

Retrospective early test for adult vigor of *Pinus pinaster* families grown under two water regimes. Implications for early selection

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Abstract – Early selection criteria for adult vigor in *Pinus pinaster* were investigated in families of Landes provenance characterized genetically for their adult vigor. One-month old seedlings were raised under two water regimes: well-watered (–0.03 MPa) and a PEG-induced water stress (–0.5 MPa). Results showed that root growth and biomass were more deeply stimulated by the drought stress in less vigorous (V–) families than in vigorous (V+) ones, but the latter had their shoots more hydrated. An osmotic adjustment was observed in both types of families with, however, values of osmotic potential in the root tip lower in V– than in V+; inversely in the upper part of the root. This study does not support the hypothesis that mimicking field limiting factors improve juvenile-mature correlations in maritime pine. Statistical parameters were not affected in such a manner which could enhance genotypic parameters, and few family-mean correlations were significant. The implications for early selection are discussed.

Pinus pinaster / retrospective test / water stress / juvenile-mature correlation

Résumé – Tests rétrospectifs pour la croissance de familles de *Pinus pinaster* soumises à deux régimes hydriques. Implications pour la sélection précoce. Des tests rétrospectifs pour la croissance ont été mis en œuvre sur des familles vigoureuses et peu vigoureuses de *Pinus pinaster* de la provenance landaise caractérisées pour leur vigueur adulte. Des semis de 1 mois ont été soumis à deux régimes hydriques en solution hydroponique : témoin (–0.03 MPa) et stress (–0.5 MPa). Les résultats obtenus ont montré une croissance racinaire des familles V– accélérée par rapport à celle des familles V+ ; mais la partie aérienne de ces dernières était plus hydratée. Un ajustement osmotique racinaire a été observé chez les deux types de familles avec, cependant, des valeurs de potentiel osmotique plus négatives dans la pointe racinaire des V– que des V+, et inversement pour la partie supérieure de la racine. Cette étude n'apporte que peu de soutien à l'hypothèse qu'une simulation des conditions limitantes en champ améliorerait les corrélations juvénile-adulte chez le pin maritime. Les paramètres statistiques issues d'analyses de variance n'ont pas été affectés dans le sens d'une amélioration des paramètres génotypiques et peu de corrélations significatives ont été enregistrées au niveau des moyennes de familles. Les implications de ces résultats quant à la sélection très précoce chez le pin maritime sont discutées.

pin maritime / test rétrospectif / stress hydrique / corrélation juvénile-adulte

1. INTRODUCTION

Height growth is a major selection criterion in forest tree breeding due to its close relationship with tree volume. However, while genotypes are usually evaluated at a relatively young age, genetic gains are expected for age at rotation, i.e. after several dozens of years. Unfortunately, the lack of correlation between early and late growth is a frequent feature in most coniferous species [4, 22, 26, 29, 35, 46] and prevents early selection to be securely successful. Three main hypotheses are usually suggested to explain the poor correlations [19, 51]: (i) the persistence of maternal effects, (ii) genotype-age interactions and (iii) genotype-environment interactions. This has resulted in the development of models which attempt to predict late performances through early performances [28, 36,

50] and the practice of retrospective tests which either accelerate seedling growth to increase the rate of maturation [26, 31] or mimic field growth conditions [1, 20, 51].

Height growth is a chief characteristic in the breeding program for maritime pine in Landes, France. This species is cultivated to an age of 50 years. Previous studies have shown that early-mature correlations remain low until an age of 12 years and that any study done at an earlier stage required the use of traits other than height growth [25]. Investigations to find proper juvenile traits for efficient early selection for height growth in maritime pine have been developed since the eighties. Height growth was subdivided in its various elementary components (annual increments, number and length of stem

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Table I. Characteristics of plant material. W is for well-watered, S for water stress. The half-sib families were obtained by polycross.

