Prospecting of popcorn inbred lines for nitrogen use efficiency and responsiveness[1]

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ABSTRACT

Large amounts of nitrogen (N) fertilizers applied to maize cropping systems support high yields but cause adverse environmental impacts. The development of cultivars with higher N use efficiency is essential to maintain sustainable production. Thus, the present study aimed to select popcorn inbred lines efficient and responsive to N fertilization expressing high popping expansion. Fifty-one popcorn inbred lines from different origins were evaluated in the field under low and high N availability, arranged in a randomized complete block design with three replications. The two main traits of economic interest in the crop were evaluated: grain yield (GY) and popping expansion (PE). A joint analysis of variance was performed and a scatter plot was generated to classify the inbred lines regarding the response to the N use, focusing on GY. For PE, a Scott-Knott grouping of means was conducted. Within the panel of evaluated popcorn inbred lines, it was possible to identify sources of favorable alleles for nitrogen use efficiency and popcorn expansion, highlighting the inbred lines L205, L206, L217, and L395. These genotypes emerge as potential parents to be included in mating blocks for the development of hybrids and/or breeding populations with high nitrogen use efficiency and popping expansion.

Keywords: *Zea mays* var. everta; abiotic stress; nutritional stress; plant breeding; sustainable agriculture.

INTRODUCTION

Nitrogen (N) is the nutrient required in large amounts by plants and is essential for growth and development. In corn, this mineral is of great importance for increasing grain yield (Hirel *et al.*, 2007; Taiz *et al.*, 2017). Thus, N deficiency is, the main abiotic stress that limits the productive potential of the popcorn crop (*Zea mays* everta). Popcorn is a special type of corn, which has great popular acceptance, providing economic gains both in the grain production and marketing sector of popcorn (Silva *et al.*, 2019). This crop has been presenting significant commercial demand in the food industry, moving about 10 billion dollars in 2020 (Serna-Saldivar, 2022).

To meet this demand, the cultivation of popcorn has increased in tropical regions, where soils generally have nitrogen availability below that required for the popcorn cultivated species to gain satisfactory yields. Thus, in order to increase agricultural production, farmers become increasingly dependent on nitrogen fertilizers. In 2019, around 107.4 million tons of nitrogen fertilizers were used worldwide. For 2022 it is estimated that 111.6 million tons will be used (FAO, 2019).

Despite the large amount of N fertilizers applied, the nitrogen utilization efficiency of the plants is low about 30 to 40%, which means that much of the applied N is not

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used (Ciampitti & Vyn, 2012). Thus, more than 60% of N is lost by leaching, volatilization, runoff, and competition with the soil microbiota, resulting in negative impacts on the environment, as well as increased crop production costs (Sharma & Bali, 2017).

In this scenario, to reduce dependence on nitrogen inputs and maintaining high levels of yields, is essential to ensure sustainable agricultural production for the future. This challenge can be overcome through the development of cultivars nitrogen use efficiency (NUE), which can be obtained by crossing inbred lines adapted to environments with low N availability and, which are responsive to N fertilization, since, the transfer of alleles favorable to descendants depends on their presence in the parent lines (Hallauer *et al.*, 2010).

The selection of parents in a breeding program aiming at the development of hybrids NUE is usually carried out by evaluating the combining ability of their parental inbred lines, via *diallel* or *top crosses*. However, prior to this stage, the selecting inbred lines by performance *per se* in environments with and without N restriction, can be an effective strategy to increase the chances of obtaining superior hybrids in the N use. Santos *et al.* (2020) observed that the hybrids classified as efficient and responsive to N use came from the crosses in which at least one of the parents demonstrated efficiency and responsiveness to N use based on their performance *per se.*

Nevertheless, in popcorn there are still few studies that address this theme, mainly in field conditions, where the cultivation environment can best be represented, especially considering the site of adaptation to which one intends to develop a breeding program. Among these studies, Santos *et al.* (2017) conducted research to identify inbred lines of popcorn with high N use efficiency (NUE) in a field setting. They evaluated a panel of 29 inbred lines originating from three different genealogies and ultimately selected 13 lines that demonstrated efficiency and responsiveness to N fertilization. Similarly, Almeida *et al.* (2018) aimed to select popcorn inbred lines with enhanced NUE. They evaluated 19 inbred lines from two genealogies and identified a specific genotype that exhibited superior NUE.

In the aforementioned studies, the number of evaluated inbred lines and their respective genealogies is low. However, by assessing a larger number of genotypes with diverse genealogical backgrounds, we can increase the likelihood of identifying sources of favorable alleles that contribute to improving NUE and, consequently, fostering the development of superior popcorn hybrids.

Thus, the present study aimed to select popcorn inbred lines efficient and responsive to N fertilization expressing high popping expansion.

MATERIAL AND METHODS

Fifty-one popcorn inbred lines belonging to the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) Germplasm Bank were evaluated. These inbred lines were extracted from 16 different genealogies, including populations adapted to tropical and temperate climates, originating from various countries such as Uruguay, Paraguay, Chile, Colombia, Bolivia, Argentina, United States, and Brazil (Table 1).

The field experiment was conducted at the Experimental Station of the State College of Agriculture “Antonio Sarlo” in Campos dos Goytacazes, Rio de Janeiro State, Brazil (latitude 21° 42’ 48” S, longitude 41° 20’ 38” W, altitude 14 m), in the summer harvest, during December 2018 to April 2019. According to Köppen (1948) classification, the climate of this municipality is considered tropical humid (Aw), with hot summers and mild winters. During the experimental period, average temperatures of 26.9 °C and precipitation of 291 mm were observed (Figure 1).

