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Evaluation of grain yield and nitrogen agronomic efficiency (NAE) in Tunisian durum wheat cultivars (*Triticum turgidum* ssp *durum*)

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Abstract - In Tunisian agriculture system, nitrogen (N) is the most important and used fertilizer for sustaining durum wheat (*Triticum turgidum ssp durum*) growth and improving yield. The aim of this study was to assess genotypic differences for N agronomic and physiologic efficiencies at three nitrogen levels (0, 75 and 150 kg N/ha). A set of four durum wheat genotypes was used and is constituted by two improved cultivars (Om Rabia and Khiar) and on two landraces (Bidi AP4 and Azizi AC2). A field trial was conducted under sub-humid climate conditions in northern Tunisia. A complete randomized block design with four replications was used. The results showed that increasing N up to 75 kg N/ha increased yield and its attributes of durum wheat. Increasing N up to 75 kg N/ha seems able to ensure the production potential of indigenous genotypes (Azizi and Bidi). On the contrary the improved varieties still respond to a greater nitrogen input. It is possible that improved genotypes require higher levels of nitrogen in order to fully express their genetic potential. In addition, we have showed that a significant genotypic variation in the N agronomic efficiency.

Keywords: durum wheat / N agronomic efficiency / N physiologic efficiency genetic variability.

1. Introduction

Durum wheat is the most extensively cultivated cereal in the Mediterranean area (Araus et al., 2013). It is the most produced crops, easily stored and transported, and an important nutritional source for humans (Arregui and Quemada, 2008). In Tunisia, wheat accounts for 60% of national cereal production (Ayadi et al., 2012) and high yielding wheat varieties need large and regular supply of N to develop high photosynthetic capacity and maintain the proper nitrogen concentration in the leaves when N large rates are required for ear growth and grain-filling period (Ali et al., 1998). However, nitrogenis the main nutrient limiting yield and it is a crucial component of the wheat production (Latiri-Soukiet al., 1992).
N use efficiency is very low and estimated to be approximately 33% (Raun and Johnson, 1999) and a substantial proportion of the remaining 67% is lost into the environment, polluting water (Pathaket al., 2008; Arnallet al., 2009; Arregui and Quemada, 2008). Therefore, scientists are challenged to develop N management strategies that guarantee increased N use efficiency (NUE) *(*Semenov *et al.*, 2006*)*. A number of experiments in winter wheat have showed that adjusting fertilizer rate and splitting of N fertilizer application are strategies to improve NUE (Alcoz*et al.*, 1993; Delogu*et al.*, 1998; López-Bellido*et al.*, 2005). Moreover, there is an urgent need to reduce the use of N fertilizer and search for plant genotypes with greater N use efficiencies, either in a strict physiological sense (increased carbon (C) gain per unit N) (Gastal and Lemaire, 2002), or in an agronomic sense (increased dry matter yield per unit plant N or per unit N applied/available to the crop) (Andrews *et al*., 2004; Good *et al.*, 2004).
In fact, nitrogen use efficiency in plants is a multi-genetic character under environmental control including soil nitrogen availability, its uptake and assimilation, photosynthetic efficiency, carbon–nitrogen flux, C allocation between organs, nitrate signaling and regulation (Raghuramet al., 2006;

