Adaptive diversity in *Castanea sativa* Mill. half-sib progenies in response to drought stress

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ARTICLE INFO

Article history:

Received 1 March 2011

Received in revised form

13 December 2011

Accepted 14 December 2011

Keywords:

Chestnut

Biomass allocation

Growth rate

Leaf water potential

Phenotypic variation

ABSTRACT

Variation patterns in phenotypic expression after cultivation in two water regimes: 50% and 90% substrate saturation were studied in progenies of chestnut (*Castanea sativa* Mill.) cultivars originating from two main origins in the Iberian Peninsula: North (specifically from Asturias and Galicia) and Central Iberian Peninsula (specifically from the Canary Islands and Andalusia in Southern Spain related to this gene pool). The restricted water supply reduced the water potential of all the families as well as their growth, both in terms of height and weight. Root development increased whilst leaf area decreased as a result of reduction in number and surface area of individual leaves as well as sprouting rather than through defoliation. Drought stress also modified the morphology of the leaves and the relationships indicating the rate of relative development of the leaves compared to the rest of the plant. A correlation with the minimum summer rainfall in the regions of provenance was observed for several traits. The phenotypic variation observed was greater than expected from progenies of selected fruit cultivars. The results of this study show that (i) phenotypic variation exists between the two origins of variability corresponding to the two identified gene pools; (ii) the Northern group might be considered more tolerant to drought stress when considering plantlets' ability to recover water potential, though important phenotypic plasticity was found in both origins of variability; and (iii) the pattern of variation found in some physiological and morphological traits suggests that Spanish chestnut stands have great potential to respond to the expected increase in drought in the Iberian Peninsula over the present century.

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1. Introduction

Decreased ecosystem productivity and increased mortality are general consequences of drought on biodiversity. The ability of forest species to withstand the rapid global warming predicted for the coming decades is uncertain. Competitive species, species adapted to cold and wet conditions, as well as those with low reproduction rates and/or limited mobility will be most affected (Archaux and Wolters, 2006).

Woody plants differ greatly in their response to, and tolerance of, water stress and this influences their metabolism, physiology

Abbreviations: Ψ_{pd} and Ψ_{md} , predawn and midday water potential; Ψ_{xil} , xylematic water potential; ΔD , increase in stem diameter; AB, aerial biomass; BW, branches dry mass; H, total height growth; ILB, index leaf lobation; LA, leaf area; LAR, leaf area ratio; LML, leaf maximum length; LMW, leaf maximum width; LN, number of leaves; LP, leaf perimeter; LW, leaves dry mass; P_s , minimum monthly rainfall in summer; RW, root dry mass; SLA, specific leaf area; SW, main stem dry mass; SWC, substrate water content; TB, total biomass; T_h , average temperature of the hottest month; TLA, total leaf area per plantlet; T_m , minimum average annual temperature.

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and morphology. In order to withstand drought, trees have developed diverse mechanisms, the cost of which may differ in terms of productivity (Ludlow, 1989). Morphological and growth responses to drought stress include reductions in total dry weight, in the number of lateral branches, in leaf production and in the expansion of both leaves and roots (Osorio et al., 1998). Plants adapt to drought by elongating and expanding their root systems and, in addition, severe drought appears to inhibit the growth of branches more severely than that of roots (Saab et al., 1990). This increase in the distribution of carbon towards the roots is thought to improve water balance within a tree and to thereby improve its chances of survival (Tschaplinski et al., 1994). Another response to limited water supply is a reduction in leaf surface area through modification of the size and number of leaves, or of the specific leaf area (SLA) (Braatne et al., 1992) or else by defoliation (Kozłowski and Pallardy, 1997). In several species, high leaf plasticity, i.e., variations in morphology, anatomy and physiology, is observed as a response to growth conditions (Witkowski and Lamont, 1991).

