

# Yield stability of soybean lines using additive main effects and multiplicative interaction analysis - AMMI

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ABSTRACT - The grain yield of 27 soybean lines was evaluated at three locations (Anhembi, Areão and Esalq) in Piracicaba, State of São Paulo, Brazil, during four crop years to study the effect of environment (E) on the adaptability and stability of the lines (G) using additive main effects and multiplicative interaction analysis (AMMI). Effects of the G, E, and GE interaction were found to be significant and accounted for 51, 12, and 36% of the variation, respectively. The first and only significant interaction principal component axis (IPCA1) accounted for 26% of the sum of squares due to original GE interaction. This concentrated the largest proportion of the pattern of GE interaction. Environments associated with Anhembi and Esalq proved more favorable, while Areão contributed negatively to the grain yield. However, Anhembi and Areão were more predictable for the crop years. USP 93-5082 and USP 93-5243 lines combined high adaptability and stability.

**Key words**: *Glycine max*, GE interaction, predictability, AMMI model.

#### INTRODUCTION

Soybean is a species of great economical interest owing to the nutritional quality of its grain, given by the high protein (40%) and oil (20%) content, as well as its high grain yield. In Brazil, soybean is nowadays cultivated in a large range of environments, from the high (southeast and southern regions) to the low latitudes (Mid-West, northeast and northern regions). In this sense, the selection of genotypes with high productivity (adaptability) and adaptation ability to a wide range of environments (stability) is a very important step in soybean breeding programs (Rocha and Vello 1999).

Depending on the genetic base and unpredictable climatic

factors prevailing at the different sites and/or years, differential responses are expected from the improved genotypes (G) tested in different environments (E). These differential genotypic responses to different environments are collectively called GE interaction (Allard and Bradshaw 1964). A significant GE interaction for a quantitative trait such as grain yield can seriously limit the genetic gain under selection. The testing of selected materials over sites and years to ensure a stability performance over a range of environments is a universal practice. In a breeding program, genotype x location interaction effects are of special interest for identifying adaptation targets, adaptative traits and test sites. These effects, generally having relatively low repeatability over years, should be studied on a multiyear basis

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in annual crops (Annicchiarico 1997).

Methodologies to analyze stability are based on the principle of an existent GE interaction, but differ in the concepts of stability they adopt and in statistical principles. The ecovalence method (Wricke 1965) is based on the decomposition of the GE interaction, on each genotype components. Joint linear regression analysis (JRA) (Finlay and Wilkinson 1963, Eberhart and Russel 1966, Verma et al. 1978, Silva and Barreto 1986) has been a commonly used technique for studying phenotypic adaptability and stability. A major criticism regarding JRA is that, usually only a small part of the interaction is explained regression. Under large environmental diversity JRA can fail. It is important to know the conditions under which this might happen (Crossa 1990).

The use of multivariate methods to study GE interaction effects has been suggested to solve the problem with JRA, which uses an additive linear model to analyze a multivariate case. The re-introduction and elaboration of the additive main effects and multiplicative interaction (AMMI) analysis by Zobel et al. (1988) has increased the interest on the principal component analysis (PCA) techniques to study GE interaction effects. The AMMI model combines the additive analysis of variance for main effects with the multiplicative PCA for the interaction (i.e., the residual from the analysis of variance).

Gauch (1990) claimed that AMMI analysis always does as well as, but frequently much better than JRA in the sum of square (SS) recovery. Preliminary results (Zobel et al. 1988) supported the hypothesis that IPCA1 in AMMI is superior to JRA in accounting for the G x E sum of squares. It seems plausible that trait stability estimated by AMMI could be more repeatable than other stability statistics because AMMI is effective at recovering even complex GE interaction patterns (Sneller et al. 1997). Recently, AMMI analysis has been applied to soybean (Zobel et al. 1988, Gauch and Zobel 1990, Sneller and Dombeck 1995, Sneller et al. 1997, Ariyo 1998, Oliveira et al. 2003).

After fitting the genotype and environmental main effects in the model, a crucial step in the analysis is the determination of the amount of pattern (portion of GE interaction variation representing real responses to genotypes and environments), and noise (random variation non-pattern resulting from microenvironments effects). Ideally, pattern is only included in the selected AMMI model by retaining the statistically significant GE interaction principal component axes (IPCA) in its multiplicative term (Annicchiarico 1997). The optimum number of IPCA to be retained in the model in order to obtain the most accurate estimation for grain yield, can be determined by two different assessments (referred to in literature as 'predictive' and 'postdictive') (Fox et al. 1997).

