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Synergistic Effect of Sulfur and Nitrogen in the Organic and Mineral Fertilization of Durum Wheat: Grain Yield and Quality Traits in the Mediterranean Environment

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Abstract: In recent years, awareness on sustainable land use has increased. Optimizing the practice of nitrogen fertilization has become crucially imperative in cropping management as a result of this current trend. The effort to improve the availability of organic nitrogen has incurred a bottleneck while seeking to achieve a high yield and quality performance for organic winter cereals. Field experiments were conducted, under rainfed Mediterranean conditions, over a period of two subsequent growing seasons. The objective was to investigate the effect of soil and foliar S application on the performance of three durum wheat cultivars fertilized with either organic or inorganic N. The hypothesis to be verified was if different S fertilization strategies could improve grain yield and quality when coupled with mineral or organic N fertilizer. There were three levels of treatment with mineral N fertilizer (120, 160 and 200 kg ha^{-1}), two levels of organic N fertilizer (160 and 200 kg ha^{-1}), two levels of S fertilizer applied to the soil (0 and 70 kg ha^{-1}), and two levels of foliar S application at flag leaf stage (0 and 5 kg ha^{-1}). Cultivars were Dylan, Iride and Saragolla. Analyzed traits were grain yield, yield components and quality features of grain. Overall, at the same N rate, grain yield and quality were markedly higher for mineral than organic N source. Cultivar × Year × N treatment interactions significantly affected grain yield and quality indices. Iride showed a high yield stability throughout the mineral N rates in the most favorable year (2011) and, in the same year, was the top performing cultivar in organic N treatments. Dylan was the top performing cultivar for protein content, while Saragolla for the SDS sedimentation test. Soil S fertilization had no effect on grain quality, whereas it significantly increased grain yield (+ 300 kg ha^{-1}) when coupled with organic rather than a mineral N source. However, foliar S application at flag leaf stage did not affect grain yield, but it significantly enhanced quality indices such as test weight (81 vs. 79.9 kg hL^{-1}), protein content (13.7% vs. 12.9 %) and SDS value (72.5 vs. 70.5 mm). A rate of 160 kg ha^{-1} of N (both mineral and organic) determined the optimal response for both grain yield and quality. Finally, soil and foliar application of S may help to contain the large yield and quality gap that still exists between mineral and organic fertilization of durum wheat.

Keywords: durum wheat; mineral N; organic N; S fertilization; grain quality; grain yield

1. Introduction

Durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) is an economically important crop cultivated worldwide. Europe-28 is by far the largest world durum wheat producer. In 2017, it was grown on 2.7 million hectares only in the European Union (EU), providing an output of about 9 million tons. The cultivation area of durum wheat in Europe is mostly concentrated in the Mediterranean region: Italy, Spain and France together account for 80% of total EU production [1]. Italy is the top EU producer country and a traditional durum wheat growing region as it dedicates half of the total EU durum wheat area to this crop, thus accounting for 45% of the entire EU production, with a yield of about 3.2 t ha^{-1}.

Grain quality has become one of the most important goals for the breeders and growers [2,3], because it is essential in obtaining premium prices and meeting markets needs for high-quality end-products of durum wheat such as pasta, couscous and burghul [4].

There is no simple and complete definition for the quality of durum wheat [4,5]. Grain protein content, color and gluten strength are considered the most important features needed for use in pasta and bread production. Grain protein content is known to be influenced by climatic parameters, genetic factors, nitrogen fertilizer rate, time of nitrogen application, residual soil nitrogen and available moisture during grain filling [6–9]. The yellow color is due to the carotenoid pigment content in the whole kernel, and it is commercially identified as the yellow index in semolina. Besides their role as an important aesthetic parameter, the carotenoids have important nutritional and health characteristics [10]. While yellow index was found to be affected by weather conditions, cultivar and N rate and timing [10–12], less is known about the effect of N source and S fertilization on this quality index.

Gluten strength contributes to the ability of dough to rise and maintain its shape as it is baked. Gluten strength is commonly estimated using the sodium dodecyl sulfate (SDS) sedimentation test that, depending on the protein quality, provides a good indicator of pasta cooking quality [13–15]. Ercoli et al. (2011) [15] found that SDS value increases with the increase of inorganic N (from 120 to 180 kg ha^{-1}) and S (from 0 to 60 kg ha^{-1}) rate.

The use of nitrogen is normally considered a key factor in cereal crops and numerous studies on the best N fertilization rates and timing have been conducted. In fact, if on the one hand it has been proved that nitrogen positively affects grain yield and quality, on the other hand N fertilizer management is pivotal to avoiding N losses caused by leaching, runoff, denitrification or volatilization [11,16,17].

After taking all this into account, the use of organic N fertilizers may be a further option, together with other cropping management and practices, to reduce nitrate pollution and improve the environmental sustainability of conventional farming systems [12]. Thus, another feature can be added to the definition of the quality of wheat products [18].

