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To cite this article: Hyejin Park, David E. Clay, Robert G. Hall, Jai S. Rohila, Tulsi P. Kharel, Sharon A. Clay & Sanghun Lee (2014) Winter Wheat Quality Responses to Water, Environment, and Nitrogen Fertilization, Communications in Soil Science and Plant Analysis, 45:14, 1894-1905, DOI: [10.1080/00103624.2014.909833](https://doi.org/10.1080/00103624.2014.909833)

To link to this article: <https://doi.org/10.1080/00103624.2014.909833>



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Published online: 10 Jul 2014.



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Winter Wheat Quality Responses to Water, Environment, and Nitrogen Fertilization

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Decreasing carbon (C) footprints by reducing nitrogen (N) and water inputs has been speculated to have negative impacts on wheat grain yield and flour processing quality. The objective of this study was to determine the impact of N and water stress on winter wheat grain yield, protein composition, and dough quality. Wheat fertilized at two N rates (unfertilized and recommended) was grown under water-stressed and well-watered environments. Nitrogen and water stress were measured using the ¹³C isotopic approach. Research showed that (1) N fertilizer and the water-management environment produced similar impacts on wheat quality and yield loss due to N stress and yield loss due to water stress (YLWS); (2) N fertilizer increased flour protein, dough stability, and relative concentration of glutenin (%Glu), unextractable polymeric protein (UPP), and relative amount of high-molecular-weight glutenin subunits (HMW-GS/LMW-GS); (3) the well-watered environment reduced protein contents when N mineralization was low, whereas it did not influence protein content when mineralization was high; and (4) the %Glu was negatively correlated with yield loss due to N stress (YLNS) and positively correlated with stability. This study showed that a clear understanding of the complex relationship between soil variability and climatic conditions should make it possible to develop adaptive management practices, increase profitability, and improve quality.

Keywords Nitrogen stress, water stress, wheat quality, winter wheat (*Triticum aestivum*), yield loss

Introduction

The most common factor used to add a premium or discount to the hard red winter wheat (HRWW) selling price is protein. In the northern Great Plains, USA, discount of −\$0.03 for

© Hyejin Park, David E. Clay, Robert G. Hall, Jai S. Rohila, Tulsi P. Kharel, Sharon A. Clay, and Sanghun Lee

Received 29 March 2013; accepted 14 May 2013.

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each 10 g kg⁻¹ less than 120 g kg⁻¹ and premiums of +\$0.015 for each 10 greater than 120 g kg⁻¹ are common (South Dakota Wheat Growers 2008). However, millers can also impose regional modifications to the selling price. These discounts may be based on perceptions of previous detailed chemical and quality analysis conducted by the buyer. For example, a buyer might discount the regional purchasing price based on perceived risk of high ash or low dough stability. Once a regional discount is imposed it might take many years to remove. Minimizing the risk for an imposed regional discount requires the development of nitrogen (N)–management practices that fully consider how water and N stress impact yields as well as dough and bread quality.

In the U.S. Northern Great Plains, wide seasonal variations in rainfall and temperature have direct impacts on yield and bread-making quality. Nitrogen and water management impacts the ability of the plant to respond to this variability. In a dry-land environment, the overapplication of N fertilizer can stimulate vegetative growth, which can result in increased water stress at grain filling. In addition, water and N management can influence the metabolic activity within the plant and the protein composition within the kernel (French and Schultz 1984; Jamieson, Stone, and Semenov 2001; Klupacs et al. 2010; Nicolas, Gleadow, and Dalling 1985). Prior to adopting N- and water-management practices that will reduce carbon (C) footprints, a clear understanding of the ramification of N and water stress on protein composition and dough quality is needed.

