Effect of Water Deficit on Gas Exchange Responses to Intercellular CO₂ Concentration Increase of *Quercus suber* L. Seedlings

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Abstract

In north Tunisia, the *Quercus suber* L. forests have shown a great decline indices as well as a non-natural regeneration. The climate changes could accentuate this unappreciated situation. In this study, the effect of water deficit on physiological behavior of *Quercus suber* seedlings was investigated. Photosynthetic responses of 15 months old Cork oak seedlings grown for 30 days under 40% and 80% soil water water content (control) were evaluated. Results showed a negative effect of water deficit and a positive effect of the intercellular CO₂ concentration increase both on photosynthesis and transpiration. Stomata conductance might play a major role in balancing gas exchanges between the leaf and its environment. Moreover, global warming could negatively affect carbon uptake of Cork oak species in northern Tunisia. Elevated CO₂ leaf content will benefit Cork oak growing under water deficit by decreasing both photosynthesis and transpiration, which will decrease either the rate or the severity of water deficits, with limited effects on metabolism. the results suggest that high intercellular CO₂ concentration could increase water use efficiency among Cork oak species.

Keywords: *Quercus suber* L., Tunisia, climate change, carbon uptake, water use

1. Introduction

The cork oak (*Quercus suber* L.) is native to western and central Mediterranean region, and appears in the coastal regions of southwest Europe and northwest Africa such as France, Italy, Portugal, Spain, Algeria, and Tunisia (Allard et al., 2013; WCSP, 2017). The most extensive forests are situated in the Atlantic coast of the Iberian Peninsula (Varela, 2008). *Quercus suber* forests cover almost 1.5 million ha in Europe (Barstow & Harvey-Brown, 2017) and 700 000 ha in North Africa (FAO, 2013). The Cork oak forest has played an important ecological and socio-economic role concerning mainly the biodiversity and sustainable forest production in these areas (WWF, 2016). In the northern Tunisia, It is the habitat of more than 400 different species (You et al., 2016).

In the last decades, the cork oak forest has showed several declining indicators (Hasnaoui et al., 2005; Stiti et al., 2005; Bellahirech et al., 2015). The intensity and frequency of drought and the extreme temperatures are considered as the main factors contributing to the degradation of the oak stands around the Mediterranean area (Amandier et al., 2006; HaeNaem et al., 2017). This situation could be intensified in near future due to different climate changes scenarios in the North African countries (IPCC, 2014). In fact, by 2050, summer drought periods within this region will be more severe while CO₂ concentration in the atmosphere will be twice as high (Smith & Myers, 2018). For sustainable ecosystem management, a comprehensive understanding of the functional traits of this species and its response to climate change is crucial (Vessella et al., 2015, 2017; Duque-Lazo, 2018). In this context, natural regeneration of *Q. suber* seedlings in montados ecosystems is necessary to maintain the forest balance. *Q. suber* seedling establishment could be improved by modifying environmental conditions, such as drought and CO₂ concentration in atmosphere, that affect physiological characteristics (Ainsworth et al., 2007; Elizabeth et al., 2007; Rzigui et al., 2015; Bouderrah et al., 2017). In fact, drought stress reduces plant growth by affecting the net photosynthesis capacity, respiration and nutrient metabolism (Jaleel et al., 2008). Net photosynthesis, stomatal conductance and transpiration decreased...
significantly (Wang et al., 2018). According to the model of photosynthesis, drought stress had a major impact on carboxylation efficiency of Rubisco (Farquhar & Sharkey, 1982; Sharkey et al., 2007). Thus, Rubisco carboxylation and RuBP regeneration might be limiting during drought stress by measuring the net photosynthesis/Ci response (Wang et al., 2018). Furthermore, drought stress significantly reduced CO₂ assimilation rates at high Ci, while only with a certain degree of lowering photosynthesis rates at low Ci. For this reason, studies of water and carbon uses and their relationships with the soil water availability can be key elements to understand the effect of climate changes on species degradation (Adach, 2014; Vialet-Chabrand et al., 2017). Furthermore, the impact of water stress on plant structure is an important determinant of physiological adaptation under severe drought. In order to provide more fundamental knowledge of Q. suber seedling establishment and the success of natural regeneration within drought conditions, the physiological responses were studied in terms of photosynthetic performance, water efficiency and responses of gas exchange to Ci increase.

