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Leaf mass per area and nitrogen content in cork oak (*Quercus suber* L.) under a range of climatic stress (drought and temperature stress)

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Key words: Cork oak (*Quercus suber* L.), climate change, kroumirie, relict areas, leaf mass per area, dendrometric parameters, nitrogen contents.

Abstract

The determination of the diversity of Cork oak tree (*Quercus suber* L.) could be a key factor in understanding the response to climate change. This experiment was carried out on 16 stands of Cork oak. Two areas were identified: The Kroumirie and Relicts areas . We try to understand through this study how these populations persist under adverse conditions (water and temperature stress) to provide valuable background information for the development of appropriate strategies for their conservation and management and to estimate the spatial variability of population growth of Cork oak belonging to two different areas. The climate effect study on leaf mass per area (LMA), dendrometric parameter (diameters in 1,30m (DBH) and height) and nitrogen content (N%) were considered, We observed a significant difference in LMA according to altitude and temperature, highest values were obtained in the sites with a high altitude and low temperatures .There are also a significant correlation between dendrometric parameters and LMA . For nitrogen content, we found negative correlations with LMA values, they are lower in drier and colder sites.

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Introduction

Mediterranean forests are subjected to a combined pressure of global climate changes and deforestation which could modify rapidly their local climatic conditions (Menzel *et al.,* 2006). Research into the adaptations of forest to stress factors has long attracted the attention of scientists because plant adaptations are crucial in determining the functioning of ecosystems (Wright *et al*., 2004).

In Tunisia, the Cork oak forest has undergone several incidents of degradation with a low regeneration rate. The regression of the Cork oak is estimated at 1.22% per annum (Ben M'Hamed *et al.,* 2002).However, its surface area has decreased dramatically, from 148.,000 to 70.,000 ha, between1920 and 2005 (Selmi., 2006), this decline has been attributed to single or combined effects of climatic extremes (winter frost, summer drought) (Thomas *et al*., 2002), insects or fungi attacks or human action by deforestation and cultivation practices as well as by bad stripping (Ben M'Hamed *et al.,* 2002).

In addition to these factors, climate changes recently observed exacerbate the alarming situation of Cork oak (*Quercus suber L.*) forest (Urbieta *et al.,* 2008), involving deteriorations and return the it's ecosystem very sensitive to natural regeneration **(**Kanouni *et al*., 2012). The ecophysiological function of the Cork oak is strongly affected by changing climatic. Among the morphological traits that undergo the most pronounced change is leaf mass per unit area (LMA), which has a huge number of implications as regards leaf productivity (Wright *et al*., 2002). The variations in LMA and other morphological leaf traits have mainly been analyzed with respect to differences in nutrient and drought stress (Turner, 1994a ; Wright *et al.* 2002, Jian *et al*., 2009; Uğurlu and Oldeland., 2010). There are some examples, such as that of Ogaya and Peñuelas (2007), who have shown in Quercus ilex that populations from colder sites tend to maintain a higher LMA than those from warmer areas. It has also been observed that temperature may affect LMA directly (Atkin *et al.* 2008; Oliveira and Peñuelas., 2000), although most of these analyses have been based on data obtained in young plants in controlled environments and this is not necessarily representative of how mature plants would respond to temperature in the field. At the same time, leaf nitrogen content (N%) is an indicator of growth and fertility that quantifies the energy and material flow in the ecosystem. Similarly, biometric parameters constitute direct indicators of the sanitary status of populations.

Therefore, assessment of the impacts of climate extremes upon in Cork oak can help us produce better forest management practices for coping with future climate change.

This work intends to evaluate the growth parameters of Cork oak populations in the Kroumirie and Relict areas, to illustrate their variabilities according to an environmental gradient. This approach will describe the ecological functioning and dynamics of Cork oak forests in response to local environmental conditions. This could eventually lead to improve predictions of the evolution of the distribution of this species in the context of climate changes. This study allowed us to classify our populations along a gradient of fertility in a perspective of reforestation and to estimate the vulnerability of these populations and to target those most sensitive and resistant to face a possible future climate hardening.

Materials and methods

Study area and sampling species

The study was conducted on adult trees of Cork oak (*Quercus suber L.*).Two areas were identified: The Kroumirie (Ten provenances studied) and Areas relicts (Six provenances studied) belonging to two different bioclimatic stages (sub-humid and semi-arid) (Table 1) and (fig.1).

Table 1. Origin and geographic position of cork oak(*Quercus suber L.*) Provenances.

