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GGE Biplot projection in discriminating the efficiency of popcorn lines to use nitrogen

Projeção GGE *Biplot* na discriminação da eficiência de linhagens de milho-pipoca ao uso do nitrogênio

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ABSTRACT

Nitrogen is essential for sustaining life on the planet, and it is the most important nutrient for obtaining high agricultural production. However, their use leads to the release of nitrous oxide with a global warming potential 296 times higher than the CO_2 molecule, making it a challenge to reduce their use in agriculture. The objective of this research was to identify efficient popcorn inbred lines and responsive nitrogen use and exhibit a good expansion volume. For this, 29 inbred lines from the Germplasm Collection of Darcy Ribeiro North Fluminense State University (UENF) were evaluated at two contrasting levels of nitrogen availability (low and ideal) at two representative locations in the north and northwest of the state of Rio de Janeiro, Brazil, arranged in a randomized block design with three replicates. These inbred lines were discriminated against efficient use of nitrogen by multivariate GGE Biplot. Selective accuracy was close to 1, showing that the genotypes were enough to provide contrasting success in selection procedures. The first two main components (PC) retained 93.82% of the total variation, and PC1 furnished an information ratio (IR) that was unaffected by noise. L77 was the most unstable line, while P7, P2, P6, P3, P5, P4, P9, P10, P8, P9, L70, L74, and L55 were efficient and responsive. The GGE biplot method is recommended for the reliable identification of popcorn lines that are efficient and responsive to the use of nitrogen.

Index terms: *Zea mays*; multivariate analysis; abiotic stress; G x E interaction.

RESUMO

O nitrogênio é indispensável à manutenção da vida no planeta, sendo o mais importante nutriente para a obtenção de elevadas produções agrícolas. No entanto, a sua utilização ocasiona a liberação de óxido nitroso, com potencial de aquecimento global 296 vezes maior que a molécula de CO_2, tornando-se um grande desafio a redução de seu uso na agricultura. O objetivo desta pesquisa foi identificar linhagens de milho-pipoca eficientes e responsivas no uso de nitrogênio e que apresentem um elevada capacidade de expansão. Para isto, avaliaram-se 29 linhagens da Coleção de Germoplasma da Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) em dois níveis contrastantes de nitrogênio (baixo e ideal) em dois locais representativos do Norte e Noroeste do Estado do Rio de Janeiro, Brasil, arranjadas no delineamento em blocos casualizados com três repetições. Estas linhagens foram discriminadas em relação a eficiência no uso do nitrogênio pela técnica multivariada GGE *Biplot*. A acurácia seletiva foi próxima de 1, revelando que os genótipos foram suficientes contrastantes para proporcionar sucesso em procedimentos seletivos. Os dois primeiros componentes principais (CP) retiveram 93,82% da variação total, sendo que o CP1 expressou relação de informação (IR) não impactada por ruídos. L77 foi a linhagem mais instável, enquanto P7, P2, P6, P3, P5, P4, P9, P10, P8, P9, L70, L74 e L55 foram eficientes e responsivas. Recomenda-se o método GGE *Biplot* na identificação fidedigna de linhagens de milho-pipoca eficientes e responsivas ao uso do nitrogênio.

Termos para indexação: *Zea mays*; análise multivariada; estresse abiótico; interação G x A.

INTRODUCTION

Increased agricultural production worldwide over the past four decades has been associated with a more than eightfold increase in the use of nitrogen (Mulvaney; Khan; Ellsworth, 2009). As a result, the use of nitrogen fertilizers in agriculture has had negative impacts on the diversity and functioning of ecosystems. The most common examples of such impacts are the eutrophication of freshwater and marine ecosystems resulting from leaching due to the high rates of nitrogen fertilizers applied in agricultural fields (Haddad et al., 2015).