The predictive assessment subdivides the data into two sets; the model data and the validation data. The former is used to construct a model, whose predictive values are then compared with the validation data, using, for example, the root mean square predictive difference (RMSPD) between the validation data and model predictions, including zero (AMMI0) to all possible N (AMMIN) IPCA, in terms of predictive accuracy (Gauch and Zobel 1988, Gauch 1992). According to Ortiz et al. (2001), when only two replications by environment are available in the trials, it was not possible to apply the cross-validation procedure. The postdictive assessment refers to a different method that uses an F-test to identify the significance of each IPCA. An early F-test devised by Gollob (1968) for the assessment of IPCA was very liberal in selecting more multiplicative terms than the true model contained (Cornelius et al. 1992). Others F-tests ( $F_{GH2}$  and  $F_R$ ) have been developed that allow a better control of type-I error rates, presenting better robustness (Cornelius 1993, Piepho 1995).

Objective of the present research was to evaluate the magnitude of the genotype by environmental interaction and to access the phenotypic adaptability and stability on grain yield of experimental soybean lines with an intermediate maturity cycle for different environments (location and year combination), using the AMMI analysis.

# MATERIAL AND METHODS

Twelve field experiments with soybean lines of intermediate maturity cycle (128-135 days) were conducted at three locations (Anhembi, Areão and ESALQ) in Piracicaba county (22° 42' lat S, 47° 39' long W and altitude 543m asl), state of São Paulo (SP), Brazil. The genotypes represents a group of experimental lines developed by the Genetic Department of the Escola de Agricultura "Luiz de Queiroz" (ESALQ), Universidade de São Paulo (USP). The lines are product of crossings among adapted parents (adapted cross) and exotic with adapted parents (mixed cross). Additional descriptions of the lines are presented in Table 1.

The Anhembi Experimental Station is located about 60 km from the ESALQ headquarters, with a plain topography. The soil type is a Typic Udifluvent (commonly found in Brazilian savannahs called "cerrados"), dystrophic alluvial and mediumsandy textured, whose acidity was neutralized by lime application; Areão has a wavy topography and a podzolic red-yellow dystrophic soil of medium-loamy texture; the area in ESALQ headquarters has a hilly relief and a high fertile soil (Kandiudalfic Eutrudox) with loamy texture. At the three sites, black oat (*Avena strigosa*) had been cultivated in the previous year and was incorporated into the soil by the end of the growing season.

The soybean genotypes were sown in November, corresponding to summer crop, in four crop years (1996/97, 1997/98, 1998/99 and 1999/2000). An incomplete block design with two complete replications of treatments was used, being each block stratified in experimental units with four common checks: 'IAC-4', 'IAC-12', 'IAC-100', and 'Stwart'. Each plot contained four five meter-long rows spaced 50cm apart. Grain yield data obtained at the three locations in the four studied years were used for the statistical analysis. The environments consisted in the location and year combination, resulting in twelve environments: Anhembi-1996/97 (AN96), Anhembi-1997/98 (AN97), Anhembi-1998/99 (AN98), Anhembi-1999/ 00 (AN99), Areão-1996/97 (AR96), Areão-1997/98 (AR97), Areão-1998/99 (AR98), Areão-1999/2000, ESALQ-1996/97 (ES96), ESALO-1997/98 (ES97), ESALO-1998/99 (ES98) and ESALQ-1999/2000 (ES99). The sowing dates were 11/20/96, 11/16/97, 11,17/98, 11/23/99 (Anhembi); 11/12/96, 11/13/97, 11/05/98, 11/30/99 (Areão); 11/06/96, 1/12/97, 11/05/98, 11/08/ 99 (ESALO).

