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Does Nitrogen Treatment Affect Leaf Photosynthetic Traits of Cork Oak (*Quercus Suber* L.) Populations?

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Abstract

The present study was conducted to assess the impact of nitrogen treatment on the physiological aspects of cork oak (*Quercus suber* L.) seedlings from acorns collected from seven sources (Kroumiry and Cap-bon). Nitrogen fertilization increased significantly specific leaf area (SLA) and leaf mass area (LMA) of *Q. suber* L grown for 2 months at nitrogen concentration 1.5 g/l, respectively. Results indicate that leaf hydraulic conductance (K_{Leaf}) increased significantly under nitrogen treatment, but no significant correlation was observed between K_{Leaf}, A and gs. Stomatal conductance (gs), transpiration rate (E), net photosynthetic rate (A) and maximum efficiency of PSII (Fv/Fm) were investigated in seedlings of *Q. suber* L. This study validated that K_{Leaf}, the stomatal conductance, transpiration rate and net photosynthetic rate of *Q. suber* were performed under increasing Nitrogen fertilizer. Stomatal conductance influences both photosynthesis and transpiration, thereby coupling the carbon and water cycles and affecting surface-atmosphere energy exchange. We found that the stomatal conductance, transpiration rate, net photosynthetic rate and maximum efficiency of PSII were showed variation in both photosynthetic traits due essentially to local genetic adaptation. However, the seven seedlings sources of cork oak (*Q. suber* L.) showed a different response of physiological aspects during nitrogen treatment. These findings suggest that nitrogen treatment affects gas exchange and the photosynthetic capacity of the cork oak. Based on the above results, we conclude that nitrogen fertilizer treatments could promote photosynthetic performance of *Quercus suber* by stimulating morphological and physiological responses.

Keywords: *Quercus suber*; Nitrogen treatment; Stomatal conductance; Transpiration rate; Net photosynthetic rate; Maximum efficiency of PSII; Leaf hydraulic conductance

Introduction

Cork oak (*Quercus suber* L.) is a typical Mediterranean resprouting species, which presents great interest for restoration in fire-prone ecosystems [1] and great socio-economic importance in the Mediterranean region [2]. Cork oak woodland (444 hectares, 70% of land utilization) is a major factor in Tunisia due to forestland with small treeless areas [3]. Attempts at restoration of cork oak stands have been largely unsuccessful because of the poor natural regeneration of this species and the high seedling mortality after transplanting [4]. In North Africa, cork oak forests extend from the Atlantic Coast in Morocco through the Algerian Coast to the North Western of Tunisia with a scattered distribution over the two Mountain ranges of Kroumirie and Mogod. Excessive human pressure is usually pointed as the main cause of the reduction in cork oak forest ecosystems [5].

As with most agricultural crop production the major driver of soil N_2O emissions is likely to be nitrogen fertilizer, added to the soil to increase biomass production [6]. Studies showed that nitrogen application exerted a significant increase in protein content and improved dough quality [7]. Several studies have investigated nitrogen fertilizer uptake, assimilation and effects on plant development [8]. Nitrogen is an essential component of chlorophyll that will reduce chlorophyll formation if lacking from the mineral nutrient supply of a plant, with concomitant effects on photosynthesis [9]. Nitrogen fertilizer plays a significant role in soil carbon sequestration by increasing crop biomass and by influencing the microbial decomposition of crop residue [10,11]. Although applications of nitrogen fertilizer consistently increase crop biomass, its effect on soil carbon content varies with the type of soil [12], which affects the flux of CO_2 into the atmosphere. Since nitrogen (N) is strongly limiting in Mediterranean forests [13], we analyzed the effects of nitrogen fertilizer on photosynthetic measurements of *Q. suber* L. Fertilizer management effects on plant water use and drought stress during production for many crops has not been evaluated fully [14]. With some woody perennial plants it is possible that growth can be enhanced more by minimizing water stress than by increasing fertility [15]. Nitrogen is a vitally important element for plant sand it profoundly influences leaf anatomical and functional traits [16]. Previous studies have shown that leaf nitrogen promotes A by increasing Rubisco content and CO_2 diffusion conductance [17]. However, the correlation of leaf nitrogen content per leaf area with K_{leaf} remains to be investigated.

