

Genetic control of agronomic efficiency of nitrogen use in maize

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Abstract: *The aim was to study the genetic control of the agronomic efficiency at low N availability in maize. Experiments were conducted in seven environments in a square lattice design with 49 hybrids, consisting of 48 crosses and one commercial hybrid, evaluated with and without application of N in top dressing. Grain yields with (GYHN) and without (GYLN) application of nitrogen in top dressing were assessed, and these traits were used to calculate the agronomic efficiency at low N availability (AELN) and the harmonic mean of the relative performance (HMRP). According to the joint analysis of variance of the diallel crosses and the estimates of the general and specific combining abilities, these traits were highly significant. Note that in general AELN was controlled by non-additive genetic effects and the hybrids H7, H3, H47 and H10 and the lines IG3-2, IG3-1, IG4-5, IG4-1 and IG4-6 show higher grain yield without top dressing.*

Keywords: *Additive and non-additive genes, general combining ability, specific combining ability, diallel crosses, nitrogen use efficiency*

INTRODUCTION

Maize (*Zea mays* L.) crop is of great economic and agricultural importance due to its diverse uses, such as in animal feed and biofuels, and it is increasingly present in human nutrition, considered one of the basic foods for food security of the population (Rahmawati et al. 2021). One of the nutrients most required in growing maize is nitrogen (N), which is supplied through nitrogen fertilizers. Global efficiency in N use in agricultural production is currently in the range of 40-50% (Cassman and Dobermann 2022). However, high provision of nitrogen fertilizers increases production costs and intensifies environmental contamination through processes such as leaching, denitrification, and volatilization, increasing greenhouse gases through emission of N₂O (Xiao et al. 2019).

One of the challenges of modern agriculture is to combine high yields with sustainable agriculture, i.e., selecting high performance cultivars that have high nutrient use efficiency. Nutrient use efficiency is defined as the ability of a genotype to obtain high yields even under conditions of low availability of nutrients (Graham 1984). High yield and low nitrogen requirements could be achieved by selecting genotypes improved for nitrogen use efficiency.

It is known that the processes of uptake, transport, and redistribution of nutrients are under genetic control and that it is necessary to optimize physiological and biochemical processes for grain formation (Prado 2020). Thus, given a

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high genetic variability in maize for nitrogen use efficiency (Bertin 2000), Gallais and Hirel (2004) showed that there is a different physiological response, in which stressful environments lead to expression of specific genes in N remobilization. Studies of the genetic control of traits can be performed through diallel analysis, which allows the effects of additive and non-additive genes to be known through general combining ability and specific combining ability, respectively. In light of that, the aim of this research was to study genetic control of agronomic efficiency at low N availability in maize.

MATERIAL AND METHODS

The experiments were conducted in seven environments, characterized by the combination of locations, crop seasons, and crop years (Table 1). These experiments were set up in two locations: the first, the São João da Montanha Farm belonging to ESALQ-USP in Piracicaba, SP, Brazil, at the Department of Genetics (lat 22° 42' 24" S, long 47° 38' 14.4" W, alt 535 m asl) and Sertãozinho (lat 22° 43' 2.8" S, long 47° 36' 33.8" W, alt 595 m asl), and the second, the Teaching, Research, and Extension Farm (Fazenda de Ensino, Pesquisa e Extensão - FEPE) (lat 21° 14' 53" S, long 48° 17' 10" W, alt 563 m asl) belonging to UNESP-Jaboticabal in Jaboticabal, SP, Brazil. Experiments were conducted in the summer crop season (1st season) in the 2013/2014 and 2014/2015 years, and the second crop season (2nd season) in the 2013/2014 year.

The experimental stations of ESALQ-USP have soils characterized as Nitisol and Acrisol, with a Cfa climate transitioning toward Cwa (Köppen 1918), mean annual temperature of 22 °C, and mean annual rainfall of 1,275 mm. The experimental station of UNESP has soil characterized as a Ferralsol, with mean annual temperature of 22.3 °C and mean annual rainfall of 1,423.9 mm.

