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Intra-specific variability in biomass partitioning and carbon isotopic discrimination under moderate drought stress in seedlings from four *Pinus pinaster* populations

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Abstract The response to drought of 20 open-pollinated families from four *Pinus pinaster* Ait. populations covering a latitudinal cline (France, Central and Southern Spain, and Morocco) was assessed. The seedlings were cultivated in a greenhouse under controlled temperature and vapor pressure deficit for 120 days, and subjected to two watering regimes for 60 days. Different biomass partitioning variables, pre-dawn water potential, and isotopic discrimination of ^{13}C in needles (Δ) as surrogate of long-term water use efficiency were estimated for each seedling at the end of the experiment. In response to the imposed drought, there was no change in the root biomass partitioning, but the overall Δ values decreased in response to water stress. All the families of the population from Morocco showed the highest investment in roots, regardless of the watering regime imposed. Inter-family differences within populations were also significant for most parameters as confirmed by the heritability values estimated (higher under the well-watered treatment). The studied *P. pinaster* populations showed different strategies of response to drought. This may represent an important mechanism by local populations in facing future climatic change. The results could be of value in forest conservation and breeding programs of maritime pine in the future.

Keywords Water stress · Maritime pine · Biomass allocation · Adaptation · Population

Introduction

Plasticity of morphological and physiological traits may be adaptive, genetically determined, and constrained by trade-offs between different traits under natural selection (Roff 2001; Pigliucci and Preston 2004; Malausa et al. 2005). Therefore, morphological and physiological adjustments may increase the adaptability of phenotypes of the same species, as a consequence of different selective pressures and evolutionary trends (Abrams et al. 1990; Cregg 1993; Abrams 1994; Zhang and Marshall 1995; Aspelmeier and Leuschner 2006; Ducrey et al. 2008).

Drought adaptation is complex, involving drought avoidance and tolerance mechanisms. Maritime pine (*Pinus pinaster* Aiton) is considered a drought-avoiding species with a high stomatal sensitivity to dry soils (Granier and Loustau 1994; Picon et al. 1996; Fernández et al. 2000). The species, however, also develops drought tolerance mechanisms such as osmotic adjustment or changes in biomass partitioning. These traits may vary among populations. Indeed, inter-population variation has been ob-

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served for traits such as biomass allocation, water use efficiency (WUE), and osmotic adjustment (Nguyen and Lamant 1989; Nguyen-Queyrens et al. 1998). Otherwise, little or no information on intra-population variation of drought tolerance is available for maritime pine (Brendel et al. 2002). It is an important forest species in the Western Mediterranean region, with high levels of variation among populations in different phenotypic traits with a likely adaptive value (Guyon and Kremer 1982; Danjon 1994; Guehl et al. 1995; Alia et al. 1997; Tognetti et al. 2000; Chambel et al. 2007). High levels of population differentiation for quantitative traits (Q_{ST}) have been reported: 0.73–0.80 for growth and survival (González-Martínez et al. 2002; González-Martínez et al. 2004), 0.15 for isotope composition, and 0.45 for height (Eveno et al. 2009), larger than the neutral levels of differentiation (F_{ST}/G_{ST}=0.08–0.10) (i.e., isozymes in Salvador et al. 2000, nuSSR in Derory et al. 2002 and Eveno et al. 2008, and CpSSR in Bucci et al. 2007).

WUE may be related to the success of a genotype in drought-prone environments (Dudley 1996; Ares et al. 2000; Franco et al. 2005), although some exceptions have also been described (Read and Farquhar 1991; Laureti et al. 1997; Nguyen-Queyrens et al. 1998; Palmroth et al. 1999). A time-integrated measurement of WUE has been readily assessed, in many cases, by determining the isotope discrimination to ^{13}C (Δ) in the plant (Farquhar et al. 1989). Frequently, Δ has been observed to be correlated to growth and biomass accumulation. The trait has been studied in comparative studies analyzing the intra-specific genetic variation (Zhang and Marshall 1995; Flanagan and Johnsen 1995; Aitken et al. 1995; Cregg et al. 2000; Grossnickle et al. 2005), and has previously been shown to be a surrogate of WUE in maritime pine seedlings (Guehl et al. 1995). However, it is still a matter of debate, the sense of the correlation between Δ and growth reported as positive, negative, or absent, depending on the species and genotypes (Roupsard et al 1998; Brendel 2001; Monclus et al. 2005, 2006).

