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ORIGINAL PAPER

Genetic variability among *Pinus pinea* L. provenances for survival and growth traits in Portugal

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Abstract *Pinus pinea* L. (Mediterranean stone pine) is an important forest species not only for its economically relevant kernel production, but also for environmental protection. The detection of genetic variability is an essential issue for Mediterranean forest species for conservation and improvement programs. Based on data collected for several years at three field sites from a *P. pinea* L. provenance trial established in Portugal in 1993, the present study aimed to evaluate the genetic variability of the adaptive traits of *P. pinea* L. and to identify a group of provenances with high performance for growth traits to be used in future plantings.

Several mixed models were fitted to survival and growth trait data to estimate provenance and provenance × site interaction variability. The empirical best linear unbiased predictors of provenance genotypic effects were used to select a superior group of provenances.

The provenance genetic variability of *P. pinea* L. was successfully detected for survival and height of different planting ages and also for diameter at breast height at age 13 years after planting. For growth traits, the most successful methods used for evaluating provenance genetic variability were based on the linear mixed spatial models. When multi-environmental analysis was performed, provenance × site interaction variability was detected for survival, but not for height at age 6 years. The existence of provenance variability in *P. pinea* L. permitted the identification of a seed lot composed of a mixture of the best provenances for height and diameter.

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Introduction

Pinus pinea L. (Mediterranean stone pine) is one of the ten *Pinus* species of the Mediterranean basin (Barbéro et al. 1998). In Portugal, it is an important forest species for its economically relevant trait of kernel production. This species is considered to be very tolerant to summer droughts and has the ability to cope with occasional low winter temperatures (Court-Picon et al. 2004). It can be found in zones where annual precipitation is less than 300–350 mm. Since ancient times, this pine has been planted along the Mediterranean basin, from Portugal to Turkey, for its edible seeds and useful timber. In the second half of the nineteenth century, reforestation programs utilizing this plastic species were initiated across the Mediterranean, often to control erosion. Such plantings have undoubtedly had a major influence on the distribution of this species in the region (Barbéro et al. 1998). Therefore, at present, it is difficult to determine if the occurrence of this species in a given location is natural or planted.

Genetic diversity is important for the maintenance of the viability and the evolutionary or adaptive potential of populations and species (Sgrò et al. 2011; Holderegger et al. 2006). The genetic diversity of *P. pinea* L. has been measured through the use of molecular markers and quantitative genetic experiments aiming to divide the phenotypic variability into genetic and environmental components. Using molecular tools, several authors report that this species has low genetic diversity (Fallour et al. 1997; Evaristo et al. 2002; Vendramin et al. 2008). Several additional studies have been conducted to evaluate the adaptive traits of *P. pinea* L. In the south of France, Court-Picon et al. (2004) developed a preliminary study on the adaptive variability of different circum-Mediterranean provenances in controlled environmental conditions and field sites and found that germination capacity, seed size and weight, and height growth varied among provenance. Mutke et al. (2005) characterized the cone yield of the clonal bank from the regional stone pine improvement program in Castile–Leon, Spain. The analysis of cone yields, from 98 different clones, demonstrated significant genetic variability. Carneiro et al. (2006) studied 25 different populations from a *P. pinea* L. provenance trial at Sines and found significant differences between provenances for plant height at ages 2 and 13 years. However, they did not detect significant differences for diameter at breast height (DBH) at age 13 years. Mutke et al. (2010) analyzed survival, growth height, and phenology in a provenance trial with 34 different populations. No significant differences in survival were found between accessions, while growth height was significantly different. Sánchez-Gómez et al. (2011) found significant variability in morphological and physiological traits using 20 clones from five different Spanish populations. In summary, the reported level of *P. pinea* L. genetic variability seems to be dependent on individual study methodologies. Even for adaptive traits, these results are inconsistent. In fact, it is important to emphasize that well-established field tests and adequate models for data analysis are critical for obtaining accurate genetic estimates of variability and for increasing the reliability of the resulting performance.

The most common experimental design used in forest genetic research has traditionally been the randomized complete block design. However, when this type of experimental design is used, the block sizes are usually large, which compromises within-block homogeneity. As a result, there may be a large reduction in the ability to identify true genetic variation. For this reason, incomplete blocks and row–column experimental designs have been progressively applied to forest trials (Fu et al. 1999b; Gezan et al. 2006). Recently, spatial methods have been more used to study patterns of site variation to overcome problems of spatial heterogeneity in forest genetic trials, such as *Pinus* sp, *Picea* sp, *Peudotsuga menziesii*, and *Castanea sativa* (Anekonda and Libby 1996; Kusnandar and Galwey 2000; Costa e Silva et al. 2001; Dutkowski et al. 2002, 2006; Ye and Jayawickrama 2008; Gapare et al. 2011, Miguez-Soto and Fernández-López 2011). Specifically related to the Mediterranean stone pine, Mutke et al. (2010) used the nearest-neighbor adjustment by the iterated Papadakis method to find significant differences in adaptive traits between provenances.

Based on data collected over a period of several years and from three field sites from a *P. pinea* L. provenance trial established in Portugal in 1993, the present study aims to contribute to the understanding of the genetic variability of this species. The specific objectives are to evaluate the genetic variability among provenances for adaptive traits (survival, total height, and diameter at breast height) and to identify a group of provenances with high performance for growth traits to be used for future plantings.