The increasing demand for cork and the low natural regeneration of this species clearly justify intensive planting with improved material. In the present study, we have investigated the possibility that nitrogen fertilizer might be beneficial for cork oak growth and development by analyzing physiological parameters including photosynthetic and gas-exchange parameters in nursery seedlings from different provenances in Tunisia.

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The study sought to compare leaf hydraulic conductance, transpiration rate, stomatal conductance, photosynthetic rate, maximum efficiency of PSII in response to nitrogen treatment on cork oak (*Q. suber* L.).

Materials and Methods

Experimental description and plant material

The study was carried out on cork oak (*Q. suber* L.) seedlings growing in the nursery located in the greenhouse of INRGREF (Tunis) under semi-arid bioclimate. *Q. suber* seedlings were collected from seven different locations in Kroumirie and Cap Bon in Tunisia (8 individuals from each populations of Sidi Zid (SZD), Kef Errand (KER), Djebel Serdj (JES), Djebel Chehid (JCH), Ain senoussi (AS), Feija (FEJ), Méjen essaf (MEJ)). The plants were growing 2 months at a nitrogen concentration of 1.5 g/l. The experiments use nitrogen fertilizer: Ammonium nitrogen (NH^4-N).

The experimental design in the greenhouse was performed with a completely randomized design of 4 replicates per treatment. Plants of each population were fertilized twice a week from July 2015 to August 2015. The experiment was conducted on 6 month-year-old plants of *Q. suber* L.

The stomatal conductance, transpiration rate and net photosynthetic rate were measured on fully expanded leaves at similar development stages with portable open gas-exchange analysis systems with leaf chambers (LI-6400, Li-Cor, Inc., Lincoln, NE, USA).

The maximum quantum efficiency of photosystem II (Fv/Fm) was measured on the same leaves as above with a portable fluorometer. The leaves were dark-adapted with clips for 20 min. The minimal fluorescence (Fo) was measured under a weak pulse of modulating light over 0.8s and maximal fluorescence (Fm) was induced by a saturating pulse of light (8000 mmol $m^{-2}s^{-1}$) applied over 0.8 s. Then, Fv/Fm was calculated, where Fv was the difference between Fm and Fo.

Leaf hydraulic conductance (k_{Leaf}; mmol $m^{-2}s^{-1}$ MPa^{-1}) for leaves was measured using the in situ evaporative flux method, with kL calculated as [18]:

$$k_{leaf} = E/\Delta\Psi_{stem\text{-}leaf}$$

Where E is the transpiration rate (mmol $m^{-2}s^{-1}$) and $\Delta\Psi_{stem\text{-}leaf}$ is the difference between stem xylem water potential (Ψ_{stem}; MPa) and leaf water potential (Ψ_{leaf}; MPa).

Statistical analysis

Effects of fertilization (N) on the morphological and physiological parameters were tested by ANOVA. Tukey's test was used to evaluate differences between the means ($P \leq 0.05$). The relationship between net photosynthesis (A), leaf hydraulic conductance (K_{Leaf}) and stomatal conductance (gs) was tested by linear regression.

Results and Discussion

For above ground plant tissue, our results show that the specific leaf area (SLA), leaf mass area (LMA) of the seedlings subjected to nitrogen fertiliser significantly increased when compared with non-N fertiliser (Figure 1) [19]. Contrary observed an exponential reduction of the total leaf area for Quercus canariensis. The specific leaf area and leaf mass area of *Q. suber* L. increased significantly in the nitrogen fertilization condition (Figure 1). The small effect of nitrogen on photosynthetic parameters suggests that the leaf photosynthetic rate remained constant, which is in agreement with studies of Lambers et al. [20] and McDonald et al. [21] showing little dependence of leaf photosynthesis on nitrogen supply. Increasing the rate of nitrogen supply increased the SLA and the organic nitrogen concentration in the leaf tissue. Nitrogen is a vitally important element for plants and it pro-foundly influences leaf anatomical and functional traits [16].