Pathaket al., 2008). This complexity makes the improvement of crop NUE a challenging multidisciplinary research mainly at the field-level.
In wheat, grain N content rather than yield components is largely influenced by the amount of N taken up after anthesis and by the amount of remobilised N originating from preanthesis uptake since these two sources of N are used for storage protein synthesis (Dupont and Altenbach, 2003). After anthesis, leaves become a source of N. N in leaves is recycled following protein hydrolysis and exported in the form of amino acids to grains (kicheyet al., 2007). As a concept, NUE includes N uptake, utilization or acquisition efficiency, expressed as a ratio of output (total plant N, grain N, biomass yield, grain yield) and input (total N, soil N or N-fertilizer applied). NUE is quantified based on apparent nitrogen recovery using physiological and agronomic parameters (Dawson et al., 2008; Pathaket al., 2008).
Studies on NUE in annual wheat have established that significant genetic variation exists for traits related to NUE (Dawson et al., 2008; Pathaket al., 2008; Singh and Arora, 2001). In wheat, significant genetic variation exists for total N uptake (McKendryet al., 1995) and for translocation efficiency (Laprecheet al., 2005).
As Tunisian wheat cultivars are characterized by a high grain yield and it is necessary to know what is the best rate is for each cultivar as well as its influence on components of yield and other agronomic parameters such as the cycle, plant height or the quality of the grain. This will also enable the identification of the most efficient genotypes for nitrogen use.
In this study we are reporting the response of four durum wheat genotypes at three levels of nitrogen fertilization. This study is intended to investigate the effects of nitrogen on wheat growth and productivity and thus determine the N agronomic and physiological efficiencies under sub humid environment.

2. Materials and methods

2.1. Plant material

Four durum wheat (*Triticumturgidum*ssp. *durum*) cultivars were included in this study. Two improved genotypes (Khiar and Om Rabia) and two landraces genotypes (Bidi AP4 and Azizi AC2) were chosen based on their genetic potential (Ben Naceur*et al.,* 2001; Deghaïs*et al.,* 1999).

2.2. Experimental site

Experiments were performed at the Experimental Farm of the Agricultural Superior School of Mateur of Tunisia (52°50'N, 1°14'W), located in the sub-humid area. The relevant soil was silty clay laom texture with the following properties presented according to Ayadi et al., 2012; 2014.

2.3. Experimental design

The experiment was designed as a randomized complete block with four replications. Plot size was 3m by 1.8 m. Three treatments were applied: two applications of N fertilizer (75 and 150 kg N ha^{-1}) and one without (0 kg N ha^{-1}). Nitrogen fertilizer was applied as ammonium nitrate (33.5% N) divided into three applications at different stage (early tillering Z13 (30%), elongation Z16 (40%) and 2nd node Z32 (30%)) and were recorded to zadoks growth stage (Zadoks et al., 1974). Seeds were sown on 26 Nov. 2008 with a target density of 300 plants m^{-2}. Planting was done manually by hand and plants were grown without supplemental irrigation in the growing seasons.At grain maturity (01 june 2009), plants from 0,5m2 from each plot were harvested. Spikes were cut and grains were separated using a wintersteiger LD180 thresher. Chaffs and remaining stalks were also collected.

2.4. Soil Mineral Nitrogen

Soil mineral nitrogen (Nmin) content was determined in soil samples taken before sowing and one week after each fertilizer application (17 Jan. 2009, 28 Jan. 2009 and 07 march 2009) and at harvest. Soil samples were taken at 0.3 m intervals to a depth of 0.9m, using augers (2.5 cm inside diam., 20 cm long). The samples were extracted with KCl (1kg of soil). Nitrate concentration in the extracts was determined by the method of reagent of Devarda's Alloy (Sims *et al.,* 1995). Ammonium was measured using the method proceeding with the distillation – titration (Rhine et *al.,* 1998).

2.5. Plant total Nitrogen

For total N determination, plant samples were washed and separated into different plant parts (namely chaffs, straw and seeds). After oven drying at 80 ºC for 24 hours, material was weighted and ground. All plant samples were digested using the Kjeldahl procedure and analyzed for N concentration using the method of Cataldo*et al.*, (1974).

2.6. Sampling and measurements

Data were recorded at maturity, number of spike per m², thousand kernels weight (TKW) and grain yields (GY) were estimated.

Various expressions were constructed and analyzed according to the method suggested by Moll *et al.*, (1982). In comparing genotypes with respect to nitrogen use efficiency the following parametersN Agronomic efficiency and N Physiological Efficiency were calculated according to Chen et al., (2014).

$$NAE=(GY_F - GY_0)/ N_F$$

$$NPE=(GY_F - GY_0)/ (Nup_F - Nup_0)$$

In the equations, GY_F is the grain yield from the plots that received N fertiliser; GY_0 **is** the grain yields in the zero N plots; N_F is the amount of N fertiliser applied; nitrogen fertilizer applied; Nup_F is the plant N uptake in the plots that received N fertiliser; Nup_0 is the plant N uptake in the zero N plots.

