Adaptation of Holm oak (*Quercus ilex* L.) to seasonal climate variations

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Abstract

Tolerance Holm oak trees (Quercus ilex L.) to seasonal climate variations and fire are related to changes in the morphological, biochemical and ecophysiological complex. This tolerance has been characterized in natural conditions (forest of Chettabah, East-Algeria). A biochemical approach for such jointly measures the accumulation of solutes (soluble sugars and total proteins) in different organs during the four seasons of the year in two different stations (fire and non-fire). Significant variations of these accumulations are observed. The different buildup of these metabolites varied depending on the body and the degree of stress, the accumulation of sugars and proteins is highest in leaves during the spring and summer seasons and the levels of these metabolites is the same in both stations. These changes in markers show that Holm oak has a capacity to adapt to thermal variability and fire in forest of Chettabah.

Key words: *Holm oak, markers, seasonal variations, fire.*

1. Introduction

Trees of the genus *Quercus* are widespread in the northern hemisphere and are significant species in many forests and woodlands (Cañellas *and al*., 2007). In the Mediterranean Basin and Middle East, the Holm Oak (*Quercus ilex* L. subsp. ballota [Desf.] Samp) occurs over an area that extends 6.000 km longitudinally, from Portugal to Syria and 1.500 km latitudinally, from Morocco and Algeria to France (Debazac, 1983). Holm Oak can occupy diverse ecological niches, and occurs in humid, subhumid, and semiarid regions (Afzal-Rafii *and al*., 1992).

All study areas are mountainous territories with a typical Mediterranean climate pattern, where hot and dry summers are a predisposing fire factor. The physical conditions are favorable to forest and/or agro-sylvo-pastoral suitability in a balanced framework.

Quercus ilex (Holm oak) is an evergreen oak species that can be found both in shrub and tree (Yaltirik, 1984), in pure or mixed stands, and is a typical Mediterranean sclerophyll species (Palacios *and al*., 2009).

Heavy degradation of vegetation in large areas of the Mediterranean basin calls for rehabilitation with native coniferous and broadleaves species (Vallejo *and al*., 2000). Mediterranean landscapes have been modified since long time by human activities. Fire, grazing pressure, shifting agriculture, and overcutting for fuel and other purposes are important degradation and/or selection factors in all the Mediterranean countries (Wessel *and al*., 2004). Moreover, it is expected that climate change could make Mediterranean ecosystems even more vulnerable (Haase *and al*., 2000; Gratani *and al*., 2012). A decrease in Mediterranean shrublands cover might accelerate soil degradation and erosion (Shakesby *and al*., 2002). The Holm oak plays a vital role in soil and water conservation and its acorns are important nourishment for many wild and domestic animals (Plieninger *and al*., 2003; Linan *and al*., 2011; Galvan *and al*., 2012). On the other hand, *Quercus ilex* is an element of maquis vegetation that can play an important role in the rehabilitation and restoration of the western and eastern Mediterranean countries. Holm oak gains more interest for Mediterranean forestry, along with other evergreen species such as *Quercus suber*, *Ceratonia siliqua*, and *Olea europaea*, especially in restoration and reforestation activities (Christensen *and al*., 2007).

Analysis of the variability of ecologically important traits, such as tolerance to drought and frost, among populations may provide valuable information on the selection criteria for breeding and reforestation programs. Climate change models for the Mediterranean area predict an increase in temperature, a decrease in rainfall, and an increase in the frequency of extreme events such as late season frosts, extreme droughts FAO (2012), and increasingly hot summers with prolonged, severe heat waves such as in 2012. This scenario is rather similar to other M.E.N.A. countries, with similar climatic and productive features, where rural populations maintain excessively high pressure on wooded lands, overexploiting firewood and over-grazing (Davis, 2004).

Changes in fire occurrence are evident throughout history of colonization, confiscation of communal land and the application of modern agricultural techniques that increased the amount of arable land (Bensaid *and al*., 2006).

The objective of this study was to compare the seasonal climate change from two stations of forest of Chettabah (Constantine, Algeria), in order to determine if physiological response to frost, hot and fire.

2. Materials and methods

2.1. The Study Area

Forest of Chettabah is located southwest of Constantine (Algeria). The estimate terrain elevation above sea level is 865 meters. The study area is located on the map topographic Constantine Scale 1/200 000 sheet N° 17 and located between the coordinates 36°19'4" north latitude and 6°28'36" East longitude (map 1). The forest of Chettabah spreads over an area of 2398 ha and 94a, and is perfectly limited and divided into six districts. Extreme altitudes of the forest is about 1104 m (maximum altitude) and 652 m (minimum altitude), corresponding to each of them respectively following map coordinates: (x = 839, y = 344), (x '= 839.9, y' = 340.3).