In this study, 49 maize hybrids were used, consisting of 48 single-cross hybrids developed by partial diallel crosses plus one commercial hybrid (DKB 390). The crosses were carried out in a partial diallel scheme, with eight and six inbred lines (S_6) extracted from the synthetic varieties IG-3 and IG-4, respectively. These synthetic varieties were developed by the maize breeding program of the Department Genetics of ESALQ/USP through one cycle of reciprocal recurrent selection with high selection intensity carried out in the BR-105 and BR-106 populations, which belong to distinct heterotic groups (Souza Júnior et al. 1993, Rezende and Souza Júnior 2000). The genealogies of these hybrids are shown in Figure 1.

Two experiments were set up in each environment of the study: one with (HN) and another without (LN) application of nitrogen in top dressing. The two experiments were set up simultaneously and followed the same crop practices; the conventional soil tillage and basal fertilization were those recommended for each environment. The source of nitrogen in top dressing in the HN experiments was urea, with 170 kg N ha⁻¹, applied between the V3 and V4 stages of plant development. Supplemental irrigation was used only in the Experimental Station of the Department of Genetics.

Both experiments (HN and LN) were sown on the same date and conducted in the same way regarding basal fertilization, soil preparation, planting, and weed, disease and pest management as recommended for the crop according to EMBRAPA (2012), differing only in top dressing, as there was no top dressing in the LN experiment.

A 7 × 7 square lattice experimental design was used, with two replications. Each plot consisted of one 4-m long row, with a spacing of 0.8 m between rows and 0.20 m between plants. Thus, 20 plants remained in the plot after thinning, representing a population of 62,500 plants ha⁻¹.

Grain yield was assessed by weighing the grains shelled from the ears harvested in each plot. Also, plant stand was evaluated by counting the number of plants in each plot at the harvest time, as well as grain moisture at harvest,

Table 1. Descriptions of the experimental environments

Environment	Acronym	Crop year	Crop season	Municipality - Experimental station
1	1E1314	2013/2014	1st	Piracicaba – Department of Genetics
2	1S1314	2013/2014	1st	Piracicaba - Sertãozinho
3	1U1314	2013/2014	1st	Jaboticabal - FEPE
4	2U1314	2013/2014	2nd	Jaboticabal - FEPE
5	1E1415	2014/2015	1st	Piracicaba – Department of Genetics
6	1S1415	2014/2015	1st	Piracicaba - Sertãozinho
7	1U1415	2014/2015	1st	Jaboticabal - FEPE

