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Performance of soft red winter wheat subjected to field soil waterlogging: Grain yield and yield components

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ABSTRACT

Soil waterlogging impacts 25% of the global area planted to wheat and the development of waterlogging tolerant cultivars lags behind progress that has been made for other abiotic stresses. This study was conducted to identify sources of waterlogging tolerance in soft red winter wheat (SRWW) adapted to the southeastern United States, a region prone to yearly waterlogging. A set of 28 SRWW genotypes were evaluated over two growing seasons in field waterlogging experiments at the Rice Research and Extension Center in Stuttgart, Arkansas. Waterlogging at the late tillering stage resulted in a mean grain yield (GY) reduction of 34%, ranging from 16 to 49% within the tested lines. Total GY was impacted by lower kernel weight spike⁻¹ resulting from reductions in kernels spike⁻¹ and 1000 kernel weight, total biomass and to a lesser extent spike density. An interaction between genotype and the waterlogging treatment (GxT) was observed for days to heading, plant height, kernel weight spike⁻¹, kernels spike⁻¹ and 1000 kernel weight with significant GxT detected for total GY during the first season only. Spectral reflectance measurements of normalized difference vegetative index were highly predictive of both GY ($R^2 = 0.77$) and total biomass ($R^2 = 0.64$) under waterlogging but not in the control, indicating potential for indirect high-throughput screening and selection. Overall, the genotypes 26R22, AR01167-3-1, Magnolia and USG 3555 had significantly higher GY than other genotypes under waterlogging, with USG 3555 also showing a non-significant GY reduction, indicating its potential as a genetic source of waterlogging tolerance.

Keywords: Waterlogging tolerance, abiotic stress, wheat, *Triticum aestivum*, NDVI

Abbreviations: GY-grain yield; Sm²-spikes m⁻²; KNS-kernel number spike⁻¹; KWS-kernel weight spike⁻¹; TB-total biomass; HI-harvest index; TKW-1000 kernel weight; DH-days to heading; PH-plant height; NDVI-normalized difference vegetative index;

1. INTRODUCTION

Soil waterlogging affects up to 10% of global land area, including 25% of the worldwide area planted to wheat (Powell et al. 2012). With global climate change predicted to result in increased winter precipitation, improving the waterlogging tolerance of wheat is a priority for plant breeders in growing regions prone to soil waterlogging such as the southeastern United States where soft red winter wheat (SRWW) is grown. During waterlogging stress, saturation of the air pore spaces in the soil results in hypoxia or anoxia due to very low or complete absence of oxygen available for the plant, respectively (Colmer and Voesenek 2009). Low oxygen availability disturbs plant physiology and metabolism, resulting in reduced growth, delayed development and lower total grain yield (GY) and yield components (Dickin and Wright 2008). A reduction in GY is physiologically explained by a lower number of molecules of adenosine triphosphate produced in the hypoxic environment, which triggers lactic acid and alcohol fermentation with fewer nutrients available for growth and development compared to aerobic conditions (Teakle et al. 2011).

Waterlogging stress duration and intensity, as well as the developmental stage at which the stress is applied, determine the extent of GY reduction. Ali et al (2002) observed GY reductions of 11%, 26% and 45% for the winter wheat cultivar “Marvdasht” waterlogged for 10, 20 and 30 days, respectively, at the 4-5 leaf stage. Collaku and Harrison (2002), evaluated the effect of five weeks of uninterrupted waterlogging initiated at the tillering stage on 15 soft red winter wheat (SRWW) cultivars and reported an average GY reduction of 44%. Dickin and Wright (2008) observed GY reductions of 20 and 24% in winter wheat exposed to waterlogging for 44 and 58 days, respectively, beginning at the tillering stage. When treated at the same stage, a 32% reduction in GY was observed with 14 days of waterlogging in the Australian spring

wheat cultivar ‘Wyalkatchem’, including a 50% reduction in tiller number (Robertson et al. 2009). Shao et al. (2013) reported GY reductions of 7.1 to 11.2% depending on the growth stage at the time of treatment, with the largest reductions observed at pre-anthesis. In addition to total GY, other studies have found reductions in yield components, including shoot weight (Khabaz-Saberi et al. 2006), spike density (Ali et al. 2012; Collaku and Harrison 2002) and kernel weight (Ali et al. 2012; Collaku and Harrison 2002; Musgrave and Ding 1998) as well as root biomass (Araki et al. 2012).

Despite the economic impacts of soil waterlogging on wheat production, particularly in the winter wheat growing region of the southeastern U.S., reports on the performance of adapted genotypes is extremely limited. Collaku and Harrison (2002) reported GY reductions ranging from 15 to 60% in 15 SRWW cultivars grown under five weeks of continuous waterlogging stress, the only such study in SRWW. A follow-up study using F₂ derived families with the tolerant cultivar ‘Tchere’ as a parent reported kernel weight to have the highest heritability and that a 17% increase in GY could be achieved by a selection index incorporating GY, kernel weight and tiller number (Collaku and Harrison 2005). Overall, the lack of information on the response of wheat genotypes to soil waterlogging stress is an obstacle toward determining genetic resources that could be integrated for developing cultivars with improved tolerance. Therefore the objective of this study was to evaluate the performance of 28 SRWW cultivars and breeding lines adapted to the southeastern U.S. in terms of their GY performance under field waterlogging stress. In addition, the contribution of yield components to total GY and the utility of normalized difference vegetative index (NDVI) as a tool to select high yielding genotypes was also explored.

