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Effect of Sulphur Fertilization on Breadmaking Quality of Three Winter Wheat Varieties

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Abstract: For study of the effect of sulphur and nitrogen on breadmaking quality of three varieties of wheat, four field experiments were carried out at four sites in north and northeast of Iran. This research carried out as factorial experiment based on randomized complete block design with three factors and four replications. Factors to be studied were, wheat varieties of Tajan, Falat, Sardari and sulphur at the rate of 0, 20 and 80 kg ha^{-1} in the form of gypsum and nitrogen at two rate of 180 and 230 kg ha^{-1} in the form of urea. Results showed that sulphur increased the loaf volume significantly and decreased the N:S ratio in grain. Grain S concentration had high correlation coefficient with grain protein percent. Sulphur increment caused increase in loaf volume, better breadmaking quality, which may be due to gluten in protein. Nitrogen fertilizer application increased protein concentration of grain, but it had no significant effect on loaf volume.

Key words: Sulphur, nitrogen, wheat, bread making quality

INTRODUCTION

It has been established that the sulphur (S) nutrition of wheat has an important influence on the breadmaking quality of flour (Thomason et al., 2007). This is due to the essential role of disulphide bonds in maintaining gluten functionally in flour (Shewry and Tarham, 1997). Although the essential role of S for plant growth and development has long been recognized, deficiency of S in agricultural crops was rare in world until about a decade ago. A considerable decrease in the inputs of S from atmospheric deposition since the early 1970s, coupled with increased crop yields and a change from the use of S-containing fertilizers to S-free fertilizers, has contribute to increased S deficiency over the last decade (Zhao et al., 1999a). Cereal crops require only a moderates amount of S (15-20 kg ha^{-1}) for optimum growth, although yield increases in response to the addition of S fertilizers have been reported by Zhao et al. (1999a). In the future the need for S fertilizers is predicted to increase as atmospheric deposition of S is likely to decrease further in industrialized countries (Al-Eid, 2006; Shewry and Tarham, 1997). In wheat, deficiency of S can result not only in yield decreases, but also in low breadmaking quality. In the 1980s Australian researchers demonstrated that S deficiency in wheat had a profound effect on the composition of gluten proteins in wheat grain, with increased synthesis of S-poor proteins (ω-gliadins and High-Molecular-Weight (HMW) subunits of glutenin) at the expense of S-rich proteins (z-and y-gliadins and low-molecular-weight) (LMW) subunits of glutenin (Shewry and Tarham, 1997; Fullington et al., 1987; Tea et al., 2004; Lerner et al., 2006; Mason et al., 2007; Thomason et al., 2007). These compositional changes were associated with decreased extensibility and increased elasticity of dough. Field experiments conducted in England before 1990 showed that breadmaking quality, as measured by loaf volume, was not affected significantly by the applications of S fertilizers (Salmon et al., 1990; Kettelwell et al., 1998), probably because S deficiency was rare at that time. Grain protein content is the most commonly studied parameter of wheat quality (Mason et al., 2007).

However, there was some evidence that when a large amount of nitrogen (N) was applied late to wheat, the quality of gluten proteins for breadmaking deteriorated owing to an imbalance of N and S (Al-Eid, 2006; Timms et al., 1981). It is important that the effect of S nutrition on the breadmaking quality of field-grown wheat are fully understood. Several recent reports suggest that environmental conditions could quantitatively affect storage protein components (Al-Eid, 2006)

This research forms part of a systematic study to evaluate the responses of breadmaking quality parameters of winter wheat to the addition of S fertilizer under field conditions in the major wheat-growing area of north and northeastern of Iran. This study further compares the responses of three breadmaking wheat varieties to S application in field experiments.