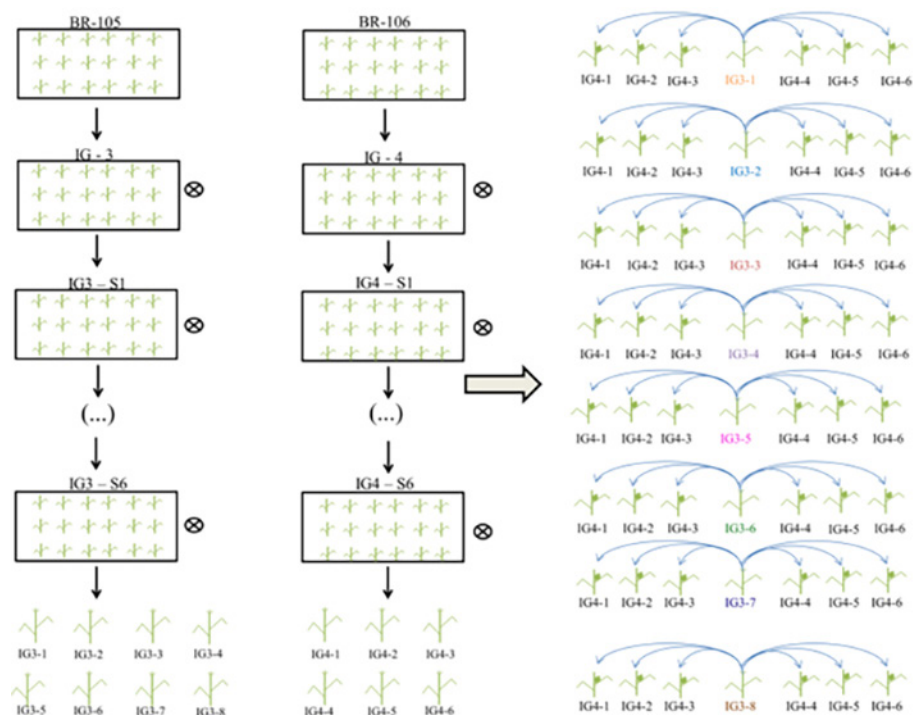


Figure 1. Flowchart of obtaining the six and eight lines and partial diallel crosses from the forty-eight single-cross hybrids.

measured in a grain sample from each plot. Grain yield was corrected to 13% moisture, adjusting it to the mean stand by covariance and converting it to $t\ ha^{-1}$.

The agronomic efficiency at low N availability (AELN) was computed by the following expression, adapted from Santos et al. (2019):

$$AELN = GYLN^2 / (GYHN * GYLN)$$

Where $GYLN$ is the grain yield without and $GYHN$ is the grain yield with application of nitrogen in top dressing.

The harmonic mean of the relative performance (HMRP) allows simultaneous observation of stability, adaptability, and yields of the hybrids (Resende 2007) and was estimated by the following expression (Resende 2007, Santos et al. 2019):

$$HMRP = \left\{ 2 / \left[\left(\frac{GYHN}{\bar{X}_{HN}} \right)^{-1} + \left(\frac{GYLN}{\bar{X}_{LN}} \right)^{-1} \right] \right\}$$

Where \bar{X}_{HN} and \bar{X}_{LN} are the overall mean of grain yield of the hybrids assessed with and without application of nitrogen in top dressing, respectively.

Through the values of the expected mean squares of the joint analyses of variance, the following values were estimated: phenotypic variance on a mean basis (σ_F^2), variance of the hybrid by environment interaction ($\hat{\sigma}_{H \times E}^2$), residual variance (σ^2), genetic quadratic component of the hybrids (\hat{V}_H), and genotypic coefficient of determination of the hybrids on a mean basis (H_H^2), in percentage, by the expressions:

$$\sigma_F^2 = [MS_H / (r * e)]$$

$$\sigma_{H \times E}^2 = [(MS_{H \times E} - MS_R) / r]$$

$$\sigma^2 = MS_R$$

$$\hat{V}_H = \{ [(MS_H - MS_{H \times E}) / r * e] \}$$

$$H_H^2 (\%) = (\hat{V}_H / \sigma_F^2) * 100$$

Where MS_H is the hybrid mean square; $MS_{H \times E}$ is the hybrid by environment interaction mean square; MS_R is the residual mean square; r is the number of replications; and e is the number of environments.

Diallel analysis was carried out according to method 4, model I of Griffing (1956), adapted to partial diallel crosses (Geraldi and Miranda Filho 1988) in multiple environments (Ferreira et al. 1993), with the following mathematical model:

$$Y_{ije} = \mu + g_i + g_j + s_{ij} + a_e + (ga)_{ie} + (ga)_{je} + (sa)_{ije} + \bar{\epsilon}_{ije}$$

Where Y_{ije} is the mean value of the hybrid from crosses between lines i and j in environment e ; μ is the overall mean; g_i is the general combining ability of line i developed from population IG-3 ($i = 1, 2, \dots, 8$); g_j is the general combining ability of line j developed from population IG-4 ($j = 1, 2, \dots, 6$); s_{ij} is the effect of specific combining ability of the cross between lines i and j ; a_e is the effect of environment e ($e = 1, 2, \dots, 7$); $(ga)_{ie}$ is the interaction of the general combining ability of line i with environment e ; $(ga)_{je}$ is the interaction of general combining ability of line j with environment e ; $(sa)_{ije}$ is the effect of the interaction of the specific combining ability of the cross of lines i and j with environment e ; and $\bar{\epsilon}_{ije}$ is the mean error.

