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Evaluation of Agronomic Traits and Drought Tolerance of Winter Wheat Accessions from the USDA-ARS National Small Grains Collection

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Abstract: Wheat accessions from the USDA-ARS National Small Grains Collection (NSGC) are a potential genetic resource for variety improvement. This study assessed the agronomic performance and drought tolerance in 198 winter wheat accessions under irrigated and terminal drought environments in the 2012–2013 season, and repeated the test under terminal drought only during the 2013–2014 season. The 198 accessions were classified into three maturity groups, early, intermediate, and late based on heading data. In all three environments, the early accessions had the best agronomic performance, produced higher grain yield, thousand-kernel weight and grain volume weight, and had earlier heading date and shorter plant height. The intermediate accessions had similar grain yield and thousand-kernel weight as the early accessions in the irrigated environment, but had lower thousand-kernel weight in the terminal drought environments. Terminal drought had significant effects on grain yield, plant height, thousand-kernel weight, and grain volume weight. The positive correlation between GY and HD suggests that the 'late early' types in the early maturity were the most successful. Out of 198 accessions evaluated, twenty-three had high yield stability and drought tolerance according to the drought susceptibility index and membership function value of drought tolerance. The eight of twenty-three accessions identified (four early and four intermediate) had high grain yield in three environments. Some of these accessions have been further used in bi-parental mapping studies and by breeders for grain yield and drought tolerance improvement.

Keywords: winter wheat accessions; maturity; agronomic traits; drought tolerance; breeding

1. Introduction

Wheat is the third largest crop in the world after maize and rice and is an important source for protein and calories in human food. The yield of wheat has shown steady growth, with an average annual production from 1998 to 2002 [1] of 586 million metric tons, partly attributed from genetic improvements, development of locally adapted cultivars with resistance to diseases and abiotic stresses [2,3], and



improved management practices [4]. However, the yield growth rate in the past two decades is insufficient to meet projected future demand on the basis of the increasing human population [3,5].

Along with the population growth, global average annual temperatures have increased in the past decades [6]. Temperature increases in agricultural regions of the world [1] can result in extreme heat exposure, which can greatly reduce wheat yield if it occurs in the reproductive period of growth [7,8]. Selection for early maturity would be a means of escaping late-season heat and drought stress [9]. Early maturing wheat germplasm showed higher grain yield than locally adapted varieties in different environments in the South Asia [10]. The timing of harvest is important for maximum production. Harvesting immature crops, including wheat, can be result in low yield [11]. In the water-limited production systems in the western United States, temperature and precipitation at flowering can greatly affect production [12]. For example, the semi-arid environment of Aberdeen, Idaho receives an average of 230 mm of annual precipitation and has the highest temperatures and lowest precipitation during June and July [13–15]. This period coincides with the flowering and grain filling stages of winter wheat. In such environments, developing the appropriate maturity of wheat cultivars is critical for maximizing yield.

Aside from the growing population and global climate change, drought stress is one of the leading abiotic constraints influencing wheat production in many arid and semi-arid areas of the world [16]. The average grain yield of wheat genotypes in Ardabil, Iran decreased by about 50% in a drought stress environment compared with a normal irrigation environment [17]. In the United States, the cultivated land is estimated to be about 113 million hectares [18], but up to 80% of this area is dependent on rainfall [19]. The wheat growing area in the U.S. will decline by over 8% by the year 2030 using projections from forecasting models [20]. In particular, 75% to 95% of wheat growing in the western United States currently is under rainfed condition and thus vulnerable to yield loss from drought [19,21]. Therefore, developing high yield and drought tolerant wheat cultivars adapted to diverse environments will help increase wheat production and stability.

The exploration and application of the novel germplasm could help plant breeders accelerate the development of the high yielding and drought tolerant wheat. The NSGC represent a large sample of the wide diversity of cereals from most regions of the world, including wheat and its wild relatives. Disease and drought tolerant wheat accessions have been identified from the NSGC [22–30]. Wheat germplasm, such as landraces, is a valuable source of many traits related to drought tolerance [31]. Some of wheat germplasm showed more stable and higher grain yield under diverse conditions compared with most improved cultivars [32,33]. The germplasm in NSGC may be useful for breeding wheat for increased grain yield stability and drought tolerance.

Drought response is a quantitative trait with a complex phenotype affected by genotype and environment [16,34]. Yield is the main index for selection in target environments. Certain indices, such as the drought susceptibility index (DSI) based on the grain yield, are a measure of yield stability but not of yield potential [14]. An alternative approach is the use of selection indices in combination with two or more traits, which could prove superior to either direct selection for either grain yield or the single traits independently [35,36]. The analysis membership function value of drought tolerance (MFVD), integrating more traits, is currently being evaluated for wheat drought tolerance selection [16,37].

The objectives of this study were to evaluate the agronomic performance and drought tolerance of winter wheat accessions from the NSGC with different maturity under irrigated and terminal drought environments and to identify germplasm sources with appropriate maturity and drought tolerance for wheat variety improvement.

