CROP BREEDING AND APPLIED BIOTECHNOLOGY

ARTICLE

Genetic evaluation of *Pinus taeda* clones from somatic embryogenesis and their genotype x environment interaction

Poliana Coqueiro Dias^{1*}, Aloizio Xavier², Marcos Deon Vilela de Resende², Márcio Henrique Pereira Barbosa³, Fabrício Antonio Biernaski⁴ and Regiane Abjaud Estopa⁴

Abstract: The objective of this study was to evaluate the genotype x environment interaction and to estimate the genetic components of variance and mean using mixed models in early selection of 238 clones of Pinus taeda propagated by somatic embryogenesis. The experiment consisted of a complete blocks design, with 12 replications, with one plant per plot, in four environments, at 1, 3, and 4 years of age. Estimates of heritability and of genetic gains in the evaluated environments showed good prospects for selection of superior genotypes. The effect of genotype x environment interaction was pronounced for all traits investigated. With the simultaneous selection for stability and adaptability, 10% genetic gain was obtained in relation to the mean of the commercial controls. This estimated gain indicates that the somatic embryogenesis technique has been effective in propagation of clones with good productive potential.

Key words: Forestry improvement, clonal silviculture, genetic selection, early selection.

INTRODUCTION

In Brazil, *Pinus taeda* has presented better development in the South and Southeast regions (Martinez et al. 2012). The increase in yield observed in *Pinus taeda* plantations is mainly due to the use of genetically superior material derived from breeding programs (Mckeand et al. 2006, Martinez et al. 2012). In view of the positive impacts of *Pinus* breeding programs on the production of raw material suitable for the manufacturing of long fiber cellulose (Mckeand et al. 2006, Martinez et al. 2012), their implementation in agricultural corporations is fundamental for yield increase. Considering the limitations of genetic gains in programs traditionally developed by seminiferous propagation, cloning tends to play important role in the consolidation of the competence of Brazilian industries in this market.

The negative effects of ontogeny have led to difficulties in clonal propagation and have consequently made the use of *Pinus taeda* clones on a commercial scale unviable (Pullman and Bucalo 2011). The cuttings collected from adult *Pinus taeda* trees are difficult to root (Alcantara et al. 2007, 2008), and the production of clonal seedlings by the minicutting technique has also presented low rooting percentages (Alcantara et al. 2007, Andrejow and Higa 2009). As an alternative, somatic embryogenesis has been developed and used in *Pinus* Crop Breeding and Applied Biotechnology 18: 55-64, 2018 Brazilian Society of Plant Breeding. Printed in Brazil http://dx.doi.org/10.1590/1984-70332018v18n1a8

> *Corresponding author: E-mail: policoqueiro@yahoo.com.br

> > Received: 01 December 2016 Accepted: 27 March 2017

 ¹ Universidade Federal Rural do Semiárido (UFERSA), Departamento de Ciências Agronômicas e Florestais, 59.625-900, Mossoró, RN, Brazil
² Universidade Federal de Viçosa (UFV), Departamento de Engenharia Florestal, 36.571-000, Viçosa, MG, Brazil
³ Universidade Federal de Viçosa, Departamento de Fitotecnia
⁴ Klabin do Paraná Produtos Florestais (KPPF), 84.279-000, Telêmaco Borba, PR, Brazil *taeda* cloning programs (Pullman et al. 2006, Alcantara et al. 2008, Andrejow et al. 2009, Pullman and Bucalo 2011). This way, plant yield can be significantly improved due to the multiplication of desirable genotypes derived from breeding programs (McKeand et al. 2008). Thus, somatic embryogenesis should be used in breeding programs of *Pinus taeda* as long as the genotype x environment interaction comes from immature zygotic embryos, given the effects related to ontogenetic age in *Pinus taeda* (Pullman et al. 2006, Pullman et al. 2011).

In a forestry-breeding program, genetic evaluation of individuals and their relations with the planting environments is a fundamental step. Due to environmental variations, phenotype variations also occur in function of the genotype x environment interaction, being one of the greatest problems of breeding programs of any species, whether at the stages of selection or recommendation of cultivars. Nevertheless, analyses of phenotypic adaptability and stability can be used, which identify cultivars responsive to environmental variations and with foreseeable behavior (Cruz et al. 2004, Resende 2007, Rosado et al. 2012). In this context, the mixed models method (REML/BLUP) is considered as more accurate (Resende 2007), since it provides better experimental accuracy, and is more efficient than analysis of variance, especially in cases with unbalanced data. Moreover, the predicted genetic values can be used to estimate the adaptability and stability of genotypes using the harmonic mean of the relative performance of genetic values (HMRPGV), which allows estimating adaptability and stability simultaneously in a single parameter (Resende 2007).

In general, forestry-breeding programs consider the results of juvenile-mature correlation analyses to carry out early selection, due to the long crop rotation cycle. In the case of *Pinus taeda*, studies have presented good results with early selection (Paludzyszyn Filho et al. 2001, 2002, 2003, Isik et al. 2005, Mckeand et al. 2006, Martinez et al. 2012). The objective of this study was to evaluate the genotype x environment interaction and to estimate the genetic components of variance and mean using the mixed models (REML/BLUP) in early selection of *Pinus taeda* individuals propagated by somatic embryogenesis.

