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Yield and nitrogen use efficiency as influenced by rates of nitrogen fertilizers of some Tunisian durum wheat cultivars

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Abstract. Nitrogen use efficiency of durum wheat grain (*Triticum turgidum* ssp. *durum*), as valuable indicator for rational N fertilization supply, was investigated in a field experiment in the subhumid area of Tunisia with four durum wheat genotypes: two improved genotypes and two landraces, grown under three mineral N fertilization treatments (0,75 and 150 kg N ha^{-1}) during two cropping seasons (2008/09, 2009/10). Total N content in grain, yield, N uptake by grain, and soil N (N-NH_4, N-NO_3) status were examined to indicate relations between N trial treatments and growing years. Analysis of variance indicated that significant interaction was noted for nitrogen x years and genotypes. Over total investigated period, above-mentioned factors significantly differ per nitrogen levels and years. The highest total N content in durum wheat grain was recorded in N150 treatment during 2009/10, but the highest yield was reached in 2008/09 growing season under 150 kg N ha^{-1}. Drought and elevated temperatures prevailing during 2009/10 cropping season were associated with lower yielding ability of most genotypes along with higher NUE per fertilization treatment, compared to 2008/09. NUE values varied from 7.85% in 2008/09 up to 24% in 2009/10 for the 150 kg N ha^{-1} treatment. During investigated growing years NUE was increased with increasing nitrogen fertilization levels.

Keywords. Nitrogen use efficiency – Fertilizer rates – Grain yield – Durum wheat.

Production et efficacité d'utilisation de l'azote influencées par le taux d'engrais azotés de certains cultivars de blé dur tunisien

Résumé. *L'efficacité d'utilisation de l'azote (EUA) des grains de blé dur (Triticum turgidum ssp. durum), comme indicateur significatif pour un apport raisonné de cet élément, a été étudiée à travers un essai en plein champ dans la zone subhumide de la Tunisie avec quatre génotypes de blé dur : deux génotypes améliorés et deux variétés locales, cultivés sous trois traitements de fertilisation azotée minérale (0,75 et 150 kg N ha^{-1}) au cours de deux saisons de culture (2008/09, 2009/10). La teneur en N total dans le grain, le rendement, l'absorption de N par le grain et la réserve azotée du sol (N-NH_4, N-NO_3) ont été examinés pour déterminer les relations entre les traitements azotés et les années de croissance. L'analyse de la variance a révélé une interaction significative entre l'azote et les années et les génotypes. Pour toute la période examinée, les facteurs évoqués diffèrent de manière significative en fonction des niveaux d'azote et des années. La teneur en N total la plus élevée dans le grain de blé dur a été enregistrée dans le traitement N150 pendant 2009/10, mais le rendement le plus important a été observé dans la saison de croissance 2008/09 avec 150 kg N ha^{-1}. La sécheresse et les températures élevées qui ont caractérisé la saison de culture 2009/10 ont été associées à une plus faible capacité de rendement de la plupart des génotypes avec une EUA plus élevée par traitement de fertilisation, par rapport à 2008/09. Les valeurs d'EUA variaient de 7,85% en 2008/09 à 24% en 2009/10 pour le traitement 150 kg N ha^{-1}. Au cours des années de croissance étudiées, on a observé un accroissement de l'EUA suite à l'augmentation des niveaux de fertilisation azotée.*

Mots-clés. *Efficacité d'utilisation de l'azote – Doses d'engrais – Rendement en grain – Blé dur.*

I – Introduction

Durum wheat is the main staple food for Mediterranean populations (Arregui and Quemada, 2008) and the major cultivated cereal in Tunisia (Latiri *et al*., 2010). N fertilizer use is one of the most important agronomic practices in cereals, particularly when crop rotations are lacking (Crews and Peoples, 2004).

Nitrogen use efficiency (NUE) is perceived as a valuable indicator for rational N supply of mineral nitrogen fertilizer in durum wheat which depends on nitrogen status in soil and plant.