The aim of the present work was (i) to evaluate the hypothesis that elevated internal CO₂ concentration influence the development (physiological and structural) of Q. suber seedlings and decrease the water stress effect; (ii) to evaluate the effect of water stress and leaf carbon increase on stomatal conductance, leaf transpiration, photosynthesis and water use efficiency of cork oak seedlings.

2. Material and Methods

2.1 Plant Material and Experimental Conditions

Cork oak seeds were collected in the Ain Snoussi site (Latitude N: 36°47'50"~36°52'40", Longitude E: 8°52'07"~8°57'01", Altitude: 640 m a.s.l.) in December, 2008 and sown in pots in a shady site of the National Research Institute in Rural Engineering, Water and Forestry (INRGREF, Tunisia) in January, 2009. The present experiment was carried out in May and June 2010 on 15 months-aged plants. Acorns of Q. suber from a population at Ain Snoussi, were sown in 10L pots containing a mixture of nursery soil, peat and sand (v/v). At 4 months of age, the seedlings were transferred from open-air conditions to greenhouses. Two contrasting treatments of water with six replicates were realized. A control treatment with a soil water content of 80% (control) and a stress induced treatment for a period of 30 days to reach water content level of 40%. The plants were grown in greenhouses in a controlled environment: with a relative humidity of about 50%, a similar temperature to the external one and in natural light conditions.

Three well-developed and healthy plants from each treatment were chosen. Gas exchanges (Net photosynthesis (PN), stomatal conductance (Gs), and leaf transpiration (LT)) were measured with a Li-Cor Li-6400XT Portable Photosynthesis System (Li-Cor, Li-6400XT and were used to characterize photosynthesis in response to intercellular CO₂ concentration (Ci) (A/Ci curves). Then, water use efficiency was calculated as a ratio of photosynthesis-transpiration (WUE = PN/LT).

The values of PAR and temperature were fixed from the beginning to 1500 μmol m⁻² s⁻¹ and 25 °C, respectively. During the experiment, the relative humidity was maintained around 60%. The needles of each branch were placed under the clamp of the chamber assimilation (6 cm²) and they were acclimatized for 35 minutes. For each curve, the sheet has reached a steady state corresponding to a CO₂ concentration of 400 mmol mol⁻¹. This measurement lasted 30 minutes to allow the plant to acclimate to the conditions of temperature. Also, PAR, ventilation speed and CO₂ concentration correspond to the first point of the curve. Then the CO₂ concentration decreased to a value of 40 mmol mol⁻¹. Several values were recorded between 100 and 40 mmol mol⁻¹, and eight additional values were then recorded between 400 and 1800 mmol mol⁻¹.

2.2 Statistic Analysis

One-way ANOVA was performed for all collected data. Means significant differences were performed using Tukey’s HSD tests at p = 0.05. All statistical analyses were realized using the SPSS.17 software.

3. Results

It was recorded among the control set of plants (80% of water field capacity) that leaf transpiration (LT) had a maximum rate around 3.5 mmol m⁻² s⁻¹, and it showed a linear decrease due to the intercellular CO₂ concentration increase (Figure 1a). Under these conditions, the relative LT value decreased by 16% when the CO₂ concentration doubled (from 380 ppm to 760 ppm). When it comes to the stressed set of plants, the maximum value of leaf transpiration recorded was 2 mmol m⁻² s⁻¹. It decreased linearly with CO₂ increasing. It decreased in this case by 25% when the CO₂ concentration increased 2-times as in the control situation (from 380 to 760 ppm) (Figure 1b).
Figure 1. Variation of net photosynthesis (PN) depending on the internal concentration of CO2 (Ci) in irrigated (1a) and stressed (1b) cork oak seedlings.