Materials and methods

Field sites and provenance samples

This study was conducted in three field sites of the first *P. pinea* L. provenance trial in Portugal that was initiated in nursery by the National Forest Station in the spring of 1992. Twenty-five seed lots from seven different countries and collected throughout the species' native range were obtained with the cooperation of the Silva Mediterranea network. Location and data information for each of the 25 *P. pinea* L. provenances' seed lots are given in Table 1. In February and March of 1993, 25 provenances were established at Sines (38°01′48″ N, 8°48′04″ W), 20 at Alcácer (38°19′30″ N, 8°32′ W), and 15 at Tavira (37°12′ N, 7°39′55″ W). Unequal numbers of seed sources within the three experimental sites were related to differences in the percentage of seed germination for each seed lot (Carneiro et al. 2000). All field sites were established according to a randomized complete block design with six replications each. The blocks were organized with a row–column arrangement per block. The number of columns per block was 25, 15, and 20 for Sines, Tavira, and Alcácer, respectively. The number of rows per block was 25 for all three field sites. Twenty-five seedlings per plot (5×5) were planted with a spacing of 3×4 m (within and between columns). For each site, as there was no border rows, the border trees were not considered in the data analysis.

Assessments

Survival, height (cm), and DBH (cm), coded as Surv_n, h_n, and d_n, respectively, in which n denotes the post-planting tree age, were evaluated. At Sines, plant survival was evaluated at ages 2, 6, and 11 years after planting; h_n was assessed at ages 2, 4, 5, 6, 10, and 11 years; and d_n was evaluated at age 13 years. At the Tavira field site, survival was assessed at 2 and 6 years after planting, and height was assessed at 2, 4, 5 and 6 years after planting. This field site was lost during the 2003 forest fire. At the Alcácer field site, only survival was evaluated at ages 2 and 4 years after planting. In 1997, this field site was abandoned due to high mortality caused by unexpected flooding. The high mortality observed at these ages did not allow an evaluation of growth trait variability for this field site.

Data analysis

Statistical models

For data analysis, the theory of mixed models was utilized (Littell et al. 2006; McCulloch et al. 2008). For the survival data (response variable with binomial distribution), a generalized linear mixed model was fitted using the GLIMMIX

Table 1 Seed sources of the *Pinus pinea* L. provenance trial established at Sines, Tavira, and Alcácer field sites

Code	Provenance name	Country	Latitude (N)	Longitude	Altitude (m)	Field site		
						Sines	Tavira	Alcácer
G2	Strophilia	Greece	38°08′	21°22′ E	5	x	x	x
G3	Mandraki	Greece	39°10′	23°24′ E	24	x		x
Is8	Monte Carmelo1	Israel	32°45′	35° E	300	x		
Is13	Monte Carmelo2	Israel	32°45′	35° E	400	x		
It4	Cecina	Italy	43°45′	10°18′ E	4	x	x	x
It6	Tomboli di Cecina	Italy	43°09′	11°17′ E	300	x	x	x
It18	Duna Felicia	Italy	42°02′	12°27′ E	70	x	x	x
M1	Koudia Hamra	Morocco	35°11′	6°10′10″ W	650	x	x	x
M7	Ain Grana	Morocco	35°16′50″	5°53′15″ W	130	x	x	x
M14	Cap Spartel	Morocco	35°47′16″	5°53′11″ W	220	x		
M20	Dunes D’ Adjir	Morocco	35°12′21″	5°53′47″ W	50	x		x
P5	Herdade Ervideira	Portugal	38°15′	8°30′ W	75	x	x	x
P9	Ponte de Lima	Portugal	41°46′	8°36′ W	300	x		x
P10	Vieira do Minho	Portugal	41°41′	8°06′ W	750	x		x
P11	Amarante	Portugal	41°18′	8°06′ W	245	x		x
P22	Viseu/Fig. Campo	Portugal	40°40′	7°54′ W	420	x	x	x
S25	Andaluzia	Spain	36°20′	6°05′ W	50	x		
S26	Sierra Morena	Spain	38°10′	4°00′ W	500	x	x	x
S27	Cordilhera Central	Spain	40°30′	4°20′ W	900	x		
Tk12	Yatagan-Katranci	Turkey	37°27′	27°55′ E	600	x	x	x
Tk15	Yalova	Turkey	48°32′38″	29°22′49″ E	500	x	x	x
Tk16	Aydin-Karine	Turkey	37°46′00″	27°23′ E	450	x	x	x
Tk17	Kumluca	Turkey	36°17′45″	30°20′02″ E	5	x	x	x
Tk19	Serik	Turkey	36°52′05″	31°01′15″ E	10	x	x	x
Tk21	Çanakkale	Turkey	40°19′36″	26°16′31″ E	20	x	x	x

x presence of the seed sources at each of the three field sites

procedure of SAS version 9.2 (SAS Institute Inc. 2008). In this model, the logit link function was used; consequently, the generalized linear mixed model for these data is as follows:

$$y* = X\beta + Zu,$$

wherein $y*$ denotes the $n \times 1$ vector whose elements are given by $\log\left[\frac{\pi_{ij}}{1-\pi_{ij}}\right]$, where π_{ij} is the probability of survival success for provenance j in the block i; X denotes the $n \times p$ design matrix for fixed effects, β denotes the $p \times 1$ vector of the fixed effects (including the overall mean and block effects), Z denotes the $n \times q$ design matrix for random effects, and u denotes the $q \times 1$ vector of random effects (provenance effects). The provenance effects were assumed to be independent and identically distributed normal random variables with an expectation of 0 and variance σ^2_{prov}.

For the height and DBH data (response variables with normal distribution), the analysis of individual traits was accomplished using the general linear mixed model:

$$y = X\beta + Zu + e,$$

wherein y denotes the $n \times 1$ vector of the observed data (phenotypic values), X denotes the $n \times p$ design matrix for fixed effects, β denotes the $p \times 1$ vector of the fixed effects (including the overall mean and block effects), Z denotes the $n \times q$ design matrix for random effects, u denotes the $q \times 1$ vector of random effects (including the provenance and plot effects), and e denotes the $n \times 1$ vector of random errors.