Wheat quality is impacted by management, environment, and genetic interactions by many factors including their impact on the length of the grain-filling periods (Kraljevic-Balalic et al. 2001). Conditions that shorten the grain-filling periods directly impact the types and amount of proteins transported to the kernel. For example, gliadin accumulates earlier in grain filling than glutenin (Gupta et al. 1996; Zhao et al. 2009), and therefore environmental conditions, such as temperature, nutrient deficiencies, and water stress, that shorten the grain-filling period tend to increase the relative concentration of gliadins (%Gli) and decrease the relative concentration of glutenin (%Glu) in the kernel (Johansson, Prieto-Linde, and Svensson 2004). Gliadins are a mixture of monomeric polypeptides that contribute to dough viscosity and extensibility (Payne 1987; Payne et al. 1982), while glutenins form large polymeric structures that impact dough strength and elasticity. After the reduction of disulfide bonds, glutenins can be divided into high-(HMW-GS; 80,000–120,000 Daltons) and low-molecular-weight-glutenin subunit (LMW-GS; 10,000–70,000 Daltons) (Wall 1979; Weegels, Hamer, and Scholfield 1996). The HMW-GS influences gluten strength and elasticity, whereas LMW-GS influences dough strength (Bietz and Wall 1972).

In the region, research is being conducted to reduce N and water inputs. Much of this research is focused on developing new N and water recommendations based on yields and protein concentration. In most cases protein composition and dough quality are not considered in these considerations. To develop a mechanistic understanding on how stress and management interact to influence yield and bread-making quality, the research needs to be expanded to include the impact of N and water stress on protein composition and flour quality. The objective of this study was to determine the impact of N and water stress on winter wheat grain yield, protein composition, and dough quality.

Materials and Methods

Plant Materials and Cultural Practices

Field study was conducted on a Lowry silt loam (coarse-silty, mixed mesic typic haplustolls) at the Dakota Lake Research Farm (99° 59' W latitude and 44° 17' N longitude)

in South Dakota, USA, in two consecutive years (2007 and 2008). Growing degree days (GDDs, base 0 °C) were summed from planting to crop maturity and were 1,891 in the first year and 2,262 in the second year. During the 2007 and 2008 growing seasons, precipitation was 457 and 450 mm, respectively. These totals were supplemented with additional water by placing a line source irrigation system in the center of the experimental area. Plots 2.3 m from the line source were identified as well watered and plots 16.0 m from the line source were identified as water stressed. In the well-watered treatment, tensiometer readings at depth of 45 and 90 cm were used for irrigation planning. In the first year, the irrigation plus natural rainfall amounts in the well-watered and water-stressed plots were 687 and 519 mm, respectively, whereas in 2008 irrigation plus natural rainfall amounts in the well-watered and water-stressed treatments were 609 and 528 mm, respectively.

The N rates in 2007 were 0 and 200 kg N ha⁻¹, whereas in 2008 the N rates were 0 and 160 kg N ha⁻¹. Nitrogen fertilizer [urea and ammonium nitrate (UAN); 28–0–0] was applied at Feekes 3.0. Each treatment was replicated four times. Winter wheat was planted at 4.44×10^6 seeds ha⁻¹ (145 kg ha⁻¹) on 21 September 2006 and 8 September 2007, respectively. The grain yield was measured following crop maturity with a plot combine with a 1.52-m header. The grain yields were adjusted to 13.5% moisture, whereas protein values were adjusted to the 12% moisture basis.

Quality Analyses

For quality analysis, bulk wheat samples were cleaned by hand and tested with an electric dockage tester (Carter Day International, Minneapolis, Minn., USA). The grain samples were milled according to the American Association of Cereal Chemists (AACC)–approved method 26-50.01 (AACC International 2011a). The milled samples were weighed and sieved through an ASTM E-11 No. 62 sieve (0.30 mm) (Fisher Scientific, Pittsburgh, Penn., USA) and then shaken on a mechanical shaker for 4 min to recover white flour.

Grain protein contents, flour protein contents, and moisture contents were determined with a near-infrared analyzer (model 6500, Foss NIR Systems, Laurel, Md., USA) according to AACC-approved methods 39-10.01 (AACC International 2011b) and 39-11.01 (AACC International 2011c). The flour-mixing characteristics were analyzed by Farinograph (C. W. Brabender Instruments, South Hackensack, N.J., USA) with a 50-g bowl following AACC-approved method 54-21.01 (AACC International 2011d). The water absorption, peak time, stability, mixing tolerance index (MTI), and breakdown time were calculated (AACC 1984).

