.6277
5	Goodstreak	3377.22	3347.12	33.57	100.07	133.41	74.20	0.6357
6	Pronghorn	3034.78	3083.65	31.43	94.02	129.28	72.71	0.6146
7	Buckskin	2920.24	2995.30	32.53	103.75	127.30	72.27	0.6558
8	Clarkscream	2359.84	2563.12	31.75	109.32	142.47	72.77	0.5614
9	Danby	3157.71	3178.48	33.16	82.16	118.86	73.83	0.5646
10	Alice	2999.31	3056.30	32.02	74.93	126.49	71.52	0.6239
11	Karl92	2954.99	3022.13	31.93	73.58	134.90	71.27	0.6155
12	Darrell	3060.80	3103.70	31.80	90.30	126.74	72.83	0.5940
13	NE99495	3353.86	3329.74	32.62	85.38	127.12	71.66	0.6332
14	Wesley	3243.35	3244.52	32.80	76.96	128.84	71.65	0.5924
15	Alliance	3249.76	3249.48	32.75	88.74	116.61	71.72	0.6058
16	Millennium	3336.54	3316.39	32.66	90.12	126.33	72.86	0.5694
17	Overland	3634.87	3546.46	32.75	87.24	121.96	72.63	0.5951
18	Expedition	3441.16	3397.07	32.34	83.91	123.11	72.31	0.5993
19	McGill	3347.62	3324.94	31.70	89.23	120.32	72.45	0.5944
20	NW03666	3503.70	3444.39	33.89	86.07	121.19	70.82	0.5848
21	NW07505	3276.68	3270.22	33.48	89.52	121.48	71.65	0.6251
22	NW03681	3087.53	3124.33	33.48	84.04	132.25	73.61	0.6028
23	NE04424	3182.92	3197.91	32.57	83.18	123.46	72.80	0.5555
24	NE05496	3364.86	3338.22	32.57	86.64	123.94	71.96	0.5846
25	NE05548	3304.58	3291.74	32.30	96.22	130.60	72.17	0.6171
26	NE08457	3441.19	3397.10	32.11	79.34	130.54	72.52	0.5837
27	NIO8708	3458.90	3410.76	32.43	82.30	121.98	70.45	0.6104
28	NE02558	3174.20	3191.19	31.20	86.81	120.86	71.57	0.5546
29	NE06545	3603.30	3520.85	31.34	82.97	117.26	71.21	0.5959
30	NE07444	3313.99	3299.00	32.16	88.70	123.52	72.00	0.5916
31	NE05425	3264.11	3260.53	31.57	83.72	126.34	71.16	0.6096
32	NE05430	3365.81	3338.94	32.80	94.60	121.71	72.04	0.5980
33	SD07165	3602.16	3521.25	31.98	85.36	120.09	72.10	0.6212
34	Hallam	3402.61	3367.32	32.75	88.63	122.36	68.91	0.5830
35	Lyman	3274.57	3268.58	33.21	91.16	135.93	73.30	0.6535
36	NX05M41806	2795.46	2899.07	31.25	80.63	117.55	68.08	0.5259
	Var_Loc		53064.15	61.91	74.47	0.00	2.89	0.0283
	Var_Entry		50016.06	0.00	53.17	32.13	1.24	0.0005
	Var_LocxEntry		29570.87	0.94	1.51	1.82	0.37	0.0005
	Var_Resid		73338.10	1.76	21.08	10.74	0.69	0.0023
	Gmean		3247.98	32.47	87.17	125.22	71.91	0.5980
	LSD		344.31	1.95	3.92	3.26	1.16	0.0520
	CV		5.32	3.01	2.25	1.31	0.81	4.3616
	Heritability		0.78	0.00	0.97	0.96	0.89	0.5945
	Nreps		6	4	6	6	6	6
	Nlocs		3	3	3	3	3	3

the biplot (Crossa et al., 2002). These results showed that the three locations present a small but relevant variability for GVW and Adate. However, for protein and height the three locations are good because they had large values for primary effects (first PC) and low values for secondary effects (second PC). This implies that the 36 genotypes are

stable for protein content and plant height in these three locations studied.

