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AMMI and SREG analysis for protein content in *Vigna unguiculata* (L.) Walp[1]

Análise AMMI e SREG para o conteúdo proteico em *Vigna unguiculata* (L.) Walp

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ABSTRACT - Identification of cowpea genotypes with high protein content for specific environments, based on the genotype-environment interaction, has a positive impact in places where access to protein for human consumption is deficient. The objective of the study was to analyze the protein content of 10 cowpea bean genotypes in five environments in the Caribbean Region of Colombia. The randomized complete block design with four replications at each site was used. The analysis of the genotype-environment interaction (GEI) was performed using the AMMI (additive main effects and multiplicative interaction) and SREG (regression in sites) models, in which the main effects of genotypes (G) + GEI are part of the bilinear term of the model. The AMMI and SREG models and their biplots were useful in the analysis and interpretation of the protein content of cowpea beans from experiments carried out in multiple environments. The AMMI model identified genotypes 1, 4 and 8 as those with the greatest adaptability and stability, and the Montería (MO7B), Mahates (MA7B) and Cereté (CE7B) environments as the most favorable. The SREG model identified a potential mega-environment constituted by the PN7B, MA7B and CE7B environments, in which genotypes 1, 2 and 3 presented greater adaptability and stability, while genotype 8 showed specific adaptability in MO7B. In both models, genotypes 6, 7 and 10 showed absence of adaptability and stability in the studied environments.

Key words: Nutritional quality, genotype-environment interaction, adaptability, stability.

RESUMO - A identificação de genótipos Vigna unguiculata com elevado conteúdo proteico, baseada na interação genótipo-ambiente, tem um impacto positivo em locais onde o acesso à proteína animal para consumo humano é deficiente e custoso. O objetivo do estudo foi analisar o conteúdo de proteínas no grão de 10 genótipos de V. unguiculata em cinco ambientes na Região Caribe da Colômbia. Foi utilizado um desenho de bloco completo aleatório com quatro repetições em cada sítio. A análise da interação genótipo-ambiente (GEI) foi realizada utilizando os modelos AMMI (efeitos principais aditivos e interação multiplicativa) e SREG (regressão em sítios) nos quais os principais efeitos dos genótipos (G) + GEI fazem parte do termo bi linear do modelo. Os modelos AMMI e SREG e os seus biplots foram úteis na análise e interpretação do conteúdo proteico de experimentos conduzidos em múltiplos ambientes. O modelo AMMI identificou os genótipos 1, 4 e 8 como os mais adaptáveis e estáveis, e os ambientes Montería (MO7B), Mahates (MA7B) e Cereté (CE7B) como os mais favoráveis. O modelo SREG identificou um potencial mega ambiente constituído pelos ambientes PN7B, MA7B e CE7B, em que os genótipos 1, 2 e 3 mostraram maior adaptabilidade e estabilidade, enquanto o genótipo 8 mostrou adaptabilidade específica no MO7B. Em ambos os modelos, **os** genótipos 6, 7 e 10 não mostraram adaptabilidade e estabilidade nos ambientes estudados.

Palavras-chave: Qualidade nutritiva. Interação genótipo-ambiente. Adaptabilidade. Estabilidade.

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INTRODUCTION

Cowpea bean (*Vigna unguiculata* (L.) Walp), is important in tropical and subtropical production systems (SINGH *et al*., 2015), because it is consumed for its protein and micronutrient content (GERRANO; RENSBURG; KUTU, 2019; MÁRQUEZ-QUIROZ *et al*., 2015). In 2018, 12.496.305 ha were harvested worldwide, with a yield of 578 kg ha^{-1} (FAO, 2020). In Colombia, this legume is important in the Caribbean region; its consumption reduces malnutrition among socio-economically sensitive populations, both urban and rural (DE-PAULA; JARMA-ARROYO; ARAMENDIZ-TATIS, 2018).