Moreover, fertility management was the identified key factor in limiting both yield and grain protein content in the organic wheat management [12,19,20]. The results on common wheat (*Triticum aestivum* L.) emphasize the importance of a sufficient supply of soils with organic fertilizers as well as the need to improve the availability of organic nitrogen [19,21]. This latter option might be accomplished by trying to regulate the degradation and mineralization of organic matter (OM) in the soils, which is the traditional role assigned to heterotrophic microbes [22]. The number of these microorganisms, and in particular those which oxidize sulfur (S), was found to be: (i) greater in some rizospheres (e.g., canola and wheat) than in bulk soil controls [23]; and (ii) stimulated by S fertilization and soil OM [24]. A recent study conducted in the Canadian prairie showed that common wheat biomass production in organic systems was positively related (among other factors) to the plant tissue S concentration [21]. Thus, S application to the soil might have a synergistic effect with organic N fertilization of durum wheat, determining higher yields and better quality. This hypothesis could be particularly verified in those agroecosystems that extend along the Mediterranean coast, in which soil temperature and water availability during the winter season do not drastically reduce mineralization capacity by the soil biota.

Sulfur is an essential element for all organisms since it is present in many molecules (amino acids, oligopeptides, vitamins and many secondary metabolites) and it is involved in several biochemical processes. Plants absorb S as sulfate ion ($SO_4{}^{2-}$) from soil solution and use it in key steps of their metabolism [25]. Furthermore, findings provide evidence for the uptake and metabolization of elemental S also at the leaf level [26,27]. However, the fact that symptoms of deficiency appear earlier in young leaves than mature ones suggests that S is relatively immobile in mature leaves and that the re-distribution from vegetative tissues to wheat kernels is noticeably less than that of N and P [28]. These are significant findings when considering foliar S application from flag leaf emergence to anthesis, aiming to reach greater efficiency in the S fertilization [29,30].

The importance of S in plant nutrition is highlighted by the fact that a limited availability of this element causes both direct (biomass reduction) and indirect production loss [28,31–33]. The indirect effects on plants productivity are attributable to the role that S plays in the synthesis of several metabolites such as Sulfur-containing Defense Compounds (SDC) involved in the physiological response to biotic and abiotic stresses [34–36]. Such considerations have led to studying grain yield and quality responses to S fertilizer and thus developing improved N and S fertilization strategies [11,16,37,38].

Although many studies have been conducted on the influence of N and S fertilization on common wheat characteristics such as growth, yield, quality and technological properties [28–30,38–40], still very little is known about the effect of S and N nutrition on grain yield and quality of durum wheat.

Studies conducted in the Mediterranean basin show different results leading to different conclusions. Garrido-Lestache et al. (2005) [11] in a three-year field experiment in southern Spain found that soil or leaf application of S had no effect on quality indices, with the exception of ash content. Conversely, Lerner et al. (2006) [41] in Argentina described a positive effect of sulfur fertilization on wheat quality traits. A similar result was observed in Southern Italy [42] and by Ercoli et al. (2011) [15] in Central Italy, even if they did not find a significant effect of S fertilization on grain protein content. Moreover, to the best of our knowledge, no studies have been conducted on durum wheat aiming to test simultaneously the interaction of S fertilization type with both mineral and organic N source.

Thus, the aims of the present study were: (1) to evaluate the effect of different N-S fertilization rates and types on grain yield and quality of three durum wheat cultivars representative of the Mediterranean region; and (2) to verify the hypothesis that soil S fertilization has a synergetic effect with organic N fertilization, on improving grain yield and quality of durum wheat.

2. Materials and Methods

2.1. Site and Experimental Design

A field experiment was set up in Tarquinia, Central Italy (42°12′ N, 11°45′ E; altitude: 22 m a.s.l.), during the 2010–2011 and 2011–2012 growing seasons. The area has a Mediterranean climate, with a mean air temperature of 15.5 °C and a mean annual precipitation of 658 mm. The weather data were retrieved from a meteorological station located in Tarquinia, at a short distance from the site. Meteorological data were characterized by a consistent difference between the growing seasons, particularly in terms of precipitation, so that in 2010–2011 rainfall was 38% higher compared to the 20-year average rainfall, while in 2011–2012 it was lower by 58%. Mean monthly temperature and total rainfall during 2010–2011 and 2011–2012 growing seasons are shown in Figure 1.

Temperatures were similar in the 2010–2011 and 2011–2012 growing seasons and, compared to the 20-year averages, were higher by 2 °C over the period from sowing to tillering stage and lower by 5.5 °C on average from February until grain ripening. Rainfall were under the average for more than five months in 2012.