MATERIAL AND METHODS

The study was carried out by genetic-statistical analysis of part of the experimental network of *Pinus taeda* of the Klabin S.A. Corporation, which is composed of 238 clones propagated by somatic embryogenesis, using megapethophytes from immature seeds of matrices selected in the company. Somatic embryos were obtained by the methodology described in the U.S. Pat. N. 5506136 A (BECWAR et al., 1996). Clonal tests were set up in the states of Parana and Santa Catarina, in 2007, using seedlings at 10 months of age (propagated by somatic embryogenesis). Seedlings were produced in 55 cm³ tubes, using decomposed pine bark as substrate, with periodic fertilizations of NPK and micronutrients solution. Subsoiling was carried out at 50 cm depth. In the field, weed control was performed with herbicide (glyphosate) in the total area, one month before planting, and 4, 12, 18, 24 and 36 months after planting. Leaf-cutting ants control was carried out using formicide baits. The experimental consisted of a complete blocks design, with twelve replications, spaced 3 m x 2 m between plants, with one plant per plot, in four environments, two in Santa Catarina and two in Parana. Three lots of commercial seeds were used as controls.

According to the Köppen climate classification, environments 1 and 2, in the state of Santa Catarina, are characterized as Cfb; and environments 3 and 4, in the state of Parana, are located in a transitional climate region between Cfa and Cfb. Environments 1 and 2 have lower average temperatures and a greater number of frosts than environments 3 and 4. The soil of environment 1 is classified as Inceptisol, with clayey texture, and slightly rolling to rolling relief. The soil of environment 2 is classified as Oxisol, with clayey texture, and slightly rolling relief. Finally, the soil of environment 3 is classified as Ochrept or Umbrept, with medium texture, with rolling to steeply rolling relief. Environment 4 is classified as Psamment, with sandy and medium light texture, and rolling to steeply rolling relief.

Diameter – DBH (in cm, measured at 1.30 m from the soil surface), total height – Ht (in m), volume – Vol (m³), and survival rate at 1, 3, and 4 years of age of Pinus taeda clones were measured. DBH was measured using a diameter tape, and height

was obtained using a relascope. For volume calculation, the following formula was used: $Vol = \left(3.1416 \times \frac{DBH^2}{4}\right) \times Ht \times 0.5$.

Survival rate was evaluated by counting the number of live trees per clone in the experiment at the time of measurements of *DBH* and *Ht* (at 1, 3, and 4 years of age).

Genetic evaluation of Pinus taeda clones from somatic embryogenesis and their genotype x environment interaction

Analyses were carried out by the estimate of variance components (Reml) and by the genetic value prediction (Blup), using the software Selegen-Reml/Blup (RESENDE 2002b). Variables were evaluated individually per environment, and in combination of environments. In evaluation of the individuals within each environment, the variables were analyzed using the univariate linear mixed model of the software Selegen-Reml/Blup, presented by Resende (2002a), according to the model: y = Xr + Zg + Wb + e, in which: $\mathbf{y} = data$ vector; $\mathbf{r} = replication$ effect vector (assumed as fixed) added to the overall mean; $\mathbf{g} = genotypic effect$ vector (assumed as random); $\mathbf{b} = block$ effect vector (assumed as random); and $\mathbf{e} = \text{error or residue value}$ (assumed as random). Uppercase letters represent the incidence matrices for the respective effects. The statistical model for the analysis of this experimental network in several environments, considering one observation per plot, is given by: y = Xr + Zg + Wb + Tge + e, in which: $\mathbf{y} = data$ vector; $\mathbf{r} = replication effect$ vector (assumed as fixed) added to the overall mean; $\mathbf{g} = genotypic effect$ vector (assumed as random); one observation per plot, is given by: y = Xr + Zg + Wb + Tge + e, in which: $\mathbf{y} = data$ vector; $\mathbf{r} = replication effect$ vector (assumed as fixed) added to the overall mean; $\mathbf{g} = genotypic effect$ vector (assumed as random); $\mathbf{ge} = genotype \times environment$ interaction effect vector (assumed as random); $\mathbf{b} = block$ effect vector (assumed as random); and $\mathbf{e} = \text{error or residue}$ value (assumed as random); $\mathbf{b} = block$ effect vector (assumed as random); and $\mathbf{e} = \text{error or residue}$ value (assumed as random); $\mathbf{b} = block$ effect vector (assumed as random); and $\mathbf{e} = \text{error or residue}$ value (assumed as random); $\mathbf{b} = block$ effect vector (assumed as random); and $\mathbf{e} = \text{error or residue}$ value (assumed as random). Uppercase letters represent the incidence matrices for the respective effects.

Stability and adaptability were simultaneously evaluated by the harmonic mean of relative performance of genetic values (HMRPGV), according to Resende (2007). All analyses were carried out using the software Selegen-Reml/Blup. With the predicted genetic values, genetic correlations were obtained between the traits evaluated in combined analysis with the environments.

