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YIELD, NITROGEN USE EFFICIENCY AND GRAIN QUALITY IN DURUM WHEAT AS AFFECTED BY NITROGEN FERTILIZATION UNDER A MEDITERRANEAN ENVIRONMENT

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SUMMARY

Limited information is available concerning the influence of nitrogen fertilization jointly on yield response, nitrogen use efficiency (NUE) and grain quality of durum wheat under semi-arid Mediterranean conditions. The study focused on evaluating, through a systematic study, over three seasons in southern Italy the effects of three nitrogen fertilization rates (0, 80 and 160 kg N ha^{-1} – N_0, N_{80} and N_{160}), on grain yield, yield components, nitrogen efficiency indices and grain quality characteristics of three durum wheat genotypes ('Creso', 'Trinakria' and 'Line 25') from different breeding eras to achieve a more sustainable fertilization management of the durum wheat crop. We found that nitrogen fertilization at 80 kg N ha^{-1} was able to maximize the yield performances (2.1 t ha^{-1} $year^{-1}$) of the crop and keep NUE index at an acceptable level (16.3 kg kg^{-1}). On the other hand, nitrogen fertilization at 160 kg N ha^{-1} improved grain quality measured through protein (up to 14.3%) and dry gluten concentration (up to 12.8%), but had a detrimental effect on grain yield and nitrogen efficiency. Among the genotypes studied, 'Trinakria' showed the greatest potential to utilize nitrogen fertilization to improve grain yield and NUE (at N_{80}) and quality (at N_{160}), 'Line 25' made good use of N_{80} both for yield and quality, whereas 'Creso' proved wholly unresponsive to nitrogen. The effect of N fertilization on grain yield and N use efficiency depends on rainfall distribution, giving the best results when about 80% of total rainfall occurred from sowing to heading. Overall, our data show that in seasons with regular rainfall in quantity and distribution, combining no more than 80 kg ha^{-1} of nitrogen fertilization with genotypes characterized by a more efficient response to nitrogen, is a useful tool to improve the agronomic and quality performance of the crop, ensuring, at the same time, a more environment-friendly nitrogen fertilization.

INTRODUCTION

Durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.] is grown on over 3.2 million ha in the European Union (EU), providing an annual output of around 5.7 million tonnes (European Commission, 2010). The durum crop area is largely concentrated in the Mediterranean countries (Italy, France, Greece and Spain), which together account for 98% of total EU production, and for about 55% of the world production. Italy is the main European producer with about 5 million tonnes per year

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(European Commission, 2010). Durum wheat provides the raw material for the pasta industry, one of Italy's most renowned products, and an increasingly greater amount is also required for the production of speciality breads and couscous (Nachit, 1998).

The Mediterranean climate is characterized by hot, dry summers and mild winters; rainfall, which is concentrated in the winter months, is extremely variable from year to year and within the year and can range from 50 to 1200 mm year^{-1}. Most cropland, represented mainly by dryland cereals, not only durum wheat, is however in the 200–600 mm year^{-1} range (Kassam, 1981). In this area, durum wheat is typically sown in late autumn or early winter and harvested in late spring–early summer. Thus, it is generally assumed that the crop is grown when rainfall and temperature are favourable until anthesis. During the grain filling period, limited rainfall and high temperatures are nonetheless frequent. This affects increasing soil water deficit, the variable presence of which may cause large fluctuations not only in grain yield but also in grain protein content and composition, with considerable effects on the rheological properties of dough (Ercoli *et al.*, 2008). With few exceptions, the soils of the Mediterranean region are low in organic matter and consequently in total nitrogen reserves, thus setting limitations on growing crops without nitrogen fertilizer or biological nitrogen fixation through legumes (Ryan *et al.*, 2009). In the Mediterranean areas, indeed, nitrogen deficiency is the major constraint in dryland cereal farming after drought (Campbell *et al.*, 1993).

Nitrogen is the major nutrient influencing wheat grain yield (Ehdaie and Waines, 2001) and is a decisive factor in improving grain quality indices mainly by its effect on grain protein concentration and composition (Gooding and Davies, 1997). Crop responses to nitrogen fertilization depend heavily on soil water availability. There are interactive effects of water deficit and additional nitrogen fertilizers in crop production (Anderson, 1985). It is widely accepted that the early developmental processes in durum wheat, such as tiller proliferation, occurring during early growth, depend on the availability of water and nitrogen in the soil (Simane *et al.*, 1993). Moreover, combined water and nitrogen shortages around anthesis are known to induce flower abortion resulting in reduced grain number per unit land area (Jeuffroy and Bouchard, 1999). A number of works in the literature refer to the relationship between irrigation regimes and nitrogen supply on durum wheat production (Albrizio *et al.*, 2010, Cossani *et al.*, 2012; Karam *et al.*, 2009). Under rainfed conditions of a Mediterranean environment, crop responses to nitrogen fertilizer depend on rainfall amount and distribution during the crop cycle, to the amount and timing of nitrogen applications as well as initial soil nitrogen levels (Abad *et al.*, 2004; Basso *et al.*, 2010; Cossani *et al.*, 2009; Lopez-Bellido *et al.*, 2012). In years with inadequate rainfall, much of the fertilizer nitrogen not absorbed by the crop may remain in the soil as available nitrate to subsequent crops; in years with normal rainfall (generally concentrated in autumn–winter), nitrates may be leached because of their mobility in the soil–plant–atmosphere system, with important consequences on energy-production costs and on the potential contribution to environmental pollution (Ercoli *et al.*, 2013; Pampana *et al* 2013). Late and split nitrogen applications are agronomical strategies to reduce N leaching and improve N utilization, more so through promoting build-up of grain protein content (Ercoli *et al.*,