Protein Fractionation and Characterization

The wheat flour samples for size-exclusion high-performance liquid chromatography (SE-HPLC) were prepared according to Singh, Donovan, and MacRitchie (1990) and Sissons et al. (2005). The flour samples (500 mg) were extracted with a buffer containing 5 mg mL⁻¹ sodium dodecyl sulfate (SDS) and 0.05 M sodium phosphate at pH 6.9 for 20 min with continuous mixing. The extract was centrifuged (10,000g) for 3 min, and the supernatant [sodium dodecyl sulfate (SDS)–soluble fraction] was removed. The residue fraction was further extracted with the same buffer by sonication for 3 min, which solubilized the large polymeric glutenins, and were then centrifuged (10,000g) for 3 min. The fractions were injected into a Phenomenex BIOSEP-SEC 4000 column (300 mm × 7.8 mm, Phenomenex, Torrance, Calif., USA) to run for 10 min at 2 ml min⁻¹ in a 1:1 mixture of deionized water containing 0.5 mg mL⁻¹ trifluoroacetic acid (TFA) and acetonitrile containing 0.5 mg mL⁻¹ TFA using a Waters HPLC system (Waters Corporation,

Milford, Mass., USA). Proteins were detected by ultraviolet (UV) absorbance at 210 nm. The relative amounts of albumins + globulins, glutenins, gliadins, and unextractable polymeric proteins (UPP) were calculated based on total SE-HPLC area of extractable and unextractable proteins.

Samples for reverse-phased-HPLC (RP-HPLC) were prepared according to Fu and Sapirstein (1996). The flour samples (100 mg) were initially extracted in 50% (v/v) 1-propanol for 15 min at room temperature with intermittent vortexing followed by centrifugation (10,000g) for 3 min. The residue was washed with 500 μ l of 50% 1-propanol to remove any remaining soluble protein. Glutenin was extracted from the residue in 0.08 M Tris-hydrochloric acid (HCl) containing 50% 1-propanol at pH 8.0 and containing 1% (w/v) freshly added dithiothreitol after a brief initial vortexing. The proteins were extracted for 30 min at 60 °C. The extract was then alkylated in 0.08 M Tris-HCl containing 50% 1-propanol, pH 8.0, and containing 4% (v/v) freshly mixed 4-vinylpyridine. The extract was incubated for 15 min at 60 °C and then centrifuged (10,000g) for 3 min. The samples were injected into a Vydac 218 TP54 column (C₁₈ 5 μ m, 250 mm \times 2.6 mm, Grace, Deerfield, Ill., USA). Protein separation was carried out using a flow rate of 0.2 ml min⁻¹ at 60 °C using a Waters HPLC system (Waters Corporation, Millford, Mass., USA). The eluents were purified water and acetonitrile, each containing 0.1% (v/v) TFA. The measured RP-HPLC fractions were HMW-GS and LMW-GS. Each analysis was conducted in duplicate.

Yield Loss Calculation

The grain samples were dried, ground on a cyclone mill, and analyzed on an isotope ratio mass spectrometer (Europa Scientific Ltd., Westchester, UK) for total N, total C, delta ¹⁵N ($\delta^{15}\text{N}$), and delta ¹³C ($\delta^{13}\text{C}$). The $\delta^{13}\text{C}$ values were used to calculate the ¹³C isotopic discrimination value with the following equation:

$$\Delta = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_s) / (1 + \delta^{13}\text{C}_s / 1000)$$

where $\delta^{13}\text{C}_a$ is the $\delta^{13}\text{C}$ value of air (-8‰) and $\delta^{13}\text{C}_s$ is the $\delta^{13}\text{C}$ value of the grain sample. The yield losses due to N stress (YLNS) and water stress (YLWS) were calculated using the relationship between Δ and grain yield. This relationship is linked by water and N stress having opposite impacts on Δ . The water stress results in stomatal closure and lower Δ whereas N stress results in greater Δ values. The YLNS and YLWS were calculated using the upper boundary line approach (Clay et al. 2001, 2005).

