# Morphological and physiological responses of seedlings of cork oak to high temperature

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## ABSTRACT

The effects of high temperature on the seedlings of cork oak were studied with respect to growth, and proline accumulation. Within this context, this work focuses on the impact of short and repetitive periods of high temperatures (38°C, 40°C, 42°C and 44°C) on certain morphological and biochemical aspects of the cork oak seedlings. Height, foliar area and number of leaves were found to be positively affected with the temperatures. Proline accumulation was found in leaves, stems and roots after thermal stress treatment. A preferential accumulation of proline was found in root system, with a maximum at 44°C. Thus, the level of proline accumulation was related to the degree of thermal stress tolerance.

**Key words:** Cork oak, thermal stress (high temperature), proline, morphological parameters

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## INTRODUCTION

Temperatures above optimum growth range can cause damages to sensitive plant species by altering patterns of gene expression, inducing changes in cellular structures, and impairing membrane function [1, 2 and 3]. Elevated temperature and the associated physiological

dehydration have been identified to accelerate senescence, diminish photosynthetic activities, and reduce yields and quality [4, 5 and 6].

Cork oak (*Quercus suber* L.) has great social and ecological importance in the Mediterranean region. In many cases, however, natural regeneration is impeded by the biotic and abiotic factors of the forest environment [7, 8, 9 and 10] as well as by grazing and management practices of the agro-forestry systems, where they exist. Due to this difficulty, the artificial regeneration may be an important alternative for the rejuvenation of cork oak stands. However, its settling is hardly a success and several assumptions were made. Rached-Kanouni *et al.* (2012) charge this failure to the transplant shock due to the abrupt change in thermal conditions when moved from standard seedbed conditions to those of plantation sites often challenged with cold or heat stress [11]. If clearing and subericulture works are done without important difficulty, the population renewal is difficult or even inexistent due to anthropozoogenic factors [12] and to the climatic changes.

Several studies [13, 14, 15, 16, and 17] with other species indicated that the morphological and physiological quality of seedlings is one of the criteria conditioning growth and seedling performance in the field. A positive relationship between seed size and seedling establishment and growth was reported for a variety of species [18], including oaks [19]. A large variability in seed size is common in oak species [20, 21] and could affect seedling quality. In Cork oak, however, up to now no attempt was done to explore the relationship between the response of seedling growth to repetitive short-tem exposure to high temperature and the effect of this abiotic constraint on the several morphological (Shoot length, foliar area and number of leaves) and on the evolution of the biochemical marker to thermal stress responses such as proline in the different organ of the plant. The objectives of this study were to evaluate how the duration and intensity of thermal stress (high temperature) would influence seedling growth and the physiological parameter (proline).

## MATERIAL AND METHODS

Our study was carried out on cork oak seedlings (*Quercus suber* L.) from acorns collected on adult subjects of the region of the province of Skikda (Filfila, at the place called Col Besbes), in a sub-humid ambient weather with soft winter (m=10.55°C, M=24.25°C and P is 830 mm). The site of collection is situated closely at the intersection of parallel 36°37 of North latitude and of meridian 7°30’ of East longitude at 500 m high. They were grown in plastic pots of a 50 cm of size and 60 cm deep, filled with peat (organic material 2%, dry material 3%, water retention 30%, pH=6.7 and resistivity 1200 ohm.cm).

The cork oak has an endogenous rhythmic growth that is expressed by the development of successive identically structural unities, called waves or flushes. Each wave is composed of an elongation period (2 or 3 weeks) and an apparent pause phase (3 weeks). This endogenous rhythmic growth was easily obtained when seedlings are placed in a culture room under semi-controlled conditions with light (98 µmol. $m^{-2}$ $s^{-1}$) at temperature 25°C ± 2°C and a 16-hr photoperiod. After the end of the second growth wave, the seedlings were successively subject to three treatments (3 x 3h) at the following temperatures: 38°C, 40°C, 42°C, 44°C, and 25°C (control). The first treatment (3 hours) was applied at the third day of the apparent rest stage of the second growth wave; the second and the third treatments were respectively at the fifth and seventh day of the same period. The choice of this short-term exposure to high

temperatures is justified by the historic weather data of the province of Skikda, indicating frequent but short period of these thermal conditions. Measurements are performed every couple of days; for each seedling in a third wave growth, the parameters that are taken into account are the temporal components (durations in days of the stem length growth phases and of the rest of apical bud) and the spatial components (leaf sets with scaly stipules, leaf sets with assimilating limb and aborted limb leaf sets). The whole foliar area is measured with a scanner of type AREA METER AM 200.

