

# Phenotypic stability via ammi model with bootstrap re-sampling

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**Abstract** - Reliable evaluation of the stability of genotypes and environment is of prime concern to plant breeders, but the lack of a comprehensive analysis of the structure of the GE interaction has been a stumbling block to the recommendation of varieties. The Additive Main Effects and Multiplicative Interaction (AMMI) Model currently offers the good approach to interpretation and understanding of the GE interaction but lacks a way of assessing the stability of its estimates. The present contribution proposes the use of bootstrap re-sampling in the AMMI Model, and applies it to obtain both a graphical and a numerical analysis of the phenotypic stability of 20 *Eucalyptus grandis* progenies from Australia that were planted in seven environments in the Southern and Southeastern regions of Brazil. The results showed distinct behaviors of genotypes and environments and the genotype x environment interaction was significant ( $p$  value  $< 0.01$ ). The bootstrap coefficient of stability based on the squared Mahalanobis distance of the scores showed that genotypes and environments can be differentiated in terms of their stabilities. Graphical analysis of the AMMI biplot provided a better understanding of the interpretation of phenotypic stability. The proposed AMMI bootstrap eliminated the uncertainties regarding the identification of low scores in traditional analyses.

**Index terms:** *Eucalyptus grandis*, confidence regions, bootstrap prediction region, GE interaction.

## Estabilidade fenotípica através da reamostragem “bootstrap” no modelo AMMI

**Resumo** - As posições críticas dos estatísticos, que atuam em programas de melhoramento genético, referem-se à falta de uma análise criteriosa da estrutura da interação do genótipo com o ambiente (GE) como um dos principais problemas para a recomendação de cultivares. A metodologia AMMI (additive main effects and multiplicative interaction analysis) propõe ser mais eficiente que as análises usuais na interpretação e compreensão da interação GE, entretanto, à dificuldade de se interpretar a interação quando há baixa explicação do primeiro componente principal; à dificuldade de se quantificar os escores como baixos, considerando estável os genótipos e/ou ambientes, além de não apresentar o padrão de resposta do genótipo, o que caracteriza os padrões de adaptabilidade, mostram-se como os principais pontos negativos. Visando minimizar esses problemas desenvolveu-se uma metodologia via reamostragem “bootstrap”, no modelo AMMI. Foram analisadas 20 progênies de *Eucalyptus grandis*, procedentes da Austrália, e implantadas em sete testes de progênies nas regiões Sul e Sudeste do Brasil, sendo a interação GE significativa (valor  $p < 0,001$ ). A metodologia “bootstrap” AMMI eliminou as dúvidas relacionadas e mostrou-se precisa e confiável. O coeficiente “bootstrap” de estabilidade (CBE), baseado na distância quadrada de Mahalanobis, obtidos através da região de predição para o vetor nulo, mostrou-se adequado para predições das estabilidades fenotípicas.

**Termos para indexação:** *Eucalyptus grandis*, região de confiança, região bootstrap de predição, interação genótipo-ambiente.

## Introduction

The presence of differential genotypic responses in different environments, known as the interaction of genotypes with environments (GE), is a natural phenomenon and part of the mechanism of species evolution. It governs the identification of stable genotypes

that are suitable for a particular environment, as well as of genotypes with a general behavior that are suitable for several environments.

Since the main objective of a breeding program is to select genotypes that are consistently and high yielding (i.e. superior genotypes) across different environments, poor efficiency in the analysis of GE interaction may present a problem to breeders as it reduces the accuracy

in the selection of environments. A stumbling block concerning the recommendation of varieties has been the lack of comprehensive analyses of the structure of GE interaction. Traditionally, the analysis of that structure was superficial and stopped short of detailing the effects of the complexity of the interaction. However, recent advances in computer science have allowed the development of interactive systems of data processing with fast and precise algorithms. Consequently, statistical methods are being developed for detailed studies of the structure and stability of GE interaction.

