



## Short communication

## Chlorophyll fluorescence and oxidative stress endpoints to discriminate olive cultivars tolerance to drought and heat episodes



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## ABSTRACT

Climate change is increasing the frequency of heat waves accompanied by drought episodes. These challenges are increasing in the Mediterranean basin, where *Olea europaea* L. has an important ecological and economic role. Olive breeding programs have been focused on highly productive cultivars, while ancient cultivars may present higher tolerance to drought and heat resilience. Therefore, it is important to select traditional cultivars that may give reliable performances under the emerging climate change scenarios. In the present work, the differential physiological response of economically important traditional Portuguese olive cultivars, Cobrançosa, Cordovil de Castelo Branco (C.C. Branco), and Cordovil de Serpa (C. Serpa), to drought combined with heat are evaluated. Stress treatment had lowest impacts on water status in Cobrançosa. Also, this cultivar was less affected regarding pigments content, maximum and effective quantum yield of photosystem II ( $F_v/F_m$  and  $\Phi_{PSII}$ ) and exhibited higher ability to trigger an antioxidant response. C.C. Branco was the most sensitive cultivar in response to pigments (carotenoids),  $F_v/F_m$  and  $\Phi_{PSII}$ , and cell membrane stability. Principal component analysis suggested that Cobrançosa has high potential to withstand climate change events, particularly drought combined with heat episodes, followed by C. Serpa and C.C. Branco.

## 1. Introduction

The emerging climate change scenarios, such as increasing frequencies of heat waves accompanied by variations in the precipitation patterns, exert a dramatic challenge for agriculture, particularly for most crop-trees of the Mediterranean region (IPCC, 2014). Olive (*Olea europaea* L.) is one of the most important crop in the Mediterranean basin, with high economic and ecological value. This species presents some physiological and morphological plasticity to respond to the climate variability of the Mediterranean region, such as an efficient osmotic adjustment, leaf anatomical modifications, a good regulation of stomata aperture, high water uptake capacity and hydraulic lift and reverse flow (Fernández, 2014). Despite these adaptive capacities, olive response to the emerging combination of several stresses remains unknown and some reports highlighted its putative negative effects on productivity, and olive and oil quality (Martinelli et al., 2013).

Drought or heat can reduce photosynthesis, promote stomatal closure, induce photosynthetic pigments loss and oxidative stress. Ultimately olive plants may undergo a decreased growth and productivity (Koubouris et al., 2015). Being specific organelles for electron transfer, chloroplasts are a major source of reactive oxygen species (ROS), and particularly susceptible to oxidative stress. Under stress conditions, light energy absorption greatly exceeds the one required for photochemistry which may increase the generation/accumulation of ROS, causing oxidative damages. However, plants have evolved several mechanisms to prevent ROS formation or scavenge the unavoidably formed ROS (Pintó-Maríjuan and Munné-Bosch, 2014). For instances, light absorption can be reduced or the extra photons' energy can be dissipated through photochemical and non-photochemical mechanisms. Also, plants possess an antioxidant system, composed by enzymatic and non-enzymatic antioxidants that can neutralise free radicals/ROS, reducing associated damages (Dias et al., 2014). Total antioxidant

**Abbreviations:** Chl, chlorophyll; CMP, cell membrane permeability; C, control plants;  $F_v/F_m$ , maximum quantum yield of photosystem II;  $F_o$ , minimum fluorescence;  $F_m$ , maximum fluorescence;  $F_s$ , steady-state fluorescence of light adapted samples;  $F_m'$ , maximum fluorescence of light adapted samples; NPQ, non-photochemical quenching; PSII, photosystem II; qP, photochemical quenching; ROS, reactive oxygen species; RWC, relative water content; S, stress treatment;  $\Phi_{PSII}$ , effective quantum yield of photosystem II

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capacity reflects the combination of these diverse antioxidant strategies. The degree of drought tolerance has been correlated with the plants antioxidant capacity (Petridis et al., 2012).

Some Portuguese ancient cultivars are originated from arid regions and their commercial value is emerging to produce olive oil of “Protected Designation of Origin – PDO” as the case of Cobrançosa and Cordovil (Amaral et al., 2010). Some studies compared cultivars’ ability for adaptation and production under drought conditions (e.g. Bacelar et al., 2004; Faraloni et al., 2011). Bacelar et al. (2004) and Pierantozzi et al. (2013) reported the susceptibility to water deficit of cultivars used in intensive production, such as Arbequina and Manzanilla, showing decreased photosynthesis and increased lipid peroxidation combined with less leaf anatomical features to prevent from water loss.

Impacts of heat stress have been less addressed (Aguilera et al., 2014; Koubouris et al., 2015), while responses to combined stresses (e.g. drought + heat) remain to unveil. Considering that some ancient cultivars, besides having a PDO importance, are identified by farmers as highly tolerant to drought and/or heat, it is important to study these cultivars for selection under an emerging climate change scenario. This work aims at characterizing and discriminating ancient olive cultivars tolerance to combined drought + heat shock, regarding impacts at the photosynthesis and oxidative stress level.

## 2. Material and methods

### 2.1. Plant material and stress exposure

Three representative Portuguese cultivars were chosen, the Cobrançosa, typically from the northeast, Cordovil de Serpa (C. Serpa) from the southern province of Alentejo and Cordovil de Castelo Branco (C.C. Branco) from the center of Portugal. Two-year-old potted (5L) plants were watered to 100% field capacity and plants with similar height were randomly divided in two groups: Group 1–control conditions (C,  $n = 6$  plants), plants well-watered; and Group 2 – water deficit and heat shock condition (S,  $n = 6$  plants), water was withholding for 20 days and in the last day plants were exposed to  $45 \pm 2$  °C for 4 h. The experiment was conducted in a climatic chamber with a relative humidity of 40% and a temperature of  $45 \pm 2$  °C. Light in the climatic chamber was provided by Osram cool white fluorescent lamps that gave an intensity of  $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ . After stress exposure chlorophyll *a* fluorescence, water status and cell membrane permeability were evaluated. Additionally, leaf samples were collected, fast-frozen in liquid nitrogen and stored at  $-80$  °C for further analysis of pigments and total antioxidant capacity.