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2011; Fuertes-Mendizábal et al 2010; Garrido-Lestache *et al.*, 2005; Lopez–Bellido *et al.*, 2005) than by an increase in grain yield (Debaeke *et al.*, 1996; Sowers *et al.*, 1994). Little information on the influence of either early or no split nitrogen application jointly on grain yield, nitrogen efficiency and grain quality of durum wheat under rainfed Mediterranean conditions is available. Furthermore, few attempts (Cossani *et al.*, 2012; Giambalvo *et al.*, 2010; Halitligil *et al.*, 2000; Lopez-Bellido *et al.*, 2006) have focused to date on defining nitrogen fertilizer efficiency in durum wheat.

The aim of the present study was to evaluate through a systematic study the effect of nitrogen fertilizer rate (an unfertilized control, a dose usually used in the semi-arid Mediterranean area matching the uptake (Todari, 1989) and a double dose) on grain yield characteristics, on NUE and grain quality in three genotypes to achieve a more sustainable fertilization management of durum wheat in a Mediterranean environment. As responses to nitrogen vary with weather conditions, tests were undertaken in three consecutive years.

MATERIALS AND METHODS

Experimental site

Field experiments were conducted over three growing seasons, 2004–2005, 2005–2006 and 2006–2007 (hereafter season I, season II and season III, respectively) in Sicily, southern Italy (Enna-Barrafranca 37° 26′ N, 14° 14′ E, 600 m a.s.l.), an area in the interior Sicilian uplands where durum wheat cultivation is common. The local climate is semi-arid Mediterranean with mild wet winters and hot, rainless summers. The deep soil was a Typic Xerorthents (USDA, Soil Taxonomy, 1999). A layer, 0.30 m thick (from −0.05 to −0.35 m) was considered for the soil analysis. Analysis made at the beginning of the experiment indicated the following characteristics: 29% clay, 22% silt, 49% sand, 1.12 ‰ total nitrogen, 59.2 kg ha^{-1} available P_2O_5 and 384 kg ha^{-1} exchangeable K_2O. The soil pH was 8.5. All soil analyses were performed according to procedures approved by the Italian Society of Soil Science (Società Italiana Scienza del Suolo, 1985).

Experimental design and crop management

In each season, a split-plot design with four replications was adopted, involving three durum wheat genotypes ('Creso', 'Trinakria' and 'Line 25') as the main plots and three N fertilization rates (0, 80 and 160 kg ha^{-1} of nitrogen, hereafter referred to as N_0, N_{80}, N_{160}, respectively) as sub-plots. The three genotypes were chosen to represent the high genotypic variability within durum wheat. 'Creso' is a durum wheat genotype that has been widely cultivated in Central Italy for the past 30 years and is still in use. It is characterized by *Rht genes* that cause a drastic reduction in plant stature and hence an increase in the harvest index (Motzo *et al.*, 2004), and by moderate but constant yield and medium grain quality. 'Trinakria' is a genotype that has been widely cultivated in Sicily for the past 30 years; it is characterized by moderate productivity, good adaptability to environments characterized by scarce water and nutrient resources and good pasta-making quality. 'Line 25' is a genotype

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developed by University of Catania, within a breeding program starting from Trinakria × Valnova; it is characterized by high yield and grain quality, above all for its high protein content and good quality protein.

Seeds were sown at a density of 400 viable seeds m^{-2} on 23 January 2005, 31 December 2005 and 28 December 2006, using a 12-row planter. The size of each plot was 4 m by 6 m and consisted of 20 rows, each 6 m long, spaced at 0.20 m. Tillage consisted of a 40 cm depth ploughing followed by harrowing in October. The experiment was in a field where the previous crop was faba bean in seasons I and III and vetch-oat lawn in season II. Fertilization with P was done before sowing, incorporating into the top 40 cm soil layer 120 kg ha^{-1} P_2O_5 as triple superphosphate (46% P_2O_5). Soil available K was adequate to crop requirements and so K fertilizers were not supplied. N was applied in the plots, two-thirds at sowing as urea (46% N) and the remaining one-third at full tillering (stage 25; Zadoks *et al.*, 1974) as ammonium nitrate (27% N). Both urea and ammonium nitrate were incorporated into the soil. Top dressing N fertilization was done on 4 April, 23 March and 21 March, in seasons I, II and III, respectively. In each season, weeding was performed with glyphosate before sowing and MCPA at first detectable node (stage 31; Zadoks *et al.*, 1974). No pesticides were used to control plant pests. Plants from ten adjacent rows of 1 m length corresponding to a 2.0 m^2 area per plot were harvested at full ripeness (stage 92 Zadoks *et al.*, 1974) during the last ten days June in the three seasons. Plants were harvested with a plot combine and were separated into culms, leaves, chaff and grain.