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MATERIALS AND METHODS

Field experiments condition: Four field experiments were conducted during 2002-2003 growing season at two sites in Golestan and two sites in Khorasan provinces. Soil properties at each experimental site are shown in Table 1. Soil samples were collected from 0-30 and 30-60 cm depth in early autumn prior to fertilizer application. There were 18 treatments in each experiment, consisting of all factorial combinations of three varieties of winter wheat (*Triticum aestivum* L.), the varieties included Tajan, Falat and Sardari, three S levels (0, 20 and 80 kg ha^{-1} in the form of gypsum) and two N levels (180 and 230 kg ha^{-1} in the form of urea). Urea applied in two splitting and gypsum applied in spring at anthesis stage. All experiments were conducted as factorial based on randomized complete block design with four replications. Fungicides and pesticides applied according to standard practices whenever needed. Plot size were 30 m^2 at each plot at different sites. At maturity, crops were harvested using one square meter quadrate with three replications from each plot. About 5 kg of grain from each plot was used for the determination of bread making quality parameter. Grains were dried at 80°C for 16 h and milled and passed through 0.5 mm sieve. The concentration of N was determined titrimetrically after Kjeldahl digestion with copper catalyst (Withers *et al.*, 1995). For determination of S, samples were digested with a mixture of HNO_3 and $HClO_4$ (Zhao *et al.*, 1994) followed by measurement of S in solution using spectrophotometer (Massoumi and Cornfield, 1963). The concentration of N and S are expressed on a dry matter basis, whereas grain protein concentration was calculated on an 86% dry matter basis. Grain N:S ratio was calculated from the N and S concentrations.

Milling and breadmaking test: Grain samples were milled on a Buhler MLU 202 mill to produce straight-run white flour. The additional flour produced was blended with the straight-run white flour for quality testing. The amount of gel protein in white flour and its elastic modulus were determined by the method of Pritchard and Brock (1994). Flour (10 g) was defatted with 25 mL of petroleum ether (at 40-60°C) for 1 h, filtered and dried. Defatted flour (5 g) was stirred with 90 mL of 1.5% sodium sulphate for 10 min

at 10°C before being centrifuged at 4×10^4 g for 40 min. The protein was measured using a 10 mg protein dissolved in 0.063 molar HCl in 6.8 and determined with HPLC. Sulphur extracted concentration determined with spectrophotometer (Massoumi and Cornfield, 1963). Total S in soil determined using Butters and Chenery (1959). Analysis of variance (ANOVA) was preformed on all data sets. Data from all sites were combined in correlation and regression analysis. The statistical package Minitab12 and Excel were used.

RESULTS AND DISCUSSION

Grain yield: Grain yields and crop S uptake data showed significant (p<0.01) yield increase in response to S application at all sites (data were not presented) but there were more response to S application in irrigated fields of both provinces. Application of N and S significantly increased flour production especially in Tajan in irrigated field of Golestan province with 0.4% at 95% level (data were not shown). Soil physico-chemical properties were presented in Table 1. The ANOVA results for all flour parameters tested are shown in Table 2. It is clear that the treatments effects varied between the four sites and interactions between variety, S and N treatments were not significant except in four cases (Table 2).

Grain protein, S concentration and N: S ratio: Results of this study showed the effects of N treatment on grain protein percent and also grain S concentration was significant (Table 2). Mean concentrations of grain protein were 12.6, 12.5 and 12.2 for Tajan, Falat and Sardari, respectively. Increasing the N rate from 180 to 230 kg ha^{-1} increased grain protein concentration significantly (Table 3), although the effect was much greater at irrigated farms than at non-irrigated farms. Significant differences were observed between the three varieties for above traits, in the order of Tajan>Falat>sardari in Golestan irrigated fields. Application of S increased grain protein concentration significantly with an average increase of 0.3% in both S_{20} and S_{80} over the S_0 treatment (Table 2-4). There were significant differences between the three varieties in the concentrations of S in grain (Table 2, 4, 5). Application of S increased grain S concentration significantly at all fields,

