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Title: Effect of nitrogen fertilization on yield and quality of durum wheat cultivated in Northern Italy and their interaction with different soils and growing seasons

Running title: *N fertilization and durum wheat yield and quality*

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Abstract

Durum wheat in temperate regions can lead to yields with higher technological quality and stability compared to Mediterranean areas. The aim of this study was to determine the effects of N fertilization on grain yield and quality, in different pedo-climatic conditions of temperate areas.

Field experiments were conducted in two sites (sandy-loam and silty-clay-loam) in Northern-West Italy for three years: five different N rates, two different types of fertilizers (ammonium nitrate, nutritional activator, slow-release fertilizer) were compared considering yield, protein content and yellowberry. N fertilizer rate had a more consistent effect on protein than yield; the response was affected by soil texture and rainfall during the growing season. The use of different types of fertilizer seem to play a minor role.

The management of N fertilization must consider mainly the effect on quality parameters of N rates and rainfall during the growing season and their interaction with soil texture.

Keywords: durum wheat, nitrogen fertilization, grain yield, grain protein, yellowberry

Introduction

The Mediterranean Basin and North America are the main durum wheat (*Triticum durum* Desf.) production areas in the world. In the European Union durum wheat is largely cultivated in Italy, (50 % of total EU production, with 1.6 milion of ha), where it is commonly used for human consumption: principally for pasta, but also for bread. In Italy, production is concentrated in southern and central areas, characterized by a warm winter and the frequent occurrence of drought, combined with heat stress during grain filling, which can lead to unsatisfactory grain yield and quality (Corbellini et al., 1997).

In recent years, improving durum wheat grain quality has become one of the main breeding and agronomic goals in many countries, due to the increase in market demand for good quality grains (De Vita et al., 2007). The grain protein content (GPC) has long been recognized as the most important factor affecting pasta making properties (Kovacs et al., 1997). Liu et al. (1996) recommended a protein concentration of 13%, because lower GPC generally resulted in a very poorly processed product. The other parameter which affects the quality of the end-product is yellowberry, expressed by the presence of farinaceous areas in a usually vitreous grain (Ammiraju et al., 2002). Poor nitrogen (N) availability is considered to be the most critical factor influencing the presence of yellowberry (Anderson, 1985; Bnejdi and Gazzah, 2008).

At present, in Italy a minimum of 13% for GPC and a maximum of 25% for yellowberry is the quality value for the value chain contract from grain to pasta (Ranieri and Bernardi, 2008). Grains in the Mediterranean are affected by severe and irregular water stress and high temperatures during grain filling, which results in high variability in terms of yield and grain quality (Dunkeloh and Jacobeit, 2003). As a result, there is considerable interest in durum wheat cultivation in more temperate regions, such as Northern Italy, where the pedo-climatic conditions could lead to a product with higher technological quality and yield stability (Rharrabti et al., 2003).

However, cooler and wetter weather conditions could result in an increase of fungal diseases (Pascale et al., 2002), particularly Septoria Tritici Blotch and Fusarium Head Blight, with the risk of deoxynivalenol (DON) contamination. Blandino et al. (2009) reported that a double fungicide treatment, is necessary to assure a higher yield and lower risk for DON contamination in durum wheat cultivated in temperate regions.

To develop durum wheat in new growing areas, characterized by more temperate conditions during the ripening stages, the agricultural techniques applied for this crop need to be reconsidered. Thus, as reported for fungal disease control, the other crop practices need to be updated in order to maximize grain yield of durum wheat, and, above all, in order to obtain kernels with high qualitative standard.

Among the main agricultural techniques N fertilization is a decisive factor to increase yield and improve the technological quality of cereals (Borghi et al., 1997; Lòpez-Bellido et al., 1998). However, the relationship between N concentration and GPC may depend on weather conditions (Troccoli et al., 1999).

Novaro et al. (1997), showed that the effect of environment, compared to genotype effect, is preponderant for grain yield, yellowberry and protein content. Moreover, the growing seasons with high rainfall could lead to N leaching in more sandy soil (Gasser et al. 2002). Thus, the optimum technical N rate in order to maximize yield and GPC of durum wheat needs to consideredN leaching in relationship of the growing season and the type of soil. For cereals, the stage of greatest sensitivity to N leaching is from the three leaf stage to the end of stem elongation (Lewan, 1994). Moreover, the risk of high yellowberry percentage could be more expressed in the cooler cropping areas, as a consequence of high N loss (Bnejdi and El Gazzah, 2008). Thus, the effect of N fertilization on this quality parameter in different growing conditions need to be verified and investigated.