Pheno-morphological and agronomical traits

Heading and full ripeness stages were recorded, respectively, when about half the plants showed emerging spikes (stage 50; Zadoks *et al.*, 1974) and very hard kernels and completely dry plants (stage 92; Zadoks *et al.*, 1974). Plant height (excluding spike) was measured during the intermediate milk maturation (stage 75; Zadoks *et al.*, 1974). Grain yield was determined on 11.2 m^2 per plot (4 central metres of 14 middle rows) to avoid border effects and was expressed at 13% moisture level. The grain moisture level was measured in a 300 g sample from each plot in a thermo-ventilated oven at 60°C until constant weight was reached. Yield components were assessed for each plot: number of spikes per square meter, number of kernels $spike^{-1}$, thousand-kernel weight (1000-kernels weight). The latter was calculated as the mean weight of three sets of 100 kernels from each plot.

Grain quality parameters

Test weight was measured on a 250 g sample with a Schopper chondrometer equipped with a 1 l container (TR Turoni, Italy); results are reported as kg hl^{-1} without reference to the moisture content. Vitreous kernel count was determined by inspecting three samples of 100 kernels sliced with a Pohl grain cutter. Shrivelled kernel was determined by counting the not-fully filled kernels on three samples of 100 kernels.

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A sample of grains was milled to flour using a laboratory mill A 11 Basic Analytical Mill (Seneco, Milan, Italy). Protein content, dry gluten content and Sodium Dodecyl Sulfate (SDS) volume sedimentation test (SDS test) were determined on flour. Protein content was calculated after multiplying Kijeldhal nitrogen determinations by 5.7. Dry gluten concentration was determined using the Glutomatic System (Perten Instruments, Huddinge, Sweden), following the standard method ICC no 137 (ICC, 1986). The SDS test (height of sediment in ml) was performed as an indicator of gluten strength according to Dick and Quick (1983).

Nitrogen efficiency indices

The efficiency of N was calculated in terms of NUE and agronomic nitrogen efficiency (N_{AE}). NUE was calculated as the ratio of grain yield per unit of available N in the soil. Available nitrogen was the sum of mineral N (NO_3-N and NH_4-N) in the 0–0.40 m soil layer at sowing, plus the N released through mineralization during the growing season, plus the N supplied through fertilization (Moll *et al.*, 1982). The initial mineral nitrogen content of the soil profile (0–0.40 m) was set at about 1% of total N, determined by the *Kjeldahl* method. N soil mineralization per month was calculated according to Gariglio *et al.* (2000), from total N content corrected by an N mineralization factor as a function of soil temperature. On the basis of this procedure, the quantity of available mineral nitrogen in the soil for the crop cycle was equal to 29.8 kg ha^{-1} for season I, 26.2 kg ha^{-1} for season II and 31.0 kg ha^{-1} for season III. The N_{AE} represents the incremental efficiency of applied N as compared to unfertilized test. It was calculated according to the following equation: $N_{AE} = (Y_N – Y_0)/N_F$ (Novoa and Loomis, 1981), where Y_N represents grain yield of N fertilized plot (expressed as kg ha^{-1}), Y_0 is grain yield of the N unfertilized plot (control) and N_F represents the amount of N applied (expressed as kg ha^{-1} in fertilized plots).

Statistical analysis

Data collected were first submitted to Bartlett's test to check the homoscedasticity, then analysed through a three-way (nitrogen rate × genotype × growing season) factorial analysis of variance (ANOVA). Provided that the *F*- test was significant, means were separated on the basis of the least significant difference (LSD) test. All calculations were performed using CoStat version 6.003 (Cohort Software, Monterey, Ca, USA).

Weather conditions

Weather conditions (maximum and minimum air temperature, relative humidity, rainfall) were monitored during the experiments by a meteorological station (Mod. Multirecorder 2.40; ETG, Firenze, Italy) sited at the experimental field. Table 1 shows the time trend of mean maximum and mean minimum temperatures and the accumulated rainfall recorded during whole-wheat cycle split into the main growth periods in the three seasons of the experiment and over the long-term 1971/2000.

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Table 1. Mean maximum and minimum air temperatures and total rainfall for season I, II and III and long term 1971–2000 average during the whole wheat cycle and main growth periods. Growth stages are defined following Zadok's scale (00 sowing, 10 emergence, 15 fifth leaf unfolded stage, 30 pseudo stem erection, 50 heading, 92 full ripeness).

