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Nitrogen fertilization and root growth dynamics in durum wheat

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Abstract

In an area of the Apulian Tavoliere (southern Italy), the effects of three levels of nitrogen fertilization (0, 50 and 100 kg N ha^{-1}) on root development, growth analysis and yield parameters of durum wheat were evaluated. The research was conducted over a four-year period (1994-97). The non-destructive mini-rhizotron method was used to study the root system at stem extension and at the beginning of heading and ripening stages. At the end of tillering and at boot and flowering stages, samples of wheat biomass were taken and subjected to growth analysis. Yield data and the main biometric parameters were collected at harvest time. The doses of nitrogen (N) fertilizer 50 and 100 kg N ha^{-1} had a greater effect on root development in the 20-30 cm soil layer and on epigeal biomass than the control test (N0) without nitrogen fertilization. In the test (N0) the growth of root and epigeal biomass was slower during the first vegetative phases, however, afterwards both of them recovered and the root system was mainly developed in the 30-40 cm soil layer. A better development of root system in deeper soil layers, without nitrogen supply, has allowed the plant to overcome more easily the water-deficit and thermal stresses during the ripening stage. The results of this research have shown that the production of grain with 50 kg ha^{-1} of N is similar to those of 100 kg ha^{-1} of N doses and higher than the test without nitrogen fertilization. In this kind of environment can be recommended a nitrogen dose of 50 kg ha^{-1} for obtaining an increase in grain production with low costs and reduced agricultural sources of pollution.

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Introduction

The knowledge of factors that affect root development is important for improving nutrient cycling in soil-plant systems. The contribution of root system to total plant weight is nearly 10-20%, and a well-developed root system is fundamental for the growth and development of plants. Root growth is genetically dependent, but it is also influenced by agronomic techniques and in particular mineral nutrition (Raun and Johnson 1999; Qiang *et al.*, 2004). In soils with adequate nutrients there are more root hairs than in those without nutrients, and the root system is mostly developed in the top layer of soil (Gregory, 1994; Cheng and Kuzyakov, 2005). The vegetative stage, at the maximum root development, varies with the species, for example at flowering in cereals and at pod-setting in legumes (Comfort *et al.* 1988; Fageria, 2009). In crop plants, generally, root and shoot growth occur in an apparent coordinated fashion and so their activities are mutually dependent (Goss and Kay, 2005, Puget and Drinkwater, 2001). When a large amount of nutrient is supplied to leaves *via* the root system, photosynthesis remains high during maturation, which ensures an adequate supply of carbohydrates to roots (Zobel, 1986). Deficiencies of many mineral elements influence plant growth and root-shoot relationships. Nitrogen (N) deficiency is one of the factors limiting crop production (Kanapiu *et al.* 1997; Johnson and Raun, 2003). The practice of adding nitrogen fertilizer to wheat is one of the farming techniques, which induces the most macroscopic effects on growth and productivity of crops (Bindrabadan, 1999; Vandeleur *et al.*, 2005; Ruggiero and Angelino, 2007). Therefore, nitrogen deficiency in plants can greatly affect plant growth and yield. The study of interactions between farming methods and root growth is very complex. The study of hypogeal growth, under real field conditions, shows greater difficulties than the one of epigeal growth, and this has considerably slowed down the understanding of the direct relationship between fertilizer applications and root growth.

In several areas of southern Italy, characterized by low rainfall, we have found low efficiency in nitrogen fertilization on durum wheat (Caliandro *et al.*, 1981; Flagella *et al.*, 1997; De Giorgio *et al.*, 2004), and applications of higher doses did not produce significant increases in grain yield. The Agricultural Research Council Research - Unit for Crop Systems in Dry Environments (CRA-SCA) of Bari (Italy) carried out a four-year research program in order to evaluate the effects of different levels of nitrogen fertilizer on root system development and biometric parameters.