The use of “special” fertilizer, such as slow-release fertilizer could be an alternative to reduce the N loss in the environment, since they minimize N leaching in sandy soils (Wang and Alva, 1996). Moreover, since, the nutritive element release is gradual during the growing season, the use of this fertilizers could simplify the operative management of N fertilization, reducing the number of field applications. Another strategy to improve the N fertilization of crop in terms of yield and quality, would be the employment of nutritional activator, which stimulates the activity of soil microorganisms responsible for the transformation of N into a form usable by the plant, resulting in higher fertilization efficiency and low volatilization losses (Giovannini et al., 2009).

Although the effect of these special fertilizers on environmental N pollution and losses are well documented, few studies have reported on grain yield and GPC as a result of their application on cereals compared to traditional fertilizers.

The objectives of this study were i) to determine the durum wheat response to N fertilization rate in term of grain yield and quality, in different pedo-climatic conditions of temperate areas, ii) to verify the possible productive and/or qualitative advantages related to the use of special, slow-release and nutritional activator, N fertilizers.

This information could be applied to implement growing tactics and set up the Good Agricultural Practices (GAP) that can drive the supply chain contracts for durum wheat cultivation in temperate environments such as Northern Italy.

Materials and methods

Experimental sites and treatments

The study was carried out in North-West Italy (Piedmont), during three growing seasons (2007-2008, 2008-2009 and 2009-2010). The experiment was performed in two sites (Table 1):

- sandy-loam (Sa-Lo) soil, Typic Hapludalfs (USDA classification), located in Cigliano, (province VC, 45° 18' N, 8° 01' E; altitude of 237 m.). The soil was characterized of low organic matter content, medium Cation Exchange Capacity (CEC), balance relation Carbonium/Nitrogen (C/N), normal mineralization velocity and sub-acid pH reaction;
- silty-clay-loam (Si-Cy-Lo) soil, Typic Udifluvents (USDA classification) located in Poirino (province TO, 44° 54' N, 7° 24' E; altitude 262 m.). The soil was characterized of medium organic matter content, medium CEC, high relation C/N, low mineralization velocity and sub-alkaline pH reaction.

The previous crop was maize for grain in every year and site.

In each locations and years, two experimental lines were performed: N fertilization rate, timing and splitting, using ammonium nitrate, and application of different N fertilization strategy based on different special N fertilizers.

In the first research line were compared: 5 increasing N rate (0, 80, 130, 170 and 210 kg N ha^{-1}) as ammonium nitrate split in the following proportions between tillering (growth stage, GS 25) (Zadoks et al., 1974), steam elongation (GS 35) and booting (GS 43): 0-0-0 (T1), 0-40-40 (T2), 50-40-40 (T3), 50-80-40 (T4), 50-120-40 (T5).

In the second research line, three types of N fertilizer were used at the same total N rate (170 kg N ha^{-1}). During the first 2 stages (GS 25, GS 35) of growth, each type of fertilizer based on established protocols with 130 kg N ha^{-1} total N application of each:

- ammonium nitrate fertilizer, split 50 kg N ha^{-1} at tillering (GS 25) and 80 kg N ha^{-1} at stem elongation (GS 35);
- nutritional activator (10 kg N ha^{-1}) added at stem elongation stage (GS 35) to an ammonium nitrate fertilization, split 50 kg N ha^{-1} at tillering (GS 25), 70 kg N ha^{-1} at stem elongation (GS 35);
- slow-release fertilizer (130 kg N ha^{-1}), applied at tillering (GS 25) and no fertilizer application at stem elongation (GS 35).

During booting (GS 45), 40 kg N ha^{-1} ammonium nitrate was applied to all plots.

The following products were used:

- ammonium nitrate fertilizer (27%, Yara S.p.A., Milan, Italy);
- nutritional activator: Rhizovit humistim® (3% N, Timac Agro S.p.A, Atessa, CH, Italy). This product is reported to have three effects: (i) it increase the microbial soil activity, since it reacts with free carboxylic acid of rhizosphere and specific activators of bacteria population; (ii) it improves the nutrient absorption of roots by increasing the prolamins concentration of roots; (iii) it increases the roots assimilation of N by managing urea nitrification and hydrolysis thus reducing N loss (Monticelli, 2008);
- slow-release fertilizer: Sulfammo 23 (23% N, Timac Agro S.p.A, Atessa, CH, Italy). The gradual release of nutrients is permitted by a double membrane calcium salt mechanism: the inner organic membrane isolate N particle and the outer calcium salt membrane excludes soil moisture (Stagnari and Pisante, 2012).

For both the research lines, the experimental design was a randomized complete block with four replicates. The plot size was 7 x 1.5 m. The wheat variety used each year was Saragolla (Produttori Sementi Bologna, Argelato, BO, Italy) a short cycle durum wheat of medium height, classified as medium quality.