Its bioclimatic is semi-arid to sub-humid. The average annual rainfall is estimated between 670 and 800 mm and the mean annual temperature of the region is 18°C, with an average of the warmest month above 35°C and the coldest month varies between 1.25 and 3.05°C.

A large plant grouping as the forest of Chettabah can be studied in its entirety, especially when it concerns hundreds of acres to be treated in the detail. Our choice is done in two stations (one fire and other not-fire) of Holm oak is one of the dominant species in this forest:

Map 1: Location of the study area.

Zone	Characteristics	
Zone fired	Thicket *Quercus ilex* (photo 1)	
	Condition of vegetation: burned in 1996	
	Altitude	989 m
	Acreage	6.72 ha
	Density	2100 tree / ha.
	Vegetation	The main essence is *Quercus ilex* recovery is 17%.
Zone not fired	Garrigue *Quercus ilex* (photo 2)	
	Vegetation status: No fire	
	Altitude	966 m
	Acreage	15 ha
	Vegetation	Tree stratum formed by a scrub of *Quercus ilex* recovery is 54%.

Photo 1. Thicket of *Quercus ilex*

Photo 2. Garrigue of *Quercus ilex*

2.2. Analytical methods

Experiment was conducted during the year of 2011-2012. The different tissues (stems, roots and leaves) were collected from two stations every season, with 4 replication per treatment.

2.2.1. Extraction and estimation of soluble proteins

Protein was determined by method described by Bradford (Bradford, 1976) , using bovine serum albumin as standard. Leaves, stems and roots (100 mg) were homogenized with 3ml extraction buffer (50mM Tris-HCl (pH: 7.5), 2mM EDTA, 1mM 2-Mercaptoethanol, 1mM DTT). Samples then were centrifuged at 14000 rpm for 25 min at 4°C and supernatants were isolated and used for protein assay.

2.2.2. Extraction and estimation of total soluble sugars

The sugar content in the extracts normally was analyzed by a phenol-sulfuric acid method (Dubois *and al*., 1956). This colorimetric method determines only the amount of total sugars. This is the most widely used colorimetric method to date for determination of carbohydrate concentration in aqueous solutions. The basic principle of this method is that carbohydrates, when dehydrated by reaction with concentrated sulfuric acid, produce furfural derivatives. Further reaction between furfural derivatives and phenol develops detectible color. The standard procedure of this method is as follows. A 2 ml aliquot of a carbohydrate solution is mixed with 1 ml of 5% aqueous solution of phenol in a test tube. Subsequently, 5 ml of concentrated sulfuric acid is added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they are vortexed for 30s and placed for 20 min in a water bath at room temperature for color development. Then, light absorption at 490 nm is recorded on a spectrophotometer. Reference solutions are prepared in identical manner as above, except that the 2 ml aliquot of carbohydrate is replaced by DDI water. The phenol used in this procedure was redistilled and 5%phenol in water (w/w) was prepared immediately before the measurements.

2.3. Data analysis

The standard errors were calculated for each point, as well as in the results were applied the variance analysis and the averages of the treatments were compared by the Tukey test at the 5% significance level, using the software XLSTAT (2010).

3. Results and discussion

The effect of environmental stresses on plants has been one of the main interests in modern biological research. This is connected with radical ecological changes and reduction in biodiversity. Changes driven by ecological stresses can be investigated at molecular and other hierarchical levels. Of particular interest is adaptation of protein biosynthesis and ultra structure to anomalous ecological factors. Such stresses as drought, salt, cold, heat, chemical pollutants and others, frequently act together and trigger adaptive and protective mechanisms (Wang *and al*., 2004).
The results of the total protein content in Holm oak trees are illustrated in table 1. They show that the high temperatures of the summer and spring seasons have led to a very large accumulation of total proteins in different organs in Holm oak trees (table 1). The highest values were recorded during the summer season while the lowest levels are assigned to the winter. The autumn season has higher levels compared to the winter.

During the winter season, the stems indicate the lowest levels and leaves the highest levels. These variations are confirmed by the analysis of variance (two criteria) that showed significant differences between the organs (p <0.004) and very highly significant differences between seasons for this characteristic (0.0001).