Materials and methods

Site

The research was carried out at the CRA-SCA experimental farm in Foggia, Southern Italy (41° 27' latitude N, 3° 04' longitude E, 90 m above sea level), over a four-year period (1994-97). The soil is a deep alluvial silty-clay textured vertisoil, typical of the flat land of the Apulian Tavoliere (southern Italy), classified as Typical Chromoxerer by the USDA Soil Taxonomy (Soil Survey Staff, 1992). The soil layer (0-40 cm) contains 42.7% clay, 27.7% silt and 29.6% sand, and the following main characteristics: total N=1.38 g kg^{-1}, available phosphorus (P)=27 mg kg^{-1}, exchangeable potassium (K)=1019 mg kg^{-1}, organic matter = 23.3 g kg^{-1}, pH =8.12 and hydraulic conductivity = 20-30 cm d^{-1}.

The climate is *accentuated thermo-Mediterranean* as classified by the UNESCO-FAO Bioclimatic Maps, characterized by hot summers with little precipitation, cold and wet winter months and with most of the rainfall concentrated in autumn and winter. The experimental field is equipped with a meteorological station which permits continual monitoring of the main climatic parameters (maximum and minimum temperatures, rainfall, wind, light radiation, *etc.*). Evaporation was measured in a Class A pan evaporimeter.

Annual rainfall for the period October-September over the four-year study was 431 mm in the 1st year, 514 mm in the 2nd, 582 mm in the 3rd and 416 mm in the 4th year (Figure 1). Compared to an average of 554 mm in the previous 42 years, measured in the same trial site, the average annual rainfall for the 1st, 2nd and 4th year was lower and slightly more than usual for the 3rd year. In addition, considering the period October-May, which is of more interest for crop growth, the values for the 1st, 2nd and 4th year decreased to 385 mm, 338 mm and 312 mm, respectively, and for the 3rd year increased to 422 mm. Low rainfall and sudden and unexpected high temperatures during the flowering and ripening stages, represent the critical limit in the efficiency of nitrogen fertilization in this type of environment.

Experimental setup

The experiment was carried out on monoculture of durum wheat (*Triticum durum* Desf.), Simeto genotype which was subjected to three levels of nitrogen fertilization (0, 50 and 100 kg N ha^{-1}, defined as N0, N50 and N100, respectively). In the test field, a randomized complete block design, with three replications in plots of 60 m^2 (10×6 m), was applied. The soil was subjected to double-share ploughing at 35-40 cm depth, rotary tillage at 15-20 cm with a disc plough and finally to rotary tillage at about 5 cm. The previous crop was sunflower. The sowing was done by mechanical drill and by hand, considering the parameters of mechanical sowing, for an area of 5 m^2 around the mini-rhizotron tubes. Every year, the soil prepared for sowing was fertilized with 100 kg ha^{-1} of P_2O_5 and received 20 kg ha^{-1} of Carbofuron geo-insecticide. The sowing was done with mechanical seed drill in late November throughout the four-year period and with 15 cm row spacing and a seed rate of 350 seed m^{-2}. The N fertilizer (ammonium nitrate) doses were applied in two equal parts at the start of tillering and steam extension at first node of stem visible (stage 2 and 6; Feekes, 1941).

Plant emergence was regular over the four-year period. The growth cycle was monitored and data collected for the main vegetative phases of the wheat (emergence, tillering, stem extension, heading and ripening). At harvest time the main bio-agronomical parameters were assessed (grain yield, 1000 seed weight, kernel moisture, stunted kernels, yellow berry, kernel spike, infertile and fertile spikes), together with the biomass (plant height, spike length, number of culms).

Biological and physical measurements

Root growth measurements

Root growth was measured with the non-destructive mini-rhizotron method. One week before sowing the mini-rhizotron tubes (transparent polybuterate), one meter in length and with an internal diameter of 50 mm, were installed in soil at a 45-degree angle to the ground surface. Soil perforations were done by pushing a probe hydraulically into the soil, and contact between the external tube surface and soil was checked carefully during the installation process. Six mini-rhizotrons were installed for each nitrogen levels (0, 50 and 100 kg N ha^{-1}) and for each replication, three of which were placed in the wheat rows and three between the rows for each plot.

Before installation the mini-rhizotrons were marked with four equidistant lines along their entire length and also with lines around their circumference every 2.5 cm. The points where the horizontal and vertical lines intersected were taken as markers and where the number of roots was counted. To facilitate the insertion of a micro webcam into the mini-rhizotrons, 5 cm of tube was left above ground level and wrapped in opaque black plastic to ensure complete darkness within the tube.