In the present work, we propose a method that uses bootstrap re-sampling in the Additive Main Effects and Multiplicative Interaction (AMMI) Model. We then apply it to obtain both a graphical and a numerical analyses of the phenotypic stability in *Eucalyptus grandis*, by using bootstrap coefficient of stability based on the squared Mahalanobis distance..

### Phenotypic stability in plants

The selection of superior genotypes is usually conducted on the basis of observations obtained of phenotypes and is, therefore, extremely dependent on the composition of the phenotypic value relative to the trait under selection. This value is affected by both the genetic effects and effects due to the environments to which the genotype was exposed during the development.

Methods for phenotypic stability analysis are applicable to the evaluation of groups of genotypes tested in several environments and are based on the existence of GE interaction. Therefore, these procedures are complementary to the single and joint analyses of variance with experimental data resulting from trials in a series of environments. In this sense, the stability concept is very important in plant breeding where interest is in obtaining varieties which perform well not only in a particular environment but also under a wide range of environments under cultivation. The environment may include many aspects, such as location (soil, climate, etc), years, seasons, farming and harvesting systems, cultivation techniques, and so on.

Stability parameters are specific to the genetic materials under study as well as to the environment analyzed. Therefore, extrapolation of results to other materials and environments may be erroneous (YATES; COCHRAN, 1938).

Although widespread in the literature, the available methods require a matching of biological and statistical approaches, and their interpretation should be treated with caution. For example, from the statistical standpoint, the dependence of the environmental index on the set of data analyzed makes the regression coefficient questionable as an estimator. Also, the assumption of linear behavior of genotypes across different environments may conflict with the biological reality. Zobel et al. (1988) and Crossa (1990) objected to the use of univariate procedures to detect interactive effects of factors. Instead, they recommended the use of the multivariate technique because the data are derived from multiple environments.

According to Crossa (1990), the data obtained from experiments performed in multiple environments show three fundamental aspects: a) structural pattern; b) non-structural noise, and c) relationships among genotypes, environments, and GE interaction. Pattern implies that genotypes respond to environments in a systematic, significant and interpretable way, whereas noise suggests that responses are unpredictable and non-interpretable, being an integral part of the random variation in the data. In this sort of approach, one does not start by assuming that deviations from the fit of the main effects arise purely from the GE interaction. Indeed, such deviations may contain noise. Removal of the noise will permit characterization of the genetic and environmental factors actually involved in the GE interaction, and will best estimate the responses of the genotypes to environments.

Many researchers have therefore turned to multivariate techniques. These techniques are statistically more complex and sometimes require specialized software or programming. However, the results generated lead to interpretations more consistent with the reality of the experimental material.

Some of these methods, such as principal component analysis (PCA) and cluster analysis (CA), do not show the limitations of linear regression analysis, and have therefore been used to elucidate the internal structure of GE interaction. Yan et al. (2001) proposed a method for evaluating phenotypic adaptability and stability based on the graphical technique called the GGE biplot (main effects of genotypes and of the GE interaction). This is built by plotting the two first components of PCA, by utilizing regression models of localities.

One of the more common methods in recent use is AMMI analysis. The main objective of this analysis is

to select models which explain the pattern of interaction, by removing any noise present in the data that does not carry agronomic interest.

### The AMMI Method

Proposed initially by Mandel (1971), the AMMI method is based upon the decomposition of the sources of variation first into additive effects of genotypes and environments in a traditional way (via analysis of variance) and then into multiplicative effects for the GEI interaction via principal components. This is expressed as the following model for the observation  $Y_{ij}$  on Genotype  $i$  in Environment  $j$ :

$$Y_{ij} = \mu + G_i + E_j + \sum_{k=1}^n \lambda_k \alpha_{ik} \gamma_{jk} + \varepsilon_{ij}$$

The second stage of the decomposition allows for a greater detailing of the sum of squares of the interaction, and consequently has advantages in the selection of genotypes when compared to other methods of analysis (ZOBEL et al., 1988). This method yields more precise estimates of the genotypic responses, and provides a graphical interpretation of the results through the biplot procedure (GABRIEL, 1971) in which the scores of interaction effects for each genotype and environment are plotted simultaneously. The interpretation is based on the magnitude and the sign of the coefficients of genotypes and environments for each principal component axis. Low values indicate genotypes and/or environments which contribute little or almost nothing to the GE interaction, thus exhibiting statistical stability.