In crop production, nitrogen application is a common practice to improve yield and grain quality. In cereals, nitrogen is applied at sowing. However, applied rates of nitrogen are usually split in two applications: at sowing and prior to anthesis to increase protein concentration (Austin *et al.*, 1977; Palta *et al.*, 1994; Fangmeier *et al.*, 1999). High nitrogen use efficiency (NUE) is a required trait in cereals, particularly when nitrogen application is made in advanced vegetative growth stage (at flowering) as compared to early application (Wuest and Cassman, 1992; Raun and Johnson, 1999; Cassman and Walters, 2002). The late application of nitrogen could alter protein composition and nitrogen accumulation in the spike (Johansson and Svensson, 2004).

Estimated NUE is within 33% in developed countries, whereas it is at lower rate in developing countries (Raun and Johnson, 1999). Field experiments indicated that no more than 45-70% of the applied N fertilizers are recovered under average growing conditions (King *et al*., 2001; Noulas *et al*., 2004). NUE is a complex trait and it is associated with N uptake efficiency (UPE) and N utilization efficiency (UTE). Moll *et al.* (1982) and Ortiz-Monasterio *et al.* (1997) noted that UPE reflects the efficiency of the crop in obtaining N from the soil, while UTE reflects the efficiency of nitrogen use of a plant in the translocation process contributing to grain yield.

Nitrogen use efficiency is also driven by genotype effects (Le Gouis *et al*., 2000) and influenced by growing conditions (Bertic *et al*., 2007). The NUE genotypic variation of durum wheat (Giambalvo *et al*., 2010) was attributed to high N uptake and/or high N use efficiency (Dawson *et al*., 2008). Selecting for high NUE genotypes would lead to the reduction of N applications and then a low environmental contamination risk (Giambalvo *et al*., 2010).

With regard to management practices, the choice of plant genotype is particularly important. In fact, several studies have shown that many crop species have genetic variability for NUE (Fageria *et al*., 2009) and that the use of the best-adapted genotype can contribute to improved efficiency in how cereal crops acquire and use soil N or fertilizer N. Foulkes et al. (1998) found that modern wheat genotypes were less efficient at recovering soil N than older genotypes, which suggests that old genotypes may be the best choice for low input and organic growing systems. In contrast, other researchers (Le Gouis *et al*., 2000; Brancourt-Hulmel *et al*., 2003; Guarda *et al*., 2004) have found that NUpE and NUE have increased with the introduction of improved genotypes, and that modern genotypes give the best result seven under limited N availability. Sylvester-Bradley and Kindred (2009) state that wheat breeding has greatly increased grain yield associated with an increase in optimum N rate. Besides, the integration of agro-physiology and molecular N pathway traits to screen wheat genotypes seem to be useful to optimize yields and NUE (Vinod, 2007). The objective of this study was to determine how different mineral nitrogen rates affect total N content in grain, yield, N uptake by grain, and grain N use efficiency during two growth cycles.

II – Material and methods

1. Description of the field experiment site

The field experiment was conducted at the experimental station of the School of Higher Education in Agriculture of Mateur (latitude: 37.04 m, longitude: 9.66 m, altitude: 51 m), located in the subhumid area of Tunisia, during two growing seasons 2008/2009 and 2009/2010. Moderate annual rainfall across the years and distribution during the cropping season were the main characteristics of this site. The area received an annual rainfall of 253 mm during the cropping season (November-June, 2009) which was lower than the mean annual rainfall of the next year

2010. Mean maximum and minimum temperatures recorded at the station during the season (November-June, 2010) were 24 and 10.76°C, respectively (Table1).

Table 1. Agro-meteorological parameters of the experimental site.

Parameter	Year	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Mai	Jun.	Season
Mean air temperature, °C										
	2009	18.3	11	9.5	9.7	11.3	15.6	17.5	19	63.1
	2010	16.5	13	10.8	12.3	13.9	14.1	24.0	24	128.6
Rainfall, mm										
	2009	58.0	55.6	141.8	99.4	80.4	190.5	2.8	1.6	252.9
	2010	68.7	53	80.5	71.5	90.4	35.7	4.8	1	405.6

2. Experimental design and treatments

The experiment was laid out in a randomized complete block design (RCBD) with three replications. A control with no N fertilizer and three nitrogen levels were applied (75 and 150 kg N ha[1]). Nitrogen was applied as ammonium nitrate (33.5% N) divided into three applications at different growth stages: early tillering Zadoks 13 (30%), elongation Z16 (40%), and 2nd node Z32 (30%). Four *Triticum turgidum* ssp. *durum*) cultivars were included in this study with two landraces (Bidi AP4 and Azizi AC2) and two high-yielding cultivars (Khiar and Om Rabia). Date of sowing was on November and December 26th, 2008 and 2009, respectively, at a rate of 300 seeds m^{-2}. Plots were 3 m long with six rows, spaced 20 cm apart. At harvest, a 0.5 m^2 portion at the center of each plot was sampled.