Every year, during the phases of stem extension (second node of stem visible) and between flowering and the beginning of ripening stage (Feekes scale 7 and 10.5-11), measurements of the root growth were taken. The last assessment was carried out before the start of the natural decline of the root system that generally coincides with the process of grain maturation. The measurements were taken by inserting a micro webcam into the mini-rhizotrons and running it in turn along the 4 vertical lines for the entire length of the transparent tube. Every 2.5 cm, where the horizontal and vertical lines intersected, images of the root system alongside the mini-rhizotron tubes were taken. The intersection points of the horizontal and vertical lines ensured that the sampling replication was always done exactly in the same position. In every mini-rhizotron tube, 152 images were taken

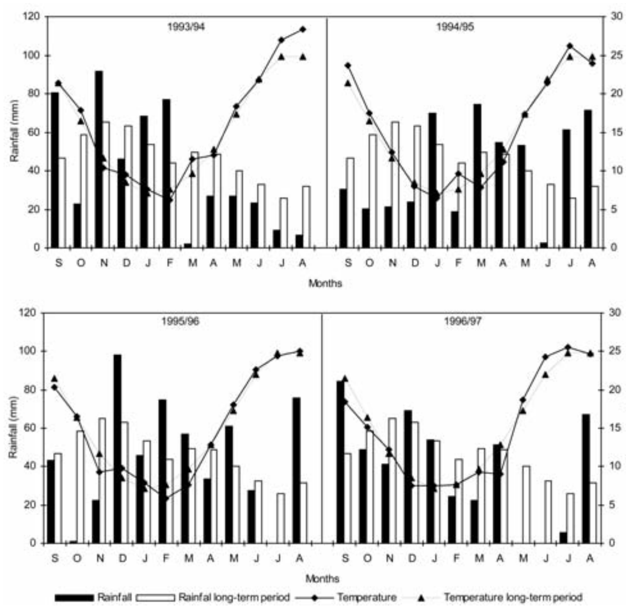

Figure 1. Monthly temperature and rainfall during plant growth to the mean of the previous 42 years.

and when they were considered all together allowed the exploration of the entire transparent surface of the tube. The images were filmed on a videotape and were later analysed in the laboratory, counting the number of root visible from the transparent tube at every 2.5 cm step. The absence of weeds was guaranteed by a thorough application of chemical herbicide (Cloquintocet-mexyl + clodinafop-propargyl and Iodosulfuron-methyl-sodium) which ensured that all the observed roots belonged to the durum wheat. The individual values at a 45° angle were grouped into 10 cm frames and recalculated vertically. The number of roots was converted into Root Length Density (RLD) (cm cm^{-3}) in accordance with Upchurch and Ritchie (1983).

Growth analysis

To have a better understanding of the effects of N fertilization on root development in wheat, growth analysis was also carried out. At the begins of tillering, strongly-erected leaf sheaths, last leaf just visible, boot and flowering stages, the plants within a 0.5 m^2 area were sampled and tested, and leaves culms and spikes were separated for measurements of dry matter weight and Leaf Area Index (LAI) with LAI 2000.

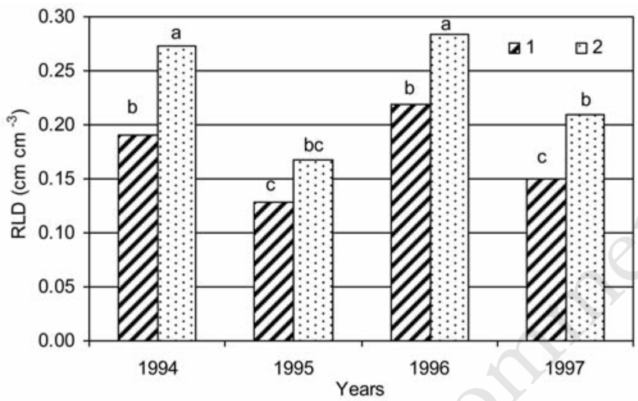

Figure 2. Average annual values of Root Length Density (RLD) at second node of stem visible (1) and at the beginning of ripening stage (2). The row values in each year with different letters are significantly different at P<0.05 (SNK test).