		Stage					
Meteorological variable	Season/long term	00–10	10–15	15–30	30–50	50–90	Whole cycle
Max temp. (°C)	I	12.3	11.9	17.1	18.6	19.7	17.2
	II	13.3	12.0	15.4	12.1	21.9	15.7
	III	10.0	11.2	12.6	16.0	23.1	15.9
	1971–2000	8.4	8.8	10.5	13.8	22.0	14.6
Min temp. (°C)	I	5.2	4.7	7.0	7.0	9.1	7.4
	II	4.7	5.3	6.5	2.2	8.1	5.6
	III	2.9	3.5	3.8	5.0	10.2	5.8
	1971–2000	3.2	3.3	4.2	6.7	12.4	6.0
Rainfall (mm)	I	11	4	26	40	118	199
	II	22	86	14	22	31	175
	III	0	100	26	53	28	207
	1971–2000	30	53	56	38	31	208

For the long-term, the periods in which the main phenophases normally occur in the area of the experiment were considered (Tuttobene *et al.*, 1985).

Mean maximum and minimum temperatures during the whole cycle from sowing to full ripeness (from 0– to 92, Zadoks *et al.*, 1974) were higher in season I than in season II (+1.5°C and +1.8 °C, respectively), than in season III (+1.3°C and +1.6 °C) and than the long-term 1971/2000 (+2.6°C and +1.4 °C). Differences were particularly evident from the fifth leaf unfolded stage to heading (from 15 to 50, Zadoks *et al.*, 1974). Total rainfall during the whole cycle of each crop ranged between 175 (season II) and 207 mm (season III) and was similar to the 30 year (1971–2000) mean rainfall (208 mm) for the area (Table 1). Rainfall distribution varied considerably among seasons; from sowing to the fifth leaf unfolded stage (from 0 to 15, Zadoks *et al.*, 1974), it was very low (15 mm) in season I, but higher in season II and III (about 100 mm) accounting for about 50% of total rainfall. From the fifth leaf unfolded stage to heading (from 15 to 50, Zadoks *et al.*, 1974) rainfall was higher in seasons I (66 mm) and III (79 mm) than in season II (36 mm). From heading to full ripeness (from 50 to 92, Zadoks *et al.*, 1974), it was about 120 mm in season I, accounting for more than 50% of total rainfall and only about 30 mm in seasons II and III.

The weather trends during the three seasons resulted in accordance with those reported by Aschman (1973) as typical of the Mediterranean climate.

RESULTS

Pheno-morphological traits

The measured pheno-morphological traits were influenced by the main factors (nitrogen rate, genotype and season), but not by their interactions. The application of either N_{80} or N_{160}, compared to the control (N_0), resulted in slight, but significantly

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Table 2. Pheno-morphological traits of durum wheat as affected by nitrogen rate, genotype and season.

Treatment	Sowing-heading interval (d)	Heading-ripeness interval (d)	Plant height (cm)
Nitrogen rate			
N_0	110 b	57 a	70 b
N_{80}	112 a	55 b	73 a
N_{160}	113 a	54 b	73 a
p	< 0.001	< 0.001	< 0.001
Genotype			
Creso	116 a	70 a	68 b
Trinakria	109 c	57 b	88 a
Linea 25	110 b	57 b	59 c
p	< 0.001	< 0.001	< 0.001
Season			
I	80 c	59 a	61 c
II	125 b	57 a	83 a
III	130 a	50 b	72 b
P	< 0.001	< 0.001	< 0.001

Different letters within main factors indicate significance at LSD test ($p \leq 0.001$).

so, longer time intervals between sowing and heading (113 vs. 110 days) as well as a shorter duration between heading to ripeness (54 vs. 57 days) (Table 2). Creso showed both a significantly longer time between sowing to heading and heading to ripeness (116 and 70 days, respectively) compared to Trinakria and Linea 25 (on average 110 and 57 days, respectively). In season I, plants showed the shortest time from sowing to heading and the longest time between heading and ripeness; whereas in season III, plants showed the longest time sowing to heading and the shortest time heading to ripeness; in season II, plants showed intermediate values.

Plant height increased significantly with nitrogen application whether N_{80} or N_{160} as compared to the control (73 vs. 70 cm). The tallest cultivar was Trinakria, the shortest Linea 25; Creso was intermediate. Plants, on average, were tallest in season II, and shortest in season I (Table 2).

Grain yield and components

The significant 'Nitrogen rate × genotype' and 'Nitrogen rate × season' interactions showed how nitrogen effects on grain yield were genotype and season dependent. Grain yield, passing from N_0 to N_{80}, increased to a greater and more significant degree in Trinakria (+19%) than in Line 25 (7%) and Creso (5%), while following a further nitrogen increase, from N_{80} to N_{160}, yield decreased more for Trinakria (−13%) than Line 25 (−5%) and Creso (−3%) (Table 3). Without nitrogen fertilization (N_0), Creso and Linea 25 gave a 21% higher grain yield (about 2 t ha^{-1}) compared to Trinakria. In seasons II and III, nitrogen fertilization, especially at rate N_{80}, significantly increased the grain yield. By contrast, in season I, nitrogen especially at rate N_{160}, showed a depressive effect on grain yield (Table 3).