In general, characteristics of resistance to stress have not been given much consideration in the application of genetic improvement programmes due to the difficulty of defining premature qualitative and quantitative criteria in adaptation (Zhu, 2002). However, this is not the case with the chestnut (*Castanea sativa*

Mill.) as its widespread distribution throughout south-east Europe means it has been exposed to a wide range of environments. This capacity for dynamic colonization has been attributed to its great adaptability (Eriksson et al., 1993), resulting from both genetic and physiological bases (Lauteri et al., 2004). These mechanisms are especially important in areas such as the Iberian Peninsula where great climatic changes are expected this century, (Ramírez-Valiente et al., 2009). Chestnut is mostly found in the western Iberian Peninsula, but with two clearly defined origins of genetic variation: one in the North (Asturias and Galicia) and a second in the Central Iberian Peninsula (Pereira-Lorenzo et al., 2010). This division roughly corresponds to contrasting climatic conditions in Spain; i.e., mesic areas (moderately humid environment) and xeric (drier environment) areas, respectively. In Spanish afforestation programmes, chestnut seeds rarely come from the scarce wild populations, and are usually collected from chestnut stands when the coppice is left to produce nuts, or from orchards. Chestnut orchards provide diverse progenies due to the high genetic variability conserved in the Iberian Peninsula, as was recently demonstrated using microsatellites (Pereira-Lorenzo et al., 2011), where diversity in orchards was mostly explained by hybridization (up to 77%) whilst mutation only accounted for 6%.

In this work, we studied variability in growth, leaf morphology and leaf water potential in different juvenile progenies of grafted trees (*C. sativa* Mill.) from the two genetic pools of the Iberian Peninsula (northern and southern Spain) when subjected to drought stress with a view to the application of this information in relation to the climate change expected in the Iberian Peninsula. Three main hypotheses were tested: (i) significant variability in adaptive traits exists between the two different genetic pools; (ii) there is more tolerance to drought stress in southern cultivars and (iii) there is phenotypic plasticity within both origins of variability.

2. Material and methods

2.1. Plant material and growth system

One and a half-year-old open pollinated seedlings of 10 half-sib progenies of the main Spanish fruit cultivars of *C. sativa* Mill were chosen from each of the two main gene pools (Pereira-Lorenzo et al., 2010); Northern (specifically from Asturias and Galicia) and Central Iberian Peninsula (specifically from the Canary Islands and Andalusia in Southern Spain).

Data from the climatological station closest to the original location of each mother tree were registered (Supplemental Table 1). Characterization of the mother plants of these progenies took place over a period of several years in order to avoid genotype–environment interaction and has been published in various works (Pereira-Lorenzo et al., 2001, 2006, 2007).

Seeds were collected from their original provenances, germinated and after a growth period of one year all plants were pruned to uniform heights (10 cm), then repotted in 21 pots containing a mixture of grade 3 peat and vermiculite (80:20, v/v, “VP BOW” fertilized with 15:8:12 (N:P:K), at 1.5 kg m^{-3} and grown on in a greenhouse. During the experiment (May–August), the average daily minimum and maximum indoor air temperature was $16 \pm 2^\circ\text{C}$ to $23 \pm 2^\circ\text{C}$ (mean $\pm$ SD), respectively. The average relative humidity was $70 \pm 2\%$ during the day and $90 \pm 4\%$ at night.

From each of the 10 families from each geographical region, 24 plantlets were randomly selected and divided into two irrigation treatments, resulting in a total of 480 plantlets (2 geographical groups $\times$ 10 families $\times$ 2 watering treatments $\times$ 12 plantlets). A nested design was devised with two factors (treatment $\times$ family), 4 blocks and three replications (plantlets) per family per block, in

which the main factor was the water treatment and the secondary factor, the family.

Plantlets were kept at 90–100% soil saturation until the drought stress experiment was started after which one of two drip-irrigation treatments were applied: (i) irrigation as required (control treatment), in which the substrate was kept at 90% of the mean saturation level; and (ii) deficit irrigation (stress treatment), which was fixed at 50% of the mean saturation level. The amount of water used for irrigation was determined from the weight of the pots every 3 days. These irrigation treatments were maintained for 9 weeks, at which time irrigation in the stress treatment was suspended for a week, with the aim of determining the vulnerability to drought of each family and of assessing the effect of the treatment on the phenotypic variation of the plants. Average substrate saturation level had decreased to 32% at the end of the stress treatment.