Statistical Analyses

Experimental design was a randomized split block with four replications. The statistical analysis approach followed the method reported by Stroup (Stroup 1989). In this analysis, a first-order autoregressive model under PROC MIXED in SAS 9.1 was used to determine the treatment differences for the individual years (SAS Institute Inc. 2008). Block was random factor whereas N and water were fixed factors.

Results and Discussion

Climate and Soil Effects

The well-watered and water-stressed environments were designed to simulate moisture differences between the summit/shoulder and footslope/toeslope areas. In addition to the

simulated water environments, temperatures were warmer in 2008 than 2007. Interactions between the moisture and temperature regimes resulted in four environmental conditions.

Greater N mineralization in 2007 (192 kg N ha⁻¹) than 2008 (99 kg N ha⁻¹) produced an apparent N response differences across years. In year 1, the greatest yields were in the fertilized well-watered environment, and lowest yields were in the unfertilized water-stressed environment (Table 1). In addition, the well-watered environment had lower YLNS and YLWS. The impact of water environment on YLNS was attributed to a synergistic relationship between N and water. The net result in year 1 was similar protein contents in both water environments. Slightly different results were observed in the second year when N fertilizer and water environment had a synergistic impact on grain yield and an

Table 1

Influences of year, water-stress environment, and N rate on grain yield (kg ha⁻¹), grain protein (g kg⁻¹), and yield loss due to nitrogen stress (YLNS) and water stress (YLWS)

Year	Treatment		Yield (kg ha ⁻¹)	Protein (g kg ⁻¹)	YLNS (kg ha ⁻¹)	YLWS (kg ha ⁻¹)
	N fertilizer	Water environment				
2007	0	Water stressed	3,280	120.3	1,513	707
	1X	Water stressed	4,241	152.5	927	333
	0	Well watered	4,255	128.5	608	637
	1X	Well watered	4,835	156.8	172	493
	<i>P</i> value		0.0002	<0.0001	0.0002	0.07
	LSD _{0.05} ^a		501	9.2	434	NS ^b
	0		3,768	124.4	1,060	672
	1X		4,538	154.6	549	413
	<i>P</i> value		0.011	<0.0001	0.068	0.015
		Water stressed	3,760	136.4	1,220	520
		Well watered	4,545	142.6	390	565
	<i>P</i> value		0.009	0.477	0.001	0.703
2008	0	Water stressed	4,277	133.5	792	1,753
	1X	Water stressed	4,701	147.8	409	1,711
	0	Well watered	4,360	118.8	1,234	1,227
	1X	Well watered	5,591	133.8	457	773
	<i>P</i> value		0.003	0.003	0.010	0.002
	LSD _{0.05}		642	12.5	478	455
	0		4,318	126.1	1,013	1,490
	1X		5,146	140.8	433	1,241
	<i>P</i> value		0.005	0.017	0.004	0.328
		Water stressed	4,489	140.6	600	1,732
		Well watered	4,975	126.3	846	1,000
	<i>P</i> value		0.142	0.019	0.279	0.001

^aLSD, least significance difference.

^bNS, not significant at *P* = 0.05.

Note. In 2007 and 2008 the recommended N rates were 200 and 160 kg N ha⁻¹, respectively.

antagonistic impact on protein (Table 1). The antagonistic impact on protein content was attributed to protein dilution.

The apparent differences between year 1 and year 2 in the relationship between N rate and protein could be explained by building a model that considered N mineralization and the N fertilizer rate (Figure 1). Differential N mineralization may have also impacted dough quality (Table 2). In the first year, peak time was generally increased by N, but it was not impacted by water environment. Nitrogen addition also increased stability and breakdown and reduced MTI. The decrease in MTI with N was attributed to the impact of protein on dough strength; that is, greater MTI values mean lower stability. Others have had similar findings (Al-Eid 2006; Ma et al. 2009).