2. MATERIAL AND METHODS

2.1 Plant material and field experiments

A set of 28 SRWW cultivars and advanced breeding lines adapted to the southern U.S. growing region was used for this study. Field waterlogging experiments were carried out at the Rice Research and Extension Center (RREC) in Stuttgart, Arkansas in the 2012-2013 (YR1) and 2013-2014 (YR2) growing seasons. Stuttgart soils are characterized by a silt loam surface layer and a clay subsoil with low permeability (NRCS 2013) and are prone to periodic waterlogging. The experimental design was a split plot with four replications in both years with the waterlogging treatment (waterlogging and non-waterlogging) as the main plot and genotypes as the subplots in a randomized complete block design (RCBD). Field plots consisted of seven rows drill seeded at a density of 280 plants m⁻² with total plot area measuring 1.25 m wide x 6 m long. In addition to pre-plant application of P and K based on soil test recommendations, all plots were fertilized with 170 kg of N as urea in a split application, with 60% applied prior to the waterlogging treatment and 40% applied post waterlogging treatment.

Field waterlogging was performed similar to Collaku and Harrison (2002), by establishing 0.30 m high levees surrounding the experimental field and using water from a nearby reservoir to apply a flood application twice weekly to maintain soil saturation for the duration of the treatment. While not continuously monitored, periodic measurements of soil moisture content indicated that soil saturation was maintained throughout the experiment. In YR1, the waterlogging treatment was started on March 20, 2013 at Feekes stage 4 and terminated on April 17, 2013 at Feekes stage 5 (28 days). In YR2, the treatment was started on April 1 at Feekes stage 4 and terminated on April 14 at Feekes stage 5 (14 days). A shorter

treatment time was used in the second year to lessen the stress impact and increase the observed genotypic variation for the measured traits not observed in the first year.

2.2 Trait measurement

Yield components including spikes m^{-2} (Sm²), kernel number per spike (KNS), kernel weight per spike (KWS), total biomass (TB) and harvest index (HI) were estimated by hand-harvesting 50 spike-bearing culms from each plot at maturity prior to whole plot harvest. A Seedburo 801 (Seedburo Equipment Company, Des Plaines, IL) was used to count 1000 kernels and determine 1000 kernel weight (TKW). Plant height (PH) was measured at maturity from the soil surface to the top of each plot, excluding awns. Days-to-heading (DH) was measured in Julian days at 50% spike emergence for each plot. Grain yield in g m^{-2} was determined on whole plots by combine harvesting, drying and weighing the grain with final GY adjusted to 13% moisture. Test weight (TW) was determined on a volume basis in kg hl^{-1} using a GAC2500AGRI DICKEY-John Grain Moisture Tester (Churchill Industries, Minneapolis, MN).

Measurements of NDVI were taken immediately following termination of the waterlogging treatment with a Greenseeker[®] Handheld Sensor (Trimble Navigation Limited, Sunnyvale, California) equipped with a Trimble[®] Nomad[®] 900 handheld computer. Readings were taken by walking between adjacent plots with the sensor held over the center row at a height of 0.50 m above the crop canopy, resulting in a plot coverage of 0.45 m. For each plot, repeated measurements (20-25 per plot) were recorded and averaged to give one measurement per plot. All measurements were taken near mid-day.

2.3 Statistical analysis

Data were analyzed both across years and in each year separately using SAS/STAT[®] software, version 9.3 (SAS Institute, Cary, NC). For across years, data were analyzed in a split-plot design in a manner similar to Collaku and Harrison (2002) according to the statistical model:

$$y_{ijklm} = u + r(y)_{l(i)} + [wr(y)]_{jl(i)} + g_k + (yw)_{ij}(yg)_{ik} + (wg)_{jk} + (ywg)_{ijk} + e_{ijklm}$$

where y_i is the effect of the i th year, $r(y)_{l(i)}$ is the effect of the l th replication within the i th year, w_j is the effect of the j th level of waterlogging; $[wr(y)]_{jl(i)}$ is the interaction effect of j th waterlogging level with the l th replication nested within year, representing the error for the waterlogging effect; g_k is the effect of the k th genotype; $(yw)_{ij}$ is the year by genotype interaction effect; $(wg)_{jk}$ is the genotype by waterlogging interaction effect; $(ywg)_{ijk}$ is the interaction effect of year by waterlogging by genotype; and e_{ijklm} is the random error for genotypes and interactions of first and second order that involve genotype, waterlogging and year effects. Waterlogging treatment, genotypes and years and their interactions were considered fixed effects (Collaku and Harrison 2002). Replications were considered random effects as were interactions which involved a random effect. Similar methods were used for analyzing years separately, without the effect of years and its interactions. To determine the percent of total variance contributed by each effect in the model, covariance parameters were estimated using Type 3 sum of squares with all effects treated as random. As dominance effects are not present in inbred lines, narrow-sense heritability (h^2), was estimated on an entry mean basis using covariance estimates and the following formula derived for a split-plot design with two treatments, two years and four replications:

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$$h^2 = \frac{\sigma_{\text{GEN}}^2}{\sigma_{\text{GEN}}^2 + \sigma_{\text{GEN} \times \text{TRT}/\text{T}}^2 + \sigma_{\text{GEN} \times \text{Year}/\text{Y}}^2 + \sigma_{\text{GEN} \times \text{Year} \times \text{TRT}/\text{YT}}^2 + \sigma_{\text{error}/\text{YTR}}^2}$$

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169 where σ_{GEN}^2 , $\sigma_{\text{GEN} \times \text{TRT}}^2$, $\sigma_{\text{GEN} \times \text{Year}}^2$, $\sigma_{\text{GEN} \times \text{Year} \times \text{TRT}}^2$ and σ_{error}^2 are the genotype variance,
170 genotype by waterlogging treatment variance, genotype by year variance, genotype by year by
171 waterlogging treatment variance and error variance, respectively; and T, Y and R are the number
172 of treatments, years and replications, respectively.

173 Pearson's correlation using PROC CORR and principal component analysis (PCA) using
174 the PRINCOM procedure in SAS v 9.3 were used to determine associations among the measured
175 phenotypic traits. For the PCA, the contributions of each variable to the first two principal
176 components are illustrated using bi-plots. Multiple regression was used to determine the
177 variation in GY explained by its components, with the independent variables (KNS, Sm2, TKW,
178 NDVI and KWS) added and removed in a step-wise manner to determine contribution based on
179 partial R^2 . A minimum value of $p \leq 0.15$ was used for determining which traits were included in
180 the final regression equation.

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182 **3. RESULTS**

183 *3.1 Impact of waterlogging stress on yield and yield components*

184 Twenty-eight SRWW genotypes were evaluated under field waterlogging beginning at
185 the late tillering stage at the RREC in Stuttgart, Arkansas during the 2012-2013 (YR1) and 2013-
186 2014 (YR2) seasons. When years were analyzed separately, waterlogging treatment (main effect)
187 accounted for the largest portion of phenotypic variance for most traits in YR1, ranging from 9%
188 for TW to 59% for GY with a significant effect ($p \leq 0.05$) observed for all traits with the

exception of TW and Sm2 (Table 1). Overall, mean GY was reduced by 42%. Likewise, in YR2 a significant treatment effect ($p \leq 0.05$) was observed for all traits with the exception of GY, DH, Sm2 and TB. Although not significant in YR2, a 13% GY reduction was observed. Overall a larger GY reduction was observed in YR1, likely due to the 28 days of waterlogging stress versus the shorter treatment of 14 days applied in YR2. Despite this difference, a significant interaction between waterlogging treatment and year was only detected for Sm2 ($p \leq 0.05$) and not for total GY or other traits (Table 2).

When analyzed across years, a significant effect ($p \leq 0.05$) for waterlogging treatment was observed for all traits with the exception of DH, Sm2 and NDVI (Table 2). Mean GY was reduced by 34%, resulting from the combined effect of reductions in KWS of 28%, KNS of 17%, Sm2 of 8% and TKW of 14% (Table 3). Narrow-sense heritability of measured traits ranged from non-heritable to $h^2 = 0.63$. Of the measured yield components, TKW was the most heritable ($h^2 = 0.63$) followed by TW ($h^2 = 0.48$) and KNS ($h^2 = 0.38$). Grain yield was lowly heritable ($h^2 = 0.27$). Biomass related traits, including TB and HI had no detectable heritability as they were strongly influenced by interactions between genotype and year.

Table 1

Means and percentage of total variance attributed to genetic, treatment, genotype x treatment and other effects for grain yield and yield components in 28 wheat genotypes evaluated under control and waterlogging treatments at the RREC in Stuttgart Arkansas from 2012-2014

Source	Yield (g m ⁻²)	Days to heading (days)	Plant Height (cm)	Test Weight (kg hl ⁻¹)	Kernel wt. spike ⁻¹ (g)	Kernels spike ⁻¹	Spikes m ⁻²	Biomass (g m ⁻²)	Harvest index (%)	1000 kernel wt. (g)	NDVI
<u>2012-2013</u>											
Means											
Waterlogging	451	117	84	73	1.03	42	25	439	844	0.54	0.83
Control	263	121	79	70	0.74	37	20	347	505	0.51	0.78
% reduction	-42	3	-6	-3	-29	-13	-19	-21	-40	-5	5.8
Percent variance											
Treatment - TRT	59*	-	16*	9	57***	32***	37*	15	52*	5.0*	30***
Genotype - GEN	0	-	0	0	0	4**	0	0	0	4	2
Rep(TRT)	24***	-	9***	6.0***	7***	1	12***	31***	25***	0	28***
GEN x TRT	4**	-	22***	28***	12***	13	11**	12**	7***	14*	1
Residual	14	-	53	56	24	49	40	41	16	77	40
<u>2013-2014</u>											
Means											
Waterlogging	306	117	70	74	0.86	37	24	362	953	0.31	0.70
Control	265	118	63	71	0.63	31	20	426	926	0.28	0.67
% reduction	-13	1	-10	-3	-26	-14	-14	17	-3	-10	4.1
Percent variance											
Treatment - TRT	5	4	33*	7.0*	58***	47***	28***	4	0	25***	0***
Genotype - GEN	14**	44***	12***	12*	11**	35***	25***	14**	24***	11*	35***
Rep(TRT)	6**	4	15***	0	0	2***	1	3	13***	0	42***
GEN x TRT	0	0	0	1	5*	5***	4	0	0	5	0
Residual	76	48	40	81	26	12	43	80	64	58	23***