2.2. Measurements

Root collar diameter and height measurements were made in all the plantlets at the beginning and end of the trial to calculate the increase in stem diameter (ΔD) and total height growth (H).

Six plantlets from each family and treatment were selected at random and weighed individually to obtain the water content of the substrate (SWC), calculated as the difference between this weight and the average dry weight of the substrate in the container without plants as determined at the beginning of the assay. For the rest of the parameters analyzed, six plantlets per family and treatment were also sampled.

Leaf water potential (predawn, Ψ_{pd} , and midday, Ψ_{md}) was measured in fully expanded leaves with a Scholander pressure chamber (PMS Instruments, Corvallis, OR).

Plant biomass was evaluated by destructive sampling. All dry mass values (leaves, LW; roots, RW; main stems, SW; branches, BW) were obtained after 48 h at 60°C . The sum of these dry weights gave the total biomass (TB) and, by excluding the weight of the root, the aerial biomass (AB).

Leaves were scanned and leaf parameters (area, LA; perimeter, LP; maximum length, LML; maximum width, LMW) and number of leaves (LN) of each plantlet were calculated with Leica Qwin vs.3.0 Image Analysis software. Total leaf blade area per plantlet (TLA) was also determined. Degree of leaf lobation (ILB) was taken as the quotient of LP and LA. Specific leaf area (SLA) was calculated as the ratio between LA and LW, and leaf area ratio (LAR) was calculated as the ratio between TLA and TB. The quotient of AB and RW was also determined. Average values were calculated from the measurements obtained from 4 fully expanded leaves per plantlet.

2.3. Statistical analysis

Statistical analysis was performed with SPSS vs 12.0 program (2003).

The following model was used to estimate differences between families and treatments:

$$X_{ijk} = \mu + F_i + T_j + (FT)_{ij} + \varepsilon_{ijk},$$

where X_{ijk} is the observation of family i ($i = 1-20$) in treatment j ($j = 1-2$) and sample k ($k = 1-12$); μ is the mean of all the observations; F_i , T_j , $(FT)_{ij}$, and $\varepsilon_{i(m)jk}$ are the effects of the family i , the treatment j , the interaction family $\times$ treatment, and the error associated with the sample k in the observation ijk , respectively.

A second model was used to estimate the effects of geographical group (Northern versus Southern Spain) and the variation of the families within each group:

$$X_{i(m)jk} = \mu + G_m + F_{i(m)} + T_j + (GT)_{mj} + (FT/G)_{i(m)j} + \varepsilon_{i(m)jk},$$

where $X_{i(m)jk}$ is the observation of the family i ($i=1-20$) within the group m ($m=1-2$) in the treatment j ($j=1-2$) and the sample k ($k=1-12$); μ is the mean of all the observations; G_m , $F_{i(m)}$, T_j , $(GT)_{mj}$, $(FT/G)_{i(m)j}$, and $\varepsilon_{i(m)jk}$ are the effects of the group m , the family i within the group m , the treatment j , the interaction group $\times$ treatment, the interaction family within group $\times$ treatment, and the error associated with the sample k in the observation $i(m)jk$, respectively.

Tukey's multiple range test was performed when significant treatment differences were detected ($\alpha=0.05$) and degree of association between variables was evaluated by means of simple regression analysis.

Relationships between minimum monthly rainfall in summer (P_s) and average values of adaptive traits by region were assessed to evaluate geographical plasticity of the genotypes in relation to macroclimatic variables. Only those relationships that fulfil the following conditions are presented: (i) $P < 0.05$ between geographical groups (North, South) and treatments (control, drought stress), and (ii) data adjusted to a straight line with a probability of over 95%.