According to the United Nations, the current world population is 7.4 billion, and it is estimated to reach 9.6 billion by 2050, which would require an increase of 50–70% in cereal production by 2050 to keep up with the

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growing demand for food (Savary; Ficke; Hollier, 2014). Thus, the use of mineral fertilizers will increase over the next few years to support these changes (Cordell; Drangert; White, 2009). In this context, projections indicate an overall increase in fertilizer consumption of approximately 69 million tons by 2030, with nitrogen accounting for 67% of this amount (Tenkorang; Lowenberg-DeBoer, 2009), which will lead to harmful economic and environmental consequences, such as high production costs, depletion of energy resources, and increasing environmental pollution (Li et al., 2015).

Under this scenario, it is extremely important that research aimed at developing efficient and response genotypes in the use of nitrogen, since this is the best option for maintaining crop yields with less impact on production costs and the environment, given that increasing linear consumption of nitrogen fertilizers has been observed over past years (Meng et al., 2016; Dresbøll; Kristensen, 2014), where most efficient genotype produce more grains in relation to other genotypes in conditions of low nutrient availability and the most responsive genotypes are those that increase productivity as the relative availability of nutrient increases (Fageria; Kluthcouski, 1980; Moll; Kamprath; Jackson, 1982).

The selection of superior genotypes for the efficient use of nitrogen has generally been performed in two or more contrasting environments. However, different responses are expected from different genotypes when subjected to specific limiting conditions, i.e., to distinct stresses that cause genotype-by-environment (GE) interactions. This is mainly due to the differential expression of genes that control a trait when faced with environmental variations. The magnitude of the GE interaction becomes more important when increasing the intensity of the nutritional stress, making it difficult to select superior individuals in breeding programs.

Thus, various methodologies have been proposed for investigating GE interactions. However, GE interactions are far too complex to be summarized using one or two stability parameters in univariate analyses. Multiplicative statistical approaches for assessing interactions, such as the GGE biplot method, are very useful for studying performance patterns in genotypes according to the environment and making predictions about the average performance of genotypes in specific environments (Karimizadeh et al., 2013).

There are few studies incorporating the GGE biplot method in the breeding of plants for the purpose of selecting genotypes that are superior in the use of nitrogen. To our knowledge, the only available studies employing such methodology are those conducted by Cirilo et al. (2009); Badu-Apraku et al. (2010) and Badu-Apraku et al. (2011), in which genotypes with potentially aggregator alleles favorable for the efficient use of nitrogen were identified.

Thus, the objective of this study was to identify efficient and responsive popcorn inbred lines to the use of nitrogen and exhibit a good expansion volume.

MATERIAL AND METHODS

Were evaluated 29 popcorn inbred lines (S_7) belonging to the germplasm bank of Darcy Ribeiro North Fluminense State University (Universidade Estadual do Norte Fluminense Darcy Ribeiro - UENF), among which seven were from the "Viçosa" population, five from the "Beija-Flor" population, seven from the "BRS-Angela" population, and ten from the base population of the program (UENF 14). As there is no definition of heterotic groups of popcorn, the populations cannot be considered to be representative of different heterotic groups.

The experiments were conducted at two levels of nitrogen availability (low N availability and ideal N availability) and at two locations: Campos dos Goytacazes, Rio de Janeiro (21º 42' 48" S and 41º 20' 38" W, at an altitude of 14 m) and the Experimental Station of Itaocara (21º 38' 50" S and 42º 03' 46" W, at an altitude of 58 m), in the north and northwest Fluminense regions, respectively. The climate of both environments is humid tropical (Aw) according to Köppen classification.

The experimental design used in both experiments was a randomized block design with three replicates. The experimental unit consisted of a row of five meters in length, with spacing of 0.90 m between rows and 0.20 m between plants. Two seeds were sown per hole and thinning was performed 21 days after emergence, leaving one plant per hole, resulting in a stand of 25 plants per plot.

For differentiation of the experiments in relation to nitrogen levels, the following strategy was adopted. For the treatment with ideal nitrogen availability, sowing fertilization was performed according to a soil analysis, with 32 kg ha^{-1} of nitrogen added, whereas topdressing for nitrogen supplementation was performed at 108 kg ha^{-1}. For the treatment with low nitrogen availability (low N), sowing fertilization was similar to the previous experiment, whereas the topdressing consisted of 30% of that applied in the environment with an ideal dosage of nitrogen.