*Indicates significance at P = 0.05

**Indicates significance at P = 0.01

***Indicates significance at P = 0.001

^a Data taken on one replication in 2012-2013

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Table 2

Split-plot ANOVA for grain yield and related traits in 28 soft red winter wheat genotypes grown under control and waterlogging treatments at the RREC in Stuttgart Arkansas from 2012-2014^a

Source of variation	df	Yield (g m ⁻²)	Days to heading (days)	Plant height (cm)	Test weight (kg hl ⁻¹)	Kernel wt. spike ⁻¹ (g)	Kernels spike ⁻¹	Spikes m ⁻²	Biomass (g m ⁻²)	Harvest index (%)	1000 kernel wt. (g)	NDVI
Genotypes (GEN)	27	16137***	9.26***	168***	25.61***	0.078***	45.31***	46667***	130050***	0.006**	129***	0.013***
Year	1	511491	88.57***	26525***	0.46	1.781**	12.49	56	7497585*	5.179***	2808***	1.402**
Treatment - TRT	1	1466956*	0.45	3676**	235.10*	7.307***	1721.15**	69423	4362821*	0.092**	3234***	0.125
Rep(Year)	6	127488	5.01*	487	12.86	0.079	35.25	151445	973287	0.001	45	0.069
GEN x TRT	27	6365	5.3***	80***	13.59	0.0508***	20.57***	8743	47374	0.004	41***	0.002
GEN x Year	27	11569***	3.04*	23	10.42	0.029**	21.71***	20094***	126136***	0.007***	20*	0.009***
TRT x Year		528820	0	104	85.72	0.217	81.89	554676*	1933695	0	19	0.004
TRT x Rep(Year)	5	96296***	0	217***	35.02	0.099***	90.59***	79041***	418570***	0.004	11	0.044***
GEN x Year x TRT	27	6333	5.38***	42	16.53	0.0206	13.81	16183**	41604	0.006**	14	0.001
Residual	325	4488	1.57	30	11.61	0.0144	9.45	8430	31840	0.003	13	0.001
h ^{2b}		0.27	0.62	0.52	0.48	0.24	0.38	0.57	0	0	0.63	0.32

*Indicates significance at P = 0.05

**Indicates significance at P = 0.01

***Indicates significance at P = 0.001

^a Column for each trait indicates mean squares from ANOVA

^b h² = narrow sense heritability calculated from: $\sigma^2_{\text{GEN}} / (\sigma^2_{\text{GEN}} + \sigma^2_{\text{GEN} \times \text{TRT}/2} + \sigma^2_{\text{GEN} \times \text{Year}/2} + \sigma^2_{\text{GEN} \times \text{Year} \times \text{TRT}/4} + \sigma^2_{\text{residual}/16})$

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Table 3

Grain yield and yield components in 28 soft red winter wheat genotypes grown under control and waterlogging treatments at the RREC in Stuttgart Arkansas from 2012-2014

