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Mediterranean stone pine (*Pinus pinea* L.) genetic variability for growth traits in a Portuguese provenance trial

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Abstract. Provenance field trials provide information on adaptability, growth and yield of the species. Results from these studies have great practical use in the improvement of forest stands, as it can assure sources of seed that provide well-adapted, productive trees in reforestation and afforestation. The aim of this study was to evaluate Mediterranean stone pine adaptive variability, using a provenance trial initiated in Portugal in 1992. The seed lots were obtained with the cooperation of *Silva Mediterranea* network. The provenance trial included twenty-five seed lots, from seven different countries, collected throughout the species' native range. In February 1993, twenty-five provenances were established at *Sines* (38°01'N, 8°42'W) and fifteen at *Tavira* (37°10' N, 7 °36' W). Total height and diameter at breast height were used as indicators of provenance variability. Mixed models were applied to data at different ages obtained from those field trials. Results showed that, at least for a significant level of 0.05, provenance genetic variability for height and diameter at btreast height was found.

Keywords. *Pinus pinea* L. – Mediterranean stone pine – Genetic variability.

Évaluation de la variabilité génétique du pin parasol* (Pinus pinea *L.) dans un essai de provenance au Portugal

***Résumé**. Un essai de provenance peut fournir des informations très importantes sur l'adaptabilité, la croissance et le rendement de l'espèce. Les résultats de ces études ont une grande utilité pratique pour l'amélioration des peuplements forestiers, car ils peuvent assurer des sources de graines bien adaptées, en vue du reboisement. Le but de cette étude repose sur l´évaluation de la variabilité du pin parasol vis-à-vis des caractéristiques adaptatives, à travers un essai de provenance établi au Portugal en 1992. Les lots de semences ont été obtenus avec la coopération du réseau Comité Silva Mediterranea. L'essai de provenance incluait vingt-cinq lots de graines, originaires de sept pays différents, recueillies dans l'aire de distribution de l'espèce. En février 1993, vingt-cinq provenances ont été plantées à Sines (*38°01'N, 8°42'W*) et quinze à Tavira (*37°10' N, 7 °36' W*). La hauteur et le diamètre ont été utilisés comme indicateurs de la variabilité entre les provenances. Les modèles mixtes ont été appliqués aux données de hauteur totale à différents âges et aussi au diamètre. Les résultats ont montré que, au moins pour un niveau significatif de 0,05, la variabilité de la hauteur et du diamètre des provenances a été significative.*

Mots-clés. Pinus pinea *L. – Pin parasol – Variabilité génétique.*

I – Introduction

Mediterranean stone pine, *Pinus pinea* L, covers about 2.6% of the pines species Mediterranean forest area (Barbéro *et al*., 1998). In Portugal, this species occupies approximately 118,000 hectares (Fig. 1) which represents 3.5% of the Portuguese total forest area (AFN, 2010). The total area devoted to Mediterranean stone pine has increased since the last inventory of about 51%, which is due to an increased interest in the species by forest land owners (Carrasquinho *et al*., 2010).

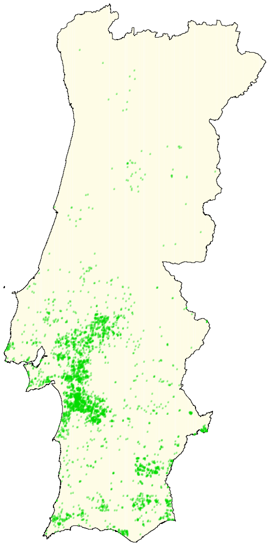

Fig. 1. Distribution of the Mediterranean stone pine (*Pinus pinea* L.) in Portugal (courtesy by Paulo Godinho-Ferreira, AFN, 2010).

Mediterranean stone pine occurs at low and medium altitudes in scattered populations throughout the Mediterranean basin in natural stands or in stands resulting from refforestation activities (Barbéro *et al*., 1998; Martínez and Montero, 2004). Concerning to its natural area, many authors recognize the difficulty to define it, as this species has been planted for its edible seeds since prehistoric times. Human impact can be expected to have strongly influenced the current distribution of it geographic and genetic diversity (Fady *et al*., 2004).

According to the Mediterranean Forest Research Agenda (EFIMED, 2009), forest owners and managers need adequate scientifically-based decision support tools for the management of this type of forest to optimize the joint production of multiple goods and services at different temporal and spatial scales in a context of increasing risks. This document, coordinated by the European Forest Institute, describes the main research priorities for the Mediterranean forest, during 2010-2020. One of the four challenges that were identified for the forest sustainability is the impact of climate and land use change on Mediterranean ecosystems. Long-term experiments, such as provenance trials, can be useful for analysing responses of plant material to changing environment. On the other hand, knowing the patterns of genetic variation among populations and proceed on its quantification can provide tools to gene resource management and, in a context of genetic improvement, the raw material for mass selection.