Less phenotypic plasticity is expected for populations enduring more stressful abiotic environments (Rehfeldt et al. 1999; Howe et al. 2003; Valladares et al. 2007).The goal of this paper is to test whether populations of maritime pine from wet sites show higher responsiveness to drought than populations from dry sites. According to the optimum allocation hypothesis, this higher responsiveness would result in (1) differential growth in terms of biomass accumulation, (2) higher investment in below-ground biomass, and (3) higher water use efficiency assessed by carbon isotopic discrimination. To address these questions, changes in carbon isotopic discrimination, growth, and biomass partitioning parameters in response to a mild drought were analyzed in 20 families from four populations of maritime pine covering a latitudinal cline across the geographic distribution of the species.

Materials and methods

Plant material

Four autochthonous populations of *P. pinaster* were selected following a latitudinal cline from France (PLEU) to Central Spain (ASPE), Southern Spain (ORIA), and Morocco (TAMR) (Table 1). In each population, seeds were collected from five mother trees with at least a 50-m separation to avoid inbreeding (González-Martinez et al. 2006), resulting in 20 open-pollinated families. The four populations had previously been tested in field provenance trials (Alia et al. 1995, 1997), although information is not available on the response at the juvenile phase. The seedling phase is of primary importance in natural stands as mortality during the first year is the main selective factor. It has been suggested that biomass allocation at juvenile stages is a fundamental aspect of the diversification of pines (Strauss and Ledig 1985), and considering growth, genetic information from early testing can be used to enhance selection efficiency at maturity (Wu 1998; Weng et al. 2008).

Experimental conditions

Seeds were sown in 400-cm^3 pots with a mixture of vermiculite and grade 3 peat (80:20 *v*:*v* peat "VP BOW", fertilized with NPK 15:8:12, at 1.5 kg m^3) on 12/04/2004 (day 0). After germination, 20 seedlings from each family (for a total of 400 seedlings) were arranged in a block design with ten blocks and a two-seedling family plot in each block. The experiment was divided into two treatment phases. Firstly, a growing phase (watering to full capacity, 20°C, and 90% of relative humidity) that lasted until the plants were 120 days old. At the end of this growing phase, the seedlings had a mean height of 29.14±16.24 mm and a diameter of 1.23±0.23 mm. Secondly, after this growing phase, plants were submitted to two watering regimes (well-watered, WW, and drought-stressed, DS, treatments) for 60 days (180 days growth in total). Each treatment was applied to five contiguous blocks (ten seedlings per family). Unpublished previous studies, using genetically diverse material, had proved the difficulty of maintaining a relationship between volumetric substrate water content and a fixed level of stress over the predicted duration of the experiment (6 months). Therefore, the two watering regimes were based on the saturation level of the substrate: well-watered treatment, in which the substrate was kept at 90–100% of the mean saturation level, and drought-stressed

Table 1 Main climatic parameters of the four seed sources used in the experiment

Code	Population	Country	Latitude and longitude	Altitude range (m)	Annual rainfall (mm)	Mean annual temperature (°C)
PLEU	Pleucaeuc	France	47° 46′ N 2° 20′ W	80	855	12.0
ASPE	Arenas de San Pedro	Spain	40° 12′ N 5° 3′ W	728	1,190	13.3
ORIA	Oria	Spain	37° 31′ N 2° 21′ W	1,150	357	15.8
TAMR	Tamrabta	Morocco	33° 36′ N 5° 01′ W	1,760	763	*

*means no data available

treatment, in which the substrate was kept at 40% of the mean saturation level across populations and families. The amount of water for each irrigation operation was determined daily by weighing ten plants randomly chosen from each treatment. The following macro- and micronutrients (milligrams per plant per week) were applied: N (9.27), P_2O_5 (4.25), K_2O (7.45), MgO (0.038), Mo (0.006), Fe (0.064), Mn (0.042), Zn (0.006), B (0.006), and Cu (0.032). During the stress period, the same program fertilization was applied twice.

The study was conducted in a greenhouse under natural light, controlled temperature, and vapor pressure deficit (1.0 kPa) with a proper mixing of the air around seedlings. Thus, similar isotopic composition of the air surrounding all the seedlings was assumed. At the end of the experiment, the seedlings reached a mean height of 135.48±29.20 mm and a diameter of 2.59±0.42 mm.