In year 2, yields and YLWS were much greater than year 1 (Table 1). These results were attributed to greater temperatures in 2008 than 2007. Associated with greater yields was an N fertilizer-induced increase in peak time. Plants growing in the water-stressed environment had greater protein contents, dough stability, and breakdown times than those growing in the well-watered environment. A comparison across environments showed that environment had an impact on the relationship between protein content and stability (Figure 2). However, this apparent difference could be explained by considering water stress [Figure 3; stability = $-11.17 + 1.34(\text{protein}) + 0.0033(\text{YLWS})$, $R^2 = 0.42^{**}$]. These finding shows that a single model could be used to explain the impacts of water and N stress on the wheat quality.

Effects of N and Water Stress on Protein Characterization

The imposed N and water treatments on winter wheat impacted protein composition, which in turn impacted dough quality. In the first year of study, N and water produced synergic impacts on protein composition (Table 3). The addition of both N and water enhanced the %Glu and UPP contained in the kernel. Opposite impacts were observed for the Gli/Glu ratio. For the HMW-GS/LMW-GS ratio in-season N increases the ratio, whereas water did not influence the ratio. In the second year of study, different results were observed. As in the first year, %Glu was increased by N and water. However, combining both the water and

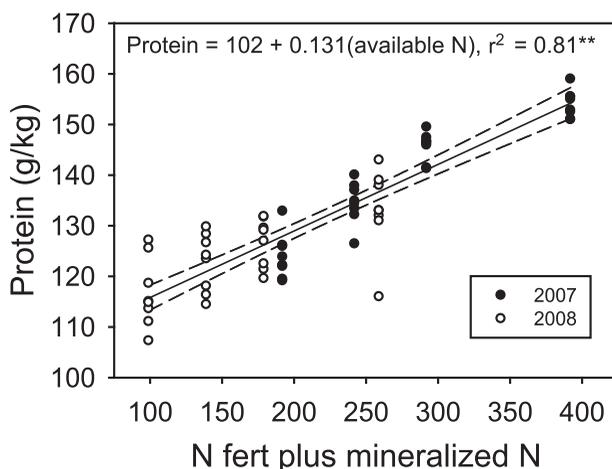


Figure 1. Relationship between N additions (N fertilizer + mineralized N) and protein content over 2 years.

Table 2
Influence of year, water-stress environment, and N rate on protein (g kg⁻¹) and selected dough quality parameters

Year	Treatment		Flour protein (g/kg)	Peak (min)	Stability (min)	MTI ^a (BU ^b)	Breakdown (min)
	N fertilizer	Water environment					
2007	0	Water stressed	104	4.90	4.9	35.5	9.25
	1X	Water stressed	139	9.50	9.5	16.0	17.9
	0	Well watered	114	5.50	5.5	31.3	11.4
	1X	Well watered	141	10.4	10.5	15.4	17.6
	<i>P</i> value		< 0.0001	0.097	0.011	0.021	0.009
	LSD _{0.05}		9.4	NS	4.13	14.01	5.46
	0		108	5.20	5.20	33.4	10.3
	1X		140	9.95	10.0	15.7	17.8
	<i>P</i> value		< 0.0001	0.018	0.001	0.004	0.001
	<i>P</i> value						
2008		Water stressed	122	7.20	7.20	25.8	13.6
		Well watered	128	7.90	8.00	23.4	14.5
	<i>P</i> value		0.496	0.609	0.544	0.461	0.724
	0	Water stressed	115	8.0	11.25	14.3	19.5
	1X	Water stressed	129	10.4	11.8	12.5	20.5
	0	Well watered	93	3.6	7.25	26.3	11.3
	1X	Well watered	109	8.4	8.88	25.6	15.0
	<i>P</i> value		0.0001	0.016	0.043	0.133	0.02
	LSD _{0.05}		11.1	3.85	4.83	NS	5.99
	0		104	5.81	9.28	20.3	15.4
1X		119	9.38	10.34	19.1	17.8	
<i>P</i> value		0.040	0.030	0.479	0.572	0.376	
<i>P</i> value							
	Water stressed	122	9.19	11.5	13.4	20.0	
	Well watered	101	6.00	8.1	25.9	13.1	
<i>P</i> value		0.001	0.056	0.013	0.018	0.003	

^aMTI, mixing tolerance index.