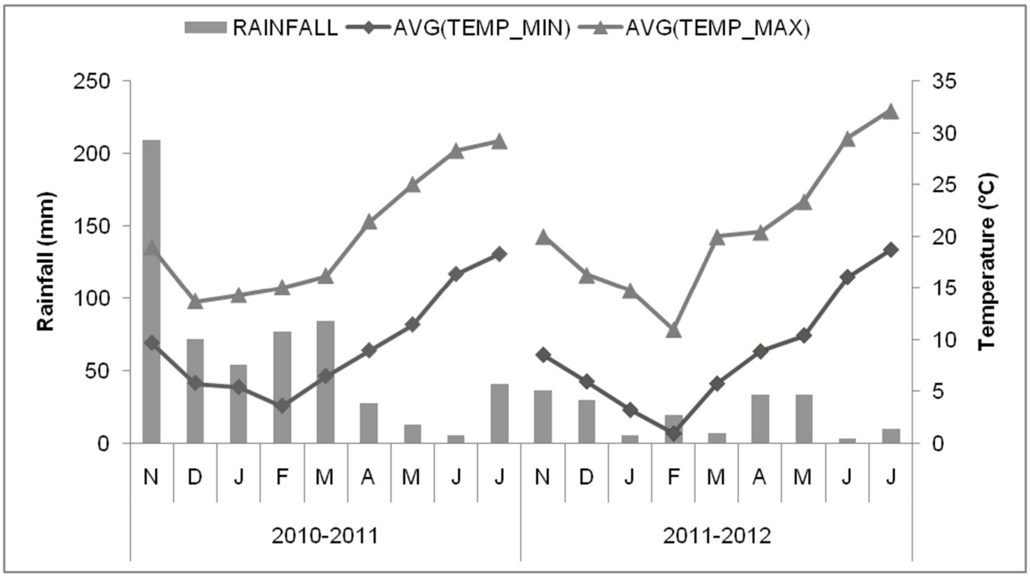

Figure 1. Minimum and maximum temperatures and rainfall recorded during the growing season (November–July) in 2010–2011 and 2011–2012.

Soil samples were collected from fields in both years before sowing. Samples, taken at 0–40 cm depth, were oven dried, grounded and then analyzed for texture and chemical analysis: pH, OM, sulfur content, total N, and total carbonate content. The soil was classified as clay according to the International Soil Science Society (ISSS) classification. The relevant soil characteristics are presented in Table 1.

Table 1. Soil characteristics of the experimental sites.

Parameter	Unit	2010–2011	2011–2012
Clay (Ø < 2 μm)	%	45.2	55.9
Silt (2.0 < Ø < 20 μm)	%	20.5	19.5
Sand (2.0 > Ø > 0.02 mm)	%	34.3	24.6
pH		7.4	7.2
Organic matter	%	1.8	1.6
Total $CaCO_3$	%	3.8	0.4
Total N	%	0.1	0.1
Available P	mg kg^{-1}	11.4	10.1
Exchangeable K	mg kg^{-1}	488.0	452.0
Available S	mg kg^{-1}	8.2	4.16

A split-split plot design with three replications was used: nitrogen fertilization levels was the main treatment, sulfur soil and foliar fertilization were the sub-treatments and varieties were the sub-sub-treatment. N-fertilization was arranged in five main plots while soil sulfur rates in two subplots in each main plot, as well as foliar sulfur rates and the three varieties were arranged in three sub-subplots in each subplot.

At the end of summer, the experimental field was ploughed at 30 cm depth and then divided into plots and subplots with three replicates for a total of 180 sub-subplots. The area of each sub-subplot was 180 m^2. Plots were sown on 20 January 2010 and 23 December 2011 at a seeding rate of 350 viable seeds m^{-2}. Three durum wheat varieties (Dylan, Iride and Saragolla) were chosen as representative of the cultivation area. They are widely adapted to different Mediterranean environments and characterized by high and constant productivity and good grain quality. Iride and Saragolla are early maturing and medium size varieties, while Dylan is medium-late maturing having a medium-taller size. All of them are relatively new varieties, released and registered in the Italian register of varieties since 1996 (Iride), 2004 (Saragolla) and 2002 (Dylan). The preceding crop was tomato (*Solanum lycopersicum* L.) in the first season and melon (*Cucumis melo* L.) in the second season.

Different nitrogen fertilizers (organic and mineral) and rates were applied: for organic fertilization 160 and 200 kg ha^{-1} of N (hereafter referred to as NO160 and NO200, respectively) and for the mineral fertilization 120, 160 and 200 kg ha^{-1} of N (hereafter referred to as NM120, NM160 and NM200, respectively). All NM plots received 92 kg ha^{-1} of P_2O_5 before sowing as diammonium phosphate. Nitrogen doses were determined by considering the minimum crop requirement of 3 kg of N per 100 kg of grain produced [43] and the more common yields recorded for the above mentioned cultivars in that environment (4–6 t ha^{-1}). In addition, subplots were treated with four combinations of sulfur fertilization: nil (hereafter referred to as SS0 or FS0), granular soil-sulfur fertilization (70 kg ha^{-1} of elemental S, hereafter referred to as SS70), foliar fertilization (5 kg ha^{-1} of S, hereafter referred to as FS5) and soil and foliar fertilization. Foliar S fertilization was applied at flag leaf emergence stage and soil S fertilization before sowing, as well as organic nitrogen fertilizer. This latter was a pelletized organic NP fertilizer (6% N; 3% P_2O_5; 30% C) derived from the fermentation of organic materials such as feather meal, bone meal, manure, etc. Nitrogen mineral fertilization was split as follows: (i) 36 kg ha^{-1} as diammonium phosphate for all rates at sowing; and (ii) 42–42, 62–62 and 82–82 kg ha^{-1} in the form of ammonium nitrate at early tillering stage and flag leaf emergence for NM120, NM160 and NM200, respectively. Weeds and diseases were chemically controlled.