The vectors u and e are mutually independent, with multivariate normal distribution with expectation 0 and variance–covariance matrices G, $q \times q$, and R, $n \times n$, respectively. The distribution of vector y is multivariate normal, with expectation $X\beta$ and variance–covariance matrix $V = ZGZ^T + R$ in which Z^T denotes the transpose of Z. The matrix G is defined as $G = G_{prov} \oplus G_{plot}$, where $G_{prov} = \sigma^2_{prov} I_{q_1}$ (σ^2_{prov} is the provenance genetic variance, I_{q1} is the $q_1 \times q_1$ identity matrix, and q_1 is the number of provenances) and $G_{plot} = \sigma^2_{plot} I_{q_2}$ (σ^2_{plot} is the plot variance, I_{q2} denotes the $q_2 \times q_2$ identity matrix and q_2 is the number of plots).

According to the structure of matrix R, three linear mixed models (models 1, 2, and 3), using single-tree data, were fitted with the MIXED procedure of SAS version 9.2 (SAS Institute Inc. 2008). In model 1 (the classical model), independent and identically distributed random errors were assumed, i.e., the matrix R was defined as $R = \sigma_e^2 I_n$, where σ_e^2 is the error variance and I_n is the $n \times n$ identity matrix. In model 2 (spatial analysis, approach 1), spatially correlated errors were considered. Specifically, the matrix R was defined as $R = \sigma_\varepsilon^2 \sum$ (σ_ε^2 is the dependent spatial error variance, and $\sum$ is the $n \times n$ spatial correlation error matrix). The numbers of rows and of columns were used as spatial coordinates, and the distance between plots was expressed as the difference in the number of rows (or columns). The spatial covariance was assumed to be equal for all blocks. The nondiagonal elements of the matrix $\sum$ were defined according to an anisotropic power correlation function (different correlations for row and column directions were assumed). Finally, in model 3 (spatial analysis, approach 2), spatially correlated errors and independent errors (nugget effect) were assumed. In this model, the matrix R was defined as $R = \sigma_\varepsilon^2 \sum + \sigma_\eta^2 I_n$ (the structure of the matrix $\sum$ is equal to model 2, σ_η^2 is the independent error variance, and I_n is the $n \times n$ identity matrix).

The evaluation of provenance × environment interaction variability was performed for survival at age 2 years, in the three field sites, and for height at age 6 years, in two field sites (Sines and Tavira). Only the 15 common provenances were considered. For this study, the provenance effects remained as random, the site as fixed, and the provenance × site interaction as random. Therefore, in the structure of the mixed models described above, the vector β of the fixed effects includes the overall mean, site, and block within site effects; the vector u of random effects includes the provenance, plot within site, and provenance × site interaction effects. The provenance × site interaction effects were assumed to be independent and identically distributed normal random variables with an expectation 0 and variance σ_{GE}^2. For height at age 6 years, the matrix R assumed different error structures for each site (the best obtained from the comparison between models 1, 2, and 3). All model assumptions were validated by graphical diagnostic tools for the residuals and for the empirical best linear unbiased predictors (EBLUPs) of random effects.

Parameter estimation, inference for covariance parameters, and comparison of models

For the h_n and d_{13} data, the model parameters were estimated using the residual maximum likelihood method (REML) (Patterson and Thompson 1971) with Fisher's scoring algorithm (Jennrich and Sampson 1976). For the survival data, a pseudo-likelihood method based on REML was used in the analysis (Littell et al. 2006).

Residual likelihood ratio tests (REMLRT) for comparing full and reduced models with respect to covariance parameters were applied. Therefore, REMLRT was performed to test the provenance genetic variance ($H_0 : \sigma_{prov}^2 = 0 \quad vs \quad H_1 : \sigma_{prov}^2 > 0$) and the provenance × site variance ($H_0 : \sigma_{GE}^2 = 0 \quad vs \quad H_1 : \sigma_{GE}^2 > 0$) as well as to compare models in terms of the analysis of growth traits. The approach was, first, to select between the two spatial models ($H_0 : \sigma_\eta^2 = 0 \quad vs \quad H_1 : \sigma_\eta^2 > 0$) and, second, to compare the best of these models with the classical one (model 1). Specifically, comparisons of model 2 with model 1 and model 3 with model 1 correspond to the testing of the null hypothesis $H_0 : \rho_{row} = 0$ *and* $\rho_{col} = 0$ and $H_0 : \sigma_\eta^2 = 0$ *and* $\rho_{row} = 0$ *and* $\rho_{col} = 0$, respectively.

The asymptotic distribution of the REMLRT statistic is assumed to be a chi-squared distribution with the number of degrees of freedom equal to the increase in the number of parameters between the two models, unless the test involved a null hypothesis where the parameter was on the boundary of parameter space, as are the cases of testing a variance component and when comparing model 3 with model 2 and model 3 with model 1. For the first two cases, the p value of the test was assumed to be half of the reported p value from the chi-squared distribution with one degree of freedom (Self and Liang 1987; Stram and Lee 1994). For the third and last case, which is an unknown mixture of chi-squared distributions, the conservative solution (the number of degrees of freedom is equal to the increase in the number of parameters between the two models) was adopted.

Evaluations of provenance performance and correlation traits in each field site

Following the mixed model equations (Henderson 1975), the empirical best linear unbiased estimator of β ($\widehat{\beta}$) and the empirical best linear unbiased predictor (EBLUP) of u ($\tilde{u}$) were obtained.