2.7. Statistical Analysis

Analysis of variance (ANOVA) was performed using the PROCGLM SAS procedure to assess the effect genotypes treatments and their interactions for all measured traits. Means were compared by Duncan's test (P < 0.05).All figures were created by SigmaPlot (software 11.0).

3. Results and discussion

3.1. Grain yield and its attributes.

Genotype grain yield is the most integrative agronomical trait since it's influenced by all known and unknown factors (Hussain, 2007). We have determined the grain yield of the four genotypes under the different N supply levels (Figure 1). Analysis of variance showed no genotypes effect on grain yield. However, nitrogen fertilisation had significantly affected this agronomical trait (Table 1).

Table 1. Effect of different levels of nitrogen on number of spike/m², TKW, GY NAE and NPE.

Values shown are the means of 9 replicates (three replicates and three treatments) of each genotype. While N treatment values are the means of 12 measurements (4 genotypes and three replicates). Means followed by different letters are significantly different by Duncan's multiple range test (*P<0.05*). Analyse of variance for the same variables is shown for the genotype (G), N treatment (T) and interaction effect (SXT). (ns, not significant).

Source of variation	**NS**	**TKW (g)**	**GY (t/ha)**	**NAE (kg/kg N)**	**NPE (kg/kg N)**
Genotype (G)					
Azizi	315.01a	51.98b	4.59b	16.30c	49.51a
Bidi	313.75a	52.61a	5.29a	30.37a	48.18a
Khiar	281.70b	49.29b	4.39b	26.24b	67.49a
Om Rabia	296.92b	45.90c	4.69ab	28.35b	50.73a
N treatment (T)					
0 kgN/ha	224.00c	49.24b	3.08c	-	-
75 kgN/ha	313.00b	49.79b	5.11b	27.11a	56.01a
150 kgN/ha	367.00a	50.70a	6.03a	23.52a	51.87b
ANOVA					
Genotype (G)	2971.52*	112.11**	1787194,53*	311.56*	657.90 ns
N Treatment (N)	83766.89**	10.04*	36467419,75**	103.46 ns	130.55*
G × T	5783.97 ns	9.10*	715771,20ns	84.14 ns	188.97 ns

ns: Test F no significant; * Test F significant at 5% level; ** test F significant at 1% level

We have found that the improved cultivars responds in a different manner to the N fertilization compared to the local landraces. In fact, the improved cultivars showed a low yield without nitrogen supply. The average yield of these cultivars was only 3.08 T/ha in these conditions, to reach up to 6.03 T/ha under N fertilization. These results agree with findings of Badraoui*et al.*, (2001), Hussain (2007) and Yunusa*et al.*, (1998) who reported that by increasing the level of nitrogen, the grain yield was also increased.
Landraces were shown to be more productive than improved cultivars under cultivation without nitrogen supply. These indigenous genotypes (AziziAC2 and Bidi AP4) showed a high yield (3.5T.ha^{-1}) without the nitrogen application. Moreover, these genotypes maximize their grain yield at only 75 kg N.ha^{-1}.The average number of spike per m² of the 4 genotypes differed between landrace (Bidi and Azizi) and improved genotypes (Khiar and Om Rabia). . N applied increased NS by 39% and 60% under 75 N rates and 150 N rates respectively compared with low N (0 N rate). The relatively small effect of N treatment observed on TKW, in comparison to the large effect on number of spike per m² and GY. However, TKW differ between cultivars. Nevertheless, these parameters had no significant difference under 75 N compared with low N (0 N), only appeared rapid increase under high N. Grain yield was higher in the improved genotypes than the landraces, which was associated mostly with a larger number of spikes per area and a shorter plant height (Araus *et al*., 2013). Ortiz-monasterio et al., (1997) stated that N efficient genotypes can be characterized by their ability to produce high grain yields under both low and high N fertility conditions, and genotypes that are N use inefficient only produce acceptably high grain yields under high N fertility conditions. Following this line of reasoning, without considering significant differences between the cultivars, the cultivars were ranked based on grain yield at the different N treatments (table 2). Ranking of the four cultivars were mostly consistent. However, it seems that the half N fertilisation level is sufficient to ensure the maximum production of landraces genotypes contrary to the improved ones which they exhibit sensitivity to the lack of nitrogen and need a greater N input. In fact, improved genotypes require higher levels of nitrogen in order to fully express their genetic potential.