3. Soil sampling and analysis

The soil of the experiments has a silt clay loam texture with a low content of organic matter (3%). The pH (H_2O) of the field was 6.7 in 2009 and 8.5 in 2010. The relevant soil characteristics at the study site are presented in Table 2. In the two years, soil samples were taken on all plots prior to sowing and after harvesting, at a depth of 90 cm. All samples were analyzed for nitrate and ammonium content according to the Devarda‟s Alloy reagent method (Sims *et al*., 1995) and ammonium was measured using the distillation–titration proceeding method (Rhine *et al*., 1998).

Table 2. Soil properties at three depths in the experimental field prior to sowing.

Soil property %	0-10 cm	10-30 cm	≥ 30 cm
Clay	22.5	21.6	18.5
Silt	57.3	57.3	52
Sand	17.3	18.3	16.1
Limestone	20.1	20.9	19.9
Mineral calcite	10.1	9.8	10.1
Organic matter	1.90	2.08	1.83
Total N	0.20	0.21	0.21
pH	8.3	8.4	8.5

4. Straw nitrogen and grain protein analysis

Straw and grain N concentration was determined using the Kjeldahl procedure and N concentration was determined using the method outlined by Cataldo *et al*. (1974). At maturity, samples of non-grain above-ground plant parts (stems, leaves and chaff) were obtained from the central unit areas of 0.5 m^2. Plant samples were oven-dried at 80 °C and the dry weight measured. The samples were ground by a rotor mill and a sample of 200 mg was used for the digestion with sulfuric acid (H_2SO_4).

5. Data measurements

Grain Yield (kg ha^{-1}): Obtained from the harvested central unit areas of 0.5 m^2. Samples were cleaned following harvesting and weighed using an electronic balance.

Nitrogen uptake (kg N ha^{-1}): Nitrogen contained in the grain was calculated as grain yield*grain protein/5.7.

Total Nitrogen uptake (kg N ha^{-1}): Total N uptake is the sum of N_{straw} and N_{grain}, whereas N accumulated in the crop residue at harvest (kg N. ha^{-1}) was calculated as the total biomass - grain yield * $\%N_{straw}$.

Crop N supply: This measure includes the sum of soil NO_3-N at sowing, mineralized N and N fertilizer (Moll *et al*., 1982). Mineralized N was estimated as the difference between pre-sowing and post-harvest plant and soil NO_3 -N in a check plot.

Nitrogen use efficiency (NUE; kg.kg^{-1}): Total N in the straw and grain samples was used to compute N use efficiency according to an expanded model of Moll *et al.* (1982) and Ortiz-Monasterio *et al.* (1997). The following N-efficiency parameters were calculated for each: N use efficiency (NUE; kg kg^{-1}), defined as grain production per unit of N in the soil.

6. Statistical analyses

Genotypes, N level and their interactions were assessed using a SAS GLM procedure (SAS Institute Inc., 1999) for all traits. The treatment means were compared by Duncan's multiple range test ($\alpha = 0.05$).

III – Results and discussion

1. Effect of N application on grain yield

The mean squares calculated for both cropping seasons (Table 3) indicated that variation for grain yield was significantly ($p \leq 0.01$) affected by the interactions of both N rate × year, N rate × cultivar and year × cultivar. The mean values for the cultivars across all N rates and years showed the lowest grain yield of the improved genotypes (Om Rabiaa and Khiar) without nitrogen supply compared with the local genotypes (Bidi and Azizi) (Table 4). The mean grain yield obtained for Khiar at 0 kg N ha^{-1} was lower than the mean yield of Bidi at the same N application rate (Table 4). Grain yield differed significantly among the two studied years. The grain yields of the landraces were significantly higher than that of the improved cultivars in 2009 and in 2010 (Table 5). This indicates differences in the genetic background of the four cultivars for yield potential. Cultivar x N application rate indicated progressive increases in grain yields ranging from 2025 kg.ha^{-1} to 6253 kg.ha^{-1} of the cultivars with increased application of nitrogen. Maximum yield increase of 208% and 179% for Khiar and Om Rabia were obtained at the highest level of N application compared to the control. There were large yield reductions for all cultivars grown without nitrogen application as compared to those fertilized plots. Huggins *et al*. (2010) reported that, in an optimal yield environment, higher levels of N fertilizer would reduce grain yield responses.