## DOSAGE OF PROLINE

The method used is that of Troll and Lindsley (1955) [22]. Each sample collected (100 mg of the vegetal substance), is immediately weighed, and then put in a test tube. A 2 ml volume of methanol at 40% is added to the sample, and then the whole is heated at 85°C in a double boiler for 1h at 85°C. After cooling 1 ml of the extraction solution is added to 2 ml of acetic acid, 25 mg of ninhydrine and 1 ml of mixture distilled water- acetic acid- orthophosphoric acid of density 1.7 (120,300, 80: v/v/v). The whole is heated up to boiling point during 30mn in a double boiler at 100°C, then let to cool down and added 5 ml of toluene. After agitation in Vortex, a pinch of sodium sulphates ($Na_2$ SO4) is added in each tube. The reading of the optic density is done at 528 nm after 48 hours (specifically for the cork oak).

## STATISTICAL ANALYSIS

Statistical analysis was performed using Excel Stat (2009). The data were subjected to ANOVA, and differences between means were determined using the Newman-Keuls's test.

## RESULTS AND DISCUSSION

All plants are subjected to a multitude of stresses throughout their life cycle depending of the species of plant and the source of stress, the plant responds in different way. The major factor that currently reduces plant productivity is thermal stress (high temperature) [23]. It is well known that higher plants accumulate some metabolites in response to osmotic stress such as salt or drought stress [24, 25] and high temperature [23]. These metabolites are termed compatible solutes or osmotic solutes (osmolytes). Compatible solutes are osmotically active, low molecular weight, and nontoxic compounds [26]. The function of these compounds is thought to be related to osmotic adjustment. Proline, an amino acid, appears to be the most widely distributed compatible solute in organisms from bacteria to plants [27]. Some researches have reported a positive correlation between proline accumulation and adaptation to environmental stress [28]. However, some questions remain as to whether the accumulation of proline actually contributes to the cellular adaptation of plant to thermal stress like high temperature or whether the function of proline is restricted to adjusting the cellular osmotic potential. In the present study, the highest quantities of proline were found in the roots compared with the leaves of the leaves of two growth flushs and stems. Increase rates in the different organs were proportional to the intensity of thermal stress (high temperature) and were statically very significant ($p < 0.000$). There were similar to the control sample up to a thermal level of 38°C then increased from 40°C (29%); the highest level was observed at 44°C (285%). For the leaves and stems submitted to high temperatures, the results increased from 38°C and the highest proline content was obtained in leaves of the $2^{nd}$ growth wave (278%) at 44°C (Fig. 1).

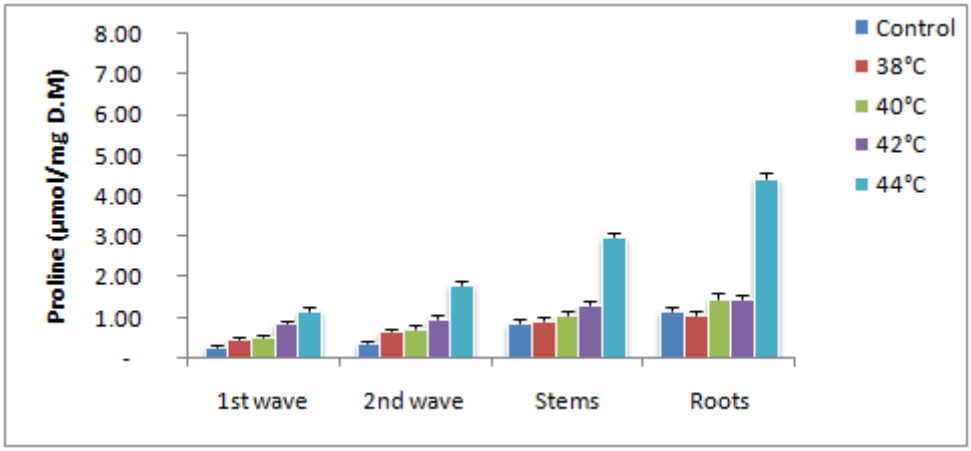


**Fig. 1:** Proline content in the different organs of cork oak seedlings (S1: 3h). Bars represent means values (n=4).

For the two stresses (S2 and S3), the measurements obtained indicate that the variation in proline content depend on the temperature and sometimes on the relevant organs (Fig. 2 and 3).

Except for stems transferred at temperatures 38°C and 40°C for 6h (S2), where the quantity of proline more or less remained constant, the largest quantities were observed at 44°C. The highest value was found in the roots (7.51 µmol/mg D.M).