Were evaluated the following traits: i) grain yield (GY), which was obtained by weighing the grains after the dehusking of each experimental unit and calculated as kg ha^{-1}; and ii) expansion volume (EV), which was obtained from the ratio between the volume of the expanded popcorn and a mass of 30 g, expressed in mL g^{-1}, using the mean of two samples per plot.

Initially, a variance analysis was performed for each environment to verify the homogeneity of the residual variance. Subsequently, combined analysis of variance was performed, considering the effects of genotype, site, and nitrogen levels, with the main objective being to determine possible interactions of genotypes with nitrogen availability. The selective accuracy was estimated as SA = $(1-1/F)^{1/2}$, where F is the F-test value for genotypes (Resende; Duarte, 2007).

The multivariate GGE biplot analysis was based on the average phenotypic information, considering the following model: $\bar{Y}_{ij} - \mu = G_i + E_j + GE_{ij}$, where $\bar{Y}_{ij}$ represents the phenotypic average of genotype i in environment j (ideal or low N availability); μ is the overall constant; G_i is the random effect of genotype i; E_j is the fixed effect of environment j; and GE_{ij} is the random effect of the interaction between genotype i and environment j.

The GGE biplot model does not separate the genotype effect (G) from the effect of the genotype x environment (GE) interaction, keeping them together in two multiplicative terms, represented in the following equation: $Y_{ij} - \mu - \beta_j = g_{1i}e_{1j} + g_{i2}e_{2j} + \varepsilon_{ij}$, where Y_{ij} is the performance expected of genotype i in environment j; μ is the overall constant from the observations; β_j is the main effect of environment j; g_{1i} and e_{1j} are the main scores for the ith genotype in the jth environment, respectively; g_{i2} and e_{2j} are the secondary scores for the ith genotype in the jth environment, respectively; and ε_{ij} is the residual not explained by either of the effects ("noise").

Thus, construction of the biplot in the GGE model is accomplished by the simple dispersion of g_{1i} and g_{i2} for genotypes and e_{1j} and e_{2j} for environments, via singular value decomposition (SVD), in accordance with the equation $Y_{ij} - \mu - \beta_j = \lambda_1\xi_{i1}\eta_{1j} + \lambda_2\xi_{i2}\eta_{2j} + \varepsilon_{ij}$, where λ_1 and λ_2 are the largest eigenvalues of the first and second principal components, PCE_1 and PCE_2, respectively; ξ_{i1} and ξ_{i2} are the eigenvalues of the ith genotype for PCE_1 and PCE_2, respectively; and η_{1j} and η_{2j} are the eigenvalues of the jth environment for PCE_1 and PCE_2, respectively. The GGE biplot analysis was performed with the aid of R software (R Development Core Team, 2014).

To assess the suitability of a biplot to display the patterns of a double-entry table, the information ratio (IR) — proposed by Yan and Tinker (2006) — was calculated. The IR can be calculated for each principal component using the proportion of the total variance explained by each principal component multiplied by *k*. If there is no correlation between the environments, all of the *k* PCs must be completely independent, and the proportion of the total variance explained by each PC must be exactly 1/*k*.

When there is a correlation between the environments, the proportion of the variation explained by the first PCs must be greater than 1/*k*, and the variation explained by other PCs must be less than or equal to 1/*k*. A PC with an IR > 1 contains patterns (associations between environments); a PC with an IR = 1 does not contain patterns but may contain some independent information; and a PC with an IR < 1 does not contain any pattern or information (Yan, 2011).

RESULTS AND DISCUSSION

There was a significant effect (P < 0.01) for all of the sources of variation, except for the site x level of nitrogen interaction (S x N). The significant difference between the genotypes indicates the existence of genetic variability among the popcorn inbred lines. The significant effect of the level of nitrogen demonstrates that the doses of nitrogen applied were sufficient to differentiate the environments, which were sufficiently contrasting to adequately select genotypes that are efficient and responsive in the use of nitrogen (Table 1).