The main drawback of the AMMI method is the difficulty of interpreting the interaction when there is a poor explanation of the first principal component and the scores are low, which could indicate statistical stability of the genotypes and/or environments. Thus care needs to be exercised in the interpretations. To researchers, the major problem is the quantification of the stability of genotypes, as well as of environments. In this sense, the difficulty of the AMMI method is in the quantification of scores which characterize stabilities. Various studies have limited to three conclusions. a) poor scores point to genotypes and/or environments which contribute little or almost nothing to the GEI interaction and thus exhibit statistical stability (genotypes and /or environments whose points on the biplot graph are furthest from the origin are those which contribute most to the GEI interaction, and, therefore, are the ones with least

stability); b) small angles among the vectors within the same quadrant show similarity among the varieties they represent, and vectors in opposite quadrants show genetic dissimilarity among the corresponding varieties; c) a genotype vector close to an environment vector on the biplot indicates that it is in this environment that the cultivar performs best as compared to other genotypes.

The main problem with AMMI method is that the researcher must draw inferences from single points plotted on biplots, leaving many margins of doubt. Therefore, we propose a systematic approach to the study and interpretation of phenotypic stability through bootstrap re-sampling of the residual matrix from fitting an AMMI model. The bootstrap technique minimizes the mentioned problems, thus making the AMMI method more precise and reliable for the characterization and selection of populations for genetic improvement programs.

### Material and Methods

The data used in this study were from a progeny trial of *Eucalyptus grandis* involving 20 families in 7 environments. The trial was established in a 10 randomised complete blocks design with 6 plants per plot at a spacing of 3.0 m by 2.0 m. The progenies were assessed by the average height per plot at five years of age (LAVORANTI et al., 2002).

The analysis was conducted by using the AMMI method in which the number of interaction components was determined by the F tests of Cornelius (1993). From the selected model, the estimates of the  $g$  genotypes in the  $e$  environments provided the fitted matrix  $\hat{\mathbf{G}}\mathbf{E}_{GE} = \left( \hat{g}_{ij}^{GE} \right) = \left[ \hat{\mathbf{Y}}_{ij}^{GE} \right]$  from which the matrix of residuals  $\left[ \hat{\mathbf{Y}}_{ij}^{GE} \right]$  was obtained.

From the latter matrix, 200 bootstrap matrices were obtained for the genotypes  $\left( \hat{\mathbf{G}}_{ij}^{GE} \right)$ ; the sampling with replacement was performed by rows, and likewise 200 bootstrap matrices were obtained for environments  $\left( \hat{\mathbf{G}}_{ij}^{GE} \right)$ , in which the sampling with replacement was performed by columns (LAVORANTI, 2003).

The effects of genotypes and environments were estimated through singular value decompositions applied to each of the 400 bootstrap matrices of deviations.

$$\tilde{\mathbf{G}}_{kl}^{+} = \tilde{\mathbf{U}}_{kl}^{+} \tilde{\mathbf{S}}_{kl}^{+} \tilde{\mathbf{V}}_{kl}^{+} = \sum_{i=1}^n \left( \tilde{\lambda}_{ikl}^{+} \gamma_{ikl} \right) \left( \tilde{\lambda}_{ikl}^{+} \alpha_{ikl} \right) = \tilde{\mathbf{G}}_{kl}^{+} \tilde{\mathbf{H}}_{kl}^{+}$$

in which: (k = g, e) for genotype and environment, respectively  $\ell = (1, 2, \dots, 200)$ ;