Table 1: Soil physical and chemical properties of study sites

Sites	pH	EC (mS cm^{-1})	Organic C (%)	Soil total S (mg kg^{-1})	Soil available S (mg kg^{-1})	Clay (%)
GPI	7.6	0.27	1.60	278	0.4	23
GPNI	8.2	0.31	1.30	311	4.6	25
KPI	8.1	0.23	0.24	234	2.7	31
KPNI	8.5	0.33	0.62	307	3.7	20

GPI: Irrigated field of Golestan, GPNI: Non-irrigated field of Golestan, KPI: Irrigated field of Khorasan, KPNI: Non-irrigated field of Khorasan

Table 2: Levels of significance of treatment effects on grain and flour properties and breadmaking quality of wheat

Sites	Variety	Grain protein (%)	Grain S Conc. (mg kg⁻¹)	Grain N:S	Flour yield (%)	Gluten protein (g/5g flour)	Loaf volume (mL)	Crumb score
GPI	Variety	***	**	***	**	***	***	**
	S	***	***	**	*	**	**	*
	N	***	***	NS	NS	NS	*	NS
	Variety×S	NS	*	NS	NS	NS	NS	NS
	Variety×N	NS	NS	NS	NS	NS	NS	NS
	N:S	NS	NS	NS	NS	NS	NS	NS
GPNI	Variety	***	**	**	**	**	**	*
	S	**	*	*	*	**	*	*
	N	***	**	NS	NS	*	NS	*
	Variety×S	NS	NS	NS	NS	**	NS	NS
	Variety×N	NS	NS	NS	NS	*	NS	NS
	N:S	NS	NS	NS	NS	NS	NS	NS
KPI	Variety	***	**	**	**	***	***	**
	S	**	*	*	*	*	*	*
	N	***	***	*	*	*	*	*
	Variety×S	NS	NS	NS	NS	NS	NS	NS
	Variety×N	NS	NS	NS	NS	NS	NS	NS
	N:S	NS	NS	NS	NS	NS	NS	NS
KPNI	Variety	*	**	*	*	*	*	*
	S	**	*	*	*	*	*	*
	N	**	*	*	*	*	*	NS
	Variety×S	**	*	*	NS	NS	NS	NS
	Variety×N	NS	NS	NS	NS	NS	NS	NS
	N:S	NS	NS	NS	NS	NS	NS	NS

*Significant at 0.05% level, **Significant at 0.01% level, ***Significant at 0.001% level, NS: Not Significant

Table 3: Effect of S and N treatments on grain S concentration, grain N: S ratio and some breadmaking quality of wheat fields of Golestan province