The soil samples were analyzed for pH, organic matter, and availability of P, K, Mg, Ca, and Al (Table 2). Liming was performed with of dolomitic limestone. Subsequently, the planting areas were prepared with mechanized tilling.

The strategy implemented to differentiate the environments regarding the level of N availability was as follows: in the sowing fertilization, the two environments received 32 $kg.ha^{-1}$ of N, 112 $kg.ha^{-1}$ of phosphorus (P_2O_5), and 56 $kg.ha^{-1}$ of potassium (K_2O), for which the formula NPK (04-14-08) was used. In the high N availability environment (HN), the topdressing provided 118 $kg.ha^{-1}$ of N, totaling an application of 150 $kg.ha^{-1}$ of N. In the low N availability environment (LN), the topdressing fertilization was 29 $kg.ha^{-1}$ of N, which corresponds to 25% of that applied in the high N dosage environment. Urea (46%) was used as the N source.

For both N conditions, the urea application was divided into two equal doses and applied to all inbred lines at the V4 (four fully expanded leaves) and V6 (six fully expanded leaves) growth stages, following the protocols described by Abendroth *et al.* (2011) and Santos *et al.* (2017). The experiments were irrigated using sprinklers, including after topdressing fertilization, and weed management was

Table 1: List of popcorn inbred lines evaluated with their respective genealogies, number of inbred lines per genealogy (N° lines), country of origin, and climate adaptation

Genealogy	ID in Germplasm Bank	N° lines	Country of origin	Climate adaptation
IAC 125	L201-L217	10	Brazil	Temperate/Tropical
BOZM 260	L236	1	Bolívia	Temperate/Tropical
PARA 172	L261-L272	5	Paraguay	Temperate/Tropical
URUG 298	L291-L298	7	Uruguay	Temperate/Tropical
Barão de Viçosa	L326-L332	3	Brazil	Tropical
PR 023	L351-L366	4	Brazil	Tropical
SAM	L381-L395	3	EUA	Temperate/Tropical
PA 170 Roxo	L413	1	Paraguay	Temperate/Tropical
SE 013	L471-L477	4	Brazil	Tropical
PA 170 Roxo	L509-L511	3	Paraguay	Temperate/Tropical
ARZM 05 083	L562	1	Argentina	Temperate/Tropical
RS 20	L592-L594	3	Brazil	Tropical
PA 091	L623-L624	2	Brazil	Tropical
ARZM 13 050	L652-L655	2	Argentina	Temperate/Tropical
Viçosa:UFV	L80	1	Brazil	Temperate/Tropical
Composto CMS-42	P2	1	Brazil	Temperate/Tropical

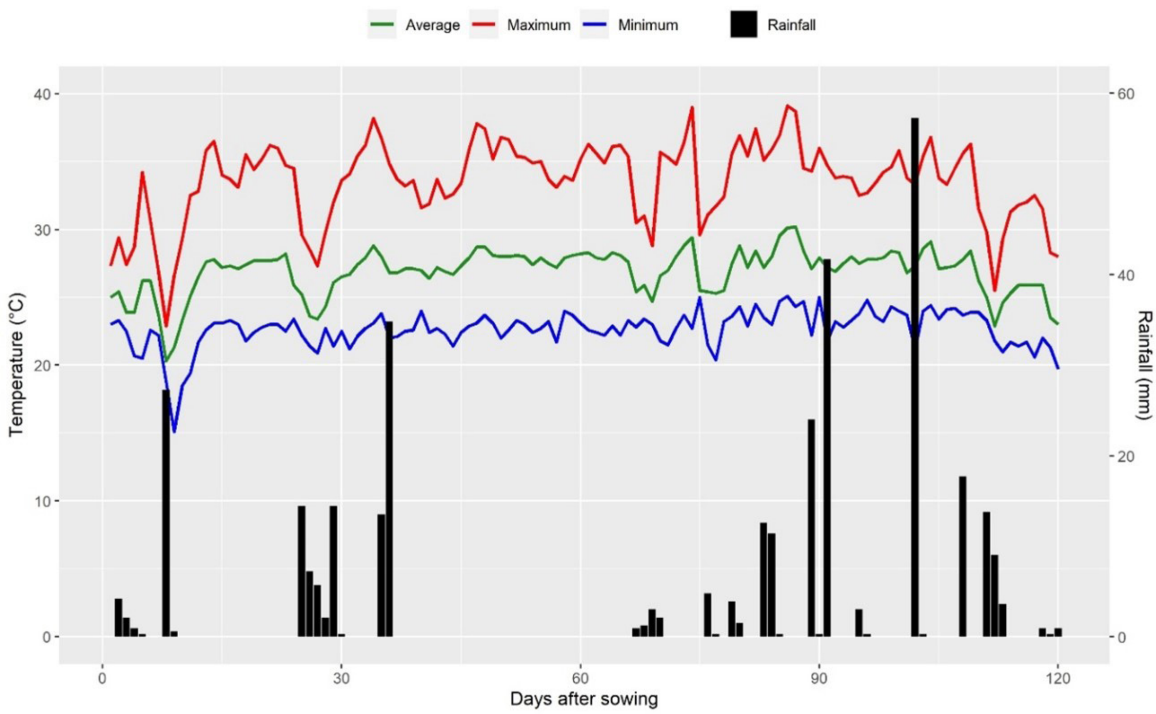

Figure 1: Rainfall (mm), maximum, average, and minimum temperature (°C) observed during the experimental period in the agricultural year 2018/2019 in Campos dos Goytacazes, Rio de Janeiro, Brazil. Sowing was carried out on December 4th, 2018, and the harvest on April 5th, 2019. Source: National Institute of Meteorology (INMET).