Families	Code	Code and adult vigor of the parents		Family type	17-year old height	Number of plantlets used
		Mother	Father(s)			
Full sibs	F1	5309 (V+)	1901 (V+)	Vigorous (V+)	9.111	19 (C) 17 (S)
	F2	4701 (V+)	5101 (V+)	Vigorous (V+)	9.268	20 (C) 20 (S)
	F3	5101 (V+)	5309 (V+)	Vigorous (V+)	9.268	14 (C) 14 (S)
	F4	0214 (V-)	1307 (V-)	Non vigorous (V-)	8.846	3 (C) 3 (S)
	F5	0820 (V-)	0214 (V-)	Non vigorous (V-)	8.760	20 (C) 20 (S)
	F6	1308 (V-)	1307 (V-)	Non vigorous (V-)	9.065	20 (C) 20 (S)
Half sibs	Fa	3801 (V+)	0007 + 0014 + 0008 + 1901 + 1319	Vigorous (V+)		20 (C) 20 (S)
	Fb	3112 (V+)	idem	Vigorous (V+)		19 (C) 20 (S)
	Fc	5301 (V+)	0137 + 0003 + 0142 + 3806	Vigorous (V+)		20 (C) 20 (S)
	Fd	0214 (V-)	0001 + 0006 + 0011 + 0054	Non vigorous (V-)		20 (C) 20 (S)
	Fe	1308 (V-)	idem	Non vigorous (V-)		20 (C) 20 (S)
	Ff	0048 (V-)	3827 + 3807 + 3820 + 0021	Non vigorous (V-)		20 (C) 20 (S)

units and cells, etc.); it was shown that the length of the first shoot and the number of stem units contribute more to the genetic variation of the composite character [24]. But such investigations finally failed to find any single component which could be an efficient early selection criterion [27]. Other studies explored the effects of accelerating the maturation of seedlings under continuous light [32]; in average, growth accelerating conditions resulted in better juvenile-mature correlations than normal conditions. A third topic was developed which consists in mimicking the adult growth conditions in Landes where a drought period occurs in summer; then, retrospective tests were undertaken on seedlings of various ages subjected to an artificial drought constraint [37]. In the present work, which was in line with the latter topic, a retrospective test for adult growth (17-year tree height in the field), using one-month-old seedlings grown under well-watered and water-stressed regimes, was performed in families of the French Landes maritime pine provenance. Growth and biomass response to the induced water stress as well as water status of seedlings was investigated along with effects on statistical parameters and juvenile-mature correlations.

2. MATERIALS AND METHODS

2.1. Plant material and culture conditions

One-month-old seedlings from six full-sib and six half-sib families of the maritime pine population of Landes (Southwestern France) were used in this work. These families have already been characterized for their adult vigor assessed in progeny tests; they have been grouped in two homogenous classes of vigor: vigorous (V+) and non vigorous (V-) according to their ranking for total height at 17 [32, 33]. Genetic material and sample sizes per family and treatment are given in Table I. Seeds were sterilized in 4% Ca(OCl)₂ for 15 min and rinsed with demineralized water before germination on mois-

tened paper in glass jars. The whole lot was placed in the darkness at room temperature. As soon as the radicles reached about 1.5 cm length (after a period of 10 days), plantlets were transferred to an aerated nutrient solution which was renewed every week and received 16 h days, the illuminance at plant height being 216 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (photosynthetic photon flux density) supplied by 6 fluorescent Gro-lux lamps (36 W). Treatments were (i) well-watered seedlings (control, $\Psi_{\text{sol}} = -0.03$ MPa), (ii) moderately water-stressed seedlings (-0.5 MPa) by adding gradually an osmoticum (polyethylene glycol 600). The final osmotic potential of the medium was reached within 5 days by steps of -0.1 MPa.

2.2. Growth, biomass and water status measurements

The seedlings, gathered by family and treatment, were individually sampled 15 days following their transfer to the nutritive solution. At the time of sampling, root, hypocotyl and epicotyl were washed with cold water, carefully swabbed with blotting paper, weighed (fresh weight) and dried for dry weight measurement. Table II lists the studied traits. However, full-sib family 4 (V-) was not considered for growth and biomass due to poor seed germination (Tab. I). The experiment as described above was replicated in April and May using the same pool of families.

Between 7 and 12 days after the transfer, seedlings were harvested from each family and treatment (well-watered and water-stressed), roots plunged in cold water for 3 s then swabbed with blotting paper. For each seedling, 5-mm-long fragments were excised from (i) the upper part of the root free of secondary roots, (ii) the middle part of the root, (iii) the tip of the root. Measurements of water potential (Ψ_w) and osmotic potential (Ψ_s) were made on two fragments from each part of the radicle with a psychrometer-hygrometer, a device constituted by a thermocouple chamber C-52 Wescor connected to a dew-point microvoltmeter HR-33T Wescor. Value of Ψ_w was read 30 min after enclosing the two fragments in the chamber; Ψ_s was measured on the same two fragments after freezing in liquid nitrogen, again 30 min after enclosing. Turgor potential (Ψ_p) is obtained by difference ($\Psi_w - \Psi_s$). For each family, considered value was the average of six such measurements.

Table II. List and abbreviations of studied traits.