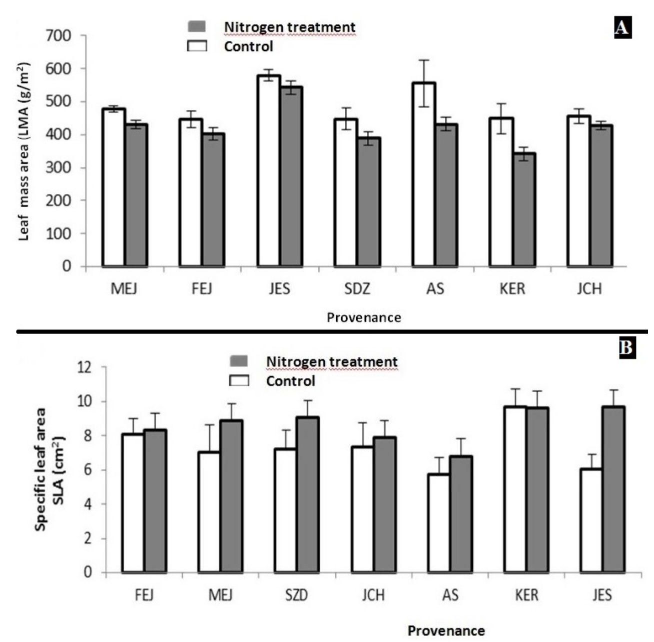

Figure 1: A. Leaf mass area (LMA) expressed as leaf m^2 per ground m^2 and; **B.** Specific leaf area (SLA) expressed as leaf m^2. Values are average of each treatment (n=8).

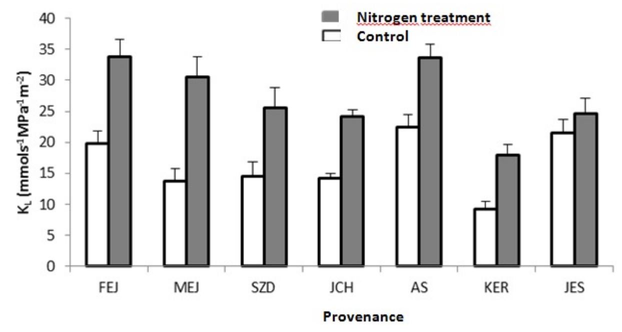

Figure 2: Leaf hydraulic conductance (K_{Leaf} mmols^{-1}MP$a^{-1}$$m^{-2}$) of cork oak (*Q. suber* L.) under nitrogen fertilizations. Mean values ± standard errors. Vertical line indicates statistical difference, while ns stands for not significant according to LSD ($p \leq 0.05$).

Leaf hydraulic conductance (K_{leaf}) is a major determinant of photosynthetic rate in plants. Results indicate that leaf hydraulic conductance increased significantly under nitrogen treatment (Figure

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2), no significant correlation was observed between K_{Leaf}, A and gs (Figure 3). Previous studies have shown correlations between A, gs and K_{Leaf} across a wide range of species [22,23]. The coupling of stomatal conductance (gs) to CO_2 and water vapour leads to strong coordination between gs and K_{leaf} [24,25]. In the present study, A and K_{Leaf} were not correlated with gs in the cork oak (Figure 3).

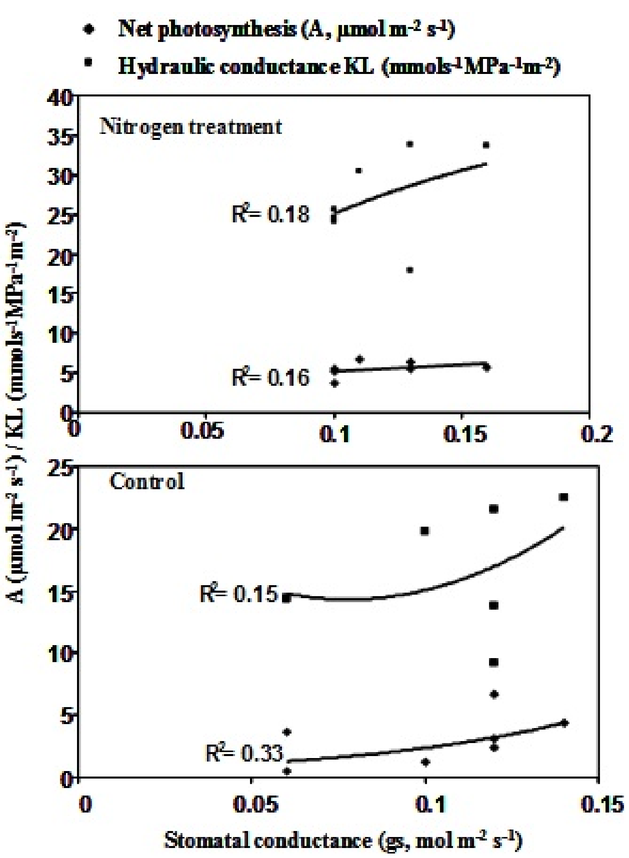

Figure 3: Relationships between net photosynthesis (A), $_{leaf}$ hydraulic conductance (KL) and stomatal conductance (gs) of cork oak (*Q. suber* L.) in control and nitrogen treatment condition. The values shown are mean ± SDand data were fitted by linear regression. Regression coefficients and significance are shown when P<0.05.