Site	N applied (kg ha⁻¹)	S applied (kg ha⁻¹)	Grain protein (%)			Grain S conc. (mg g⁻¹ DW)			Grain N:S ratio			Loaf volume (mL)			Crumb score		
			Tajan	Falat	Sardari	Tajan	Falat	Sardari	Tajan	Falat	Sardari	Tajan	Falat	Sardari	Tajan	Falat	Sardari
GPI		0	12.4	12.2	11.2	1.28	1.35	1.54	16:01	15:01	15:01	1715	1712	1710	8.50	8.00	6.80
	180	20	12.6	12.3	11.8	1.32	1.50	1.56	15:01	16:01	15:01	1725	1735	1732	8.00	7.30	7.00
		80	12.3	11.6	11.5	1.52	1.63	1.41	14:01	14:01	14:01	1738	1728	1728	7.70	5.80	6.60
		0	13.1	13.4	13.2	1.67	1.72	1.65	17:01	16:01	16:01	1654	1745	1714	7.60	7.60	7.10
	230	20	12.8	13.7	12.4	1.87	1.65	1.43	15:01	15:01	15:01	1753	1743	1765	7.40	7.80	7.30
		80	12.9	12.2	13.2	1.68	1.87	1.41	14:01	15:01	15:01	1758	1768	1745	7.30	6.90	6.90
Mean			12.6	12.5	12.2	1.55	1.62	1.50	15:01	15:01	15:01	1725	1738	1732	7.75	7.23	6.95
LSD 0.05	For S or variety				0.47		0.065				0.44			22.5		0.25		
LSD 0.05	For N			0.35			0.053			0.36			17.2			0.20		
GPNI		0	11.5	11.4	11.2	1.45	1.42	1.32	16:01	17:01	15:01	1702	1667	1586	7.50	7.5	7.10
	180	20	11.9	11.6	11.4	1.56	1.56	1.45	15:01	16:01	14:01	1698	1712	1677	7.70	8.0	7.30
		80	11.6	11.4	11.5	1.67	1.69	1.64	14:01	14:01	14:01	1738	1735	1675	7.80	8.0	7.60
		0	11.8	11.8	11.6	1.44	1.38	1.35	16:01	17:01	15:01	1725	1689	1605	7.50	7.4	7.50
	230	20	12.0	12.2	12.2	1.57	1.55	1.55	15:01	15:01	16:01	1720	1722	1688	7.60	7.5	7.40
		80	12.1	12.3	12.4	1.67	1.65	1.65	14:01	14:01	15:01	1735	1726	1685	7.50	8.1	7.70
			11.81	11.78	11.71	1.56	1.54	1.49	15:01	16:01	15:01	1719	1708	1652	7.60	7.6	7.43
Mean			0.17			0.034			0.4			17.2			0.28		
LSD 0.05	For S or variety		0.15			0.028			0.35			14.4			0.30		
LSD 0.05	For N																	

the response being much greater at irrigated field of Golestan province. Increase the N rate also increased grain S concentration. Grain N: S ratio was significantly affected by variety and S treatments (Table 2). Grain N: S ratio in plots received N and S fertilizes was below the 16 and Grain N: S ratio decreased significantly in other treatments compared to control (Table 2). Significant mean differences between varieties were found only in Sardari compare two other varieties (Table 3, 4). Loaf volume was increased significantly by variety, S and N addition in all farms except GPNI in later case (Table 2).

The results showed application of S significantly increased the loaf volume in all treatments, due to the fact that increased grain S concentration cased greater grain protein (Al-Eid, 2006; Zhao et al., 1999b). Application of S in early spring increased the grain S concentration which decreased the grain N:S ratio in all as shown in (Table 5) sites especially in irrigated field of both

Table 4: Effect of S and N treatments on grain S concentration, grain N: S ratio and some breadmaking quality of wheat in fields of Khorasan province

Site	N applied (kg ha⁻¹)	S applied (kg ha⁻¹)	Grain protein (%)			Grain S conc. (mg g⁻¹ DW)			Grain N:S ratio			Loaf volume (mL)			Crumb score		
			Tajan	Falat	Sardari	Tajan	Falat	Sardari	Tajan	Falat	Sardari	Tajan	Falat	Sardari	Tajan	Falat	Sardari
KPI		0	11.8	11.50	11.30	1.44	1.39	1.35	16:1	17:1	16:1	1566	1667	1586	7.20	7.50	7.10
	180	20	12.2	11.80	11.40	1.56	1.41	1.45	15:1	16:1	15:1	1687	1723	1675	7.40	7.80	7.40
		80	11.9	11.90	11.80	1.65	1.62	1.56	14:1	14:1	14:1	1697	1745	1654	7.10	7.70	7.30
		0	12.1	12.20	12.30	1.57	1.72	1.41	15:1	16:1	16:1	1603	1685	1623	7.80	7.40	7.20
	230	20	12.3	12.30	12.10	1.64	1.41	1.59	16:1	15:1	17:1	1645	1721	1523	7.60	7.60	7.50
		80	12.0	12.20	11.60	1.67	1.55	1.68	14:1	14:1	15:1	1673	1634	1586	7.20	7.40	7.20
Mean			12.1	11.98	11.75	1.58	1.51	1.50	15:1	15:1	16:1	1645	1677	1607	7.38	7.56	7.28
LSD 0.05	For S or variety			0.17			0.065			0.49			10.6			0.23	
LSD 0.05	For N			0.13			0.043			0.37			12.6			0.32	
KPNI		0	10.5	10.10	9.50	1.40	1.45	1.40	15:1	14:1	15:1	1667	1645	1713	8.40	7.6	7.2
	180	20	10.6	10.30	10.20	1.34	1.47	1.42	16:1	14:1	15:1	1698	1634	1734	8.60	7.4	7.5
		80	10.2	10.80	9.60	1.51	1.43	1.51	16:1	14:1	14:1	1656	1642	1721	7.40	7.7	8.0
		0	11.6	10.70	10.20	1.53	1.52	1.52	16:1	15:1	14:1	1675	1623	1716	8.30	7.8	7.6
	230	20	11.4	10.60	10.80	1.52	1.49	1.47	16:1	15:1	16:1	1686	1648	1719	7.80	7.4	7.4
		80	11.3	10.80	10.20	1.50	1.48	1.49	17:1	15:1	15:1	1683	1649	1707	7.70	7.5	7.3
Mean			10.9	10.50	10.16	1.46	1.47	1.46	16:1	14:1	15:1	1677	1640	1718	8.03	7.43	7.5
LSD 0.05	For S or variety			0.17			0.065			0.49			10.6			0.23	
LSD 0.05	For N			0.12			0.043			0.37			12.4			0.32	