Entry no.	Genotypes	Yield (g m ⁻²)			Kernel wt spike ⁻¹ (g)			Kernels spike ⁻¹			Spikes m ⁻²			1000 kernel wt. (g)		
		Control	Wlog.	Red (%)	Control	Wlog.	Red (%)	Control	Wlog.	Red (%)	Control	Wlog.	Red (%)	Control	Wlog.	Red (%)
1	AGS 2035	334	171	49	1.04	0.67	36	22.4	18.9	16	315	247	22	46	35	25
2	AGS 2038	419	219	48	1.15	0.63	45	27.3	18.9	31	363	349	4	42	33	21
3	AGS 2060	356	235	34	1.00	0.66	34	26.8	20.4	24	348	337	3	37	32	12
4	AR01163-3-1	412	225	45	0.80	0.60	25	24.7	20.2	18	528	370	30	32	30	7
5	AR01167-3-1	425	305	28	0.82	0.65	21	21.9	18.8	14	516	492	5	38	34	9
6	AR01179-4-1	379	203	47	0.94	0.62	34	26.6	20.9	21	392	329	16	35	30	14
7	Baldwin	340	240	29	0.99	0.73	26	22.6	20.4	10	370	325	12	44	36	18
8	GA031086-10E29	410	280	32	0.92	0.66	28	25.0	19.9	21	444	438	1	37	33	10
9	LCS BRAND 343	363	251	31	0.81	0.67	17	22.1	19.7	11	456	386	15	37	34	6
10	GA04570-10E46	365	236	35	1.12	0.72	36	27.3	21.8	20	314	333	-6	41	33	20
11	Jamestown	333	264	21	0.69	0.66	5	20.5	19.6	5	475	441	7	34	33	2
12	Terral LA754	291	245	16	0.93	0.70	24	18.5	18.7	-1	296	364	-23	50	38	24
13	AGS 2040	327	243	26	0.86	0.67	22	23.3	20.2	13	363	370	-2	37	33	11
14	LA0320DE-2	340	199	41	0.81	0.63	22	21.4	18.0	16	407	312	23	37	35	8
15	Pioneer 26R10	444	263	41	1.09	0.79	27	26.8	22.7	15	407	336	17	42	35	17
16	Pioneer 26R20	428	260	39	1.04	0.71	32	25.7	21.2	18	405	382	6	41	33	19
17	Pioneer 26R22	409	311	24	0.89	0.62	30	23.8	18.6	22	455	514	-13	37	34	8
18	Pioneer 26R41	390	240	38	1.06	0.75	29	24.5	21.8	11	358	312	13	44	34	22
19	Pioneer 26R53	401	281	30	0.82	0.69	16	23.0	20.8	10	480	421	12	36	33	6
20	Pioneer 26R87	334	182	46	1.00	0.64	36	21.6	18.0	17	325	277	15	46	36	22
21	Syngenta CK9553	363	232	36	1.01	0.67	34	26.3	19.2	27	351	352	0	39	35	10
22	Syngenta Magnolia	405	288	29	1.08	0.70	35	25.6	20.6	20	377	421	-12	42	34	19
23	Syngenta Oakes	402	255	36	0.88	0.64	28	26.5	20.6	22	452	392	13	33	31	7
24	Syngenta SY Harrison	405	262	35	1.00	0.68	31	26.6	21.2	20	406	381	6	37	32	14
25	Terral LA841	336	256	24	0.81	0.70	14	21.6	21.0	2	407	357	12	38	33	12
26	Terral TV8861	462	266	42	1.06	0.70	34	29.4	20.9	29	431	388	10	36	34	6
27	USG 3120	364	245	33	0.92	0.73	21	21.9	19.4	11	388	339	13	42	38	11
28	USG 3555	355	285	20	0.89	0.84	5	22.5	22.3	0	397	346	13	39	38	4
	Mean	378	248	34	0.94	0.68	28	24.2	20.2	17	401	368	8	39	34	14
	LSD within treatment	30			0.045			1.2			36			1.2		
	LSD between treatment	23			0.041			1.0			31			1.2		

3.2 Genotypic variation and interactions with waterlogging treatment

The effect of genotype was highly significant for all traits (Table 2) and despite the highly significant effect of treatment, genotypes showed a differential response to waterlogging (Table 3). Within the non-waterlogging treatment, TV8861 and 26R10 had the highest GY, followed by 26R20, AR01167-3-1 and AGS 2038. Within the waterlogging treatment 26R22, AR01167-3-1, Magnolia and USG 3555 had significantly higher GY than other genotypes. Reduction in GY as a result of waterlogging ranged from 16% for LA754 to 49% for AGS 2035, with Jamestown, LA754, AGS 2040, LA841 and USG 3555 the only genotypes not significantly reduced by the waterlogging treatment ($p \leq 0.05$).

A significant interaction effect between the waterlogging treatment and genotype was detected for DH, PH, KWS, KNS and TKW but not for total GY or biomass related traits (Table 2). A significant interaction effect between genotype and year was also detected for all traits with the exception of PH and TW, indicating that relative genotype performance differed between the two crop seasons and thus analyzing years individually was warranted.

When years were analyzed separately, genotype by treatment interaction was significant for all traits in YR1 with the exception of TKW, indicating that genotypes performed differently under waterlogging versus non-waterlogging conditions and an indication of variation for tolerance within the tested lines (Table 1). This was in contrast to YR2, with interaction between genotype and waterlogging treatment only significant for KWS and TKW and more of the phenotypic variance explained by genotype, ranging from 11 to 44% of the total variance.

3.3 Trait associations and contribution to grain yield

Both the PCA (Fig. 1) and Pearson correlations (Table 4) showed yield components to be positively correlated with total GY, although the magnitude of the correlations differed between treatments. Under waterlogging, biomass related traits were most strongly correlated with GY, including TB ($r = 0.94$), Sm2 ($r = 0.86$) and NDVI ($r = 0.88$). This was in contrast to the non-waterlogging control, where in addition to TB ($r = 0.81$) and Sm2 ($r = 0.52$) KNS was strongly correlated with GY ($r = 0.70$). While plant height showed a moderate correlation under control ($r = 0.42$), a slightly negative correlation was observed under waterlogging ($r = -0.14$), albeit non-significant. The PCA biplots distinguished four clusters (groupings) under waterlogged conditions, 1) DH, 2) TKW, HI, TKW, KNS, 3) NDVI, TW, GY, and 4) Bio, PH and Sm2. However, no clear clustering was observed in the non-waterlogging treatment. In addition, both the PCA and regression showed a strong association in the waterlogging treatment between NDVI and both GY ($R^2 = 0.77$) and TB ($R^2 = 0.64$) that was not observed in the control (Figure 2).

Multiple stepwise regression analysis was used to further explore the relationship between GY and the traits KNS, Sm2, TKW, NDVI and KWS. In the control treatment, the total R^2 explained was 0.96 with the contribution of individual traits KNS, Sm2, TKW and NDVI included in the final regression. The contribution of these traits to GY ranged from 0.75 to 51.4% with KNS the largest contributor. In the waterlogging treatment, the total R^2 explained was 0.98 with only Sm2, KNS and KWS included in the final regression. The contribution of these traits ranged from 0.33 to 75.3%, with Sm2 explaining the greatest amount of variation.