$\tilde{\mathbf{G}}_{k\ell}^+$  : bootstrap effects of genotypes;  
 $\tilde{\mathbf{H}}_{k\ell}^+$  : bootstrap effects of environments;  
 $\tilde{\mathbf{H}}_{k\ell}^+$  : square roots of the i-th eigenvalue of the bootstrap  
 $\lambda_{ik\ell}$  matrices  $\left( \tilde{\mathbf{G}}_{k\ell}^+ \right) \left( \tilde{\mathbf{G}}_{k\ell}^+ \right)'$  e  $\left( \tilde{\mathbf{H}}_{k\ell}^+ \right) \left( \tilde{\mathbf{H}}_{k\ell}^+ \right)'$  of equal  
 non-null eigenvalue;  
 $\gamma_{ik\ell}$  : i-th vector (related with genotype i) of the k-th  
 eigenvector of  $\left( \tilde{\mathbf{G}}_{k\ell}^+ \right) \left( \tilde{\mathbf{G}}_{k\ell}^+ \right)'$  associated with  $\lambda_{ik\ell}^{2+}$   
 and  
 $\alpha_{ik\ell}$  : i-th vector (related with environment j) k-th eigenvector  
 of  $\left( \tilde{\mathbf{H}}_{k\ell}^+ \right) \left( \tilde{\mathbf{H}}_{k\ell}^+ \right)'$  associated with  $\lambda_{ik\ell}^{2+}$ .

Each bootstrap matrix produced scores for each genotype and environment, and each of these 200 sets of scores was plotted in a two-dimensional graph. Denote the two axes of the representations by  $\text{IPCA}_1$  and  $\text{IPCA}_2$  respectively. The graphs are interpreted by declaring the genotypes and environments whose points lie close to the origin, i.e. with practically null scores on both  $\text{IPCA}_1$  and  $\text{IPCA}_2$ , and bootstrap coefficient of stability (BCS) above the third quartile, to be consistently stable; the genotypes and environments whose scores include the origin and have low spread across the graphs (BCS between the second and third quartile) are declared to have high stability; those with scores including the origin and having medium spread across the graphs (BCS between the first and second quartile) are declared to have medium stability; those with scores which include the origin and have high spread across the graphs (BCS below the first quartile) are declared to have poor stability; and those whose scores do not include the origin are considered to lack stability.

In order to conduct this interpretation,  $100(1-\alpha)\%$  confidence regions for the mean  $\left( \bar{\theta}^+ = \left[ \overline{\text{IPCA}_1^+}, \overline{\text{IPCA}_2^+} \right] \right)$  were calculated by using the bootstrap technique. Given the unbiased estimate of the bootstrap covariance matrix  $\mathbf{S}_{\theta}^+$  for a pair of coordinates,  $\theta^+ = \left[ \text{IPCA}_{1kl}^+, \text{IPCA}_{2kl}^+ \right]$  the limits of the  $100(1-\alpha)\%$  bootstrap confidence regions for  $\bar{\theta}^+$  in the  $\left( \text{IPCA}_1^+, \text{IPCA}_2^+ \right)$  plane can be found from the

the equation (WEINBERG et al., 1984):

$$\left( \bar{\theta}^+ - \theta^+ \right)' \left( \mathbf{S}_{\theta}^+ \right)^{-1} \left( \bar{\theta}^+ - \theta^+ \right) \leq \frac{2(B-1)}{B(B-2)} F_{\left( 2, B-2 \right)}^{\alpha}$$

where:

$$\mathbf{S}_{\theta}^+ = \begin{bmatrix} \mathbf{S}_{\text{IPCA}_1}^{2+} & \mathbf{r}_{(\text{IPCA}_1 \times \text{IPCA}_2)}^+ \mathbf{S}_{\text{IPCA}_1}^+ \mathbf{S}_{\text{IPCA}_2}^+ \\ \mathbf{r}_{(\text{IPCA}_1 \times \text{IPCA}_2)}^+ \mathbf{S}_{\text{IPCA}_1}^+ \mathbf{S}_{\text{IPCA}_2}^+ & \mathbf{S}_{\text{IPCA}_2}^{2+} \end{bmatrix}$$

and (2, B-2) indicates the numerator and denominator degrees of freedom, respectively, of the F distribution.