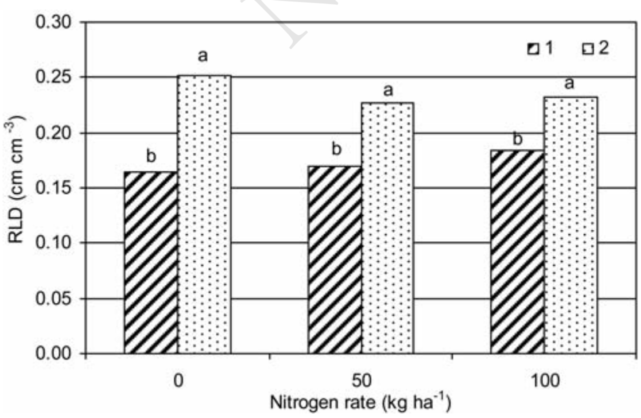

Figure 3. Average Root Length Density (RLD) values for the four-year period for each nitrogen level at second node of stem visible (1) and at the beginning of ripening stage (2). The row values in each year with different letters are significantly different at P<0.05 (SNK test).

Soil moisture

Samples were taken from the trial areas to determine soil moisture content (gravimetric method).

The section analysed (0-60 cm) was subdivided into three layers (0-20, 21-40 and 41-60 cm). The samples were taken at sowing, the start of stem extension and at the beginning of heading and ripening stages. The soil samples were weighed and then put in an oven at 105°C for 48 h before being weighed again to establish the moisture content.

Statistical analysis

Statistical analysis was performed using SAS procedures software (SAS, 2006). The experimental data were submitted to analysis of variance (ANOVA), considering the years on a random basis and fertilization levels as fixed. Differences between treatments were compared using the Student-Newman-Keuls (SNK) test at P≥0.05.

Results

Root length density

In order to have a more reliable evaluation of the RLD, the 0-10 cm soil layer was eliminated, because it is more likely to be subjected to external factors (small weeds, trampling, *etc.*) that can alter the natural growth of the most superficial roots. During the four-year period the average annual RLD values (Figure 2) were higher in the 1st and 3rd year (0.23 and 0.25 cm cm^{-3}, respectively) followed by the 4th (0.18 cm cm^{-3}) and the 2nd year with the lowest result (0.15 cm cm^{-3}).

The annual climate variability did not show significant differences

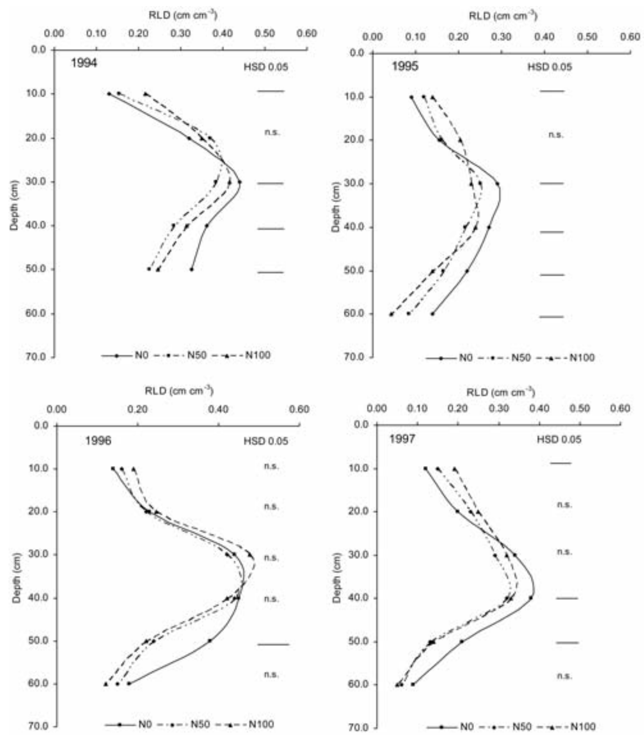

Figure 4. Root Length Density (RLD) of durum wheat on three nitrogen rates beginning of ripening stage (HSD 0.05 bar statistical significant difference between the treatment for every step of soil profile).

between the effects of three levels of nitrogen (N0, N50 and N100) on the average RLD results over the four-year period. In particular, the RDL values increased with the increasing N availability (Figure 3) at the time of the first sampling at the middle of stem extension, whereas the values were in inverse order and the control sample (N0) had the highest RLD (Figure 4) at the time of the second sampling when the wheat reached the maximum vegetative growth.