	Trait	Abbreviations
Growth and biomass	Root length (mm)	RL
	Hypocotyl length (mm)	HL
	Epicotyl length (mm)	EL
	Total dry weight	TDW
	Root dry weight (mg)	RDW
	Hypocotyl dry weight (mg)	HDW
	Epicotyl dry weight (mg)	EDW
	Root dry weight/root length ratio	RDW/RL
	Hypocotyl dry weight/hypocotyl length ratio	HDW/HL
	Epicotyl dry weight/epicotyl length ratio	EDW/EL
	Root/shoot length ratio	RL/SL
	Root/shoot dry weight ratio	RDW/SDW
	Water content	Root water content (%)
Hypocotyl water content (%)		HWC
Epicotyl water content (%)		EWC
Water status	Water potential (MPa)	$\Psi_w(u)$, $\Psi_w(m)$, $\Psi_w(t)$
	Osmotic potential (MPa)	$\Psi_s(u)$, $\Psi_s(m)$, $\Psi_s(t)$
	Turgor potential (MPa)	$\Psi_p(u)$, $\Psi_p(m)$, $\Psi_p(t)$

2.3. Statistical analysis

Analysis of variance was made (i) to test main effects of treatment (well-watered vs. water-stressed), type of families (vigorous vs. non vigorous) and family within type of family, and (ii) interaction effects between factors. The subsidiary factor (replicate) was included in the analysis. The overall linear model is as follows:

$$X_{ijklm} = \mu + r_i + \theta_j + \phi_k + f(\phi_k)l + (r\theta)_{ij} + (r\phi)_{ik} + (\theta\phi)_{jk} + (rf(\phi_k))_{il} + (\theta f(\phi_k))_{jl} + (r\theta\phi)_{ijk} + e_{ijklm},$$

where X_{ijklm} = the measured value on a given plantlet; μ = the overall mean; r_i = the random effect of the i th replicate ($i = 1, 2$); θ_j = the fixed effect of the j th treatment ($j = 1, 2$); ϕ_k = the fixed effect of the k th type of families ($k = 1, 2$); $f(\phi_k)l$ = the random effect of the l th family within the k th type of families; $(r\theta)_{ij}$ = the interaction between the i th replicate and the j th treatment; $(r\phi)_{ik}$ = the interaction between the i th replicate and the k th type of families; $(\theta\phi)_{jk}$ = the interaction between the j th treatment and the k th type of families; $(rf(\phi_k))_{il}$ = the interaction between the l th family within the k th type of families and the i th replicate; $(\theta f(\phi_k))_{jl}$ = the interaction between the l th family within the k th type of families and the j th treatment; $(r\theta\phi)_{ijk}$ = the interaction between the i th replicate, the j th treatment and the k th type of families; e_{ijklm} = the random error associated with each plantlet.

Effect and variance estimates were carried out in two steps using the following submodels:

$$X_{ijkl} = \mu + r_i + \theta_j + \phi_k + (r\theta)_{ij} + (r\phi)_{ik} + (\theta\phi)_{jk} + (r\theta\phi)_{ijk} + e'_{ijkl}$$

and,

$$X_{kl} - \hat{r}_i = \mu + \phi_k + f(\phi_k)l + e''_{kl},$$

where e'_{ijkl} and e''_{kl} are random errors. The latter submodel was run separately (after removing replicate effects \hat{r}_i) for the control and

water-stressed treatments with a view to assessing statistical parameters (F tests, family and error variances, coefficients of correlation) under both conditions and to see whether they were or not affected by the drought constraint.

3. RESULTS

F tests from analyses of variance (Cross and hierarchical classifications) are presented in Table III. Results are reported only for the experiment with full-sib families. Although the effect of the water stress on the growth of the plantlets was comparable in the two groups of families, half-sib families, either vigorous or non vigorous, were not differentiated neither for growth and biomass nor for the water status of the seedlings. This is likely due to the more heterogeneous genetic constitution of this kind of families.

There were significant differences between replicates for 7 traits of the 15 studied (Tab. III); this was, probably, attributable to differences in room temperature and relative humidity at the time they were implemented; conditions during replicate 2 (May) were warmer and drier than during replicate 1 (April). The effect of treatment was significant for all but root length (RL) and total and epicotyl dry weights (TDW and EDW). Water stress caused a significant increase of root-shoot ratios (Root-shoot length, RL/SL, and root-shoot dry weight, R/S) of plants (Tab. IV). In root, dry weight (RDW) and dry linear weight (RDW/RL) were substantially increased by the water deficit contrary to water content (RWC) which was reduced. In hypocotyl, water content (HWC) was lower in water-stressed than in well-watered plants, at the opposite of dry weight (HDW) and dry linear weight (HDW/HL) which both were higher in the latter. Epicotyl was the more sensitive organ to the water stress; water content (EWC) was significantly reduced, when dry linear weight (EDW/EL) was raised (Tab. IV). Globally, shoot growth and biomass were affected by the moderate water stress relative to root growth and biomass which were maintained or even stimulated.