2. Results

2.1. Genetic Variation and Broad Sense Heritability (H²) of Traits

Genotypic effect were significant (p < 0.001) for all traits (i.e., grain yield (GY), heading date (HD), plant height (PH), thousand-kernel weight (TKW) and grain volume weight (GVW)) evaluated

in individual environments and in the combined analysis of variance across environments, and environmental effects of the all traits except HD were significant in the combined analysis (p < 0.0001) (Table 1). The interaction effect of genotype and environment was not significant for all the traits measured in this study. GVW showed the highest H^2 of 0.92, followed by HD (0.90) and PH (0.80).

Table 1. The analysis of variance (ANOVA) and broad-sense heritability (H^2) of winter accessions (198 accessions and five checks in 1213IR and 1213TD, three replications in 1314TD) for the traits in three environments.

Variable	1213IR			1213TD		1314TD	Across Environments		
Vallable	DF	MS	DF	MS	DF	MS	DF	MS	
GY									
Block/Replication	11	2.87 ****	11	1.38 *	2	46.01 ****			
Genotype (G)	202	1.53 ****	202	1.50 ***	197	2.77 ****	197	3.28 ****	
Environment (E)							2	67.57 ****	
$G \times E$							394	0.34	
Error	44	0.49	42	0.63	394	0.55	396	0.78	
H^2								0.74	
HD									
Block/Replication	11	9.28 **	11	1.27	2	4.25			
Genotype (G)	202	18.18 ****	202	23.53 ****	197	78.45 ****	197	101.13 ****	
Environment (E)							2	2.25	
$G \times E$							394	6.07	
Error	44	3.04	44	1.20	394	5.22	396	5.21	
H^2								0.90	
PH									
Block/Replication	11	193.15 **	11	139.42 **	2	8549.62 ****			
Genotype (G)	202	200.01 ****	202	205.05 ***	197	476.04 ****	197	674.81 ****	
Environment (E)							2	6120.15 ****	
$G \times E$							394	41.97	
Error	44	68.38	44	45.52	394	85.62	396	128.37	
H^2								0.80	
TKW									
Block/Replication	11	7.72 *	11	11.31 *	2	1996.55 ****			
Genotype (G)	202	18.22 ****	202	19.41 ****	197	37.19 ****	197	52.27 ****	
Environment (E)							2	1918.95 ****	
$G \times E$							394	12.34	
Error	44	2.89	43	4.57	394	4.59	396	14.65	
H^2								0.66	
GVW									
Block/Replication	11	223.13 ***	11	746.07 **	2	126318.6 ****			
Genotype (G)	202	624.85 ****	202	1676.65 ****	197	2443. 17 ****	197	3584.66 ****	
Environment (E)							2	63472.23 ***	
$G \times E$							394	317.94	
Error	44	52.97	42	215.80	394	374.98	396	398.84	
H^2								0.92	

1213IR, irrigated in 2012–2013 seasons; 1213TD, terminal drought in 2012–2013 seasons; 1314TD, terminal drought in 2013–2014 seasons; DF, degree of freedom; MS, Mean squares; $G \times E$, the interaction effect of genotype and environment; GY, grain yield; HD, heading date; PH, plant height; TKW, thousand-kernel weight; GVW, grain volume weight; * p < 0.05; ** p < 0.01; **** p < 0.0001; **** p < 0.0001.

2.2. Plant Maturity Effect on Grain Yield and Agronomic Traits

The plant maturity had significant (p < 0.0001) effect on all five traits assessed (Table 2). Environmental effects were not significant for HD, but the maturity × environment interaction was significant on HD (p < 0.0001), reflecting it was caused by the changes in maturity ranking rather than magnitude changes across the environment. The early accessions had significantly higher GY, much earlier HD (as expected), significantly shorter PH, significantly heavier TKW, and greater GVW than the late accessions in all three environments ($\alpha = 0.05$) (Table 3). In 1213IR, despite the longer growing period of the late accessions, the early accessions exhibited higher GY, which might likely be due to high temperatures instead of soil moisture deficit. The intermediate accessions had similar GY and TKW to the early accessions in the 1213IR, but significantly later HD, higher PH, and lower GVW in all three environments. TKW in particular was lower for the intermediate versus early accessions in the 1213TD and 1314TD ($\alpha = 0.05$). The agronomic performance of the intermediate accessions was significantly better than the late accessions ($\alpha = 0.05$).

Sourceof Variation	GY		HD		PH			TKW	GVW	
	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
Maturity (M)	2	54.15 *****	2	7102.90 ****	2	14,773.46 ****	2	1196.29 ****	2	140,005.40 ****
Environment (E)	2	67.66 ****	2	5.23	2	5985.44 ****	2	1889.76 ****	2	98,056.50 ****
$M \times E$	4	3.87 **	4	102.72 ****	4	305.97	4	78.47 **	4	6382.97 ****
Error	981	1.11	981	10.99	981	199.40	981	19.53	981	1068.50

Table 2. The analysis of variance (ANOVA) on the three maturity groups for each trait across the three environments

DF, degree of freedom; MS, Mean squares; $M \times E$, the interaction effect of genotype and environment; GY, grain yield; HD, heading date; PH, plant height; TKW, thousand-kernel weight; GVW, grain volume weight; ** p < 0.001; **** p < 0.001.

Table 3. Mean value \pm standard deviation (SD) of traits investigated among winter wheat of three maturity groups in three environments.