2.2. Sampling and Measurements

Grain yield was determined at 13% moisture content, harvesting 15 m^2 sampling areas. At the same time, one square meter of plants was cut and then processed to obtain the following grain yield components: number of spikes, number of kernels per spike, and mean kernel weight. From each main sample, a sub-sample of grains was taken for the following measurements: test weight, vitreousness, thousand kernels weight, protein content, sodium dodecyl sulfate (SDS) sedimentation test and yellow index. Grain test weight was measured by the Schopper chondrometer. To determine protein content and yellow index and perform the SDS sedimentation test, samples were ground and analyzed using a Foss NIR System 6500 monochromator (Foss NIR Systems Inc., Silver Spring, Laurel, MD, USA), equipped with a sample transport module and a small ring cup. Prior to taking the measurements, the instrument was validated according to the diagnostic procedure of Win ISI II (InfraSoft International, LLC., Port Mathilda, PA, USA) software. The calibration equation developed at the Società Produttori Sementi Spa (Bologna, Italy) in accordance with the NIR guidelines for prediction model development [44] was used in this study. The range of wavelength used for analyses was set from 400 to 2500 nm and recorded at 2-nm intervals as log (1/R), where R represents decimal fraction transmittance [45]. Each sample was analyzed twice.

2.3. Data Analysis

All data were processed using R statistic software (R Development Core Team, 2006). Statistically significant differences among means were detected by the least significant difference test [46] after analysis of variance (ANOVA). The main effect of Year, N rate, S application and variety and their interactions were tested. Means were separated at 95% probability level. Each set of data was checked for normality and appropriate transformations were used, when necessary, prior to the ANOVA to improve normality [47].

3. Results

3.1. Yield and Yield Components

The analysis of variance for yield, protein content, test weight, SDS test, yellow index, vitrousness, and yield components are presented in Table 2. Treatments differently affected the measured traits.

Fourth and fifth order interaction was never significant. Third order interactions were significant only for V × N × Y.

For the same nitrogen treatment, grain yield was significantly lower in 2012 as compared to 2011 in all tested cultivars (Table 3). This may be largely attributed to the dramatic reduction of rainfall amount in 2012 which had a negative effect on the number of kernels per spike and mean kernel weight. A decrease in the number of kernels per spike were detected for Dylan in NO160 (−37%), Iride in both NO160 and NO200 (−36% and −40%, respectively) and Saragolla in NO200 treatment (−47%). On the contrary, the mean kernel weight showed a lower variation in the NO treatments (5–10%) than in the NM ones (18–20% in NM200) for all cultivars. Considering these results and the number of spikes per unit area, more yield decreases were registered for Iride in NM120 and NO200 plots (−52.6% and −52.5%, respectively) and for Saragolla in NM160 plots (−53.4%). In contrast, the lower differences in grain yield between 2011 and 2012 were found for Dylan in both NM160 and NO160 plots (−29.8%). Iride showed a high yield stability throughout the NM rates in the most favorable year (2011) and, in the same year, was the top performing cultivar in NO treatments. However, when limiting weather conditions occurred (2012), Dylan had a grain yield significantly higher than that of Iride and Saragolla, both in NO160 (+21% and +19%, respectively) and NO200 (+23% and +19%, respectively). In general, at the same N rate, yield responses were dramatically higher for mineral than organic N because of a significantly higher number of both spikes per unit area and kernels per spike. Cultivars differently responded to the increase of N rate, both for mineral and organic form. Particularly, 2011 grain yield significantly increased in Dylan by 11% from NM160 to NM200, while it did not change significantly between NO rates. By contrast, grain yield did not significantly vary in Iride through NM rates but it increased significantly from NO160 to NO200 (+11%). Finally, Saragolla showed the best yield performance at 160 kg ha^{-1} of N, both mineral and organic form, since no significant increases were detected at the higher rate. NM160 was also the best solution in 2012 with the exception of Saragolla which yielded significantly higher with NM200 (+25%).

Regarding the effect of S fertilization, SS × N interaction significantly affected grain yield, highlighting a positive effect of S soil application in both organic nitrogen fertilization rates, while no significant effect was detected for mineral nitrogen (Table 4). This was due to a significant increase in both the number of spike per unit area and the number of kernels per spike (this latter significant only for NO160).

S foliar treatment had no significant effect on yield (Table 5), as it increased the number of kernels per spike (+3.4%) but decreased the mean kernel weight (−2.4%).

3.2. Quality Traits

As expected from such an assorted collection of cultivars and fertilization treatments, quality characteristics of grain varied considerably.

As for yield, V × N × Y interaction was significant for all quality traits.

For the same nitrogen treatment, test weight significantly decreased in 2012 in all tested cultivars (Table 6). This was due to kernel shriveling caused by the severe drought and heat stress which occurred in the second year during grain filling. NM200 treatment resulted in the highest test weight decrease between the two years for all cultivars (from 14.3% of Dylan to 18.2% of Saragolla). On the contrary, organic fertilization showed the lowest differences in the test weight values between the two years (from 4.3% of Dylan in NO200 to 8.7% of Iride in NO160). Moreover, Dylan was the only cultivar which accomplished market request for test weight in 2012, overcoming 80 kg hL^{-1} in NO treatments. Soil sulfur fertilization had no significant effect on the test weight (data not shown), whereas foliar S application slightly increased this trait from 79.9 to 81 kg hL^{-1} (Table 5).

Table 2. Results of the ANOVA on different grain yield and quality traits.