The seed bed was set after ploughing (30 cm) and harrowing. The sowing season extended from the latest days of October to the beginning of November (27 October – 8 November) depending on weather conditions.

Planting was conducted in 12 cm wide rows at a seeding rate of 450 seeds m^{-2}. The experimental fields received 115 kg ha^{-1} of K_2O each year. The weed control was conducted with isoproturon and diflufenican at wheat tillering (GS 31).

All plots were treated with two application of fungicide: mixture of azoxystrobin and cyproconazole (Amistar Xtra®, Syngenta Crop Protection S.p.A., Milan, Italy) applied at 0.2 kg + 0.08 kg active ingredient (AI) ha^{-1} at steam elongation (GS 35) to control foliar disease and mixture of cyproconazole and procloraz (Tiptor Xcell®, Syngenta Crop Protection S.p.A., Milan, Italy) applied at 0.02 kg + 0.17 kg AI ha^{-1} at heading (GS 55) to avoid Fusarium Head Blight infection and protect the flag leaf. The fungicides were applied with a three nozzle precision sprayer (T-Jet 110/04) using a fine mist at a slow walk to ensure effective coverage. The delivery pressure at the nozzle was 324 KPa.

Grain yields were obtained by harvesting with a Walter Wintersteiger cereal plot combine-harvester. Harvesting was conducted at the beginning of July (2 - 11 July). A subsample was taken from each plot to determine the grain moisture. The grain yield results were adjusted to a 120 g kg^{-1} moisture content.

The harvested grains were accurately mixed, and 2 kg grain samples were taken from each plot to analyse for GPC and yellowberry.

Flag leaf greenness

A chlorophyll meter, Hydro N-Tester® (HNT) (Hydro-Agri, now Yara), was used to measure the relative flag leaf greenness for the different N fertilizer application during ripening. HNT is a hand-held instrument that measures the light transmitted by a plant leaf at two different wavelengths (650 and 960 nm) (Arregui et al., 2006). The ratio of the light transmitted at these wavelengths, in addition to the ratio determined with no sample, is processed by the instrument to produce a digital reading. The HNT values are numerical, dimensionless values that are proportional to the amount of total chlorophyll present in the leaf. Readings were taken using the HNT at midlength of the flag leaf from 30 randomly selected plants per plot.

Since the longevity and photosynthesis activity of the flag leaf is closely correlated with accumulation of protein in the grain (Pepler et al., 2005) the HNT measurements were carried out at the following growth stages: heading (GS 59), early dough (GS 83) and hard dough (GS 87). This last stage was used to distinguish between plots in which the flag leaves were still photosynthezing and those in which N translocation to grains was almost complete.

Grain quality analysis

Grain samples from each plot were milled into white flour using a Brabender Quadrumat Junior Mill (Brabender, Duisberg, Germany), fitted with a 200 μm aperture sieve. GPC was determined by near-infrared reflectance spectroscopy, using a NIRSystems 6500 monochromator instrument (Foss-NIRSystems, Silver Spring, MD, USA) and presented on a dry matter basis.

The percentage of yellowberry was determinate by taking a reading of 4 slabs of 40 grains, cut with a Pohl grain cutter.

Statistical analysis

For both the experiments, normal distribution and homogeneity of variances were verified by performing the Kolmogorov–Smirnov normality test, and the Levene test, respectively.

For the first research study, related to N rate and timing, an analysis of variance (ANOVA) was utilized to compare the HNT readings, grain yield, GPC and percentage of yellowberry, keeping separate each growing season and sites, and using a completely randomized block design, in which the N treatment was the independent variable.

For the second research study, related to the type of N fertilizer, ANOVA was performed on the previous reported parameters, using a completely randomized block design, in which the type of N fertilizer was the independent variable and the combination of growing season and site was the random variable.

Multiple comparison tests were performed according to the SNK test on treatment means. The statistical package SPSS for Windows, Version 18.0 (SPSS Inc., Chicago) was used for the statistical analysis.

Results

Weather conditions

Monthly rainfall and growing degree days (GDD) observed at the two experimental sites over the three growing seasons, are reported in Table 2. The three growing seasons differ mainly in the total rainfall that occurred in the period between the durum wheat sowing (November) and flowering (May). The 2008-2009 growing season experienced the highest total rainfall (> 700 mm in the 2 sites), followed by 2009-2010 and finally 2007-2008 (< 500 mm). For further considerations to N fertilization effect on grain yield and quality, the three growing seasons have been reported as high (2008-2009), medium (2009-2010) and low rainfall season (2007-2008). The GDD varied between years, with the greatest number in 2008-2009, followed by 2007-2008 and the least in 2009-2010.