Table 5: Correlation coefficient between various grain and flour protein and breadmaking quality parameter

Parameters	Grain S concentration	Grain N:S ratio	Grain protein (%)	Loaf volume	Gluten protein (g 5⁻¹g flour)
Grain S concentration	1.00				
Grain N:S ratio	-0.50	1.00			
Grain protein (%)	0.70	0.33	1.00		
Loaf volume	0.53	-0.30	0.37	1.00	
Gluten protein (g 5⁻¹g flour)	0.41	0.14	0.56	0.25	1

provinces. Although the effect of S on breadmaking quality is significant, but increased 50 kg ha⁻¹ N application from 180 to 230 kg ha⁻¹ has no significant effect on breadmaking quality of the grain (Table 2). Apart from being an indicator of the quantity of protein in grain, the S concentration also reflects the quality of proteins. This is demonstrated by the profound influence of S nutrition on the concentration of gluten proteins similar result were obtain by Al-Eid (2006) and Zhao *et al.* (1999a). An imbalance of N and S in wheat grain, as indicated by a grain N: S ratio of more than 16 in this study reflects the poor breadmaking quality. A slightly higher critical N: S ratio of 17 was reported by Byers *et al.* (1987).

The three varieties used in this study differed greatly in the properties of gel protein and dough rheology. Tajan contain larger amounts of gel protein than Falat and Sardari (data were not shown). Despite these differences, the responses of loaf volume to S application were similar among all three varieties at all sites. On average, there were some small differences in loaf volume between the three varieties, but these differences were not consistent at all sites.

Table 5 shows simple correlation coefficient between grain S concentration, Grain N:S ratio, Grain protein percentage, Loaf volume and grain gluten protein,

using the whole data set of all varieties pooled from the four sites. There were close and significant correlation between S concentration of grain and grain protein percentage (Table 5). Because more than 90% of the total S in wheat grain is bound in organic forms, mainly as protein (Byers *et al.*, 1987), it is not surprising that the concentration of S correlated strongly with grain protein concentration. Positive correlations were observed between grain S concentration and loaf volume and also with grain gluten protein (Table 5).

In conclusion, an increase in grain S concentration in response to S fertilization benefited the breadmaking quality of three wheat varieties, whereas application of an extra 50 kg ha⁻¹ N increased a little grain protein concentration without improving breadmaking quality. This study revealed that addition of S fertilizer is required in many wheat-growing area of north and northeastern of Iran to maintain breadmaking quality.

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