The percentages of protein in *V. unguiculata* range from 20,9 to 24,7 g/100g, with higher contents of arginine, proline, glutamic acid and methionine than the common bean (*Phaseolus vulgaris*) and similar in other amino acids (BAPTISTA *et al*., 2017).

The stability and adaptability of cultivars are important in the final phase of genetic improvement and their evaluation allows recommendations to be made for specific environments or sets of environments. Adaptability refers to the ability of genotypes to take advantage of environments, while stability refers to predictable behavior in the face of environmental conditions, even different ones (SANTOS *et al*., 2015); both parameters allow us to know if a cultivar has general or specific adaptability (ROCHA *et al*., 2017).

One of the genetic improvement efforts in cowpea beans is aimed at the selection of genotypes with a higher content of mineral elements and proteins in the grain (GERRANO; RENSBURG; ADEBOLA, 2017). For protein, cultivar adaptation to specific environments has been reported in Uganda (DDAMULIRA *et al*., 2015), in Brasil (SILVA; SANTOS; BOITEUX, 2016), and in South Africa (GERRANO *et al*., 2018).

Knowledge of genotype-environment interaction is relevant for crop improvement according to the variability of the environment (SANTOS *et al*., 2015). Different methods have been used to analyze genotype-environment interaction and estimate stability and adaptability for yield and nutrient content, such as linear regression analysis, non-parametric methods and others (SILVA; SANTOS; BOITEUX, 2016). However, multivariate methods have been more efficient, such as the additive main effects and multiplicative interaction (AMMI) model and the genotype main effects plus genotype-environment interaction (GEI) model, based on site regression (SREG), applied on cowpea beans (MELO *et al*., 2020; OLIVEIRA *et al*., 2017; SOUSA *et al*., 2018).

The purpose of AMMI analysis is to understand the complexity of Genotype-Environment interaction for the delimitation of environments, groups of environments, selection of better adapted genotypes, and increasing the accuracy of recommendations (GAUCHJUNIOR, 2013).

The GGE biplot, based on SREG, makes it possible to visualize: the behavior of genotypes in a specific environment, relative adaptability of a genotype in a variety of environments, identification of the best genotype in each environment, stability of genotypes, and discrimination of environments, constituting an effective tool in plant improvement (MOUSAVI; HEJAZI; KHALKHALI, 2016).

Both models make it possible to estimate stability, evaluate localities and classify environments by means of a two-dimensional plot (biplot) of genotypes and environments (GAUCH; PIEPHO; ANNICCHIARICO, 2008).

The objective of this study was to analyze the genotype - environment interaction for the protein content of 10 genotypes of *V. unguiculata* in five environments of the Caribbean region of Colombia with the AMMI and SREG models.

MATERIALS AND METHODS

Nine cowpea bean lines selected for their protein content in the grain were evaluated: 1. LC-029-16; 2. LC-002-016; 3. LC-036-016; 4. LC-009-016; 5. LC-021-016; 6. L-019; 7. LC-006-016; 8. LC-005-016; 9. L-014-016, plus the commercial control Caupicor 50. These lines were obtained from the genetic breeding program of the University of Córdoba. The nine lines identified with LC were obtained by genealogical method in the segregating population of the crossing between the genotypes IT86 and LCPM.35 and selected for desirable agronomic characteristics, such as precocity to flowering, number of pods per plant between 22 and 29, grain yield between 944 to 1189 kg ha^{-1}, medium grain of cream color and semiprostrate growth habit. The L-019 line was obtained by individual selection of the homozygous heterogeneous population, Criollo-Córdoba; it is early flowering, forms 29 pods per plant and has a grain yield of 1536 kg ha^{-1}, medium cream-colored grains and a semi-prostrate growth habit, while Caupicor 50 is similar to L-019, but with grain yield of 1362 kg ha^{-1}.