The significant effects of the genotype x level of nitrogen (G x N), genotype x site (G x S), and triple (G x S x N) interactions for GY show that the mean GYs are distinct and that the genotype classifications may have changed between the experiments with low and ideal nitrogen availability. Thus, the selection of genotypes that efficiently use nitrogen based on GY must be specific for the environment and site and must not be performed in terms of the average performance because, under conditions of low nutritional supplementation, the alleles that control the expression of a particular trait differ at least to some extent from the alleles that control that trait under ideal supplementation conditions (Souza et al., 2008).

For EV, the significant effect (P < 0.01) of the genotype as a source of variation indicates the existence of variability in this parameter among the evaluated lines. However, the environment had no influence on EV, considering the significant absence of the G x S x N triple interaction, which is in line with Lu, Bernardo and Ohm (2003), who indicated that EV is little influenced by the environment. This result then leads to the inference of oligogenic expression for EV — as previously noted by Doffing, D'croz-Mason and Buck (1991) — with EV representing a trait for which additive effects are the principal components of genetic variance (Pereira; Amaral Junior, 2001).

Table 1: Summary of the combined analysis of variance for the grain yield (kg ha^{-1}) and expansion volume (mL g^{-1}) of the 29 S_7 popcorn lines.

Sources of variation	DF	Mean squares	
		Grain yield	Expansion volume
(B/N)/S	8	34,193.47	19,931
Genotype (G)	28	2,745,565.46**	228,078**
Site (L)	1	2,266,088.28**	4,604 ^{ns}
Level (N)	1	1,843,703.73**	8,714 ^{ns}
Genotype x Site (G x S)	28	99,393.97**	38,805**
Genótipo x Level (G x N)	28	106,969.80**	18,904 ^{ns}
Site x Level (S x N)	1	16,634.59 ^{ns}	9,079 ^{ns}
Genotype x Site x Level (G x S x N)	28	233,316.90**	13,505 ^{ns}
Error	224	23,274.88	18,538
Accuracy selective	-	0.99	0.96
Means	-	955.56	30.25

**, ^{ns}: significant at P < 0.01, not significant at the 5% probability level, respectively, in accordance with the F-test.

The experimental precision was evaluated through the estimation of selective accuracy (SA). This parameter reflects the quality of the information and the procedures used in the prediction of genetic values (Pimentel et al., 2014). Thus, a suitable parameter for evaluating the quality of experiments can be summarized in terms of a single statistic that includes the coefficient of experimental variation, the number of replications, and the coefficient of genotypic variation (Resende; Duarte, 2007). In this context, there is a significant level of accuracy, which represents higher precision in the identification and likelihood of success in the selection of individuals who are efficient in the use of nitrogen.

The first two principal components account for 93.82% of the total variation (Table 2). Thus, the GGE biplot method explained a large proportion of the sum of squares of genotypes and the GE interaction, indicating that there is a high level of confidence in the results produced using the multivariate technique. Regarding the IR, any PC with an IR value substantially less than 1.0 expresses little information, while a PC with an IR value greater than 1.0 — as observed for PC1 — exhibits important patterns. Therefore, the biplot was considered to be appropriate for representing the patterns related to the interaction.

In relation to the productive performance, the genotypes allocated on the vertex of the polygon were farther from the origin than the other genotypes within the sector bounded by them, and they are classified as the most responsive. These may be the genotypes showing better or worse performance in some or in all of the environments and, therefore, they can be used to identify possible mega-environments. In turn, the genotypes located within the polygon are the least responsive to the stimuli of the environments (Figure 1A).

Table 2: Singular value, explained variation, cumulative proportion, and information ratio (IR) for the four principal components (PCs) of the GGE biplot analysis.