	Site name	***Provenance***	***Altitude (m)***	***Geographic coordinates***		***N***
				Width	***length***	***Individuals***
Kroumirie populations	Bizerte	Bellif(BL)	158	37°04'N	9,07'E	10
	Nefza	Ghorgalia (Gh)	166	37,07'N	9,04'E	10
	Nefza	Tabbouba (Ta)	353	36,88'N	9 ,08'E	10
	Tbarka	Djbel Kroufa (Dk)	357	36,47'N	8,24'E	10
	Tbarka	Wechtata (W)	151	36,49'N	8,26'E	10
	Ain Drahem	Beni Metir (BM)	373	36,67'N	8,78'E	10
	Ain Drahem	Dar Fatma(DF)	862	36,65'N	8,63'E	10
	Ain Drahem	Mejen Sef (Ms)	552	36,70'N	8,67'E	10
	Ain Drahem	Ain Zana (AZ)	690	36,82'N	8,77'E	10
	Ghardimaou	Feija(F)	793	36,89'N	8,77'E	10
Relict population	Grombalia	J.bouchoucha (JB)	364	36°50'N	10.44'E	10
	Sliman	J.Abrehaman (JA)	466	36.85'N	10.78'E	3
	Zagaouan	Jbel zid (JZ)	933	36.47'N	10.31'E	10
	Siliana	Jbel sarj (JS)	709	35.92'N	9.51'E	10
	Kasserine(Thala)	Jbel Mgila (JM)	1087	35.40'N	9.22'E	5
	Kasserine(sbitla)	Jbel Biranou (JBi)	1266	35.47'N	8.63'E	5

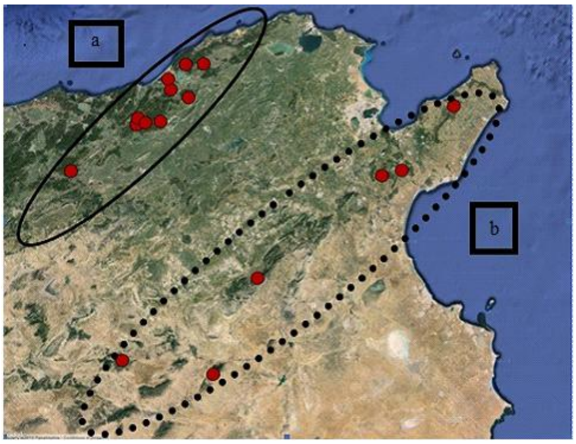

Fig. 1. Collection sites of the sixteen of cork oak provenances in Tunisia Kroumirie (a) and Relict (b) populations.

Regional climate analysis

Annual daily data from the station nearest to each plot were provided by the National Meteorological Institute of Tunisia (INM).

Regional climate analysis was made using data of daily temperatures recorded via buttons Thermo Type 22L (-40 / +85 ° C) located in the kroumirie.

Measurement of dendrometric parameters

The measurements were made on the individuals of the entire plot. The dendrometric parameters (diameter at breast height (DBH) and tree height (H) on the basis of which wood volume was calculated for each provenance.

Measurements of leaf mass per area

At each site, a sampling of Cork oak leaves was performed in August 2012, on the seven representative trees within each population. Mature leaves were collected randomly on each sampling. The foliar surface masses are obtained by estimation of the average surface of the leaves and measure of the dry weight (The total projected leaf areas were determined by an image analysis system (Delta-T Image Analysis System, Delta-T Devices Ltd., Great Britain).

LMA (g / m^2) = dry mass (g) / foliar Surface (m^2).

Chemical parameters

Nitrogen content (%N): is directly correlated to photosynthetic capacity, were obtained by the Kjeldahl method (1883) consists of three steps.

Extraction, Distillation and Titration

Extraction is based on the digestion of 0.4 g of leaves, the samples were digested with a hydrochloric acid so that it releases nitrogen. The samples were weighed and digested by the sulphuric acid, and anhydrous sodium sulphate. Digestion converts nitrogen into ammonia, CO_2 and H_2O. The ammonia gas liberated from the solution moves into the receiving flask which contains excess of boric acid. The low pH of the solution converts ammonia gas into ammonium ion, and simultaneously converts the boric acid to the borate ion. The nitrogen content was estimated by titration of the ammonium borate formed with hydrochloric acid...

N(%) = Quantity HCL * 0.14 *6.25 / Dry weight (0.4g)

Data analysis

The relationships between leaf traits, Nitrogen content (%N), and dendrometric parameters (DBH, H) were described using the software STATITCF (ver.F). The measures were the object of an analysis of the variance to one or two factors following the case, for all statistical determinations, significance levels were established at $P<0.05$.