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Phenotypic correlations (r) of measured traits with total grain yield in 28 soft red winter wheat genotypes grown under control and waterlogging treatments at the RREC in Stuttgart Arkansas from 2012-2014			
Trait	Control Treatment	Waterlogging treatment	
Days to heading	0.42*	-0.14	
Plant height	0.14	0.36	
Test weight	0.05	0.26	
Kernel weight per spike	0.54	0.29	
Thousand kernel weight	0.32	0.08	
Kernel number per spike	0.70***	0.30	
Spike m ⁻²	0.52**	0.86***	
Total biomass	0.81***	0.94***	
Harvest index	0.26	0.31	
NDVI	0.48	0.88***	
*Indicates significance at P = 0.05			
**Indicates significance at P = 0.01			
***Indicates significance at P = 0.001			

Table 5
Multiple linear regression of grain yield (GY) with yield components in 28 wheat genotypes grown under control and waterlogging treatments.

Treatment	R ^{2a}	% Contribution ^b
<u>Control</u>		
GY = -619.31 + 14.63 KNS + 0.69 Sm2 + 6.77 TKW+ 135.79 NDVI + 0.00 KWS	0.96***	
Kernels spike ⁻¹ (KNS)	0.50***	51.4
Spikes m ⁻² (Sm2)	0.26**	27.8
1000 kernel wt. (TKW)	0.12 ^{ns}	20.2
NDVI	0.23*	0.75
Kernel wt spike ⁻¹ (KWS)	0.10 ^{ns}	0.00
<u>Waterlogging</u>		
GY = -358.25 + 2.03 KNS + 0.55 Sm2 + 0.00 TKW + 0.00 NDVI + 283.70 KWS	0.98***	
Kernels spike ⁻¹ (KNS)	0.09 ^{ns}	0.33
Spikes m ⁻² (Sm2)	0.74***	75.3
1000 kernel wt. (TKW)	0.00 ^{ns}	0.00
NDVI	0.77***	0.00
Kernel wt spike ⁻¹ (KWS)	0.08 ^{ns}	24.3

^a Coefficient of variation of each independent variable using simple linear regression
^b Percent of the total R² contributed by each independent variable based on partial R² values

Fig. 1. PCA bi-plots of phenotypic traits measured under control and waterlogged treatments for 28 soft red winter wheat genotypes evaluated at the RREC in Stuttgart, Arkansas from 2012-2014.

Fig. 2. Relationship of NDVI with grain yield (a) and biomass (b) under control and waterlogged treatments for 28 soft red winter wheat genotypes evaluated at the RREC in Stuttgart, Arkansas from 2012-2014.

4. Discussion

4.1 Impact of soil waterlogging on grain yield and yield components

Few studies have evaluated the impact of soil-waterlogging on wheat GY, particularly under field conditions (Ballesteros et al. 2015; Boru et al. 2001; Collaku and Harrison 2002, 2005; Dickin et al. 2009; Kahlowan and Azam 2002; Musgrave and Ding 1998; Setter et al. 2009; Zhang et al. 2007). A 34% reduction in mean GY was observed for the 28 SRWW genotypes evaluated over two seasons in this study with field waterlogging beginning at the late tillering stage. Differences in the severity of the treatment were observed between the two years, resulting from both a reduced duration of stress and overall lower yields and productivity in year 2 (Table 1). While reducing the duration of the stress did result in a larger amount of observed genotypic variation for GY and yield components, it also reduced the treatment effect and interaction between genotype and treatment which is valuable for identifying lines that perform differently between treatments. Overall, the results presented here are similar to previous studies in winter wheat that have reported reductions of 44% (Collaku and Harrison 2002) and 45% (Musgrave and Ding 1998). In addition to total GY, significant reductions ranging from 8 to 28% were observed in yield components, with KWS the most affected due to the combination of reductions in both KNS and TKW. Previous studies in winter wheat have shown spike density to be most significantly impacted by waterlogging (Collaku and Harrison 2002; Dickin et al. 2009; Musgrave and Ding 1998) when the stress is applied closer to seedling stage, in contrast to late tillering as shown in this study. Reductions in KNS are often reported as well (Collaku and Harrison 2002; Musgrave and Ding 1998).

4.2 Genetic variation for waterlogging tolerance

Despite most studies pointing to a high level of susceptibility in wheat to soil waterlogging, genotypes having a high level of tolerance were identified. A range in reduction from 16 to 49% was observed, with Jamestown, LA754, AGS 2040, LA841 and USG 3555 not significantly reduced by the waterlogging treatment ($p \leq 0.05$). Despite the potential tolerance of these lines in terms of lower GY reduction from stress, their overall GY performance was generally low. Similar results were reported by Collaku and Harrison (2002). The lone exception was USG 3555, which in addition to not showing a significant reduction in GY, was also in the highest yielding group. Overall, USG 3555 showed low reductions in KWS (5%), KNS (0%) and TKW (4%) which contributed to its high level of performance under waterlogging. Other genotypes showing high GY potential under waterlogging included 26R22, AR01167-3-1, and Magnolia, with these lines generally having higher Sm2 compared to lower yielding genotypes. The importance of high Sm2 are in agreement with previous waterlogging studies in both spring (Zhang et al. 2007) and winter wheat (Collaku and Harrison 2005).