The bootstrap coefficient of stability (BCS) based on the squared Mahalanobis distance was determined as a function of the number of scores included inside the bootstrap prediction region for the stability, i.e. the predictive region for  $\bar{\theta}^+ = \left[ 0, 0 \right]$  as:

$$\text{BCS} = B^{-1} \# \left[ \left( \bar{\theta}^+ - \theta^+ \right)' \left( \mathbf{S}_{\theta}^+ \right)^{-1} \left( \bar{\theta}^+ - \theta^+ \right) \leq \frac{2(B-1)}{B(B-2)} F_{\left( 2, B-2 \right)}^{\alpha} \right]$$

Therefore, by using the bootstrap technique, it was possible to estimate the precision and the confidence regions for the stability.

## Results and Discussion

First, a traditional variance analysis was performed (Table 1) and significant effects were detected at 1% level for genotype (G), environment (E) and GEI interaction effects. The environment effect was responsible for the greatest part of the variation, followed by the interaction and genotype effects in that order. The genotypes were differentially influenced by the environments. This hinders the recommendation of varieties for the whole area embraced by the study. It is therefore necessary to conduct a detailed study of that interaction.

The multiplicative GE interaction effect was disentangled by decomposing the interaction sum of squares (SS(GE)) into interaction principal component axes (IPCA). The “postdictive” criterion (use of tests of hypotheses) was adopted for the selection of AMMI models through the  $F_R$  test of Cornelius et al. (1992).

The  $F_R$  test showed the two first axes ( $\text{IPCA}_1$  and  $\text{IPCA}_2$ ) to be significant, with p values < 0.01 for the first axis and < 0.05 for the second axis (Table 1). These axes accounted for 55.53% of SS(GE).

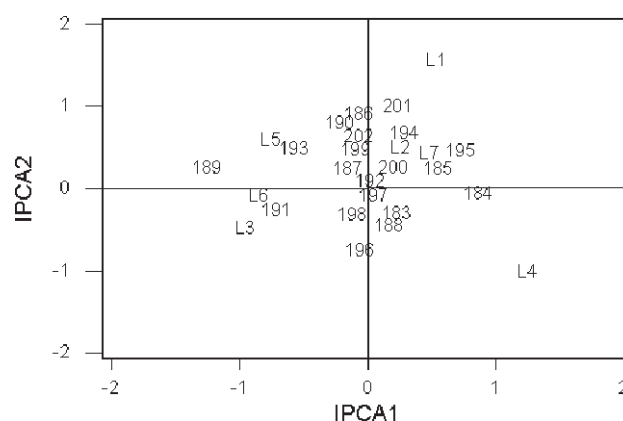


Although this is a relatively low proportion of SS(GE) accounted for by the first two axes, according to Gauch (1988), the first axis captures the largest percentage of “pattern” while subsequent axes showed a decrease in the percentage of “pattern” and an increase in the percentage of “noise”. Thus, a graphical evaluation of interaction using a two-dimensional (AMMI<sub>2</sub>) biplot is worthwhile.

**Table 1.** Eigenvalue ( $\lambda_k^2$ ) and percentage of the accumulated sum of squares (ASS) per singular axis. Joint analysis of variance, including the decomposition of the GE interaction for height (m) at five years of *Eucalyptus grandis* genotypes.