For GVW the genotypes with the best performance (positive response in terms of genotype and GE for the three locations) were Lyman, NW03681, Danby and

Goodstreak with means equal to 73.30, 73.61, 73.83 and 74.20 $kg\ b\ l^{-1}$ respectively (Fig. 1E and Table 4). While the genotypes Hallam and NX05M41806 are located on the opposite side of the biplot, this mean that they have a negative response in all sites with means of 68.91 and 68.08 $kg\ b\ l^{-1}$ respectively. On the other hand, for Adate all the genotypes are located in the center of the biplot and the three locations are spread to all sides. This mean that the behavior of the 36 genotypes is very similar in each location, which can be corroborated with Table 4 (for Adate) where the range of the means is between 31.2 to 33.9 days.

While for plant height, genotypes Goodstreak, Buckskin, and Clarkscream have the superior performance in terms of genotype and GE for the three locations because they are in the same direction and their corresponding means are 100.07, 103.75 and 109.32 cms respectively. Contrarily, genotypes Karl92, Alice and Wesley are located on the opposite side of the biplot, this mean that they have a negative response in all sides with means of 73.58, 74.93 and 76.96 cms respectively. For protein content genotypes Goodstreak, Karl 92, Lyman, and Clarkscream have the best performance in terms of genotype and GE in the three locations because they are in the same directions with means 133.41, 134.90, 135.93 and 142.47 grams per kg of flour respectively. While genotypes Alliance, NX05M41806, Danby and NE02558 have the worst performance (on the opposite side of the biplot) with means 116.61, 117.55, 118.86 and 120.86 grams per kg of flour respectively.

Genetic and phenotypic correlations

For traits there was a large range in the genetic correlation among all pairwise comparisons between five of the six traits measured (Table 5). The lowest genetic correlation was 0.009 between GVW and grain yield, while the largest was -0.542 between protein content and yield. This is in agreement to the results given by (Marinciu et al., 2009)

Table 5: Genetic (lower triangle) and phenotypic (upper triangle) correlation coefficients among five traits averaged over three locations

Trait	Yield	NDVI	Height	GVW	Protein
Yield	1	0.3	-0.04	0.06	-0.3
NDVI	0.1	1	0.1	0.05	0.1
Height	-0.2	0.3	1	0.2	0.2
GVW	0.009	0.4	0.3	1	0.2
Protein	-0.5	0.4	0.3	0.4	1

Table 6: Phenotypic correlation coefficients among the three locations (Stae the Counties) for five traits (State the Traits)

Location	Clay	Dixon	Saunders
Clay	1	0.3	0.5
Dixon		1	0.3
Saunders			1

that found a negative relationship between protein content and yield. The second largest correlation was 0.470 between NDVI and GVW. The correlation between GVW and protein content was 0.446, while between height and GVW was 0.378; and between height and protein content was 0.360. The genetic correlation between NDVI vs plant height, GVW and protein content was 0.324, 0.367 and 0.434 respectively. Finally, the genetic correlation between grain yield and height was negative (-0.282) and with regard to NDVI was positive but very low 0.157. On the other hand, the phenotypic correlations had only two negative correlations. Similar to the genetic correlation, grain yield vs plant height (-0.045) and grain yield vs protein content (-0.353) were negatively correlated using phenotypic values. The phenotypic correlation between grain yield, and GVW and NDVI was 0.066 and 0.305 respectively. The phenotypic correlation between NDVI and plant height, GVW and protein were 0.199, 0.056 and 0.118 respectively. The phenotypic correlation between plant height and GVW and protein content was 0.238 and 0.228, respectively. Finally the correlation between GVW and protein content was 0.269.

For locations we can see in Table 6 that the largest phenotypic correlation was 0.5175 between Clay and Saunders, while the lowest correlation was 0.35258 between Clay and Dixon, while the correlation between Dixon and Saunders was 0.39249.