The experiments were conducted in five environments in the humid Caribbean region of Colombia in the second half of 2017, identified as 1. Cereté-Córdoba (CE7B), 2. Mahates-Bolivar (MA7B), 3. Montería-Córdoba (MO7B), 4. Polonuevo-Atlántico (PN7B), 5. Sampués-Sucre (SA7B). The region is located between 07º41’16’’ and 10º52’14’’ North latitude and 72º53’27’’ and 74º08’28’’ West longitude. It has a tropical climate, a typical savanna subtype

Aw, according to the Köppen classification, with a temperature variation between 24 and 28 °C and annual rainfall between 800 and 1800 mm. Soils differ from area to area with fertility ranging from high to very low, depending on rainfall and fluvial influence.

The experimental design used in each location was randomized complete blocks with four repetitions. The plots or experimental units consisted of six rows of five meters long and 0.80 m between rows. The separation between plants in each row was 0.40 m for a plantation density of 31,250 plants ha^{-1}.

The nitrogen content was determined by digestion, distillation and titration according to the Kjeldahl method for grains AOAC 979.09 (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 2005). A Buchi K-355 equipment (Flawil - Switzerland) was used and the protein content was quantified by multiplying the nitrogen content by the factor 6.25 (BENTON-JONES, 1989).

Individual and combined analyses of variance were performed for all environments, with parameter estimates, according to the sources of variation based on the linear models commonly used for such purposes. From the individual and combined analyses, only the protein averages of the genotypes in each locality and in each of the five environments were extracted.

The analysis of genotype-environment interaction (GEI) was performed using the AMMI model based on estimation of the additive main effects and multiplicative interaction explained by principal component analysis (EBDON; GAUCH JUNIOR, 2002; ZOBEL; WRIGHT; GAUCH, 1988) and the site regression model (SREG). In addition to estimating the main additive effects, the main effects of genotypes (G) plus the GEI (G + GE) are part of the bilinear term of the model and is abbreviated as GGE in the biplot (CROSSA; CORNELIUS; YAN, 2002). Both models are useful in interpreting the responses of cultivars in experiments carried out in multiple environments (SAMONTE *et al*., 2005).

The AMMI model is expressed by the following equation (GAUCH JUNIOR, 2013):

$$Y_{ger} = \mu + \alpha_g + \beta_e + \sum_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \kappa_{r(e)} + \varepsilon_{ger} \quad (1)$$

where, Y_{ger} is the protein content of genotype g in environment e and in repeat r; μ is the general mean; α_g are the mean deviations of the genotypes (mean minus the general mean); β_e are the mean deviations of the environments; λ_n is the singular value for the interaction principal component $(IPC)_n$ and, consequently, λ^2_n is its eigen value; γ_{gn} is the value of the eigenvector for genotype g and component n, δ_{en} is the eigenvector for environment e and component n, with both eigenvectors scaled as unit vectors, ρ_{ge} is the residual; $\kappa_{r(e)}$ is the block effect for repetition r within environment e; and ε_{ger} is the error. Commonly the interaction scores are obtained as $\lambda^{0.5}_n \gamma_{gn}$ and $\lambda^{0.5}_n \delta_{en}$ so that their products directly estimate the interactions. The AMMI model first applies an analysis of variance to divide the variation in the genotype (G), environment (E) and genotype-environment interaction (GEI) factors, and then applies a principal component analysis (PCA) for the GEI (GAUCH JUNIOR, 2013).

The SREG model is expressed by the following equation (CROSSA; CORNELIUS; YAN, 2002):

$$\bar{\gamma}_{ij} = \mu + \beta_j + \sum_{n=1}^{t} \lambda_n \alpha_{in} \gamma_{jn} + \bar{\varepsilon}_{ij} \quad (2)$$