For the survival data, the predicted survival percentage ($\widehat{\pi}$) was obtained from the inverse of the link function as in the following equation:

$$\widehat{\pi} = \frac{\exp\left(X\widehat{\beta} + Z\widetilde{u}\right)}{\left[1 + \exp\left(X\widehat{\beta} + Z\widetilde{u}\right)\right]}.$$

For the interpretation of results, the mean of the predicted survival percentage ($\overline{\widehat{\pi}}$), the minimum predicted survival percentage ($\widehat{\pi}_{\min}$), and the maximum predicted survival percentage ($\widehat{\pi}_{\max}$) were used.

For the growth traits, provenance selection was based on the EBLUPs of the provenance effects for the traits evaluated. The predicted genetic gain (PGG) was obtained for the four top-ranked provenances from the ages in which the selected group remains stable. PGG was computed as the mean of the EBLUPs of the selected provenances and the respective approximated prediction standard error (SE) as in the following equation:

$$SE = \sqrt{\frac{\sum_{i=1}^{k} PEV_i}{k^2}},$$

where k is the number of selected provenances and PEV_i is the prediction error variance of the EBLUP of the provenance i. The PGG and SE are given as percentage of the mean.

Spearman's rank correlation coefficient (r_s) was used to measure the correlation between provenance rankings for survival (predicted percentage), height, and DBH (EBLUPs). For this analysis, the CORR procedure of SAS version 9.2 (SAS Institute Inc. 2008) was used.

Results

Provenance genetic variability for survival and growth traits

The survival provenance variance was highly significant ($p<0.0001$) for all field sites and for all evaluated ages (Table 2). The highest predicted survival rate means were observed at Sines and the lowest at Alcácer. When multi-environmental analysis was performed for survival at age 2 years, the provenance×site interaction variability was significant ($\widehat{\sigma}^2_{GE} = 0.16, p=0.0002$).

Concerning growth traits, the results for the single-site analysis obtained for the three models are reported in Table 3. First, considering the two spatial models, model 3 (spatial model with nugget effect) was selected for the Sines data sets h_2, h_4, and d_{13} ($p<0.0001$). In this case, the nugget variance represented 59.22, 49.58, and 61.40 % of the total residual variance. In contrast, for h_5, h_6, h_{10}, and h_{11}, it was verified that the spatial model without the nugget effect (model 2) was adequate for those four data sets ($p>0.05$). Considering the Tavira data, the selected spatial model was always model 3 ($p<0.0001$). The proportion of the nugget variance compared to the total residual variance ranged from 42.65 % (h_6) to 58.84 % (h_2).

Table 2 Provenance survival results obtained at different ages in the three field sites

Site analyses	Trait	$\widehat{\sigma}^2_{prov}$ (p value)	$\overline{\widehat{\pi}}$	$\widehat{\pi}_{\min}$	$\widehat{\pi}_{\max}$
Sines	$Surv_2$	0.43 (<0.0001)	96.8	92.3	98.7
	$Surv_6$	0.30 (<0.0001)	95.1	91.0	98.0
	$Surv_{11}$	0.26 (<0.0001)	93.6	89.0	96.1
Tavira	$Surv_2$	0.26 (<0.0001)	93.1	86.8	96.4
	$Surv_6$	0.23 (<0.0001)	90.1	82.2	95.2
Alcácer	$Surv_2$	0.15 (<0.0001)	74.1	59.3	83.8
	$Surv_4$	0.17 (<0.0001)	50.8	31.0	61.9

$\widehat{\sigma}^2_{prov}$ survival provenance variance estimate (logic scale), $\overline{\widehat{\pi}}$ predicted provenance survival percentage mean, $\widehat{\pi}_{\min}$ minimum predicted provenance survival percentage and $\widehat{\pi}_{\max}$ maximum predicted provenance survival percentage

For both field sites, the best spatial model was always better than model 1. In fact, comparing model 3 with model 1 through REMLRT for traits h_2, h_4, and d_{13}, at Sines and for all heights at Tavira, model 1 was rejected ($p<0.0001$). For h_5, h_6, h_{10}, and h_{11}, at Sines, when models 2 and 1 were compared, model 1 was also rejected ($p<0.0001$).

In Sines, the provenance genetic variability was always found to be significant for growth traits in all models ($p<0.05$; Table 3). Considering each evaluated age, the provenance genetic variance estimated with model 1 was higher than the value estimated with the spatial models, except for height at age 2 years. In Tavira, the provenance genetic variability was only detected ($p<0.01$) with the best spatial model (model 3).

Regarding both field trials, the plot variance estimate ($\widehat{\sigma}^2_{plot}$) was reduced when the spatial models were fitted, particularly when the model with the nugget effect was considered.

The autocorrelation coefficient values were always higher in the column direction than in the row direction. When model 3 was selected, the autocorrelation coefficient estimates in the column direction ranged from 0.70 (h_4) to 0.80 (d_{13}) for the Sines field site but were equal for all traits in the Tavira field site (0.87). Considering the row direction, the estimates ranged from 0.63 (h_4) to 0.77 (d_{13}) in the Sines field site and from 0.67 (h_2) to 0.71 (h_4) for the Tavira field site. When model 2 was chosen (Sines, h_5, h_6, h_{10}, and h_{11}), this autocorrelation coefficient ranged from 0.25 (h_5) to 0.28 (h_{10} and h_{11}) for the column direction and from 0.21 (h_5) to 0.24 (h_6 and h_{10}) for the row direction.

When multi-environmental analysis was conducted for height at age 6 years, the provenance × site interaction variance estimate was 0, thus, it was not significant. However, the provenance variance estimate was significant ($\widehat{\sigma}^2_{prov}$ = 48.60, $p<0.0001$).