The increase of photosynthesis (PN) with increasing leaf CO2 concentration had a logarithmic shape, saturating its value of 25 mol m\(^{-2}\) s\(^{-1}\) in irrigated plants and 10 mol m\(^{-2}\) s\(^{-1}\) in stressed plants set (Figure 2a). PN increased by 50% (from 10 to 15 mol m\(^{-2}\) s\(^{-1}\)) when the intercellular CO2 concentration increased 2-times (from 380 to 760 ppm) in irrigated plants. PN increased from 4 to 6 mol m\(^{-2}\) s\(^{-1}\) in stressed plants which was lower than irrigated ones (Figure 2b).
Figure 2. Relationships between leaf transpiration (LT) and leaf CO2 concentration (Ci) in the irrigated (2a) and stressed (2b) cork oak seedlings

The mean values of water use efficiency (WUE) were higher under stressed conditions but they increased with increasing CO2 (Figure 3a). In the control conditions, WUE increased very slightly. An increase of the current CO2 concentration (by 2-times) could affect an increase of WUE value in stressed plants more than in the control (Figure 3b).
A decreasing trend in stomata conductance (GS) with CO₂ increases was observed for Cork oak seedlings under irrigated and stressed conditions (Figure 4). GS value was maximal at the lowest Ci concentration of 40 mmol mol⁻¹ in the irrigated plants. This value reached 700 mmol m⁻² s⁻¹ but decreased quickly to 200 mmol mol⁻¹ for a CO₂ concentration of 760 ppm. However, the trend in stressed plants was less evident with lower values recorded in stressed set. Furthermore, positive and high correlation coefficients between internal CO₂ concentration and the net photosynthesis and water use efficiency were obtained (Table 1).
4. Discussion

The present study showed a positive effect of the increased CO₂ concentration in leaves on water stress adaptation of Cork oak seedlings. In fact, several studies showed that limited water availability often reduces photosynthesis in many species (Tenthunen et al., 1987; Chaves, 1991) such as Quercus sp. (Peña-Rojas et al., 2003). Several studies showed that water status has a stronger impact on gas exchange than changes in leaf CO₂ concentration (Morgan et al., 2004; Xu et al., 2014; Chen et al., 2015). Thus high Rubisco activity during drought may also indicate the alleviation of metabolic limitations caused by drought damages rather than stomata limitations imposed by elevated CO₂ (Chen et al., 2015). Within a climate changes context, the decrease in stomatal conductance (GS) under elevated atmospheric CO₂ conditions may limit the CO₂ fixation rate but promote the water use efficiency (WUE) to benefit plant growth (Leakey et al., 2009; Sreeharsha et al., 2015). Such behavior of oak was observed within other Mediterranean environments. Stomata conductance could be the key element for explaining the combined effects of water shortage and CO₂ assimilation increase (Gao et al., 2015; Xu et al., 2016). Stomata closure, which decreases in carbon dioxide (CO₂) availability to mesophyll cell chloroplasts, has been considered as the main limiting factor for CO₂ assimilation in response to water deficit (Lawlor, 2000; Lawlor & Cornic, 2002). Stomata CO₂ responses differs from one species to another (Bierhuizen & Slatyer, 1964; Warrit, Landsberg, & Thorpe, 1980). Differences are not limited to interspecific contrasts but occurred also within the same species under environmental conditions, such as water deficit. The current findings showed that stomata conductance in responses to Ci values for stressed plants were lower than those of irrigated plants; so, it is modified by water availability (van Oosten et al., 1994). It was shown also that Gs
decreased with CO₂ increase of internal CO₂ concentration (Van Oosten et al., 1994). Therefore, most species showed that the degree of stomata opening decreases with the CO₂ concentration increasing either around or inside the leaf (Brodribb et al., 2009). In fact, stomata conductance of stressed plants at 40 mmol mol⁻¹ of intercellular CO₂ concentration averaged 50% less compared with the irrigated ones. Drought conditions, showed stomatal closure, as indicated by lower Gs, marked a decline in photosynthesis, especially at high Ci concentrations (Kimball et al., 1993; Morison & Lawlor, 1999). Increasing internal CO₂ concentration lead to an increase of net photosynthetic rate while, under drought stress, this elevation is less important. Therefore, in such conditions, plants close their stomata to avoid further water loss (Dulai et al., 2006). The decrease in P(n) with water deficiency was related to lower Rubisco activity rather than to ATP and RuBP contents (Tezara, 2002).