4.3 Trait associations

In general, correlations between GY and yield components under waterlogging stress were positive, as has previously been reported (Collaku and Harrison 2002; Musgrave and Ding 1998; Zhang et al. 2007). In addition, measurements of NDVI were found to be highly correlated with GY in the waterlogging treatment (Fig. 1 and Fig. 2). The relationship of NDVI with other abiotic stresses has previously been established for drought (Babar et al. 2006b; Gutierrez-Rodriguez et al. 2004; Reynolds et al. 2007) and heat (Gutierrez et al. 2010; Hazratkulova et al. 2012; Lopes and Reynolds 2012; Reynolds et al. 2007) in addition to irrigated or well-watered

environments (Aparicio et al. 2000; Babar et al. 2006a; Christopher et al. 2014; Erdle et al. 2013; Freeman et al. 2003; Gutierrez-Rodriguez et al. 2004; Prasad et al. 2007; Reynolds et al. 1999). However, this study is the first report of the relationship of NDVI and waterlogging stress. The strong association with GY ($R^2 = 0.77$) observed here indicates that NDVI could be utilized for indirect selection of GY under waterlogging likely by selecting for increased biomass ($R^2 = 0.64$), a trait previously identified as a target for waterlogging tolerance in spring wheat (Zhang et al. 2007).

5. Conclusions and breeding implications

Waterlogging tolerance is a complex trait and these results reinforce the importance of screening breeding lines in waterlogged soils within a target environment. Furthermore, the observed interactions between genotype and the waterlogging treatment and with years suggest the importance of multi-environment testing across years. While improving GY is the ultimate goal, the higher heritability of yield components and the strong association between NDVI and GY make these traits potential targets that could collectively increase GY performance under waterlogged conditions. Tolerance information obtained from this research suggests potential cultivars that could be incorporated as parents for future crosses in breeding programs, particularly for soft red winter wheat cultivars targeted toward the southeastern United States.

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REFERENCES

- Ali R, Mohammad-Eghbal G, Saeid J-H, Mokhtar G, Mohsen S (2012) Impacts of waterlogging on shoot apex development and recovery effects of nitrogen on grain yield of wheat. *European Journal of Experimental Biology*, 2012 2:8.
- Aparicio N, Villegas D, Casadesus J, Araus JL, Royo C (2000) Spectral vegetation indices as nondestructive tools for determining durum wheat yield. *Agronomy Journal* 92:83-91.
- Araki H, Hossain MA, Takahashi T (2012) Waterlogging and Hypoxia have Permanent Effects on Wheat Root Growth and Respiration. *J Agron Crop Sci* 198doi:10.1111/j.1439-037X.2012.00510.x
- Babar MA, Reynolds MP, Van Ginkel M, Klatt AR, Raun WR, Stone ML (2006a) Spectral reflectance to estimate genetic variation for in-season biomass, leaf chlorophyll, and canopy temperature in wheat. *Crop Sci* 46:1046-1057. doi:10.2135/cropsci2005.0211
- Babar MA, van Ginkel M, Klatt A, Prasad B, Reynolds MP (2006b) The potential of using spectral reflectance indices to estimate yield in wheat grown under reduced irrigation. *Euphytica* 150:155-172. doi:10.1007/s10681-006-9104-9
- Ballesteros D, Mason RE, Addison C, Andrea Acuña M, Nelly Arguello M, Subramanian N, Miller R, Sater H, Gbur E, Miller D, Griffey C, Barnett M, Tucker D (2015) Tolerance of wheat to vegetative stage soil waterlogging is conditioned by both constitutive and adaptive QTL. *Euphytica* 201:329-343. doi:10.1007/s10681-014-1184-3
- Boru G, van Ginkel M, Kronstad WE, Boersma L (2001) Expression and inheritance of tolerance to waterlogging stress in wheat. *Euphytica* 117:91-98. doi:10.1023/A:1003929803920
- Christopher JT, Veyradier M, Borrell AK, Harvey G, Fletcher S, Chenu K (2014) Phenotyping novel stay-green traits to capture genetic variation in senescence dynamics. *Functional Plant Biology* 41:1035-1048. doi:10.1071/fp14052
- Collaku A, Harrison SA (2002) Losses in wheat due to waterlogging. *Crop Sci* 42:444-450.
- Collaku A, Harrison SA (2005) Heritability of waterlogging tolerance in wheat. *Crop Sci* 45:722-727.
- Colmer TD, Voesenek LACJ (2009) Flooding tolerance: suites of plant traits in variable environments. *Functional Plant Biology* 36:665-681. doi:<http://dx.doi.org/10.1071/FP09144>
- Dickin E, Bennett S, Wright D (2009) Growth and yield responses of UK wheat cultivars to winter waterlogging. *J Agric Sci* 147:127-140. doi:10.1017/s0021859608008289

Dickin E, Wright D (2008) The effects of winter waterlogging and summer drought on the growth and yield of winter wheat (*Triticum aestivum* L.). *European Journal of Agronomy* 28:234-244. doi:<http://dx.doi.org/10.1016/j.eja.2007.07.010>

