Genotype-environment-attribute interaction in an advanced cashew population through joint biplot graphical analysis

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ABSTRACT: Despite the progress achieved, the cashew cultivar (*Anacardium occidentale* L.) breeding program at Embrapa Agroindústria Tropical (CNPAT) has not yet peaked in terms of primary and secondary yield components, and the quality properties of the peduncles. Significant improvement is expected with the breeding program in obtaining new elite clones (cultivars). This this study investigated genotype x environment x attribute interaction in cashew trees using principal component analysis with multiple data matrices. The experimental design was completely randomized, in a factorial scheme with repeated measures over time, applying the confounding technique, with 3 treatments and 4 replications, totaling 48 plots. The total number of plants was represented by 30 plants of each clone in each environment. Around 30 ripe cashew peduncles of each genotype were harvested early in the morning and sent to the post-harvest laboratory of Embrapa Agroindústria Tropical (CNPAT). The factors considered for joint biplot graphical analysis were three genotypes (G1, G2, G3), four environments (combination of two locations, Alto Santo and Beberibe, municipalities in the state of Ceará, two agricultural years (2013 and 2014), and eight physicochemical peduncle properties. The study was implemented using principal component analysis (PCA), with a three-way data array (genotypes x environments x variables). The genotypes did not exhibit phenotypic stability for the variables assessed and environments studied because these cultivars are located far from the center of the graph, that is, away from the origin. The three genotypes showed positive and negative phenotypic adaptations for different variables and environments, qualifying them for consumption of pseudofruits as table fruits, industrially processed products, or even displaying dual purpose consumption suitability.
Key words: *Anacardium occidentale* L., elite clones, phenotypic adaptability, phenotypic stability.

Interação genótipo-ambiente-atributos em uma população avançada de cajueiro por meio da análise gráfica *joint biplot*

RESUMO: O programa de desenvolvimento de cultivares de cajueiro (*Anacardium occidentale* L.) da Embrapa Agroindústria Tropical (CNPAT) a despeito dos avanços obtidos ainda não se obteve atingir o seu patamar máximo para seus componentes produtivos primários e secundários, como também para os caracteres de qualidade dos pedúnculos, sendo esperado uma evolução significativa com o programa de melhoramento genético na obtenção de novos clones elite (cultivares). O objetivo deste trabalho foi estudar a interação genótipo 'x ambiente x atributo em cajueiro empregando-se a análise de componentes principais, com múltiplas matrizes de dados. O delineamento adotado foi o inteiramente casualizados, no esquema fatorial com medida repetida no tempo, aplicando-se a técnica de confundimento, com três tratamentos e quatro repetições, totalizando 48 parcelas, o número total de plantas foi representado por 30 plantas de cada clone em cada ambiente. Cerca de 30 cajus maduros, de cada genótipo, foram colhidos nas primeiras horas do dia e encaminhados para o laboratório de pós-colheita da Embrapa Agroindústria Tropical (CNPAT). Os fatores considerados para a análise gráfica *joint biplot* (*plot*) foram: três genótipos (G1, G2, G3), quatro ambientes (combinação de dois locais, Alto Santo e Beberibe, municípios do estado do Ceará, e dois anos agrícolas, 2013 e 2014) e oito atributos físico-químicos dos pedúnculos. O estudo foi implementado por meio de análise de componentes principais (CP), com arranjo de três entradas (genótipos x ambientes x variáveis). Os genótipos não apresentaram estabilidade fenotípica em relação às variáveis avaliadas e os ambientes estudados em função dessas cultivares se posicionarem distantes do centro do gráfico, isto é, afastados da origem. Os três genótipos mostraram adaptações fenotípicas positivas e negativas com as diferentes variáveis e ambientes credenciando-os para o consumo dos pseudofrutos como frutos de mesa, ou a partir, de produtos processados industrialmente, ou ainda, apresentando dupla aptidão de consumo.
Palavras-chave: *Anacardium occidentale* L., clones elite, adaptabilidade fenotípica, estabilidade fenotípica.