Analysis of variance (ANOVA) was used to test the differences among the lines (G), environments (E), as well as to test the magnitude of the GE interaction. AMMI analysis was performed by removing additive effects for genotypes and environments using the analysis of variance procedure and then fitting multiplicative effects for GE interaction by PCA. The statistical analysis was performed by SAS software (SAS Institute Inc 1997) according to the program elaborated by Duarte and Vencovsky (1999). The AMMI model is

$$\boldsymbol{Y}_{ij} = \boldsymbol{\mu} + \boldsymbol{g}_i + \boldsymbol{e}_j + \sum_{n=1}^{N} \boldsymbol{\lambda}_k \boldsymbol{\gamma}_{ik} \boldsymbol{\delta}_{jk} + \boldsymbol{\rho}_{ij} + \boldsymbol{\epsilon}_{ij}$$

where  $Y_{ij}$  is the grain yield of the  $i^{th}$  genotype in the  $j^{th}$  environment;  $\mu$  is the grand mean;  $g_i$  and  $e_j$  are the effects of genotype and environment deviations from the grand mean, respectively;  $\lambda_k$  is the singular value of the PCA axis k;  $\gamma_{ik}$  and  $\delta_{jk}$  are, respectively, the genotypic and environmental elements of singular vectors associated to  $\lambda_k$  of the matrix of interaction; N is the number of principal components retained in the model:  $\rho_{ij}$  is the GE interaction residual; and  $\epsilon_{ij}$  is the mean error. The interpretation was given by a graphic biplot analysis (Gabriel 1971) with the aid of a table containing the means predicted by the model AMMI selected for each combination genotype and environment.

AMMI generates a family of models. AMMI0 uses the additive genotypic and environmental effects only to describe the data matrix and thus ranks genotypes identically for each environment, ignoring GE interaction. The second model, AMMI1, considers the main effects as well as the IPCA1 to interpret the residual matrix. AMMI2 considers the main effects plus two axes, IPCA1 and IPCA2. The higher order multiplicative components that are not significant can be ignored, resulting in a 'reduced' model (Fox et al. 1997).

**Table 1**. Genealogies of 27 experimental soybean lines

0.1	Lines									
Order	USP Number	Genealogies								
L1	93-2258	Bossier x IAC-12 <sup>1</sup>								
L2	93-2266	Bossier x IAC-1 <sup>1</sup>								
L3	93-2514	Viçoja x FT 81-27061								
L4	93-2530	IAC-10 x IAC-12 <sup>1</sup>								
L5	93-2643	IAC-10 x FT 81-2706 <sup>1</sup>								
L6	93-2722	IAC-12 x GO 81-11.646 <sup>1</sup>								
L7	93-2725	IAC-12 x GO 81-11.646 <sup>1</sup>								
L8	93-2753	IAC-12 x SOC 81-228 <sup>1</sup>								
L9	93-2802	IAC-1 x GO 81-11.646 <sup>1</sup>								
L10	93-2825	IAC-1 x FT 81-2706 <sup>1</sup>								
L11	93-2870	BR-1-Fosca x FT 81-27061								
L12	93-5082	GO 81-11.646 x SOC 81-2281								
L13	93-5243	FT 81-2706 x PI 371610 <sup>2</sup>								
L14	93-5423	SOC 81-76 x Foster <sup>2</sup>								
L15	93-5539	Paranagoiana x Jackson-40282								
L16	93-5544	Paranagoiana x Jackson-40282								
L17	93-5549	Jackson-4028 x FT 81-21292								
L18	93-5552	Jackson-4028 x FT 81-21292								
L19	93-5582	Cobb x BR-8 <sup>2</sup>								
L20	93-5585	Cobb x BR-8 <sup>2</sup>								
L21	93-5586	Cobb x BR-8 <sup>2</sup>								
L22	93-5597	Cobb x BR-8 <sup>2</sup>								
L23	93-5690	Bienville x UFV-Araguaia1								
L24	93-5692	Foster x FT 79-3408 <sup>2</sup>								
L25	93-5843	BR 80-15725-B x Planalto <sup>1</sup>								
L26	93-5860	BR 80-15725-B x Planalto <sup>1</sup>								
L27	93-5884	Planalto x GO 81-11.0941								

<sup>1</sup>adapted cross (adapted parent x adapted parent) <sup>2</sup>mixed cross (adapted parent x exotic parent)

## RESULTS AND DISCUSSION

The additive main effects and multiplicative interaction analysis showed that environments, genotypes (G) and GE interaction were highly significant (P < 0.01) and accounted for 41, 10 and 29% of the total sum of squares (SS), respectively (Table 2).