^bBU, Brabender unit.

Note. In 2007 and 2008 the recommended N rates were 200 and 160 kg N ha⁻¹, respectively.

N treatment decreased the differences. Opposite impacts were observed for %Gli. On the HMW-GS/LMW-GS ratio N fertilizer increased the ratio, whereas water addition did not impact the ratio. Nitrogen had a similar impact on the HMW-GS/LMW-GS ratio, but water stress did not affect the HMW-GS/LMW-GS ratio in both years. Contrasts between the 2 years were attributed to (1) greater N mineralization rates in the first year than the second year of study and (2) water enhancing the uptake of soil and fertilizer N in the first year of study (Kharel et al. 2011). Others (Gupta, Batey, and MacRitchie 1992; Jia et al. 1996; Johansson, Prieto-Linde, and Svensson 2004; Triboi et al. 2000) have produced similar and different results. For example, Johansson, Prieto-Linde, and Svensson (2004) reported % Glu increased with N application, while Triboi et al. (2000) reported that N increased the Gli/Glu ratio. Differences between the studies may be related to the impact of N on

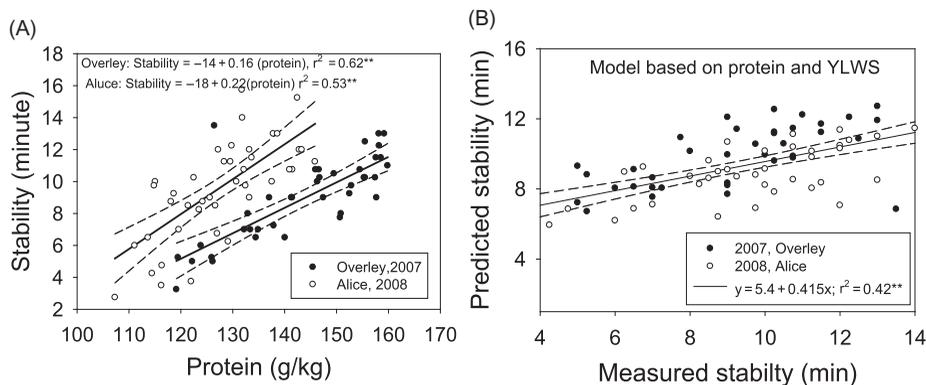


Figure 2. Relationship between protein and dough stability (A) and a comparison between predicted stability, based on protein content and water stress, and measured dough stability (B).