By analyzing the EBLUPs of provenance rankings according to growth traits at the Sines field site, it can be observed that the Moroccan, M1, the two Italians, It4 and It18, and the Israeli, Is13, were among the top four provenances from an age of 5 years after planting (Table 4). The predicted genetic gain obtained for this superior group, ranged from 6.74 % (h_5) to 7.98 % (h_{11}) for height and was 9.89 % for the diameter at age 13 years. The Tk12 and Tk19 provenances and the G3 provenance showed a low growth performance not only for height, but also for d_{13}. Analyzing the provenance rankings for height at the Tavira field site (Table 4), provenances M1,

Table 3 Parameter estimates obtained with the fitting of the three models and REMLRT for model comparison for growth traits in the two field sites: Sines and Tavira

Site/trait		Model	$\widehat{\sigma}^2_{plot}$	$\widehat{\sigma}^2_{e}$	$\widehat{\sigma}^2_{\varepsilon}$	$\widehat{\rho}_{col}$	$\widehat{\rho}_{row}$	$\widehat{\sigma}^2_{\eta}$	REMLRT		REMLRT		$\frac{\widehat{\sigma}^2_{\eta}}{\widehat{\sigma}^2_{\varepsilon}+\widehat{\sigma}^2_{\eta}}$ (%)	$\widehat{\sigma}^2_{prov}$ (*p* value)
									D_1[a]	*p* value	D_2[b]	*p* value		
Sines	h_2	1	12.95	55.89										2.65 (0.0080)
		2	9.84		57.64	0.23	0.12		62.5	<0.0001	259.8	<0.0001		2.73 (0.0039)
		3	*0.84*		*27.00*	*0.73*	*0.64*	*39.23*					*59.22*	*2.95 (<0.0001)*
	h_4	1	118.91	377.34										21.15 (0.0127)
		2	88.38		392.26	0.24	0.21		81.6	<0.0001	388.7	<0.0001		19.45 (0.0113)
		3	*13.36*		*231.11*	*0.70*	*0.63*	*227.24*					*49.58*	*12.55 (0.0057)*
	h_5	1	215.02	611.11										37.06 (0.014)
		2	*164.86*		*636.88*	*0.25*	*0.21*		*0.2*	*0.3917*	*308.0*	*<0.0001*		*34.11 (0.0134)*
		3	164.68		635.87	0.25	0.21	1.05					0.17	34.09 (0.0113)
	h_6	1	442.93	1,161.61										65.26 (0.0256)
		2	*318.93*		*1,219.31*	*0.27*	*0.24*		*0.1*	*0.4834*	*363.7*	*<0.0001*		*62.11 (0.0180)*
		3	318.80		1,218.39	0.27	0.24	1.07					0.09	62.11 (0.0180)
	h_{10}	1	984.81	2,212.30										189.70 (0.0076)
		2	*688.75*		*2,340.61*	*0.28*	*0.24*		*0.1*	*0.4834*	*367.7*	*<0.0001*		*184.65 (0.0031)*
		3	688.53		2,339.74	0.28	0.24	1.11					0.05	184.61 (0.0031)
	h_{11}	1	1,211.00	2,585.44										235.87 (0.0064)
		2	*863.89*		*2,737.62*	*0.28*	*0.23*		*0*	*1*	*355.2*	*<0.0001*		*233.92 (0.0025)*
		3	863.56		2,736.83	0.28	0.23	1.02					0.04	233.89 (0.0007)
	d_{13}	1	2.73	6.34										0.40 (0.0241)
		2	2.31		6.54	0.18	0.15		84	<0.0001	140.6	<0.0001		0.41 (0.0169)
		3	*0.48*		*3.08*	*0.80*	*0.77*	*4.90*					*61.40*	*0.35 (0.0011)*
Tavira	h_2	1	15.16	58.02										0.72 (0.2919)
		2	9.96		60.80	0.26	0.15		84.9	<0.0001	225.1	<0.0001		0.79 (0.2635)
		3	*0*		*29.51*	*0.87*	*0.67*	*42.19*					*58.84*	*1.48 (0.0076)*
	h_4	1	78.95	287.02										0 (1)
		2	50.91		302.42	0.29	0.18		108.9	<0.0001	278.6	<0.0001		0.49 (1)
		3	*0*		*157.31*	*0.87*	*0.71*	*200.53*					*56.04*	*8.97 (0.0031)*
	h_5	1	144.88	427.77										0 (1)
		2	80.88		460.11	0.36	0.22		109.2	<0.0001	364.8	<0.0001		5.09 (0.2919)
		3	*0*		*279.19*	*0.87*	*0.68*	*268.31*					*49.01*	*22.76 (<0.0001)*
	h_6	1	311.27	747.56										0 (1)
		2	187.97		806.85	0.40	0.22		116.2	<0.0001	430.2	<0.0001		0 (1)
		3	*0*		*575.98*	*0.87*	*0.68*	*428.35*					*42.65*	*35.68 (0.0009)*

$\widehat{\sigma}^2_{plot}$ plot variance estimates, $\widehat{\sigma}^2_{prov}$ provenance variance estimate, $\widehat{\sigma}^2_{e}$ error variance estimate for the classical model, $\widehat{\sigma}^2_{\varepsilon}$ spatial dependent error variance estimate, $\widehat{\sigma}^2_{\eta}$ nugget variance estimate, $\widehat{\rho}_{col}$ and $\widehat{\rho}_{row}$ autocorrelation estimates for the column and row directions, respectively, *REMLRT* residual likelihood ratio tests. The italicized data identify the best model.

