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Effect of sulphur and nitrogen fertilization on bread-making quality of wheat (*Triticum aestivum* L.) varieties under Mediterranean climate conditions

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Summary

Turkey has applied for EU-membership, but still faces problems of lacking quality standards for bread wheat. Studies on the influence of S-fertilization on grain yield and bread-making quality of wheat (*Triticum aestivum* L.) in the region haven't been carried out until today. This research was conducted for two growing seasons (2008-2009 and 2009-2010) at Adnan Menderes University Research and Experimental Farm located in the Western Turkey (Aegean region) at 37º 44' N 27º 44' E in order to determine the effects of nitrogen (0, 70, 140, 210 kg ha^{-1}) supplemented with sulphur (0 or 40 kg ha^{-1}) with respect to yield and bread-making quality of the varieties Golia and Sagittario, grown primarily in Western Turkey. S-fertilization had positive effects on grain yield and some quality parameters under Mediterranean conditions; however, significant differencess were rather rare. Particularly the gluten-index and the sedimentation value promoted by S fertilization were among the tested parameters. Therefore, S-fertilization in improving bread-making quality of wheat in the region should not be disregarded. Grain yield and quality could be promoted simultaneously with increasing N-doses.

Introduction

The area cultivated with wheat (*Triticum spp.* L.) has reduced in the last years in Turkey and today covers approximately 8.0 million ha (FAO, 2010). About 75% of the wheat produced in Turkey is bread wheat type. In the Aegean region of western Turkey, the wheat yield is mostly higher than the other wheat-cultivated areas (Dinc and Erekul, 2010). Despite the large extent of wheat cultivation in Turkey the locally produced wheat hardly achieved sufficient yield and barely met the standards of food processing industry, thus high quality wheat has to be imported (Erekul et al., 2009).

Yield and bread-making quality is influenced by genotype, growth conditions and fertilization regime (Johansson et al., 2004). Responses of wheat to nitrogen application alone have been well recognized for many varieties under different climates (Erekul et al., 2009; Tea et al., 2007; Guarda et al., 2004; Johanson et al., 2004). Besides nitrogen, the essential role of sulphur in plant growth and development is well known (Zhao et al., 1999a; Steinfurth et al., 2012). Deficiency of sulphur can decrease grain yield but has a stronger influence on bread-making quality (Fullington et al., 1987; Shahsavani and Gholami, 2008), due to the essential role of disulphide bonds in maintaining gluten functionality (Zhao et al, 1999b). Function of N and S are closely interrelated and an optimal N/S ratio improves bread-making quality. Interactions of nitrogen and sulphur fertilization have not been widely investigated (Zhao et al., 1999c; Tea et al., 2007). Although wheat require only a modest amount of sulphur (about 15-30 kg/ha) for optimum growth and grain yield (Zhao et al., 1999a; Zhao et al., 1999b), deficiency of sulphur has become increasingly widespread in Europe in recent years (Schnug, 1991; Zhao et al., 1999c).

Reports on the response of cereals to sulphur nutrition, especially in the Mediterranean climate, are not numerous. This research was conducted for two growing seasons, (2008-2009 and 2009-2010) firstly to determine to our knowledge in Aegean region about the effects of different doses of nitrogen combined with sulphur fertilizing under field conditions with respect to yield and bread-making quality of two wheat varieties, widely grown in western parts of Turkey.

Materials and methods

Trials were performed in 2008-2009 and 2009-2010 at Adnan Menderes University Research and Experimental Farm located in western Turkey at 37º 44' N 27º 44' E and at 65 m above sea level. Mean annual precipitation and temperature (1970-2005) was 648.9 mm and 17.6 ºC, respectively. Average monthly temperatures were lower in 2008-2009 than in 2009-2010 with 14.8 ºC and 15.4 ºC, resp. The soil texture is classified as a sandy loam and the soil type is a Calcaric Fluvisol (FAO-classification). Soil samples were taken to determine the amount of extractable sulphate-S (Tab. 1), which was extracted with 0.016 M KH_2PO_4 and determined with ion chromatography (Zhao and Grath, 1994). Total N was determined by Kjeldahl method (Bremner and Mulvaney, 1982), available P by the method of Olsen et al. (1954) and available K was analyzed in 1M NH_4OAc extract by flame emission.