Environments	Maturity Group	GY (t/ha)		HD (days)		PH (cm)		TKW (g)		$GVW (kg/m^{-3})$	
Environments	Maturity Gloup	Range	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range	$Mean \pm SD$	Range	Mean \pm SD
1213IR	Early	2.53-6.97	$5.14~^{\rm a}\pm1.05$	146.0-157.6	156.3 c \pm 3.13	66.5–116.4	88.8 $^{\rm c} \pm 12.67$	30.5-55.5	$41.6\ ^{a}\pm 4.66$	735.7-818.3	774.9 a \pm 15.90
	Intermediate	2.97-6.55	$5.01~^{\rm a}\pm0.75$	158.8-161.4	159.6 $^{ m b}$ \pm 2.38	63.0-121.3	95.0 $^{ m b}$ \pm 13.18	33.0-61.1	$40.7\ensuremath{^a}\xspace \pm 4.60$	693.4-805.7	760.9 $^{ m b}$ \pm 23.82
	Late	2.16-6.92	$4.54^{\ b} \pm 1.12$	162.6-170.6	$166.2\ ^{a}\pm 3.71$	65.6–114.1	107.1 $^{\rm a}$ \pm 14.14	30.1-46.4	$38.2^{\ b} \pm 3.76$	690.2–783.2	742.0 c \pm 23.09
1213TD	Early	2.36-6.26	$4.04~^a\pm0.98$	144.2–157.2	155.2 $^{\rm c} \pm 3.15$	64.0-103.7	$80.6 \text{ c} \pm 10.26$	29.5–55.0	$37.6 \ ^{a} \pm 4.42$	690.1-800.5	755.7 ^a ± 25.11
	Intermediate	1.76-5.91	$4.02~^a\pm1.05$	158.6-161.2	159.9 $^{ m b}$ \pm 2.31	65.4-112.2	82.6 $^{ m b}$ \pm 12.37	27.2-49.5	$34.8~^{b}\pm 4.72$	638.5-783.3	731.5 $^{\rm b}$ \pm 33.37
	Late	1.62 - 5.90	$3.12^{\ b}\pm 0.89$	163.4–170.6	$167.4\ ^{a}\pm 3.49$	59.4-118.4	94.9 $^{\rm a}\pm14.61$	24.1-37.6	$31.0\ ^{c}\pm2.84$	580.5–773.9	$685.3\ ^{c}\pm 39.20$
1314TD	Early	2.08-6.57	$4.92~^{\rm a}\pm1.18$	146.3-157.0	$155.4 ^{\mathrm{c}} \pm 3.39$	66.9–107.5	87.2 $^{\rm c} \pm 11.67$	29.3-44.7	$37.2\ ^{a}\pm 4.85$	725.2–785.6	756.9 ^a ± 14.56
	Intermediate	2.46-6.18	$4.31^{\text{ b}} \pm 1.10$	158.0-161.7	159.9 $^{ m b}$ \pm 2.58	66.9–115.1	90.9 $^{ m b}$ \pm 15.20	29.1-44.5	$35.9 \ ^{\mathrm{b}} \pm 4.61$	666.0-784.1	732.4 $^{\mathrm{b}} \pm 24.05$
	Late	2.21-5.53	$3.73\ ^{c}\pm1.03$	163.7-170.3	166.4 $^{\mathrm{a}}$ \pm 3.53	58.4-125.3	99.3 $^a\pm16.88$	29.0-41.5	$34.1\ ^{\text{c}}\pm4.16$	634.0-761.9	713.6 $^{c} \pm 26.87$

Values in a column followed by the same letter are not significantly different status at α = 0.05. 1213IR, irrigated in 2012–2013 seasons; 1213TD, terminal drought in 2012–2013 seasons; 1314TD, terminal drought in 2013–2014 seasons; GY, grain yield; HD, heading date; PH, plant height; TKW, thousand-kernel weight; GVW, grain volume weight.

2.3. Correlation Analysis of Traits

In all three environments, GY was negatively correlated with HD and PH, but was significantly and positively correlated with TKW and GVM (p < 0.01) (Table 4). For the early accessions, GY had significantly positive correlations with TKW and GVW in all three environments and with HD in the 1213TD and 1314TD (p < 0.05). The significant and positive correlation was observed between GY and TKW for the intermediate accessions in the 1213IR and 1213TD, while GY was significantly and positively correlated with GVW in 1213TD and 1314TD (p < 0.05). GY of the late accessions had significant and positive correlations with TKW (p < 0.05) in the 1213IR and 1213TD, but had highly significant and negative correlations with PH in 1213IR and 1314TD.

Environment	r	All the Lines	Early	Intermediate	Late	
Environment	,	GY	GY	GY	GY	
	HD	-0.25 ***	0.13	-0.09	-0.22	
	PH	-0.32 ****	-0.08	0.02	-0.53 ****	
1213IR	TKW	0.30 ****	0.21 *	0.26 *	0.26 *	
	GVW	0.19 **	0.26 *	0.12	-0.06	
	Ν	198	61	71	66	
	HD	-0.24 ***	0.15 *	-0.09	-0.12	
	PH	-0.07	-0.12	0.18 *	-0.08	
1213TD	TKW	0.37 ****	0.37 ****	0.27 ****	0.27 ****	
	GVW	0.55 ***	0.43 ****	0.47 ****	0.48 ****	
	Ν	198	61	71	66	
		GY	GY	GY	GY	
	HD	-0.44 ****	0.26 *	-0.14	-0.22	
1014	PH	-0.42 ****	-0.12	-0.16	-0.56 ****	
1314TD	TKW	0.36 ****	0.34 **	0.19	0.09	
	GVW	0.45 ****	0.34 *	0.26 *	0.12	
	Ν	198	61	71	66	

Table 4. Pearson's correlation coefficients (*r*) between grain yield (GY) and traits investigated in the accessions of three maturity groups grown in three environments.