$\bar{\gamma}_{ij}$ is the mean of the i-th genotype in the j-th environment for g genotypes and s environments (i = 1,2,…, g; j = 1,2,…, s); μ is the general mean; βj is the effect of the site (environment); λ_n ($\lambda_1 \geq \lambda_2 \geq \ldots \geq \lambda_g$) are constants (singular values) that allow the imposition of orthonormality restrictions on genotype vectors, α_{in}= ($\alpha_{1n,}$ α_{2n}, …, α_{gn}) and the environments, γ_{jn} = (γ_{1n}, γ_{2n}, …, γ_{en}), such that $\sum_i \alpha^2_{in} = \sum_j \gamma^2_{jn} = 1$ and that $\sum_i \alpha^2_{in} \cdot = \sum_j \gamma^2_{jn} \cdot = 0$ for $\eta \neq \eta'$, α_{in} y γ_{jn}, for n = 1, 2, 3, …, they are called primary, secondary, tertiary effects, ..., of the i-th cultivar and the j-th environment, respectively; $\bar{\varepsilon}_{ij}$ is the error of the model assumed to be normal and independently distributed (0, σ^2/r); σ^2 is the combined variance and r the number of repetitions. The number of bilinear terms is $t \leq min$ (g, e). In this model, bilinear terms absorb the main effects of genotypes plus the genotype-environment interaction (TOLESSA; GELA, 2014).

For AMMI and SREG analyses, the GEA-R software (Genotype x Environment Analysis With R for Windows) Version 4.1. was used in the International Corn and Wheat Improvement Center CIMMYT (PACHECO *et al*., 2017).

RESULTS AND DISCUSSION

In the AMMI model, the mean squares of the main additive effects of environments (E), genotypes (G) and GEI for protein content were highly significant ($p < 0.01$) (Table 1). The protein percentages of the genotypes were higher than those reported by Baptista *et al*. (2017). The significance of the GEI indicates that the genotypes expressed differential response depending on the environment where they were cultivated, which agrees with Ddamulira *et al*. (2015), and obeys the characteristics of the soil and climatic conditions. The variation of protein content by A and G is explained in 54.99 and 23.69%, respectively, but the GEI contributed 21.31% of the total variation of these three sources of variation. These results showed a similar trend with research conducted in cowpea beans by Santos *et al*. (2015).

The GEI analysis, with the multiplicative component of the AMMI model, shows that the first principal component explains 64.56% of the interaction and is highly significant; the other principal components were not significant. It is evident that these other components make up the residual of the interaction with SS of 40.74 and 24 degrees of freedom, which represents 7.55% of the AMMI model and 35,44% of the interaction. Consequently, the AMMI1 model, based on the first principal component, represents 92.45% of the SS of the model and significantly explains the GEI.

In the SREG model, the first principal component (PC1) that includes the primary effects of genotypes and environments, explains 75.94% of the variation of G + GEI (bilinear term of the model) and is highly significant ($p < 0.01$), while the second principal component (PC2), which includes secondary effects, explains 12.96% with significance ($p < 0.07$) and both components add up to 88,90% of the SS of the model (Table 1). In addition, the SS of G is 52.6% of the SS of G + GEI, which indicates a high correlation, greater than 0.95 and, therefore, meets the criteria established by Gauch Junior (2006) to separate in the GGE biplot, the adaptability and stability of the genotypes.

The biplot of the AMMI1 model (Figure 1) shows the means of the estimated protein content for genotypes and environments on the abscissa axis, while on the ordinate axis the scores of the first principal component (PC1) are indicated with their corresponding percentage equivalence (64.56%) of the SS of the interaction and 92.45% of the variation of the three sources of the additive effect of the AMMI model. In addition, the SS of PC1 is 58.07% of the SS of G, which emphasizes the importance of taking the GEI into consideration when estimating important traits in different environments or sites (SAMONTE *et al*., 2005).

The environments, where above-average protein content was estimated, were Montería (MO7B), Mahates (MA7B) and Cereté (CE7B), while in Sampués (SA7B) and Polonuevo (PN7B) the lowest and below-average contents were estimated, which can be corroborated in Table 2. However, the environments with the greatest contribution to the interaction were Montería (MO7B), Mahates (MA7B) and Sampués (SA7B), evidenced by the greater distances of each vector in relation to the point of origin (Figure 1). This result could be due to differences in nitrogen absorption in soils, favored by higher soil fertility and moisture in environments such as MO7B and MA7B, and disadvantaged as in SA7B, on the basis that the intake of nitrate and ammonium anions increased with an adequate regime of soil moisture, as reported by Alidu, Asante and Mensah (2020), because it facilitates the uptake of nitrate anion and ammonium cation by the roots.