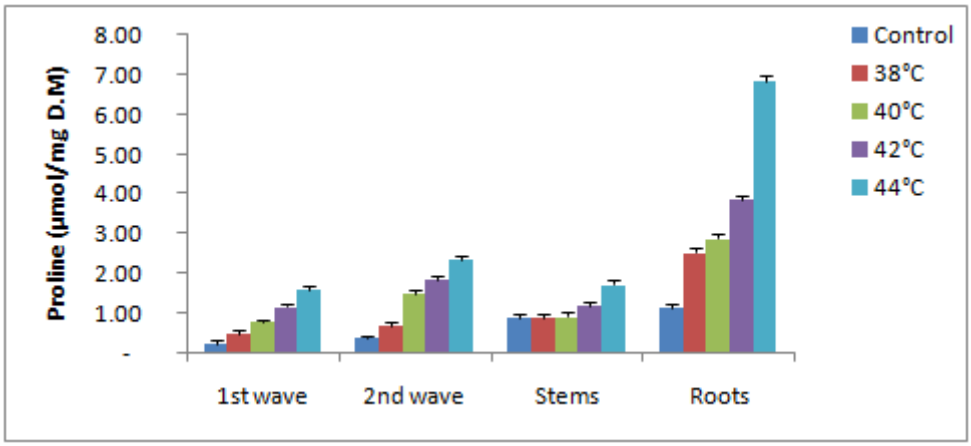


**Fig. 2:** Proline content in the different organs of cork oak seedlings (S2: 6h). Bars represent means values (n=4).

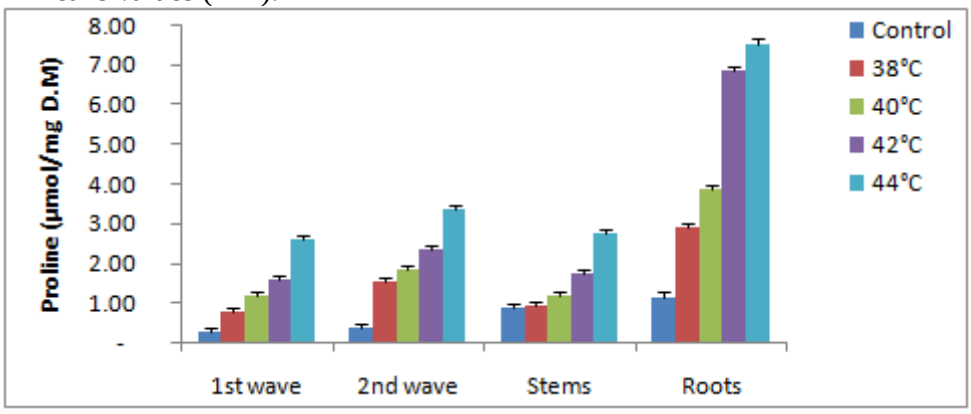


**Fig. 3:** Proline content in the different organs of cork oak seedlings (S1: 3h). Bars represent means values (n=4).

The analysis of variance for proline indicated a very significant difference between treatments, organs and stresses. Every parameter was significantly different from the other. In fact, the Newman-Keuls test attributed set different averages for each treatment, i.e, for the temperature 44°C that characterizes the highest average of the proline and the level was on the roots. The value in proline according to stresses presents the following sequence: [S3] > [S2] > [S1].

The foliar area was measured on the total leaves of the 3rd growth flush for each stress. The statistical analysis displayed no significant difference ($p<0.028$) in terms of foliar area for the two stresses (S1: 3h and S2: 6h). For the third stress, the same analysis indicated that the treatment 44°C had negative effect by reducing the foliar area to 976.4 $mm^2$ (Fig. 4).

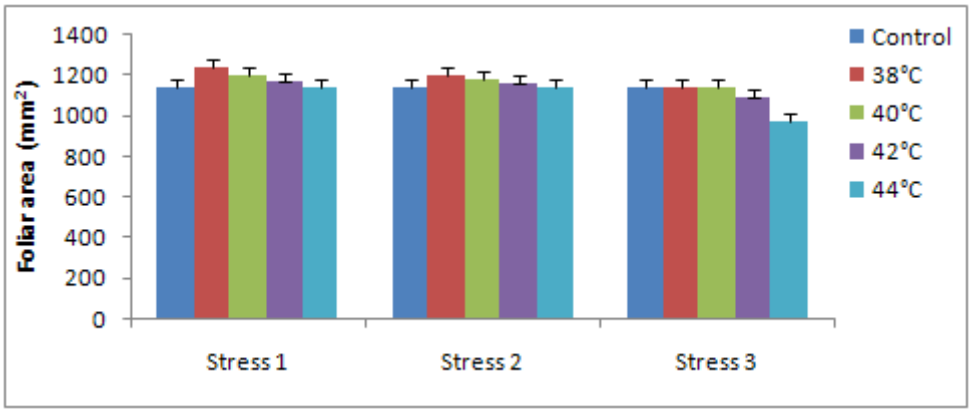


**Fig. 4:** Effects of high temperature on foliar area of cork oak seedlings.

The results obtained for growth number of leaves are illustrated in figure 5. The analysis of variance showed a significant difference between the different temperatures. The treatments 38°C marked always the highest number of leaves for all stresses. For the two stresses (S1 and S2), the number of leaves was important for the stressed leaves than the control; however the results obtained were similar to the control for the third stress (S3: 9h).