The positive effects of nitrogen rate (N_{80}) on grain yield were due mainly to a higher number of spikes m^{-2} in Creso and Linea 25 and to a higher number of

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Table 3. Yield components of durum wheat as affected by the interactions 'nitrogen rate × genotype' and 'nitrogen rate × season'.

	Nitrogen rate											
	N_0	N_{80}	N_{160}	N_0	N_{80}	N_{160}	N_0	N_{80}	N_{160}	N_0	N_{80}	N_{160}
	Grain yield (t ha^{-1})			Spikes m^{-2} (n)			1000-kernel weight (g)			Kernels spike^{-1} (n)		
Genotype												
Creso	2.0	2.1	2.0	235	258	227	43.8	40.1	40.2	19.8	20.5	22.5
Trinakria	1.7	2.0	1.9	210	213	249	41.3	42.1	42.0	19.5	22.7	17.9
Linea 25	2.1	2.2	2.2	237	268	254	40.8	39.6	39.8	21.4	20.8	21.7
P		<0.001			<0.001			<0.001			<0.001	
LSD interaction ($p < 0.001$)	0.14			15.0			1.67			2.9		
Season												
I	1.2	1.1	1.0	199	175	165	36.7	36.4	36.4	17.1	17.4	17.3
II	2.2	2.7	2.7	234	237	265	45.9	46.3	48.0	20.5	24.3	21.5
III	2.4	2.5	2.3	251	326	300	42.4	39.1	37.6	22.2	19.8	20.8
P		< 0.001			< 0.001			< 0.001			< 0.001	
LSD interaction ($p < 0.001$)	0.1			15.0			1.7			2.9		

Table 4. Nitrogen efficiency indices of durum wheat as affected by the interactions 'nitrogen rate × genotype' and 'nitrogen rate × season'.

	Nitrogen rate					
	N_0	N_{80}	N_{160}	N_0	N_{80}	N_{160}
	NUE (kg kg^{-1})			NAE (kg kg^{-1})		
Genotype						
Creso	40.8	16.3	9.8	–	0.97	0.06
Trinakria	34.0	15.6	8.9	–	4.14	1.09
Linea 25	41.4	17.0	10.4	–	1.69	0.77
p		< 0.001			< 0.001	
LSD interaction ($p < 0.001$)		3.1			1.25	
Season						
I	24.8	8.5	5.0	–	− 1.05	− 1.27
II	44.2	20.5	13.0	–	5.81	3.30
III	47.2	19.4	11.1	–	2.05	− 0.10
p		< 0.001			< 0.001	
LSD interaction ($p < 0.001$)		3.1			1.25	

kernels spike^{-1} in Trinakria (Table 4). For this latter genotype, the higher rate (N_{160}) led to a marked and significant decrease in the number of kernels spike^{-1}. The better yield performance of nitrogen fertilization in season II was brought about largely by the number spikes m^{-2} and 1000-kernel weight (N_{160}) and number of kernels spike^{-1} (N_{80}) (Table 3). In season III, only supply of 80 kg ha^{-1} of nitrogen increased the number spikes m^{-2}.

Nitrogen efficiency

The negative effect of high nitrogen rate on NUE was marked. It, significantly decreased overall as the N supply increased from N_0 to N_{160}. The decrease was more evident in both Creso and Linea 25 than in Trinakria (Table 4). Nevertheless, in the absence of nitrogen fertilization (N_0), Trinakria showed a lower NUE (34 kg kg^{-1}) than Creso and Linea 25 (on average about 41 kg kg^{-1}) (Table 4). As the N supply increased from N_{80} to N_{160}, the N_{AE}, significantly decreased, but this decrease was higher in Creso (−94%) than in Trinakria (−74%) and the decrease in the latter was greater than in Line 25 (−54%) The low nitrogen rate N_{80} significantly improved N_{AE} especially in Trinakria (4.14 kg kg^{-1}). The values of the N_{AE} showed that N_{AE} was higher in season II than in season III, whereas NUE values were similar in seasons II and III. Further, regardless of nitrogen rate, NUE in both season II and III was about double that of season I (on average about 46 vs. 25 kg kg^{-1}) (Table 4).

Grain quality

Some quality variables such as test weight, vitreous kernel, shriveled kernel and SDS test were almost always influenced by the main factors (nitrogen rate, genotype and season), but not by their interactions. Regardless of the other factors, N_{80} or N_{160} irrespectively compared to N_0, determined a slight but significant decrease in test weight (78 vs. 79 kg hl^{-1}) and a significant increase in the percentage of vitreous

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Table 5. Test weight, vitreous kernels, shriveled kernels and SDS test sedimentation of durum wheat as affected by nitrogen rate, genotype and season.