The relative contributions of the general combining ability (GCA) and of the specific combining ability (SCA) were estimated by the following expressions, respectively:

$$GCA(\%) = [(SS_{GCA(IG3)} + SS_{GCA(IG4)} / SS_{GCA(IG3)} + SS_{GCA(IG4)} + SS_{SCA}) * 100]$$

$$SCA(\%) = [(SS_{SCA} / SS_{GCA(IG3)} + SS_{GCA(IG4)} + SS_{SCA}) * 100]$$

Where $SS_{GCA(IG3)}$ and $SS_{GCA(IG4)}$ are the sum of squares of the general combining abilities of the inbred lines from the IG-3 and IG-4 populations, respectively, and SS_{SCA} is the sum of squares of the specific combining ability.

The estimates of the general combining abilities of the inbred lines from IG-3 (\hat{g}_i) and IG-4 (\hat{g}_j) populations and the specific combining abilities (\hat{s}_{ij}) were computed by:

$$\hat{g}_i = \{(Y_i/N_1) - [Y_t/N_1 * N_2]\}$$

$$\hat{g}_j = \{(Y_j/N_2) - [Y_t/N_1 * N_2]\}$$

$$\hat{s}_{ij} = \{Y_{ij} - (Y_i/N_1) - (Y_j/N_2) + [Y_t/(N_1 * N_2)]\}$$

Where Y_{ij} is the value of the hybrid developed from cross of lines i and j ; Y_t is the sum of all hybrids; Y_i is the sum of the combinations of line i with lines j ; Y_j is the sum of the combinations of line j with lines i ; N_1 is the number of lines i ; and N_2 is the number of lines j .

The diallel analyses were performed in R software (R Core Team 2019) through the “lm()” function, and the figures were generated by the “ggplot2” package (Wickham 2011).

RESULTS AND DISCUSSION

There was a significant effect of the general combining ability on the agronomic efficiency at low N availability for the lines of group IG4 (Table 2). In addition, both groups (IG3 and IG4) showed significance for GCA for grain yield with and without application of N in top dressing and HMRP. General combining abilities are function of additive effects, so inbred line selection for this trait could be efficient.

The effects of hybrids and hybrid by environment interaction were significant ($p < 0.01$) for all variables, thus showing that there is at least one hybrid with different behavior from the others and that hybrids behave differently in relation to environments (Table S1). For example, in environments 1, 4, 6 and 7 the AELN was not significant, whereas in environments 2, 3, and 5 the AELN was significant at $p < 0.01$ (Table S2).

The hybrids and the interaction of the hybrids with the environments were highly significant ($p < 0.01$) for grain yield when considering the environments with and without application of nitrogen in top dressing (Table S3). When the experiments were analyzed separately, there was only significance in the interaction between hybrid and environment for yield in experiments 3, 5 and 6 (Table S4). Biplot analysis was considered to rank the environments, which made it possible to observe that the conditions with and without N in top dressing are far

Table 2. Analysis of variance for diallel hybrids with the mean squares and respective significance for the traits of grain yield without (GYLN) and with (GYHN) application of nitrogen in top dressing in t ha⁻¹, agronomic efficiency at low N availability (AELN), and harmonic mean of the relative performance (HMRP)

SV	df	GYLN	GYHN	AELN	HMRP
GCA IG3	7	4.04**	15.09**	0.072	0.16**
GCA IG4	5	10.96**	26.12**	0.153**	0.34**
SCA	35	2.22**	2.10**	0.074*	0.035**
GCA IG3* ENV	42	4.70**	2.15**	0.091**	0.055**
GCA IG4* ENV	30	3.20**	1.62*	0.085**	0.050**
SCA* ENV	210	2.04**	1.26	0.071**	0.025**
REP	1	0.82	0.63	0.066	0.066
REP*ENV	12	240.92**	246.99**	2.72**	4.73**
Residual	329	1.24	1.07	0.044	0.015
GCA (%)		52	76	33	70
SCA (%)		48	24	67	30

** and * significant at 1%, 5%, and non-significant probability, respectively, by the F test.

from the ideal environment (arrow and circle), confirming that, despite changing only the application of N, they are contrasting environments (Figure S1).