Table 1- Analysis of variance and Gollob test of the AMMI and SREG models for the protein content of 10 genotypes (G) of cowpea beans evaluated in five environments (E) of the Colombian Caribbean

MODEL	SV	DF	SS	PSS	PSCS	MS	F	P > F
ADDITIVE	E	4	296.55	54.99	54.99	74.14	42.27	0.0000
	G	9	127.77	23.69	78.69	14.20	8.09	0.0000
	E*G	36	114.93	21.31	100.00	3.19	1.82	0.0069
AMMI	PC1	12	74.19	64.56	64.56	6.18	3.57	0.0001
	PC2	10	27.46	23.90	88.45	2.75	1.59	0.1158
	PC3	8	8.72	7.59	96.04	1.09	0.63	0.7516
	PC4	6	4.55	3.96	100.00	0.76	0.44	0.8525
	PC5	4	0.00	0.00	100.00	0.00	0.00	1.0000
SREG	PC1	12	184.31	75.94	75.94	15.36	8.76	0.0000
	PC2	10	31.46	12.96	88.90	3.15	1.79	0.0622
	PC3	8	20.03	8.25	97.15	2.50	1.43	0.1819
	PC4	6	5.83	2.4	99.55	0.97	0.55	0.7606
	PC5	4	1.07	0.45	100.00	0.27	0.15	0.9609
	Error	150	263.11	0.00	0.00	1.75	NA	NA

SV: Source of variation; DF: degrees of freedom; SS: Sums of squares; PSS: Percentage of the sum of squares; PSCS: Percentage of the sum of cumulative squares; MS: mean squares; ADDITIVE: conventional model; AMMI: AMMI Model; SREG: SREG Model; PC1, PC2, ..., PC5: Principal component 1, 2, ..., 5, respectively. NA: it does not apply

Genotypes 1, 2, 3, 4, 5, 8 and 9 presented above average protein contents, while 6, 7 and 10 were below, which can be attributed to differential capacity in the deepening of the roots to increase the extraction of water and nutrients from the soil (POLANIA *et al*., 2009) and the conditions of fertility and humidity during the crop cycle. Likewise, genotypes 1, 4 and 8 stood out as those with the greatest adaptability and stability to environments, as they are closer to the point of origin, while 5 and 2 showed specific adaptability to favorable environments such as MA7B, while, 6,7 and 10, despite being closer to SA7B, did not present specific adaptation, which is consistent with the interpretation of Ochoa-Cadavid, Preciado-Ortíz and Bayuelo-Jiménez (2019). In addition, genotypes 5 and 2 presented average protein values higher than 27.0% and close to those of 1, 4, 8, 9 and 3, with similar magnitudes to those reported by Baptista *et al.* (2017) in this species, so they represent a good alternative due to the greater consistency and predictability of their behavior.

When a genotype and an environment have the same sign in the scores, their interaction is positive, but if the signs are different, their interaction is negative. The magnitude of the product of the scores determines how strong or weak the interaction is. In Figure 1 and Table 2, the effects of GEI on genotypes 5 and 2 are positive and high, while for 6, 7 and 10 the scores are negative and high, and, overall, they were the least stable. In contrast, for genotypes 1, 4 and 8 the interaction is small, positive for 1 and 4, and negative for 8, which suggests adaptability and stability in protein content in both cases.

An estimate of the GEI effect for any genotype-environment combination is the product of its PC1 scores, corresponding to genotype and environment, respectively. For any combination of GEI, the main effect is equal to the genotype mean plus the environment mean minus the general mean (ZOBEL; WRIGHT; GAUCH, 1988). For example, Caupicor 50 in CE7B had a main effect for protein content of 26.11 + 27.22 – 26.97 = 26.36%, and the interaction effect was -0.9050 x 0.3991 = - 0.36%. Therefore, the AMMI model gives an estimate of 26.36 – 0.36 = 26.00%, a value only 0.50% above that observed (25.50%), but contrasting with the additive ANOVA estimate (26.36%) (Table 2).