The differences between provenances for the investigated variables were tested with a Principal Component Analysis (PCA). All calculations were done by the using of XLSTAT 2011.

Results

Regional climate analysis

Lower temperatures during the colder months and higher temperatures for the hot months are registered in Kasserine and jendouba, lower temperatures during the warmer months is observed in Aindrahem. The average temperature is also showed in Tbarka and Grombalia (fig.2A).

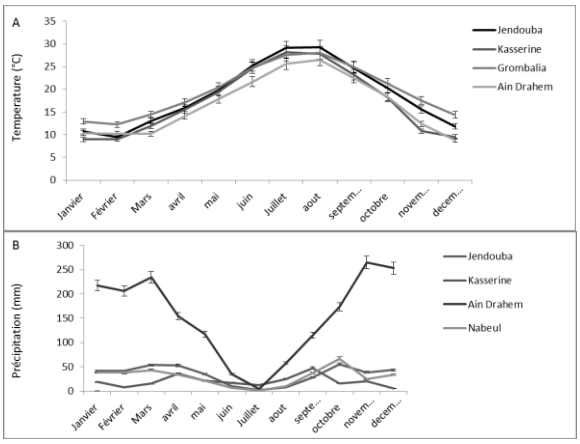

Fig. 2. Average monthly temperature (A) and rainfall (B). Data were obtained from the nearest meteorological station.

Nevertheless, there is a trend to have higher rainfall levels in the sites with a bioclimate Sub-wet in the cold months as Ain Drahem, minimum at Tbarka and Nabeul : very lower rainfall in the coldest sites (jendouba and kasserine) which helps to reduce the differences in the intensity of drought stress among cold and hot sites (fig.2B).

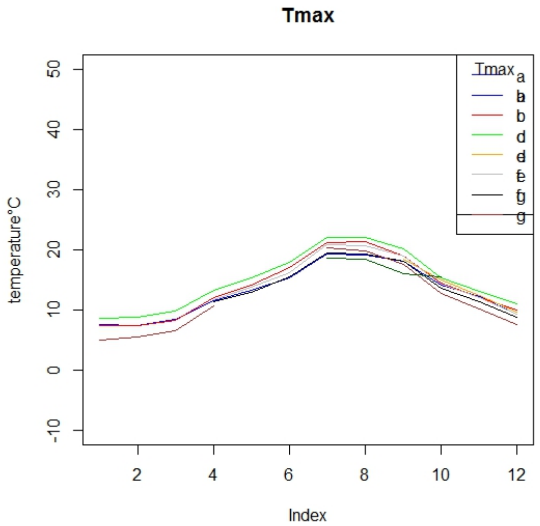

Fig. 3. Average Daily temperature for kroumérie provenances at August 2010 to June 2013.

For the average, daily temperature for kroumérie provenances, the highest temperature is found in Tabouba(d) and wechtata(b) (32,5 °C in the summer and 12.9 °C in the winter) (fig.3) and the minimum is recorded at Feija (g) (5,6°C). The average of temperature is registred in Beni metir (e) and Dar fatma(f) during the winter(9°C) or summer (20°C) .

Measurement of leaf mass per area, Nitrogen content and dendrometric parameters.

The results of analyses variances is shown in (Table 2), whiles graphs for each dimension are shown in figure 4 and 5. The statistical analysis shows a highly significant difference for different structural parameters (Table 3).

Table 2. Quantitative parameters scored of the sixteen populations of Cork Oak.

Population	*LMA (g/m²)*	*(% N)*	*Height (m)*	*DBH (m)*
Bellif(BL)	138,06 ±10,63	18±1,07	8,29±1,40	1,52±0,31
Ghorgalia (Gh)	131,52±41,35	14,5 ±1,15	6,51±0,42	0,89±0,17
Tabbouba (Ta)	121,91±25,86	16,5 ±1,25	11,9±1,91	1,32±0,38
Djbel Kroufa (Dk)	131,04±16,60	16,5 ±1,35	13,17±1,97	1,74±0,48
Wechtata (W)	205,38±57,21	13±1,41	5,72±1,29	0,64±0,14
Beni Metir (BM)	118,97±17,39	20±2,05	14,58±0,95	1,98±0,28
Dar Fatma(DF)	167,7±41,39	15,5±0,62	13,28±1,90	1,73±0,28
Mejen Sef (Ms)	128,87±20,97	16,5±0,92	7,58±1,36	0,97±0,25
Ain Zana (AZ)	157,43±37,28	13,5±1,42	9,11±1,65	0,66±0,20
Feija(F)	125,7±10,55	21±1,29	11,55±1,51	1,62±0,33
J.bouchoucha (JB)	164,7±20,34	16±1,15	6,65±1,11	0,57±0,14
J.Abrehaman (JA)	250,87±66,17	14,9±1,01	3,86±0,30	0,25±0,07
Jbel zid (JZ)	181,96±21,93	19±0,70	6,43±1,007	1,08±0,31
Jbel sarj (JS)	248,38±98,14	18,7±1,59	6,9±1,74	0,84±0,22
Jbel Mgila (JM)	271,07±93,14	15,1±1,08	-	-
Jbel Biranou (JBi)	228,12±45,35	14,9± 1,73	-	-