[a] Test statistic $D_1 = (-2l_R \text{ model } 2) - (-2l_R \text{ model } 3)$

[b] Test statistic $D_2 = (-2l_R \text{ model } 1) - (-2l_R \text{ Best spatial model})$

M7, It4, and It8 were among the top four provenances from an age of 4 years after planting. The correspondent PGG for this selection ranged from 2.99 % (h_4) to 4.33 % (h_6). For all of the evaluated ages, the Greek provenance, G2, ranked the lowest. Globally, considering both field sites and all the evaluated adaptive traits, the provenances M1, It18, and It4 consistently were among those with the best ranking positions.

Correlations between traits

Table 5 provides Spearman's correlation (r_s) between all analyzed traits within each field site. Considering the 25 provenances at Sines at 2 years after planting, the height was positive and significantly correlated ($p<0.05$) with h_4 ($r_s = 0.75$), h_5 ($r_s = 0.67$), h_6 ($r_s = 0.60$), and d_{13} ($r_s = 0.47$). From age 4 years after planting, the height (h_4) was significantly correlated with all the studied growth traits. These correlation values ranged from 0.54 (h_{11}) to 0.95 (h_5). Height at age 5 years after planting was positive and significantly correlated ($p<0.05$) with survival at ages 6 and 11 years; height at age 6 years was also correlated with survival at age 11 years. However, in Tavira, where there were 15 provenances under study, the total height was not found to be correlated with survival for any of the evaluated ages. In this field site, h_2 was significantly correlated ($p<0.05$) only with h_4 ($r_s = 0.54$). Comparatively, from age 4 years, Spearman's correlation values were higher, ranging from 0.86 (h_4 and h_6) and 0.96 (h_4 and h_5). In each of the three field sites, Spearman's correlation for survival at an evaluated year was always highly positive and statistically significant ($p<0.01$).

Discussion

The provenance genetic variability for *P. pinea* L. in terms of height at different planting ages, DBH at age 13 years, and survival at different ages was successfully detected in this study. Carneiro et al. (2006) found genetic variability among provenances for height at ages 2 and 13 years, but not for DBH at age 13 years. The study was conducted in Sines, the same provenance field site as this study. In a *P. pinea* provenance trial established in Spain, Mutke et al. (2010) found significant differences in terms of tree height, but not in survival. In the current study, survival variability was successfully detected with a generalized linear mixed model using the logit link function, which is now an adequate approach for analyzing binary data (McCulloch et al. 2008). According to Ræbild et al. (2004), survival is considered to be one of the key variables when analyzing tree provenances as it indicates the adaptability of the each provenance to the environment at the trial site. By analyzing plants 2 years after planting, information about the effects of transplantation from nursery to field site can be obtained. Of the three field sites, Alcácer was found to have the greatest transplantation effects, expressed through its lower survival rates. However, for this species, survival rate was not a constraint in a biological point of view. In fact, the lower values for Sines and Tavira were 89.0 and 82.2 %, respectively. For the Alcácer field site, the lower survival rate observed (31 %) resulted from an unfavorable environmental condition—the flood occurred 3 years after planting. When a multi-environmental analysis was performed, the biological consequences of the significant provenance × site interaction variability are also not relevant.

The most successful methods for assessing growth traits in terms of provenance genetic variability were based on linear mixed spatial models. In particular, for the Tavira field site, the provenance genetic variability was only detected when the spatial model with the nugget was fitted. The classical model (model 1) and the spatial model without the nugget effect (model 2) both underestimated the provenance genetic variability. However, for Sines, the provenance variance was actually overestimated with the nonspatial model (model 1), except for h_2. The observed importance of spatial models in improving the accuracy and precision of the *P. pinea* provenance data analysis is in accordance with results obtained from other forest species (Anekonda and Libby 1996; Kusnandar and Galwey 2000; Costa e Silva et al. 2001; Dutkowski et al. 2002, 2006; Ye and Jayawickrama 2008; Gapare et al. 2011; Miguez-Soto and Fernández-López 2011). Generally, all these studies have stated that spatial modeling is an important data analysis approach, especially in trials designed with large blocks. In fact, site heterogeneity needed to be accounted for because the Mediterranean stone pine is usually planted in marginal soils using large spacing. Nevertheless, as some authors have claimed, the advantage of spatial modeling decreases with the use of adequate experimental designs, such as incomplete blocks and row–column designs (Fu et al. 1999; Gezan et al. 2006; Gonçalves et al. 2010).

No particular spatial model seemed to be suitable for all of the traits and trials studied, as discussed by Gilmour et al. (1997). In fact, the existence of nugget variance differed according to each trial and analyzed trait. A high level of independent error variance (nugget variance) was observed in some data sets, reaching 61.40 % for DBH at the Sines field site. This is in accordance with the normal outcome of forest trials. For example, in their assessment of height and DBH, Dutkowski et al. (2006) reported that the independent component was generally larger than the spatial component. In a *Castanea sativa* progeny trial, the spatial structure explained between 40.2 and 42.1 % of the total residual variation in height and between 38.1 and 39.4 % of the total residual variation in diameter (Miguez-Soto and Fernández-López 2011). However, the nugget effect was present only during the first 2 years after planting (2 and 4 years), and for

Table 4 EBLUPS for genetic provenance effects obtained with the selected model for growth traits (h_n and d_{13}), their respective rankings, the overall mean estimate, PGG and respective approximated prediction SE obtained for the four top-ranked provenances as percentage of the mean, at Sines and Tavira field sites