The aim of this study was to evaluate Mediterranean stone pine variability for adaptive traits, such as total height and diameter at breast height, using a provenance trial established in Portugal in 1992.

II – Material and methods

In 1993, National Forest Station, a Portuguese forest research institute, established a provenance trial. In this study, the field sites of *Sines* (38^{o}01'N, 8^{o}42'W) and *Tavira* (37^{o}10'N, 7^{o}36'W) were evaluated.

Twenty five seed lots, from seven different countries, Portugal, Spain, Italy, Greece, Morocco, Israel and Turkey, were obtained throughout the species' native range, with the cooperation of Silva Mediterranea network. Table 1 indicates the location and climate information of these provenances seed lots that are represented in the field sites. The provenances are considered to be a sample from different ecological conditions (latitude, longitude, altitude, rainfall and temperature). The altitude ranges from sea level to 1007 meters (seed lot from Spain, Cordillera Central). The lower annual precipitations are 324 mm, 373 mm and 411 mm from Spain, Sierra Morena (S_{26}) and Cordillera Central (S_{27}) and Greece, Mandrakii (G_3), respectively. Ponte de Lima (P_9), Vieira do Minho (P_{10}), and Amarante (P_{11}), three locations from the north of Portugal, correspond to the higher mean annual precipitation.

Twenty-five and fifteen provenances were established at *Sines* and *Tavira* field sites, respectively (Carneiro *et al*., 2000a). Unequal distribution of seed sources within the two experimental sites was related to different seed germination per seed lot.

The two field sites were established as a randomized complete block design with six replicates for each provenance. Twenty-five seedlings per plot (5 x 5) were planted at a spacing of 3 x 4 meters, within and between rows, respectively.

Growth traits, total height (h_n) and diameter at breast height (d_{13}), were used for evaluation of provenance genetic variability. In *Sines* field site, h_n (cm) was assessed at age (n) two, four, five, six, ten and eleven, and d_{13} (centimeters) at age thirteen, for each individual tree. In *Tavira*, total height was assessed at age two, four, five, and six. To avoid border effects, the border line trees of the trial were not considered in data analysis.

The methodology for statistical analysis followed a linear mixed model, using MIXED Procedure of SAS version 9.2 (SAS Institute Inc. 2008). Single tree data of total height (h_n) and diameter at breast height (d_{13}) were used. The analysis was performed assuming fixed effects for the overall mean and block effects, and random effects for provenances and plots. Spatial correlated random errors were modelled according to a power correlation function and different correlations for row and column directions were assumed. This is equivalent to a separable first order autoregressive model (Gilmour *et al*. 1997). When necessary, an independent error term was also added. Model parameters were estimated by residual maximum likelihood method (REML, Patterson and Thompson 1971), using the Fisher-Scoring algorithm (Jennrich and Sampson, 1976).

A residual likelihood ratio test (REMLRT) was used to test the provenance genetic variance component associated to each trait. Because the null hypothesis (provenance genetic variance equal to zero) specifies a value on the boundary of the parameter space, the p-value was assumed to be half of the reported p-value from a chi-squared distribution with one degree of freedom (Self and Liang, 1987 and Stram and Lee, 1994).

For a better comparison of provenance variability among traits and between sites, the coefficient of genetic provenance variation was computed (ratio between the estimates for the provenance genetic standard deviation and the estimates for the overall mean).

Following the mixed model equations (Henderson, 1975), the empirical best linear unbiased predictor (EBLUP) of genotypic effect for the total height at age six (the maximum common age for both field sites) was obtained for each provenance. Provenance performance evaluation was then based on the ranking of the predicted height at age six (EBLUP of the provenance genetic effects plus overall mean estimate).

Table 1. Location and climate information for *Pinus pinea* L. provenances represented in Sines and Tavira field trials

