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Study of morphological and phenological diversity in chestnut trees ('Judia' variety) as a function of temperature sum

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ABSTRACT

Morphological and histological adaptation of chestnut leaves at the different altitudes and edaphoclimate conditions were shown. The study was carried out on *Castanea sativa* Mill. var. 'Judia'. The growth range altitudes of 'Judia' were between 709 m a.s.l. and 860 m a.s.l. (above sea level), corresponding to a variation in the sum of temperatures (expressed in degree-day values – °D) of 2751 °D to 2316 °D in 2006 and 2338 °D to 1700 °D in 2007, between May and October. In 2007 the thickest leaves (319.9 μm), the highest stomata density (469.1 stomata mm^{-2}), one of the largest leaf areas (69.2 cm^2) and the highest fruit size (71.7 fruit kg^{-1}) were observed in Alfândega da Fé (759 m a.s.l., 2186 °D, during the May–October period) whereas in 2006 the highest fruit size (86.8 fruit kg^{-1}) was observed in Valpaços (860 m a.s.l., 2316 °D, during the same period).

Additionally, the leaves of the trees grown in this locality (Valpaços) displayed one of the largest areas (57.0 cm^2). Overall results suggest that annual climate conditions do significantly influence both fruit and leaf biometric characteristics, and that the range of temperatures from 2100 °D to 2300 °D (between May and October) correspond to the optimal temperature sum, which can be ascribed to different places, depending on the year in question. Concerning the molecular characterisation using nuclear microsatellites, the individuals more distant are those from Macedo de Cavaleiros and Alfândega da Fé (genetic distance = 0.227), while the ecotypes with closer similarities were those collected in Murça and Vinhais (genetic identity = 1.171). The current results suggest that the morphological and phenological differences among ecotypes are not related to the small genetic differences, but are simply phenotypic adaptations to diffent climatic conditions.

1. Introduction

In many Mediterranean countries the European chestnut (*Castanea sativa* Mill.) is an economically important resource for wood and fruit (Míguez, 2000). The North of Portugal, and specifically the Trás-os-Montes region, is the main Portuguese chestnut production area, generating almost 85% of the total national production. The chestnut variety 'Judia' is one of the most important cultivated varieties, and is found in both old and new orchards of two protected designations of origin (PDO) areas, namely "Castanha da Terra Fria" and "Castanha da Padrela". Therefore 'Judia' grows under quite distinctive climate conditions, between 500 m a.s.l. (above sea level) and 1020 m a.s.l., which results in distinctive fruit production in terms of both quantity (fruit yield) and quality. 'Judia' presents one of the highest fruit sizes (50–80 fruits kg^{-1}) among all Portuguese native varieties, which places it extremely well in the market either for industrial transformation or for fresh seasonal consumption (Gomes-Laranjo et al., 2009; Pimentel-Pereira et al., 2007).

Nut and leaf descriptors are usually very useful for characterizing chestnut varieties, although these morphological observations, as referred by Beccaro et al. (2005), are not considered effective enough and are prone to environmental and developmental factors.

Among all plant organs, the leaf is the most flexible in its response to environmental conditions. Since leaves must fulfil critical and sensitive roles in the life-cycle of the plant, the structure of the leaf must maximize a diverse set of functions. Consequently, its structure reflects more clearly, than that of the stem and roots, the effect of environmental stress and heterogeneity (Levitt, 1980). Leaf structure has important implications for the performance of plants in specific habitats (Garnier et al., 1999). Both between and

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within species, leaves have differences in structure, including plastic responses such as leaf size and mass per unit leaf area (Gutschick, 1999).

Specialisation has allowed plant species to co-exist in the same environment, and species with similar morphological and physiological traits reflect their evolutionary adaptations. Species with different leaf morphology and physiology are able to tolerate different levels of stress, e.g. drought and heat (Kloeppel et al., 1993).

Correlations between the number of stomata in leaves and plant varieties, cold and drought resistance and strength of rootstocks have been established in previous studies performed on apricot varieties (Gulcan and Misirli, 1990; Tanzeralla et al., 1984). According to Gomes-Laranjo et al. (2008), 'Judia' leaves from the northern side of the tree canopy have 254.8 stomata mm$^{-2,}$ less 6.2% than those from the southern side, where there are 2.7 times more available sunlight. Likewise, evolution of phenological phases depends on climate conditions as was, already, studied for chestnut (Proietti et al., 2000).

'Judia' leaves are simple, elliptic to lanceolate, 15–17 cm long and 5–6 cm wide and petiole length 2.6–2.8 cm. The leaves are defined as members of the flat leaf shape variety group, since the length/width ratio is 2.8–2.9. Leaves are sharply pointed and have widely spaced teeth with shallow rounded sinuate concavities between them. Typically they have 16–17 teeth (Pimentel-Pereira et al., 2007).

European chestnut's catkins are astamine, brachystamine, mesostamine and longistamine (Bounous, 2002); 'Judia' catkins are brachystamine (stamen length, 1.30 mm) and belong to the long length catkin class (193.1 mm to 202.1 mm). The shortest catkins are very frequently found in the ancestral Longal variety (around 150 mm) (Pimentel-Pereira et al., 2007). Compared to other Portuguese varieties, 'Judia' catkins bloom quite late, almost one week after the rather precocious 'Aveleira'.