3. Results

3.1. Availability of water, growth, and allometric relationships

Water potential at midday (Ψ_{md} , Fig. 1a) and at predawn (Ψ_{pd} , Fig. 1b) in relation to the amount of water in the container

Fig. 1. Response of predawn (a) and midday (b) leaf water potential (Ψ_{md} and Ψ_{pd}) to the substrate soil water content (SWC). Empty symbols represent the stress treatment and full symbols the control one: (○) Galicia; (▽) Andalusia; (□) Canary Islands; and (◇) Asturias.

Fig. 2. Leaf water potential at predawn and at midday (Ψ_{pd} and Ψ_{md}) for each family studied and for the watering treatments applied: stress (a) and control (b). Solid fill bars, Ψ_{pd} ; and shaded, Ψ_{md} .

(SWC) closely adjusted to an exponential model (Ψ_{md} , $R^2 = 0.7113$, $P < 0.0001$; Ψ_{pd} , $R^2 = 0.7614$, $P < 0.0001$). Data for family 18 is not included in Fig. 1 since, under stress treatment, individuals of this family exhibited signs of permanent wilting and therefore measurement was not possible.

Both water potentials, Ψ_{md} and Ψ_{pd} , had more negative values in the stress treatment (Fig. 2a) than the control (Fig. 2b), and only families 135 ($F = 135.172$, $P < 0.001$), 40,010 ($F = 9.178$, $P = 0.014$), 40,029 ($F = 6.573$, $P = 0.028$), 50,207 ($F = 22.696$, $P = 0.001$), 50,226 ($F = 177.277$, $P < 0.001$) and 50,238 ($F = 16.024$, $P = 0.004$) recovered levels of Ψ_{pd} to a significant degree ($P < 0.05$). In the case of the control treatment all the families showed significant differences ($P < 0.05$) between Ψ_{md} and Ψ_{pd} , except for family 10024 ($F = 3.607$, $P = 0.087$).

The average values of all parameters evaluated were lower under drought stress (Fig. 3a) than in the control treatment (Fig. 3b); a decrease of 10% for ΔD ($F = 3.346$, $P = 0.068$), 22% for H ($F = 112.658$, $P < 0.001$), 17% for TB ($F = 22.806$, $P < 0.001$), 24% for LN ($F = 27.801$, $P < 0.001$), 8% for RW ($F = 3.896$, $P = 0.05$), 41% for TLA ($F = 175.86$, $P < 0.001$), 68% for SWC ($F = 363.440$, $P < 0.001$), 63% for Ψ_{md} ($F = 46.911$, $P < 0.001$), 405% for Ψ_{pd} ($F = 64.993$, $P < 0.001$). However, the range of response for individual families was consistently greater in the drought stress treatment, reaching the most extreme limits in Ψ_{pd} , with values from 116% to −80% (families 220 and 40,010, respectively). In addition, average values of all leaf parameters were also significantly lower in drought stress (data not

Fig. 3. Family ranking in increase in diameter (ΔD , mm), height (H , cm), total biomass (TB, g), number of leaves (LN), root weight (RW, g), total leaf area per plant (TLA, cm²), content of water in the substrate (SWC, g), water potential at midday (Ψ_{md} , MPa) and water potential at predawn (Ψ_{pd} , MPa) after the stress treatment (a) and irrigation as required (b). Symbols colour gather geographic origins: black, Galicia; clear grey, Andalusia; dark grey, Canary Islands; and white, Asturias. *These parameters could not be measured for family 18.

shown), with perimeter falling by 16%, length by 15%, width by 14% and average leaf area by 27%.

Differences between treatments and between families were significant for all morphological and water variables ($P < 0.05$) except ΔD (Supplemental Table 2). Interaction between treatment and family was only significant in relation to Ψ_{pd} . Differences between treatments for the allometric relationships calculated (LAR, SLA, ILB and AB/RW) were significant in all cases ($P < 0.05$), but not between families or for the interaction between the two factors, with the exception of ILB.