The significance between environments and genotypes indicated that these showed enough variability while the significance of the magnitude of the GE interaction revealed differential response of genotypes across environments. The interaction was partitioned in eleven interaction principal components axis (IPCA) along with their contribution to the SS. It was not possible to adopt the criterion predictive by

Table 2. Additive main affects and multiplicative interaction analysis of variance for grain yield (kg ha<sup>-1</sup>) including the first interaction principal component axis (IPCA1)

Source of variation	df	SS	MS	$\mathbf{R}^{2}$ (%) <sup>1</sup>
Total	635	190684921	300291	
Treatments	323	151083385	467750**	79.23
Environments (E)	11	77813175	7073925**	51.50
Genotypes (G)	26	18757752	721452**	12.42
GE Interaction	286	54512458	190603**	36.08
IPCA1	36	14109120	391920**	25.88
Residual	250	40403500	161614	74.12
Error	312	39601536	126928	

<sup>\*\*</sup>P < 0.001, by *F*-test

cross-validation for the selection of the AMMI model because there were only two replications by environment. The criterion of postdictive success for AMMI using all the data (both replications) and  $F_R$ -test proposed by Cornelius (1993) and Piepho (1995) indicated the inclusion of the IPCA1 and the selection of the AMMI1 model because its residue was not significant at the probability level 0.01 (Table 2).

IPCA1 explained 26% of the G x E sum of squares. This value is smaller than that obtained by Zobel et al. (1988), Gauch and Zobel (1990), Sneller et al. (1997), Ariyo (1998) and Oliveira et al. (2003), who found 71%, 70%, 47%, 86%, and 36%, respectively. However, it was larger than the value obtained by Sneller and Dombek (1995), where the IPCA1 explained 23% of the total GE interaction SS.

Although the variation for IPCA1 was slow in the present work, it is very important because it represents the significant portion of the interaction pattern. This is confirmed by the non significance of the axes remainders that were included as residue; and hence, much of the variability accounted for by the remaining axes presents more noise than the pattern. According to Lavoranti et al. (2001), the graphic evaluation for the biplot becomes valid as the AMMI analysis has the main characteristic of capturing most of the pattern in the first axes,.

Figure 1 presents a biplot of the AMMI analysis results. It shows the line and environment means (additive mean effects) in the abscissa, and scores of the IPCA1 (multiplicative interaction), in the ordinate. When a line and an environment have the same sign on the IPCA, their interaction is positive; if different, their interaction is negative. When a line or an environment has a IPCA score close to zero, interaction effect is small (and, hence, can be fitted well by an additive model) that is considered as stable.

For the sake of result interpretation regarding adaptability and stability, in the present work the term high adaptability will be used as synonym of high grain yield, and wide adaptability as synonym of high stability, according to Freire Filho et al. (2003).

Biplot AMMI1 shows that the lines had a relatively similar performance to the interaction (homogeneous variation for the multiplicative effects in the vertical sense), except for the line 7 which had a different performance in relation to the other lines. Environmental effects were more variable, showing that the location x year interaction was very strong. The same locations in different years they were quite distant to each other in the biplot. A total of 18 lines (67% of the lines) presented means above the grand mean of the checks (2177 kg ha<sup>-1</sup>). Among these, lines USP 93-2643 (L5), USP 93-5082 (L12), USP 93-5243 (L13), USP 93-5582 (L19), and USP 93-5843 (L25) combined high grain yield and stability, since they presented low scores for the GE interaction axis. It is important to highlight the behavior of line L 13 which presented the largest grain yield and high stability. This line can be recommended for all three locations because it presented strong stability across the environments.

The environments in the surroundings of the ESALQ location presented higher interactions, mainly ES98 and ES99, shown by the broader scores for the GE interaction. Environments near the Areão location (AR96, AR97, AR98, and AR99) were more predictable (smaller and low variation scores for the GE interaction), but showed association with the lowest means. It was observed that AR96, AR97 and AR99 exhibited similar performance in terms of additive effects of environments. The environments associated with the Anhembi location showed medium stability and high adaptability (high grain yield).

The lines USP 93-5423 (14), USP 93-5692 (24) and USP 93-5860 (26) were more adapted to environment ES98. Lines USP 93-2722 (L6), USP 93-55539 (15) and USP 93-5549 (L17) presented positive adaptation with the environments ES97, AR98 and AN96, respectively (Figure 1), as shown by the means in Table 3. Line L 17 can be recommended for the Anhembi location, while line L 6 can be indicated for the ESALQ location.