Table 2: Chemical attributes of the soil in the experimental area in Campos dos Goytacazes-RJ, at layers of 0-10 and 10-20 cm

Layers	pH	P	K	Ca	Mg	Al	H+Al	Na	C	MO
	H_2O	mg/dm^{-3}			mmol$_c$/dm^{-3}				g dm^{-3}	
0 - 10 cm	5.9	27	3.3	15.8	8	0	28.5	1.1	12.4	23.38
10 - 20 cm	5.8	28	2.4	17.6	8.6	0	38.1	0.8	13.4	21.1

performed using mechanical weeding techniques. Pest and disease control were implemented through the appropriate application of agrochemicals specific to the crop.

The HN and LN experiments were grown side by side in the same field, and each trial was laid out in a completely randomized block design with three replications. Plots were 3 m long, with rows spaced 0.9 m apart. Three seeds were sown per hole and, 21 days after emergence, thinning was performed, leaving only one plant per hole, resulting in a density of 55,555 plants per hectare. The open-pollinated population "UENF-14" was used as a border along each experiment, including a 6.00 m gap separating both N conditions.

To evaluate the performance of inbred lines, the two main traits of economic interest in the crop were measured: i) grain yield (GY), expressed in kg ha^{-1}, evaluated around 120 days after sowing, was obtained through the grain production of the plot corrected to 13% moisture and, ii) popping expansion (PE), expressed in mL.g^{-1}, obtained by the relation between the volume of expanded popcorn and the mass of 30 g, using the average of two samples per plot.

The classification of genotypes regarding their efficiency and responsiveness to N use was based on the deviations of the mean grain yield of each inbred line from the overall mean of each environment. These deviation values were then plotted on a scatter plot, with the X-axis representing the deviations under high N levels (responsiveness in N use) and the Y-axis representing the deviations under low N availability (efficiency in N use). Based on this classification, the lines were grouped into four categories: efficient and non-responsive (ENR), efficient and responsive (ER), inefficient and non-responsive (INR), and inefficient and responsive (IR).

Data were tested for normality of residuals (Shapiro-Wilk test) and no transformation was needed. Individual analysis of variance was performed for each N level separately to verify the homogeneity of residual variances (O'Neill and Mathews test), followed by joint analysis of variance, considering genotype and environment (N level) as fixed effects.

The genetic parameters were estimated using the mean squares of the individual analysis of variance. The coefficient of genotypic determination ($\hat{H}^2$) was estimated using the following expression: $\hat{H}^2=(MSg-MSe)/MSg$, in which *MSg:* corresponds to the mean square of the genotype; *MSe:* corresponds to the mean square of the error; genotypic coefficient of variation (CVg), obtained by $CV_g = 100\,x\frac{\sqrt{(MSg - MSe)/r}}{\mu}$, where r and μ correspond to replicate and general average, respectively; environment coefficient variation (CV_e), obtained by $CV_e = 100\,x\frac{\sqrt{QMr}}{\mu}$; and Index variation ($\hat{I}_v$), estimated by $\hat{I}_v=CV_g/CV_e$.

For the PE trait, means were clustered by the Scott & Knott (1974) algorithm at 5% probability and represented on a circular bar chart. The statistical analyses were executed using the GENES (Cruz, 2013) computer program.

RESULTS AND DISCUSSION

The genotype (G) effect for the traits GY and PE was significant ($p < 0.01$), revealing the existence of genetic variability, which is an essential condition for obtaining genetic gains through selection (Table 3). Genetic variability for agronomically important traits has also been reported among popcorn inbred lines under contrasting N levels in other studies (Santos *et al.*, 2017; Almeida *et al.*, 2018).

Significant effects of N level ($p < 0.01$) and G x N interaction ($p < 0.01$) were observed for GY (Table 3). The significant effect of N levels demonstrates that the applied N doses were satisfactory in providing differentiation between environments and, consequently, correctly discriminating between efficient and inefficient inbred lines in terms of N use.

The significant G x N interaction for GY (Table 3) suggests that the classification of popcorn inbred lines varied between the two N levels. Therefore, the selection of the most efficient genotypes for N use should be performed specifically for each environment and not based on the overall performance, as the alleles controlling the expression of the trait under N stress are, at least in part, different

Table 3: Summary of the analysis of joint variance for grain yield (GY) and popping expansion (PE) evaluated in 51 popcorn inbred lines under conditions of high and low nitrogen availability in the soil

SV	DF	Means squares	
		GY ($kg.ha^{-1}$)	PE ($mL.g^{-1}$)
Block/N	4	46905.78	1.47
Nitrogen (N)	1	1670663.24**	10.83^{ns}
Genotype (G)	50	1522316.24**	191.58**
G x N	50	142666.58**	0.45^{ns}
Error	200	15299.11	2.10
Average		748.80	22.92
CV (%)		16.52	6.32
Genetic parameters – High N			
$\hat{H}^2$		0.98	0.98
CVe (%)		14.03	5.70
CVg (%)		70.83	24.14
IV		5.05	4.24
Mean		822.69	23.11
Genetic parameters – Low N			
$\hat{H}^2$		0.96	0.97
CVe (%)		19.47	6.91
CVg (%)		67.13	24.68
IV		3.45	3.57
Mean		674.91	22.73

$^{**, ns}$: significant at P < 0.01, not significant at the 5% probability level, respectively, in accordance with the F-test. $\hat{H}^2$: coefficient of genotypic determination; CVg: genotypic coefficient of variation; CV_e: envarioment coefficient variation; $\hat{I}_v$: Index variation.

from the alleles controlling the same trait under optimal N supplementation conditions (Fritsche-Neto *et al.*, 2010).