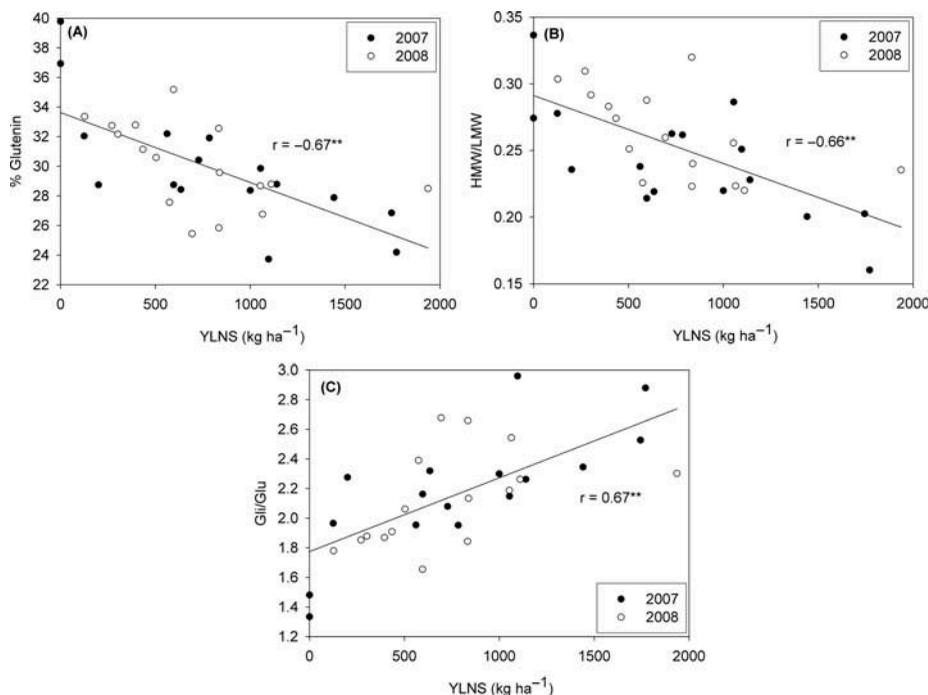


Figure 3. Relationships between yield loss due to nitrogen stress and percentage of glutenin (A), gliadin/glutenin ratio (B), and HMW-GS/LMW-GS ratio (C) in 2007 and 2008.

the length of the grain-filling period and water and N stress. Jiang et al. (2009) reported that water stress after anthesis increased the accumulation of HMW-GS during the early grain-filling stage. Konopka et al. (2007) reported that wheat grains obtained under stress conditions were less abundant in LMW- and HMW-GS.

The protein fraction analysis across years showed that %Glu and HMW-GS/LMW-GS ratios were negatively correlated with YLNS and positively correlated with stability whereas the Gli/Glu ratio was negatively correlated with stability and positively correlated with YLNS (Figures 3 and 4). These results show that the protein composition was

Table 3
Influences of water-stress environment and N rate on protein characterization in 2007 and 2008

Year	Treatment		Glutenine (g/kg)	Gliadin (g/kg)	Ratio of gliadin to glutenine	UPP (g/kg)	Ratio of HMW-GS to LMW-GS
	N fertilizer	Water environment					
2007	0	Water stressed	255	685	2.71	165	0.20
	1X	Water stressed	301	638	2.13	263	0.27
	0	Well watered	286	647	2.27	179	0.22
	1X	Well watered	352	584	1.68	354	0.28
	<i>P</i> value		0.0003	0.004	0.0002	0.0001	0.002
	LSD _{0.05}		3.36	4.64	0.33	6.55	0.04
	0		270	666	2.49	172	0.21
	1X		327	621	1.90	308	0.28
	<i>P</i> value		0.002	0.011	0.002	0.0001	0.0002
		Water stressed	278	662	2.42	214	0.24
		Well watered	319	616	1.97	266	0.25
	<i>P</i> value		0.043	0.039	0.031	0.238	0.464
2008	0	Water stressed	264	677	2.57	202	0.23
	1X	Water stressed	317	610	1.93	253	0.27
	0	Well watered	289	642	2.22	182	0.24
	1X	Well watered	335	59.57	1.78	272	0.31
	<i>P</i> value		<0.0001	<0.0001	<0.0001	0.182	0.0002
	LSD _{0.05}		1.45	2.05	0.15	NS	0.03
	0		277	659	2.39	192	0.24
	1X		326	603	1.86	263	0.29
	<i>P</i> value		<0.0001	<0.0001	<0.0001	0.028	0.0001
		Water stressed	290	644	2.25	227	0.25
		Well watered	312	619	2.00	227	0.27
	<i>P</i> value		0.146	0.149	0.131	0.993	0.240

Notes. Glu, glutenin; Gli, gliadin; UPP, unextractable polymeric protein; HMW-GS/LMW-GS, ratio of high-molecular-weight-glutenin subunits to low-molecular-weight-glutenin subunits. In 2007 and 2008 the recommended N rates were 200 and 160 kg N ha⁻¹, respectively.

influenced by N stress, and that across the 2 years, glutamine was greatest when N stress was the lowest. These results are attributed to gliadin accumulating earlier in the kernel than glutenin and stress reducing the length of the grain-filling period (Panozzo and Eagles 2000). For example, Panozzo and Eagles (2000) reported that high temperatures (>30 °C) increased the %Gli concentrations. The protein composition impacted dough stability because gliadins are a mixture of monomeric polypeptides that contribute to dough viscosity and extensibility (Payne 1987), whereas glutenins are large polymeric structures that impact dough strength and elasticity. Dough stability was not correlated with %Glu, the Gli/Glu ratio, or the HMW-GS/LMW-GS ratio. However, Wang and Yu (2009) reported that when N was added, the grain protein and flour protein contents and the %Glu increased whereas the %Gli and the Gli/Glu ratio decreased, which induced the increase of stability.