Figure 1 - GEI biplot of the AMMI1 model for the protein content of 10 cowpea bean genotypes evaluated in five environments of the Colombian Caribbean

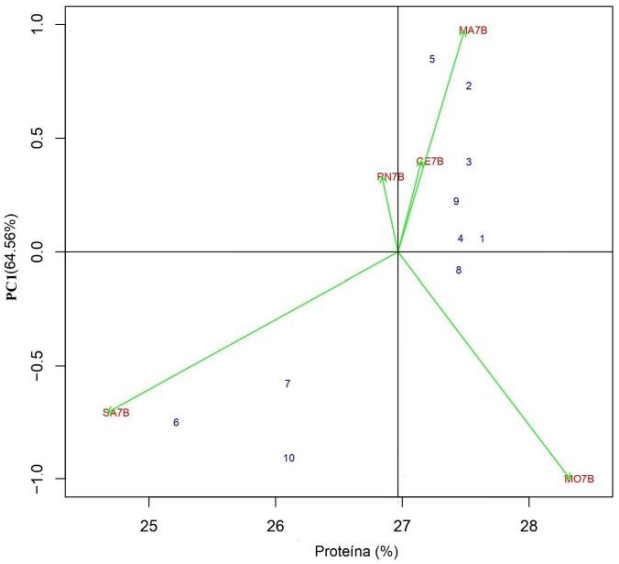


Table 2 - Protein content (%) for 10 genotypes (GEN) of cowpea beans in five environments, means, scores of the first principal component (PC1) of the interaction analysis of the AMMI model and of the first two principal components (PC1 and PC2) of the SREG model

GEN	ENVIRONMENT						AMMI	SREG	
	CE7B ns	MA7B*	MO7B*	PN7B*	SA7B ns	MEDIA	PC1	PC1	PC2
1. LC-029-016	27.65	27.55	28.85	28.68	25.18	27.63	0.0582	-0.3017	-0.0648
2. LC-002-016	27.93	29.35	27.65	27.75	24.95	27.53	0.7307	-0.4649	-0.2196
3. LC-036-016	28.50	28.60	28.65	27.35	24.53	27.53	0.3951	-0.3646	0.0259
4. LC-009-016	27.80	27.55	28.43	28.23	25.30	27.46	0.0594	-0.2189	-0.1853
5. LC.021-016	28.00	29.00	27.13	27.55	24.50	27.24	0.8476	-0.3754	-0.4049
6. L-019	25.68	24.30	27.23	24.68	24.20	25.22	-0.7491	1.0000	-0.3085
7. LC-006-016	25.93	25.63	28.15	26.18	24.60	26.10	-0.5780	0.5566	-0.0756
8. LC-005-016	27.00	29.00	30.05	26.60	24.58	27.45	-0.0807	-0.2103	0.7157
9. LC-014-016	28.23	28.50	28.83	26.85	24.73	27.43	0.2220	-0.2671	0.1511
10. Caupicor 50	25.50	25.88	29.03	25.25	24.88	26.11	-0.9050	0.6464	0.3661