Carbohydrates act as nutrient and signaling molecules, modulating the expression of a large number of genes and they are also involved in the response to abiotic stresses. The results of soluble sugar contents of Holm oak trees during the four seasons of the year are shown in table 2. There is a difference in accumulation of soluble sugars in the different organs manifested by thermal fluctuations. The highest values were recorded during the summer season, specificity, at the leaves. The lowest levels are those posted during the autumn and winter, respectively in zone not fire and zone fire. These changes are confirmed by the analysis of variance of two criteria of classification that shows very highly significant differences in the characteristic according to seasons ($p < 0.001$) and organs ($p < 0.001$).

Table 1: Mean squares from analysis of variance of Data for total proteins (mg/g FW) in different organs.

Zone 1	Zone not fire (ZNF)		
Saesons	Leaves	Stems	Roots
Autumn	0.82±0.17[b]	1.01±0.16[b]	0.53±0.17[b]
Winter	0.49±0.09[c]	0.21±0.05[c]	0.31±0.09[c]
Spring	1.66±0.27[a]	1.61±0.23[a]	1.31±0.27[a]
Summer	1.67±0.23[a]	1.75±0.25[a]	1.36±0.23[a]
Zone 2	**Zone fire (ZF)**		
Saesons	Leaves	Stems	Roots
Autumn	0.73±0.13[b]	0.85±0.09[b]	0.62±0.08[b]
Winter	0.29±0.05[c]	0.25±0.04[c]	0.52±0.05[c]
Spring	1.59±0.21[a]	1.61±0.19[a]	1.22±0.23[a]
Summer	1.86±0.18[a]	1.65±0.20[a]	1.35±0.21[a]

Table 2: Mean squares from analysis of variance of data for total sugar (μmol/mg DW) in different organs.

Zone 1	Zone not fire (ZNF)		
Saesons	Leaves	Stems	Roots
Autumn	510±17[c]	530±16[c]	530±17[b]
Winter	650±9b[c]	500±15[c]	540±19[b]
Spring	780±27[b]	620±23[b]	630±27[a]
Summer	1200±23[a]	920±25[a]	680±23[a]
Zone 2	**Zone fire (ZF)**		
Saesons	Leaves	Stems	Roots
Autumn	640±13[b]	630±19[b]	610±18[b]
Winter	590±15[b]	450±14[c]	540±15[c]
Spring	680±21[b]	670±19[b]	660±23[b]
Summer	910±18[a]	880±20[a]	860±21[a]

There are earlier reports on carbohydrate accumulation during various abiotic stresses in the temperate grasses and cereals from the Gramineae family where long term carbohydrate storage occurs during reproductive development (Zhang *and al*., 1997). Accumulation of sugars in different parts of plants is enhanced in response to the variety of environmental stresses (Sturgeon, 1990; Zhu *and* Xiong 2002). There are few studies on carbohydrate status in germinated seeds and its early developmental stages under stress conditions. The metabolism of these compounds can be affected by a number of environmental factors such as irradiance, temperature, salinity and type of ion present (Prado *and al*., 2000). Thus the variation that occurs in carbohydrate level, during early developmental stages of seedling under different abiotic stresses is not well understood and information on physiological events involved in this process is scarce.

Table 3. Pearson correlation between the soluble sugar and soluble protein content of Holm oak organs.

Trait	Zone not fire (ZNF)		Zone fire (ZF)	
	Sugar	Protein	Sugar	Protein
Sugar **(ZNF)**	1	0.935**	0.761*	0.818*
Protein **(ZNF)**	0.935**	1	0.826*	0.867**
Sugar **(ZF)**	0.761*	0.826*	1	0.996**
Protein **(ZF)**	0.818*	0.867**	0.996**	1

A correlation analysis demonstrated significant correlation of the soluble protein content with soluble sugar concentration (0.935^{**}, 0.761^{*}) respectively in zone not fire and zone fire (Table 3). Soluble protein one of the important mechanism can increase cold tolerance with synthesis of specific proteins (Wang *and al*., 2004). Proteins are involved in several processes, i.e., signal transduction, RNA processing, translation protein processing, photosynthesis, photorespiration, redox homeostasis and metabolisms of carbon, nitrogen, sulfur and energy (Bohnert, 1995).

4. Conclusion

The effect of seasonal variation is very significant on the biochemical parameters. Our results show that the Holm oak trees are suitable for different osmolytes accumulated as soluble sugars and proteins in these organs. A significant accumulation of soluble sugars and total proteins was obtained at different organs under natural conditions (fire and non fire station), this accumulation has been labeled by the leaves during the summer season when the temperature is very high.

In conclusion, we found that Holm oak actually manifest morpho-biochemical adaptation traits response to thermal fluctuations and fire, which explains the xerophytic character of the species.

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