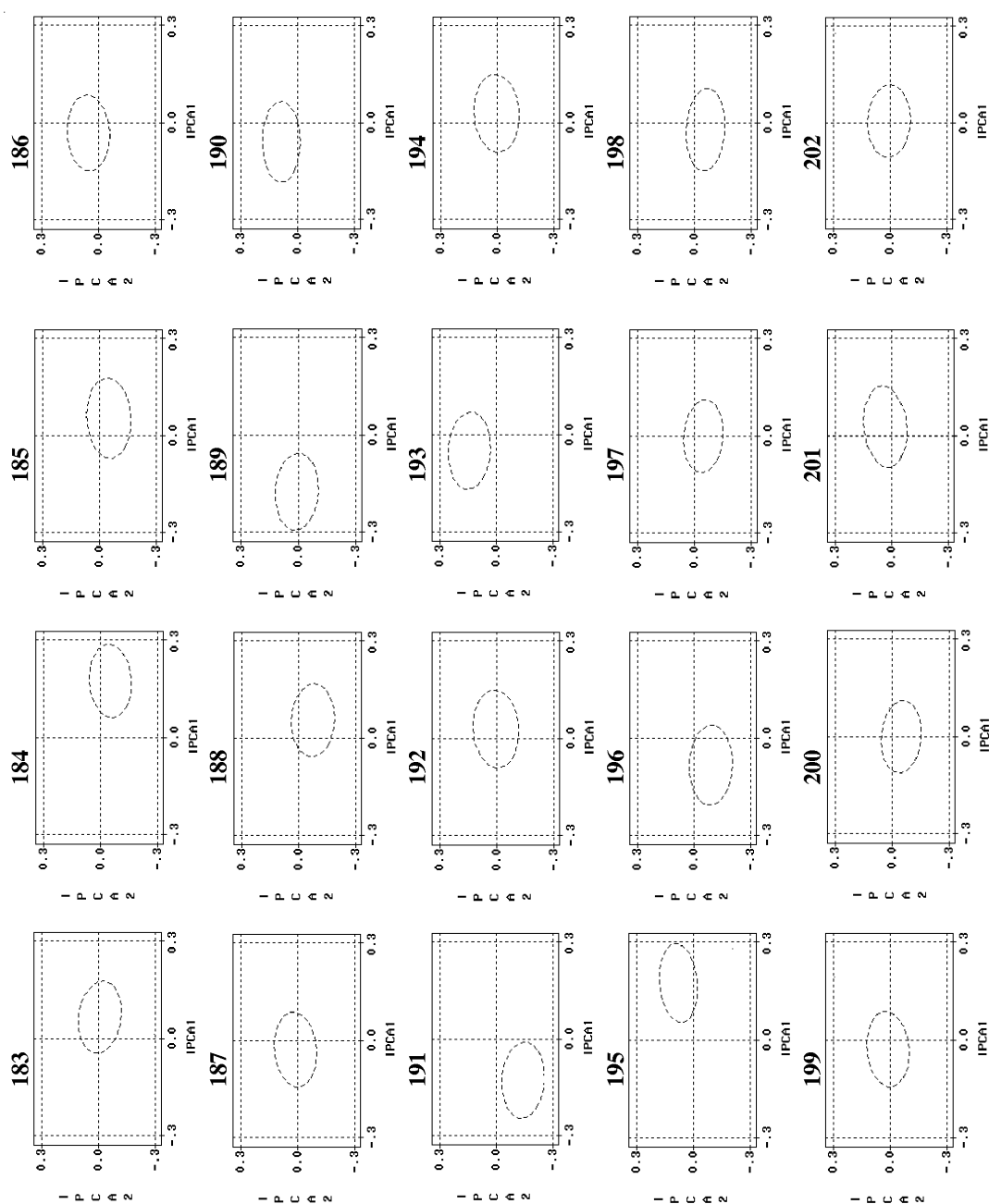
Singular Axis	$\lambda_k^2$	ASS	Source of variation	Cornelius DF	F <sub>R</sub>
			Genotypes (G)	19	10.10**
			Environments (E)	6	392.77**
			G×E	114	66.06**
1	18.9016	29.03	IPCA <sub>1</sub>	90	64.91**
2	17.2607	55.53	IPCA <sub>2</sub>	68	1.38*
3	13.3854	76.09	IPCA <sub>3</sub>	48	1.04ns
4	7.3940	87.44	IPCA <sub>4</sub>	30	0.88ns
5	5.6835	96.18	IPCA <sub>5</sub>	14	0.57ns
6	2.4844	100.00	IPCA <sub>6</sub>		
			Error mean	216	