Variables measured

Two harvests were made, at times $t_1=30$ and $t_2=60$ after the beginning of the water stress cycle, when seedlings were 150 and 180 days old, respectively. One seedling per plot and block was harvested (five seedlings/family, or 25 seedlings/population, for each of the two watering treatments, for a total of 200 seedlings in each harvest). Therefore, for each of the two harvests, we can assume that a one-seedling plot was used in the experiment. In each harvest, pre-dawn water potential was determined by a Scholander pressure chamber, the plant was collected and oven-dried at 70°C for 48 h, and three fractions (root, needles, and stem) were weighed (±0.01 g). The following variables were subsequently derived: root biomass ratio (RBR—dry root biomass/total seedling biomass, g g^{-1}), stem biomass ratio (SBR—dry stem biomass/total seedling biomass, g g^{-1}), and needle biomass ratio (NBR—dry needle biomass/total seedling biomass, g g^{-1}). The roots from the harvested seedlings were not pot-bound, enabling us to deduce that the size of the rooting media had not excessively limited root development.

At the final harvest (t_2), several needles from the apical third of the seedlings were collected, dried, and ground into a fine, homogenous powder and the isotopic ratio $^{13}C/^{12}C$ was analyzed in an elemental analyzer (Carlo Erba 1108) coupled to a mass spectrometer IRMS (Isochrom, Micromass) at the SIDI of the Autonomous University of Madrid. The $^{13}C/^{12}C$ composition of samples was expressed as $\delta^{13}C$ and isotopic discrimination (Δ) calculated according to:

$$\Delta(‰) = \frac{\left(\delta^{13}{}_{air} - \delta^{13}{}_{sample}\right)}{\left(1{,}000 + \delta^{13}{}_{sample}\right)} \times 1{,}000$$

where $\delta_{\text{sample}}{}^{13}$ refers to $\delta^{13}C$ of needles and $\delta_{\text{air}}{}^{13}$ is the $\delta^{13}C$ of the air acting as carbon source (it was assumed to be −8‰).

Statistical analysis

Given the experimental design and independent and normally distributed residuals (0, V_e), the following model was established for all the variables:

$$y_{ijklm} = \mu + t_i + p_j + tp_{ij} + \varphi_{k(j)} + t\varphi_{ik(j)} + \beta_l + \varepsilon_{ijklm} \quad (1)$$

where μ is the overall mean of the variable, y_{ijklm} is the value of the variable for the m-th seedling from the k-th family within j-th population, located in the l-th block in the i-th treatment, t_i is the effect of the i-th treatment (1–2), p_j is the effect of the j-th population (1–4), $\varphi_{k(j)}$ is the effect of the k-th family within the j-th population (1–5), β_l is the effect of the l-th block (1–10), and ε_{ijklm} is the residual.

The model was analyzed as a mixed model with fixed (block, watering treatment, and population) and random (family within population and error) effects, where the components of variance were obtained by restricted maximum likelihood. A significant watering regime by population interaction for a variable would indicate significant differences in the phenotypic plasticity between populations (Schlichting 1986).

The change in above-ground (needles and stem) dry biomass with regard to below-ground (root) dry biomass was tested by an allometric analysis, using log-transformed data from each of the two harvests. Differences in slopes and intercepts for the four populations and two watering regimes were tested by a parallelism test (Poorter and Nagel 2000).

Heritability (narrow sense) over populations, h^2, was calculated for each variable after removing the population component from each family and the treatment effect:

$$h^2 = \frac{V_\mathrm{A}}{V_\mathrm{P}} = \frac{4V_\mathrm{fam}}{V_\mathrm{fam} + V_\mathrm{e}} \tag{2}$$

where V_P is the phenotypic variance, V_A is the additive variance, V_fam is the family variance, and V_e is the error variance. A value of 4 can be assumed as the mean value of the coancestry coefficient of maritime pine families has been estimated as 0.130 (Gaspar et al. 2009), i.e., a coefficient of relationship among open-pollinated sibs of 0.260, similar to that obtained in natural populations (de Lucas et al. 2008) and to the values expected for half-sib families (0.250). The outcrossing rate for this species is close to 1.0 (t_m and t_s ~0.96) (de Lucas et al. 2008).