3.2. Nitrogen Agronomic Efficiency (NAE)

The ratio of produced grain to N applied fertilizer may be used to estimate NUE, and this ratio (kg grain/kg N) has been defined as agronomic efficiency (NAE) (Ladha*et al*., 2005; Stevens *et al*., 2005). As this calculation subtracts the yield of the control from the yield of the N treatment plot, this difference method assumes that N fertilization has had no additional positive effects on plant uptake of soil N, and that all other agronomic factors are considered equal between the respective treatments (Stevens *et al*., 2005).
The NAE average of improved genotypeOm Rabiaunder 150 kg N. ha^{-1} was significantly the highest (23.36 kg/kg N applied) mainly because of high grain yield (6.28 T/ha). Also,Om Rabia under75 Nkg ha^{-1} was significantly the highest (41 kg grain/kg N applied). The cultivar Khiar showed the same NAE at 75 kg N/ha and 150 kgN/ha. This result prove that it is possible to improve the grain yield and NAE of the cultivar Khiar by increase the rate of nitrogen over 150 kgN/ha.
Our study have shown that for durum wheat cultivars, the NAE average was more efficient at 75 kgN/ha and less efficient at 150Kg N/ha (fig.). Lóppezbellido*etal.* (2001) obtained similar results with the cultivars of bread wheat at 50Kg N/ha and 150Kg N/ha.

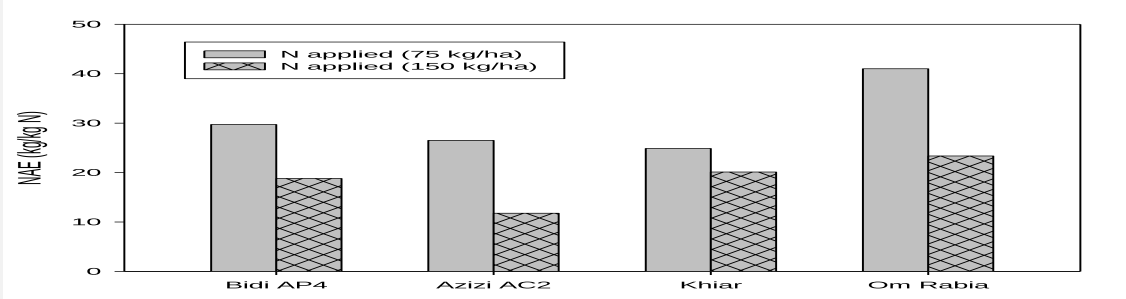

Figure 1: Wheat cultivar differences in nitrogen agronomic efficiency (kg/kg N)

3.3. Nitrogen Physiologic Efficiency (NPE)

In this trial, the mean NPE was only 53.9 kg grain/kg N uptake. Neither the cultivars nor the N treatments showed significant effects on NPE. However, the NPE values of the cultivars varied from 48.18 kg/kg N uptake with landraces cultivars (Bidi AP4) to67.49 kg/kg N uptake with the improved (Khiar). The NPE values of the N treatments ranged between 56.01 kg grain/kg N uptake at 75 kgN/ha to 51.87 kg/kg N uptake at 150 kgN/ha.