RESULTS AND DISCUSSION

Evaluation in each location

Considering the evaluations in the third and in the fourth years, heritability values for clones means in relation to the traits height, *DBH*, and volume were of high magnitude (from 60% to 82%), and significant by the likelihood ratio test at 5% significance. This fact results in high accuracies in the selection of clones propagated by somatic embryogenesis, indicating expressive genetic control for these traits in *Pinus taeda* clones (Table 1). These estimates are in agreement with those reported for *Pinus taeda* by Isik et al. (2003) for volume (0.70), and by Isik et al. (2005) for growth traits (0.50 to 0.75). Since the family structure is considerably different between these studies, it is inferred that growth traits in *Pinus taeda* are under moderate to strong genetic control, and that the somatic embryogenesis technique did not affect the expression of these traits.

The estimates of broad-sense individual heritability were lower than those obtained at the mean level of the clone, and varied according to the environment and year of evaluation (Table 1). Heritability estimates of low to moderate magnitude have been observed in other species propagated by somatic embryogenesis, such as in *Pseudotsuga menziesii* at five and a half years after planting (height = 0.25 ± 0.01 ; *DBH* = 0.21 ± 0.01 ; and volume = 0.20 ± 0.01) (Dean 2008); and in *Picea glauca* at four years after planting (height = 0.137 ± 0.041) (Wahid et al. 2012).

The lowest values for heritability, accuracy, and coefficient of genotypic variation at all the ages were observed in environment 1, in Santa Catarina (Table 1). The other environments presented better conditions for the development and expression of the genetic potential of clones, providing, in these cases, better conditions to detect existing variation and, consequently, greater possibilities of genetic gains with selection. Environment 1 presented edaphic traits inferior to those of the other environments, and this may have influenced gene expression of the clones propagated by somatic embryogenesis, which negatively reflected in the genetic parameters evaluated in this study.

The coefficient of genotypic variation (CV_{gi}) of the traits evaluated in this study had little variation, considering the three ages of study and the four environments. Environment 1 in Santa Catarina had the lowest coefficients of genotypic variation at the three ages of evaluation (ranging from 6.2 % to 10.5 % for height; 9.2% to 11.7 % for *DBH*; 0.6% to 5.5% for survival rate; and 21.8% to 28.8% for volume), as observed in Table 1. Of the traits evaluated, volume had the greatest coefficients of genotypic variation at all ages (greater than 20%). The presence of considerable genetic variability, as observed in this study, indicates the possibility of practicing selection among clones, especially for volume (Resende 2007). Thus, it is possible to obtain genetically significant gains in selection of *Pinus taeda* clones propagated by somatic embryogenesis.

No great variation was observed among the different ages for survival rate (Table 1), indicating good ability of

aec	
ıs t	
int	
μ	
n ³ i	
ц.	
ć	
20	
e	
In	
2	
рц	
), a	
sur	
te (
E L	
iva	
2n	
, si	
ter	S.
ш	est
'nti	al t
8	lon
.⊑	гc
BH)	fو
<u>ם</u>	he
ter	Sr t
nei	5
liar	age
s, C	of
ter	ars
me	Ye
.⊑	'n
ť),	1 5
E	anc
ght	e)
hei	hre
its	e, t
tra	õ
he	at
ir tl	sis,
s fc	ne
ter	9gc
.eu	ž
araı	цр
ğ	с С
etic	lati
ene	on
of g	y s
S O	dr
ate	ate
ti M	Jag
ESI	rop
1.	s p
ble	DUE
Ta	ы