The effects of the N doses on root growth were more evident during the period of stem-extension (second node of stem visible) and the RDL values were much higher in nitrogen-fertilized areas than in the control (Figure 5). Although root growth was visible with both levels of N doses, only with N100 it showed significant differences when compared with the control but not between N50 and N100. This trend remained constant throughout the four-year period. In the later phases, heading and ripening, the root system of wheat developed further and reached the maximum root concentration to a depth of 30-40 cm while the maximum root length was reached at a depth of 60 cm (Figure 5). The N50 and N100 doses produced the largest concentration of roots around 30 cm while the control showed a strong recovery and moved the maximum intensity of RLD to a depth of 40 cm. The highest root growth of the control sample was constant up to the maximum depth sampled compared to the N doses N50 and N100, and with significant differences sometimes only with N50 and other times with both N50 and N100. To sum up, it can be said that if the wheat finds a good level of nitrogen such as in the upper soil layers, it is not stimulated to explore the deeper soil layers, whereas in the absence of nitrogen fertilizer, in order to satisfy its own nutritional needs it explores a larger volume of soil and reaches the largest concentration of roots at deeper levels of soil.

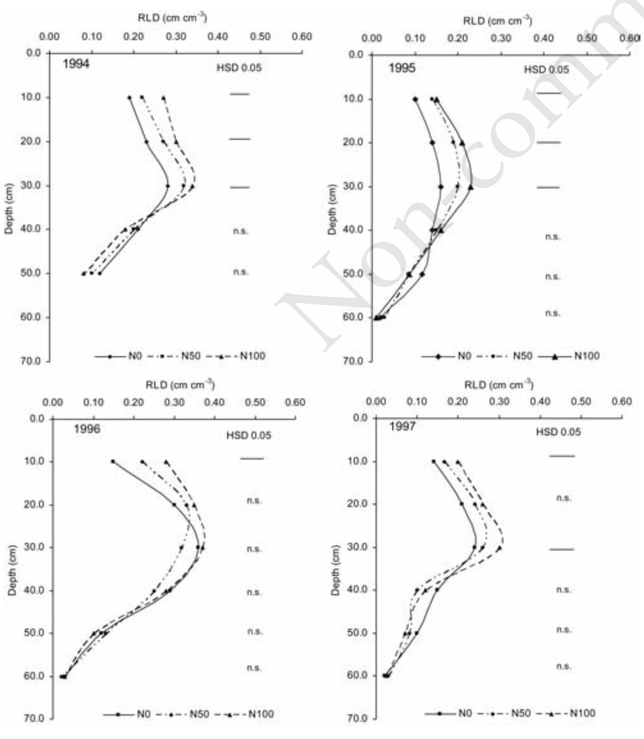

Figure 5. Root Length Density (RLD) of durum wheat on three nitrogen rates at second node visible stage (HSD 0.05 bar statistical significant difference between the treatment for every step of soil profile).

Soil moisture

Soil moisture content varied in relation to rainfall and the natural progression of seasons. During the four-year period at sowing time, the values range between 12% in the second year and 18% in the fourth year, and this is due to low rainfall in the summer-autumn period. In the last year, short precipitation before sowing led to higher levels of soil moisture. The highest water content was found at the end of tillering and at the start of stem-extension. No significant differences were noted on the variations of soil moisture in relation to the three levels of nitrogen analysed at the three soil depths (0-20, 21-40 and 41-60 cm).