In an AMMI<sub>2</sub> biplot, the statistically stable genotypes and environments are shown by points which lie close to the origin, that is with scores practically null for the two interaction axes (IPCA<sub>1</sub> and IPCA<sub>2</sub>) (DUARTE; VENCOSKY, 1999). The genotypes which met this condition were 187, 192, 197, and 200 and were considered to have high stability. A little further from the origin were the genotypes 183, 198, and 199 which may be considered to have medium stability, while those falling further away may be considered to have less stability. The genotypes with the largest interaction scores (that lack stability) were 184, 189, 191, 195, 196, and 201. Environments L2 and L7 may be considered to have high stability, while others can be considered to have low stability.

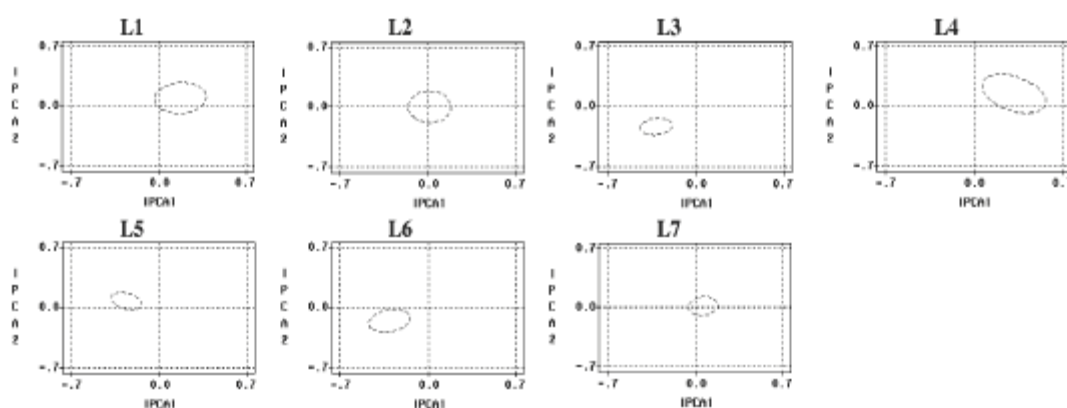


**Figure 1.** AMMI<sub>2</sub> biplot for scores of the genotypes and environments for height (m) of *Eucalyptus grandis* at five years of age .

These criteria are subjective and each researcher may interpret differently. However, by using the AMMI bootstrap method, the analyses of stability of genotypes (Figure 2) and environments (Figure 3) allow us to declare as consistently stable the genotypes and environments whose 99% confidence region includes the origin and whose bootstrap coefficient of stability (BCS) is above the third quartile (Table 2). The genotypes which satisfy these conditions were 185 and 199. Somewhat less stable (BCS lying between the second and the third quartiles), but with origin still within the confidence region were the genotypes 186, 188, 202, 192, and 197, and the environments L2 and L1; these can be characterised as having high stability. The scores with medium spread across graphs (BCS lying between the first and second quartiles) and including the origin were of genotypes 194, 198, and 201 which can be characterized as having medium stability.



**Figure 2.** Confidence regions (99%) for bootstrap scores of genotypes for height (m) of *Eucalyptus grandis* at five years of age.