PC	Singular Value	Explained (%)	Cumulative (%)	IR
1	5,082.73	86.73	86.73	3.47
2	1,453.16	7.09	93.82	0.28
3	1,040.79	3.64	97.46	0.15
4	870.47	2.54	100.00	0.10

The P7 and P6 lines represent the vertices of the polygon in which the environments are contained; thus, they are the most productive in the environments with both low and ideal availability of nitrogen (Figure 1A). Therefore, these genotypes are likely sources of alleles that are favorable for efficient nitrogen use, showing potential for use as parents in genetic recombination programs aimed at increasing the frequency of favorable alleles.

This option will be much facilitated if the influence of additivity on the expression of efficiency in nitrogen use becomes higher. However, there are currently no available studies on the inheritance of nitrogen use efficiency in popcorn. On the other hand, in common maize, it has been reported that additive effects are more important for the components of nitrogen use efficiency (NUE) (DoVale et al., 2012; Worku et al., 2008). Therefore, selection can be accomplished based on the performance of the lines *per se*, as the hybrid combinations obtained from crosses of these lines will also be superior in relation to NUE.

When genotypes give rise to vertices of the polygon but do not contain any clustered environments, they are considered to be unfavorable in relation to the groups of environments tested, revealing low responsiveness and yields. Thus, the individuals located in the sectors composed of them are also unfavorable for recommendation as parents in breeding programs aimed at increasing the expression of the trait under stress conditions. Therefore, the lines located in these sectors can be considered to be inefficient and not responsive to the use of nitrogen, which include lines L66, L65, P1, L51, L53, L71, L63, L61, L75, L80, and L52.

The GY values and the stability of the genotypes were evaluated based on the average environment coordination (AEC) (Figure 1B). In this method, an ideal environment is defined based on the mean score of principal components 1 and 2 (PC1 and PC2) for all of the environments, which is represented in the graph by a small circle. The line that passes through the origin of the biplot and through the ideal environment is referred to as the axis of the ideal environment, which represents the AEC abscissa, whereas the small arrow points to the highest genotypic value with a main effect. The AEC ordinate is the axis that passes through the origin of the biplot — it is perpendicular to the AEC abscissa and indicates a greater effect of the genotype x environment interaction and lower stability, and it separates the genotypes that are superior and inferior to the overall mean (Yan; Tinker, 2006).

Thus, the genotypes that exhibited a higher GY than the mean were P3, P7, P6, P2, P5, P9, P4, P10, P9, P8, L70, L74, and L55. Genotypes L54, L76, and L77 presented a yield similar to the overall mean yield, and the other genotypes showed a lower yield than the overall mean. In addition to the mean yield, genotypic stability is a crucial point to be analyzed. The greater the projection of the genotype on the axis of the AEC ordinate, higher the instability of the genotype, which represents a greater interaction with the environment. Thus, it can be seen that the L77 genotype was the most unstable.

The "ideal" genotype should exhibit an average-to-high GY in all of the evaluated environments. This ideal genotype is defined graphically by the longest vector in PC1 and PC2 without projections, represented by the arrow at the center of the concentric circles (Figure 2A). Although this genotype represents one more representative model, it is used as a reference for identifying the ideotype. Thus, lines P3 and P2, allocated to the first and second concentric circles, respectively, are the closest to ideal in terms of GY and phenotypic stability.