With regard to allometric relationships (Supplemental Table 3), in the majority of cases in the drought stress condition families showed a similar tendency, characterized by a reduction in LAR (30%; $F = 74.501$, $P < 0.0001$), SLA (14%; $F = 22.413$, $P < 0.0001$) and

AB/RW (21%; $F = 22.148$, $P < 0.0001$) and an increase in ILB (18%, $F = 41.141$, $P < 0.0001$). Exceptions were families 40,050 and 50,226, in which the SLA relationship increased, though not significantly. It is worth highlighting that families from the Canary Islands (40,010, 40,011 and 40,029) frequently exhibited significant differences in relation to many parameters, in particular family 40,010, where significant differences were found in all 4 relationships calculated. Six of the 20 families studied showed no significant differences in any of the allometric relationships evaluated, 2 from Galicia (18 and 172), 3 from Andalusia (10,009, 10,019 and 10,032) and 1 from Asturias (50,226).

The relationship between average leaf area (LA) and the degree of lobation (ILB) of the adult leaves for each family was strong, with ILB decreasing as LA values increased ($R^2 = 0.9512$; $P < 0.0001$).

Fig. 4. Relationships between height (H , a), leaf blade area (LA , b), leaf width (LMW , c) and leaf lobation index (ILB , d) with the minimum monthly rainfall in summer (P_s) recovered in each geographic region. Empty symbols represent the stress treatment and full symbols the control one: (○), Galicia; (▽), Andalusia; (□) Canary Islands; and (◇) Asturias.

3.2. Northern versus southern geographic variation

Significant differences between both geographical groups, northern Spain versus Andalusia and the Canary Islands, were found for most morphological and growth traits, i.e., for ΔD , H , LN , LML , LMW , LA and ILB (Supplemental Table 4). Additionally, variation between families within geographical groups was also strong and was significant for most traits. The treatment $\times$ group and treatment $\times$ family within group interactions were not significant. Only the degree of ILB showed significant differences at all statistical levels of variation.

Linear relationships were obtained for H , LA , LMW and ILB parameters – grouped according to average values for each geographic region (Northern and Southern Spain) – with respect to the corresponding minimum summer rainfall value in the area where the mother plants were located (P_s) (Fig. 4), most markedly, ILB and LA in the drought stress treatment ($R^2 = 0.3671$, $P = 0.0046$) (Fig. 4d), ($R^2 = 0.2321$, $P = 0.0315$) (Fig. 4b) and LMW in the control ($R^2 = 0.2339$, $P = 0.0307$). H , LA and LMW values increased in proportion to P_s (Fig. 4a–c, respectively). Moreover, these values were always higher in the control treatment. However, ILB values were inversely proportional to P_s (Fig. 4d) and higher under stress. In general, for each treatment, the lowest values of H , LA and LMW and the highest degree of ILB corresponded to the *C. sativa* families from the southern group (Andalusia and Canary Islands).

4. Discussion

The drought stress applied to the progenies of the main Spanish fruit cultivars of *C. sativa* Mill. used in this study significantly

modified ($P < 0.05$) their water potential, growth, morphology and several allometric relationships.

As would be expected, lower values of Ψ_{pd} and Ψ_{md} were obtained in plantlets subjected to stress than in those of the irrigation treatment. Likewise, most of the control plantlets demonstrated recuperation of their water potential at predawn compared to that at midday, indicating that the plantlets reached maximum stress level in the middle part of the day (Costa e Silva et al., 2004). However, most of the families studied did not show this recovery under drought stress, and some families, from both Northern and Southern Spain (220, 10,009, 10,024, 50,050 and 50,152), even maintained the same water potentials, indicating that they were at critical matric potential, i.e., the soil potential at which predawn and midday plant potential are equal (Sun et al., 1995). When critical matric potential is reached, hydraulic conductivity from the soil to the leaves falls abruptly (Tyree et al., 1992) and there is a consequent reduction in xylematic potential (Ψ_{xil}) until the leaves become desiccated. This situation may or may not result in the plant dying, depending on its capacity to withstand the Ψ_{xil} that causes desiccation, thus limiting the lethal potential below which the plant does not recover (Sun et al., 1995). Following the conclusion of the trial, the majority of the progenies recovered their water potential after being re-watered to field capacity (data not shown).