The experimental design was a split-split plot with randomized complete block and four replications. Wheat (*Triticum aestivum* L.) varieties Golia and Sagittario were sown between mid-November and the beginning of December (04.12.2008; 26.11.2009) with a rate of 500 seeds m^{-2}. For the phenological growth stages, the BBCH-scale was used (BBCH Monograph, 2001). N treatments (Ammonium nitrate) were 0 kg ha^{-1} N, 70 kg ha^{-1} N (at sowing), 140 kg ha^{-1} N (70 kg sowing, + 35 kg beginning of tillering, + 35 kg beginning of shooting) and 210 kg ha^{-1} N (70 kg sowing, + 70 kg, beginning of tillering, + 70 kg beginning of shooting).

Sulphur treatment 0 and 40 kg ha^{-1} S were applied as gypsum be-

Tab. 1: Chemical properties of soil (Ap-horizon) at the study site in Aydin

Horizon	Soil Texture (%) Sand	Silt	Clay	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	S[1] (mg/kg)	OM[2]	pH
Ap	65.2	23.2	11.6	0.13	18.0	383	2897	379	6.3	1.50	8.0

[1] Available sulphur
[2] Organic matter

tween the end of February and the beginning of March (beginning to end of tillering). Further field management (P and K fertilization, weed control and pest management) were carried out in accordance to the nutrient state of the soil and crop development. The sampling area for grain yield was 4.8 m^2 in the centre of the plot (plot size: 12.0 m^2, grain test weight was measured on a 250 g sample and expressed as kg hl^{-1}).
Grains were milled by the AMG (Ardic Medical Gida, Ankara, Turkey) laboratory mill. Grain N-content was determined by Kjeldahl method. Starch content was determined on a dry weight basis by near infrared reflectance spectroscopy (NIRS), using a Perten DA-7200 instrument (Perten Co., Huddinge, Sweden). Bread-making quality analyses were evaluated by the Standard Method of the International Association of Cereal Chemistry (ICC, 1986): wet gluten and gluten index by ICC 137, 155 and 158, using a Glutomatic 2200 instrument; sedimentation values by Zeleny Sedimentation Test (ICC 116); the falling number according to ICC 107. The data were analysed by using statistical software SPSS, version 14.0, univariate model and Least Significant Difference (LSD) among means of cultivars and N and S doses in each year were tested at the $P<0.05$ level of probability.

Results and discussion

Nitrogen and year as main effect showed a significant influence on yield (univariate model; data not shown). However, the main effect yield was not different between the cultivars and independend from the sulphur application. The grain yield ranged in 2009 from 2590 kg ha^{-1} for the control to 4081 kg ha^{-1} for the highest N-application for Golia and for Sagittario from 2909 kg ha^{-1} to 4172 kg ha^{-1} (Tab. 2). The applied sulphur resulted in higher yield up to 4322 and 4379 ha^{-1} for Golia and Sagittario, resp., but the increase was not significant. The grain yield was higher in 2010 and ranged from 3015 kg ha^{-1} for the control to 4698 kg ha^{-1} for Golia and for Sagittario from 2963 kg ha^{-1} to 4627 kg ha^{-1}. No remarkable benefits were noticed at a higher yield level in 2010.
In Aegean Region, grain yield average under rain fed conditions is 1,5 times higher than the average of Turkey. The difference in effectiveness of nitrogen and sulphur fertilization in the two years was firstly and primarily caused by different weather conditions during the growth periods. In this study the varieties remained below their yield potential of about 8000 to 9000 kg ha^{-1} (Dinc and Erekul, 2010). Intensive rains in 2009 particularly in January and February (total 428.2 mm) caused reducing effects during tillering period (BBCH 20- BBCH 29) and later on ear density (ears m^{-2}; data not shown). Also the amount and distribution of rain in Aegean Region in May can affect the yield formation remarkably. This was more advantageous in May 2010, rain which were higher than 2009, led to remarkably higher yield than 2009. Positive effects of sulphur fertilizer on wheat yield have been noticed also in other studies (Rasmussen and Kresge, 1986; Zhao et al., 1999c). Although in this study, which is the first study in our region in terms of the effects of nitrogen and sulphur fertilization and its combinations on quality parameters of wheat, gave progressive results, the acquired differences haven't found to be statistically significant. Similar results in terms of yield were mentioned in a study by Paulsen (1999). When both years were compared, it can be concluded that Sagittario is more susceptible to sulphur fertilization than Golia.
The measured parameter of the internal grain quality, like grain protein, flour protein, gluten index, sedimentation value, falling number, with the exeption of the gluten content, were significantly influenced by the cultivar, nitrogen application and year. Considerably influenced by the sulphur application was the protein content in the flour, the gluten index and the sedimentation value (univariate model; data not shown).
As one of the bread making qualities, protein content in both wheat varieties increases in paralel with the increasing nitrogen application. These increases are significant especially between the control (0 kg ha^{-1} N) and 70/140 kg ha^{-1} N and 210 kg ha^{-1} N (Tab. 3). Protein content ranged between 11.5% and 16.1% and between 12.3% and 15.3 %, in 2009 and 2010, resp., without any distinctive effect of sulphur fertilization.
In Aegean Region decreasing rainfall in the second half of May and increasing temperatures triggers shortening of grain filling period (BBCH 61-BBCH 87), decreasing carbohydrate accumulation, thus increasing the protein content (Gooding et al., 2003; Erekul et al., 2011). When compared to results of other studies in Mediterranean climates (Guarda et al., 2004), the protein contents determined in our investigation were either similar or higher.
The protein content in flour is the main quality criterion for wheat, especially for bread making (Triboi et al., 2006) and is generally lower than the amount in the grain (Zhao et al., 1999b). The protein content in the flour was about 0.4% to 1.3% lower than the protein content in the whole grain and ranged between 10.8% and 15.3% in 2009 and 2010. Effects of increasing nitrogen doses on protein content in flour have been similar to the effect on protein content in grain. The application of sulphur did not affect the protein content; therefore our results are in accordance with Steinfurth et al., 2012 and Zhao et al., (1999b), but not with Shahsavani and Gholami (2008). The effect of sulphure fertilizer on flour protein content in comparison with the grain protein content was principally the same in Sagittario however the differences are not significant. Different nitrogen doses led to the known effect on protein content (Borghi et al., 1997). Sulphur fertilization can have a comparatively low and unstable effect on wheat flour protein content (Järvan et al., 2008). When the protein content in flour has been examined it can be seen that sulphure application in both wheat varietes is more distinctive