There was a significant effect of the hybrids × environment interaction, indicating that there was differentiated expression of GCA for the lines of both groups in the different environments when evaluated with and without N application. The differential response of the hybrids and of the lines regarding N use efficiency in different locations is noteworthy, and it indicates a favorable situation for use of the selection tool (Table 2).

Specific combining ability (SCA) was significant for all the traits (Table 2). Specific combining ability is related to the non-additive genes (dominance and epistasis) and is important for obtaining hybrids and exploiting heterosis (Hallauer et al. 2010). In general, N use efficiency is controlled by non-additive genetic effects and, in that scenario, the use of heterosis is recommended with synthesis of hybrids through the groups evaluated.

HMRP was significant for all sources of variation, so it is understood that there is a variation of this trait in the hybrids. By means of the harmonic mean of the relative performance (HMRP) it is possible to select highly productive hybrids under conditions of high and low availability of N. The index leads to the simultaneous selection of stability and adaptability and can be used for above average and stable yield selection (Santos et al. 2019). It is thus understood that there is a variation of this trait in the hybrids.

GCA for grain production with low nitrogen content is more important than SCA (52% vs. 48%); however, for the high content condition GCA was more important than SCA (76% vs. 24%), showing that for the same trait evaluated under stress and non-stress conditions, the genetic effects were altered, that is, for the LN condition the additive effects are more important than the non-additive ones, the opposite being true for the HN condition. For the AELN trait, SCA is more important than GCA (67% vs. 33%), indicating that non-additive effects are more important than additive effects for its expression; therefore, selection can only be efficiently performed in hybrids (Table 2). These traits, despite being related, behave differently in relation to genetic control.

The phenotypic variance was 0.32 and 0.48 for GYLN and GYHN, respectively. Furthermore, the genetic quadratic component of the hybrids (\hat{V}_H) was 0.37 for GYHN, and the genotypic coefficient of determination was 77.41% and 66.33% for GYHN and HMRP, respectively (Table 3). According to the variance components, it is possible

Table 3. Components of phenotypic variance on a mean basis (σ_F^2), variance of the hybrid by environment interaction ($\sigma_{H \times E}^2$), residual variance (σ^2), genetic quadratic component of the hybrids (\hat{V}_H), and genotypic coefficient of determination of the hybrids on a mean basis (H_H^2)

Components of phenotypic variance	GYLN	GYHN	AELN	HMRP
σ_F^2	0.32	0.48	0.0064	0.007
$\sigma_{H \times E}^2$	0.77	0.32	0.019	0.010
σ^2	0.99	0.89	0.039	0.012
\hat{V}_H	0.14	0.37	0.0008	0.0046
H_H^2 (%)	43.15	77.41	12.36	66.33

GYLN - grain yield without application of nitrogen in top dressing in t ha⁻¹, GYHN - grain yield with application of nitrogen in top dressing in t ha⁻¹, AELN - agronomic efficiency at low N availability, HMRP - harmonic mean of the relative performance

to select hybrids with greater gains from selection for GYHN and HMRP, due to the high values for the coefficient of determination, greater heritability and greater presence of genetic variance, which contribute to the greater additive effect (GCA). These effects are passed on to the progeny, with greater predictability in selection (Hallauer et al. 2010). This, therefore, indicates that it is better to select lines for GYHN and HMRP.

The effect of general combining ability is classified in the graph by the axes, colors, and sizes. The GCA of group IG3 for the GYLN trait, shown on the ordinate axis, was greater for lines IG3-2 and IG3-1, with effects of 2 and 3 t ha⁻¹, respectively (Figure 2a). The lines of greatest contributions to the GYHN trait were IG3-2 and IG3-1, which showed