INTRODUCTION

Breeding programs typically involve at least three stages: beginning, where the aim is to form a base population to improve good performance, with wide variability, low genetic load, and good adaptation; middle, where the focus is on managing segregating families in order to maximize direct, indirect, or simultaneous gains in important traits; and end, where improved genetic material is available

and recommended for large or specific regions, making studies of genotype versus environment interaction(GEI), adaptability, and stability essential (CRUZ et al., 2007).

The phenomenon of cross-interaction between genotypes and environments (GEI) can be investigated by dividing it into adaptability (responsiveness to environmental stimuli) and stability parameters (behavior predictability). Additionally, yield and/or fruit quality can be jointly analyzed for more accurate interpretations and inferences; consequently, obtaining better results. In regard to the contribution of different environments to GEI, their variation is attributed to a predictable (fixed) and unpredictable (random) portion. The fixed part is the variation that can be measured in magnitude and direction, being systematic in nature, which facilitates reasonable control, while stochastic variation is uncontrolled and indeterminate, with uncertain direction, contributing significantly to experimental error (MAIA et al., 2014).

According to DUARTE & ZIMMERMAN (1995), in order to take advantage of these positive GEI effects, appropriate statistical methods are needed to estimate and explore the interaction, thereby allowing regionalized recommendations. More accurate and robust genetic-statistical procedures, such as principal component analysis (PCA) with multiple data matrices (three-way), can ideally be used to elucidate the cross-interaction and the productive and qualitative behavior of species with high breeding levels, such as cashew trees in the final stages of breeding programs (MAIA et al., 2013).

In breeding programs, when there are several matrices and the aim is to describe the most important aspects of observations through graphs, PCA with multiple data matrices (three-way PCA) can be used to construct joint biplots. The concept of correlation is also incorporated, and the most important aspects of the information can be summarized with few components. The joint biplot graph facilitates the understanding of triple interaction and provides researchers with more accurate information than AMMI (Additive Main effects and Multiplicative Interaction) modeling with a two-way data structure (ARAÚJO et al., 2010). Generalized PCA is carried out by decomposing the data array into three-component matrices (generalization of matrices with eigenvectors) and a core matrix (generalization of the matrix with eigen values). Models used to analyze three-way data structures include those proposed by TUCKER (1966). The number of components to be retained in these matrices can be determined by the TIMMERMAN & KIERS process (2000).

In practical situations where data are organized in several matrices, such as the assessment of different attributes in multi-environments, and the aim is to describe the most important aspects of the data using graphs, classical PCA cannot be applied because the concept of decomposition into singular values and vectors is applicable only in the case of a single matrix. In this respect, the three-way PCA approach proposed by KROONENBERG & DE LEEUW (1980) is more appropriate. This is a generalization of classical PCA and biplots (MAIA et al., 2014).

This study investigated genotype × environment × variables interaction in an advanced cashew population using graphical principal component analysis with multiple data matrices.

MATERIALS AND METHODS

The genetic material involved an advanced population of genotypes: elite clones (CCP 09, BRS 265, and PRO 555-1) obtained from the cashew breeding program at CNPAT due to their higher yield. The aim was to study phenotypic behavior in multiple environments while simultaneously considering the physicochemical properties of the peduncles.

The experiments were conducted in two municipalities of Ceará State, Brazil: Alto Santo and Beberibe, under significantly different climatic conditions. The climate of Beberibe (Ceará coast) is classified as mild semi-arid warm tropical, and Alto Santo (inland of Ceará) as warm semi-arid tropical (Fundação Cearense de Meteorologia e Recursos Hídricos – FUNCEME) and Instituto de Pesquisa e Estratégia Econômica do Ceará (IPECE).