Erdle K, Mistele B, Schmidhalter U (2013) Spectral high-throughput assessments of phenotypic differences in biomass and nitrogen partitioning during grain filling of wheat under high yielding Western European conditions. *Field Crops Research* 141:16-26. doi:10.1016/j.fcr.2012.10.018

Freeman KW, Raun WR, Johnson GV, Mullen RW, Stone ML, Solie JB (2003) Late-season prediction of wheat grain yield and grain protein. *Communications in Soil Science and Plant Analysis* 34:1837-1852. doi:10.1081/css-120023219

Gutierrez-Rodriguez M, Reynolds MP, Escalante-Estrada JA, Rodriguez-Gonzalez MT (2004) Association between canopy reflectance indices and yield and physiological traits in bread wheat under drought and well-irrigated conditions. *Australian Journal of Agricultural Research* 55:1139-1147. doi:10.1071/ar04214

Gutierrez M, Reynolds MP, Raun WR, Stone ML, Klatt AR (2010) Spectral Water Indices for Assessing Yield in Elite Bread Wheat Genotypes under Well-Irrigated, Water-Stressed, and High-Temperature Conditions. *Crop Sci* 50:197-214. doi:10.2135/cropsci2009.07.0381

Hazratkulova S, Sharma RC, Alikulov S, Islomov S, Yuldashev T, Ziyaev Z, Khalikulov Z, Ziyadullaev Z, Turok J (2012) Analysis of genotypic variation for normalized difference vegetation index and its relationship with grain yield in winter wheat under terminal heat stress. *Plant Breeding* 131:716-721. doi:10.1111/pbr.12003

Kahlown MA, Azam M (2002) Individual and combined effect of waterlogging and salinity on crop yields in the Indus basin. *Irrig Drain* 51:329-338.

Khabaz-Saberi H, Setter TL, Waters I (2006) Waterlogging induces high to toxic concentrations of iron, aluminum, and manganese in wheat varieties on acidic soil. *Journal of Plant Nutrition* 29:899-911. doi:10.1080/01904160600649161

Lopes MS, Reynolds MP (2012) Stay-green in spring wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology. *J Exp Bot* 63:3789-3798. doi:10.1093/jxb/ers071

Musgrave ME, Ding N (1998) Evaluating wheat cultivars for waterlogging tolerance. *Crop Sci* 38:90-97.

NRCS (2013) "Stuttgart" the Arkansas State Soil. February 14 2013 edn. Natural resources conservation service (NRCS), http://www.ar.nrcs.usda.gov/soils/state_soil.html, p 1

Powell N, Ji X, Ravash R, Edlington J, Dolferus R (2012) Yield stability for cereals in a changing climate. *Functional Plant Biology* 39:539-552. doi:<http://dx.doi.org/10.1071/FP12078>

Prasad B, Carver BF, Stone ML, Babar MA, Raun WR, Klatt AR (2007) Potential use of spectral reflectance indices as a selection tool for grain yield in winter wheat under great plains conditions. *Crop Sci* 47:1426-1440. doi:10.2135/cropsci2006.07.0492

Reynolds MP, Rajaram S, Sayre KD (1999) Physiological and genetic changes of irrigated wheat in the post-green revolution period and approaches for meeting projected global demand. *Crop Sci* 39:1611-1621.

Reynolds MP, Saint Pierre C, Saad ASI, Vargas M, Condon AG (2007) Evaluating potential genetic gains in wheat associated with stress-adaptive trait expression in elite genetic resources under drought and heat stress. *Crop Sci* 47:S172-S189. doi:10.2135/cropsci2007.10.0022IPBS

Robertson D, Zhang H, Palta JA, Colmer T, Turner NC (2009) Waterlogging affects the growth, development of tillers, and yield of wheat through a severe, but transient, N deficiency. *Crop & Pasture Science* 60doi:10.1071/cp08440

Setter TL, Waters I, Sharma SK, Singh KN, Kulshreshtha N, Yaduvanshi NPS, Ram PC, Singh BN, Rane J, McDonald G, Khabaz-Saberi H, Biddulph TB, Wilson R, Barclay I, McLean R, Cakir M (2009) Review of wheat improvement for waterlogging tolerance in Australia and India: the importance of anaerobiosis and element toxicities associated with different soils. *Ann Bot* 103:221-235. doi:10.1093/aob/mcn137

Shao GC, Lan JJ, Yu SE, Liu N, Guo RQ, She DL (2013) Photosynthesis and growth of winter wheat in response to waterlogging at different growth stages. *Photosynthetica* 51:429-437. doi:10.1007/s11099-013-0039-9

Teakle NL, Armstrong J, Barrett-Lennard EG, Colmer TD (2011) Aerenchymatous phellem in hypocotyl and roots enables O₂ transport in *Melilotus siculus*. *New Phytologist* 190:340-350. doi:10.1111/j.1469-8137.2011.03655.x

Zhang HP, Turner NC, Poole ML, Asseng S (2007) High ear number is key to achieving high wheat yields in the high-rainfall zone of south-western Australia. *Aust J Agric Res* 58:21-27. doi:10.1071/ar05170