Table 3. Results of ANOVA for analysed variables depending the altitude.

	DL	*MC*	*F*
DBH	81	11799	10,287**
H	81	3,1423	17,467**
LMA	81	1893,6	7,819**
Teneur en N	81	186,36	7,412**

** Significant at the 5%

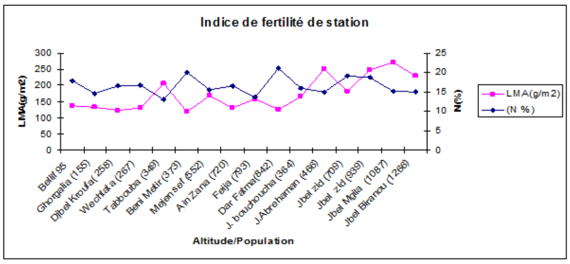

Fig. 4. Relationship between LMA (leaf per mass area) of Q. suber and nitrogen contents (%N).

In Kroumirie, The values of LMA show a significant variation between different populations. From the results of analysis of the variance, the values of LMA shows a significant variation which it is evident that the provenance Tabouba has a statistically significant higher LMA (205,38g / cm ²), while the provenances Beni metir (118,97g/ cm ²) have a statistically significant lower LMA (fig.4). Besides the lowest LMA values, this provenance also showed the highest value of nitrogen contents N (20%). The lowest value of nitrogen content was seen in the provenance Tabouba (13%) (fig. 5).

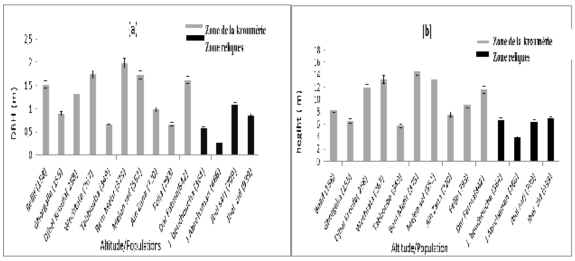

Fig. 5. Morphological characteristics of Cork Oak : (a)= DBH and (b)= averaged-height.

For relicts' areas, J.Mgila and J.Abderhaman present the highest values of LMA. The minimum values are in J.Bouchoucha (fig.4). These results indicate a wide variety of situations sampled. Highest values were observed in areas with high altitudes , dry climate and low water reserves as J. Mgila and J. biranou ; in areas that are in the limits of Cork oak distribution (Tabouba) as well as in areas with a low density (3 feet only) (J.abderahman). For nitrogen contents (% N), we found a wide variability from 15% in J.Migila population to 25% in J. Sarj population (fig.4). Moreover, a significant variation was identified and was negatively correlated with the Leaf Mass per Area (LMA), in some populations where conditions estimated unfavorable to the Cork oak growth (fig.4). Otherwise LMA was positively correlated with total annual rainfall. DBH analysis showed that the mean diameter for all Cork oak provenances amounts to 1.35 m in Kroumérie , ranging from 0.64 m for the provenance Tabouba and 0,66m for the provenance of Feija up to 1.98 m for the provenance Béni métir (fig.5a). The mean height of the analyzed provenances amounts to 10.16 m. The best height was shown by the provenance Béni métir (14.58 m) which originates from lower altitudes, while the lowest height was shown by the provenance Tabouba (5.72 m) (areas that are within in the limits of Cork oak distribution) and the provenace of Ain Zana (7,58m) from higher altitudes (fig.5b).At relicts Zones, the lowest values of circumferences and heights of Cork oak trees were observed in J.Aberrahman and the highest values are identified in j.Bouchoucha and J.sidi Zid. Fig.5 (a.b). Dendrometric parametres shows that the populations that have significant weaker values are those where the climatic conditions are the worst.