Plantlet growth parameters, except ΔD , fell significantly under stress in all chestnut families. Reduction in TB , one of the first responses to water stress, has also been described in wild progenies of chestnut (Eriksson et al., 2005; Pliura and Eriksson, 2002) as well as in other species (Black-Samuelsson et al., 2003; Ngugi et al., 2003).

Plants adopt different strategies to control their consumption of water and hence withstand drought though decrease in leaf area is one of the main response mechanisms in situations of water stress (Givnish, 1979; Villar-Salvador et al., 2004). In this study, TLA reduction in *C. sativa* families under drought stress conditions was due to a decrease in the average LA combined with a reduction in LN, but it was not the result of leaf fall. This strategy is also adopted in other drought-tolerant species such as certain clones of *Populus* (Chen et al., 1997) and *Eucalyptus* (Valdés et al., 2005a,b) in order to reduce water loss through transpiration. Reduced water supply may lead to a lower absorption of nutrients and this could, in turn, reduce leaf growth.

In addition, leaf shape was also found to change because ILB increased under drought conditions. Nevertheless, leaf proportions were not modified since LMW was linearly correlated to LML ($R^2 = 0.8405$; $P < 0.0001$) for all families under both watering regimens. ILB and LA were related in both treatments in that the lower the leaf area, the higher the lobation. Similar results have been found for *Quercus* in response to different levels of ambient light and to nutritional stress (Balaguer et al., 2001; Valladares et al., 2000). Sisó et al. (2001) have shown that leaf hydraulic resistance was negatively related to ILB in broadleaf *Quercus* species, suggesting that the lower hydraulic resistance in deeply lobed leaves may constitute a mechanism for improving water balance under dry atmospheric conditions.

All the progenies of *C. sativa* responded by reducing SLA values when grown with limited availability of water, though only some progenies (40,010, 40,011, 40,029 and 50,238) showed significant differences between treatments. Fischer and Turner (1978) indicated that low SLA values might be associated with high resistance to drought. For example, certain clones of *Eucalyptus globulus*, with low SLA values, were able to withstand drought periods (Pita and Pardos, 2001).

Considering the grouping of the plant materials according to their family origin, mesic or xeric, we have not detected any significant differences ($P < 0.05$) between the families assigned to each area for this index (data not shown), unlike the results of Abrams (1994) who concludes that the xeric genotypes generally produce leaves with a lower SLA than do mesic ones.

LAR index is another parameter closely related to the growth rate of a plant. This index fell significantly in plants under drought stress, as has been described by other authors for *Eucalyptus globulus* Labill (Costa e Silva et al., 2004; Pita and Pardos, 2001) and *Populus kangdingensis* Wang et Tung (Yin et al., 2005). In our case, reduction in area is the most influential parameter in altering this index (41% decrease in TLA compared to 17% in TB), which would indicate that this is one strategy of morphological adaptation because the leaf surface is responsible for the loss in water.

An increase in root:shoot ratio is a common observation under water stress since drought inhibits growth of the stem more severely than that of the root (Saab et al., 1990), and biomass distribution changes in response to the water content of the soil (Nagakura et al., 2004). In our study, the relationship AB/RW showed a common general tendency for all families, with lower values in those plants subjected to drought stress. Low values of this parameter are associated with resistance to periods of drought (Nagakura et al., 2004; Villar-Salvador et al., 2004; Yin et al., 2005), indicating a greater investment in root biomass and hence an increased capacity to absorb water from the soil. These changes in the relationship are of great interest in studies of drought tolerance in woody species (Bernier et al., 1995; Farrell et al., 1996) and are regarded as an acclimatization mechanism to avoid dehydration (Guan et al., 2004; Yin et al., 2005).

In summary, water stress had a great effect on growth and most of the morphological traits studied in these *C. sativa* juvenile progenies, all but one of the families studied demonstrated the capacity

to respond to drought. Moreover, in spite of the strong and significant between family variation within both geographical groups for most traits, we also found variation between Northern and Southern Spain in terms of height of the plants, as well as LMW, ILB, LA, LN, LML, and ΔD . This variation is in line with the genetic differentiation found by Mattioni et al. (2008) using ISSRs, indicating that the gene pool in the north was different from that of the rest of Spain, reflected in the increased vigour of progenies from the Northern Iberian Peninsula. Martin et al. (2010) and Pereira-Lorenzo et al. (2010, 2011) found the same using EST-SSRs and SSRs, respectively.