DISCUSSION

The grain yield and NDVI responses were highly significant and highly responsive. Their performance varied across locations which is reflected in the lowest broad sense heritability estimates. The average difference in the grain yield was 473.61 $kg\ b\ a^{-1}$ and the location with the highest grain yield was Saunders while Dixon had the lowest grain yield. For NDVI the average difference among locations was 0.3131 where again Saunders reported the highest values and Dixon the lowest. Although, GVW and Adate had little variability compared with grain yield and NDVI across locations we have not elements to guarantee that these trait are stable across locations, because there are also a highly significant effect of environment and GE. In this case for GVW the average difference between locations was of 3 $kg\ b\ l^{-1}$ where Saunders had the lowest value. While for Adate, the average difference was of 15 days where Dixon had the highest value and Saunders the lowest Adate value. For protein content the average difference was of 1.83 $g\ kg^{-1}$ between Dixon (lowest) and Saunders (highest). For plant height the average difference was of 17.28 cms between Dixon (lowest) and Saunders (highest). The site regression biplots (Fig. 1) show that the

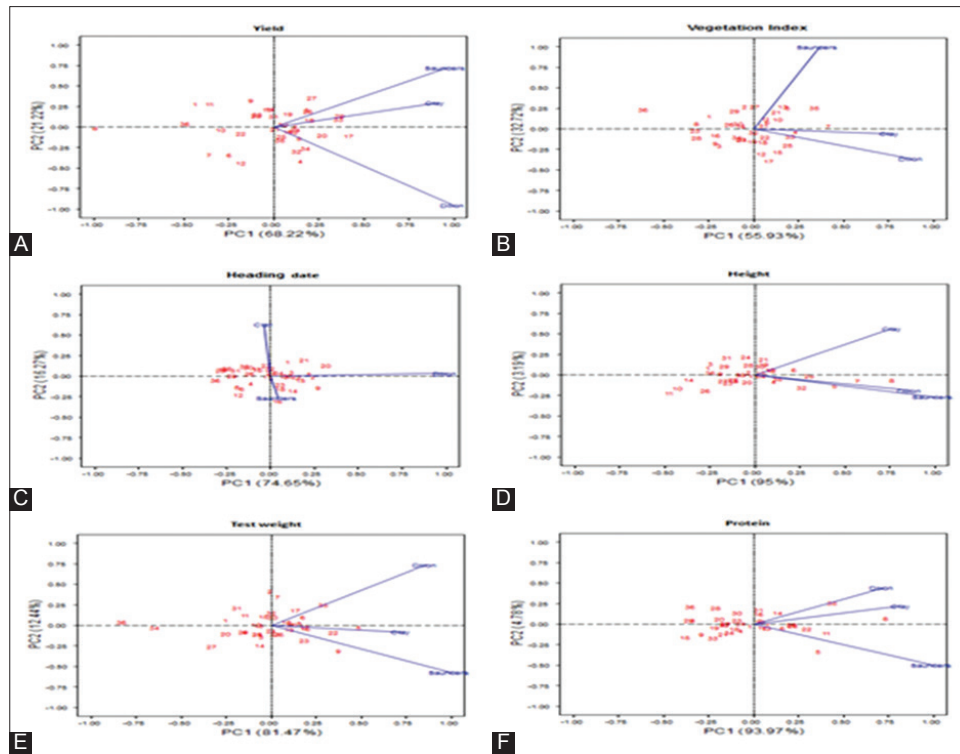


Fig 1. Side regression biplot of number of locations of 36 wheat lines in three environments in Nebraska, USA, for A) Yield B) the NDVI index, C) Adate, D) Height, E) GVW and F) protein.

Table 7: Analysis of variance for yield NDVI, Adate, height, GVW and protein in 36 lines of winter wheat across three environments in Nebraska 2011. GE means genotype by environment interaction