Total rainfall and GDD differed also between sites with the highest level of rainfall and GDD consistently observed in Cigliano (Sa-Lo) for all the three years.

Flag leaf greenness

The HNT reading clearly described the greenness status of the flag leaves during different GSs (Figure 1), which was primarily influenced by the pedo-climatic conditions across the compared experiments.

For both sites, the HNT values of the unfertilized control (T1) at heading stage (GS 58) was highest during low rainfall years. This indicates a higher natural N occurrence in the soil during these years and confirming a probable lower N leaching during the growing season.

Moreover, in each growing season and at each site, the HNT flag leaf values were lower for unfertilized control (T1) compared all other fertilized plots. The greatest difference in unfertilized control and the fertilized treatments was observed in growing seasons with high total rainfall and in the site with the lightest soil.

Overall, HNT values increased proportionally with the increase of N rate, although in all the experiments there were slight differences between treatments T3, T4 and T5 for HNT values from heading stage (GS 58) to the end of dough stage (GS87) . Only in the experiment conducted in the Sa-Lo soil in the growing season with high rainfall it is possible to observe at GS87 a clear lower HNT value for treatment T3 (130 kg N ha^{-1}) compared to T4 (170 kg N ha^{-1}) and T5 (210 kg N ha^{-1}).

In each site and growing season, the evolution of HNT values during the ripening stage was very similar for the different fertilization strategies, based on traditional or special fertilizers (data not shown).

Grain yield and quality

ANOVA showed a significant effect of N fertilization on grain yield rate in each experiment (Table 3), but the productive response of durum wheat to N rate was different in the different growing season and site ($P<0.05$). In both sites the grain yield of unfertilized control (T1) increased when rainfall decreased that occurred during growing season, confirming a probable higher natural N occurrence.

The grain yield increased significantly with rising N rate of 80 kg N ha^{-1} (T2) compared to the untreated control (T1) in all the experiments conducted in the Sa-Lo soil, while in the Si-Cy-Lo soil only in the growing season with high rainfall did we observe significant difference between these treatments. The overall trend is an increase in yield between T1 and T2 with increased rainfall; the highest in the wettest growing season (+85%), compared to the medium (+61%) and driest (+25%).

In all the experiments, with the exception of Sa-Lo soil in the high rainfall growing season and Si-Cy-Lo soil in the low rainfall growing season, a significant further increase in grain yield was observed for the application of 130 kg N ha^{-1} (T3) compared to treatment T2. Otherwise, only in the high rainfall season in the Sa-Lo soil, did the yield responded significantly to N rates up to 170 kg N ha^{-1} (T4) compared to the treatment T3. The increase of N rate (T5) did not lead to a significant increase in the grain yield at any site or year.

Across all experimental treatments, an increase N fertilization rate increased GPC significantly ($P<0.01$), however, the difference between the compared N fertilizer rate result to be higher moving from the Si-Cy-Lo to the Sa-Lo soil and from the growing season with low rainfall to that with high rainfall (Table 4).

When comparing GPC from unfertilized control (T1) and the fertilization with 80 kg N ha^{-1} (T2), the significant change was found in all conditions except in Sa-Lo soil in the wettest growing season. In the Sa-Lo soil, a significant further increase in GPC was observed, with the fertilization of 130 kg N ha^{-1} (T3), compared to the treatment T2 in the medium and low rainfall growing seasons. No significant difference between T3 and T2 have been observed in the Si-Cy-Lo soil. The higher N rate application of 170 kg N ha^{-1} (T4), compared to the 130 kg N ha^{-1} (T3) resulted in a significant increase in GPC in all conditions except Si-Cy-Lo soil in the low rainfall growing season. In the Sa-Lo soil, this increase was 6, 9 and 8% in the growing season with high, medium and low rainfall, respectively; while in the Si-Cy-Lo soil the increase in the high and medium rainfall season was 4%. A significant advantage of the highest N rate of 210 kg N ha^{-1} (T5), was only observed in the Sa-Lo soil during a high rainfall growing season. Under these conditions there was a significantly increase in the GPC of 6% compared to the N rate application of 170 kg N ha^{-1} (T4).

In all the experiments conducted in the Sa-Lo soil, ANOVA showed a significant effect of N fertilizer rate on yellowberry percentage in kernels ($P<0.05$), while no significant difference was observed in any experiment conducted in Si-Cy-Lo soil (Table 5). In the Sa-Lo soil, the control without N fertilization (T1) always resulted in the highest percentage of kernels with yellowberry. In all growing seasons, fertilization with 80 kg N ha^{-1} (T2), significantly decreased the yellowberry percentage compared to T1. As far as the Sa-Lo soil is concerned, with the exception of the driest growing season, a significant further decrease in yellowberry percentage was observed with the N rate application of 130 kg N ha^{-1} (T3) compared to T2.