					San	ta Catarini							4	araná			
Parameter	Year		Sit	te 1			Sit	e 2			Site 3				Site	4	
		Ht	рвн	sur	lov	Ħ	DBH	sur	vol	Ht	DBH	sur	vol	Ht	DBH	sur	lov
	1	0.095	0.058	0.004	0.067	0.311	0.228	0.004	0.274	0.332	0.234	0.025	0.289	0.274	0.225	0.004	0.247
h²g	ŝ	0.241	0.183	0.145	0.177	0.364	0.314	0.004	0.340	0.305	0.421	0.027	0.417	0.269	0.314	0.031	0.331
	4	0.224	0.201	0.193	0.192	0.231	0.366	0.004	0.375	0.134	0.255	0.012	0.336	0.321	0.271	0.008	0.326
	1	0.056	0.046	0.001	0.033	0.001	0.024	0.000	0.007	0.005	0.063	0.009	0.035	0.016	0.037	0:030	0.027
c²bloc	ŝ	0.042	0.064	0.002	0.066	0.006	0.008	0.001	0.009	0.132	0.004	0.014	0.019	0.060	0.034	0.021	0.024
	4	0.025	0.040	0.000	0.032	0.022	0.003	0.000	0.002	0.425	0.279	0.076	0.081	0.058	0.125	0.022	0.058
	7	0.438^{*}	0.311^{*}	0.027	0.341^{*}	0.720*	0.637	0.022	0.685*	0.763*	0.685*	0.172	0.733*	0.669*	0.617^{*}	0.024	0.642^{*}
h^2mc	ŝ	0.681^{*}	0.608*	0.029	0.599*	0.764*	0.724	0.023	0.746*	0.774*	0.820*	0.173	0.822*	0.656*	0.695*	0.173	0.822*
	4	0.667*	0.640*	0.062	0.625*	0.640^{*}	0.765	0.022	0.771^{*}	0.706*	0.808*	0.118	0.818^{*}	0.722*	0.692*	0.051	0.771^{*}
	Ļ	0.654	0.581	0.164	0.600	0.830	0.785	0.148	0.810	0.841	0.795	0.415	0.823	0.812	0.785	0.155	0.797
Acclon	ŝ	0.796	0.757	0.170	0.752	0.855	0.832	0.152	0.846	0.849	0.878	0.416	0.879	0.817	0.837	0.416	0.844
	4	0.782	0.767	0.249	0.758	0.786	0.856	0.148	0.860	0.781	0.850	0.344	0.856	0.844	0.829	0.226	0.847
	-	0.805	1.828	0.993	0.083	0.873	1.905	0.991	0.083	1.172	2.679	0.952	0.083	1.176	2.545	0.853	0.083
Overall mean	ŝ	3.855	5.925	0.982	3.333	3.946	6.103	0.991	3.611	4.086	7.247	0.943	5.833	5.570	5.782	0.839	4.444
	4	5.145	9.753	0.981	8.750	5.253	10.423	0.991	10.208	6.976	10.430	0.904	13.958	7.872	7.976	0.839	8.958
	1	31.302	36.039	8.513	105.646	21.294	19.993	11.314	60.404	22.435	23.128	20.546	78.949	22.910	24.711	39.101	61.176
CVe (%)	ŝ	15.599	23.769	10.795	54.799	13.225	21.073	11.309	46.125	16.493	20.120	22.386	56.055	22.747	19.488	39.811	49.734
	4	11.379	19.372	11.280	43.742	9.905	16.722	11.868	35.710	18.788	18.662	31.007	42.612	20.433	19.235	41.319	44.924
	1	10.471	9.165	0.553	28.801	14.302	11.044	0.738	37.259	15.866	13.345	3.463	51.534	14.231	13.666	2.537	35.680
CVgi (%)	ŝ	9.042	11.737	4.446	26.514	10.044	14.336	0.739	33.324	12.134	17.221	3.789	48.210	14.416	13.535	7.190	35.675
	4	6.222	9.979	5.513	21.766	5.511	12.738	0.757	27.684	10.231	13.813	3.492	32.391	14.675	12.897	3.846	32.699
h^2g : Coefficients of	broad-se	ense individ	dual herit	ability, free	of interaction	on; c ² bloc: cc	befficient o	f determina	ation of the bl	ock effects; h ²	mc: heritabili	ty of clone	mean; <i>Accloi</i>	n: accuracy o	f selection o	of genotype	:s; CVe (%):

the clones in surviving under the conditions in which the experiments were developed. Survival rate in all the experiments was high, near 100%; this is probably because the matrices selected for cloning by somatic embryogenesis were adapted to the environmental conditions of the experiments. For this reason, there was not enough variability for selection in this trait. These results indicate the stability of the clones propagated by somatic embryogenesis in relation to survival ability in different environments. The high survival rates for Pinus taeda clones confirm that the clones propagated by somatic embryogenesis may be established in different environments. In addition, this result demonstrates that the cloning of Pinus taeda by somatic embryogenesis is viable, producing genetically stable individuals with good development in the field.

Combined analysis of environments

The heritability estimates reported in the combined analysis lead to expressive selective accuracies for the studied traits, especially for volume (Table 2). Nevertheless, these heritability estimates were low when compared with those found in the individual analysis per environment (Table 1). This indicates that individuals should be selected. The relatively low estimates for heritability in the traits evaluated in the combined analysis of environments (Table 2) suggest that other factors, besides genetics, strongly affect these traits, such as the environmental effects of sites and the genotype x environment interaction.

Corroborating the data obtained in this study, Xiang et al. (2003) observed in full-sib families of *Pinus taeda* that the ideal age for early selection, considering volume and the *DBH*, is from 4 to 5 years. Gwaze et al. (2001)