The soils of the experimental area exhibit significantly different properties, with Beberibe predominantly characterized as Clay-Sandy Orthic Quartz Arenite Neossol (BRASIL, 1973), and Alto Santo as Eutrophic Red Argisol (BRASIL, 1971). The trials were conducted over two agricultural years (2013, 2014), using the management system recommended for cashew crops (SERRANO, 2016).

The experimental design was completely randomized, in a factorial scheme with repeated measures over time, applying the confounding technique, with 3 treatments and 4 replications, totaling 48 plots. The plants consisted of 30 individuals from each clone in each environment. Around 30 mature cashew peduncles (pseudofruits) of each genotype were collected in the early morning and sent to the post-harvest laboratory of Embrapa Agroindústria Tropical (CNPAT).

The following variables were assessed: pH, soluble solids - SS (°Brix), titratable acidity - TA (%), SS/TA (dimensionless), vitamin C - Vit C (mg/100g), total soluble sugars - TSS (%), total extractable polyphenols - TEP (mg/100g), and trolox equivalent antioxidant capacity - ABTS (µmoltrolox/g).

The data analysis was conducted through the generalization of the principal components model for two-input data. To a arrangement of the three-inputs X, every factor can be written as: $x_{ijk} = \sum_{p=1}^{P} \sum_{q=1}^{Q} \sum_{r=1}^{R} a_{ip} b_{jq} c_{kr} g_{pqr} + e_{ijk}$, being that e_{ijk} is a factor of the residual arrangement $\underline{E}$ $(I \times J \times K)$; a_{ip}, b_{jq} and c_{kr} are factors of the components matrices $A(I \times P)$, $B(J \times Q)$ e $C(K \times R)$; e g_{pqr} is a factor of the matrix core $\underline{G}$ $(P \times Q \times R)$. This is the model Tucker3 of $\underline{X}$ (P, Q, R), wherein P, Q, R indicate the number of components in each input.

The graphical representation of Tucker3 models is presented by KROONENBERG (1983), which is denominated a joint biplot. This representation is similar to a standard biplot (GABRIEL, 1971), and the principles of interpreting joint biplots can be reported in VARELA et al. (2006) and ARAÚJO et al. (2010). The difference between these graphs lies in the construction, where the joint biplot is built as a biplot for two factors given the component matrix of the Tucker3 model related to the third factor (third input). In other words, each joint biplot is constructed using different slices of the core matrix, that is, to construct a joint biplot after fitting a Tucker3 model, it is necessary to obtain a matrix Δr = AGrBt = ArBrt of size I×J, with r = 1, 2, ..., R, and through Singular Value Decomposition (SVD), Δr is represented by a biplot, obtaining the joint biplot. For each matrix C component, there is a Gr slice associated with the core, and thus, for each matrix C component, it is necessary to construct a joint biplot for the component matrix.

The interpretation of the joint biplot (VARELA et al., 2006; ARAÚJO et al., 2010) depends on the sign and magnitude of the component weights used to design the graph. Thus, assuming that matrix C has a high positive value associated with the k-[th] level of the third input, then proximities between the first and second input levels indicate that the interaction (or association) between genotypes and variables at the k-[th] level of the third input (environments) is positive. Conversely, if the i-[th] level of the first factor is far from the j-[th] level of the second factor, this indicates that the interaction associated with these three factors is negative. If matrix C has a high negative value associated with the k-[th] level of the third factor, the interactions will be opposite

to when the value is positive. In general, levels of an input located at the center of the joint plot are considered a set that exhibits average performance in all the other modes.

All analyses were implemented using the R SOFTWARE (2022) version 4.2.2.