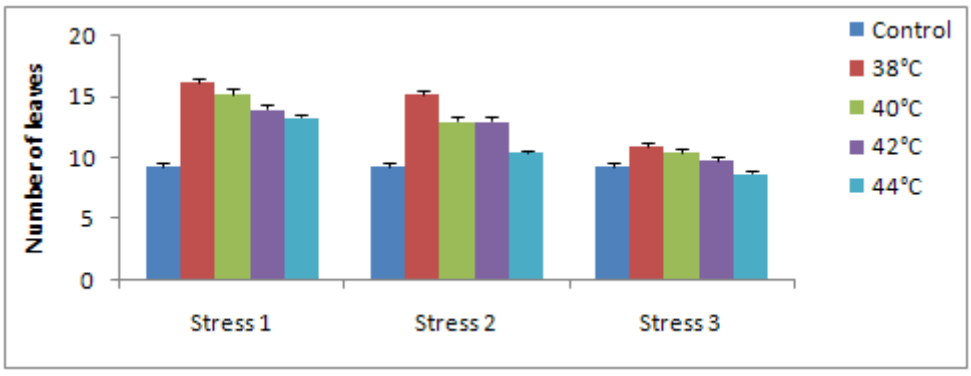


**Fig. 5:** Effects of high temperature on number of leaves.

For the growth in height for the seedlings transferred 3h (S1) at the following temperatures: 38°C, 40°C, 42°C and 44°C, the analysis of the variance ($p< 0.002$) had a positive effect by increasing height growth at all treatments for the third growth flush compared with the

control. However, the results obtained for the seedlings which subjected to a thermal stress of 6h (S2). It shows a rather different evolution according to the temperatures, where 38°C marked the highest growth rates and the other temperatures were similar to those of the control (Fig. 6). Contrary to the preceding results, the stem of seedlings which underwent three successive stresses (9h: S3) was most important at 25°C (control), and the temperature 38°C, 40°C and 42°C were similar to the control whereas a reduction was noted at 44°C. Such conditions impose strong stresses on leaves of tree species like oaks [29]. High temperatures above 40°C may under circumstances induce leaf necroses and yellowing [30]. Among cellular functions, photosynthesis is frequently regarded as one of the most sensitive to high temperatures. The reversible impact of moderately high temperature (i.e., 35-40 ◦C) on photosynthetic.

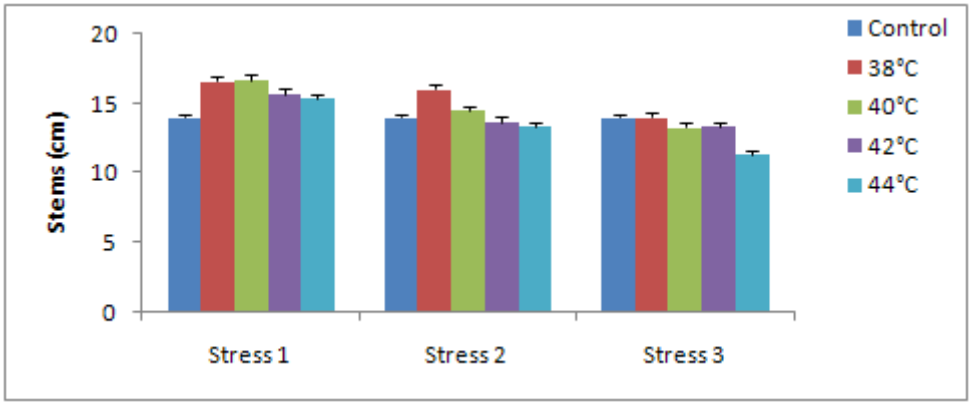


**Fig. 6:** Effects of high temperature on height growth of cork oak seedlings.

It is interesting to note that in cork oak seedlings, proline accumulation increases following its passage at high temperatures. In fact, although proline content markedly increases in roots. These results show that the proline may play a leading part in the response of cork oak seedlings organs to short-term exposure to high temperatures. The seedling growth rate was positively affected by high temperature.

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