Dissimilarity of Pearson based on Aggregation method was found to separate the provenances into three clusters at a mean distance of 0.35: cluster 1included population: w, Dk and BM, cluster 2 linked population GH , BL and cluster 3 included the rest of populations. It can be divided into two sub-clusters: sub cluster 1 with three populations (JB, Tb andJA) and sub cluster 2 with eight populations (the most distant one) (F, AZ, DF, Ms, JZ, JS, JM and JBi) (Fig 6). According to the amount of variation of some parameters, population from Jbi et JM was clearly separated from the rest of populations. These areas have an unfavorable climatic conditions and we find some Cork oak trees (very low density). It was distinguishable by the High LMA , low nitrogen contents and high altitude.JS has a very similar conditions to that of JBi and JM. These observations have allowed us identify the populations most fertile , most resistant face future climates hardening (cluster 1 and cluster 2) and the most vulnerable populations (cluster 3) (Fig.6)

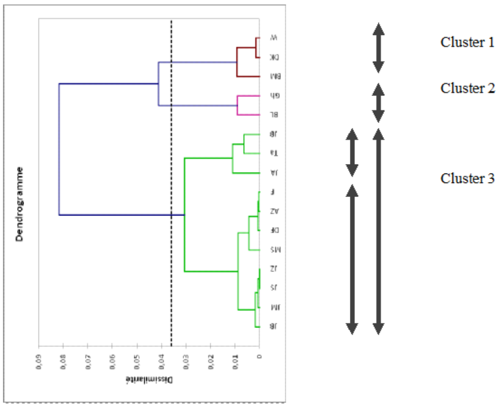

Fig. 6. Dendrogramme of Cork Oak L. populations clustered based on Dissimilarity of Pearson Method = Aggregation.

Discussion and conclusion

This study aims to elucidate the impact of climate and environmental conditions on the distribution of Cork oak (*Quercus suber* L.). Under harder climatic conditions such as lower water availabilities and high temperatures; Cork oak tends to have more leaves with higher LMA, which is more efficient to maximize photosynthetic gain and invest more resources in strong and rigid leaves to resist climatic adversities (Ogaya *et al.* 2011). High LMA values in Mediterranean vegetation are often related to leaf resistance to dry conditions (Niinemets, 2001), and to high vapour pressure deficit and potential evapotranspiration (Wright *et al.*, 2004). The greater LMA normally shown by the leaves at the drier sites have been interpreted as a mechanism that allows leaves to increase their resistance to drought and improve their water use efficiency (Niinemets 2001; Turner 1994b).The relationship between LMA and temperature could indirectly explain a part of the inverse relationship between LMA and (%N), because highest LMA values were found at colder sites and (%N) was inversely correlated with temperature (Ogaya and Penuelas , 2007).

Our results confirm these observations, LMA values increased also with temperature, despite of the high evaporative demand of the warmest sites .For nitrogen content, we found negative correlations with LMA values, they are lower in drier and colder sites , and in areas with high altitudes like J.Mgila, Jbiranou and Tabouba , as well as in areas with a low density (J.abderahman), these provenance showed a lowest value of DBH and H.

The best provenances in Kroumirie are Beni métir and Dar fatama were also generally superior in total values of DBH, H and %N with a lower LMA. This suggests that provenances from bioclimate subhumide, have adapted well in new bioclimatic conditions of the different geographic longitude and altitude, moisture, temperature and precipitation, presents a weather conditions which are favorable for the growth of Cork oak.

For relict population :J. Bouchoucha is the best provenances with a low altitude, highest % N; DBH and H and a weak LMA, is considered the most fertile population that could be considered as an ecotype of reforestation in the future.

According to Mediavilla *et al* (2012), LMA and leaf thickness were the two traits that showed the most pronounced response to changes in temperature. In general, climate influence on leaf traits was proved and LMA values increased when temperature increased (Wright *et al.*, 2004). The morphological changes were also accompanied by changes in the nitrogen content per unit leaf area, with lower values at the drier and colder plots, where the LMA was also greater (Mediavilla *et al* .2012).

So ,This study provides a background information for the conservation and management of population growth of Cork oak , which we can classify our populations along a gradient of fertility for the protection of our oak forest. The results of provenance tests are used in regular practice. Therefore, our recommendation on the basis of the presented results is also to include it into the practical management of forest cultures in Tunisia. Long-term selection can lead to the development of adaptations to the local environment, generating ecotypic differentiation. This study allowed us to classify our populations along a gradient of fertility in a perspective of reforestation the most vulnerable areas.

Therefore, bringing ecological, evolutionary, physiological and molecular perspectives together, we hope to provide clear directives for future research when we provide a toolbox with definitions of key theoretical elements and a synthesis of the current understanding of the molecular and genetic mechanisms underlying to climate change.

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