RESULTS AND DISCUSSION

The Tucker3 model that presented the best solution, according to the method of TIMMERMAN & KIERS (2000), was the (2 x 3 x 2), which explained 90.58% of total data variability (Table 1). The two components (p1 and p2) of matrix A explain, to a greater extent, 72.44 and 18.14% of variance, respectively. The three components (q1, q2, and q3) of matrix B explain 62.28, 21.86, and 6.44% of variance, respectively, while in matrix C, the two components r1 and r2 explain 68.58 and 22.00%, respectively, explaining alarge extent, the standard portion of the GxA interaction. Indeed, for this population, the model was precise and accurate, given that a high portion of the variance was extracted from the total sum of squares. This result revealed that there is excellent predictive capacity for the distinct behavior of genotypes with a relatively simple model, that is, consisting of few multiplicative terms.

It is important to note that matrix C (Table 1) is useful for interpreting the joint biplot results when the data are organized into three factors or inputs (genotypes, environments, and variables), projecting these elements or the first two inputs into the third input. In the present study, the third input considers the environments. The factors of Model II are two principal components for factor A (genotypes), three factors or three principal components for the variables, and two principal components for the environments. Thus, the standard statistical effect of the data can be simplified and theoretically explained with only a few principal components, which is desirable in multivariate analysis.

It is undeniable that the graphical representation of data in a multivariate scatter diagram, such as a joint biplot, has the important property of summarizing the data, allowing for better visualization of the data distribution and outlier trend, consequently facilitating interpretation (MAIA et al., 2013).

Based on the above, the principal components of genotypes and variables that will be projected into the principal components of environments are considered. Since there are two principal components (R1 and R2) for matrix C, two graphs are consequently generated. For example, if

Table 1 - Fit of the Tucker3 model that presented the best solution for genotypes (Matrix A), variables (Matrix B), environments (Matrix C), and core matrix (G) for cashew genotypes.

Matriz A (Genotypes)	$p1^{\#}$	$p2^{\#}$		Matriz B (Variables)	$q1^{*}$	$q2^{*}$	$q3^{*}$
CCP 09	-0.82	-0.04		pH	-0.39	-0.23	-0.02
BRS 265	0.45	-0.68		SS	0.40	-0.20	-0.23
PRO 555-1	0.37	0.73		SS/AT	-0.11	-0.68	-0.35
				AST	0.27	-0.51	-0.07
Matriz C (Environments)	$r1^{\ddagger}$	$r2^{\ddagger}$		ACIDITY	0.39	0.28	-0.18
Altos Santo 2013	-0.71	0.47		VIT.C	-0.22	0.31	-0.88
Beberibe 2013	-0.42	-0.20		PET	0.44	0.01	-0.11
Altos Santo 2014	-0.41	-0.83		ABTS	0.45	0.02	-0.02
Beberibe 2014	-0.39	0.24		-	-	-	-
	----------$r1^{\ddagger}$----------			----------$r2^{\ddagger}$----------			
G	$q1^{*}$	$q2^{*}$	$q3^{*}$	$q1^{*}$	$q2^{*}$	$q3^{*}$
$p1^{\#}$	2.18	-0.19	-0.05	-0.27	-0.86	0.42
$p2^{\#}$	0.38	0.46	0.57	0.00	0.87	0.10