Provenance code	Sines													
	h_2		h_4		h_5		h_6		h_{10}		h_{11}		d_{13}	
	EBLUP	*R*	EBLUP	*R*	EBLUP	*R*	EBLUP	*R*	EBLUP	*R*	EBLUP	*R*	EBLUP	*R*
G2	−2.69	25	−3.81	24	−4.86	22	−5.50	22	−5.06	17	−3.71	16	−0.31	18
G3	−0.84	19	−3.28	23	−6.52	24	−7.70	24	−18.79	25	−19.65	25	−0.76	25
Is8	−0.43	13	−2.29	21	−5.48	23	−5.64	23	0.82	11	1.77	10	−0.01	11
Is13	0.84	8	3.29	2	5.66	3	7.70	4	15.09	4	15.53	4	0.60	4
It4	1.07	6	2.01	6	5.33	4	8.32	3	15.61	3	18.52	3	0.64	3
It6	−0.19	11	−0.40	15	−0.56	15	−0.99	15	0.39	14	0.95	13	0.32	5
It18	2.05	3	3.05	3	5.92	2	9.03	2	17.60	2	21.93	1	1.12	1
M1	3.10	1	6.19	1	7.46	1	10.69	1	20.20	1	20.29	2	0.69	2
M7	−1.26	22	0.30	12	−0.92	16	−1.21	16	0.46	13	1.37	11	−0.06	14
M14	−0.64	16	−2.61	22	−3.64	20	−5.17	21	−7.76	19	−8.28	19	−0.38	19
M20	−0.25	12	1.28	8	3.11	6	4.19	5	4.80	6	4.49	7	0.17	9
P5	1.52	4	2.30	5	2.32	8	−0.20	14	−5.97	18	−7.19	18	−0.01	12
P9	−0.62	15	0.49	10	1.48	10	0.51	12	0.01	15	−1.64	15	0.15	10
P10	−0.98	21	−0.80	17	0.60	12	0.93	11	3.53	9	2.89	9	−0.26	17
P11	−0.72	17	−1.97	19	−0.11	14	0.05	13	3.81	8	3.67	8	−0.14	16
P22	0.94	7	−0.33	14	−1.27	17	−4.83	20	−8.76	20	−11.40	20	−0.50	22
S25	−0.78	18	−0.48	16	−1.86	18	−2.79	17	−4.12	16	−4.47	17	−0.02	13
S26	−1.30	23	−0.16	13	0.99	11	1.54	9	4.26	7	5.21	6	0.28	7
S27	−0.09	10	0.47	11	−0.08	13	1.03	10	7.67	5	12.11	5	0.30	6
Tk12	−1.82	24	−5.04	25	−7.71	25	−10.28	25	−16.50	24	−16.78	24	−0.52	24
Tk15	−0.54	14	1.25	9	1.58	9	1.71	8	0.62	12	1.13	12	−0.11	15
Tk16	3.10	2	3.02	4	2.68	7	2.84	7	−8.78	21	−12.86	22	−0.49	21
Tk17	0.17	9	−2.29	20	−4.04	21	−4.65	19	−9.66	22	−11.64	21	−0.45	20
Tk19	−0.87	20	−1.63	18	−3.48	19	−3.36	18	−10.37	23	−13.12	23	−0.51	23
Tk21	1.24	5	1.43	7	3.43	5	3.75	6	0.92	10	0.90	14	0.24	8
Mean estimate (cm)	27.40		69.00		91.55		129.60		217.13		239.07		7.71	
PGG (SE) (%)					6.74 (2.34)		6.89 (2.26)		7.89 (2.15)		7.98 (2.18)		9.89 (2.48)	

Table 4 (continued)

Provenance code	Tavira							
	h_2		h_4		h_5		h_6	
	EBLUP	*R*	EBLUP	*R*	EBLUP	*R*	EBLUP	*R*
G2	−1.10	14	−5.37	15	−8.70	15	−10.72	15
G3								
Is8								
Is13								
It4	−1.04	13	0.92	4	4.48	3	6.13	2
It6	−0.27	8	0.29	6	1.01	5	1.75	6
It18	0.70	5	3.74	2	6.76	1	9.03	1
M1	1.46	1	3.92	1	5.54	2	5.72	3
M7	1.40	2	2.45	3	4.20	4	2.79	4
M14								
M20								
P5	−0.37	9	−0.48	9	−1.22	10	−3.09	12
P9								
P10								
P11								
P22	0.96	4	0.13	8	−0.27	8	−2.60	10
S25								
S26	−0.58	11	−1.36	13	−1.56	11	−0.71	8
S27								
Tk12	−1.23	15	−1.21	11	−1.15	9	−2.01	9
Tk15	−0.67	12	0.19	7	0.00	7	1.06	7
Tk16	1.02	3	−0.55	10	−2.71	12	−3.42	14
Tk17	−0.51	10	−1.31	12	−2.98	13	−3.22	13
Tk19	0.03	7	−2.14	14	−3.72	14	−3.08	11
Tk21	0.20	6	0.79	5	0.33	6	2.36	5
Mean estimate (cm)	34.59		92.13		110.62		136.69	
PGG (SE) (%)			2.99 (1.08)		4.74 (1.26)		4.33 (1.34)	

EBLUPs Empirical best linear unbiased predictors, *R* ranking, *PGG* predicted genetic gain, *SE* standard error

Table 5 Spearman's rank correlation coefficient for the analyzed traits h_n, d_{13}, and Surv$_n$