Main Effect	Significance								
	Grain Yield	Grain Protein	Test Weight	SDS Test	Yellow Index	Vitreous Kernels §	Spikes m^{-2}	Kernels $Spike^{-1}$	Kernel Weight
Year (Y)	***	**	**	***	***	***	***	***	***
Nitrogen (N)	***	***	***	***	***	***	***	***	***
Variety (V)	***	***	***	***	***	***	***	***	***
Soil sulfur (SS)	n.s.	n.s.	n.s.	n.s.	n.s.	***	n.s.	*	***
Foliar sulfur (FS)	n.s.	***	***	***	n.s.	***	n.s.	***	***
Two-way interactions									
N × Y	***	***	***	***	n.s.	***	***	***	***
V × Y	***	***	***	***	***	***	**	***	**
V × N	n.s.	***	*	***	n.s.	**	***	***	*
SS × N	***	n.s.	*	n.s.	n.s.	*	***	***	***
FS × SS	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	***
Three-way interactions									
V × N × Y	***	***	**	***	*	**	***	***	**

ANOVA signif. codes: '***' < 0.001; '**' < 0.01; '*' < 0.05; '.' < 0.1; n.s.: not significant. Other interactions are not reported since they are not significant. § transformed data were used to perform the ANOVA.

Table 3. Yield related traits. Variety × Nitrogen × Year interaction.

Yield Related Traits		NM120			NM160			NM200			NO160			NO200		
		Dylan	Iride	Saragolla	Dylan	Iride	Saragolla	Dylan	Iride	Saragolla	Dylan	Iride	Saragolla	Dylan	Iride	Saragolla
Grain yield (t ha^{-1})	2011	5.95	6.46	5.81	6.07	6.47	6.83	6.75	6.79	6.72	5.26	5.54	5.19	5.53	6.06	5.32
	2012	3.55	3.06	3.38	4.26	4.43	3.18	4.13	3.91	3.96	3.69	3.06	3.11	3.54	2.88	2.98
	LSD	0.36 ***														
Kernels $spike^{-1}$ (*n*)	2011	39.22	41.05	38.10	41.95	43.16	42.92	41.95	41.97	39.20	35.72	40.13	36.49	38.72	39.18	41.20
	2012	28.02	26.34	26.01	36.30	35.37	30.36	35.88	30.93	32.62	22.51	25.55	26.47	27.88	23.35	21.71
	LSD	1.97 ***														
Spikes m^{-2} (*n*)	2011	405.2	464.1	441.9	437.4	477.2	497.8	484.1	543.6	520.3	403.9	401.5	415.8	398.8	455.5	378.8
	2012	458.8	468.4	484.9	473.8	495.6	510.3	492.6	544.4	509.2	462.7	438.6	409.4	405.9	442.2	472.6
	LSD	13.44 ***														
Mean kernel weight (mg)	2011	47.65	42.32	43.88	46.35	40.56	43.99	44.61	41.28	43.76	51.18	46.14	47.18	51.00	46.35	48.35
	2012	39.37	37.75	40.06	39.10	36.40	34.47	35.62	33.68	35.32	48.19	42.50	44.82	46.81	43.78	43.68
	LSD	1.83 ***														

ANOVA signif. codes: '***' < 0.001; LSD: least significant difference ($p < 0.05$).

Table 4. Yield related traits. Soil Sulfur × Nitrogen interaction.

Trait		NM120	NM160	NM200	NO160	NO200
Grain yield (t ha^{-1})	SS0	4.63 cd	5.28 ab	5.34 a	4.17 e	4.23 e
	SS70	4.77 c	5.13 b	5.41 a	4.45 d	4.54 d
Kernels spike^{-1} (n)	SS0	32.13 d	39.46 a	36.60 b	30.22 e	31.72 d
	SS70	34.12 c	37.23 b	37.58 b	32.07 d	32.30 d
Spikes m^{-2} (n)	SS0	455.4 c	477.2 b	516.5 a	411.6 e	417.2 e
	SS70	452.3 c	486.8 b	514.9 a	432.4 d	434.0 d
Mean kernel weight (mg)	SS0	42.25 d	40.24 e	39.38 ef	48.33 a	46.35 b
	SS70	41.43 d	40.05 e	38.71 f	45.01 c	46.97 b

For each trait, numbers followed by the same letter are not significantly different at $p < 0.05$.

Table 5. Yield and quality related traits. Foliar sulfur fertilization mean values.

Treatment	Grain Yield (t ha^{-1})	Kernels Spike^{-1} (n)	Spikes m^{-2} (n)	Mean Kernel Weight (mg)	Test Weight (kg hL^{-1})	Protein Content (%)	SDS Test (mm)	Yellow Index
FS0	4.76	33.77	457.7	43.39	79.88	12.86	70.53	24.81
FS5	4.83	34.91	462.0	42.35	80.97	13.69	72.53	24.72
ANOVA signif.	n.s.	***	n.s.	***	***	***	***	n.s.

ANOVA signif. codes: '***' < 0.001; n.s.: not significant.

Table 6. Quality related traits. Variety × Nitrogen × Year interaction.