### 2.2. Leaf water status and chlorophyll content determination

Plant water status was assessed through the measurement of the leaf relative water content,  $\%RWC = 100 \times (\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight})$ . Pigments were quantified as described in Dias et al. (2014). Briefly, frozen leaves were homogenized with acetone:Tris 50 mM (80:20, v/v, pH of 7.8), centrifuged and the absorbance was read in a spectrophotometer Genesys 10-uvS (Thermo Fisher Scientific Inc., Waltham, USA) at 663, 537, 647 and 470 nm.

### 2.3. Chlorophyll *a* fluorescence parameters

Photosynthetic activity was assessed through Pulse Amplitude Modulation (PAM) fluorometry using a portable PAM fluorometer Portable Junior-PAM, Gademann Instruments GmbH, Germany as described by Serôdio et al. (2013). Leaves were adapted to dark during 30 min, and then a weak-intensity modulated light pulse (blue light emitted by a LED-lamp peaking at 470 nm, half-bandwidth of 31 nm) was applied to determinate the minimum fluorescence ( $F_o$ ) followed by a saturating pulse ( $> 5000 \mu\text{mol m}^{-2} \text{s}^{-1}$  for 0.7 s) to determine the maximum fluorescence ( $F_m$ ). Then, leaves were adapted to light for

5 min and steady-state fluorescence ( $F_s$ ) and the maximal fluorescence level  $F_m'$  were determined. Actinic light was turned off and the minimal fluorescence level  $F_o'$  was measured. Maximum and effective quantum yield of photosystem II (PSII) [ $F_v/F_m = (F_m - F_o)/F_m$  and  $\Phi_{PSII} = (F_m' - F_s)/F_m'$ , respectively], photochemical quenching [ $qP = (F_m' - F_t)/(F_m' - F_o')$ ] and non-photochemical quenching [ $NPQ = (F_m - F_m')/(F_m')$ ] were calculated.

### 2.4. Cell membrane permeability and total antioxidant capacity

Electrolyte leakage was used to determine cell membrane permeability (CMP) according to Farooq and Azam (2006). Briefly, leaves were immersed in de-ionized water and incubated overnight on a rotary shaker. The electrical conductivity ( $L_t/L_o$ ) of solutions were read before ( $L_t$ ) and after autoclaving ( $L_o$ ) the samples (121 °C, 10 min). For total antioxidant capacity (TAC), leaf frozen samples ( $\sim 100$  mg) were homogenized with methanol (1.25 ml), sonicated in a 40 °C bath over 30 min and centrifuged ( $15,000 \times g$ , 15 min, 4 °C). Then, the supernatant was added to an ABTS (2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) solution and the absorbance recorded at 734 nm (Re et al., 1999). TAC was calculated using a gallic acid standard curve ( $y = 0.00036x + 0.057$ ,  $r^2 = 0.99$ ).

### 2.5. Statistical analysis

Data were analysed by One-Way Analysis of Variance followed by a multiple comparison test (Holm-Sidak Test). Principal component analysis (PCA) was performed with CANOCO for Windows v4.02.

## 3. Results

### 3.1. Effect of stress on relative water content, pigments, photosynthesis, cell membrane permeability and total antioxidant activity

No significant changes were found on the RWC between control and stress treatment for Cobrançosa (Fig. 1A). However, for the other cultivars, stress treatment induced a significant reduction of the RWC (C. Serpa: 22% and C.C. Branco: 20%) in comparison to the respective control.

Carotenoids decreased significantly only in C.C. Branco plants when exposed to stress (Fig. 1B). Under control conditions, C. Serpa showed the lowest ( $P < 0.05$ ) level of carotenoids. Stress exposure induced a significant decline in the content of chlorophylls in all cultivars when compared to the respective control (Fig. 1C, D).

Maximum quantum yield of PSII ( $F_v/F_m$ ) in Cobrançosa and C. Serpa were not affected ( $P > 0.05$ ) by stress treatment, but in C.C. Branco, stress decreased significantly this parameter when compared to the respective control (Fig. 1E). However, for all cultivars, stress treatment reduced significantly  $\Phi_{PSII}$  (Fig. 1F). When compared to the respective control, the  $\Phi_{PSII}$  drop was higher in C.C. Branco (61%) than in Cobrançosa or C. Serpa (41% and 45%, respectively). Also,  $qP$  decreased significantly in all cultivars when exposed to stress (Fig. 1G). No significant differences were found in the NPQ between control and stress treatment (Fig. 1H).

Cell membrane permeability increased ( $P < 0.05$ ) in all cultivars (C.C. Branco: 38%, Cobrançosa: 53% and C. Serpa: 62%) after stress exposure (Fig. 1I). However, plants from the C.C. Branco cultivar showed the highest ( $P < 0.05$ ) values of CMP. Also, stress increased ( $P < 0.05$ ) the TAC in all cultivars: Cobrançosa and C.C. Serpa increased  $\sim 70\%$ , while in C.C. Branco increased only 46%, comparatively to the respective control (Fig. 1J).

### 3.2. Principal component analysis (PCA)

Principal component analysis was carried out to identify parameters that can best describe olive cultivars physiological performance upon

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