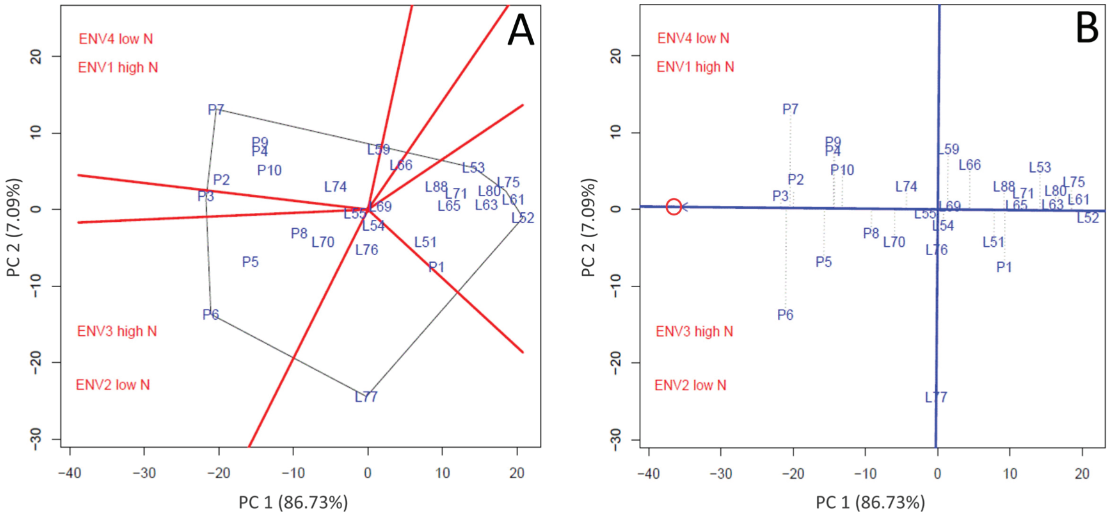

Figure 1: GGE biplot representing: (A) the "which-won-where" graph and (B) means x stabilities, indicating the yield rankings of the 29 popcorn lines, with their respective production stabilities.

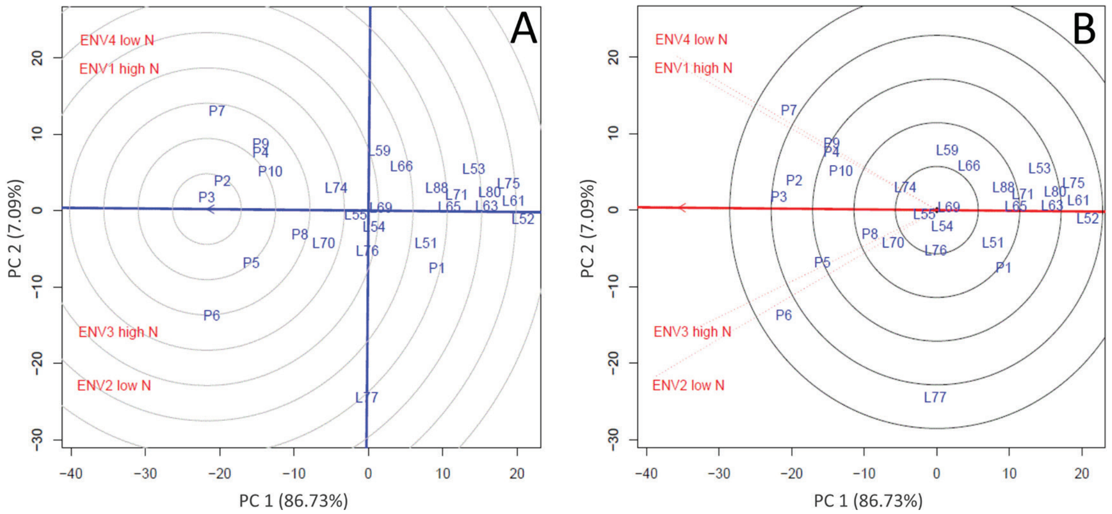

Figure 2: GGE biplot comparing the 29 S_7 lines of popcorn evaluated with (A) the estimate of an ideal genotype and (B) the discrimination and representativeness of the environments for grain yield (kg ha^{-1}).

An ideal test environment should discriminate superior genotypes and be representative of all test environments; that is, it should present a high PC1 score because it has a greater ability to discriminate the genotypes and zero score for PC2, thereby demonstrating a greater ability to represent all the other environments. Thus, the proportions explained by PC1 (86.73%) and PC2 (7.09%) were appropriate for reliable selection of the most representative environments (Figure 2B).