In general, grain yields in the cropping season 2009-2010 were lower than those in 2008-2009. This was attributed to the drought and the high temperatures during 2010. It is apparent, that elevated temperatures and drought prevailed during the vegetative growth stages during both cropping seasons. Johnson and Raun (2003) noted that temporal yield variability was greatly affected by differences in temperature and cumulative precipitation.

Table 3. Mean squares of grain yield (GY), nitrogen uptake (NUp), total nitrogen content (TNc), crop N supply (CNS) and nitrogen use efficiency (NUE) of four wheat cultivars grown under three Nitrogen levels and two years in subhumid conditions of northern Tunisia.

Sources of variation	DF	GY kg ha^{-1}	NUE kg kg^{-1}	NUp kg N ha^{-1}	TNc kg N. ha^{-1}	CNS kg N. ha^{-1}
Years (Y)	1	27280880**	3872**	94	70	254643**
Blocks in Year	4	320719	3	453	955*	649
Cultivars (C)	3	327545	13	608	631	928
Nitrogen (N)	2	70952346**	89**	34453**	38974**	38564**
N x C	6	1857441**	13	682*	847*	702
YxC	3	1649772**	15	969*	926*	928
YxN	2	3997202**	26	3330**	3966**	31184**
YxNxC	6	602299	7	473^{s}	392^{s}	702
Error	44	348842	9	235	290	634

, significant differences at α = 0.05 and α = 0.01 respectively.*

Table 4. Two-year mean durum wheat grain yields (kg.ha^{-1}) under three nitrogen levels.

Cultivars	0 N	75 N	150 N	Mean
Azizi	2305G	4434DE	5174CD	3971A
Bidi	2496G	4588DE	5358BC	4147A
Khiar	2024G	3395F	6253A	3891A
Om Rabia	2112G	3498F	5908AB	3839A
Mean	2235C	3979B	5673A	

LSD (0.05) = 396.8 and 370.6 for comparison of cultivar means and treatment means, respectively. Different letters indicate significant differences between cultivars (within-row) and treatments (within-column) at α = 0.05.

Table 5. Means of NUE components of durum wheat cultivars of two years (2009 and 2010). Data represent means of three nitrogen levels.

NUE component	Cultivars	2009	2010	Mean	LSD
GrainYield (kg.ha^{-1})	Azizi	4361B	3581C	3971A	† : 396.8
	Bidi	5133A	3163C	4148A	‡ 370.6
	Khiar	4238B	3544C	3891A	
	Om Rabia	4580AB	3099C	3840A	
N uptake (kg.ha^{-1})	Azizi	74.3B	85.3A	84.8AB	† 10.29
	Bidi	88.2A	76.8AB	88.7A	‡ 13.93
	Khiar	64.6B	73.9AB	74.9B	
	Om Rabia	88.4A	70.1B	85.5AB	
Total N uptake (kg.ha^{-1})	Azizi	78.8ABC	90.8AB	84.8BA	
	Bidi	93.7A	83.5ABC	88.6A	† 11.45
	Khiar	71.0C	79.0ABC	74.9B	‡ 20.23
	Om Rabia	94.4A	76.5BC	85.5BA	

† For comparison of cultivar means; ‡ For comparison of year means.
Different letters indicate significant differences between genotypes (within-row) and treatments (within-column) at α =0.05.