Three groups of lines can be distinguished as the similarity for magnitude of the GE interaction: group 1 (most stable lines): L1, L3, L4, L5, L8, L9, L10, L11, L12, L13, L16, L18, L19, L20, L21, L22, L25 and L27; group 2 (intermediate stability): L14, L17, L23, L24, and L26; and group 3 (least stable): L2, L6, and L15. Line 7 presented larger interaction (instability) with the environments. This indicates that 67% of lines were more stable across the environments. In relation to the environments, two group types are observed: group 1 (most stable environments): AN97, AN98, AN99, AR97, AR98, AR99, ES96, and ES97; and group 2 (least stable): AR96 and AN97. The environments AN96, ES98 and ES99 grouped isolatedly, showing different performances among them and also in relation to the other environments (Figure 1).

The lines' grouping was not related to the cross origin (adapted or mixed) and adaptability. However, among the five

<sup>&</sup>lt;sup>1</sup>Fraction of sum of squares associated to each term or interaction

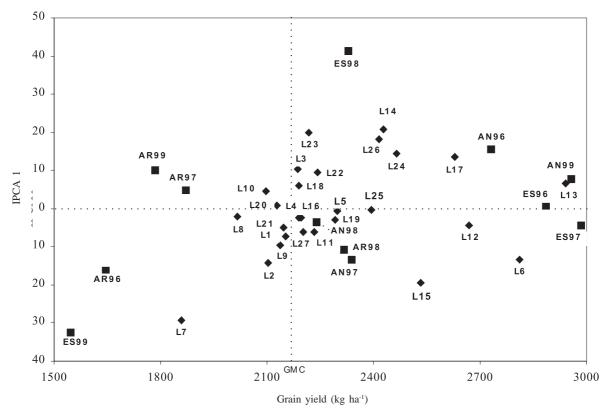


Figure 1. Biplot AMMII: grain yield x first interaction component principal axis (IPCA1) for 27 experimental soybean lines with an intermediate maturity cycle, grown in 12 environments (combinations of three locations and four years). Lines are represented by lozenges and environments by squares. The vertical line in the center represents the grand mean yield of the checks (GMC = 2177 kg ha<sup>-1</sup>)

most productive lines, four lines were originated from mixed crosses. The line 13, USP 93-5243, that presented high adaptability and stability, belongs to a mixed cross (FT 81-2.706 x PI 371.610). This result had not been expected, because the probability of obtaining superior lines is a function of genic frequency in the population, meaning that new improved lines are more easily obtained in basic populations formed through the recombination of elite cultivars.

Vello et al. (1988) comment that, in spite of the negative effect that exotic materials may have on the mean of the population derived from mixed crosses, it is possible to obtain superior genotypes even in these crosses since the exotic parents present good adaptation to the cultivated environments. Probably, these lines should have concentrated, with the advancing of the selfing generations, a larger number of genes for adaptation from adapted parent, in detriment of the exotic parents, resulting in lines with high stability.

Anhembi and ESALQ locations showed the most favorable performance for grain yield (2567 kg ha<sup>-1</sup> and 2436 kg ha<sup>-1</sup>, respectively). The Areão location was unfavorable, since it

presented the lowest mean for grain yield (Table 3). These results agree with those reported by Rocha and Vello (1999) in a study on the genotype x location interaction in the crop year of 1996/97 at the same locations.

The AMMI method allowed an easy graphic interpretation of the results regarding adaptation and stability. The analysis allowed capturing the pattern underlying to the GE interaction, removing the largest part of the noise present in the GE sum of squares, resulting in better accuracy in the estimates of the genotypic responses across environments. More precise yield estimates will increase the probability of making successful selections (Crossa 1990).

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Table 3. Grain yield (kg ha<sup>-1</sup>) means as predicted by model AMMI1 for 27 experimental soybean lines evaluated in 12 environments (three locations and four years)