Several studies have investigated the CO₂ responses of stomata conductance in leaves (Brodribb, 1996; Kelly et al., 2016). Physiological traits, leaf structural and biochemical characteristics may also play an essential role in plant response to high CO₂ concentration (Ayub et al., 2011). Some suggestions estimated that Gs increase in response to the reduction of the intercellular CO₂ concentration caused by mesophyll photosynthesis (Marchi et al., 2008; Jin et al., 2011). In fact, Studies reported that Ci was correlated to light intensity, mesophyll assimilation and stomata conductance (Xiong et al., 2018). Ci declined as Gs increased. Abid et al. (2016) showed that drought application at Alfalfa leaves led to a high reduction on net photosynthetic rate, stomata conductance and chlorophyll content per cons leading to an increase on the the WUE and on intracellular CO₂ concentration (Ci). Previous studies indicated that the fixation of CO₂ in the Calvin cycle is sensitive to environmental conditions (Greer et al., 1986; Altaweel et al., 2007). Under these stressful environmental conditions, the inhibition of protein synthesis do to interruption of the fixation of CO₂ might be expected to accelerate the decrease in photosynthesis.

In the same context, Wang et al. (2018) had found that under drought conditions, photosynthetic rate (PN), stomata conductance (Gs), transpiration rate (E) and intercellular CO₂ concentration (Ci), showed a strong positive correlation with the water potential state.

Comparing different Mediterranean oaks, Ksontini et al. (2007) showed a higher reduction of stomata conductance, related to the decrease of predawn leaf water potential, occurred earlier with Q. faginea, Q. coccifera kept its stomata partly open at a water potential of -3 MPa. Q. suber behaved somewhat intermediate, while both photosynthesis and stomata conductance were correlated and showed a slight decrease.

Picon (1996) showed that transpiration rate was lower under elevated Ci, reflecting compensation between leaf area and stomata control of transpiration. However carbon isotope discrimination decreased in drought state and increased in Ci. While, Aranda et al. (2007) had indicated a relationship between stomata conductance, water and net photosynthesis on leaf area basis confirming that seedlings in higher irradiance conditions maintained a higher rate of carbon uptake at a particular stomata conductance, implying that shaded seedlings have lower water use efficiency independently of the water availability.

5. Conclusion

This study confirmed the hypothesis that Q. suber seedling development is positively influenced by an increase in internal CO₂ concentration, as indicated by an increase in photosynthesis, which also induced the higher production of assimilates for growth. However, net photosynthesis is negatively affected by water stress even with the increase of intercellular CO₂ concentration.

In addition, the reduction in stomata conductance and transpiration promoted higher water use efficiency especially in stressed plants. An increase in the intensity and frequency of summer drought with climate change might have a negative impact on natural Q. suber regeneration, due to the suppression of the positive effects of elevated intercellular CO₂ concentration. Therefore WUE is the most interesting criteria to monitor the cork oak forest. Further research on the water use efficiency on stand level and of different provenances is probably an important way to understand and to evaluate the effect of climate changes on the cork oak species.

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