2. Effect of N-application on grain N-uptake and total N-uptake

Nitrogen uptake in grain and total nitrogen uptake had a significant response to nitrogen and years and different genotypes and years (Table 3). Grain N-uptake increased significantly with N supply during the two years. The highest grain N-uptake was noted for Om Rabia (88.42 kg ha^{-1}). Om Rabia accumulated the maximum N content in grain (88.42 kg ha^{-1}) which may be associated with maximum yield. Fageria *et al*. (2003) and Shinano *et al.* (1995) reported that in cereals, N accumulation is associated with dry matter production and shoot yield and grain representing the total biomass. Nitrogen uptake in the straw increased significantly with N (data not shown). An application of 150 kg N ha^{-1} caused the highest content of N uptake (104.55 kg ha^{-1}) in 2009 and in 2010 (129.46 kg ha^{-1}) (Table 6). Fageria *et al.* (2009) argued that this response could be associated with maximum yield of shoot yield.

Total N uptake differed significantly among years (Table 3). For the two years study as a whole, total mean N uptake of111.23 kg.ha^{-1} and 138.90 kg.ha^{-1} for both cropping seasons and suggesting that N uptake was proportional to yield: 5859 and 5487 kg N. ha^{-1} in 2009 and 2010, respectively. However, in their study of bread wheat, Limaux *et al.* (1999) reported a significant effect on partitioning of added N between soil and plant.

Differences between species in N uptake were noticeable at heading and maturity. Barley took up 70-73% of the total N before heading, whereas wheat and oat averaged 64% (Peltonen-Sainio *et al*., 2007b). Our results for durum wheat are comparable to those of earlier studies (Bulman and Smith, 1994; Delogu *et al*., 1998). These results indicated that wheat had much lower N uptake up to heading than the 90 to 100% reported by Clarke *et al.* (1990) and Heitholt et al. (1990). According to our results, wheat had up to 69% higher heading N uptake than barley, possibly because they require much longer growing period under northern growing conditions than barley (Peltonen-Sainio *et al*., 2007b). These results did not support those of earlier studies which suggested that higher N uptake of wheat would contribute to improved NUE in wheat (Van Sanford and MacKown, 1986; May *et al*., 1991; Le Gouis *et al*., 2000).

Table 6. Means of NUE components of durum wheat cultivars of two years. Data represent means at three nitrogen levels.

NUE component	N rate	Year				
	kg ha^{-1}	2009	2010	Mean	LSD1	LSD2
Grain yield	0	2898C	1572D	2235C	370.6	343.6
(kg.ha^{-1})	75	4977B	2981C	3979B		
	150	5859A	5488A	5674A		
N uptake	0	49.6E	33.2F	41.4C	13.93	8.91
(kg.ha^{-1})	75	82.4C	66.9D	74.6B		
	150	104.6B	129.5A	117.0A		
Total N uptake	0	53.7E	35.5F	44.6C	20.23	9.91
(kg.ha^{-1})	75	88.4C	72.9D	80.7B	† :	
	150	111.2B	138.9A	125.1A		
Crop N supply	0	35.2D	79.3C	57.2	6.67	14.65
(kg.ha^{-1})	75	39.2D	164.0B	101.6		
	150	43.3D	231.2A	137.2		

LSD1 and LSD2 for comparison of year means and treatment means, respectively.
Different letters indicate significant differences between cultivars (within-row) and treatments (within-column) at α = 0.05.

3. Effect of N application on N use efficiency under different N treatments

NUE refers to the total nitrogen available to the plant either from the soil or from the fertilizers. It has been shown that under suboptimal yields, NUE increases with the increase of the total available N (Raun and Johnson, 1999). NUE was significantly affected by N fertilizer rates and years (Table 3).The NUE has been increased under nitrogen application (Figure1). Maximum NUE (15.67%) was observed at 150 kg N/ha; while the lowest efficiency (11.87%) was noted in the control. In addition, no significant difference was noted between genotypes for the NUE of durum wheat genotypes cultivated in subhumid growing conditions of northern Tunisia. These results would imply that sufficient available nitrogen for cereal exists in the soil of the experimental station or it has been leached. In fact, the soil total N content of the experimental field was very low (Table 2). The quite high yield levels obtained at N control could be attributed to the high averages

higher levels of nitrogen to fully express their genetic potential. Nitrogen fertilization level of 150 kg N. ha^{-1} leads to the best average use efficiency (Giambalvo *et al*., 2010). To improve durum wheat NUE, more information is required on seasonal soil changes and its impact on crop utilization.

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