Data revealed significant differences between types of vigor for all of traits in the plantlet as a whole (Tab. III). TDW, RL/SL and R/S were higher in non vigorous (V-) than in vigorous (V+) families (Tab. IV). In root, RDW was significantly higher in V- than in V+, which is a clear indication that dry matter accumulation in root is greater in the V- than in the V+ families; RWC was not different between the two types. In hypocotyl, the only trait markedly differentiating V+ and V- types was HWC (Higher in V+). In epicotyl, EDW and EDW/EL were traits greater in V-, at the opposite of EWC which was higher in V+.

On the plantlet as a whole, family (within vigor type) was a significant effect only for TDW (Tab. III). In root, differences between families were recorded for root length (RL). In hypocotyl, they were marked for all of traits, as they were, in epicotyl, except for EWC (Tab. III).

Replicate \times treatment interaction was not significant for any of traits; replicate \times family type interaction was important only for RL/SL; treatment \times family type interaction do not at all contribute to variation (Tab. III). Third order interaction was a negligible source of variation (Tab. III). Interactions, when recorded, express differential behaviours of the two types of families when faced with a situation of a water deficit.

Table III. F-test values from cross and hierarchical analyses of variance. Cross analysis was made on replicate (1), treatment (2) and family type (3) classifications, hierarchical analysis on family type and family within type (4) classifications. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

(df)	Main effects				Interaction effects			
	1 (1, 176)	2 (1, 176)	3 (1, 176)	4 (3, 179)	1*2 (1, 176)	1*3 (1, 176)	2*3 (1, 176)	1*2*3 (1, 176)
RL	16.68***	~ 1	25.36***	4.44**	3.39	~ 1	~ 1	~ 1
RDW	1.53	19.42***	18.98***	2.31	~ 1	~ 1	~ 1	~ 1
RDW/RL	2.65	34.83***	2.83	2.30	~ 1	~ 1	~ 1	~ 1
RWC	~ 1	41.53***	2.64	1.03	~ 1	~ 1	2.37	3.51
HL	~ 1	~ 1	~ 1	6.63***	~ 1	~ 1	~ 1	~ 1
HDW	2.12	11.08**	3.60	8.04***	~ 1	~ 1	~ 1	~ 1
HDW/HL	1.44	9.61**	3.43	4.67**	~ 1	3.11	~ 1	2.60
HWC	4.82*	49.17	4.03*	4.98**	~ 1	~ 1	1.90	2.60
EL	~ 1	48.76***	3.03	6.39***	~ 1	10.12**	~ 1	2.25
EDW	13.51***	2.28	5.51*	4.43**	1.66	~ 1	2.04	~ 1
EDW/EL	~ 1	25.47***	8.57**	8.58**	~ 1	15.23***	~ 1	2.14
EWC	9.12**	59.67***	8.52**	~ 1	~ 1	~ 1	8.41**	~ 1
R/S	5.41*	30.53***	6.40*	~ 1	~ 1	~ 1	~ 1	~ 1
RL/SL	14.55***	5.24*	45.41***	~ 1	3.13	7.93**	2.48	~ 1
TDW	10.77**	~ 1	9.23**	5.06**	~ 1	1.57	~ 1	~ 1

Table IV. Mean values of traits under well-watered (control) and water-stressed (stress) conditions. Stress effect: not significant (\approx), significant increase (\blacktriangleright), significant decrease (\blacktriangleleft).