Quality Related Traits		NM120			NM160			NM200			NO160			NO200		
		Dylan	Iride	Saragolla	Dylan	Iride	Saragolla	Dylan	Iride	Saragolla	Dylan	Iride	Saragolla	Dylan	Iride	Saragolla
Test weight (kg hL^{-1})	2011	85.35	85.05	84.43	84.69	85.47	84.86	84.60	86.13	84.95	85.43	84.98	83.79	85.15	85.33	84.68
	2012	75.09	74.82	73.74	78.11	73.71	71.50	72.53	71.94	69.46	80.94	77.61	78.83	81.49	79.70	78.39
	LSD								1.61 ***							
Protein content (%)	2011	14.01	12.00	11.59	14.52	12.72	13.30	15.01	12.49	13.49	11.28	10.23	10.44	11.49	10.54	11.55
	2012	14.80	14.67	15.22	16.29	16.58	16.86	16.45	15.58	16.35	12.69	12.29	11.85	11.19	11.23	11.44
	LSD								0.72 ***							
SDS (mm)	2011	71.28	67.88	70.78	69.55	68.07	74.60	74.76	66.64	75.47	58.59	59.65	66.79	62.19	62.22	71.45
	2012	76.91	73.36	82.19	73.04	79.36	85.50	81.27	83.15	90.75	64.58	67.85	72.68	60.42	64.91	70.00
	LSD								3.69 ***							
Yellow index	2011	23.52	21.66	23.78	23.59	21.49	23.87	23.78	20.98	23.92	22.84	21.10	23.77	22.85	21.10	23.46
	2012	27.84	25.44	27.06	27.43	25.79	27.83	28.02	26.17	27.09	27.13	25.40	26.79	27.23	24.96	27.05
	LSD								0.57 ***							

ANOVA signif. codes: '***' < 0.001; LSD: least significant difference ($p < 0.05$).

Grain protein content was significantly higher in 2012 than 2011 for all cultivars and N treatments with the exception of NO200, in which it remained substantially unchanged (Table 6). This increase between the two years was due to the fact that in 2012 grains were shriveled, with low starch accumulation, resulting in higher protein concentration [4]. Dylan showed a protein content significantly higher than Iride and Saragolla for each N fertilization treatment in 2011 with the exception of NO200. Even with lower rates, mineral nitrogen fertilization always showed a significantly higher protein content with respect to NO treatments, with the exception of NM120, where the protein content was similar to that of NO200 for Saragolla in 2011.

Even though grain protein content was not affected when sulfur was applied to the soil (data not shown), foliar S fertilization had a positive effect, increasing this trait significantly from 12.9% to 13.7% (Table 5).

As for grain protein content, the sedimentation values (SDS test) increased markedly in 2012 over 2011, with the exception of NO200 treatments (Table 6). Generally, mineral fertilization showed higher values than NO and the best performing cultivar for this trait was Saragolla in both years.

Similar to grain protein concentration, S fertilization applied to the soil had no effect on the sedimentation values (data not shown), while foliar application significantly increased this trait, especially when coupled with soil application (Figure 2).

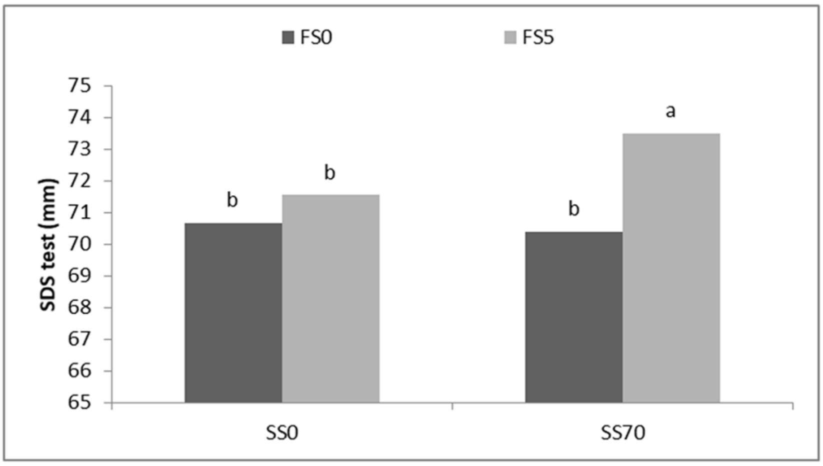

Figure 2. SDS sedimentation test. Foliar and soil sulfur interaction. Bars sharing the same letter are not significantly different ($p < 0.05$).

Values for the yellow index from 2012 were significantly higher than those from 2011 for all cultivars and N treatments (Table 6). In general, Dylan and Saragolla showed similar results for each N treatment while Iride always highlighted a significantly lower yellow index. Moreover, the increase of N rate had a significant effect on this trait just in two circumstances (from NM120 to NM200 for Iride and from NM120 to NM160 for Saragolla, both in 2012).

Sulfur fertilization had no effect on this trait.

Similar to the other quality traits, vitrousness was also affected by N × V × Y interaction, showing significantly higher values in 2012 than 2011 for all cultivars and N treatments (Figure 3). Compared to the other cultivars, Dylan showed a significantly higher percentage of vitrous kernels in 2011 NM treatments, while it performed similar to Iride and Saragolla in 2011 NO200 fertilization and in all 2012 N treatments. Mineral nitrogen fertilization always caused a significantly higher percentage of vitrous kernels than NO treatments, with the exception of NM120 compared with NO 200 for Saragolla in 2011.

Consistent with the result on grain protein concentration, vitrousness of kernels increased with foliar sulfur application from 80.8% to 83.5% (data not shown).