In the low N environment, there was an 18% reduction in the overall average of GY compared to the high N environment (Table 3). According to Bolaños & Edmeades (1996), a yield reduction of 20 to 30% in abiotic stress conditions, compared to non-stress conditions of the same genetic set, is considered indicative of stress. Although the reduction observed in the present study was slightly lower than the recommended range, the results obtained were higher than those reported by Guedes *et al.* (2015) and Heinz *et al.* (2019), who observed yield decreases of only 13% and 16%, respectively.

Popping expansion (PE) was not influenced by N levels or the G x N interaction (Table 3), indicating that the selection of superior inbred lines can be based on the average performance in both environments. A similar result was reported by Santos *et al.* (2017) when evaluating a set of 29 inbred lines of popcorn in environments with contrasting N availability in the soil.

Although popping expansion (PE) shows a pattern of quantitative inheritance, this trait may be little influenced by the environment, as it exhibits high heritability and is influenced by three to five genes with large effects (Robbins & Ashman, 1984; Lu *et al.*, 2003). Babu *et al.* (2006), mapping quantitative trait loci (QTLs) for PE, identified a total of four QTLs distributed on chromosomes 1, 3, 8, and 10, explaining together 62% of the phenotypic variation.

To perform the selection of individuals under specific environmental conditions, it is initially interesting to understand the magnitude of genetic and environmental influence on the phenotypic variation of the main traits of interest (Falconer & Mackay, 1996). Thus, the coefficient of genotypic determination ($\hat{H}^2$) was estimated, which reveals the proportion of phenotypic variance due to genotypic variability among genotype means (Cruz *et al.,* 2014). High estimates of $\hat{H}^2$ ($\hat{H}^2$ > 0.96) were found for both traits in both N levels (Table 3). This result indicates that genetic effects were predominant over residual effects, suggesting promising prospects for genetic gains in the two key economically important popcorn traits through a phenotypic selection of superior individuals in both N availability conditions.

It is often reported in maize experiments conducted in contrasting N availability environments that heritability estimates, a parameter similar to the coefficient of genotypic determination, are lower in stressful environments due to lower precision under those experimental conditions (Bänziger *et al.*, 1997; Gallais & Coque, 2005; Soares *et al.*, 2011; Heinz *et al.*, 2019; Ertiro *et al.*, 2020). However, the estimates of $\hat{H}^2$ were similar in conditions with and without N stress (Table 3). Similar results were reported by Presterl *et al.* (2003), Rodrigues *et al.* (2017), and Ertiro *et al.* (2017).

The coefficient estimates of experimental variation (CV_e) obtained under N stress were higher than those observed under conditions of high N availability for GY and PE, as shown in Table 3. This increase in CVe due to N stress is consistent with findings from other studies on corn (Bänziger *et al.,* 1997; Santos *et al.,* 1998; Guedes *et al.,* 2015; Almeida *et al.,* 2018; Torres *et al.,* 2019) reported in the literature. One possible explanation for this difference in CV_e is that in environments with a high supply of N, soil deficiencies are corrected through fertilization. However, in environments where this nutrient is not provided, soil fertility issues may arise, leading to greater environmental variation. Additionally, under stress conditions, the average values are generally lower, which can result in a higher estimate of the coefficient of experimental variation (Badu-Apraku *et al.,* 2013).

The variation index ($\hat{I}_v$) is an important parameter as it provides breeders with a meaningful understanding of the trait's status for the breeding program. This index indicates the proportion of genetic effects relative to the experimental error. According to Vencovsky (1987), this estimate should approach or exceed a value of one. For all traits in both nitrogen conditions, we observed $\hat{I}_v$ values higher than one, indicating that the genetic effects were larger than the experimental error. Specifically, under HN condition, the Iv values were 46% and 19% higher than those obtained under LN condition for GY and PE, respectively (Table 3). These results suggest that despite the higher experimental errors in the low N environment, it is still feasible to successfully select popcorn inbred lines with high agronomic potential for both levels of N availability in the soil.

The identification of genotypes that are adaptable to environments with a lower supply of N fertilizers and show positive responses to potential environmental improvements is a crucial objective of breeding programs focused on developing NUE cultivars. In this regard, a scatter plot was created to classify the inbred lines based on their performance at contrasting levels of soil N availability