Code	Provenance name	Country	Latitude (N)	Longitude	Altitude (m)	Mean precipitation (mm)			Mean temperature (^{0}C)		
						Annual	Driest month	Wettest month	Annual	Warmest month	Coldest month
G2	Strophilia	Greece	38º08’	21º22’ E	5	673.2	1.2	129.2	16.8	24.9	9.6
G3	Mandraki	Greece	39º10’	23º24’ E	24	411.3	2.8	60.5	15.6	25.0	7.2
Is8	Monte Carmelo1	Israel	32º45’	35º E	300	490.2	0	139.5	17.1	26.4	11.6
Is13	Monte Carmelo2	Israel	32º45’	35º E	400	490.2	0	139.5	17.1	26.4	11.6
It4	Cecina	Italy	43º45’	10^{o}18’ E	4	774.1	18.3	104.9	14.7	23.2	7.0
It6	Tomboli di Cecina	Italy	43º09’	11º17’ E	300	591.6	20.5	82.8	13.7	22.6	5.9
It18	Duna Felicia	Italy	42º02’	12º27’ E	70	684.2	11.9	101.2	15.2	23.7	7.5
M1	Koudia Hamra	Morocco	35º11’	6º10’10’’ W	650	545.5	0	100.0	17.3	22.8	12.2
M7	Ain Grana	Morocco	35º16’50’’	5º53’15’’ W	130	565.7	0.1	101.0	17.3	23.2	12.1
M14	Cap Spartel	Morocco	35º47’16’’	5º53’11’’ W	220	573.6	0.1	107.4	17.5	23.5	12.4
M20	Dunes D’ Adjir	Morocco	35º12’21’’	5º53’47’’ W	50	540.4	0	95.9	17.6	23.4	12.4
P5	Alcácer do Sal	Portugal	38º15’	8º30’ W	75	500.2	0.2	75.7	16.9	23.2	11.2
P9	Ponte de Lima	Portugal	41º46’	8º36’ W	300	1317.4	15.6	194.6	13.9	19.6	8.6
P10	Vieira do Minho	Portugal	41º41’	8º06’ W	750	1256.8	15.9	180.8	11.8	18.9	5.7
P11	Amarante	Portugal	41º18’	8º06’ W	245	1035.8	9.5	148.0	13.7	20.4	7.7
P22	Viseu	Portugal	40º40’	7º54’ W	420	919.5	5.8	131.1	14.3	21.3	8.1
S25	Andalucia	Spain	36º20’	6º05’ W	50	514.1	0.1	97.4	17.8	24.1	12.3
S26	Sierra Morena	Spain	38º10’	4º00’ W	500	372.9	0.7	50.4	17.0	27.1	8.8
S27	CordilleraCentral	Spain	40º30’	4º20’ W	1007	324.2	5.4	40.5	13.1	23.1	5.2
Tk12	Yatagan-katranci	Turkey	37º27’	27º55’ E	600	737.2	3.6	142.5	14.7	24.8	5.6
Tk15	Yalova	Turkey	48º32’38’’	29º22’49’’ E	500	629.4	15.0	102.1	13.5	22.1	4.7
Tk16	Aydin-Karine	Turkey	37º46’00’’	27º23’ E	450	620.6	2.3	142.1	16.4	25.5	8.2
Tk17	Kumluca	Turkey	36º17’45’’	30º20’02’’ E	5	750.2	1.1	177.8	17.4	26.1	9.7
Tk19	Serik	Turkey	36º52’05	31º01’15’’ E	10	514.0	6.3	81.3	10.1	20.6	-0.2
Tk21	Çanakkale	Turkey	40º19’36’’	26º16’31’’ E	20	535.6	3.9	85.3	14.8	24.0	6.0

Note: Climate data were obtained from the International Water Management Institute (http://wcatlas.iwmi.org/results.asp) using latitude and Longitude data.

III – Results and discussion

As it can be observed, in Table 2, the REMLRT for the provenance genetic variance component indicates that the provenance genetic variability was always significant (p - value<0.05) for total height at all ages, in both field sites and also for diameter at breast height in *Sines* at age 13.

Table 2. Estimates for the overall mean and for the provenance genetic variance ($\hat{\sigma}^2_{prov}$), residual likelihood ratio test (REMLRT) for the provenance genetic variance component, and coefficient of genetic variation for provenances (CV_{prov}) for the traits h_n and d_{13} and for the two field sites: Sines and Tavira

Trait	Overall mean estimate (cm)	$\hat{\sigma}^2_{prov}$	$-2l_R$	$-2l_{R0}$	REMLRT		CV_{prov} (%)
					D	p-value	
Sines							
h_2	27.40	2.95	23200.3	23216.9	16.6	<0.0001	6.27
h_4	69.00	12.55	29303.2	29309.6	6.4	0.0057	5.13
h_5	91.55	34.11	30499.2	30504.1	4.9	0.0134	6.38
h_6	129.60	62.11	32326.8	32331.2	4.4	0.0180	6.08
h_{10}	217.13	184.65	34409.4	34416.9	7.5	0.0031	6.26
h_{11}	239.07	233.92	34935.5	34943.4	7.9	0.0025	6.40
d_{13}	7.72	0.35	14909.6	14943.9	9.3	0.0011	7.68
Tavira							
h_2	34.55	1.48	13076.8	13082.7	5.9	0.0076	3.52
h_4	92.13	8.97	15643.0	15650.5	7.5	0.0031	3.25
h_5	110.62	22.76	16167.8	16181.7	13.9	<0.0001	4.31
h_6	136.69	35.68	18697.0	17027.5	9.7	0.0009	4.37

Note: $-2l_R$ and $-2l_{R0}$ are the minus twice the residual log-likelihood for the full model and for its variant without provenance effect, respectively; $D=[(-2l_{R0})-(-2l_R)]$.