[#]: p1 and p2 are the components of the genotypes factor; [*]: q1, q2 and q3 are only components of the variables factor; [‡]: r1 and r2 are components of the environments factor.
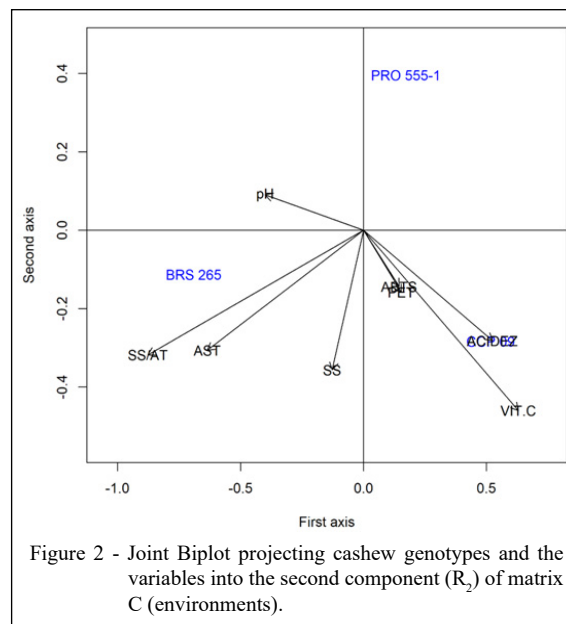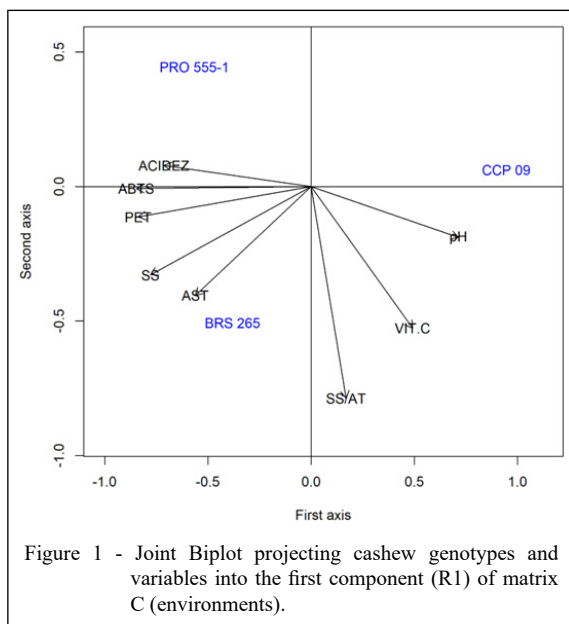
we considered the first principal component of an environment (column R1 of matrix C), and if these values were all positive, the interpretation of the joint biplot would be similar to the classic biplot. Since the sign is negative, the circumstances are reversed. For example, in the classic biplot, if two variables are in the same direction and close to each other, it means they have a positive relationship, while opposite directions indicate a negative correlation. Given that the coefficient of the first principal component of matrix C is negative, this interpretation is reversed (ARAÚJO et al., 2010). In other words, when two points are in the same direction, they are negatively correlated. When they are in opposite directions, they have a positive relationship.

Another question to consider is the magnitude of the coefficients of each principal component in each column of matrix C. By comparing PC1 and PC2, it is observed that for the Alto Santo/2013, the largest coefficient is in PC1 (absolute value) (Table 1). Thus, if considering the first environment (Alto Santo/2013) in module or absolute value, being more associated with component R1, Alto Santo/2013 should be investigated in the first graph. For Beberibe/2013, the absolute value (0.42020) related to R1 (the first component) should be considered, and also observed in the first graph. Alto Santo/2014, with an absolute value of (0.83), is associated with the second component. As such, Alto Santo/2014 should be considered in the second graph

that refers to the projection of this second component. For Beberibe/2014, since the absolute value of 0.39 > 0.24 is related to the first principal component, interpretation should be made in the first graph.

The first component of matrix C is characterized by the Alto Santo environments in 2013 (-0.71), Beberibein2013 (-0.42), and Beberibe in 2014 (-0.39), and the second component by the Alto Santo environment in 2014 (-0.83) (Table 1). Thus, when constructing a joint biplot that projects genotypes and variables into the first component of matrix C, conclusions will be directed towards Alto Santo in 2013 and Beberibe in 2013 and 2014 (Figure 1), but when projecting genotypes and variables into the second component of matrix C, conclusions will be valid for the Alto Santo environment in 2014 (Figure 2).

It is important to mention that the behavior of the joint biplot projected into the first component of C (Figure 1) exhibits all rl loadings as negative. Thus, for Alto Santo in 2013, Beberibe in 2013, and Beberibe in 2014, CCP 09 showed a positive relationship (positive adaptation) with acidity, ABTS, TEP, SS, and AST. For products derived from the industrial processing of cashew pseudofruits, there is a reduction in the requirement to add artificial preservatives (>acidity), they are rich in antioxidants and have direct benefits for human health, the presence of phenolic compounds providing a binary relationship, that is, it provides an unpleasant taste for the consumption of fresh pseudofruits. Conversely,

Figure 1 - Joint Biplot projecting cashew genotypes and variables into the first component (R1) of matrix C (environments).