Treatment	Test weight (kg h l^{-1})	Vitreous kernels (%)	Shriveled kernels (%)	SDS test (ml)
Nitrogen rate				
N_0	79.0 a	94.1 b	20.1 b	51.5 a
N_{80}	78.1 b	96.2 a	21.6 a	52.5 a
N_{160}	77.8 b	96.3 a	20.5 b	52.3 a
p	< 0.001	< 0.001	< 0.001	< 0.02
Genotype				
Creso	79.0 a	95.2 c	20.4 b	53.7 a
Trinakria	77.6 c	95.6 b	20.3 b	53.8 a
Linea 25	78.3 b	95.8 a	21.6 a	47.5 b
p	< 0.001	< 0.001	< 0.001	< 0.001
Season				
I	75.6 c	98.5 a	26.1 a	54.9 a
II	81.3 a	94.6 b	16.5 c	54.7 a
III	78.0 b	93.5 c	19.7 b	45.3 b
p	< 0.001	< 0.001	< 0.001	< 0.001

Different letters within main factors indicate significance at LSD test ($p < 0.001$).

Table 6. Grain protein and dry gluten content of durum wheat as affected by the interactions 'nitrogen rate × genotype' and 'nitrogen rate × season'.

	Nitrogen rate			Nitrogen rate		
	N_0	N_{80}	N_{160}	N_0	N_{80}	N_{160}
	Protein (%)			Dry gluten (%)		
Genotype						
Creso	12.4	13.2	13.2	11.2	11.8	11.8
Trinakria	12.9	13.4	14.8	11.6	12.3	12.8
Linea 25	12.9	14.6	15.0	11.8	13.4	13.8
P		< 0.001			< 0.001	
LSD interaction ($p < 0.001$)		1.10			0.47	
Season						
I	13.3	14.1	14.7	13.6	14.2	14.9
II	10.8	11.7	11.9	10.0	10.7	11.2
III	14.0	15.3	16.6	11.3	11.7	13.0
p		< 0.001			< 0.001	
LSD interaction ($p < 0.001$)		1.10			0.47	

kernels (94 vs. 96%). Compared to Creso and Trinakria, Line 25 showed the highest percentage of vitreous and shriveled kernel and the lowest SDS test, whereas Creso showed the highest test weight and the lowest percentage of vitreous kernel. With respect to seasons, season I led to the lowest test weight and the highest percentage vitreous kernel and shriveled kernel compared to seasons II and III (Table 5).

The significant 'Nitrogen rate × genotype' and 'Nitrogen rate × season' interactions showed how nitrogen effects on protein and gluten content were genotype- and season-dependent. Nitrogen fertilization up to N_{160} increased the protein and dry gluten content, but more markedly in Line 25 and Trinakria than in Creso (Table 6). For

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both protein and dry gluten content, the increase in response to N fertilization up to N_{160} was more evident in season III than in seasons I and II (Table 6).

DISCUSSION

Nitrogen fertilization has proved an effective means of improving grain yield and quality of durum wheat in the Mediterranean environment. Grain yield from 1.9 t ha^{-1} in the unfertilized plot (N_0) increased up to 2.1 t ha^{-1} in response to 80 kg N ha^{-1} applied; a similar response of grain yield to N fertilization has been reported by other authors in rainfed conditions of a Mediterranean environment (Caliandro *et al.*, 1981; Ercoli *et al.*, 2011; Garrido-Lestache *et al.*, 2005). Increasing nitrogen rate resulted in a decrease in N_{AE} and NUE. Moreover, we found lower values of NUE at both the two studied N rates, compared to values with similar doses of N and in similar environments by Cossani *et al.* (2012), Lopez-Bellido *et al.* (2005 and 2006). This was likely due to a low soil-water availability in our experiment, rainfall in the three seasons being about 180 mm in comparison to about 280 mm in Cossani *et al.* (2012), and 300 and 550 mm in Lopez-Bellido *et al.* (2006).

The response of grain yield and N use efficiency to N fertilizer rate was genotype-dependent. Both Creso and Linea 25, without supplemental nitrogen fertilization, showed better performances than Trinakria in terms of grain yield (about 2.0 vs. 1.7 t ha^{-1}) and NUE (about 40 vs. 34 kg kg^{-1}). However, Creso and Trinakria seem to have the same capacity to take up nitrogen from the soil; indeed at N80 and N160, they had similar yields and NUE, in agreement with Motzo *et al.* (2004), who found that among cultivars belonging to intermediate (Trinakria) and modern (Creso) breeding eras the total nitrogen uptake is not significantly different.