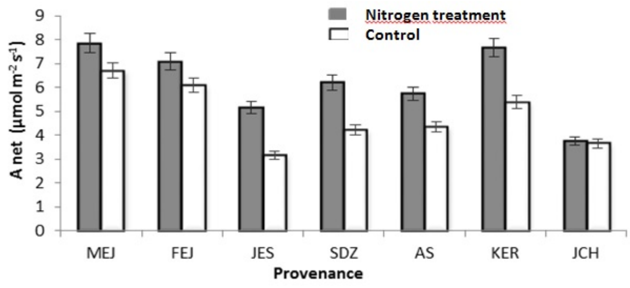

Figure 4: Net photosynthesis (A, µmol $m^{-2}s^{-1}$) of cork oak (*Q. suber* L.) under nitrogen fertilizations. Mean values ± standard errors. Vertical line indicates statistical difference, while ns stands for not significant according to LSD ($p \leq 0.05$).

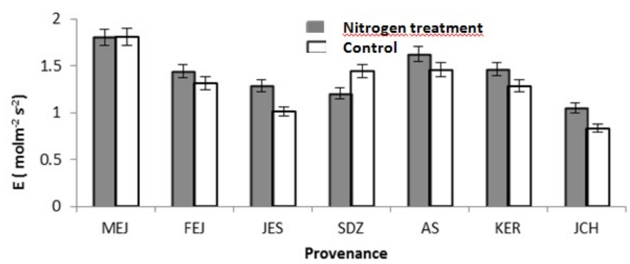

Figure 5: Transpiration rate (E, mol $m^{-2}s^{-1}$) of cork oak (*Q. suber* L.) under nitrogen fertilizations. Mean values ± standard errors. Vertical line indicates statistical difference, while ns stands for not significant according to LSD ($p \leq 0.05$).

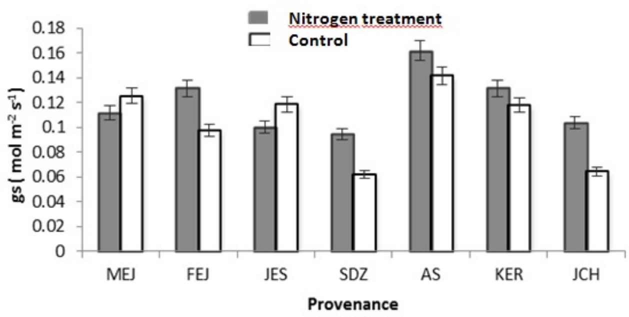

Figure 6: Stomatal conductance (gs, mol $m^{-2}s^{-1}$) of cork oak (*Q. suber* L.) under nitrogen fertilizations. Mean values ± standard errors. Vertical line indicates statistical difference, while ns stands for not significant according to LSD ($p \leq 0.05$).

E, gs, A and FV/Fm differed from cork oak between sites. Nitrogen fertilization and source of cork oak no significantly influenced E, gs, A and Fv/Fm. Indeed A, E, FV/Fm and gs showed similar trends for cork oak (*Q. suber* L.) from the seven sources (Kroumirie and Cap-bon). The highest A values were measured for cork oak (*Q. suber* L.) in the nitrogen fertilization condition. Maximum A values (7.87 µmol CO_2 $m^{-2}s^{-1}$) were found in MEJ, while the lowest in JCH with 3.76 µmol CO_2 $m^{-2}s^{-1}$, respectively (Figure 4). Similarly, the highest E values were measured in MEJ, 1.80 mol H_2O $m^{-2}s^{-1}$, the lowest in JCH, 1.05 mol H_2O $m^{-2}s^{-1}$ (Figure 5).

The highest gs in the nitrogen fertilization condition was in AS (0.16 mmol $m^{-2}s^{-1}$) and the lowest in SDZ (0.1 mmol $m^{-2}s^{-1}$). The lowest values were registered from SDZ (0.06 mmol $m^{-2}s^{-1}$), while in AS gs approached the highest values (0.14 mmol $m^{-2}s^{-1}$), mostly for the control (Figure 6).