	Total full-sib families			Within the V+ families			Within the V- families		
	Control	Stress	Stress effect	Control	Stress	Stress effect	Control	Stress	Stress effect
RL (mm)	196.5	192.6	\approx	187.83	180.85	\approx	210.57	209.70	\approx
RDW (mg)	8.31	10.00	\blacktriangleright	7.74	9.16	\blacktriangleright	9.14	11.12	\blacktriangleright
RDW/RL	0.042	0.052	\blacktriangleright	0.040	0.050	\approx	0.043	0.053	\blacktriangleright
RWC (%)	91.64	89.44	\blacktriangleleft	91.63	89.95	\blacktriangleleft	91.60	88.87	\blacktriangleleft
HL (mm)	35.92	36.60	\approx	35.39	36.66	\approx	36.71	36.42	\approx
HDW (mg)	7.61	8.36	\blacktriangleright	7.43	8.20	\blacktriangleright	7.87	8.64	\blacktriangleright
HDW/HL	0.213	0.229	\blacktriangleright	0.211	0.223	\approx	0.216	0.240	\blacktriangleright
HWC (%)	88.53	86.61	\blacktriangleleft	88.60	86.99	\blacktriangleleft	88.42	86.03	\blacktriangleleft
EL (mm)	10.18	6.70	\blacktriangleleft	10.75	6.94	\blacktriangleleft	9.41	6.39	\blacktriangleleft
EDW (mg)	34.88	32.77	\approx	34.60	30.80	\blacktriangleleft	35.79	35.69	\approx
EDW/EL	29.30	34.00	\blacktriangleright	3.75	5.21	\blacktriangleright	4.55	6.24	\blacktriangleright
EWC (%)	86.04	83.66	\blacktriangleleft	86.02	84.44	\blacktriangleleft	86.01	82.53	\blacktriangleleft
R/S	0.197	0.246	\blacktriangleright	0.18	0.24	\blacktriangleright	0.21	0.25	\blacktriangleright
RL/SL	4.27	10.00	\blacktriangleright	4.09	4.15	\approx	4.58	4.96	\blacktriangleright
TDW (mg)	50.80	51.13	\approx	49.77	48.16	\approx	52.80	55.45	\approx

Table V. Family (σ_f^2) and error (σ_e^2) variances and mean-family correlations (r). The ratio $\sigma_f^2/(\sigma_f^2 + \sigma_e^2)$ estimates the proportion of genotypic (family) effects in the phenotypic (total) variance. C (control), S (stress). Stress effect on the strength of parameters: increasing (+), decreasing (-), no effect (0).

	σ_f^2			σ_e^2			$\sigma_f^2/(\sigma_f^2 + \sigma_e^2)$ (%)			r		
	C	S	Effect	C	S	Effect	C	S	Effect	C	S	Effect
RL	12.49	389.47	+	1520.4	802.3	-	0.8	32.7	+	-0.48	-0.67	+
RDW	0.00	1.21	+	6.22	6.33	0	0.0	16.1	+	-0.86	-0.85	0
RDW/RL	4×10^{-6}	9×10^{-6}	+	95×10^{-6}	131×10^{-6}	+	4.0	6.4	0	-0.09	-0.01	0
RWC	0.71	0.00	-	2.70	7.57	+	20.8	0.0	-	0.87	0.15	-
HL	8.61	6.48	-	15.03	23.96	+	36.4	21.3	-	0.27	0.30	0
HDW	0.04	1.12	+	1.68	2.43	0	21.5	31.6	+	-0.46	-0.43	0
HDW/HL	165×10^{-6}	223×10^{-6}	+	1375×10^{-6}	1426×10^{-6}	+	10.7	13.5	0	-0.75	-0.60	-
HWC	0.05	1.10	+	3.90	2.81	-	1.4	28.0	+	-0.07	0.15	0
EL	6.83	21.50	+	92.51	49.54	-	6.9	30.3	+	0.39	-0.13	-
EDW	5.47	1.48	-	12.18	5.72	-	31.0	20.5	-	-0.54	-0.69	+
EDW/EL	1.20	1.06	0	3.02	5.09	+	28.5	26.5	0	-0.37	-0.14	-
EWC	0.00	0.33	+	4.58	5.22	+	0.0	6.0	0	-0.03	0.42	+
R/S	0.000	0.000	0	0.002	0.004	+	0.0	0.0	0	-0.76	-0.21	-
RL/SL	0.008	0.000	0	0.525	0.423	-	1.5	0.0	0	-0.97*	-0.76	-
TDW	5.32	47.52	+	151.74	85.76	-	3.4	35.6	+	-0.71	-0.70	0

3.1. Comparative response of V+ and V- families under water stress

3.1.1. Growth, biomass and water content

Mean values are given in Table IV along with the effect of the stress. Root/shoot dry weight ratio (R/S) was raised similarly in the two types, whereas Root/shoot length ratio (RL/SL) was significantly increased in V- but not in V+. TDW was not significantly affected by the stress in both types. Stress did not affect RL in both types of families when RDW was increased more in V- than in V+; accumulation of dry matter in root was, as a result, manifest in V- under water stress. Hypocotyl was an organ whose length (HL) was achieved prior to the application of the stress; this trait was not, therefore, influenced by the stress. On the other hand, a significant, and similar, increase was observed in its dry weight (HDW) in both types of families, while HDW/HL was significantly raised in V- families solely. A marked drop in HWC was recorded in either type but hypocotyl in V+ remained slightly more hydrated (Tab. IV). Epicotyl was apparently the more affected organ in both types of families. Reduction in length and water content of this organ was drastic as well as in vigorous and in non vigorous families; however epicotyl still was more hydrated in V+ than in V- whereas its dry weight was significantly decreased in V+ but not in V- families. An increase in epicotyl linear dry weight was observed as a result of the water stress in either type (Tab. IV).