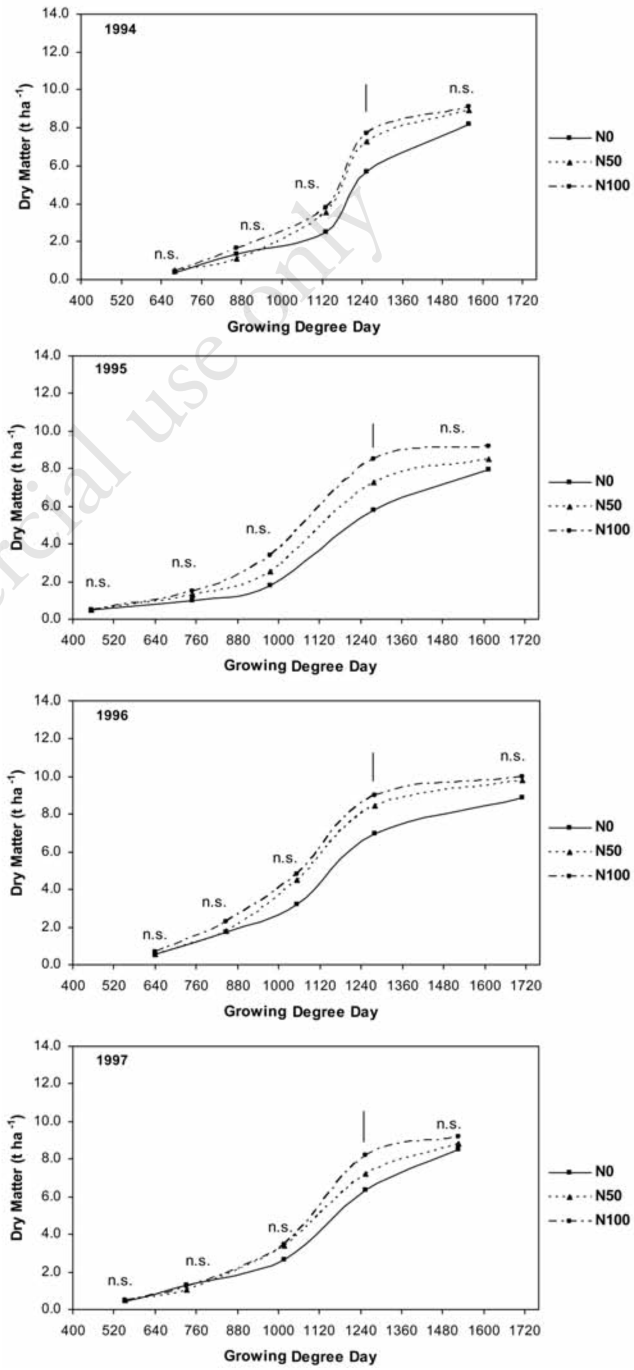

Figure 6. Soil water content during the durum wheat growth (Days After Sowing) of 1994-97 at the three steps of the 0-60 cm profile.

Dry matter and leaf area index

Both levels of nitrogen fertilizer (N50 and N100) produced an increase in dry matter (Figure 6). Significant differences were observed during stem extension and heading stages which are the periods of the greatest vegetative growth. Although the control sample shows lower values throughout the entire vegetative cycle, in the final phase it showed a recovery and the differences diminished to the extent that its growth overtook that of the nitrogen-fertilized plants. The effects on LAI (Figure 7) were similar to those of dry matter, with higher levels in the two nitrogen doses, more in the N100 than in the N50. During the vegetative cycle only in few stages were found significant differences between N50 and N100 compared to N0, and sometimes with both nitrogen doses and other times only with N100. The control had continuous low values, but it recovered well in the heading and ripening stages (Feekes' scale 10.5-11). This can be attributed to a premature yellowing of the leaves in nitrogen-fertilized plants when compared to the control. The recovery of RLD and the accumulation of dry matter produced a prolongation of the vegetative stage in durum wheat. The only significant differences between the nitrogen-fertilized plants and the control were shown to be in the boot stage, in the first year with both N50 and N100 nitrogen levels whereas in the remaining three years only with N100.