Continuation Table 2

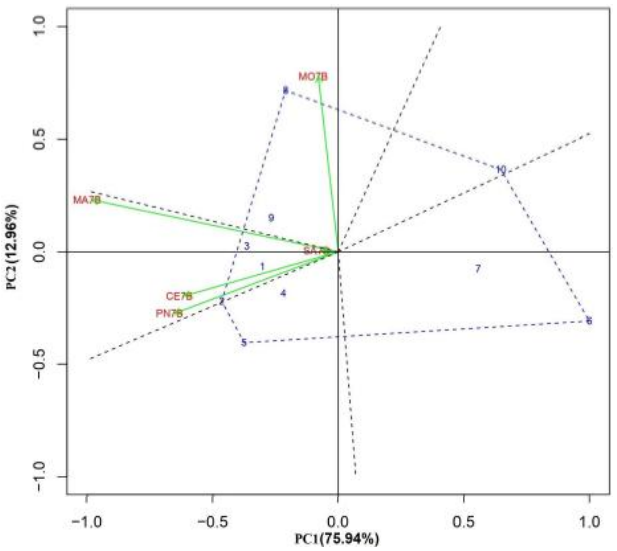
MEAN	27.22	27.56	28.40	26.91	24.74	26.97[+]
PC1 (AMMI)	0.3991	0.9749	-1.0000	0.3304	-0.7044	
PC1 (SREG)	-0.6336	-1.0000	-0.0994	-0.6726	-0.0865	
PC2 (SREG)	-0.1965	0.2310	0.7784	-0.2742	0.0061	

* and ns: significant and non-significant, according to Tukey's 5% test, vertical comparison in each environment; [+]: general mean

The SREG analysis for protein percentage is a methodology that allows the identification of mega-environments associated with genotypes having greater adaptability to each of them. In the SREG biplot (Figure 2) also known as GGE biplot, PC1 represents the proportion of protein content that is due only to genotypes, while PC2 represents the proportion due to genotype-environment interaction. Two groups of genotypes with opposite primary (α_{i1}) effects were observed; the first was formed by genotypes 1, 2, 3, 4, 5, 8, 9, with effects directed towards the environments and, the second group was formed by genotypes 6, 7 and 10, with primary effects without direction to any environment, a result similar to that estimated with the AMMI model.

Cultivars 2, 5, 6, 8 and 10 located at the vertices of the irregular polygon contributed more to the interaction in the environments of their respective sectors and are considered 'marker genotypes' of each sector. A sector is understood as the region delimited by two dotted rays with vertex at the origin of the coordinate system, whose angle is less than 90° or greater than 270° (CROSSA; CORNELIUS; YAN, 2002). However, marker genotypes 6 and 10 do not have any environment in their corresponding sectors, which indicates that they were not the best in any of them. The most stable genotypes were 1 and 3 due to their high primary effects (in absolute value) and their secondary effects (α_{i2}) close to zero as can be seen in the biplot of the SREG model (Figure 2 and Table 2). However, 1, 2 and 3 showed greater adaptability in the PN7B, MA7B and CE7B environments, and could be cultivated or used as parents in a breeding program, while genotype 8 showed specific adaptability in MO7B, much more evident than that visualized in the AMMI biplot. The CE7B and MA7B environments were the most favorable for all genotypes, while SA7B, whose primary (α_{i1}) and secondary (γ_{i2}) effects are close to zero, did not discriminate any genotype, since they all presented similar protein content. The MO7B environment did not properly discriminate genotypes because it had relatively small primary effect and relatively large secondary effect values; predicted a high interaction in the genotype response when compared to other environments. A potential mega-environment consists of CE7B, PN7B and MA7B for the protein content of the genotypes studied.

Figure 2 - GGE Biplot of the SREG model for the protein content of 10 cowpea bean genotypes evaluated in five environments of the Colombian Caribbean



CONCLUSIONS

1. The AMMI and SREG models and their biplots were useful in the analysis and interpretation of the protein content of cowpea beans from experiments performed in multiple environments;
2. The AMMI model identified genotypes 1, 4 and 8, with above average protein contents, as those with the greatest adaptability and stability, while 6, 7 and 10 did not show specific adaptation; the most favorable environments were MO7B, MA7B and CE7B;
3. The SREG model identified a mega-environment consisting of PN7B, MA7B and CE7B in which genotypes 1, 2 and 3 stood out as those with the greatest adaptability and stability, while genotype 8 showed specific adaptability in MO7B;
4. In both models, genotypes 6, 7 and 10 showed no adaptability and stability in the environments studied.

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