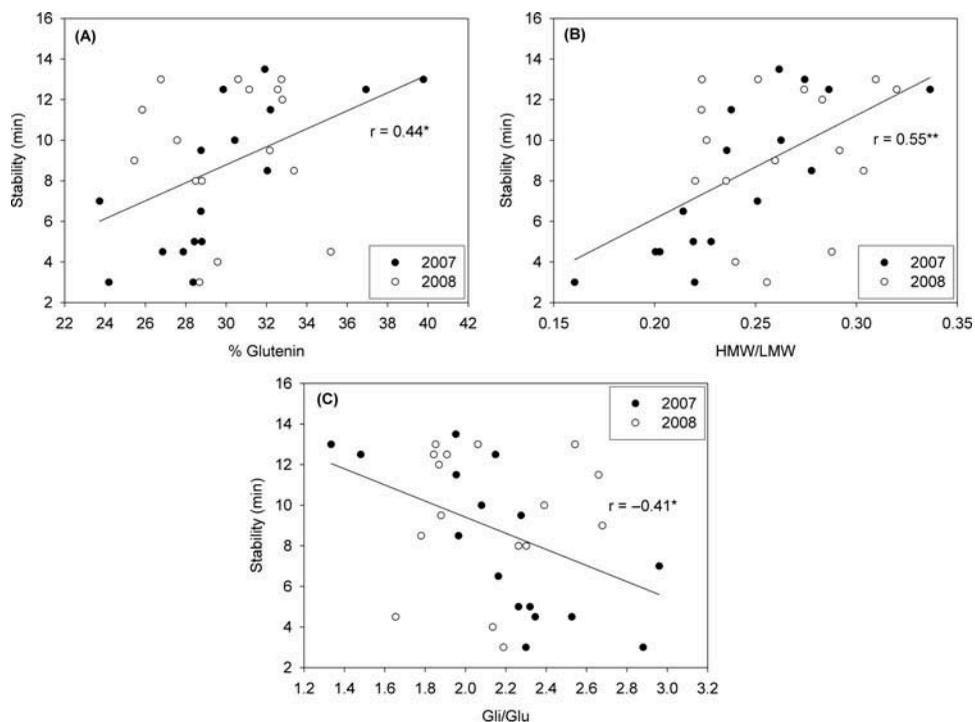


Figure 4. Relationships between protein fraction [percentage of glutenin (A), gliadin/glutenin ratio (B), and HMW-GS/LMW-GS ratio (C)] and stability in 2007 and 2008.

The composition of the glutenin protein was impacted by stress. Across the 2 years the HMW/LMW-GS ratio decreased with increasing N stress (Figure 3), which in turn impacted stability (Figure 4). These results agree with the findings by both Bayoumi and Demardash (2008) and Luo et al. (2000).

Conclusions

This experiment produced four uniquely different environments. In year 1, temperatures were lower and N mineralization was greater than year 2. This environment resulted in greater N mineralization, lower yields, lower yield losses due to water stress, and synergistic relationships between water and N use. For both years, N fertilizer generally improved dough quality, and the relative glutenin amount and HMW-GS/LMW-GS ratio were negatively correlated with and positively correlated with stability. A single model could be used to explain protein accumulation and the relationship between protein content and stability.

This article had three major conclusions. The first conclusion was that soil and climate variability could impact yields, protein composition, and dough quality. To quantify these impacts, N mineralization was measured. The second conclusion was that by understanding complex relationships between climatic conditions and soil variability it should be possible to develop adaptive management practices to increase profitability and reduce greenhouse gas footprints. The third conclusion was that routine measurements of dough quality and protein composition should be integrated into management studies.

Funding

Support was provided by South Dakota Wheat Commission, the SD Drought Tolerance Center, South Dakota 2010 grant program, South Dakota Experiment Station, and USDA-NIFA-AFRI.

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