Statistical analysis was performed using SAS 9.1 (SAS/STAT® Software; SAS Institute). The mixed model means, heritability, and standard error of the estimates were calculated by the ASReml software (Gilmour et al. 2001).

Results

Watering regime, population, the family within population, and, on some cases, their interaction had significant effects depending on the traits under consideration (Table 2). Watering had a significant effect on most of the traits (except in RBR). To characterize the drought stress imposed, we can use the differences in pre-dawn water potential and Δ. Pre-dawn water potentials in the first harvest were −0.24±0.15 MPa in the WW treatment and −0.27±0.15 MPa for the DS treatment. In the second harvest, these values were respectively −0.38±0.21 and −0.45±0.21 MPa. Differences for Δ were close to 1‰ among the two treatments in the second harvest (23.07±0.71‰ in WW and 22.25±1.32‰ in DS).

It is interesting to note that no significant treatment by population interaction was detected for any of the analyzed traits except Δ, i.e., no differences in plasticity have been observed in those traits.

While biomass in the different fractions (root, needles, and stem) was similar for both drought levels at the time of the first harvest, there was a clear reduction in biomass of stressed seedlings in comparison to the well-watered ones in the second harvest (Fig. 1). The differences in biomass as consequence of the induced mild drought stress were significant only at the end of the experiment.

There were significant population differences in RDB, NDB, and SDB but not in TDB (Table 2). These differences were observed on both watering treatments. When considering biomass fractioning, there were no statistical differences among populations in NBR, being only significant for RBR and SBR. In this respect, population TAMR showed the lowest SBR and the highest RBR of the populations tested (Table 3).

The allometric analysis showed a higher intercept in the regression between $\log_{10}$ (RB) and $\log_{10}$ (SB+NB) for TAMR (Fig. 2) in comparison to the other three populations, but no differences in the slope. Drought induced a reduction in the dry stem biomass for the four populations and an increment in root dry biomass (P=0.08). TAMR and ORIA showed a low plasticity for Δ (P<0.05 for the interaction term), the plasticity in morphological parameters being similar among the four populations (P>0.05 for the interaction term).

Table 2 Mean square and significance level for the different factors

	Mean square; *F* value and significance level				h^2 (SE)[a]
	Watering	Population	W×Pop	Block	
Total dry biomass—TDB (g)	6.18411; 170.22***	0.1576; 2.54 ns	0.0347; 0.13 ns	0.26320; 1.02 ns	0.89 (0.397)
Root dry biomass—RDB (g)	6.0198; 103.72***	1.0375; 18.13***	0.0572; 1.45 ns	0.07087; 1.79*	0.99 (0.361)
Stem dry biomass—SDB (g)	13.576; 525.39***	0.8803; 98.91***	0.0089; 0.98 ns	0.01033; 1.13 ns	0.77 (0.378)
Needle dry biomass—NDB (g)	6.0198; 105.24***	1.0375; 18.13***	0.0572; 0.69 ns	0.08294; 1.01 ns	0.99 (0.389)
Root biomass ratio—RBR	0.00028; 0.10 ns	0.0567; 20.25***	0.0028; 0.87 ns	0.00555; 1.73 ns	0.07 (0.173)
Stem biomass ratio—SBR	0.0642; 71.33***	0.0431; 47.89***	0.0009; 1.43 ns	0.00082; 1.31 ns	0.04 (0.051)
Needle biomass ratio—NBR	0.0284; 9.16***	0.0057; 1.83 ns	0.0031; 1.20 ns	0.00427; 1.66 ns	0.11 (0.183)
Isotopic discrimination—Δ (‰)	26.451; 9.02***	12.334; 4.20***	2.9313; 3.75*	0.02300; 0.03 ns	0.07 (0.050)

Narrow sense heritability (h^2) for the quantitative traits studied in both watering treatments were also estimated

[a] h^2 for the different traits was calculated from Eq. (1)