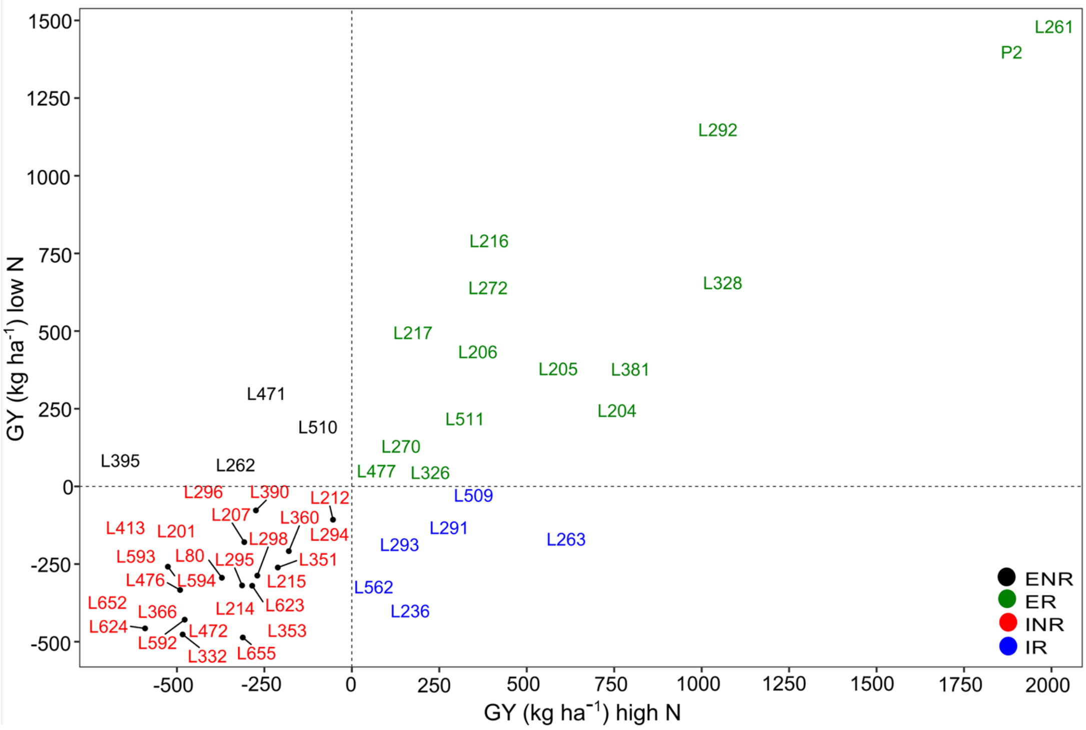

Figure 2: Classification of 51 popcorn inbred lines for efficiency and responsiveness to the N use. ENR: efficient and non-responsive; ER: efficient and responsive; INR: inefficient and non-responsive; and IR: inefficient and responsive.

(Figure 2).

Among the analyzed inbred lines, the following genotypes were classified as efficient and responsive (ER) since they exhibited mean grain yield values above the means of environments with low and high nitrogen availability: L261, P2, L292, L216, L328, L272, L217, L206, L205, L381, L204, L511, L270, and L326 (Figure 2). Therefore, these genotypes are noteworthy as potential sources of favorable alleles for improving grain yield under high and low nitrogen conditions.

The inbred lines L263, L509, L291, L293, L562, and L236 showed specific adaptation to the HN environment, that is, they were inefficient and responsive to the use of N since they yielded below the overall average of LN and increased productivity when N was applied. On the other hand, it was observed in lines L510, L262, L471, and L395, which showed means whose deviations were positive with respect to the average of the LN environment and negative with respect to the average of the HN environment, thus demonstrating potential adaptative to the stress condition (Figure 2).

It was observed that 51% of the evaluated inbred lines exhibited grain yield (GY) averages below the overall average of both high and low N availability environments. Consequently, these lines were classified as inefficient and non-responsive (INR) to nitrogen use (Figure 2). Therefore, these genotypes are considered unfavorable for inclusion as parents in breeding programs aimed at improving grain yield under N stress conditions.

We would like to highlight that inbred line P2 was classified as efficient and responsive (ER), while inbred line L80 was classified as inefficient and non-responsive (INR) (Figure 2). This classification aligns with the findings of Santos *et al.* (2017) who evaluated 29 popcorn lines in a field study with two contrasting N levels.

Similarly, Khan *et al.* (2020), evaluating the same inbred lines (P2 and L80) and the F1 hybrid resulting from their cross, in experiments with different doses of nitrogen