A major response to N fertilization was noted in the lower yielding genotype 'Trinakria', instead, which proved more able to maximize N_{AE} especially at N_{80}. N_{AE} represents the incremental efficiency of applied N as compared to the unfertilized test and is indicative of the cost-benefit ratio from investment in N inputs (Ierna *et al.*, 2012). The positive effects of N fertilization on grain yield were mainly due to a higher number of spikes m^{-2} in Creso and Linea 25 in agreement with Albrizio *et al.* (2010) and Caliandro *et al.* (1981) and to a higher number of kernels $spike^{-1}$ in Trinakria in agreement with Karam *et al.* (2009).

'Trinakria' was the only variety with higher yields in season III than in season II, likely due to the greater rainfall from the fifth leaf unfolded stage to heading in the former (79 vs. 36 mm), which allowed N to act upon the main components of the yield (number of spikes m^{-2} and grain with high 1000-kernel weight) (data not shown). Indeed, in tests made over several years in the same environment characterized by varying rainfall, 'Trinakria' responded to nitrogen fertilization (N80) only in those years in which the aridity index measuring rainfall and mean temperatures were higher and the rainfall was concentrated in the period before heading (Lombardo and Tuttobene, 2000).

The response of grain yield and N use efficiency to N fertilizer rate was season-dependent, concurring with the weather conditions of each season, given that the

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available N in the soil in the three different years was similar, namely 29.8, 26.2 and 31.0 kg ha^{-1} for the three seasons. In season I, owing to both higher temperatures and irregular rainfall which was absent or nearly so until heading and mainly concentrated from heading to full ripeness, nitrogen fertilization did not have any effect on grain yield. In addition, in that season sowing date was delayed by about one month, compared to seasons II and III, and this meant a reduction in the growth cycle (140 vs. 180 days). Therefore, in rainfed conditions in a Mediterranean environment, nitrogen fertilization should be commensurate to the sowing date and rainfall distribution. When sowing date is late and water is the limiting factor for plant growth, it is not appropriate to supply N fertilizers because root growth is restricted and fertilizer is not effectively dissolved in the soil water, so applied N will not be absorbed efficiently and may, if unused by the crop, be damaging to the environment.

In seasons II and III, nitrogen fertilization, especially at rate N_{80}, significantly increased grain yield due to ideal weather conditions (optimal temperature and well-distributed rainfall) throughout the cycle. In particular, rainfall was high (about 100 mm) in the two seasons, accounting for about 50% of total rainfall. It was intermediate accounting for about 30% of total rainfall from the fifth leaf unfolded stage to heading and was low accounting for about 16% of total rainfall from heading to full ripeness. This rainfall distribution enabled a positive impact of the applied N, expressed in season II with an increase in the number of kernels $spike^{-1}$ (N_{80}) and number of spikes m^{-2} and 1000-kernel weight (N_{160}) and in season III, with an increase in the number of spikes m^{-2} (N_{80}). Nitrogen fertilization showed greater efficiency in season II than in season III, as the higher values of N_{AE} demonstrate. This may be attributed to the negative effect of higher rainfall from the fifth leaf unfolded stage to heading, equal to 38% (season III) compared to 20% (season II), which probably stimulated a tiller proliferation (Simane *et al.*, 1993), leading to a higher number of spikes m^{-2} but not accompanied by an adequate number of kernels $spike^{-1}$ and 1000-kernel weight. Nitrogen application at N_{80} or N_{160} reduced test weight compared to N_o in agreement with Garrido-Lestache *et al.* (2005), though it was always above the minimum acceptable level (76 kg $h\ l^{-1}$) and increased vitreous kernel percentage, in accordance with Abad *et al.* (2004), Anderson (1985), Ryan *et al* (1997). The percentage of vitreous kernels by far exceeded the quality standards for most commercial agreements, which is 80% (Mahaut, 1997). Vitreous kernels together with grain protein content, are recognized as being influenced mainly by N application (Oweis *et al.*, 1999), but also by cultivar, due to different uses of available soil N and by season, due to variability in weather trends. The SDS test, an indicator of gluten strength, was not influenced by the nitrogen fertilization, but by genotype as observed previously (Boggini *et al.*, 1997). Other authors found only little variation of SDS with N fertilization (Lerner *et al.*, 2006).

Nitrogen fertilization had a positive effect on both grain protein and dry gluten content, increasing on average by 14.3 and 12.8% respectively in response to 160 kg N ha^{-1} applied. Grain protein and dry gluten response to N fertilization was similar to that reported by other authors in rainfed Mediterranean environment conditions (Garrido-Lestache *et al.*, 2005). They found a satisfactory yield response up to 100

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kg N ha^{-1}, but higher N input was necessary to achieve good quality standards. We also found a different pattern of response of grain yield and grain protein content to N fertilization: up to 80 kg N ha^{-1} both grain yield and grain protein increased in parallel, but from 80 to 160 kg N ha^{-1} grain protein content continues to increase, while grain yield decreased. This differential response of grain yield and grain protein content to fertilizer N may be attributed to competition between vegetative and reproductive growth, and also to the shortage of water and to the typically high temperatures of Mediterranean areas during the grain filling stage, which can reduce grain carbohydrate accumulation rather than N accumulation (Gooding and Davies, 1997).