Source	Yield		Vgeindex		Adate		Height		GVW		protein	
	DF	Mean square	DF	Mean square	DF	Mean square	DF	Mean square	DF	Mean square	DF	Mean square
Environment (E)	2	2178.4**	2	4.4**	2	8598.4**	2	1908.5**	2	331.7**	2	1.3**
Block (R)	12	175.8**	12	0.03**	7	0.960	12	18.6**	12	1.4**	12	3.8**
Lines (G)	35	208.8**	35	0.01**	35	3.897	35	125.9**	35	12.0**	35	4.7**
McGill vs experimentals	1	0.9	1	0.0002	1	2.986	1	26.3**	1	6.0**	1	1.0**
Lyman vs experimentals	1	4.2	1	0.05**	1	4.63	1	67.5**	1	24.2**	1	21.3**
Lyman-McGill vs experimentals	1	0.5	1	0.03**	1	0.08	1	84.1**	1	25.9**	1	6.3**
Buckskin-Clarkscream vs experimentals	1	2656.7**	1	0.009*	1	1.460	1	1790.2**	1	13.3**	1	35.2**
Released vs experimental	1	506.9**	1	0.02**	1	0.425	1	159.5**	1	23.7**	1	16.9**
Karl92 vs experimentals	1	434.3**	1	0.008	1	1.545	1	299.7**	1	0.899	1	18.3**
Camelot vs experimentals	1	6.8	1	0.003	1	5.7	1	2.3	1	1.5	1	1.4**
McGill-Camelot vs experimentals		6.0	1	0.002	1	0.2	1	21.0*	1	6.3**	1	0.01
Expedition vs experimentals	1	42.4	1	0.001	1	0.2	1	5.0	1	4.4**	1	0.005
Lyman-Expedition vs experimentals	1	9.3	1	0.03**	1	1.2	1	16.8*	1	23.6**	1	10.0**
GE	70	47.8**	70	0.005**	70	4.6**	70	4.3*	70	1.5**	70	0.1**
Error	416	16.1	418	0.002	245	1.8	420	3.2	408	0.4	412	0.1
CV(%)		8.3		7.8		4.0		5.2		1.1		2.6
Mean		48.3		0.6		33.0		34.5		57.5		12.5
R-square		0.7		0.9		0.9		0.8		0.8		0.8

Table 8: Mean performance (blups and blues) and heritability for each trait in location Clay for the 36 lines studied

Entry	Blup		Blue					
	Pedigree	Yield	Yield	Adate	Height	Protein	GVW	NDVI
1	Hatcher	2856.34	2806.57	31.00	81.70	125.17	72.25	0.6563
2	Camelot	3195.28	3196.62	29.50	86.36	126.83	73.15	0.6758
3	Ne03490	3351.11	3375.95	30.00	82.97	118.17	73.00	0.6498
4	Wahoo	3090.09	3075.57	29.50	87.63	123.33	72.15	0.6830
5	Goodstreak	3203.07	3205.58	30.00	95.25	130.00	74.44	0.7047
6	Pronghorn	3132.95	3124.88	28.50	93.13	129.50	72.77	0.7075
7	Buckskin	2896.28	2852.52	29.50	99.48	127.50	74.12	0.7292
8	Clarkscream	2557.34	2462.47	29.00	104.56	141.33	74.08	0.6427
9	Danby	3156.32	3151.78	28.50	82.13	119.83	75.85	0.6453
10	Alice	3089.12	3074.45	30.00	72.81	127.33	71.96	0.7158
11	Karl92	3068.67	3050.91	30.00	70.70	135.00	71.77	0.7068
12	Darrell	2774.53	2712.42	28.50	89.75	128.50	73.62	0.7015
13	Ne99495	3270.28	3282.92	29.50	84.67	126.83	72.88	0.7053
14	Wesley	3119.31	3109.19	28.50	76.20	128.83	72.75	0.6803
15	Alliance	3425.14	3461.13	29.00	88.05	116.00	72.00	0.6943
16	Millennium	3348.19	3372.59	28.00	89.75	127.17	73.69	0.6520
17	Overland	3480.65	3525.02	30.00	85.94	121.67	73.48	0.6910
18	Expedition	3313.13	3332.24	28.50	82.13	124.33	72.71	0.6852
19	McGill	3400.79	3433.11	30.50	90.17	119.50	73.67	0.6982
20	NW03666	3457.06	3506.30	30.50	83.82	119.49	72.42	0.6532
21	NW07505	3360.85	3387.16	31.00	91.44	120.67	73.17	0.6883
22	NW03681	2969.32	2936.58	30.00	82.55	131.50	74.71	0.6623
23	NE04424	3155.35	3150.66	29.00	81.28	122.83	73.56	0.6102
24	NE05496	3241.06	3249.30	30.00	89.32	124.00	72.94	0.6475
25	NE05548	3357.93	3383.80	31.00	93.98	130.00	73.04	0.7140
26	NE08457	3391.05	3421.90	30.00	76.20	131.00	73.56	0.6375
27	NIO8708	3274.17	3287.40	30.50	80.86	122.67	71.98	0.6533
28	NE02558	3117.36	3106.95	30.00	88.05	121.67	72.35	0.6410
29	NE06545	3471.72	3523.65	29.50	84.24	117.17	71.85	0.6717
30	NE07444	3387.15	3417.42	30.50	89.32	123.67	72.88	0.6600
31	NE05425	3179.70	3178.68	30.00	86.78	128.49	72.58	0.6913
32	NE05430	3249.82	3259.38	30.50	90.17	120.89	73.42	0.6675
33	SD07165	3605.32	3668.49	30.50	84.67	118.49	73.21	0.6685
34	Hallam	3165.09	3161.87	30.00	87.21	122.49	69.95	0.6508
35	Lyman	2988.80	2959.00	30.00	88.90	136.29	73.59	0.6985
36	NX05M41806	2495.98	2391.86	29.50	80.86	119.29	70.19	0.5373
	Var_Entry		69848.43	0.12	41.61	30.11	1.09	0.0010
	Var_Resid		63200.64	0.99	27.68	13.17	0.68	0.0015
	Gmean		3183.23	29.74	86.20	125.21	72.94	0.6716
	LSD		288.08	2.02	5.99	4.22	0.98	0.0438
	CV		4.59	3.35	3.52	1.71	0.68	3.3068
	Heritability		0.87	0.20	0.90	0.93	0.91	0.8084
	nreps		6	2	6	6	6	6