ła

Damanatan	Veee	San	ta Catarina	(SC)		Paraná (PR))	Combined SC/PR		
rarameter	rear	Ht	DBH	vol	Ht	DBH	vol	Ht	DBH	vol
	1	0.064	0.077	0.080	0.171	0.100	0.121	0.099	0.074	0.076
h^2g	3	0.114	0.100	0.086	0.087	0.133	0.161	0.079	0.119	0.133
	4	0.154	0.116	0.106	0.088	0.126	0.144	0.067	0.124	0.132
	1	0.076^{*}	0.006	0.016	0.068*	0.063*	0.084*	0.115*	0.063*	0.125*
$c^2 int$	3	0.169*	0.125*	0.146*	0.109*	0.094^{*}	0.129*	0.142*	0.118^{*}	0.160*
	4	0.074^{*}	0.143*	0.156*	0.142*	0.105*	0.152*	0.174^{*}	0.127*	0.173*
	1	0.336*	0.471*	0.463*	0.615*	0.499*	0.504*	0.540*	0.533*	0.470^{*}
h^2mg	3	0.384^{*}	0.387^{*}	0.335^{*}	0.417^{*}	0.551^{*}	0.528^{*}	0.479^{*}	0.593*	0.528^{*}
	4	0.548^{*}	0.411*	0.378^{*}	0.387^{*}	0.522^{*}	0.484^{*}	0.387^{*}	0.527^{*}	0.484^{*}
	1	0.579	0.686	0.680	0.784	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	0.735	0.730	0.686	
Acgen	3	0.620	0.622	0.579	0.646	0.742	0.727	0.692	0.770	0.727
	4	0.740	0.641	0.615	0.622	0.722	0.696	0.622	0.726	0.696
	1	0.455	0.927	0.837	0.715	0.615	0.590	0.463	0.540	0.378
rgloc	3	0.403	0.445	0.371	0.444	0.588	0.555	0.358	0.502	0.455
	4	0.657	0.448	0.404	0.388	0.547	0.487	0.278	0.494	0.433
	1	0.847	1.883	0.083	1.238	2.761	0.083	1.040	2.314	0.083
Overall mean	3	3.913	6.024	3.611	4.959	6.757	5.556	4.441	6.399	4.722
	4	5.224	10.106	9.583	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	6.108	9.956	11.042		
	1	7.973	9.523	28.071	12.478	10.099	30.599	9.824	9.104	27.974
<i>CVge</i> (%)	3	5.990	8.439	17.628	8.992	12.108	30.418	7.483	10.493	26.948
	4	4.921	7.375	15.294	8.155	11.349	25.700	6.094	9.537	22.474
	1	27.482	30.859	89.212	21.429	22.576	64.290	24.071	26.131	76.953
CVe (%)	3	14.686	22.718	50.877	19.169	19.803	50.780	17.888	21.138	52.364
	4	10.826	18.242	39.647	17.325	18.745	44.366	15.694	18.534	43.398

Table 2. Estimates of genetic parameters for diameter (DBH), height (Ht) and volume (vol) of Pinus taeda clones propagated by somatic embryogenesis, at four ages, in relation to the four clonal tests (two in Santa Catarina and two in Parana)

h²g: broad-sense herdability of individual plots; c²int: coefficient of determination of the effects of genotype x environment interaction; h²mg: adjusted heritability to genotypes mean; Acgen: accuracy of selection of genotypes; rgloc: genotypic correlation between performance in the different environments; CVge (%): coefficient of genotypic variation; CVe (%): coefficient of residual variation; overall mean of the experiment. *Significant by the likelihood ratio test at 5% probability.

reported similar results when evaluating *Pinus taeda* families at 5 to 25 years of age. These results, once again, show that 1 year of age is not adequate for selection. This is because early selection does not reveal the presence of competition among plants, which is manifested in the evaluations at 3 and 4 years, in addition to the lower heritability.

Significant genetic variability is observed by the likelihood ratio test at 5% significance among the clones evaluated in the combined analysis in the state of Santa Catarina, Parana, and in all environments, as shown by the heritability estimates and their standard deviations (Table 2). The values of the coefficient of genotypic variation (CV_{gl}) for *DBH* and height in the three years of study were of approximately 7%; however, considering the combined analysis among the environments, the volume showed values greater than 22% in the three years of evaluation. The coefficient of genotypic variation for volume demonstrates that selection of genotypes is possible; this is because the CVgi is greater than 10%, which is enough to practice effective selection among clones (Resende 2002).

Genotypic correlation among the environments (r_{gloc}) was moderate to high for almost all the traits evaluated in the first year of the study, ranging from 0.38 to 0.93. However, in the third and fourth years, genotypic correlation among the environments was of low to moderate magnitude for almost all the traits evaluated, ranging from 0.28 to 0.65 (Table 2). According to Table 2, the experiments revealed low coefficients of determination of the effects of the genotype x environment interaction at the ages evaluated.

Genotypic correlation greater than 0.67 are considered as high, and indicates that a single breeding program simultaneously and satisfactorily meets the demands of all the environments evaluated in the present work (Resende 2002a). In this study, the combined analysis of all environments was moderate, which requires differentiated selection for the different environments, indicating that some genotypes may have superior performance in one environment,

but not in another (Cruz et al. 2004, Resende 2007). In general, clones are more unstable than families; thus, there is a tendency of lower genotype x environment correlation in clonal tests. Nunes et al. (2002) reported that the response correlated by selection in an environment and gain in another environment has always been lower than the gain from direct selection in the environments when significant interaction is observed.

Genetic correlation among the environments indicates that selection of specific clones for each environment is recommended. In addition, from these results, adaptabilities and stabilities of clones should be taken into account when selecting these clones (Resende 2007). For analyses of genetic gain, stability, and adaptability in each environment, only the volume data will be discussed, since this trait tends to be the most representative at the initial stage of clone selection (Mckeand et al. 2006, Santos et al. 2006, Beltrame et al. 2012), and is of great commercial interest.

Genetic gains

Genetic gain in relation to the overall mean of the experiment, using the five best clones according to the genotypic values, was of approximately 50% in selection in the combined analysis of environments; greater than 69% in environment 1; 57% in environment 2; and greater than 100% in environments 3 and 4, in the state of Parana (Table 3). However, when compared with the mean value of the controls (matrices 161, 162, and 163), the genetic gain using the same five best clones decreased to values from 9 to 19% in the combined analysis of environments; 7 to 13% in environment 1; 8 to 18% in environment 2; 30 to 70% in environment 3; and 15 to 30% in environment 4 (Table 3). Genetic gain, in relation to the overall mean of the experiment, indicates good possibility of gain with selection under these conditions, especially for the environments located in Parana. However, in relation to the controls, lower possibility of genetic gain was observed, when compared with the gain of the overall mean of all the clones of the experiments.