The response of grain protein and dry gluten content to N fertilization rate was genotype- and season-dependent.

In unfertilized control, compared to Trinakria, Creso showed a lower content of protein (12.4 vs. 12.9%) and consequently of gluten (11.2 vs. 11.7%), in agreement with Motzo *et al.* (2004) and Fois *et al* (2011) that report a clear trend of decreasing average protein content from old to modern cultivars, presumably owing to the dilution effect caused by their heavier grains. Both Linea 25 and Trinakria were able to exploit N fertilization to improve grain quality: the former up to N_{80}, the second up to N_{160}, whereas Creso did not usefully use nitrogen fertilization in this study, to improve both grain yield and quality. This knowledge on the differential response of genotypes to use N fertilizers and available soil N is crucial for choosing genotypes in relation to various agricultural systems: with zero, low or high N fertilizer input and with the priority towards very high quality grain. The choice therefore falls on the modern Creso when it is preferred not to use N fertilization and the quality is not a priority; vice versa, Trinakria and Linea 25 are better choices when N fertilization up to 160 kg ha of N can be applied and the aim is high grain quality.

Grain protein and dry gluten content respond to progressively increasing N rate from N_0 to N_{160} in the three growing seasons, consistently with findings of Ercoli *et al.* (2011), Garrido-Lestache *et al.* (2005) and Labuschagne *et al.* (2006), but with a greater increase from N_0 to N_{80} than from N_{80} to $N_{160.}$ Grain protein and dry gluten content are important quality parameters for durum wheat, as the former is widely used as a criterion in determining premium prices and the latter as an indicator of gluten strength for pasta quality. According to UNI EN ISO 9001 quality management system, a threshold of 11.5% for protein content as the lower limit for class 3 grain is indicated and a threshold of 12% for dry gluten to ensure acceptable pasta quality (Landi, 1995). In this experiment, N fertilization at N_{80} would be necessary to accomplish the limit for protein content in the season II and to accomplish the limit for dry gluten in the season III, thus proving an important strategy to improve the grain quality of durum wheat.

Apart from the dose of nitrogen, the timing is also important to optimize wheat quality under rainfed Mediterranean conditions, given that it is recognized that application between tillering and stem elongation is more efficacious (Ercoli *et al.*, 2011; Fuertes-Mendizàbal *et al.*, 2010). What is vital is not to apply the nitrogen later than stem elongation and to avoid the typical water stress period in spring in Mediterranean

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conditions (Lopez-Bellido *et al.*, 2005). With split N fertilizer applications, smaller N rates than usual can be used (Fuertes-Mendizàbal *et al.*, 2010), resulting in better translocation of pre-anthesis assimilates to the grain and in decreasing N losses from the soil–plant system, which may have important consequences for energy-production costs and on the potential reduction of environmental pollution.

Given the high variability in weather conditions, especially with rainfall in the Mediterranean environment, and the differential response of genotypes to nitrogen fertilization and weather conditions, further research will be necessary in our environment to evaluate the effect of timing, splitting and rate of N, including less than 80 kg ha^{-1} which is the normal dose used.

CONCLUSION

Nitrogen fertilization has proved to be effective in improving grain yield and quality of durum wheat. In comparison to unfertilized plots, N_{80} was able to maximize the yield performances of the crop up to 2.1 t ha^{-1}, keeping the NUE index at an acceptable level (16.3 kg kg^{-1}); whereas N_{160} further improved grain quality measured through grain protein (up to 14.3%) and dry gluten content (up to 12.8%), but had a detrimental effect on grain yield and nitrogen efficiency. Genotypes responded differently to N fertilization in our experiment. Creso proved wholly unresponsive to nitrogen both for grain yield and quality, Trinakria showed the greatest potential to use nitrogen fertilizer both in terms of grain yield and NUE at 80 kg ha^{-1} of N and grain quality at 160 kg ha^{-1} of nitrogen, while 'Line 25' used the 80 kg ha^{-1} well, for yield but above all for the much improved quality. This knowledge on the ability of the different cultivars to use N could be used to choose the cultivar in relation to the agricultural systems: with zero, low or high N fertilizer input and with the priority towards very high quality grain.

However, the effect of N fertilization on grain yield and N use efficiency depended on weather conditions during the crop cycle, in particular rainfall distribution. The best responses in terms of yield and efficiency of use was when about 80% of total rainfall occurred from sowing to heading and no effect instead when rainfall was very low in this first period and abundant from heading to full ripeness.

Overall, in seasons with regular rainfall in quantity and distribution, our data show that combining no more than 80 kg ha^{-1} of nitrogen fertilization with genotypes characterized by a more efficient response to nitrogen fertilization, is a useful tool to improve the agronomic and qualitative performances of the crop, ensuring a more environment-friendly nitrogen fertilization at the same time.

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