The circle indicated by the arrow represents the average environment. If the angle formed between the test environment and the line passing through the average environment is small, it means that this test environment is representative, and the larger the vector for each environment, greater the discrimination capability (Yan; Tinker, 2006). Thus, it can be observed that the environments with low nitrogen availability showed a greater capacity to discriminate the genotypes. Hence, it is recommended that the evaluation and selection of superior genotypes that are efficient in nitrogen use be performed under stress conditions. And further evaluation of their responsiveness in an environment with optimal availability of nitrogen.

It should be noted that environments that are both discriminating and representative favor the selection of genotypes with broad general adaptation. On the other hand, discriminating but non-representative environments may be useful for discarding unstable genotypes. Non-discriminating and non-representative environments are of little use in experimental test networks and should be discarded (Yan; Tinker, 2006; Yan et al., 2007).

The fact that the environments with low nitrogen availability have a greater ability to discriminate genotypes may be related to the fact that under stress conditions, in addition to a plant being efficient in absorbing scarce soil nitrogen, it must be efficient in using nitrogen and channeling it for the production of grains (Hirel et al., 2007). Thus, an inbred line that has a highest grain yield in low nitrogen concentration in the soil consequently will be more eficiente in the use of this nutrient under stress conditions. One of the reasons for this is that the inbred line will be efficient in absorbing the scarce nitrogen and will use the nutrient for grain production (Chen et al., 2014).

Regarding the relationship between the environments, the cosines of the angles between the vectors of each environment are related to the correlation coefficients between them. All of the environments are positively correlated because the cosine of the angle between them is positive; that is, the angle is less than 90°. This consistent association between the evaluated environments suggests that the same information about the genotypes may be obtained in the same environments, thus enabling a reduction in the costs of evaluating the genotypes (Yan; Holland, 2010).

Consequently, it can be inferred that the predominant interaction is of the simple type, and there is, therefore, a correlation between the environments, indicating that there are only differences in variability between the genotypes in the environments in which they are being evaluated. This favors the reliable selection of superior genotypes, as their rankings - based on the performance in the different environments - remain coincident.

To study the performance of the genotypes under nitrogen stress, the genotypes were represented in a Cartesian plane using the ratio between the GY values obtained at different nitrogen levels (Figure 3). The P7, P2, P6, P3, P5, P4, P9, P10, P9, P8, L70, L74, and L55 lines were classified as efficient and responsive (ER). Therefore, these inbred lines were placed in the first quadrant; i.e., they exhibit a mean GY greater than the means for the environments with low and ideal nitrogen availability. It should be noted that these inbred lines were the same present in the two mega environments (Figure 1A), thus confirming the efficiency of the GGE biplot method in the identification of genotypes that are efficient in the use of nitrogen.

The inbred lines L59 and L77 exhibited yields above the overall mean in the environments with low nitrogen availability, thus showing potential for adaptation to stress conditions. The inbred lines L54, L76, and L69 displayed specific adaptation to the environments with high availability of nitrogen (IR). The other inbred lines can be classified as inefficient and non-responsive (INR) to the use of nitrogen.

In the cultivation of popcorn, GY and EV are a reflection of a historically established problem: the existence of negative correlations between these two main traits (Hallauer, 2001; Rangel et al., 2011; Willier; Brunson, 1927). In selective studies of popcorn genotypes, both traits must be evaluated and addressed according to biometric procedures that favor the selection of superior genotypes. With this in mind, phenotypic evaluation of EV was conducted in the inbred lines (Table 3) for the purpose of monitoring the advantageous selection of genotypes that are both efficient in the use of nitrogen and demonstrate adequate expression of EV.

The inbred lines were allocated to three distinct groups by Scott-Knott grouping, which confirmed the existence of differences for this trait. The inbred lines that were efficient and responsive to nitrogen belonged to the group with higher means, except for P2, P3, and P4, which were included in the second group, showing potential for selection, because according to Galvão, Sawazaki and Miranda (2000), by American standards, expandability values above 25 mL g^{-1} are acceptable. In Brazil, according Zinsly and Machado (1987), the minimum value of expandability of the commercial population should be 15 ml g^{-1}.