N, the number of accessions is used for calculating each correlation coefficient. 1213IR, irrigated in 2012–2013 seasons; 1213TD, terminal drought in 2012–2013 seasons; 1314TD, terminal drought in 2013–2014 seasons; HD, heading date; PH, plant height; TKW, thousand-kernel weight; GVW, grain volume weight. * p < 0.05; ** p < 0.01; **** p < 0.001; **** p < 0.001.

2.4. Identification of Drought Resistant Accessions by Drought Susceptibility Index (DSI) and Membership Function Value of Drought Tolerance (MFVD)

All traits except HD had the highest values in the 1213IR (Table 5). The mean values of the other four traits decreased significantly in the terminal drought environments (1213TD and 1314TD). To quantify the response of accessions to drought, the DSI was calculated for each accession using 2012–2013 data GY differences between TD and IR plots. The DSI values of the 198 accessions varied from 0 to 2.55 (Table S1). Among these accessions, 109 had smaller DSI values (DSI < 1) in the 2012–2013 seasons, including 40 early, 44 intermediate and 25 late accessions (Table S1). Greater DSI values (DSI > 1) were observed in 89 accessions, indicating poor tolerance to drought. The cluster analysis of DSI in the 2012–2013 season generated four clusters, cluster 1 (0.00-0.52, 57 accessions), cluster 2 (0.56-1.26, 75 accessions), cluster 3 (1.30-1.81, 44 accessions) and cluster 4 (1.85-2.55, 22 accessions), respectively (Table S1).

Traits		1213IR	1213TD	1314TD
GY (t/ha)	Range Mean \pm SD	$\begin{array}{c} 2.166.97 \\ 4.89 ^{\text{a}} \pm 1.01 \end{array}$	$\frac{1.626.26}{3.72} ^{\text{c}} \pm 1.06$	$2.086.57 \\ 4.30^{\text{ b}} \pm 0.96$
HD (days)	Range Mean \pm SD	$\begin{array}{c} 146.0170.6 \\ 160.7 \text{ a} \pm 5.11 \end{array}$	$\begin{array}{c} 144.2170.6 \\ 160.8 ^{\text{a}} \pm 6.04 \end{array}$	$\begin{array}{c} 146.3170.3 \\ 160.7 ^{\text{a}} \pm 5.12 \end{array}$
PH (cm)	Range Mean \pm SD	63.0–141.1 97.1 ^a ± 15.27	55.4–118.4 85.9 ^c ± 13.85	$58.4{-}125.3 \\92.6^{\text{ b}} \pm 12.60$
TKW (g)	Range Mean \pm SD	30.1-61.1 $40.1 \text{ a} \pm 4.56$	24.1–55.0 34.4 ^c ± 4.87	29.0–44.7 35.7 ^b ± 3.52
GVW (kg/m ^{-3})	Range Mean \pm SD	$\begin{array}{c} 690.2818.3 \\ 758.9 \text{ a} \pm 25.11 \end{array}$	580.5–800.5 723.6 ^c ± 43.90	$\begin{array}{c} 634.0785.6 \\ 733.7 ^{\text{b}} \pm 28.50 \end{array}$

Table 5. Mean value and standard deviation (SD) of traits measured in three environments.

Values in a row followed by the same letter are not significantly different status at $\alpha = 0.05$. 1213IR, irrigated in 2012–2013 seasons; 1213TD, terminal drought in 2012–2013 seasons; 1314TD, terminal drought in 2013–2014 seasons; GY, grain yield; HD, heading date; PH, plant height; TKW, thousand-kernel weight; GVW, grain volume weight.

The MFVD of each accession was estimated according to the primary trait GY and two correlated traits, PH and GVM, to further study the consistency of these identified accessions for high drought tolerance and high yield. Five classes were made according to the mean \pm SD of MFVD obtained for the traits (Table S1). Eleven accessions were highly drought tolerant (A, $U_i \ge 0.84$) and 21 accessions were drought tolerant (B, $0.75 \le U_i < 0.83$).

Among the 198 accessions, 23 had both smaller DSI values (cluster 1) and higher MFVD values ($0.86 \le U_i < 0.96$), including 12 early, nine intermediate, and two late accessions. The GY of the 23 accessions identified with good yield stability and high drought tolerance was distributed across the range of the 198 accesses by comparing the GY of those accessions among all the accessions (Table 6). Of the 23 accessions, 8 accessions had high GY in three environments, comprising of four early and four intermediate accessions. Five accessions were classified into the group of low-yield in the three environments, comprised of three early and two late accessions. The yield performance of the other ten accessions, including five early and five intermediate accessions, was inconsistent across environments.