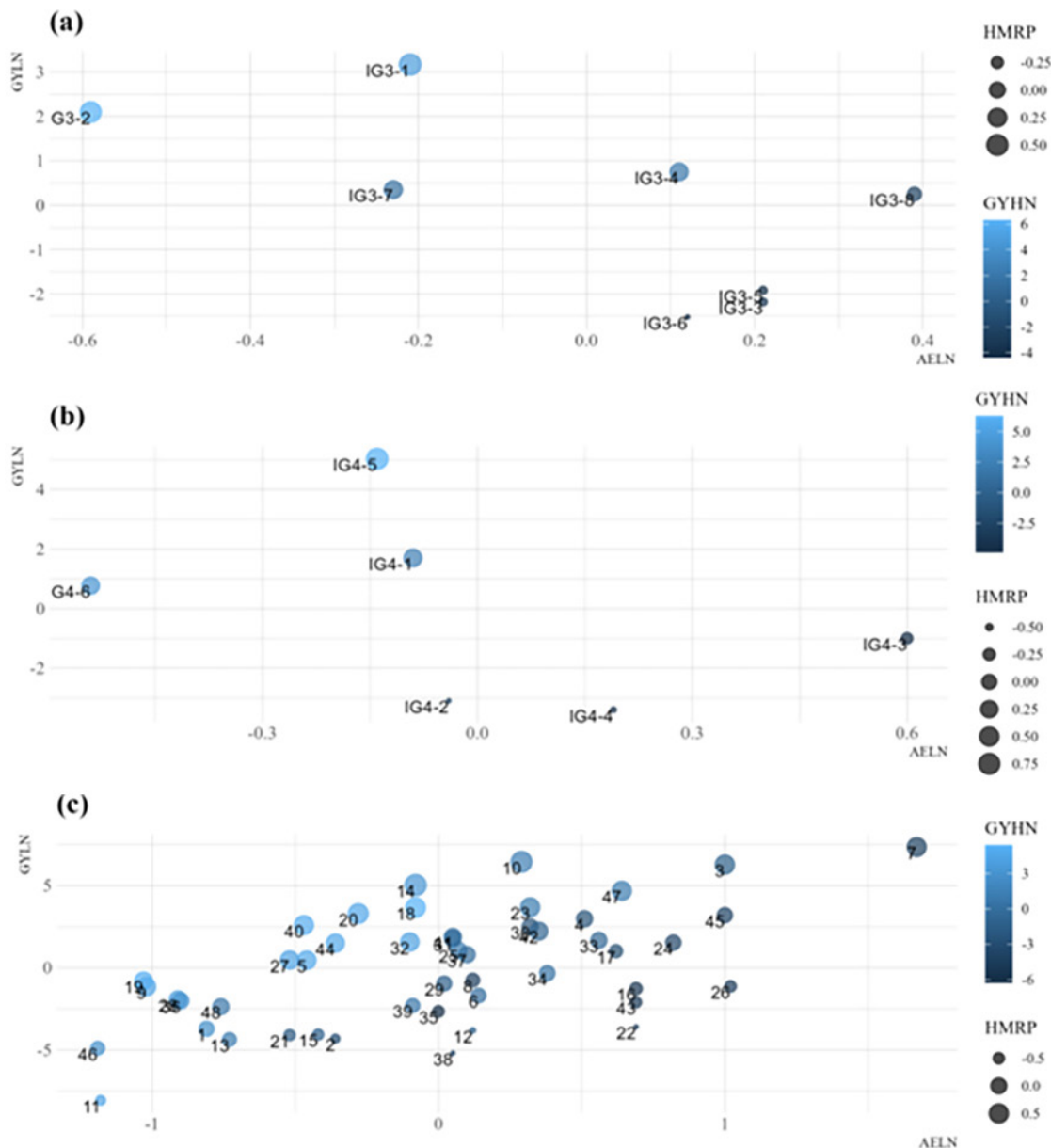


Figure 2. Effect of general combining ability of group IG-3 (A), of group IG-4 (B), and of specific combining ability (C) for the following traits: grain yield without application of nitrogen in top dressing in t ha⁻¹ (GYLN), grain yield with application of nitrogen in top dressing in t ha⁻¹ (GYHN), agronomic efficiency at low N availability (AELN), and harmonic mean of the relative performance (HMRP).

the largest contributions of 6 and 4 t ha⁻¹, respectively. For the HMRP trait, lines IG3-2, IG3-1, IG3-7, and IG3-4 have a greater effect. For AELN, line IG3-8 made the largest contribution, with small contribution to the other traits (Figure 2a).