Differentiation among wild chestnut populations has also been highlighted by Fernández-López et al. (2005), who defined different models for phenological and growth characteristics according to a north-south geographic gradient. In our work (Supplemental Table 1), in the northern gene pool region, minimum average annual temperature (T_m) does not exceed 14 °C, average temperature of the hottest month (T_h) is below 19 °C and minimum monthly rainfall in summer (P_s) exceeds 17 mm. In contrast, for the southern group, T_m exceeds 14 °C, T_h is over 19 °C and summer rainfall is less than 2 mm (Allué-Andrade, 1990). The climatic conditions of the Southern gene pool are less suitable for chestnut growth, principally because of the low minimum monthly rainfall in summer. In particular, Conedera et al. (2010) concluded that this species does not seem to have an effective mechanism to protect against over-transpiration in extremely hot and dry weather, and suggested studying the ecological plasticity of the chestnut in more depth in view of possible climate change.

Taking Turkey as one of the supposed centres of origin of the European chestnut, Villani et al. (1992, 1994, 1999) confirmed genetic, morphological and physiological differentiation among wild chestnut populations spread along a Turkish transect spanning from the Black Sea coast to the Mediterranean area. Genetic differentiation of these populations was associated with macroclimatic variables, specifically, with amount of rainfall (Pigliucci et al., 1990). Moreover, it has been stated that chestnut populations from Northern Spain display a lower xerothermic index than the populations located in the Mediterranean basin (Lauteri et al., 2004). In our work, H, LMW, LA and ILB all show a significant linear relationship with respect to the corresponding minimum summer rainfall value (P_s) in the area of origin of the mother plants, which could conceivably play a role in adaptation to water shortage. This phenotypic variation corresponds to the genetic variation found using molecular markers indicating adaptive variation due to climatic conditions. Hybridization is an important factor in the diversification process of chestnut cultivars and has contributed greatly to their high genetic variability. Furthermore, it explains how they have adapted to local conditions.

Analysing the differences between both geographical groups, we found that only two families from Andalusia and the Canary Islands (20%) compared to four from the northern group (40%) were able to recover their water potential at predawn. This fact might be interpreted as the former being less tolerant to drought stress, counter to our hypothesis and to the results of Lauteri et al. (2009) and Villani et al. (2010), who suggested that populations from drought regions seem to have more phenotypic plasticity, indicating that they might also be less vulnerable to possible climate changes. However, the fact that one of the families from the north in fact died after the stress condition, points to the question of the relationship between progeny and drought tolerance being more complex than it seems at first glance.

5. Conclusion

This study shows evidence for considerable variation in adaptive traits among *C. sativa* families when they were grown under

water deficit. The variation between progenies within the northern and southern groups would suggest that these chestnut production areas offer valuable experimental material for studies of characteristics and processes involved in divergence and speciation, as well as for the safeguarding and development of genetic resources. In addition, a significant geographical trend related to P_s was found for certain morphological traits. These findings provide support for the notion that the geographical adaptation of F_1 half-sib progenies of Spanish chestnut cultivars mirrors the two Spanish gene pools, though important phenotypic plasticity was found in both origins of variability. This information will be useful in responding to the challenges of climate change expected in the Iberian Peninsula.

Acknowledgements

We would like thank D. Ríos and A.J. González for providing seeds from the Canary Islands. Thanks are also to Ernesto, Antonio, Belén, Lucía and Inma for assistance with measurements and plant maintenance. Our acknowledgements also to R. Lendrum for helping with the English review. This work was supported by I+D Project (Ministerio Ciencia y Tecnología, AGL-2003-09874-CO2-02).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.envexpbot.2011.12.018](https://doi.org/10.1016/j.envexpbot.2011.12.018).

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