Tab. 2: Effect of nitrogen and sulphur fertilization on grain yield of two wheat varieties.

Year/	2009				2010			
Variety	Golia		Sagittario		Golia		Sagittario	
	Grain yield (kg ha^{-1})							
N / S Doses (kg ha^{-1})	0	40	0	40	0	40	0	40
0	2590	2923	2909	2978	3015	3016	2963	3383
70	3481	3634	3397	3534	4218	4006	3890	4026
140	3854	4242	3846	3955	4540	4280	4240	4196
210	4081	4322	4172	4379	4698	4615	4627	4813
	LSD (0.05) = 432				LSD (0.05) = 464			

Tab. 3: Effect of nitrogen and sulphur fertilization on protein content in grain and flour of two wheat varieties

Year / Variety	2009				2010			
	Golia		Sagittario		Golia		Sagittario	
	Grain protein content (%)							
N / S Doses (kg ha^{-1})	0	40	0	40	0	40	0	40
0	12.0	11.5	13.1	12.4	12.3	12.7	13.0	12.5
70	14.0	14.3	14.4	14.0	13.8	13.5	13.8	14.2
140	15.2	14.9	15.1	15.7	14.2	14.4	14.0	14.8
210	15.8	15.9	15.9	16.1	14.9	15.1	15.1	15.3
	LSD (0.05) = 0.9				LSD (0.05) = 0.6			
	Protein content flour (%)							
0	40	0	40	0	40	0	40	
0	11.1	10.8	12.2	11.8	11.8	11.9	12.6	11.9
70	13.2	13.3	13.1	13.6	13.0	12.8	13.2	13.7
140	14.2	14.1	14.5	14.8	13.2	13.9	13.2	14.0
210	14.5	15.0	15.0	15.3	13.7	14.3	14.3	14.6
	LSD (0.05) = 0.8				LSD (0.05) = 0.4			

than on grain protein content and the differences in some cases have been recorded significant (Tea et al., 2007). The grain quality is a complex of physical and chemical characteristics and its expression depends on their genetic potential and is influenced by environmental conditions (Johansson, 2002; Johansson et al., 2004). In our study, there is found to be no dilution effect between protein content and grain yield (Acuna et al., 2005; Erekul et al., 2009).

In 2009 and 2010 starch content ranged between 60.1-64.8% (data not shown), which is in the range of values for wheat given by Gooding and Davies (1997) and Johansson (2002). It is known that in developed kernels proteins occur first and thereafter starch is produced (Sowers et al., 1994). Therefore, high crude protein contents were accompanied by lowering starch content in both cereal varieties. Protein and starch contents were found to be inversely correlated, which is in line with Gooding and Davies (1997) and Viswanathan and Khanna-Chopra (2001).