The data presented by Isik et al. (2005) corroborate those reported in this study. According to the authors, the volumes of *Pinus taeda* clones selected at four years of age in each environment were of 27% and 31% greater than the mean volume of all the clones tested by Isik et al. (2005). Nevertheless, when the authors compared the gain with the families used as control, the former were around 4% to 13%. *Pinus taeda* breeding programs have increased volumetric yield by 10-30% in relation to the sources not subjected to breeding (Mckeand et al. 2003, Mckeand et al. 2006).

Table 3 shows the small difference between the genotypes used as controls (matrices 161, 162, and 163) and the best clones of the experiments. This indicates that these matrices have good performance in the mean of the environments, and may be considered as plastic and reasonably adapted to the different edaphic and climatic growing conditions.

The comparison of predicted genotypic gains in relation to the commercial control is essential, since the goal of a breeding program is to always improve the mean value of the genetic materials (clones) currently planted for commercial purposes, and not only to improve the mean of the population over time. Therefore, an important challenge is to develop genetic materials and selection criteria that maximize the genetic gain of new materials that surpass the mean value of the commercial control.

Stability and adaptability

The five best clones based on the HMRPGV (Table 4) do not totally coincide with the five best clones according to the order of genotypic values predicted by combined analysis of the environments (Table 3). Coincidence was of 80% among the five best clones, and the order among the coinciding clones was inverted. This lower estimate is associated with selection of the clones that show good performances in both environments, but which are not necessarily the best clones of each environment. The interaction reduces the correlation between the genotypic and phenotypic values, and also reduces the genetic gains with selection (Nunes 2002). This was expected, since the greatest gain is obtained with direct selection for the trait of interest and for the specific environment. The present results corroborate those reported by Martinez et al. (2012) in *Pinus taeda* families.

When comparing the gains obtained from HMRPGV in relation to the controls (matrices 161, 162, and 163), the mean superiority of these five genotypes was of 33.3% (Table 4). When compared with that predicted in the order of genotypic values of combined analysis of environments (Table 3), also in relation to the control, gain was of 10%.

Individual selection, considering the selection by harmonic mean of the relative performance of genotypic predicted

values (HMRPGV), is advantageous for considering the three attributes (productivity, adaptability and stability) (Table 5), taking into account that these new attributes or selection criteria will lead to a more accurate selection (Resende 2007).

Results show that simultaneous selection by adaptability and stability of the genotypic values (HMRPGV) generates 10% additional gain in relation to the control. According to Resende (2007), this occurs because simultaneous selection in the new genetic materials takes advantage of the gain from mean interaction between the environments, which does not occur with the genetic material used as control, since they go through many replications in the trials, and their heritability at mean level tends to be equal to 1.0 in each trial. According to Anputhas et al. (2011), the recommendation

Table 3. Ordering of *Pinus taeda* clones, propagated by somatic embryogenesis, according to their genotypic values and predicted gains for volume (m³ ha⁻¹ year⁻¹), in combined analysis of environments and in each environment at four years of age

Environ-ment	Order	Genotype	g	u+g	Gain	New mean	Relative gain (overall mean) %	Relative gain (mean of controls) %
	1	47	6.875	17.917	6.875	17.917	62.264	19.444
	2	149	5.625	16.667	6.250	17.292	56.604	15.278
	3	132	5.208	16.250	5.833	17.083	54.717	13.889
All anvironmente	4	238	4.583	15.625	5.625	16.667	50.943	11.111
All environments	5	146	4.375	15.417	5.417	16.458	49.057	9.722
	6	163	4.167	15.417	5.208	16.250	47.170	8.333
	20	162	2.708	13.750	3.750	14.792	33.962	-1.389
	50	161	1.458	12.708	2.708	13.750	24.528	-8.333
	1	112	6.458	15.417	6.458	15.417	76.190	13.846
	2	132	6.042	15.000	6.250	15.208	73.810	12.308
	3	163	6.042	15.000	6.250	15.208	73.810	12.308
Environment 1	4	139	5.208	14.167	6.042	14.792	69.048	9.231
	5	143	4.375	13.333	5.625	14.583	66.667	7.692
	10	162	3.542	12.500	4.792	13.750	57.143	1.538
	43	161	1.458	10.417	2.917	11.875	35.714	-12.308
	1	16	7.292	17.500	7.292	17.500	71.429	18.310
	2	7	6.875	16.875	7.083	17.292	69.388	16.901
	3	72	5.208	15.417	6.458	16.667	63.265	12.676
Environment 2	4	73	5.208	15.417	6.042	16.250	59.184	9.859
Environment 2	5	37	5.000	15.208	5.833	16.042	57.143	8.451
	7	162	4.375	14.583	5.417	15.625	53.061	5.634
	11	163	3.542	13.750	5.000	15.000	46.939	1.408
	30	161	1.875	11.875	3.542	13.750	34.694	-7.042
	1	47	23.750	38.958	23.750	38.958	179.104	70.000
	2	146	12.917	28.125	18.333	33.542	140.299	46.364
	3	149	12.917	28.125	16.458	31.875	128.358	39.091
Environment 2	4	80	11.458	26.667	15.208	30.417	117.910	32.727
Environment 3	5	132	11.042	26.458	14.375	29.792	113.433	30.000
	17	163	6.458	21.875	10.000	25.208	80.597	10.000
	44	162	3.333	18.750	6.667	21.875	56.716	-4.545
	49	161	3.125	18.333	6.458	21.667	55.224	-5.455
	1	238	10.625	20.625	10.625	20.625	130.233	30.837
	2	167	9.375	19.375	10.000	20.000	123.256	26.872
	3	173	6.875	16.875	8.958	18.958	111.628	20.264
Environment 4	4	222	6.875	16.875	8.542	18.542	106.977	17.621
Environment 4	5	233	6.667	16.667	8.125	18.125	102.326	14.978
	8	163	6.250	16.250	7.500	17.500	95.349	11.013
	14	162	3.333	13.333	6.042	16.042	79.070	1.762
	39	161	1.875	11.875	3.542	13.750	53.488	-12.775