Figure 2 - Joint Biplot projecting cashew genotypes and the variables into the second component (R_2) of matrix C (environments).

it translates into relative and effective tolerance to plant pathogens (>TEP), savings in the addition of sugars in the industrial processing of pseudofruits (> SS and AST), and a negative relationship (negative adaptation) with pH.

Genotypes PRO 555-1 and BRS 265 exhibited a negative relationship (negative adaptation) with acidity, ABTS, TEP, SS, and AST, which translates into a genotype with potentially low acidity content (natural preservatives), reduced antioxidant proportion, low phenolic compound content, and low sugar content. Furthermore, the PRO 555-1 clone showed a positive relationship (positive adaptation) with pH, Vit C, and SS/AT, in addition to being rich in Vit C and having a high SS/AT ratio, indicating a taste that directly translates into a balance between sugars/ acids. In other words, a high SS/AT ratio results in a pleasant taste for the consumer's palate, especially if consumed as fresh fruit (table fruit). The BRS265 clone exhibited no relationship with these variables.

It is important to underscore that since all environments are associated with the first or second component of matrix C, there is no phenotypic stability for any elite clone in terms of the variables evaluated, meaning they are phenotypically unstable for the environments considered. Given that the genotypes are far from the center of the plot, as reported by ARAÚJO et al. (2010), no phenotypic stability was found. On the other hand, they were adaptable to the variables. For example, in figure 1, the CCP 09 genotype shows negative adaptability,

reducing the pH in environments related to the first vector of the first joint biplot. In other words, this clone will produce a lower pH value, showing an acidic relation; consequently, with good amounts of natural preservatives in its peduncles in Alto Santo/2013, Beberibe/2013, and Beberibe/2014 environments. By contrast, the PRO 555-1 genotype exhibited positive adaptability and consequently will increase the pH value in these environments.

These results highlighted the relationship of the joint biplot projected into the second component of C (Figure 2). All r2 loads for Alto Santo in 2014 are negative, and, according to ARAÚJO et al. (2010), markers in opposite directions show a positive relationship, and those in the same direction a negative relationship. The CCP 09 genotype exhibits a positive relationship (positive adaptation) with pH, SS/AT, and AST, and a negative relationship with acidity, Vit C, ABTS, and TEP. Thus, in Alto Santo/2014, the CCP 09 genotype, despite having low acidity, low levels of Vit C, and being poor in antioxidants and phenolic compounds, shows a high SS/AT ratio, qualifying it for fresh consumption. The PRO 555-1 clone showed a positive relationship with SS, AST, and SS/AT and no correlation with the other variables. In Alto Santo/2014, the PRO 555-1 clone exhibited good of SS - °Brix concentrations and are also rich in antioxidants (Vitamin C and TEP).

Thus, it has a high ABTS content and, mainly, showed a good balance between sugars and acids, which may indicate dual suitability of the

pseudofruits of this clone, qualifying it for use by the processing industry and consumption as table fruits, allowing various marketing alternatives, which is interesting from the producer's perspective. The BRS 265 clone showed a negative relationship (negative adaptation) with pH, SS/AT, and AST, a positive relationship with acidity and high levels of Vit C, with the SS variable exhibiting no relationship.

CONCLUSION

The genotypes did not display phenotypic stability in relation to the variables evaluated and the environments studied because these cultivars are far from the center of the graph, that is, away from the origin. The three genotypes showed both positive and negative phenotypic adaptations with different variables, qualifying them for the consumption of pseudofruits as table fruits, processed products, or even exhibiting dual-purpose consumption suitability.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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