**Figure 3.** Confidence regions (99%) for bootstrap scores of environments for height (m) of *Eucalyptus grandis* at five years of age.

**Table 2.** Bootstrap coefficients of stability (BCS) of genotypes (Gen) and environments (Env) for height (m) of *Eucalyptus grandis* at five years of age.

Consistently stable				High stability				Medium stability			
Gen	Mean	StDev	BCS	Gen	Mean	StDev	BCS	Gen	Mean	StDev	BCS
185	17.04	6.67	0.140	186	16.42	3.48	0.075	194	17.24	3.60	0.065
199	17.74	3.47	0.090	188	16.87	3.65	0.075	198	17.42	3.40	0.065
				202	15.89	3.34	0.075	201	15.06	3.11	0.060
				192	17.65	2.93	0.070				
				197	17.10	2.63	0.070				
Low stability				Unstable							
Gen	Mean	StDev	BCS	Gen	Mean	StDev	BCS				
183	18.59	3.77	0.050	184	17.86	3.62	0.065				
187	15.93	3.42	0.050	193	17.05	3.35	0.065				
200	17.11	3.03	0.050	195	16.87	3.58	0.055				
190	18.11	3.60	0.045	191	17.18	3.85	0.035				
196	17.87	3.71	0.045	189	15.37	3.09	0.015				
High stability				Low stability				Unstable			
Gen	Mean	StDev	BCS	Gen	Mean	StDev	BCS	Gen	Mean	StDev	BCS
L2	22.68	1.31	0.110	L7	13.39	0.97	0.025	L5	12.75	0.96	0.045
L1	16.67	0.93	0.095					L6	18.55	1.12	0.020
								L3	16.88	1.07	0.010
								L4	18.21	1.53	0.000

For recommendations concerning varieties, a stable genotype should also present a desirable performance in terms of averages (Table 2). Genotypes 185, 186, 188, 201, and 202 showed poor average height, but the other five genotypes mentioned above all perform well. These genotypes will therefore have wide adaptability to the studied environments.

Environmental stability implies reliability of the ranking of the genotypes in particular environments, relative to the ranking for the average over all environments. Thus the ranking of genotypes in environments L2 and L1 should be consistent with the ranking across all environments. Therefore, the test of genetic materials in L2 and L1 should produce more consistent and reliable ranking for recommendation of varieties. As environment L2 has the bigger average and the smaller

variance, it should be preferred for future breeding studies.

Although their origins are included in the confidence region (BCS lying below the first quartile), genotypes 183, 187, 200, 190, and 196, and environments L7, may be characterized as of low stability. However, with the exception of genotype 187, all present high averages so should not be discarded in a breeding programme aimed at selecting for high productivity. Genotypes 184, 193, 195, 191 and 189, may be characterised as unstable because their bootstrap confidence regions do not include the origin. The same can be said for environments L5, L6, L3, and L4.

More detailed investigations are needed before fixing the levels of stability, through the prediction regions for the null vector. Also, different correction factors could

be tried for the Wishart distribution in order to increase the BCS coefficient. Nevertheless, the bootstrap enhancements to the AMMI model proposed above show that a better quality can be achieved in the prediction of phenotypic stability than by the traditional AMMI analysis alone.

### Conclusions

a) The confidence regions obtained through bootstrapping enhance the interpretation of the phenotypic stability through graphical analysis of AMMI biplots;

b) The bootstrap coefficient of stability based on the squared Mahalanobis distance of the AMMI<sub>2</sub> model allows genotypes and environments to be distinguished in terms of stability;

### Acknowledgments

The authors thank Professor Roland Vencovsky of the Department of Genetics of the ESALQ/USP, for valuable scientific discussions which have greatly contributed to the development of this work, and Embrapa Florestas, who furnished the data and provided technical assistance.

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Received on November 25, 2005 and accepted on March, 2007