genotype performance in each location was quite diverse and depended strongly of the trait under study.

We found that yield is negatively correlated with protein content and plant height. In addition, we observed that in general the genetic correlation produced the largest influence in the genotype performance, although the GE interaction was also significant and needs to be considered to avoid mistakes in selection. Therefore, the genotypes showed variation in their degree of stability from one trait

to another suggesting that the genetic factors involved in the GE differed between traits (Grausgruber et al., 2000). The highly significant effects of environments and GE found in this study are in agreement with those reported by other studies (Fufa et al., 2005; Campbell et al., 2004; Peterson et al., 1992; Mengistu., 2010) conducted for winter wheat grain yield in Nebraska.

The best genotypes for grain yield across the three environments were genotypes NW03666, SD07165,

NE06545 and Overland. It is important to point out that only the line Overland was released, the other three were experimental lines. For NDVI the best genotypes are: Lyman and Buckskin, both were released. For GVW the best genotypes are: Lyman, NW03681, Danby and Goodstreak, only line NW03681 is experimental. For Adate all genotypes are similar. The best genotypes for plant height were: Goodstreak, Buckskin and Clarkscream, all released. For protein content the best genotypes are: Goodstreak, Karl92, Lyman, and Clarkscream, all released. While the worst genotypes are: Hatcher, NE03490 and Clarkscream (for yield), NX05M41806 (for NDVI), Hallam and NX05M41806 (for GVW), Karl 92, Alice and Wesley (for height) and Alliance, NX05M41806, Danby and NE02558 (for protein content). In general the average yield of the experimental lines were better than the released lines which is to be expected due to the released lines including older lines and breeding progress should occur. For Adate, the performance was the same between experimental and released lines which is expected because selecting for adaptation in Nebraska means that there is an optimum Adate and both release and experimental lines select for that Adate. However, for NDVI, height, GVW and protein, the average performance of the experimental lines was lower than the released lines. Also, it is important to point out that we found a negative correlation between grain yield and protein content, and between yield and plant height.

CONCLUSIONS

We found that in general the average grain yield of the experimental lines were better than the released lines. The best genotypes for grain yield across the three environments are genotypes NW03666, SD07165, NE07444 and Overland. For vegetation index the best lines were: Lyman and Buckskin. For grain volume weight the best lines were: Lyman, NW03681, Danby and Goodstreak. For anthesis date all genotypes were similar. For plant height, the best lines were Goodstreak, Buckskin and Clarkscream. For protein content, the best lines were Goodstreak, Karl92, Lyman, and Clarkscream. For anthesis date, the performance was similar between experimental and released lines. However, for vegetation index, plant height, grain volume weight and protein content, the average performance of the experimental lines was lower than the released lines. Also, it is important to point out that the linear mixed models and site regression model implemented were very effective for reaching the objectives of the present research.

Authors' contributions

Richard S. Little and P. Sephen Baenziger conducted the experiments and collected the data. Osva Antonio

Montesinos-López, Eliel Martínez-Cruz and Emeterio Franco-Pérez did the analysis and write the paper and we work all approximately the same amount of time.

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