Wet gluten content ranged between 27% and 33.9 % in 2009 (Tab. 4). Increasing nitrogen have caused significant increases in wet gluten contents which was also found by Pechanek et al. (1997). Application of sulphure in 2009 has caused increase in wet gluten content in the doses of 70 kg ha^{-1} N, 140 kg ha^{-1} N and 210 kg ha^{-1} N in Golia and in the doses of 140 kg ha^{-1} N and 210 kg ha^{-1} N in Sagittario. However these increases have not been significant in general. Wet gluten content in Sagittario was higher than in Golia in 2009. These differences are significant in the control (0 kg ha^{-1} N) with and without sulphur.

Increase in nitrogen fertilization in 2010 led to higher wet gluten content but not as pronounced as the amount in 2009. S-fertilization resulted in the variety Golia in all N levels in higher gluten content, however the differences were not significant. In Sagittario variety ultimately the opposite was observed at all nitrogen levels. The decreases occured here have not become in significant levels. In the second year as well, Sagittario general produced more wet gluten than Golia however in any case, the difference was not significant.

Tab. 4: Effect of nitrogen and sulphur fertilization on wet gluten content and gluten index of two wheat varieties

Year / Variety	2009				2010			
	Golia		Sagittario		Golia		Sagittario	
	Wet gluten content (%)							
N / S Doses (kg ha^{-1})	0	40	0	40	0	40	0	40
0	27.4	27.0	30.7	28.9	28.9	29.0	29.3	29.1
70	30.1	31.8	31.9	30.4	30.2	31.2	31.6	30.9
140	31.6	32.4	32.1	32.8	30.6	31.8	32.4	31.7
210	32.8	33.1	33.5	33.9	31.9	32.5	33.3	33.1
	LSD (0.05) = 1.2				LSD (0.05) = 2.0			
	Gluten index (%)							
	0	40	0	40	0	40	0	40
0	85	86	76	79	86	86	79	80
70	88	88	80	81	88	90	80	85
140	90	90	82	81	91	92	83	83
210	89	90	80	81	90	91	81	82
	LSD (0.05) = 1.8				LSD (0.05) = 1.9			

Generally, we found wet-gluten to be mostly over 28%, which we assume to be between high and very high values (EREKUL and KÖHN, 2006). The effect of sulphur fertilizing on wet gluten content has not been so distinctive when both years have been considered but much rather the increases of wet gluten content have been found in Golia after sulphur fertilizing. WIESER et al. (2004) concluded that the effect of the sulphur fertilizer on gluten proteins in pot experiments were not remarkable. However the results of JÄRVAN et al. (2008) are in accordance with our study.

Gluten index which indicates the quality of the gluten has ranged between 76% and 90% in 2009. The increasing doses of nitrogen in both wheat varieties have caused the gluten index parameters to increase up to 140 kg ha^{-1} N (Tab. 4). These increases are almost significant at all N-levels. Application of 210 kg ha^{-1} N had no influence on the gluten index. Application of sulphur fertilizer have resulted positively in both wheat varieties, but except for one N application obtained for Sagittario, all differences have not been found significant. Similar results about the efficiency of sulphur fertilizer application have been observed also in 2010. The gluten index reached the highest values when 70 kg ha^{-1} N and 140 kg ha^{-1} N was applied in 2010. Golia achieved significantly higher gluten index values than Sagittario in terms of all nitrogen and sulphur doses and in both years. The gluten index has been recently introduced as a better measure of wheat processing quality, rather than wet gluten content (DENG et al., 2005). However the gluten index is a new, and yet seldom used parameter for wheat bread-making quality; little information is available on the relationship between the gluten index and other indices, or about the influence of environment on the gluten index (JOHANSSON et al., 2002). CURIC et al. (2001) reported optimum values between 75% and 90% for gluten-index of wheat. In our study in both years, the gluten index values have been above 76%. These results are in accordance with JOHANSSON et al. (2004). In the same fertilizing doses in both trial years the gluten index values of Golia have significantly higher values than Sagittario. This implies that the gluten quality and its dough characteristics of Golia is higher (KAHRIMAN, 2007). It is stated that in many studies done with sulphur fertilizer application, the gluten index among the quality parameters is affected positively at most (SCHÄFER and HONERMEIER, 2007; JÄRVAN et al., 2008).