***P<0.001; **P<0.01; *P<0.05

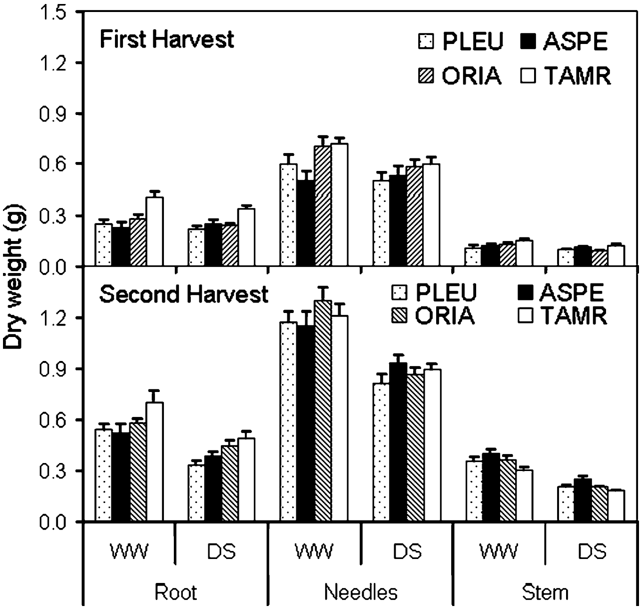

Fig. 1 Total dry biomass in root, needles, and stems of seedlings for four maritime pine populations (*ASPE*, *PLEU*, *ORIA*, *TAMR*). No significant differences among populations were observed in total dry biomass and the different fractions for the first harvest (**a**). However, differences were observed between treatments and populations in the second harvest (**b**). *Asterisks* depict significant differences among provenances within treatment after log-transformation of data ($P<0.05$)

The effect of families within populations is significant for all the biomass traits and isotopic discrimination but not in the biomass partitioning parameters. It is worth noting that, for TDB, SDB, NDB, and RDB, the heritability was high, but the heritability for discrimination and biomass partitioning traits was close to 0. When analyzing each watering regime independently, the same pattern of the variation within families was observed in all traits (no significant watering by family interactions, data not shown) except for isotopic discrimination. Under the drought-stressed watering regime, the families differ significantly ($h^2=0.69\pm0.31$).

Total dry biomass for all families was positively correlated between the well-watered and drought treatments (Fig. 3). A significant interaction was observed between the watering regime and family within population for Δ, indicating differences in plasticity among populations and families for this parameter (Fig. 4).

PLEU showed the highest inter-family variability in Δ ranging from 22.46±0.62‰ to 23.08±0.22‰ for well-watered seedlings and 20.05±0.35‰ to 22.41±0.34‰ in water-stressed ones. TAMR was the origin that had lowest within-population variability in Δ that ranged from 22.85±0.18‰ to 23.86±0.20‰ and 22.69±0.61‰ to 23.51±0.27‰ for well-watered and water-stressed seedlings, respectively (Fig. 4). Most families from PLEU and ASPE showed a significant decrease in Δ in response to water stress, whereas differences were not noticeable in TAMR and only significant in three of the five families from ORIA. This suggests that the Δ values measured in well-watered plants were positively correlated with those under drought stress conditions but with a low weight of the correlation coefficient ($r=0.4$, $P<0.05$).

A plot of family means for the different dry biomass fractions versus Δ (Fig. 5) reveals a negative genetic relationship which is stronger and has a higher slope in the WW treatment (Fig. 5). The weight of the relationship between Δ and the different plant fractions decreased from stems ($P<0.05$) to needles ($P<0.1$) and roots, being not significant in the last case ($P>0.1$). When overall growth was considered (TDB), the relationship was only significant for well-watered seedlings (data not shown).

Discussion

In the present study, we have induced drought stress in maritime pine seedlings to test the performance of populations and families at the juvenile stage. The pre-dawn water potential measured at the end of the experiment did not reflect the average water stress endured by plants throughout the experiment. This was clear as the imposed stress caused a reduction of close to 30% in biomass traits relative to the unstressed (WW) treatment by the end of the experiment (30.3% for TDB, 41.4% for NDB, and 28.4% for RDB) and close to 1‰ in Δ. One of the most important results was the absence of treatment by population interaction (except for Δ), i.e., the lack of differences in plasticity between provenances. The highly significant interaction between watering and population for Δ indicated differences in phenotypic plasticity among the four populations (Correia et al. 2008). Furthermore, h^2 was high under water stress but not under well-watered conditions. The two populations from the south (ORIA and TAMR) showed a low responsiveness of Δ to drought, whereas PLEU and ASPE displayed a great change. Guyon and Kremer (1982) also reported low plasticity in populations of maritime pine from harsh environments, with respect to seasonal drought intensity or low growing season length for high-altitude populations.