3.1.2. Water status of plants

Well-watered seedlings were characterized by the existence of a water potential gradient ($\Delta\Psi_w = \Psi_w(t) - \Psi_w(u)$) from the tip ($\Psi_w(t)$) to the upper part of the root ($\Psi_w(u)$), more markedly however in V- than in V+ families, in which this gradient inflects at the middle part of the root ($\Psi_w(m)$). Turgor potential in the root (Ψ_p) reached values of about 0.20 to 0.25 MPa in all of families; the highest values being recorded at the root tip, this means that this part of the root was more turgescient than the others.

A generalized drop of Ψ_w to values lower than that of the solution (-0.5 MPa) was observed in response to the PEG-induced water stress. In addition, a decrease, of a similar magnitude, in Ψ_w and Ψ_s , at the three parts of the root (tip, middle and upper parts), allowed turgor potential to be maintained at values close to those recorded in well-watered plants, especially in the root tip. The water potential gradient, already observed in the root of the well-watered plants, was also observed in the stressed ones, and the difference between V- and V+ was even more pronounced.

3.2. Effect on statistical parameters and juvenile-mature correlations

Estimates of family and error variances and family variance/total phenotypic variance ratio $[\sigma_f^2/(\sigma_f^2 + \sigma_e^2)]^{(1)}$ for

(1) Rigorously, classical heritabilities cannot be calculated for full-sib families (these would be, moreover, too inaccurate due to the small number of families); nevertheless, this ratio may bring some information on the strength of genotypic effects in determining phenotypic characteristics of seedlings.

15 juvenile traits and family-mean correlations between these traits and 17-year tree height are given in Table IV.

Percentage of family variance to total phenotypic variance was enhanced by the water stress for RDW, RL, HDW, HWC, EL, EWC and TDW; it went down for RWC, HL and EDW while it remained of the same magnitude for RDW/RL, HDW/HL, EDW/EL, RL/SL and R/S. In well-watered plants, the highest value was found for HL (36.43%); at the opposite, percentage was nil for those traits as RDW, EWC or R/S. In water-stressed plants, the largest percentage was recorded for TDW (35.65%); it was zero for RWC, RL/SL and R/S.

Neither in the well-watered nor in the water-stressed treatment were the one-month-old traits highly correlated with 17-year tree height; the only significant correlation ($r = -0.97$, $p < 0.01$) was recorded for root-shoot length ratio (RL/SL) in the well-watered treatment (Tab. V). The other values of juvenile traits-mature height correlations were generally of the same magnitude; water stress treatment had little or no effect on the strength of juvenile-mature correlations. Nevertheless, coefficients of correlation higher than 0.5 (but non significant due to the small number of degrees of freedom) were obtained for RDW ($r_w = -0.86$, $r_s = -0.85$), RL ($r_s = -0.67$), RWC ($r_w = 0.87$), HDW/HL ($r_w = -0.75$, $r_s = -0.60$), EDW ($r_w = -0.54$, $r_s = -0.69$), TDW ($r_w = -0.71$, $r_s = -0.70$), RL/SL ($r_w = -0.97^{**}$, see above, $r_s = -0.76$) and R/S ($r_w = -0.76$) (r_w are correlations under the well-watered treatment, r_s , correlations under the water stress). It is not lacking of interest to note that most of the mean-family correlation values were negative, above all those related to root growth and biomass. If confirmed on greater samples of families, negative correlations would mean that one-month-old seedlings with a lower growth and biomass attributes will develop, in average, a taller bole at 17, and vice versa.

4. DISCUSSION

4.1. Response of V+ and V- families to water stress

4.1.1. Growth, biomass and water content

The present work showed that a water constraint imposed to roots resulted in a growth reduction of the shoot but not of roots in maritime pine, as often observed in response to moderate droughts [5, 23, 37, 42]. However, such a reaction is more marked in xeric than in mesic plants [2, 33, 40, 45]. In maritime pine, when faced to a water stress, responses in non vigorous families were larger than in vigorous ones. Plants of non vigorous families develop a deeper root system and exhibit a higher root-shoot ratios than do plants of vigorous families. Globally, V- families were less affected by the stress than were V+ ones.