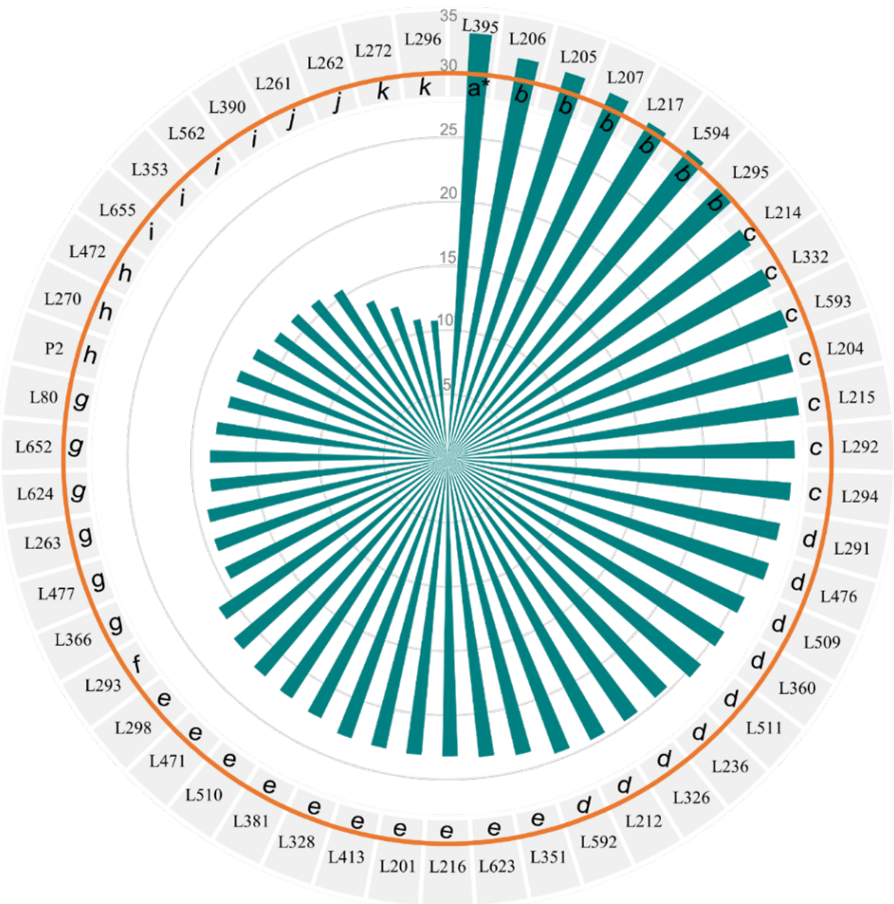

Figure 3: Means clustering of popping expansion ($mL.g^{-1}$) of popcorn inbred lines evaluated.*Bars followed by the same letters belong to the same group according to Scott-Knott test at 5% probability.

and two vegetative stages (V6 and VT), observed the superiority of the hybrid and the P2 line over L80 in terms of efficiency in N uptake, utilization, and use. This superiority was independent of the N dose and the vegetative stage at which the plants were evaluated. Therefore, the results obtained in the present study confirm the contrasting performance of these two inbred lines regarding N use and, at the same time, demonstrate the reliability of the discrimination made among the set of genotypes in terms of N use.

The development of hybrids and/or breeding populations of popcorn adapted to environments with and without nitrogen supply through cross between superior inbred lines selected for their performance *per se* in N use, can constitute a feasible selection strategy, especially when there is a greater influence of additivity in NUE expression.

Santos *et al.* (2019) studied the inheritance and genetic effects involved in the efficiency and responsiveness of N use based on grain yield in popcorn. These authors found that additive gene effects were more important than dominance effects in the expression of NUE in popcorn. Therefore, selecting superior inbred lines for NUE will contribute to obtaining progenies with superior performance. In addition to these results, these authors reported that extrachromosomal genes can influence the expression of NUE in popcorn, as significant reciprocal effects were observed for grain yield. Therefore, the prior evaluation of inbred lines for nitrogen use efficiency allows the breeder to better plan the composition of mating blocks, as superior lines for NUE should be restricted as female parents.

In corn, NUE is usually evaluated considering only grain yield as the main trait. However, in popcorn breeding programs aimed at improving adaptation to low N availability environments, special attention should also be given to popping expansion, as it is the main quality characteristic of the crop. Selecting NUE genotypes associated with high popping expansion (PE), however, is not an easy task. This challenge is well known historically, as grain yield and popping expansion are negatively correlated (Dofing *et al.*, 1991; Daros *et al.*, 2004; Rangel *et al.*, 2011; Cabral *et al.*, 2016). Therefore, popping expansion should be carefully evaluated to select NUE genotypes that simultaneously perform satisfactory for PE.

The selection of inbred lines with high means for popping expansion (PE) is an important criterion to be adopted in popcorn breeding programs, as additive effects are the main components of genetic variation for this trait (Larish & Brewbaker, 1999; Pereira & Amaral Junior, 2001; Scapim *et al.*, 2006). Thus, it is expected that hybrids with high PE can be obtained by crossing superior inbred lines, as in this situation, the F1 mean is equal to the mean of the parents (Lyerly, 1942; Cruz *et al.*, 2014). In this context, for the present study, inbred lines that exhibited PE values equal to or higher than 30 $mL.g^{-1}$ were considered desirable, as a popcorn cultivar to be released should have a minimum volume expansion of 30 $mL.g^{-1}$ (Matta & Viana, 2001).

The average performance for popping expansion (PE) of the set of inbred lines, considering the average of HN and LN, is shown in Figure 3. It was observed that the PE means varied from 10.8 (L296) to 33.2 $mL.g^{-1}$ (L395), forming 11 distinct mean groups, indicating significant genetic variability that can be further explored. Among the inbred lines classified as efficient and responsive (ER) to N use (Figure 3), only L205, L206, and L217 displayed means higher than 30 $mL.g^{-1}$ for PE. Furthermore, the inbred line L395, despite being non-responsive to N, exhibited adaptation to LN environments. This adaptation led to higher efficiency in N use, associated with a higher mean popping expansion (33.2 $mL.g^{-1}$).

Therefore, these genotypes emerge as promising sources of favorable alleles for both NUE and popping expansion, making them excellent candidates for inclusion as parents in breeding programs aimed at developing superior cultivars for these traits. These findings are consistent with the results reported by Santos *et al.* (2017), who also suggested the feasibility of selecting inbred lines of popcorn that exhibit efficient N use and high popping expansion simultaneously.

It is worth noting that the aforementioned lines identified as superior for NUE and popping expansion were derived from the testcross hybrid IAC-125 (L205, L206, and L217) and the open-pollinated variety SAM (L395), which are cultivars known for their good agronomic performance. These cultivars likely contributed favorable alleles for grain yield and popping expansion in the process of developing their respective inbred lines.