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ACNO	Maturity _	HD (days)			DSI	1213	MFV	D1213		GY (t/ha)	
Actio		1213IR	1213TD	1314TD	Value	Cluster	Value	Class	1213IR	1213TD	1314TD
PI622701	Early	157.0	157.2	157.0	0.40	1	0.88	В	6.51	5.89	6.50
PI573732	Early	156.6	157.0	156.3	0.12	1	0.90	В	5.52	5.37	5.98
PI559524	Early	156.8	156.2	156.7	0.02	1	0.96	А	5.28	5.25	5.25
PI361982	Early	156.6	157.2	156.7	0.07	1	0.89	В	5.02	4.94	5.45
PI595379	Early	152.6	152.2	150.3	0.15	1	0.90	В	4.62	4.45	5.00
PI361980	Early	154.6	156.2	154.7	0.12	1	0.96	А	4.23	4.11	5.00
PI406530	Early	155.6	157.2	153.0	0.23	1	0.95	А	4.76	4.5	4.88
PI548139	Early	156.6	156.2	156.0	0.05	1	0.96	А	4.22	4.17	4.77
PI314558	Early	155.6	156.2	156.7	0.08	1	0.90	В	4.02	3.94	4.77
PI195545	Early	156.8	156.4	155.3	0.23	1	0.95	А	4.37	4.13	3.84
PI57159	Early	155.2	156.4	153.7	0.04	1	0.92	А	3.25	3.22	3.43
PI414577	Early	150.2	153.2	153.0	0.28	1	0.92	А	2.53	2.36	3.35
PI620751	Intermediate	158.8	159.2	158.0	0.09	1	0.88	В	6.04	5.91	6.18
PI622644	Intermediate	158.8	159.6	159.0	0.33	1	0.90	В	5.96	5.49	5.44
PI622586	Intermediate	159.8	159.0	159.3	0.28	1	0.94	А	5.84	5.44	5.22
PI627480	Intermediate	158.8	159.6	159.7	0.52	1	0.87	В	5.76	5.05	5.18
PI84527	Intermediate	160.6	159.6	159.3	0.06	1	0.95	А	5.45	5.37	4.23
CItr11363	Intermediate	159.6	158.6	159.3	0.30	1	0.91	В	5.33	4.95	4.13
PI428660	Intermediate	159.4	160.2	158.7	0.32	1	0.89	В	5.02	4.64	5.09
PI372344	Intermediate	159.4	159.0	159.3	0.00	1	0.95	А	4.91	4.91	4.63
PI627303	Intermediate	160.8	162.2	160.7	0.23	1	0.92	А	4.82	4.56	5.22
CItr16642	Late	168.8	167.6	168.0	0.04	1	0.86	В	4.14	4.10	4.22
PI192716	Late	164.4	170.2	167.3	0.24	1	0.88	В	2.16	2.03	2.21
Range of population Average value and standard deviation [#] Norwest553 (PI 655030) [#] IDO444 (PI 578278) [#] Settler (PI 6538833) [#] Yellowstone (PI 643428) [#] Hatcher (PI 638512) All the checks									2.16-6.97 4.89 $^{a} \pm 1.01$ 6.38 $^{a} \pm 0.74$ 5.63 $^{a} \pm 0.90$ 6.11 $^{a} \pm 1.00$ 5.64 $^{a} \pm 1.05$ 6.79 $^{a} \pm 1.17$ 6.11 $^{a} \pm 1.04$	$\begin{array}{c} 1.626.26\\ 3.72\ ^{c}\pm1.06\\ 5.05\ ^{b}\pm1.17\\ 4.91\ ^{b}\pm0.98\\ 5.31\ ^{b}\pm0.62\\ 5.11\ ^{b}\pm0.75\\ 5.83\ ^{b}\pm0.78\\ 5.24\ ^{b}\pm0.91\end{array}$	$\begin{array}{c} 2.086.57 \\ 4.30 \ ^{\text{b}} \pm 0.96 \end{array}$

Table 6. Performance of Grain yield (GY) for high drought resistant winter wheat accessions by drought susceptibility index (DSI) and membership function value of drought tolerance (MFVD) and five checks [#].

1213IR, irrigated in 2012–2013 seasons; 1213TD, terminal drought in 2012–2013 seasons; 1314TD, terminal drought in 2013–2014 seasons; HD, heading date; Values in a row followed by the same letter are not significantly different status at $\alpha = 0.05$. Italic and bold showed high yield in three environments. Boldface and Italic indicated low yield in three environments.

3. Discussion

Maintaining wheat production is a challenge under drought and high temperatures. Improving GY will almost certainly require employing the underused genetic resources of wheat collections [38]. Some of wheat germplasm from the International Maize and Wheat Improvement Center has exhibited excellent adaption in environments with drought and heat stress, as these materials were selected in Ciudad Obregon, northwestern Mexico, where temperatures increased steadily from January to July to a maximum of about 40 °C [10,39,40]. Heat stress escape via early maturity is an excellent adaptation approach for wheat in regions with terminal and continual high temperature stress, such as in South Asia [41,42].