Traits		h_4	h_5	h_6	h_{10}	h_{11}	d_{13}	Surv$_2$	Surv$_4$	Surv$_6$	Surv$_{11}$
Sines	h_2	*0.75* $p<0.0001$	*0.67* $p=0.0002$	*0.60* $p=0.0016$	0.34 $p>0.05$	0.29 $p>0.05$	*0.47* $p=0.017$	0.36 $p>0.05$		0.22 $p>0.05$	0.16 $p>0.05$
	h_4		*0.95* $p<0.0001$	*0.90* $p<0.0001$	*0.61* $p=0.0011$	*0.56* $p=0.0033$	*0.68* $p=0.0002$	0.36 $p>0.05$		0.40 $p>0.05$	0.34 $p>0.05$
	h_5			*0.97* $p<0.0001$	*0.73* $p<0.0001$	*0.68* $p=0.0003$	*0.73* $p<0.0001$	0.37 $p>0.05$		*0.43* $p=0.03$	*0.40* $p=0.045$
	h_6				*0.80* $p<0.0001$	*0.74* $p<0.0001$	*0.75* $p<0.0001$	0.33 $p>0.05$		0.39 $p>0.05$	*0.44* $p=0.027$
	h_{10}					*0.99* $p<0.0001$	*0.88* $p<0.0001$	0.14 $p>0.05$		0.13 $p>0.05$	0.33 $p>0.05$
	h_{11}						*0.87* $p<0.0001$	0.12 $p>0.05$		0.12 $p>0.05$	0.34 $p>0.05$
	d_{13}							0.12 $p>0.05$		0.08 $p>0.05$	0.23 $p>0.05$
	Surv$_2$									*0.86* $p<0.0001$	*0.73* $p<0.0001$
	Surv$_6$										*0.88* $p<0.0001$
Tavira	h_2	*0.54* $p=0.0396$	0.38 $p>0.05$	0.26 $p>0.05$				0.36 $p>0.05$		0.38 $p>0.05$	
	h_4		*0.96* $p<0.0001$	*0.86* $p<0.0001$				0.30 $p>0.05$		0.39 $p>0.05$	
	h_5			*0.93* $p<0.0001$				0.29 $p>0.05$		0.39 $p>0.05$	
	h_6							0.39 $p>0.05$		0.45 $p>0.05$	
	Surv$_2$									*0.95* $p<0.0001$	
Alcácer	Surv$_2$								*0.84* $p<0.0001$		

The italicized data identify the significant correlation ($p<0.05$)

DBH, it was present at age 13 years in Sines and, in Tavira, it was present for all evaluated ages. Concerning height, it seems that during the initial ages, the measures were affected by uncontrollable environmental effects. Moreover, these data reflect the effects of planting in particular sites. The effect on DBH may be partially justified by the fact that, in this species, branch insertion may cause stem deformation, which may in turn affect the individual tree evaluation in terms of this trait and, consequently, increase the independent error variance.

In this study, the autocorrelation coefficients were always positive, indicating that competition was not present as this study addresses young trees. According to Fox et al. (2001), young pre-canopy closure stands usually exhibit positive spatial dependence, and these authors argue that this can be attributed to the dominance of microsite effects and the absence of competition. Additionally, the moderate values obtained for autocorrelation coefficients (up to 0.4 for models without the nugget and until 0.87 for models with the nugget) show that the autoregressive process was mainly useful in modeling small patches of soil fertility. The range of obtained values for row and column autocorrelation coefficients is consistent with those obtained in other forest trials. For models without a nugget effect, Magnussen (1990) found autocorrelations for height between 0 and 0.4; Anekonda and Libby (1996) found nearest-neighbor correlation values around 0.3 to 0.41 for tree height. For models with the nugget, Costa e Silva et al. (2001) observed that for tree height, the spatial autocorrelations were larger than 0.60. For DBH, Dutkowski et al. (2002) found values above 0.9 for the row and column directions. For height, Kusnandar and Galwey (2000) obtained values of 0.66 and 0.74 for the column and row directions, respectively. In a study conducted by Ye and Jayawickrama (2008) assessing both height and DBH, more than 95 % of the dataset yielded autocorrelation coefficients of 0.6 or above. In fact, 11 % of the autocorrelation coefficients were larger than 0.9.

When a multi-environment analysis for height at age 6 years was performed, some reasons can justify the nonexistence of provenance × site interaction variability. For example, one may be related to the number of sites involved in these analyses, as only two sites were available. Similar conditions shared by both field sites or lower sensitivity of this species to environmental changes can also justify the obtained results. As the provenance × site interaction is an important issue to be considered in a context of selection, research on this subject should, therefore, be implemented comprising the entire Mediterranean basin.

The opportunity of selection for growth traits at an age earlier than 11 years is indicated by the positive, large, and significant age-to-age correlations obtained between heights at different ages after planting in both field sites. Additionally, the ranking in DBH at age 13 years for the Sines field site was similar to that for height since age 6 years after planting. This result is reflected by the positive correlations that were obtained between growth traits (heights and DBH), further suggesting that a selection of the best provenances in terms of tree height would also yield a favorable outcome in terms of diameter. Thus, selecting plants according to height is desirable because it can be easily measured at a young age. On the other hand, according to Carrasquinho et al. (2010), for *P. pinea*, the two biometrical traits, total height and diameter, are related to cone production. Therefore, the selection for growth traits at an early age would represent a critical achievement as it could contribute indirectly to the increase of cone/kernel production, the ultimate goal in the genetic improvement program for this species. Based on the results of PGG in both field sites, the selection would be more efficient in Sines. However, these results should be compared with caution, because the selected group for each field site differs in one provenance and the proportion of selection is different. At each field site, the same group was selected from the ages 5 and 4 years onwards for Sines and Tavira, respectively.

The global results of this study for the evaluated traits offer some insight into the general field of afforestation. An adequate selection strategy would be to advise the use of a seed lot composed of a mixture of the best provenances. Future studies on cone/kernel productivity should be implemented in the unique field site available from this provenance trial.

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