Analyzing the genealogical context along with the performance of the lines regarding N use and popping expansion, different strategies of gene recombination can be adopted to develop hybrids and/or breeding populations that simultaneously express the two main economically important traits for the crop, GY, and PE. In this scenario, crossing L395, which showed merit for both PE and GY in LN, and belongs to a distinct gene pool from the other elite lines (L205, L206, and L217), holds high potential for heterosis exploitation and, consequently, generating superior hybrids in terms of NUE and popping expansion.

CONCLUSIONS

Within the panel of evaluated popcorn inbred lines, it was possible to identify sources of favorable alleles for nitrogen use efficiency and popcorn expansion, highlighting the inbred lines L205, L206, L217, and L395. These genotypes emerge as potential parents to be included in mating blocks for the development of hybrids and/or breeding populations with high nitrogen use efficiency and popping expansion.

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REFERENCES

Abendroth L, Elmore RW, Boyer MJ & Marlay SK (2011) Corn growth and development. Ames, Iowa State University. 50p.

Almeida VC, Viana JMS, Oliveira HM, Risso LA, Ribeiro AFS & DeLima RO (2018) Genetic diversity and path analysis for nitrogen use efficiency of tropical popcorn (*Zea mays* ssp. everta) inbred lines in adult stage. Plant Breeding, 137:839-847.

Babu R, Nair SK, Kumar A, Rao HS, Verma P, Gahalain A, Singh IS & Gupta HS (2006) Mapping QTLs for popping ability in a popcorn x flint corn cross. Theoretical Applied Genetics, 112:1392-1399.

Badu-Apraku B, Oyekunle M, Fakorede MAB, Vroh I, Akinwale RO & Aderounmu M (2013) Combining ability, heterotic patterns and genetic diversity of extra-early yellow inbreds under contrasting environments. Euphytica, 192:413-433

Bänziger M, Betrán FJ & Lafitte HR (1997) Efficiency of High-Nitrogen Selection Environments for Improving Maize for Low-Nitrogen Target Environments. Crop Science, 37:1103-1109.

Bolaños J & Edmeades GO (1996) The importance of the anthesis - silking interval in breeding for drought tolerance in tropical maize. Field Crops Research, 48:65-80.

Cabral PDC, Amaral Júnior AT, Freitas ILJ, Ribeiro RM & Silva TRC (2016) Cause and effect of quantitative characteristics on grain expansion capacity in popcorn. Revista Ciência Agronômica, 47:108-117.

Ciampitti IA & Vyn TJ (2012) Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: A review. Field Crops Research, 133:48-67.

Cruz CD (2013) GENES - a software package for analysis in experimental statistics and quantitative genetics. Acta Scientiarum Agronomy, 35:271-276.

Cruz CD, Carneiro PCS & Regazzi AJ (2014) Modelos biométricos aplicados ao melhoramento genético. Viçosa, Editora UFV. 668p.

Daros M, Amaral Júnior AT, Pereira MG, Santos FS, Scapim CA, Freitas Júnior SP, Daher RF & Ávila MR (2004) Correlações entre caracteres agronômicos em dois ciclos de seleção recorrente em milho-pipoca. Ciência Rural, 34:1389-1394.

Dofing SM, D'Croz-Mason N & Buck JS (1991) Inheritance of expansion volume and yield in two popcorn x dent corn crosses. Crop Science, 31:715-718.

Ertiro BT, Beyene Y, Das B, Mugo S, Olsen M, Oikeh S, Juma C, Maryke L & Prasanna BM (2017) Combining ability and testcross performance of drought-tolerant maize inbred lines under stress and nonstress environments in Kenya. Plant Breeding, 136:197-205.

Ertiro BT, Olsen M, Das B, Gowda M & Labuschagne M (2020) Efficiency of indirect selection for grain yield in maize (*Zea mays* L.) under low nitrogen conditions through secondary traits under low nitrogen and grain yield under optimum conditions. Euphytica, 216:134-145.

Falconer DS & Mackay TFC (1996) Introduction to quantitative genetics. 4ª ed. London, Longman Group. 480p.

FAO - Food and Agriculture Organization of the United Nations (2019) World fertilizer trends and outlook to 2022. Available at: <http://www.fao.org/3/ca6746en/CA6746EN.pdf?eloutlink=imf2fao>. Accessed on: December 3rd, 2020.

FritscheNeto R, Miranda GV, DeLima RO, Souza LV & Silva J (2010) Herança de caracteres associados à eficiência de utilização do fósforo em milho. Pesquisa Agropecuária Brasileira, 45:465471.

Gallais A & Coque M (2005) Genetic variation and selection for nitrogen use efficiency in maize: a synthesis. Maydica, 50:531-547.

Guedes FL, Ferreira Junior EJ, Castro CEC, Pereira CH, Prado PER & Souza JC (2015) The behavior of maize hybrids generated from contrasting progênies regarding the use of nitrogen. Acta Scientiarum. Agronomy, 37:45-50.

Hallauer AR, Carena MJ & Miranda Filho JB (2010) Quantitative Genetics in Maize Breeding. 3ª ed. New York, Springer. 663p.

Heinz R, Ribeiro LP, Gonçalves MC, Bhering LL & Teodoro PE (2019) Selection of maize top-crosses for different nitrogen levels through specific combining ability. Bragantia, 78:208-214.

Hirel B, Le Gouis J, Ney B & Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. Journal of Experimental Botany, 58:2369-2387.