g: total genotypic effects; u + g: predicted genotypic value. The highlighted genotypes correspond to the controls.

PC Dias et al.

Ordering	Stability and Adaptability		
Ordering	Genotype	HMRPGV	HMRPGV* OM
1	238	2.062	22.917
2	<u>149</u>	1.844	20.417
3	<u>132</u>	1.707	18.958
4	222	1.678	18.542
5	233	1.659	18.333
6	47	1.604	17.708
7	219	1.566	17.292
8	143	1.534	17.083
9	163	1.507	16.667
10	146	1.415	15.625
11	139	1.409	15.625
12	95	1.404	15.625
13	68	1.371	15.208
14	16	1.370	15.208
15	184	1.359	15.000
16	69	1.351	15.000
17	112	1.344	15.000
18	148	1.339	14.792
19	162	1.338	14.792
20	125	1.331	14.792
-	-	-	-
-	-	-	-
-	-	-	-
-	161	1.181	13.125
Overall Mean (OM)			11.042
Genetic gain in relation to the mean of t	he experiment (five best clones)		79.60%

Table 4. Stability and adaptability of genotypic values (HMRPGV) predicted by the BLUP analysis for volume (m³ ha⁻¹ year⁻¹) at four years of age

Obs: The underlined clones are the five best in order of predicted genotypic values in combined selection of environments, and are also present in the order of selection for stability and yield.

33.38%

Genetic gain in relation to the mean of the controls (five best clones)

of cultivars with broad adaptability and stability is essential for regions with different productive environments, or with distinct climatic seasons.

The selection of the 20 best *Picea glauca* clones based on height at four years after planting generated mean genetic gain of 4.3% (Wahid et al. 2012), which is lower than that obtained in the present study. According to the authors, this result is considered as important for selection, taking into account that the genetic gain is static and any increase generates gain in selection. Thus, for the present study, selection that considered simultaneously adaptability and stability generated gains close to 10%, and may be used for the recommendation of new clones within the breeding program of the company. Similar result was reported by Sun (2004) when evaluating the adaptability and stability of introduced families of *Pinus taeda*.

Results show that selection using the volume may be practiced from the fourth year after planting *Pinus taeda* clones propagated through somatic embryogenesis. Due to the high magnitude of the "g x e" interaction involving *Pinus taeda* clones propagated by somatic embryogenesis in the two states, a single selection program cannot be adopted, requiring selection of specific clones for the different environments, unless the attributes of adaptability and stability of the clones are used in their selection. Estimated gains confirm the efficiency of the somatic embryogenesis technique in propagation of clones with good yield, aggregating better results to *Pinus* breeding programs.

Genetic evaluation of Pinus taeda clones from somatic embryogenesis and their genotype x environment interaction

ACKNOWLEDGEMENTS

The authors thank Klabin S.A for providing the data for the research. This work was supported by Capes and CNPq.