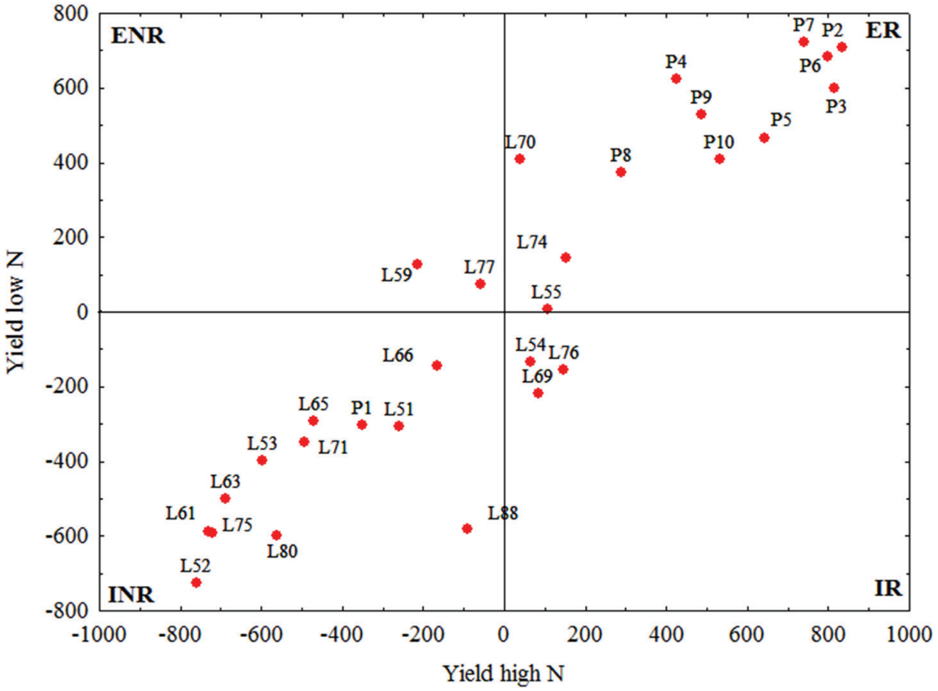

Figure 3: Performance in relation to the efficiency use nitrogen and the response to nitrogen fertilization in the 29 S_7 lines of popcorn. ER: efficient and responsive; ENR: efficient and non-responsive; IR: inefficient and responsive; and INR: inefficient and non-responsive.

Table 3: Mean expansion volumes (mL g^{-1}) of the 29 popcorn inbred lines evaluated at two sites.

Lines	Campos dos Goytacazes	Itaocara
	Expansion volumes	Expansion volumes
L70	34.38 a	34.27 a
L76	32.78 a	33.91 a
L80	23.57 c	21.32 c
L66	34.77 a	32.46 a
L54	32.71 a	34.80 a
L53	30.05 a	33.63 a
L88	28.77 b	29.96 a
L69	28.10 b	30.27 a
L65	25.96 b	24.24 c
L77	30.68 a	30.35 a
L52	26.10 b	21.70 c
L75	25.96 b	24.41 c
L51	21.43 c	25.71 b
L59	28.60 b	30.96 a
L74	31.94 a	35.41 a
L55	29.27 b	33.05 a
L61	28.91 b	25.19 b
L63	28.85 b	23.30 c
L71	33.66 a	31.71 a
P1	32.06 a	28.18 b
P2	29.41 b	29.30 b
P3	27.99 b	26.88 b
P4	25.57 b	26.18 b
P5	37.07 a	36.32 a
P6	32.66 a	32.46 a
P7	35.17 a	38.27 a
P8	33.18 a	37.96 a
P9	33.82 a	36.38 a
P10	35.88 a	36.93 a

Means followed by the same letter in the column belong to the same group, in accordance with the Scott-Knott test, 1974 ($P < 0.05$).

CONCLUSIONS

It is possible to select Individuals who are efficient in the use of nitrogen and exhibit a good expansion volumes. The GGE biplot method is recommended for the reliable identification of popcorn lines that are efficient and responsive to the use of nitrogen.

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