Chestnut cultivar identification is a major concern in Mediterranean countries, especially in the major chestnut cultivation countries such as Spain (Pereira-Lorenzo et al., 2006), Portugal (Costa et al., 2005a) and Italy (Boccacci et al., 2004). The Trás-os-Montes region of Northern Portugal has a high number of different genotypes indicating the importance of this region as a centre of biodiversity (Pereira-Lorenzo et al., 2010). SSR molecular markers of genome polymorphism have increasingly been used to analyze genetic stability (Martins et al., 2004).

This work is part of the 'Judia' improvement program which is being implemented and its aim is to study the influence of weather conditions on selected leaf descriptors of the 'Judia' variety and their relationship to fruit characteristics.

2. Material and methods

2.1. Plant material and growth conditions

The study was carried out in 2006 and 2007 on a total of 25 adult *C. sativa* Mill. var. 'Judia' trees. Tree selection was based on their fruit size (fruit kg^{-1}). Analyses were performed using samples from collected from orchards at different altitudes – from 709 m a.s.l. to 860 m a.s.l. in the Trás-os-Montes region: Alfândega da Fé, Bragança, Chaves, Valpaços and Vinhais (4 trees from each location), Macedo de Cavaleiros (3 trees), Murça (2 trees). All trees were between 30 and 40 years old and based on visual assessment they were in good sanitary and nutritive health.

The edaphoclimatic conditions were based on the soil and climatic conditions of each locality. Degree-days were estimated (sum of temperature, °D) according to Cesaraccio et al. (2001) using the formula $\sum$Temperature (°D) = $(Tx - t_0) \times n$: where 'x' is the average temperature of each month, 't_0' the base temperature, which is 6 °C for chestnut, and 'n' total days of each month. Weather conditions were recorded between May and October, which corresponds to the period between leaf emergence and leaf fall. Concerning soil analysis (Table 1B), extractable phosphorous (P_2O_5) content was extracted by the Egner–Riehm solution (ammonium lactate, pH 3.6) and determined by molecular absorption spectrophotometry (Egner et al., 1960). The pH was determinated by potentiometry in suspensions (1:2.5) of soil in H_2O (ISRIC, 1993). Exchangeable metal ions (Ca, Mg, K and Na) were extracted with 1 M ammonium acetate 1 M pH 7 (Sparks et al., 2001) and determined by atomic absorption spectrophotometry (Ca and Mg) or by flame emission spectrophotometry (K and Na). The organic matter (OM) was determinate as organic carbon (C) using an elemental analyzer with NIR (near infrared) detector and applying the factor 1.724 (Nelson and Sommers, 1996).

2.2. Leaf morphological characterization

In June of both years, from the Southern side of the crown of each tree, five randomly selected leaves (third leaf down from the extremity of the different branches bearing burrs approximately 2 m above the ground) were harvested. Each leaf was weighed (fresh weight, FW) and the width (W), length (L), length of the petiole (LP), distance from the foliar base to the first tooth (DT) of each leaf was measured. The number of teeth (NT) per leaf was also recorded. Leaf areas (A) were measured in a leaf area meter (Delta-T Devices-Windias, UK). Shape factor (f) was then calculated, $f = A/L \times W$, according Sesták et al. (1971) and Serdar and Demirsoy (2006). Shape factor represents the correction factor that is used to estimate leaf area from the maximal leaf length and width values. This factor requires frequent checking, mainly when leaf shape changes with position on the tree and with tree age (Sesták et al., 1971). Finally, leaves were submitted to a drying process in an oven at 60 °C (USM/ULM 500 model, Memmert, Schwabach, Germany) for 48 h, and afterwards samples were weighed so that dry weight could be determined and leaf mass per unit area (g m^{-2}) calculated, according to Dijkstra (1989).

2.3. Leaf histology

Anatomical tissue measurements were performed on three healthy leaves, collected from each tree crown facing south, in June 2007. In each leaf, five cuts (5 μm of thickness) were done (n = 15 per tree). The thickness of whole leaf blade (Lth), palisade and spongy layer (Pl and Sl, respectively), upper and lower epidermis (UE and LE, respectively) were measured by micrometer in leaf cross sections of fresh material prepared for inverse optical microscope analysis (OLYMPUS Mod. 1X 51), according Deysson (1965), with minor modifications. Stomatal density (number of stomata per unit leaf area, Sd) and ostiole (O, is the stomatal pore length) of three leaves from each tree were measured using an environmental scanning electronic microscope (FEI QUANTA SEM/ESEM) at 15 KV and 5 TORR of pressure in the chamber (adaptation of Liodakis et al., 2007).

2.4. Fruit biometry

One week after the beginning of fruit fall (October), twenty complete burrs were randomly collected directly from each tree. The following biometric characteristics were recorded i. number of well developed and aborted fruits per burr and ii. total fruit size (fruit kg^{-1}, TF) for all the fruits (good, aborted, rotten or wormy fruits) and iii. corrected fruit size (fruit kg^{-1}, CF) which refers only to undamaged/good fruits.