The effect of general combining ability for IG4 is classified in the graph in the same way – by axes, colors, and sizes. For GYLN, GYHN, and HMRP, the lines of greatest effect were IG4-5, IG4-1, and IG4-6, respectively. Line IG4-3 showed a greater effect on AELN, followed by line IG4-4; nonetheless, these lines made small contributions to the other traits (Figure 2b).

We can make a selection of the hybrids, grouping them in relation to the traits and effects. For greater effects on GYLN, AELN, and HMRP, we can select the hybrids H7, H3, H47, H10, H45, H24, H23, H4, H33, H17, H30, H42, H41, H31, H25 and H37, that is, 33.3% of the hybrids of these crosses. For greater effects on GYLN, GYHN, and HMRP, we can select the hybrids H14, H18, H20, H40, H44, H32, H27 and H5, that is, 16.6% of the hybrids (Figure 2c).

The hybrid IG3-2 × IG4-1 exhibited a contribution to AELN greater than 1.5. Thus, we can understand that the non-additive effects have greater importance for agronomic efficiency of nitrogen use, because there was efficient allelic complementarity. Allelic complementarity occurs when there is dissimilarity between the hybrids and a heterosis effect (Santos et al. 2019), that is, lines IG3-2 and IG4-1 showed negative effects for AELN and, when crossed, they exhibited excellent hybrid vigor. Thus, with greater control of non-additive genes, interpopulation methods should be used in plant breeding programs, with the aim of increasing N use efficiency (DoVale et al. 2012), thus corroborating the method used in this maize breeding program.

Derera and Musimwa (2015), who studied the effects of heterosis on the hybrid, noted that both the additive gene effects and the non-additive gene effects act in the control of grain yield and associated traits, with greater contribution of dominance effects. That study corroborates our results, because the AELN trait is associated with grain yield and with greater effect of the dominance genes.

A study of heterosis for N use efficiency conducted by Li et al. (2022) showed greater yield for hybrids than for lines when there is low availability of nitrogen and showed that the increase in N use efficiency is mainly due to the lower concentration of nitrogen in the grain and increase in internal nitrogen use efficiency, indicating greater efficiency in N transport and in N redistribution during post-silking.

For GYHN and HMRP, additive genetic effects are more important and *per se* selection is recommended for these traits, because there was predominance of GCA (%). The non-additive effects of the genes are important for control of greater agronomic efficiency at low N availability in maize, and hybrid formation is suggested. For GYLN, the additive and non-additive effects are important. Thus, we can select lines IG3-2, IG3-1, IG4-5, IG4-1, and IG4-6 together for GYLN, GYHN, and HMRP. The hybrids IG3-2 × IG4-1 (H7), IG3-1 × IG4-3 (H3), IG3-8 × IG4-5 (H47), and IG3-2 × IG4-4 (H10) exhibited high effects of SCA for GYLN, AELN, and HMRP together, thus indicating that they are efficient and have stability and adaptability for these traits.

The lines IG3-1, IG3-2, IG4-5 and IG4-6 and hybrids 5, 9, 14, 18, 19, 20, 27, 32, 40 and 44 were superior for GYHN, demonstrating that they have increased responsiveness to nitrogen fertilizers. Responses to nitrogen application vary depending on the genotype, the environment and the amount of N available in the soil, and these environmental interferences may affect the genetic control of this response, silencing or expressing different genes in the plant. For this reason, it is important to identify the genetic control of this trait, in order to carry out the selection with greater accuracy.

Additive genetic effects are greater for GYHN and HMRP, indicating the selection of lines; for AELN there was greater importance of non-additive effects, being indicated to obtain hybrids, and for GYLN the additive and non-additive effects are important. Aiming at the characteristics GYLN, GYHN and HMRP, the lines IG3-2, IG3-1, IG4-5, IG4-1 and IG4-6 are indicated and, for GYLN, AELN and HMRP, the hybrids H7, H3, H47 and H10 are indicated.

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