ANOVA did not show a significant effect of the fertilization with nutritional activator or slow-release fertilizer compared to ammonium nitrate on grain yield, GPC and percentage of yellowberry (Table 6). The interactions between the independent variable (N fertilizer) and the random factor (trial) were never significant.

Discussion

The data from this research highlights that the cultivation of durum wheat in environment such as the Northern Italy can lead to considerably high grain quality, in terms of GPC and low yellowberry percentage. The observed GPC under ordinary N fertilization rates (from 130 to 210 kg N ha^{-1}) averaged 1.5 g kg^{-1}, with the highest value (1.7 g kg^{-1}), observed in the low rainfall growing season and with the highest N fertilization rate. These data confirm that GPC is influenced by soil fertility, cooler climatic conditions and rainfall during grain filling which delay crop ripening, that positively influence this qualitative parameter in temperate areas (Rharrabti et al., 2003). Belocchi et al. (2012) and Codianni et al. (2012), report a mean of quinquennial results (2007-2011) of 4.07 t ha^{-1} and 12.7 % in yield and protein, respectively, for Southern and Central Italy and 5.47 t ha^{-1} and 13.8%, respectively for Northern Italy.

Moreover, the response of grain yield and quality parameters to N rate varied depending on different soils texture and the total rainfall occurring during the growing season. The slight grain yield response to N rates higher than 130 kg N ha^{-1} was similar to that reported by Hogg and Ackerman (1998) for durum wheat in the irrigated areas of Canada. Lòpez-Bellido et al. 1996, in Andalusia region (Spain), reported that the wheat yield was influenced by the N fertilizer rate only in the years with a total amount of rainfall which exceeded 450 mm during the growing season. With the exception of Sa-Lo soil in the wettest season, where the N leaching could be more intense, the application of the N rates of 170 and 210 kg N ha^{-1} did not increase grain yields compared to 130 kg N ha^{-1}. On the other hand, the effect of N fertilization rate on GPC in the compared different pedo-climatic conditions, was always higher than the effect on grain yield.

Thus, N fertilizer rate had a more consistent effect on durum wheat quality than on grain yield. These results are consistent with experiments conducted by Garrido-Lestache et al. (2004), in irrigated Typic Xerofluvent soils of Catalonia region (Spain), which showed that GPC of durum wheat increased with a fertilization of 170 kg N ha^{-1} compared to 100 kg N ha^{-1}, even if no grain yield increase was observed.

In all growing seasons, the Sa-Lo soil resulted in a higher response of GPC to N fertilization compared to Si-Cy-Lo soil, since it is characterized by a lower C.E.C and organic matter content. In the wettest growing season at the Sa-Lo soil, GPC was not significantly different in N fertilization rate lower than 170 kg N ha^{-1}. In agreement with the findings reported by a study in southern Spain by Garrido-Lestache et al. (2004), GPC increased with the application of 210 kg N ha^{-1}.

Yellowberry in durum wheat was reduced by increasing the N fertilization rate in the Sa-Lo soil, only. Gianibelli et al. (1991) reported that N application decreased the percentage of yellowberry in durum from 80% to 7%, depending on the rate and growth stage of application.

As far as the type of fertilizer and the adopted N fertilization strategy is concerned, the application of slow release fertilizer at tillering (GS 25) or nutritional activator at stem elongation (GS 35) instead of only ammonium nitrate fertilization, did not lead to any advantage in terms of yield or grain quality, confirming experiments conducted on soft wheat in Alberta region (Canada) by McKenzie et al. (2007) and in Spain on durum wheat by Diez et al. (1997). On the other hand, Cowling et al. (2001), showed that the application of N slow-release fertilizer, instead of urea significantly increased the N fertilizer use efficiency and the GPC of durum wheat. Kochba et al. (1990) and Shoji and Gandeza (1992) showed that N release from these type of fertilizers strongly depends on soil temperature and moisture content. In agreement with McKenzie et al. (2007), the use of slow-release fertilizer at tillering could be adopted to simplify the agronomical management of crop, with only one fertilizer application, although it would be necessary to take in consideration the higher cost of this fertilizer compared to the traditional ones. As far as the addition of nutritional activator to traditional N fertilizer is concerned, a study carried by Antisani et al. (1996) demonstrated that the effectiveness of the activator was strongly influenced by soil characteristics.