At the provenance level, a significant differentiation was found for growth and physiological adaptations to water stress or drought-like stress [14, 15, 17]. Relating growth to drought adaptation, early work [16] found that Tamjoute, a non vigor-

ous southern provenance from Morocco, was more resistant to drought than vigorous northern provenances from France or Spain. This provenance stopped transpiration as soon as the drought set in; and, histological observations revealed that wax cuticle in this provenance was thicker than in others. Later, investigations were pursued at a very juvenile stage in growth chamber or in greenhouse as well as at a more advanced age in field trials. Results highlighted larger shoot-root ratios in the Moroccan provenance than in Landes when a drought or a drought-like treatments were applied on seedlings [34, 37]; in addition, water use efficiency differentiated between these two provenances in the context of Landes climate [38]. Results from Spain on Iberian and Landes provenances confirmed that vigor and other physiological parameters were related to drought resistance in maritime pine [15].

At the family level, a high variability for growth was recorded within Landes provenance. Studies on *plus tree*⁽²⁾ offsprings at the adult stage revealed existence of vigorous and non vigorous families [31, 32]. It seems to be a general fact, in maritime pine, that vigorous and non vigorous genotypes develop different strategies for allocation of matter; the former invest in shoot growth while the latter give priority to root development. These differential behaviours are accentuated in a situation of moderate droughts.

4.1.2. Water status of seedlings

Our results showed that seedlings under water stress reacted by a generalized drop of water potential in the root at a level below that of the medium. Such a drop of Ψ_w in the root relative to that of the nutrient solution creates a gradient of water potential ($\Delta\Psi_w$) which allows to maintain a water flux at the benefit of roots. Water potential fell more in the tip of the root. The fall in root water potential was paralleled with a decrease in root osmotic potential (Ψ_s), such that turgor (Ψ_p) was maintained at a high level in this organ. This highlighted an efficient osmotic regulation in the root of the plants under the water constraint. However, osmotic potential values were likely slightly underestimated (more negative than really) due to dehydration of samples in the interval (10 min) between Ψ_w and Ψ_s measurements. Nevertheless, such an osmoregulation was also observed in provenances [37], where a slight increase of Ψ_p in the root tip of water-stressed plants aged one month were recorded for a similar water stress intensity.

Plants of non vigorous families exhibited a more pronounced decrease in root water and osmotic potentials than did plants of vigorous families, suggesting that the former have a greater ability for osmotic adjustment when faced with a drought. Similarly, it was found [37] a deeper osmotic adjustment in the non vigorous Moroccan provenance of Tamjoute than in the vigorous French provenance of Landes. These results pointed out that turgor was maintained in all of genotypes, but at a higher level in non vigorous than in vigorous ones. So, root growth of non vigorous genotypes would be greater in a situation of a moderate water stress (-0.5 MPa). As it was observed for plants under water stress, seedlings of Tamjoute provenance exhibited a higher capacity for

⁽²⁾ A phenotypically superior tree compared to the neighbouring trees.

osmoregulation under saline stress (a drought-like effect) than seedlings of Landes (and Iberian) provenance [34].

Similar responses were observed in many other coniferous species when plants were subjected to a water or a drought-like constraint (*Larix decidua* [6], *Pinus halepensis* [10], *Thuja occidentalis* [12], *Pinus radiata* [39], *Chamaecyparis lawsoniana* [52]). In some of these species, the ability of osmoregulation seemed to be related to geographic origin; generally, genotypes from dryer regions exhibited faster and deeper osmotic regulation when subjected to a drought.

In maritime pine, root water status of plants enough clearly differentiated vigorous and non vigorous genotypes at a very early age; then, it could represent a candidate trait for early selection for adult growth in this species.

4.2. Statistical parameters and juvenile-adult correlations

The efficiency of early selection in forest trees depends on the strength of the statistical and genetic parameters of juvenile traits and on the significance of the juvenile-mature correlations [51]. Optimal environments and appropriate juvenile traits were investigated to improve these parameters and thus reinforce the effectiveness of early selection [8, 21, 29, 43, 49]. It was found that genotype-environment interactions were one of the major causes of low juvenile-mature correlations suggesting that these could be enhanced by mimicking adult growth-limiting factors in the field [41].

