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Study of the temperature effect in three chestnut (*Castanea sativa* Mill.) cultivars' behaviour

José Gomes-Laranjo[a,*], Francisco Peixoto[b], Harro W. Wong Fong Sang[c], José Torres-Pereira[a,†]

[a]*Centre for Technological Studies on Environment and Life, University of Trás-os-Montes and Alto Douro, 5001-911 Vila Real, Portugal*
[b]*Department of Chemistry, University of Trás-os-Montes and Alto Douro, 5001-911 Vila Real, Portugal*
[c]*Department of Structural Biology, BioCentrum Amsterdam, Faculty of Earth and LifeSciences, Free University of Amsterdam, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands*

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Summary

The aim of this work was to analyse the effect of temperature in three chestnut cultivars, Aveleira, Judia and Longal. For this purpose, gas exchange, thylakoid membrane potential, photosynthetic pigment and lipid content data in July, September and October under different temperatures (31, 26 and 18 °C) were determined. With respect to gas exchanges, significant changes in photosynthesis rate of Aveleira were observed between July and September (7 μmol $CO_2\,m^{-2}\,s^{-1}$). In contrast, Judia and Longal showed a strong increase in this period, 6.1–8.5 and 4.9–6.7 μmol $CO_2\,m^{-2}\,s^{-1}$, for Judia and Longal, which represent an increase of about 15% and 43%, respectively. Similar patterns were detected in daylight photosynthesis measurements for Judia and Longal, in which an almost 60% decrease was observed, in contrast to 40% for Aveleira, from morning to midday, when temperatures increased from 27 to 34 °C. In addition to high photosynthetic rates in the hottest month, Aveleira was also the sunniest cultivar according its highest value on chlorophyll a/b ratio (3.65). Cultivars also presented maximal thylakoid membrane potential at different temperatures, with their values being 20.8, 17.8 and 17.2 °C for Aveleira, Longal and Judia, respectively. These results were also supported by thylakoid fatty acid composition which indicated that the unsaturation index of Aveleira (158) was the

Abbreviations: A, net photosynthesis; ACMA, 9-amino-6-chloro-methoxyacridine; Chl, chlorophyll; Ci, internal CO_2 concentration; *E*, transpiration; g_s, stomatal conductance; MV, methylviologen; PPFD, photosynthetic photon flux; *Q*, ACMA fluorescence; *T*, air temperature; UI, unsaturation index; WUE, water use efficiency

*Corresponding author.
E-mail address: jlaranjo@utad.pt (J. Gomes-Laranjo).
†In memory of.

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lowest in comparison with other two cultivars, 168 and 175, for Longal and Judia, respectively.

Introduction

Portugal is one of the most important producers of chestnuts of Europe. Here, most of the chestnut stands are planned for nut production either for immediate consumption or for export to Brazil, the USA and France, which are the main importers of Portuguese chestnuts (Cortizo et al., 1996).

As a consequence of its economic value, many cultivars are grown indiscriminately, with Aveleira, Judia and Longal the most cultivated in the NE of Portugal, on Trás-os-Montes. The importance of Aveleira comes from its early ripening in the first fortnight of October, and in consequence, its fruits do not keep well, since it has the lowest dry matter content (Ferreira-Cardoso, 2003). Maturation of fruits from Judia and Longal normally occurs at the end of October. Fruits from Judia are the largest, with a caliber of about 50–60 fruits per kilo and those from Longal and Aveleira are around 70–80 fruits per kilogram. However, fruits from Longal, which are easy to peel and keep well, have the highest starch content, giving them a very good flavour (Ferreira-Cardoso et al., 1999; Pimentel-Pereira et al., 1999).

However, very little is known about the thermotolerance of the chestnut cultivars for the vegetative cycle, particularly during the summer period where in Trás-os-Montes, the temperatures often reach over 30 °C, where the adverse conditions throughout this period, low water soil availability and high temperatures are referred as the destabilising factor for normal chestnut growth, inducing a loss of plant vigour, and consequently inducing the susceptibility of the trees to the ink pathogen (Gomes-Laranjo 2001; Gomes-Laranjo et al., 2004).

The overall process of photosynthesis is temperature dependent, since it is a biophysical and biochemical process and elevated temperatures are usually regarded as one of the most important external influences affecting the overall photosynthetic capacity of intact photosynthesising tissues and the specific functions of various parts of photosynthetic apparatus (Bukhov and Mohanty, 1999).

The purpose of this study was to characterise the effect of temperature in three different chestnut cultivars, Aveleira, Judia and Longal, using parameters related to gas exchange and plant–water relations. This work was complemented with an analysis on the influence of the temperature on generation of thylakoid membrane potential and an analysis of their fatty acids contents.

Materials and methods

The assay was carried out on a slightly south-facing and non-watered orchard, 30 years old, located in Carrazedo de Montenegro, Trás-os-Montes, NE Portugal (Latitude: 1°42′02″; Long: 41°34′08″ and 600 m above sea level). Soils are well drained, with textures from loamy sand to silty loam, and the parent materials are base-poor (Portela et al., 2003).

Gas exchanges were determined with an infrared gas analyser (IRGA, mod. LCA-2 Analytical Development Co., Hoddesdon, UK). Rates of stomatal conductance (g_s), transpiration (E), net photosynthesis (A) were obtained. The analyser is also equipped with sensors to measure the photosynthetic photon flux density (PPFD) and temperature (T). Water use efficiency (WUE) was calculated according to Salisbury and Ross (1991). Leaves were selected up to 3 m high, from the crown of chestnut trees. Data were collected during 1995, 1996 and 1997 from six chestnut trees (*Castanea sativa* Mill.) of Aveleira, Judia and Longal cultivars, during three periods of the year, designated here as: 1st, 2nd and 3rd season, corresponding to July ($n = 1110$), September ($n = 1140$) and October ($n = 1234$), respectively.

For determination of photosynthetic pigment content, 12 sunlight leaves were selected from south-side canopy of same plants. From each leaf, six 8 mm disc samples were punched out and left on 10 ml of 80% (w/v) acetone (pH = 7.5, buffered by 25 mM Hepes) (Porra et al., 1989) during 48 h. Chlorophyll (Chl) and carotenoid were spectrophotometrically quantified using the equations of Lichtenthaler (1987).

Isolations of chestnut thylakoids were performed according Torres-Pereira et al. (1974) and Packer et al. (1975), with some modifications, derived from the nature of the leaves. The grinding and washing medium was composed of 20 mM sorbitol, 10 mM tricine-NaOH (pH = 8.4), 30 mM KCl, 5 mM $MgCl_2$, 0.75 mM EDTA, 0.1% (w/v) BSA, 1% (w/v) ascorbic acid and 0.4% Polyvinylpyrrolidone. The storage medium was composed of 165 mM sorbitol, 10 mM