The increasing nitrogen doses have brought significant increases of sedimentation values in both wheat varieties and sulphure doses. In general there has been more impact of the application of sulphure fertilizer on sedimentation values in both wheat varieties and years (Tab. 5). The application of sulphure fertilizer has resulted in equal or higher sedimentation values in all nitrogen applications in both varieties and years. Compared to the gluten index, Sagittario has produced significantly higher sedimentation value in all nitrogen doses than Golia. Impacts of the applications of nitrogen and sulphur on sedimentation values of both wheat varieties have been similar in the second year of the trial. Furthermore in the second year of the trial as well, the Sagittario variety has produced significantly higher sedimentation values in all nitrogen and sulphur levels than the Golia variety.

The sedimentation value of the wheat varieties, characterizing the swelling capacity of gluten, exceeded the nominal value (20 ml) for bread-making wheat in both years. In all applications in both years, the minimum limit of 20 ml has been eminently exceeded. The positive effect of the sulphur fertilizer has been seen more on sedimentation value rather than on gluten index parameter. SCHÄFER and HONERMEIER (2007) reported that sulphur application created positive effects on sedimentation value but the results obtained were also not significant. WEBER at al. (2008) put forward that the effect of additional sulphur application on sedimentation value is extremely limited. The sedimentation value of the Sagittario has higher values in both years than the Golia and based mainly on genotypic characteristics. Increasing nitrogen doses caused the protein contents to increase and due to this, the sedimentation values increase significantly in both varieties and in both years. Similar results have also been reported in previous studies (GOODING et al., 2003).

The falling number has been measured in order to determine impacts of nitrogen and sulphur fertilizer on alpha amylase activity in wheat flour. The falling number values ranged between 333 s and 396 s in 2009 (Tab. 5). In 2009, any distinct impact of nitrogen and sulphur fertilizer has not been determined on the falling number. Sagittario showed substantially lower falling number values than the Golia variety. In 2010 the falling number values have ranged from 298 s to 351 s and showed lower values than 2009 without impact of nitrogen and sulphur fertilizer. The falling numbers, which allow for conclusions to the enzymatic state of the grain, to the expected baking volume, and to the grade of outgrowth, have an optimum range of 220 to 250 s for winter wheat (DIEPENBROCK et al., 2005). Falling number values in both varieties and years have been above

Tab. 5: Effect of nitrogen and sulphur fertilization on sedimentation value and falling number of two wheat varieties

Year/ Variety	2009				2010			
	Golia		Sagittario		Golia		Sagittario	
	Sedimentation value (ml)							
N / S Doses (kg ha^{-1})	0	40	0	40	0	40	0	40
0	26	28	29	33	27	28	30	32
70	30	30	35	35	30	31	33	35
140	31	33	38	38	32	33	36	36
210	36	37	40	42	34	36	37	39
	LSD (0.05) = 1.6				LSD (0.05) = 2.0			
	Falling number (s)							
	0	40	0	40	0	40	0	40
0	373	352	347	333	324	298	312	323
70	389	348	361	371	347	340	304	329
140	358	381	343	340	329	351	324	311
210	370	396	353	387	341	338	339	333
	LSD (0.05) = 17				LSD (0.05) = 24			

the optimum values. Falling number values are affected by the climate conditions during the grain filling period (BBCH 61-BBCH 87) and primarily by rainfall more than the fertilizing (GOODING et al., 2003). When evaluated from that perspective, decrease in rainfalls and increase in temperatures in grain filling period in our region caused the falling number values to increase. This situation has caused enyzme activity of the flour obtained to decrease. Similar results have also been reported by JOHANSSON (2002) and GOODING et al. (2003). It may also be possible to see differences among genotypes (EREKUL et al., 2009). In our study, Sagittario generally has lower falling number values than Golia. No significant effect of nitrogen and sulphur fertilizing applications on falling number values have been found and these findings are in accordance with the results of WEBER et al. (2008).

Conclusion

Quality characters of bread wheat have not adequately been examined on varieties grown in Western Turkey. Due to lack of information, wheat varieties have not been classified into quality groups which cause inability to compare them with international wheat varieties. Under more favorable climatic conditions the grain yield could increased up to 210 kg ha^{-1} N without substantially losses in the grain quality. The results indicate that S-fertilization showed benefical effects in grain yield and in some bread-making quality parameters. S-fertilization on protein content was more remarkable in flour than in grain. The most benefited quality parameter by sulphur was the gluten-index and especially the sedimentation value. In this respect, S-fertilization in improving bread making quality of the region should not be disregarded. However, an S fertilization of > 40 kg ha^{-1} would be advisable to prove more clearly effects on yield and quality parameters.

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