Carneiro *et al.* (2006) found significant differences among provenances for total height at ages 2 and 13, but not for diameter at breast height at age 13. However, these authors fitted a classic model, assuming fixed effects for provenances and independent and identically distributed errors. In addition, only the central trees inside each provenance plot were considered and the plot factor was not included.

The studies concerning *Pinus pinea* L. variability are few and contradictory (Carneiro *et al.*, 2006; Mutke *et al.*, 2010). It seems that, the variability evaluation for *Pinus pinea* L. using provenance trials depends on the methodologies of data analysis.

Comparing the two field sites, at the same ages, we verified that, for each age, the overall mean height at *Tavira* was always higher than at *Sines*. The ecological conditions at *Tavira* promoted the stone pine growth, as it was also verified by Carneiro *et al*. (2000b). They have evaluated flowering and fructification at age five, in *Tavira* and *Sines* field sites and concluded that the amount of the flowers and the cones were the double at *Tavira*.

Considering the coefficient of genetic variation for provenances, for total heights at different ages, the values ranged from 5.13% to 6.40% in *Sines* and from 3.25% to 4.37% in *Tavira*. That is, the higher values for the CV_{prov} were obtained for *Sines*, as a larger number of provenances, 25, were used in this field site, while in *Tavira*, only 15 were studied. Therefore, it is reasonable to state that with larger number of provenances becomes easier to detect variability among them.

For each of the twenty-five provenances from the Sines field site and the fifteen from the Tavira site, the predicted height at age 6 are shown in Table 3.

Table 3. Predicted height (cm), at age six, of the provenances and their respective rankings in the two field sites: Sines and Tavira

Provenance code	Predicted height (cm)			
	Sines	Rank	Tavira	Rank
G2	124.10	22	125.97	15
G3	121.90	24		
Is8	123.96	23		
Is13	137.30	4		
It4	137.92	3	142.82	2
It6	128.61	15	138.44	6
It18	138.63	2	145.72	1
M1	140.29	1	142.41	3
M7	128.39	16	139.48	4
M14	124.43	21		
M20	133.79	5		
P5	129.40	14	133.60	12
P9	130.11	12		
P10	130.53	11		
P11	129.65	13		
P22	124.77	20	134.09	10
S25	126.81	17		
S26	131.14	9	135.98	8
S27	130.63	10		
Tk12	119.32	25	134.68	9
Tk15	131.31	8	137.75	7
Tk16	132.44	7	133.27	14
Tk17	124.95	19	133.47	13
Tk19	126.24	18	133.61	11
Tk21	133.35	6	139.05	5

At Sines field site, it can be observed that the Moroccan provenance M1, the two Italians provenances It18 and It4, the Israeli provenance Is13, and the Moroccan provenance M20, were, respectively, the top five provenances. The Portuguese provenances P5, P9, P10 and P11 performances were around the overall mean estimate (129.60 cm), but the P22 fell below this mean estimate. The Turkish provenances Tk17 and Tk19, displayed similar behavior as the latter Portuguese provenance. The Turkish provenance Tk12, the Greek provenances G3 and G2, and the Israeli provenance Is8 showed a low growth performance. At the Tavira field site, provenances It18, It4, M1 and M7 were also found to be in the top five of the ranking. On the contrary, Tk16 anf Tk17 were among the lowest performers. As it happened in Sines, the Greek provenance G2 ranked the lowest also in Tavira. The only Portuguese provenance included in this site, P22, showed the same tendency as in Sines (below the overall mean estimate, 136.69 cm).

The common assumption that local seed sources will outperform other provenance of the same species (Zobel and Talbert, 1984) was not verified in this study, for Sines field site. The performance of the local seed source, P5, was lower than the performance of foreign provenances.

IV – Conclusions

Provenance variability for growth traits was detected in both field sites, *Sines* and *Tavira*. However, since genetic variability results on *Pinus pinea* L. are inconsistent among the available studies, it is strongly advised to improve research on statistical models for this type of data.

The existence of provenance variability for growth traits permits not only to perform mass selection, but also to hope to detect genetic variability in other economically important traits, such as, kernel production and/or kernel quality.

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