1	Lines	Environments										Mean/		
Order	USP	AN96	AN97	AN98	AN99	AR96	AR97	AR98	AR99	ES96	ES97	ES98	ES99	Line
L1	93-2258	2466	2283	2110	2749	1607	1680	2238	1557	2724	2862	1877	1626	2148
L2	93-2266	2310	2333	2090	2648	1675	1599	2267	1440	2672	2847	1538	1810	2102
L3	93-2514	2775	2082	2084	2920	1364	1804	2089	1771	2777	2822	2642	1092	2185
L4	93-2530	2586	2265	2140	2831	1579	1750	2235	1652	2775	2888	2121	1518	2195
L5	93-2643	2718	2344	2237	2949	1653	1863	2319	1774	2881	2983	2300	1563	2299
L6	93-2722	3035	3028	2796	3366	2368	2314	2966	2160	3383	3552	2290	2486	2812
L7	93-2725	1836	2292	1900	2292	1669	1283	2182	1047	2416	2670	679	2051	1860
L8	93-2753	2413	2084	1962	2656	1397	1575	2054	1477	2598	2709	1955	1332	2018
L9	93-2802	2422	2300	2107	2722	1630	1659	2249	1525	2711	2860	1780	1683	2137
L10	93-2825	2597	2070	2016	2787	1367	1687	2061	1624	2683	2758	2315	1190	2096
L11	93-2870	2568	2352	2191	2842	1673	1771	2310	1653	2810	2941	2010	1673	2233
L12	93-5082	3030	2764	2620	3290	2082	2214	2728	2106	3247	3369	2516	2053	2668
L13	93-5243	3471	2889	2853	3645	2181	2540	2885	2487	3528	3594	3238	1972	2940
L14	93-5423	3180	2180	2288	3241	1438	2098	2219	2118	3027	3017	3319	992	2426
L15	93-5539	2664	2830	2538	3041	2183	2005	2750	1821	3098	3299	1765	2400	2533
L16	93-5544	2584	2258	2135	2828	1571	1747	2228	1649	2771	2882	2123	1508	2190
L17	93-5549	3268	2482	2516	3388	1757	2264	2499	2247	3223	3251	3217	1433	2629
L18	93-5552	2712	2144	2104	2891	1437	1787	2139	1732	2777	2845	2467	1236	2189
L19	93-5582	2676	2366	2237	2924	1680	1844	2334	1744	2871	2985	2201	1626	2291
L20	93-5585	2572	2153	2061	2790	1458	1700	2132	1619	2711	2806	2193	1342	2128
L21	93-5586	2498	2252	2101	2764	1572	1690	2214	1578	2725	2851	1965	1555	2147
L22	93-5597	2818	2154	2146	2971	1439	1858	2158	1819	2834	2885	2659	1183	2244
L23	93-5690	2955	1983	2081	3024	1244	1883	2019	1898	2816	2811	3068	813	2216
L24	93-5692	3114	2303	2346	3227	1576	2100	2322	2088	3056	3080	3085	1237	2461
L25	93-5843	2815	2437	2332	3045	1746	1959	2412	1870	2975	3078	2400	1653	2394
L26	93-5860	3127	2204	2285	3209	1468	2072	2234	2079	3013	3016	3197	1065	2414
L27	93-5884	2536	2323	2160	2810	1645	1739	2281	1621	2779	2911	1974	1646	2202
Mean/ei	nvironment	2731	2339	2238	2957	1647	1870	2316	1784	2884	2984	2329	1546	
Mean/lo	ocation		Anh	embi			Ar	eão			ESA	LQ		
	ambi location:		25				19	04			24	36		

AN: Anhembi location; AR: Areão location; ES: ESALQ location

# CONCLUSIONS

The lines and environments presented high variability both in additive and multiplicative effects. Environments associated with Anhembi and ESALQ locations were more favorable than those associated with the Areão location for grain yield. Anhembi and Areão locations were more predictable for the crop year although the Anhembi location associated high adaptability and predictability. USP 93-5082 and USP 93-5243 lines combined high adaptability and stability. The AMMI

method allowed an easy graphic interpretation of the results regarding adaptation and stability.

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# Estabilidade produtiva de linhagens de soja utilizando análise de efeitos principais aditivos e interação multiplicativa - AMMI

**RESUMO** - Avaliaram-se 27 linhagens de soja em três locais (Anhembi, Areão e Esalq) em Piracicaba, São Paulo, durante quatro anos, com o objetivo de verificar o efeito do ambiente (E) sobre a adaptabilidade e estabilidade das linhagens (G), usando a análise AMMI (additive main effects and multiplicative interaction). Os efeitos de G, E e da interação GE foram significativos e explicaram 51, 12 e 36% da variação, respectivamente. O primeiro e único componente principal da interação (IPCA1) explicou 26% da soma de quadrados da interação GE; este concentrou a maior porção do padrão da interação GE. Os ambientes associados com Anhembi e Esalq mostraram-se mais favoráveis, enquanto aqueles relacionados com Areão contribuíram negativamente para a produtividade de grãos. No entanto, Anhembi e Areão foram mais previsíveis com os anos agrícolas. As linhagens USP 93-5082 e USP 93-5243 reuniram alta adaptabilidade e estabilidade.

Palavras-chave: Glycine max, interação GE, previsibilidade, AMMI model.

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