Khan S, Amaral Júnior AT, Ferreira FRA, Kamphorst SH, Gonçalves GMB, Freitas MSM, Silveira V, Souza Filho GA, Amaral JFT, Smith REB, Hussain KI, Vivas JMS, Souza YP & Peçanha DA (2020) Limited Nitrogen and Plant Growth Stages Discriminate Well Nitrogen Use, Uptake and Utilization Efficiency in Popcorn. Plants, 9:893.

Köppen W (1948) Climatologia: conun estúdio de los climas de La Tierra. México, Fondo de Cultura Econômica. 479p.

Larish LLB & Brewbaker JL (1999) Diallel analyses of temperate and tropical popcorns. Maydica, 44:279-284.

Lu HJ, Bernardo R & Ohm HW (2003) Mapping QTL for popping expansion volume in popcorn with simple sequence repeat markers. Theoretical Applied Genetics, 106:423-427.

Lyerly PJ (1942) Some genetic and morphological characters affecting the popping expansion of popcorn. Journal American Society of Agronomy, 34:986-995.

Matta PP & Viana JMS (2001) Testes de capacidade de expansão em programas de melhoramento de milho-pipoca. Scientia Agricola, 58:845-851.

Pereira MG & Amaral Júnior AT (2001) Estimation of Genetic Components in Popcorn Based on the Nested Design. Crop Breeding Applied Biotechnology, 1:03-10.

Presterl T, Seitz G, Landbeck M, Thiemt EM, Schmidt W & Geiger HH (2003) Improving nitrogen-use efficiency in European maize: Estimation of quantitative genetic parameters. Crop Science, 43:1259-1265.

Rangel MM, Amaral Júnior AT, Gonçalves LSA, Freitas Júnior SP & Candido LS (2011) Análise biométrica de ganhos por seleção em população de milho pipoca de quinto ciclo de seleção recorrente. Revista Ciência Agronômica, 42:473-481.

Robbins WA & Ashman RB (1984) Parent-offspring popping expansion correlations in progeny of dent corn x popcorn and flint corn x popcorn crosses. Crop Science, 24:119-121.

Rodrigues MC, Rezende WM, Silva MEJ, Faria SV, Zuffo LT, Galvão JCC & DeLima RO (2017) Genotypic variation and relationships among nitrogen-use efficiency and agronomic traits in tropical maize inbred lines. Genetic and Molecular Research, 16:gmr16039757.

Santos A, Amaral Júnior AT, Fritsche-Neto R, Kamphorst SH, Ferreira FRA, Amaral JFT, Vivas JMS, Santos PHAD, Lima VJ, Khan S, Schmitt KFM, Leite JT, Junior DRS, Bispo RB, Santos TO, Oliveira UA, Guimarães LJM & Rodriguez O (2019) Relative importance of gene effects for nitrogen-use efficiency in popcorn. PLoS One, 14:e0222726.

Santos A, Amaral Júnior AT, Kurosawa RNF, Gerhardt IFS & Fritsche-Neto R (2017) GGE Biplot projection in discriminating the efficiency of popcorn lines to use nitrogen. Ciência Agrotecnologia, 41:22-31.

Santos A, Amaral Júnior ATD, Kamphorst SH, Gonçalves GMB, Santos PHAD, Vivas JMS, Mafra GS, Khan S, Oliveira FTD, Schmitt KFM, Santos Junior DRD & Mora F (2020) Evaluation of popcorn hybrids for nitrogen use efficiency and responsiveness. Agronomy-Basel, 10:485.

Santos MX, Guimarães PEO, Pacheco CAP, França GE, Parentoni SN, Gama EEG & Lopes MA (1998) Improvement in the synthetic elite NT for soils with low nitrogen content. Pesquisa Agropecuária Brasileira, 33:55-61.

Serna-Saldivar SO (2022) Popcorn and Other Puffed Grains. In: Serna-Saldivar SO (Ed.) Snack Foods: Processing, Innovation, and Nutritional Aspects. Boca Raton, CRC Press. p.201-220.

Scapim CA, Pinto RJB, Amaral Júnior AT, Mora F & Dandolini TS (2006) Combining ability of white grain popcorn populations. Crop Breeding Applied Biotechnology, 6:136-143.

Scott AJ & Knott M (1974) Cluster Analysis Method for Grouping Means in the Analysis of Variance. Biometrics, 30:507-512.

Sharma LK & Bali SK (2017) A Review of Methods to Improve Nitrogen Use Efficiency in Agriculture Sustainability, 10:01-23.

Silva TRC, Amaral Júnior AT, Almeida Filho JE, Freitas MSM, Guimarães AG & Kamphorst SH (2019) Contrasting phosphorus environments as indicators for popcorn breeding lines. Functional Plant Breeding Journal, 1:01-15.

Soares MO, Miranda GV, Guimarães LJM, Marriel IE & Guimarães CT (2011) Parâmetros genéticos de uma população de milho em níveis contrastantes de nitrogênio. Revista Ciência Agronômica, 42:168-174.

Taiz L, Zeiger E, Moller IM & Murphy A (2017) Fisiologia e desenvolvimento vegetal. 6ª ed. Porto Alegre, ArtMed. 954p.

Torres LG, Caixeta DG, Rezende WM, Schuster A, Azevedo CF, Silva FF & DeLima RO (2019) Genotypic variation and relationships among traits for root morphology in a panel of tropical maize inbred lines under contrasting nitrogen levels. Euphytica, 215:01-18.

Vencovsky R (1987) Herança quantitativa. In: Paterniani E & Viegas GP (Eds.) Melhoramento e produção do milho. Campinas, Fundação Cargill. p.135-214.