In the present study, as in Fernández et al. 2006, total dry biomass and biomass of the different compartments of young seedlings varied among families within population. The additive variance was high, resulting in significant variation among families and substantial heritabilities for most traits under both watering treatments. However, drought was the main factor promoting a decrease in overall dry biomass, although no effect was observed in patterns of dry biomass partitioning (Osório et al. 1998). It is noteworthy that carbon allocation to the different plant

Table 3 Means (with ±SE) of allocation biomass parameters: NBR (needle dry biomass ratio), SBR (stem dry biomass ratio), and RBR (root dry biomass ratio)

	ASPE		ORIA		PLEU		TAMR	
	WW	DS	WW	DS	WW	DS	WW	DS
NBR (g g^{-1})	0.56±0.01 aA	0.60±0.01 bX	0.58±0.01 aA	0.58±0.02 aX	0.57±0.01 aA	0.61±0.01 bX	0.55±0.01 aA	0.57±0.01 aX
SBR (g g^{-1})	0.19±0.01 bA	0.16±0.01 aZ	0.16±0.01 bC	0.13±0.01 aY	0.17±0.01 bB	0.15±0.01 aZ	0.13±0.01 bD	0.12±0.01 aX
RBR (g g^{-1})	0.25±0.01 aA	0.24±0.01 aX	0.26±0.01 aA	0.29±0.02 aY	0.25±0.01 aA	0.24±0.01 aX	0.31±0.01 aB	0.31±0.01 aY

Capital letters indicate differences among populations in a watering treatment. Different lowercase letters depict significant differences between treatments in a specific population

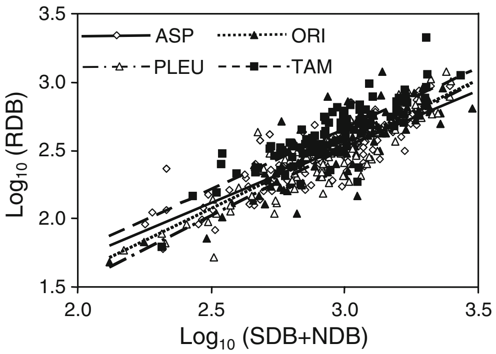

Fig. 2 Allometric regressions between $\log_{10}$ root dry biomass (*RDB*) and $\log_{10}$ stem plus needle dry biomass (*SDB+NDB*). Data from drought-stressed and well-watered seedlings were pooled. Intercepts were significantly different according to population ($P<0.05$). Populations: TAMR (*black squares* and *black dashed line*—Log (RDB)=0.893 Log(SDB+NDB)−0.021 $r^2=0.70$), ORIA (*black triangles* and *dotted line*: Log(RDB)=0.944 Log(SDB+NDB)−0.284 $r^2=0.71$), PLEU (*white triangles and hybrid line*—Log(RDB)=0.988 Log(SDB+NDB)−0.443 $r^2=0.78$), and ASPE (*white diamonds* and *solid line*—Log(RDB)=0.826 Log(SDB+NDB)+0.051 $r^2=0.74$). All the regressions were significant ($P<0.01$)

compartments had a genetic basis, and populations had contrasting patterns of dry biomass partitioning. Indeed, the four populations are from the three different *P. pinaster* meta-populations described previously (Burban and Petit 2003). Populations from the south of Spain (ORIA) and Morocco (TAMR) showed a higher overall dry root biomass, whereas ASPE and PLEU from central Spain and southern France, respectively, showed a higher overall dry stem biomass and height (data not shown). Drought promoted a decrease in total dry biomass in the four populations. The mild water stress applied during our experiment was not enough to cause a more evident shift in allocation towards below-ground organs as previously reported. However, it was noticeable that dry biomass partitioning was to a large extent under genetic control, as described in other species (Tschaplinski et al.