In conclusion, the cultivation of durum wheat, in temperate areas such as Northern Italy, clearly allows for higher technological quality. The pasta industry is interested to mix lots with these high GPC with those produced in Mediterranean areas, characterized by a general lower protein and variable technological quality, in order to increase the overall quality of their product.

The diffusion of durum wheat in temperate regions first requires the development of varieties with higher resistance to winter stress and diseases. Moreover, since the cultivation of durum wheat in these environment requires adequate N fertilization in order to assure the assimilation of nutrients for protein accumulation in the kernels, it is necessary to optimize the fertilization tactics to both maximize grain yield and quality. Thus, the N fertilization strategy should not be based solely on grain yield, but should take into account quality parameters which are more strongly influenced by N fertilization. Under the experimental conditions of our study, the application of 170 kg N ha^{-1} resulted in the best result for the qualitative purpose (GPC), while the optimum technical dose for grain yield purpose was 130 kg N ha^{-1}. Moreover, the management of N fertilization needs to take into account also the rainfall during the growing season and its interaction with soil texture for the risk of N leaching, while the application of different N fertilization strategies based on different type of fertilizer seem to play a minor role.

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- **TABLE 1**

- Main physical and chemical characteristics of the top soil layer (0–30 cm) of the two experimental sites.

Parameters	Cigliano (VC)	Poirino (TO)	Measure unit
Sand (2mm-50 μm)	54.3	15.2	%
Silt (50-2 μm)	35.8	53.0	%
Clay (<2 μm)	9.9	31.8	%
pH	6.6	7.5	
Total carbonate	(*)	1.4	%
Organic matter	1.3	1.8	%
Organic carbon	0.8	1.0	%
Total nitrogen	0.078	0.068	%
C/N	10.8	11.8	
Cation Exchange Capacity (C.E.C.)	10.8	14.6	meq/100g
Exchangeable Potassium	71	66	ppm
Phosphates	20	20	ppm

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- Soil was sampled from 0-30 cm using Eijkelkamp cylindrical augers.

- (*) total carbonate is not determine for value of pH lower 7.

- **TABLE 2**

- Monthly rainfall and growing degree days (GDD 0s) from the sowing (November) to the flowering (May) in 2008-2010 period in the research sites.

Site	Month	2008/2009		2009/2010		2008/2009	
		Rainfall (mm)	GDD 0s[a] (°C d^{-1})	Rainfall (mm)	GDD 0s[a] (°C d^{-1})	Rainfall (mm)	GDD 0s[a] (°C d^{-1})
Sa-Lo Cigliano	November	199	238	103	236	85	222
	December	250	126	53	105	2	152
	Janury	46	88	47	58	77	141
	Febrary	74	155	96	115	23	177
	March	113	292	67	245	10	307
	April	253	420	56	398	133	357
	May	91	599	185	510	167	539
	November - May	1026	1916	607	1667	496	1894
Si-Cy-Lo Poirino	November	160	225	79	223	63	206
	December	188	112	35	92	1	124
	Janury	50	62	56	39	71	127
	Febrary	37	151	79	96	16	163
	March	85	286	58	228	7	281
	April	218	379	45	381	129	320
	May	26	584	108	487	101	501
	November - May	764	1798	460	1545	388	1721

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- [a] Accumulated growing degree days for each month using a 0°C base.

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- **TABLE 3**

- Effect of N fertilization rate with ammonium nitrate on durum wheat grain yield (t ha^{-1}).

Soils[a]	Treatments[b]	Rainfall of growing season[c]		
		high	medium	low
Sa-Lo	T1	1.5 c	3.3 c	3.4 c
	T2	3.0 b	5.4 b	4.3 b
	T3	3.6 b	6.4 a	5.2 a
	T4	4.9 a	6.5 a	5.4 a
	T5	5.1 a	6.9 a	5.3 a
	P (F)	<0.001	<0.001	0.014
	sem[d]	0.26	0.29	0.61
Si-Cy-Lo	T1	1.2 c	1.0 b	3.3 b
	T2	2.1 b	1.6 b	4.1 ab
	T3	3.1 a	3.0 a	4.0 ab
	T4	3.5 a	3.9 a	4.0 ab
	T5	3.6 a	3.6 a	4.2 a
	P (F)	<0.001	0.039	0.041
	sem[d]	0.26	0.31	0.28

- Means followed by different letters are significantly different (the level of significance is shown in the table). Reported values are based on 4 replications.
- [a] soils: Sa-Lo = sandy-loam soil (Cigliano); Si-Cy-Lo = silty-clay-loam soil (Poirino); see table 1.
- [b] treatments: 0-0-0 (T1), 0-40-40 (T2), 50-40-40 (T3), 50-80-40 (T4), 50-120-40 (T5) kg N ha^{-1} as ammonium nitrate
- [c] season rainfall: see table 2.
- [d] sem: standard error of mean.