Chlorophyll fluorescence (Fv/Fm) not differed among seedling sources as well as among nitrogen fertilization treatment. Fv/Fm represents the maximum photochemical efficiency of PSII, which is an important index of the degree of environmental stress. Fv/Fm of each treatment slightly decreased when compared with the control (Figure 7). For all cork oak (*Q. suber* L.) sources, nitrogen fertilization treatments showed no effect in Fv/Fm, however, there were no significative differences in the induction of Fv/Fm among the seven seedling sources (Figure 7). Under well watered condition, quantum efficiency of photosystem II (Fv/Fm) ratio in bioregulators treated plants was not showing significant variation. Plants treated with N fertilizer maintained higher quantum yield of photosystem II (Fv/Fm) than the control just in JCH and AS seedling sources. Chlorophyll fluorescence dynamics are an ideal internal measure of photosystem injury from various stresses [26,27] and can rapidly and sensitively reflect the relationship between the environment and the plant's photosynthetic physiological state.

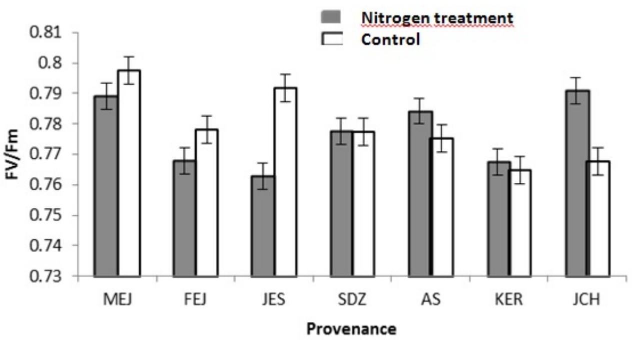

Figure 7: Maximum efficiency of PSII of cork oak (*Q. suber* L.) under nitrogen fertilizations. Mean values ± standard errors. Vertical line indicates statistical difference, while ns stands for not significant according to LSD (p ≤ 0.05).

The above-mentioned photosynthetic changes may play an important role in determining how cork oak plants adjust their photosynthetic. Photosynthetic traits decreased due to a biochemical and morphological acclimation and environmental constraints, such as water and nutrient supply [28,29]. Those changes are associated with decreases in the nitrogen concentrations and the increase of LMA due to the assimilation of photosynthetic products [28]. Plants are therefore expected to make an optimal nitrogen allocation into leaf photosynthetic proteins to achieve the maximum carbon gain in their own light environment [30]. These physiological changes are also associated to morphological modifications during stress, as for example a reduced leaf area [31].

Moreover, photosynthesis is aptly regarded as the most important physiological process in plants which is particularly sensitive to effects of water deficiency [32]. Even a small decrease in the water potential of a plant causes its stomata to closeand decreases the intensity of photosynthetic assimilation of CO_2 [33]. The reduction in photosynthetic activity under stress takes place due to decline in CO_2 availability caused by the restriction of CO_2 diffusion [23] and inhibition of ribulose-1,5-bisphosphate (RuBP) synthesis [33,34]. Our results suggest that more detailed anatomical and structural studies are needed to elucidate the impacts of leaf feature traits on K_{leaf} and gas exchange in cork oak.

Conclusion

The present study was motivated by the need to examine the nitrogen treatment and genetic sources influences on photosynthetic traits variation among seedlings of cork oak (*Quercus suber* L.) sources to assist reforestation decisions. Our study revealed that (i) The specific leaf area (SLA) and leaf mass area (LMA) increased significantly in the nitrogen fertilization condition (ii) the seedling sources of *Q. suber* L. differed in maximum efficiency of PSII (Fv/Fm), photosynthetic rate (Anet), transpiration rate (E) and stomatal conductance (gs) (iii) the seedling sources demonstrated different responses of photosynthetic traits under nitrogen treatment, suggesting that the observed variation in growth among seed sources resulted largely from genetic variation in functional traits rather than from photosynthetic traits. Present results may have practical applications to help maximize physiological performances and optimized resource use efficiency but the physiological mechanisms need further elucidation. The genetic basis of photosynthesis affected photosynthetic traits in various plants. However, the genetic relationship sources and photosynthesis remains to be elucidated.

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