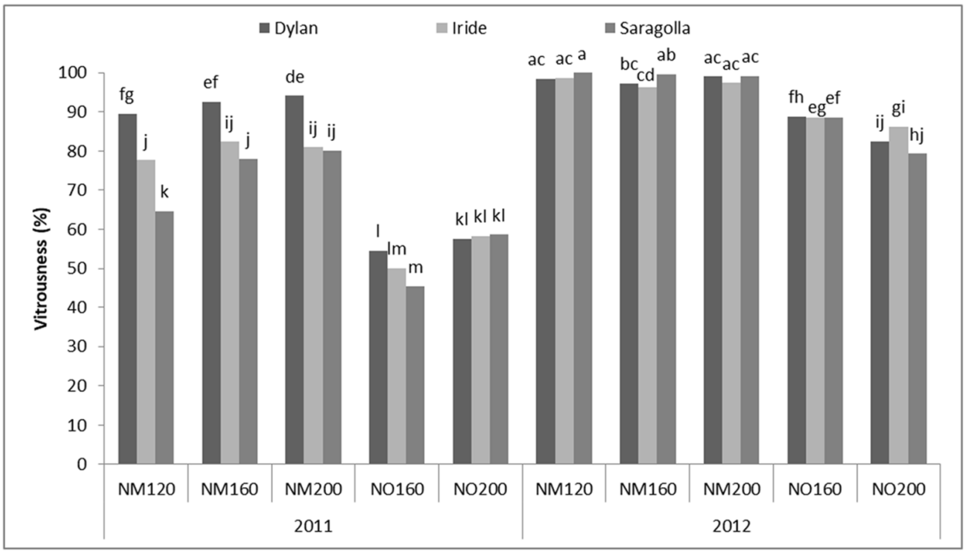

Figure 3. Grain vitrousness. Nitrogen × variety × year interaction. Bars sharing the same letter are not significantly different ($p < 0.05$).

4. Discussion

Results of this study demonstrate that fertilization type and rate have a strong influence on durum wheat yield, yield components and quality characteristics of grain. However, as also found by other authors [6,11,17], crop growth, yield and quality traits are mainly a function of environmental conditions. In fact, significant second and third order interactions were found which confirm the year on year variations for durum wheat production. This variability may be due to changes in the rainfall amount and distribution throughout the growing seasons [48].

Overall, organic fertilization was a determining factor in the observed reduction of grain yield and quality. As other studies confirm, mineral N fertilization gives better results as compared to organic fertilization, either in terms of yield and protein content [49,50]. These studies reported that winter wheat receiving organic fertilization had yields up to 19% lower than that fertilized with mineral N, on average. In our study, considering only the N effects and the same N rate, mineral nitrogen fertilization gained significantly higher grain yield than NO, ranging from +21% to +23%.

Similarly, protein content and SDS test values were higher for mineral fertilization as compared to the organic one, and they increased with the increase of nitrogen rate. These results are consistent with those by other authors and corroborate the issue of N availability in the case of organic fertilization, which is limited during the crop reproductive phases and always lower when compared to mineral nitrogen [12,50]. Systems that are based on organic fertilization usually have very different seasonal N cycle and availability than those that use mineral fertilizers. Thus, reliance on organic N sources requires an understanding of organic N mineralization–immobilization and turnover patterns in relation to crop N demands and N loss pathways. Besides the dependence of mineralization on pedoclimatic conditions [51], it usually takes many years to mineralize past organic fertilizer and support crops with appropriate N availability [52]. Moreover, even if a balance is reached, winter crops generally suffer significant yield reduction due to slow mineralization during their growth cycle. Even though commercial organic fertilizer, such as that used in this study, contains this reduction due to its low C/N ratio and higher nutrient availability, the yield gap was found to be significant even after six years [53]. However, a long-term study would better clarify if a multi-year application of an organic fertilizer may reduce this yield gap, thanks to the positive effects that organic matter has on physical, chemical and biological properties of the soil.

In this study, yield gain for NM fertilization treatments was at 14.5% between 120 and 200 kg ha^{-1} while it was just at 3% from 160 to 200 kg ha^{-1}. On the contrary, no significant increase was detected with the increase of NO rate (from 160 to 200 kg ha^{-1}). Ercoli et al. (2011) [15] found an increase of 20% (from 120 to 180 kg ha^{-1} of NM) in similar climatic conditions. Other authors in Spain reported no grain yield response to NM rates of up to 100 kg ha^{-1} [11,54,55]. Such a large difference is probably because wheat yield is influenced by N rate only when the amount of rainfall exceeds 450 mm during the growing season, as reported by Lopez-Bellido et al. (1996) [48] in a long-term experiment. In fact, in the wetter year (2011), we obtained significantly different yields at each N rates (both NM and NO), whereas in the drier year (2012), NM160 and NM200 yields were similar. In 2012, only the NM120 yield was significantly lower, supporting the finding that the crop fertilized with 120 kg ha^{-1} of N, often has sub-optimal yield performance [15].