- **TABLE 4**

- Effect of N fertilization rate with ammonium nitrate on durum wheat GPC (%).

Soils[a]	Treatments[b]	Rainfall of growing season[c]		
		high	**medium**	**low**
Sa-Lo	T1	13.2 c	10.2 d	11.9 d
	T2	13.2 c	12.7 c	13.7 c
	T3	13.1 c	13.3 b	14.6 b
	T4	13.9 b	14.5 a	15.7 a
	T5	14.7 a	14.9 a	15.9 a
	P (F)	<0.001	<0.001	0.005
	sem[d]	0.16	0.28	0.02
Si-Cy-Lo	T1	14.20 c	11.1 c	15.0 c
	T2	14.40 b	12.7 b	15.8 b
	T3	14.40 b	12.8 b	16.8 ab
	T4	15.00 a	13.3 a	16.8 ab
	T5	15.00 a	13.8 a	17.1 a
	P (F)	<0.001	<0.001	<0.001
	sem[d]	0.14	0.57	0.38

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- Means followed by different letters are significantly different (the level of significance is shown in the table). Reported values are based on 4 replications.
- [a] soils: Sa-Lo = sandy-loam soil (Cigliano); Si-Cy-Lo = silty-clay-loam soil (Poirino); see table 1.
- [b] treatments: 0-0-0 (T1), 0-40-40 (T2), 50-40-40 (T3), 50-80-40 (T4), 50-120-40 (T5) kg N ha^{-1} as ammonium nitrate
- [c] season rainfall: see table 2.
- [d] sem: standard error of mean.

- **TABLE 5**

- Effect of N fertilization rate with ammonium nitrate on durum wheat yellowberry (% of kernels).

Soils[a]	Treatments[b]	Rainfall of growing season[c]		
		high	medium	low
Sa-Lo	T1	40 a	25 a	16 a
	T2	26 b	13 b	6 b
	T3	20 c	6 c	4 b
	T4	18 c	6 c	4 b
	T5	10 c	5 c	3 b
	P (F)	0.036	0.039	<0.001
	sem[d]	0.08	0.06	0.19
Si-Cy-Lo	T1	28 a	25 a	20 a
	T2	24 a	23 a	16 a
	T3	24 a	13 a	13 a
	T4	20 a	14 a	16 a
	T5	21 a	11 a	20 a
	P (F)	0.542	0.134	0.120
	sem[d]	0.16	0.22	0.17

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- Means followed by different letters are significantly different (the level of significance is shown in the table). Reported values are based on 4 replications.
- [a] soils: Sa-Lo = sandy-loam soil (Cigliano); Si-Cy-Lo = silty-clay-loam soil (Poirino); see table 1.
- [b] treatments: 0-0-0 (T1), 0-40-40 (T2), 50-40-40 (T3), 50-80-40 (T4), 50-120-40 (T5) kg N ha^{-1} as ammonium nitrate
- [c] season rainfall: see table 2.
- [d] sem: standard error of mean.

- **TABLE 6**

- Effect of the type of fertilizer on grain yield, GPC and yellowberry of durum wheat.

Factor	Source of variation	Yield t ha^{-1}	GPC %	Yellowberry %
Trial[a]	Site Sa-Lo, 2009	4.4	13.6	20
	Site Sa-Lo, 2010	6.6	14.3	5
	Site Si-Cy-Lo, 2008	4.0	16.8	15
	Site Si-Cy-Lo, 2009	3.4	15.0	24
	Site Si-Cy-Lo, 2010	3.7	13.2	15
Type of fertilizer[b]	ammonium nitrate	4.7 a	14.9 a	13 a
	nutritional activator	4.6 a	14.6 a	14 a
	slow-release	4.4 a	14.5 a	15 a
	P (F)	0.353	0.170	0.556
	sem[c]	0.4	0.4	2.5
Fertilizer X trial	*P* (F)	0.179	0.616	0.719