In Landes, juvenile-mature correlations lacked in maritime pine when assessed prior to 12 years. Among other hypotheses, genotype-environment interactions were thought to have a chief effect on such a lack [26]. So, it was of importance to determine what field elements contribute strongly to genotype-environment interaction in maritime pine and thus reduce juvenile-mature correlations in this species. In Landes, drought occurs in summer months and was suspected to be a major factor which could explain, at least in part, genotype-environment interactions. Then, mimicking a drought when early testing could be a way to optimize early selection in Landes maritime pine. Pioneer works in this field, showed that changes in morphological or physiological juvenile traits induced by a water deficit were related to adult growth in the field [37]. However, no statistical or genetic parameters were estimated within the framework of these studies. In the present study, some strong correlations were recorded disregarding the water regime, but no sound argument can be lent for the hypothesis that mimicking field summer drought in Landes should improve the efficiency of very early selection in maritime pine. Statistical parameters were not always affected in such a manner that could enhance family effect (and then heritabilities); and, the only one-month-old trait significantly correlated with the 17-year tree height was the root-shoot length ratio in the well-watered treatment. Average juvenile-mature family-mean correlations between growth chamber traits and 17-year old tree height in the field were roughly the same under both treatments (-0.26 for the well-watered treatment and -0.23 for the drought). Family-mean correlations integrate both genetic and environmental causes and, when testing correlations between sites, family mean-correlations are always lower than genetic correlations as long as heritabilities are not

perfect [9]; in our maritime pine case, it does not have significant meaning to estimate such genetic parameters because of the family structure (full sibs) and the limited number of individuals per family. Therefore, subsequent works in this field must be laid out in such a manner which maximizes such parameters.

Some studies support the hypothesis that juvenile-mature correlations would be improved by mimicking limiting growth factors in the field while others do not. In loblolly pine (*Pinus taeda*), strongest family-mean correlations were found when the nursery conditions were comparable to field environments [11]; on the other hand, shoot-root biomass ratio of well-watered seedlings was the better predictor of later performance than other traits of seedlings disregarding the water regime [47]. In lodgepole pine (*Pinus contorta* ssp. *latifolia*), 2-year-seedling traits in the greenhouse were correlated with 9-year tree height on the only site (amongst four) whose growth conditions were closer to those in greenhouse; it was stated that early tests should be conducted under simulated conditions [51]. In Scots pine (*Pinus sylvestris*), growth chamber data were found to be more genetically correlated with older field data for the higher fertilization (N) regime in one study [13], whereas, several strong juvenile-mature correlations between low nitrogen treatment and northern field sites (where N was more limiting) were obtained in an other one [1]. Also, mimicking the competition for light by a dense or a wide spacing in growth chamber was not efficient to improve juvenile-mature correlations in Scots pine [18]. Mimicking field conditions did not influence the relative efficiency of early selection in Douglas-fir (*Pseudotsuga menziesii*); juvenile-mature correlations were evenly consistent under different nursery testing regimes [3]. In Norway spruce (*Picea abies*), it was found that heritabilities for biomass and height of 2-year-old plants were moderate to high in the well-watered treatment and weaker in the drought treatment; on average, juvenile-mature (24-year-old growth traits in the field) correlations were stronger in the drought-treatment than in the well-watered treatment [41]. Authors stated that their work supported the hypothesis that higher juvenile-mature correlations can be obtained from simulated-field conditions in growth chambers. Many studies highlighted that low juvenile-mature correlations were often associated with high genotype-environment interactions in forest trees [7, 18, 22, 30, 48, 51]. Genotype-environment interaction effects generally reduce statistical and genetic parameters as variance family and heritability. Extreme early conditions that are highly limiting generally result in higher genotype-environment interaction effects and then reduce juvenile-mature correlations. Therefore, comparisons of statistical and genetic parameters should be interpreted with caution because they are highly dependent on the base population and testing environments.

It can be concluded from this work, that candidate traits for early selection for adult growth in maritime pine could be found at a very juvenile age. Indeed, some biomass traits and root water status of one-month-old seedlings discriminated between vigorous and non vigorous genotypes. In addition, certain large correlations in both water regimes, although non statistically significant, raise hopes of recording significant ones if powerful trials were implemented, especially by use of larger samples of families and individuals. The most questionable

thing is whether mimicking a moderate drought at a very early age in growth chamber should really improve the efficiency of retrospective tests in this species. Field conditions are a complex of interlinked factors, and summer drought probably is not the only major factor for the lack of juvenile-mature correlations in maritime pine; indeed, interactions also exist between silvicultural practice, pedoclimatic variations and the capacity of trees to tolerate drought episodes [44]. In that situation, further investigations and refinement on the field and at the growth chamber are required to find adequate experimental conditions as well as appropriate juvenile traits and to build sound deductions from these kind of researches in maritime pine.

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