Concerning varieties, all the tested genotypes responded similarly to the year-on-year variations of climatic conditions, but were differently sensitive to N fertilization in each year. Particularly, all the three cultivars were markedly sensitive to water shortage which especially reduced the number of kernels per spike and the mean kernel weight. This behavior was also verified by Ercoli et al. (2011) [15] in medium and late-maturing varieties (Claudio and Creso, respectively), while in early or medium-early varieties they did not find any yield difference between wet and dry season. However, other authors also found short-cycle cultivars decreasing grain yield with the decrease of the rainfall amount in the Mediterranean environment [11,56]. The different behavior of cultivars in response to climatic fluctuations is of crucial importance for Mediterranean environments, because of the high year-to-year variability in rainfall and temperature pattern existing in the climate.

In our study, the yield performance of the cultivar Dylan in the drier year was unexpected. This medium-late variety was expected to yield poorly in the most limiting environmental conditions of the second year, while it performed similarly to early-maturing cultivars with NM treatments and even better with NO fertilization. This finding is consistent with the results from the Italian durum wheat network, which show that, in the last seven years (from 2011 to 2017) and in an environment comparable to that of this study, Dylan yielded similarly to Iride and/or Saragolla in five different seasons (2012, 2013, 2014, 2015, and 2017). Although Iride had the same yielding performance of Dylan, it did not have the same quality of grains, showing a significantly lower protein content, yellow index, test weight and grain vitrousness. Saragolla was the lowest yielding variety but that with the highest SDS value.

Concerning the effect of sulfur, it is known that elemental sulfur (ES) has to be oxidized to SO_4^{2-} before it is available for plants and that the response to sulfur fertilization can be very variable in wheat. Sulfur uptake and metabolization depends on soil N and S balance, water supply, timing and rates of N and S application [28,57,58]. With regard to grain yield, we found that, for a given N rate, the S application to the soil had a synergistic effect with organic rather than mineral N fertilization. Yield gains obtained from S fertilization ranged between 280 and 310 kg ha^{-1} (+7%) within a same NO rate and were caused by a higher number of both spikes per unit area and kernels per spike. Consistently with our findings, several studies reported a similar grain yield increase and suggested that S deficiency leads to a reduction of the number of spikelets or to an increase of floret mortality [28,39,59,60]. The synergistic effect of S with NO fertilization may be attributed to the higher rates of OM degradation achieved by the improved activity of the heterotrophic S-oxidizing microorganisms and the resulting release of other nutrients [61,62]. In fact, there is evidence which shows that organic amendments to the soil promote ES oxidation rates and that some specific rhizosphere (e.g., wheat and canola) may stimulate the proliferation of heterotrophic ES oxidizing microorganisms and arbuscular mycorrhyzal fungi [21,23,24,63–66]. Moreover, in a recent study, regression analysis showed that initial soil pH was the most important factor affecting ES oxidation, followed by OM content [67]. Specifically, in soils with pH above 6.65 and higher S and OM content, the ES oxidation rate was found to be significantly higher than that of the other soils. In our study, pH after fertilization treatments may have played an important role in the ES oxidation dynamic, considering that ammonium nitrate (used to fertilize the NM plots) has soil acidifying potential while organic fertilization often resulted in an increased soil pH [68].

Even though some authors found that S deficiency may have a significant effect on the synthesis and accumulation of proteins [29,69], generally, the S nutrition of wheat has a marked effect on the composition of the seed storage protein, rather than the concentration of total proteins in seeds [28,70]. Although we did not find any effect of S application to the soil on grain protein concentration and SDS test [15,71,72], it must be observed that foliar S application significantly increased both these traits. This effect could be due to a better assimilation of N and S, as previously reported by Tea et al. (2007) [30]. In that same study, the authors demonstrated that S applied by foliar spray was mainly assimilated in the grain and here it may favor N accumulation. Furthermore, many studies, as reviewed by Zhao et al. (1999) [28], demonstrated that the higher S accumulation in grain determines an increase in disulfide groups (polymeric glutenins), which are related to a higher gluten strength that means a higher SDS sedimentation value. Our results are consistent with those by Ercoli et al. (2011) [15], who showed that SDS sedimentation values and alveograph W were the quality indices highlighting the highest correlation with S concentration. The same authors argued that a high S concentration in grain is a key factor in obtaining a high quality pasta.

5. Conclusions

Our results evidenced the clear advantage of the mineral nitrogen fertilization when compared with the organic one in a relatively short time frame (two-year study). Nevertheless, for those cropping systems that base soil fertility on organic sources, soil S fertilization may be a winning strategy to improve grain yield in the Mediterranean environment. Even though future long-term studies should be conducted under strict organic conditions, also involving organic weed and disease control, results from our study may help to contain the large yield gap that farmers usually experience during the transition from a conventional system to an organic one or when they aim to improve the environmental sustainability of conventional farming systems. In general, we did not find any effect of soil S fertilization on the quality indices of durum wheat, whereas foliar S application proved to be a key factor in determining higher protein content and SDS value. Further studies are needed to verify if this finding results in improved rheological characteristics of grain. Concerning varieties, Iride and Dylan yielded similarly under both mineral and organic fertilization but Dylan had higher quality indices. However, all tested genotypes responded similarly to the year-on-year climatic fluctuations, which remain the most limiting factors for yield and quality performance of durum wheat in the Mediterranean region. Finally, from our results, a rate of 160 kg ha^{-1} of N (both mineral and organic) determined the optimal response in terms of both grain yield and quality.

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