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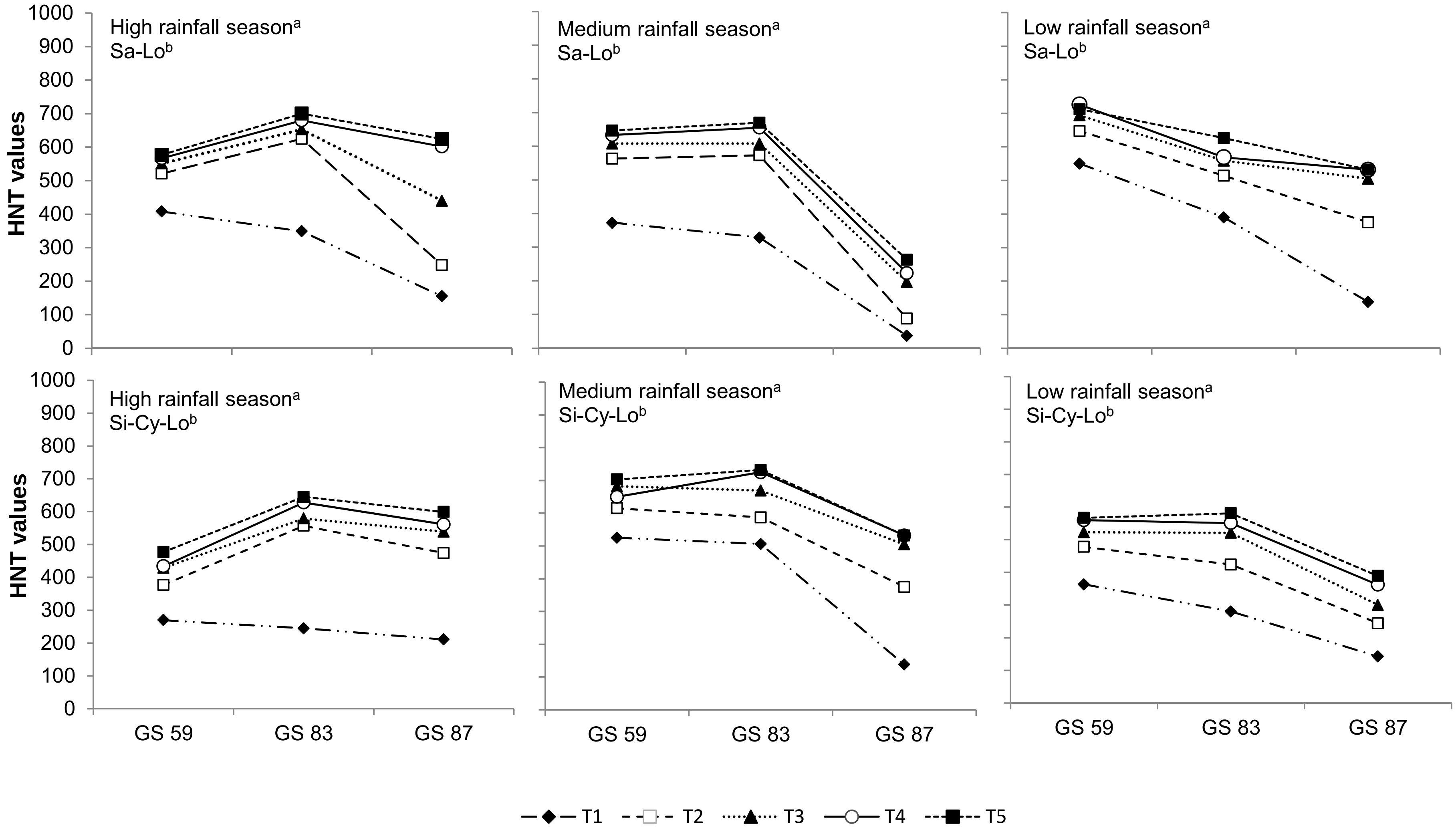
- Means followed by different letters are significantly different (the level of significance is shown in the table). Reported values for each type of fertilizer are based on 4 replications X 6 trials.
- [a] soils: Sa-Lo = sandy-loam soil (Cigliano); Si-Cy-Lo = silty-clay-loam soil (Poirino); see table 1. Year with different rainfall during the growing season; see table 2.
- [b] type of fertilizer: ammonium nitrate, splits 50-80-40 at GS 25, 35 and 45, respectively; nutritional activator (10 kg N ha^{-1}) at GS 35 and ammonium nitrate splits 50-70-40 at GS 25, 35 and 45, respectively; slow-release fertilizer (130 kg N ha^{-1}) at GS 25 and ammonium nitrate splits 0-0-40 at GS 25, 35 and 45. All the compared N fertilization strategy supplies a total N rate of 170 kg N ha^{-1}.
- [c] sem: standard error of mean.



[a] season rainfall: see table 2.

[b] Sa-Lo = sandy-loam soil (Cigliano); Si-Cy-Lo = silty-clay-loam soil (Poirino); see table 1.

[c] Growth stage (Zadocks et al., 1974)

FIGURE 1

Effect of N fertilization rate with ammonium nitrate on flag leaf greenness (HNT) of durum wheat in different field experiments.