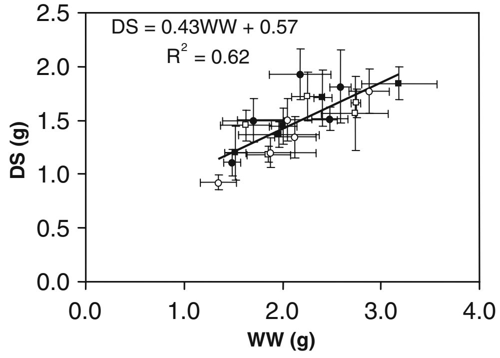

Fig. 3 Relationship between total dry biomass for drought-stressed (*DS*) and well-watered (*WW*) maritime pine seedlings. Each *point* represents the average value of one open-pollinated family ($N=5$) from four provenances: PLEU (*open circles*), ORIA (*open squares*), ASPE (*filled circles*), TAMR (*filled squares*)

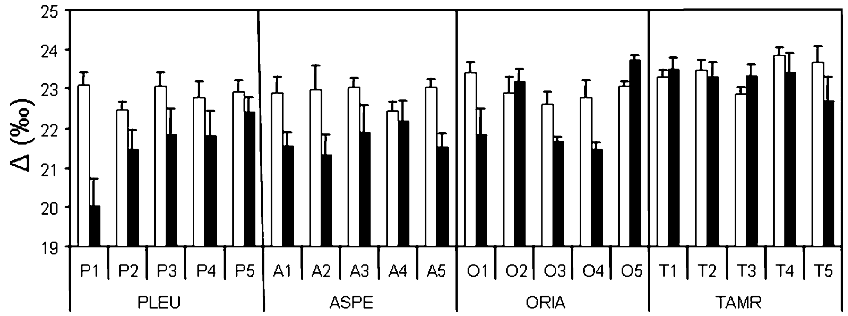

Fig. 4 Average isotopic discrimination in needles (Δ—‰) for the different open-pollinated families of maritime pine studied. Needles were collected in the last harvest from the apex of seedlings. Each *bar* depicts the mean value for each family: *black*—drought-stressed seedlings, *white*—well-watered seedlings (n=5, ±SE)

1998). RBR was higher for most families in the southernmost populations (ORIA and TAMR), irrespective of water availability. This was to the detriment of investment in dry stem biomass (lower SBR), and resulted in higher needle packing along stems. NBR was almost the same for all populations, without any change with watering. The below-ground growth was favored relative to above-ground growth, but preferentially by promoting a decrease in stem growth. Thus, seedlings from TAMR had a higher needle area per unit of stem together with a lower height. Investment in the root system has been traditionally viewed as a mechanism of drought tolerance that would increase the uptake of water and therefore the possibility of improved water status of plants under stress conditions (Ibrahim et al. 1997; Joslin et al. 2000; Pinheiro et al. 2005).

Isotopic discrimination in needles was positively correlated with above-ground biomass growth in well-watered and drought-stressed seedlings. The low significance of the relationship was explained by the high levels of differentiation in the phenotypic plasticity among populations and families. There was significant genetic variation both in Δ among and within populations as found previously by Brendel et al. (2002). The southernmost population showed the highest isotopic discrimination. While the overall phenotypic response to water stress was a decrease in the isotopic discrimination in two of the four populations, as noted by Guehl et al. (1995) reflecting the adaptive potential of plasticity in delta, this does not translate into populations from dry sources actually having lower delta values (Roupsard et al. 1998). This is in agreement with previous results in