REFERENCES

- Alcantara GB, Ribas LLF, Higa AR and Ribas KCZ (2008) Efeitos do ácido indol-butírico (AIB) e da coleta de brotações em diferentes estações do ano no enraizamento de mini estacas de *Pinus taeda* L. Scientia Forestalis 36: 151-156.
- Alcantara GB, Ribas LLF, Higa AR, Zuffellato-Ribas KC and Koehler HS (2007) Efeito da idade da muda e da estação do ano no enraizamento de miniestacas de *Pinus taeda* L. Revista Árvore 31: 399-404.
- Andrejow GMP and Higa AR (2009) Potencial de enraizamento de miniestacas de *Pinus Taeda* L. provenientes de brotação apical de mudas jovens. **Floresta 39**: 897-903.
- Anputhas M, Samita S and Abeysiriwardena DSZ (2011) Stability and adaptability analysis of rice cultivars using environment-centered yield in two-way ANOVA model. **Communications in Biometry and Crop Science 6**: 80-86.
- Becwar MR, Chesick EE, Handley LW and Rutter MR (1996) Method for regeneration of coniferous plants by somatic embryogenesis. US n. 5506136 A, Apr. 9.
- Beltrame R, Bisognin DA, Mattos BD, Cargnelutti A, Haselein CR, Gatto DA and Santos GA (2012) Desempenho silvicultural e seleção precoce de clones de híbridos de eucalipto. Pesquisa Agropecuária Brasileira 47: 791-796.
- Cruz CD, Regazzi AJ and Carneiro PCS (2004) Modelos biométricos aplicados ao melhoramento genético. Editora UFV, Viçosa, 480p.
- Dean CA (2008) Genetic parameters of somatic clones of coastal douglasfir at 5 1/2-years across Washington and Oregon, USA. **Silvae Genetica 57**: 269-275.
- Gwaze DP, Bridgwater FE, Buram TD and Lowe WJ (2001) Genetic parameter estimates for growth and wood density in loblolly pine (*Pinus taeda* L.). Forest genetics 8: 47-55.
- Isik F, Goldfarb B, Lebude A, Li B and McKeand S (2005) Predicted genetic gains and testing efficiency from two loblolly pine clonal trials. Canadian Journal of Forest Research 35: 1754-1766.
- Isik F, Li B and Frampton J (2003) Estimates of additive, dominance and epistatic genetic variances from a clonally replicated test of loblolly pine. Forest Science 49: 77-88.
- Martinez DT, Resende MDV, Costa RB, Higa AR, Santos Gados and Fier ISN (2012) Estudo da interação genótipo x ambiente em progênies de *Pinus taeda* por meio da análise de parâmetros genéticos. Floresta 42: 539-552.
- McKeand S, Mullin T, Byram T and White T (2003) Deployment of genetically improved loblolly and slash pines in the South. Forestry 101: 32-37.

- McKeand SE, Jokela EJ, Huber DA, Byram TD, Allen HL, Li B and Mullin TJ (2006) Performance of improved genotypes of loblolly pine across different soils, climates and silvicultural inputs. Forest Ecology Management 227: 178-184.
- McKeand SE, Li B, Grissom JE, Isik F and Jayawickrama KJS (2008) genetic parameter estimates for growth traits from diallel tests of loblolly pine throughout the southeastern United States. Silvae Genetica 57: 101-110.
- Nunes GHS, Rezende GDSP, Ramalho MAP and Santos JB (2002) Implicações da interação genótipos x ambientes na seleção de clones de eucalipto. Revista Cerne 8: 49-58.
- Paludzyszyn Filho E, Fernandes JSC and Resende MDV (2002) Avaliação e seleção precoce para crescimento de *Pinus taeda*. **Pesquisa Agropecuária Brasileira 37**: 1719-1726.
- Paludzyszyn Filho E, Mora AL and Maestri R (2001) Interação de genótipos de *Pinus taeda* L. com locais no sul-sudeste do Brasil. **Revista Cerne** 7: 90-100.
- Paludzyszyn Filho E, Shimoyama VRS and Mora AL (2003) Seleção precoce para incremento simultâneo do crescimento e da qualidade da madeira em *Pinus taeda* L. **Boletim de Pesquisa Florestal Embrapa Florestas 46**: 31-46.
- Pullman GS and Bucalo K (2011) Pine somatic embryogenesis using zygotic embryos as explants. Methods in Molecular Biology 710: 267-291.
- Pullman GS, Chopra R and Chase KM (2006) Loblolly pine (*Pinus taeda* L.) somatic embryogenesis: improvements in embryogenic tissue initiation by supplementation of medium with organic acids, vitamins B₁, and E. **Plant Scince 170**: 648-658.
- Resende MDV (2002a) Genética biométrica e estatística no melhoramento de plantas perenes. Editora Embrapa, Colombo, 975p.
- Resende MDV (2002b) Software SELEGEN-REML/BLUP. Editora Embrapa, Colombo, 65p.
- Resende MDV (2007) Selegen–Reml/Blup: sistema estatístico e seleção genética computadorizada via modelos lineares mistos. Editora Embrapa, Colombo, 359p.
- Resende MDV and Duarte JB (2007) Precisão e controle de qualidade em experimentos de avaliação de cultivares. **Pesquisa Agropecuária Tropical 37**: 182-194.
- Rosado AM (2012) Seleção simultânea de clones de eucalipto de acordo com produtividade, estabilidade e adaptabilidade. **Pesquisa Agropecuária Brasileira 47**: 964-971.
- Santos GA, Xavier A and Leite HG (2006) Desempenho silvicultural de clones de *Eucalyptus grandis* em relação às árvores matrizes. **Revista Árvore 30**: 737-747.
- Sun X (2004) The adaptability and stability evaluation on introduced

PC Dias et al.

families of *Pinus taeda*. Journal of Anhui Agricultural University **31**: 63-367.

Wahid N, Rainville A, Lamhamedi MS, Margolis HA, Beaulieu J and Deblois J (2012) Genetic parameters and performance stability of

white spruce somatic seedlings in clonal tests. Forest Ecology and Management 270: 45-53.

Xiang L, Li B and Isik F (2003) Time trend of genetic parameters in growth traits of *Pinus taeda*. Silvae Genetica 52: 114-121.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.