Agronomic traits, including GY, HD, PH, TKW and GVW, of 198 winter wheat accessions of early, intermediate, and late maturity were evaluated in irrigated and terminal drought conditions in the present study. The significant effects of environment on these five traits (p < 0.0001) resulted from factors such as water treatments and temperature. Large differences for the five traits were found between the maturity groups under different conditions. The early accessions produced higher GY and heavier TKW than the late accessions in all three environments, which was consistent with previous studies [10,39]. Early accessions headed earlier, were shorter, and had greater GVW than late maturity accessions, likely because the early accessions escaped late high temperature stress [42]. These results show the value of higher GY (under both irrigation and terminal drought conditions) of the early winter wheat accessions from the NSGC.

The effect of drought on GY and other agronomic traits depends on the timing and severity of drought occurrence. GY is particularly sensitive to water stress [43] and agronomic trait such as PH, TKW and GVM decreased under drought stressed conditions [13,16,29,43]. In the present study, terminal drought was induced when 95% of the plots had headed, and wheat accessions under stressed conditions received irrigation before induction of terminal drought, so as the stress could be regarded as moderate. However, drought stress of some accessions of the early maturity group was less serious than the other two groups, because some accessions of this group had passed anthesis. GY, PH and TKW were significantly affected by terminal drought, which was consistent with that reported by others [17]. HD did not respond to drought conditions as reported previously [17], which was due to the drought stress occurring during the heading stage. GY, PH and GVW exhibited marked reductions under the terminal drought conditions, suggesting that these traits are sensitive to water stress and could be used to evaluate drought tolerance in wheat.

GY has commonly been used as a direct selection index for drought tolerance, as represented by the DSI, which is based on the GY difference between the well-watered and water-stressed conditions and is used to identify more stable genotypes [14,29]. However, GY had lower heritability and high genotype by environment interaction. Traits with higher genetic variation and heritability, such as PH, could be a reliable selection criterion for drought tolerance in wheat breeding [16]. A number of secondary traits such as PH, GVW and HD have been investigated to provide relevant information for drought tolerance evaluation [17,29,37]. Selection criteria based on secondary traits may vary significantly with different germplasm [44]. Therefore, it is necessary to evaluate different selection criteria in wheat germplasm with a broad geographic origin and diverse genetic background like the accessions used in the present study.

In the present study, 109 accessions were identified with smaller DSI values (DSI < 1) across different environments, suggesting these accessions produced better yield stability. However, analysis on the performance of GY showed some of drought tolerant accessions identified by DSI displaying lower GY Thus, aside from DSI, other indices of drought tolerance should be considered when evaluating the performance of GY.

The MFVD index integrates the drought-tolerant coefficient (DC) of different traits [16,37]. In the present study, the H^2 of the five traits was calculated using the average mean of three environments. HD had the highest heritability, as the trait was least influenced by the environment. The fact that the heritability values for the five traits were relatively high shows that variation was mostly

a result of genetic differences. By examining the heritability of the five traits and the correlation between other four them, GY, PH and GVW were selected as the best traits for evaluating drought tolerance. Through calculating MFVD values of the selected traits, eleven accessions were identified with high drought tolerance, including seven early and four intermediate accessions. Twenty-one accessions were identified with drought tolerance, comprising eleven early, seven intermediate and three late accessions.

The GY of the drought tolerant accessions identified by DSI and MFVD did not always produce high GY. This result is not surprising because high GY and drought adaptation may be based on conflicting mechanisms [45]. In addition, in most cases, drought stress mainly resulted in a reduction of GY rather than the plant death [46]. Thus, when breeding cultivars with drought tolerance, GY performance should be considered, including both yield stability and GY potential [47]. The wheat accessions with good yield stability and high drought-tolerance identified in the present study could be used as breeding materials for crossing with high-yield but drought-sensitive lines to generate lines with both high drought tolerance and high GY. The eight accessions identified with high GY under the terminal drought environments could be further investigated for their potential application in the improvement of drought tolerance of wheat accessions with high yield under the moderate drought stress conditions. In addition, since 9K SNP data is available for these accessions, further studies should undertake GWAS analysis of the traits assessed in the present study.

4. Material and Methods

4.1. Plant Materials

The 198 winter wheat accessions used in this study originated from 46 countries were classified into three maturity groups according to heading data (HD, Julian days), including 61 early, 71 intermediate, and 66 late accessions. The early group had HD of 148–157 days, intermediate 158–162 days, and late 163–171 days. The HD of cultivars adapted to the region is from 152 to 161 days. The accessions number, name, country and state (if available), improvement statue, and maturity classification of these accessions are listed in Table S2.