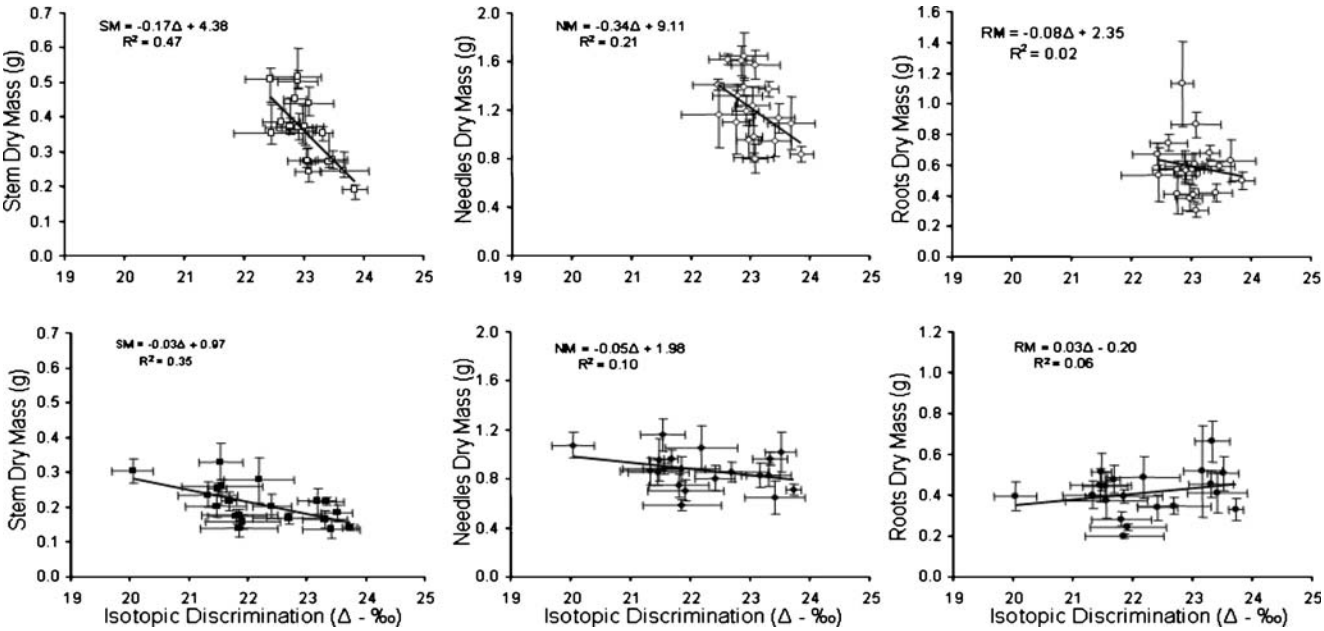

Fig. 5 Genetic relationships between isotopic discrimination in needles (Δ—‰) and dry biomass in stem, needle, and roots (from *right* to *left*) in well-watered (*upper graphs*) and drought-stressed seedlings (*lower graphs*). Each *point* represents one family average value (±SE). The regressions were only significant for Δ and dry biomass in stem (P<0.05), marginally for dry biomass in needle (P<0.1), and not significant for dry biomass in roots

maritime pine (Guehl et al. 1995; Nguyen-Queyrens et al. 1998) and other forest species such as *Pinus ponderosa* (Zhang et al. 1997; Guy and Holowachuk 2001), *Nothofagus* sp. (Read and Farquhar 1991), or *Betula pendula* clones (Aspelmeier and Leuschner 2004, 2006). Higher values of Δ for populations from drier sources have also been found in other species. Schulze et al. (2006) showed, for a ranking of *Eucalyptus* species covering a rainfall gradient, a higher variability in the response of Δ to drought for species originating from dry areas. In this sense, and within some species, populations from more xeric sites were not those with the lowest Δ, the isotopic signal being more strongly modulated by other species-specific traits such as specific leaf area or N content. The TAMR population comes from a high altitude with a short growing season and where access to soil water at the beginning of the growing season may be more important than efficient water use (Benowicz et al. 2000). A similar trend was observed by Guy and Holowachuk (2001) with *Pinus contorta* populations.

The highly significant interaction between watering and population for Δ indicated differences in phenotypic plasticity among the four populations. Furthermore, isotopic discrimination showed a high h^2 under water stress but not under well-watered conditions. The two populations from the south (ORIA and TAMR) showed a low responsiveness of Δ to drought, whereas PLEU and ASPE displayed a great change.

Conclusions

Maritime pine (*P. pinaster* Aiton) is a species which exhibits high differentiation among populations, both in neutral and adaptive traits. Our study showed large differences among populations and families in biomass partitioning, isotopic discrimination, and in the plasticity to water stress at the seedling state. This would point out to different strategies among populations, particularly for isotope discrimination. Therefore, the two southern populations from drier sources were less plastic in their response of delta to drought than the two northern ones. However, the long-living nature of forest tree species necessitates caution when generalizing from experiments carried out with very young materials. The results should be viewed with care as changes in the expression of the traits studied are to be expected with age. It is known that seedlings may behave differently from adult trees, and that differences in traits observed in seedlings do not necessarily translate to mature trees. To what extent the present results can be generalized to mature trees in the field deserves future study. However, studies of the effect of water stress on plants that consider the response at early ages are important, considering that early developmental stages are especially sensitive to selective pressures imposed by perturbations such as drought.

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