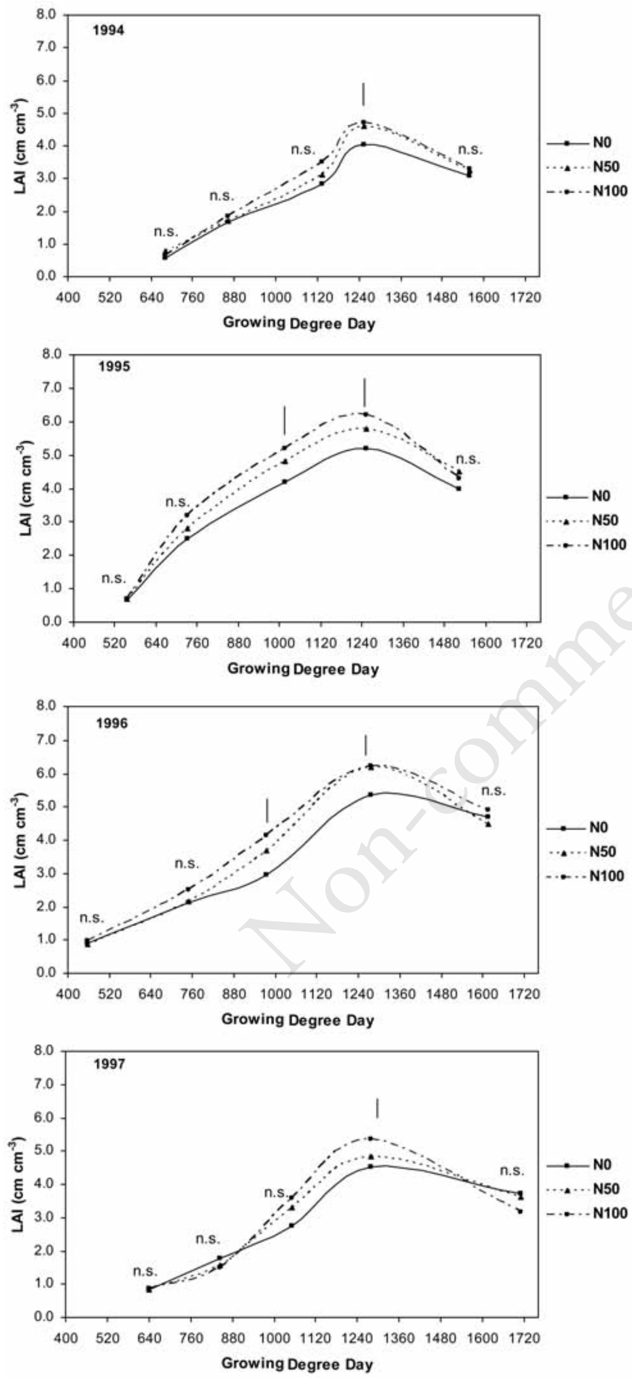

Figure 7. Dry matter of nitrogen treatments during wheat growth during four years (HSD 0.05 bar statistical significant difference between the treatment for every step of soil profile).

Yield component and grain production

Both levels of nitrogen fertilizer (N50 and N100) had positive effects on grain yield and percentage of yellow berry grain with significant differences compared to the control treatment N0 (Table 1). The highest nitrogen dose (N100) caused an increase in the percentage of stunted kernels compared to both the N50 and the control sample which had similar percentages. The number of kernels increased progressively with increasing of N doses, however, significant differences were only noted between N100 and N0. Spike length and plant height, measured to the last leaf, increased with higher doses of nitrogen and showed significant differences, the former with N100 compared to N50 and N0, the latter between N100 and N0. The weight of 1000 seeds in the trial area did not show significant increases with nitrogen inputs. The high incidence of stunted kernels with N100 caused a reduction in the 1000 seed weight. In brief, N fertilization led to an increase in wheat growth in terms of a higher number of kernels per spikes and a greater grain yield, but the highest nitrogen dose (N100), which limited its stimulating action on production, produced the highest percentage of stunted kernels and a yield similar to that produced by N50. In trial conditions, the wheat fertilized with 100 kg ha^{-1} of N, which had a more superficial root system, was more affected by abiotic stress that led to a precocious yellowing of leaves and a reduction in the vegetative activity. As a result there was a rapid slow in kernel's growth and an increase in stunted kernels.

Table 1. Grain yield and biometric parameters of durum wheat (four-year average) 1994-1997.

Parameters	Nitrogen levels		
	N0	N50	N100
Grain yield (t ha^{-1})	3.02^{B}	3.62^{A}	3.78^{A}
Yellow berry (%)	29.7^{A}	10.5^{B}	7.3^{B}
Stunted kernels (%)	6.8^{B}	6.4^{B}	11.0^{A}
Kernels (n m^{-2})	4998.0^{B}	5862.0^{AB}	6555.0^{A}
Harvest index (%)	39.6^{A}	37.3^{A}	37.0^{A}
1000 seed weight (g)	52.5^{A}	52.2^{A}	50.5^{A}
Spike length (cm)	5.4^{B}	5.6^{B}	5.9^{A}
Plant height at last leaf (cm)	54.5^{B}	57.5^{AB}	59.3^{A}

^{A,B}The row values in each year with different letters are significantly different at P<0.05 (SNK test).