4.2. Experimental Design

Field experiments were carried out in two growing seasons, 2012-2013 and 2013-2014, in Aberdeen, Idaho, USA (42.96° N, 112.83° W, and elevation 1342 m). Drought tolerance was evaluated in the 2012–2013 growing season under irrigated (1213IR) and terminal drought (1213TD) environments using an un-repeated augmented complete block design. This augmented design was useful for screening large numbers of new and untried treatments [48]. The 198 accessions were planted side by side in two irrigation treatments without replication and were randomly assigned to twelve sub-blocks. The five checks were replicated and assigned randomly within each of twelve blocks. Therefore, each sub-block comprised 45 un-replicated wheat accessions to be evaluated and the five check cultivars. The five checks were all high yielding cultivars at the released time, Norwest553 (PI 655030), IDO444 (PI 578278), Settler (PI 6538833), Yellowstone (PI 643428) and Hatcher (PI 638512). Accessions in each treatment were divided into early, intermediate, and late maturity groups and both terminal stress and no stress (irrigated) treatments were applied in the 2012–2013 season. In the 2013–2014 growing season, all accessions were planted with three replications under terminal drought (1314TD). An aluminum sprinkler pipes system was used to irrigate each treatment. The 1213IR was irrigated once per week between May and July. To induce terminal drought stress, in each year these treatments were irrigated until 95% of plots had headed. Table 7 and Figure S1 show the distribution of precipitation, irrigation, and maximum and minimum mean daily temperature in both growing seasons. Precipitation was 129.5 mm during the 2012-2013 growing season and 1062 mm of irrigation water was applied in the 1213IR for a total of 1191.5 mm. For 1213TD, a total amount of 708 mm, 826 mm, and 944 mm of irrigation water was applied for the early, intermediate

and late maturity groups, respectively. The precipitation in the 2013–2014 growing season was 248.7 mm and 705 mm of irrigation water was applied for a total of 953.7 mm. Sowing density in each environment was adjusted by thousand kernels weight to provide 0.48 million seeds per hectare. The plot dimensions were $3.0 \text{ m} \times 1.5 \text{ m}$ with seven rows and 25 cm between rows. Herbicide and fungicide were sprayed as needed throughout the growing season. The application of fertilizer was adjusted based on a soil test before planting to provide a rate of 207 kg N ha⁻¹.

Years	Treatment	Maturity Groups	Precipitation (mm) Irrigation (mm)		- Total (mm)	Averag	e High Ter	np. (°C)	Average Low Temp. (°C)				
leals	Ireatment	Maturity Groups	September~July	May	June	July	- 10tal (11111) -	May	June	July	May	June	July
2012-2013	1213IR	Early/Intermediate/Late	129.5	354.0	590.0	118.0	1191.5	19.3	26.3	31.7	5.4	8.4	12.4
	1213TD	Early	129.5	354.0	354.0	/	837.5	19.3	26.3	31.7	5.4	8.4	12.4
		Intermediate	129.5	354.0	472.0	/	955.5	19.3	26.3	31.7	5.4	8.4	12.4
		Late	129.5	354.0	590.0	/	1073.5	19.3	26.3	31.7	5.4	8.4	12.4
2013-2014	1314TD	Early/Intermediate/Late	248.7	376.0	329.0	/	953.7	20.9	24.1	31.2	5.2	7.3	11.8

Table 7. Description of the test environments.

1213IR, irrigated in 2012–2013 seasons; 1213TD, terminal drought in 2012–2013 seasons; 1314TD, terminal drought in 2013–2014 seasons. Average high or low temperature was estimated based on daily maximum or minimum temperature for the month.

4.3. Trait Measurements

Heading date (HD, Julian days) was recorded as the number of days from 1 January until 50% of the spikes in the plot were fully emerged from the boot. At maturity (Feekes 11.3–11.4) [49], plant height (PH, cm) was measured as the distance between the soil surface and the tip of the spike excluding awns. Plots were harvested using a Wintersteiger Classic small plot combine (Wintersteiger Inc., Salt Lake City, UT, USA) equipped with a Harvest Master weighing system (Juniper Systems, Inc., Logan, UT, USA). Grain yield (GY, t/ha) was calculated by the grain weight of each accession in each plot at the moisture content of 12%. Single kernel weight was obtained from cleaned grain samples with the single-kernel characteristics system (SKCS 4100; Perten Instruments Inc., Springfield, MO, USA) and was converted into thousand-kernel weight (KWT, g). Grain volume weight (GVW, kg/m⁻³) (test weight) was measured from cleaned grain samples by a one-pint container ($5.5 \times 10^{-4} \text{ m}^3$) using a grain analyzer (Inframatic 9100; Perten Instruments Inc., Springfield, IL, USA).

4.4. Data Analysis

All the data was analyzed using JMP (Version 12.0, SAS Institute Inc., Cary, NC, USA). The adjusted means of each trait measured in 1213IR and 1213TD were estimated for block differences which were measured by the cultivar checks [50]. The adjustment of un-replicated accession values (T_{adj}) for block effects was calculated following the equation:

$$T_{adj} = T_{orig} - \left(\overline{T_i} - \overline{T_J}\right) \tag{1}$$

Here, T_{orig} is the original value of un-replicated accessions, $\overline{T_i}$ is the mean of the replicated checks in block *i*. $\overline{T_I}$ is the overall mean of the replicated checks.

Analysis of variance for 1213IR and 1213TD was performed by the random effects of blocks and fixed effects of genotypes [50,51]. The two-way analysis for the 1314IR was performed with random environment effects and fixed genotype effects by the least square mean estimated [52]. PROC GLM was used in the analysis of variance, where genotypes were treated as fixed effects and environments and the interaction of genotypes and environments and blocks nested in environments were all treated as random effects. The inverse of the variance of the individual environments were treated as weights. The broad sense heritability H_B^2 was calculated as:

$$H_B^2 = \sigma_g^2 / \left(\sigma_g^2 + \sigma_{ge}^2 / r + \sigma_{\varepsilon}^2 / er\right)$$
⁽²⁾

Here, σ_g^2 is the genetic variance, σ_{ge}^2 is the interaction variance of genetic and environment, δ_{ϵ}^2 is the error variance, *r* is the number of replicates, and *e* is the number of environments.