Neither, the cultivars nor the N treatments affected the NPE values. There were also no obvious trends in the NPE values. On average higher NPE values realized in the improved genotypes (Khiar and Om Rabia) than in the landraces, mainly because of the higher grain yields measured for khiar genotype. Generally, either Bidi AP4 or Azizi AC2 had the lowest NPE values and Khiar genotype the highest NPE. Hence, the response of landraces genotypes to N can be regarded as poor. Song et al., (2007) reported that the NPE and NAE values of the super rice variety were greater than that of the rice cultivars at higher N application and lesser at lower N application. Another study compared the NPE and NAE of super rice showed a similar result with our results (Chen et al., 2014). In our study, more N was accumulated in N75 than in the rate of 150 kgN/ha in Om Rabiaa genotype, but no difference was found between bidi AP4 and Azizi AC2 cultivars at N75, indicating that the yield increase of Om Rabiaa resulted from high N inputs. Similar results were also found for NAE. These results suggest that the improved genotypes have an advantage at NUE when high N is applied.

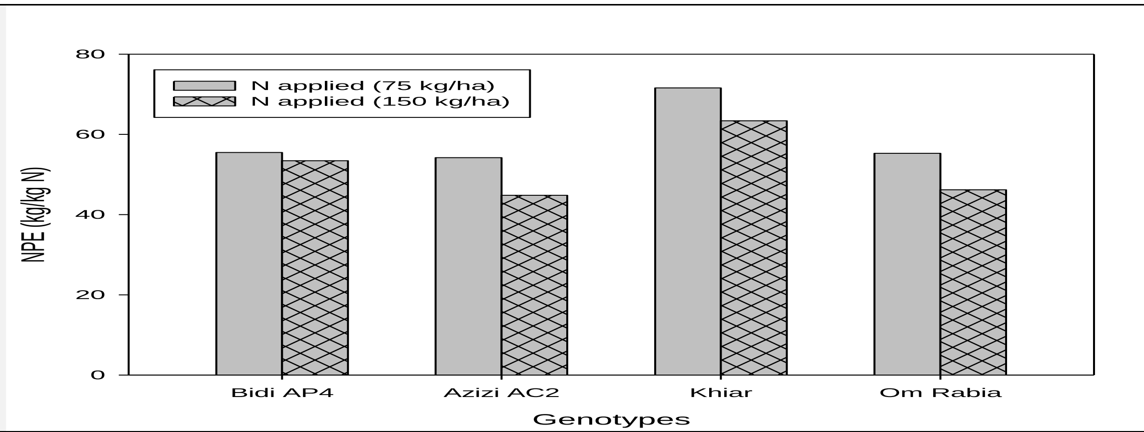

Figure 2: Wheat cultivar differences in nitrogen physiological efficiency (kg/kg N).

4. Conclusion

The NAE evaluation between the non fertilized assay and the half dose or the full N dose confirmed the previous findings. In fact, we have found the NAE at the half dose was lower in the landraces. These landraces respond to a big nitrogen fertilization level to reach their genetic production potential. Higher nitrogen doses are more suitable for improved genotypes. Indeed, the NAE of theses cultivars was higher for 150Kg N/ha than for the local accessions.

The values of NAE of our result (around 23 Kg grains/Kg N) were more higher than the values of NAE (around 6 Kg grains/Kg N) obtained by LóppezBellido*et al.*,(2001). This point was explained in this present experiment by the presence of high nitrogen nutriment on the soil. So, excessive N nutriment on the soil affected wheat yield and the N agronomic efficiency. The durum wheat cultivars showed good response in the grain yield and NAE. However, the results showed a significant genotypic variation with grain yield and NAE.

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Abbreviations:

GY: grain yield; **GY_0**: grain yield at plots without nitrogen; **GY_F**: grain yield at plots with nitrogen applied; **NS**: number of spike/ m2; **TKW**: thousand kernels weight; **NAE**: nitrogen agronomic efficiency for grain yield; **NPE**: nitrogen physiological efficiency for grain yield; **G**: genotype; **N**: nitrogen treatments.