Discussion

The results of this research have shown that, in the trial area with the highest dose of nitrogen (100 kg N ha^{-1}), the wheat root system is not stimulated to explore deep soil layers. The effect of N fertilization can be seen throughout the growth process until heading stage (see notes), but in the following ripening stage it dies away. The meteorological monitoring, in the same site of the experimental field, shows the negative effects of low rainfall and rapid rises in temperature between the heading and ripening stages. These conditions lead to a rapid slow in kernels growth and a higher incidence of stunted kernels, which have obvious negative effects on both the quantity and quality of grain. Similar results were found by Fageria and Moreira (2011). They carried out a study on the role of mineral nutrition on root growth and reported that the N fertilization at different soil depths can influence the development of root system, and consequently can vary the ability of plant to respond to the stress factors (water deficit, thermal condition, *etc.*).

The durum wheat, fertilized with 100 kg N ha^{-1} and so not stimulated to deepen the root system, reduces the capacity to take up water and nitrogen from deeper soil layers, therefore it is more subjected to water-deficit stress. Other studies conducted by Hoad *et al.* (2001) and Svoboda and Haberle (2006) found that higher application rates of nitrogen fertilizer reduced root growth in deeper soil layers while increased the root density in the soil surface.

Different results both on root growth and epigeal biomass have been achieved in areas where the issue of water-deficit is less critical or where there is a combination of nitrogen fertilizer with different conditions of water availability. The variability of root growth in relation to water and nitrogen availability and thermal time during tillering and stem elongation and between the years has been the subject of numerous studies (Katterer *et al.*, 1993; Brady *et al.*, 1995; Houlès *et al.*, 2007). Without the addition of fertilizers in the first phases of growth, wheat shows stunted growth but afterwards, in order to recover water resources and the necessary nutrition to satisfy its needs, the plant pushes its roots down deeper. This allows the plant to overcome water-deficit and temperature stresses and complete the process of grain ripening with greater uniformity. Other studies, conducted in different environmental conditions, reported that the development of root system can be influenced by the availability of nutrients and water at different soil depths (Mi *et al.*, 2000; Ahmadi and Baker, 2001; Talbert *et al.*, 2001; Egli, 2004).

The relationships between soil, water stress, farming methods and more specifically nitrogen fertilization have an influence on the root system development, and their understanding is essential in order to decide which farming technique needs to be applied (Robinson *et al.*, 1994; King *et al.*, 2003, Smith and Groenwold, 2005). In the trial site with low rainfall during stem extension and heading stages and with the use of 50 kg ha^{-1} N dose, the wheat showed a low root density between tillering and stem extension stages, but its subsequent recovery ensured good development of root system and allowed it to reach levels of grain yield similar to those of higher N doses.

The results of this research can contribute to better understand the effects of nitrogen fertilization on the root system development of durum wheat in hot-arid conditions.

Conclusions

The wheat plant, without or with limited inputs of N fertilizers, pushes its roots down deeper and so allowing better plant growth. With the nitrogen dose of 100 kg ha^{-1}, the root system develops more in the 20-30 cm soil layer and wheat suffers the most water-deficit and thermal stresses with an increase in stunted kernels and with no significant increases in grain yield. To sum up, in the areas with similar conditions to the trial area, taking into account the critical issues arising from the use of 100 kg ha^{-1} N dose and conversely the ability of the root system to react where nitrogen is less available, it is not recommended to use high doses of nitrogen fertilizers. Wheat uses its own energy system very economically and develops a root system of the right size to respond to its own needs, it was shown that the use of 50 kg ha^{-1} of N can be a good solution for the right balance between productivity and agronomical quality of durum wheat and environmental protection. In our environmental conditions it was shown that with the use of 50 kg ha^{-1} N dose, the level of grain yield is similar to that of 100 kg ha^{-1} N dose.

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