The drought tolerance of the genotype in the 2012–2013 season was evaluated with the drought susceptibility index (DSI) [53] following the equation:

$$DSI = \frac{1 - \frac{\overline{GY}_{1213TD}}{\overline{GY}_{1213IR}}}{D}$$
(3)

Here \overline{GY}_{1213TD} and \overline{GY}_{1213IR} are the mean GY for each accession under terminal drought (1213TD) and under irrigated (1213IR) environments, respectively, and $D = 1 - \frac{\overline{GY}_{1213TD}}{\overline{GY}_{1213IR}}$, here \overline{GY}_{1213TD} and \overline{GY}_{1213IR} are the mean GY of all the accessions under terminal drought (1213TD) and under irrigated (1213IR) environments, respectively. The cluster analysis of DSI was conducted with JMP.

The drought-tolerant coefficient (DC) for each trait in 2012–2013 season was calculated [54,55].

$$DC_{j} = \frac{X_{j1213TD}}{X_{j1213IR}}$$
(4)

Here, DC_j was the drought-tolerant coefficient of the trait (*j*) for the accession; $X_{j1213TD}$ and $X_{j1213IR}$ were the values of the trait (*j*) for the accession evaluated under 1213TD and 1213IR conditions, respectively.

The drought tolerance of wheat was evaluated by using the membership function value integrating multiple traits. The membership function of a fuzzy set was used for a comprehensive assessment [56]. The first step uses the membership function to assign each indicator a corresponding value in the unit interval [0,1], then single-factor membership function value was calculated to assess each indicator. Next a weighted arithmetic mean of all the single-factor membership function values was calculated as the comprehensive membership function value. According to the DC, the modified membership function value of drought tolerance (MFVD) in 2012–2013 season was calculated as:

$$U_{ij} = \frac{DC_{ij} - DC_{jmin}}{DC_{jmax} - DC_{jmin}}$$
(5)

$$U_{i} = \frac{1}{n} \sum_{j=1}^{n} U_{ij}$$
(6)

Here, U_{ij} is the membership function value of the trait (*j*) for the genotype (*i*) for drought tolerance; DC_{jmin} is the minimum value of DC_{ij} for all the genotypes; DC_{jmax} is the maximum value of DC_{ij} for all the genotypes; U_i is the average value of the membership function of all the traits for the genotype (*i*).

Drought tolerance was divided into five classes (A, B, C, D and E) according to the average value (\overline{U}) and standard deviation (SD) by value of MFVD. (A) $U_i \ge \overline{U} + 1.64$ SD, highly drought tolerant; (B) $\overline{U} + 1$ SD $\le U_i < \overline{U} + 1.64$ SD, drought tolerant; (C) $\overline{U} - 1.64$ SD $\le U_i < \overline{U} - 1$ SD, moderately drought tolerant; (D) $\overline{U} - 1$ SD $\le U_i < \overline{U} + 1$ SD, susceptible; (E) $U_i < \overline{U} - 1.64$ SD, highly susceptible [16].

Significant differences among different maturity groups were determined using Fisher's protected LSD at $\alpha = 0.05$. Analysis of variance (ANOVA) was conducted for the traits in winter accessions of different maturity groups in each environment (Table S3). Significant differences for ANOVA and correlations were detected as * p < 0.05, ** p < 0.01, *** p < 0.001, and **** p < 0.0001.

5. Conclusions

The present study evaluated agronomic traits (GY, HD, PH, TKW and GVW) and drought tolerance for a set of 198 winter accessions from USDA-ARS NSGC in one irrigated and two terminal drought conditions. Wheat accessions were classified into early, intermediate, and late maturity groups. Considerable variation in agronomic traits was observed among three maturity groups. Comparing agronomic traits among the three maturity groups, early accessions exhibited the best agronomic performance with higher GY, heavier TKW, greater GVW, and shorter PH. Terminal drought had significant effects on GY, PH, TKW and GVW. Twenty-three accessions were identified with high yield stability (DSI \leq 0.52) and drought tolerance using the DSI and MFVD indices and these accessions may be useful materials for drought tolerance improvement. Analysis of the GY indicated that drought resistant accessions did not always have high GY potential. Of the 23 accessions, 8 accessions had both drought tolerance and higher GY, and these 8 are the most promising for improving both yield and drought tolerance.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4395/7/3/51/s1, Figure S1: Maximum and minimum daily air temperature at two meters and precipitation in the 2012–2013 and 2013–2014 growing seasons. The weather data were from AgriMet Cooperative Agricultural Weather Network and National Oceanic and Atmospheric Administration, Table S1: Drought Susceptibility Index (DIS) and Membership function value of drought tolerance (MFVD) and grain yield (GY) of the 198 accessions, Table S2: The number of accessions (ACNO), name, country, state, improvement status, and maturity group of 198 winter wheat accessions, Table S3: The analysis of variance (ANOVA) of winter accessions (198 accessions and five checks in 1213IR and 1213TD, three replications in 1314TD) for the traits in three maturity groups (early, intermediate and late) in three environments.

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