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# Efficiency, responsiveness and stability of maize hybrids under low nitrogen availability conditions

## Eficiência, responsividade e estabilidade de híbridos de milho sob condições de baixa disponibilidade de nitrogênio

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#### Abstract

Growing maize has required ever greater use of nitrogen fertilizers, increasing costs and possible environmental contamination. In this respect, the aim of this study was to characterize single cross maize hybrids under conditions of low and high nitrogen availability to identify them in regard to efficiency, responsiveness, and stability in relation to N. A total of 49 single-cross maize hybrids were evaluated in seven environments in square lattice design. Two experiments were conducted: one with application of nitrogen in top dressing and the other without application. Grain yield without application of nitrogen and grain yield with application of nitrogen were evaluated and, from these estimates, the agronomic efficiency at low N availability and the harmonic mean of the relative performance (HMRP) were obtained. Analysis of variance, the Scott-Knott test, and characterization of the efficiency and responsiveness of the hybrids were carried out. Hybrids with greater stability were identified by HMRP, and 23% of the hybrids were classified as efficient and responsive. Of the hybrids evaluated, 67% can be used to increase nitrogen use efficiency in breeding programs.

Additional keywords: Zea mays L.; agronomic efficiency of nitrogen use; genotype by environment interaction; harmonic mean of the relative performance.

#### Resumo

O cultivo do milho tem exigido cada vez mais o uso de fertilizantes nitrogenados, aumentando os custos e a possível contaminação ambiental. Neste sentido, o objetivo deste estudo foi caracterizar híbridos simples de milho em condições de baixa e alta disponibilidade de nitrogênio para identificá-los quanto à eficiência, responsividade e estabilidade em relação ao N. Um total de 49 híbridos simples de milho foi avaliado em sete

1

ambientes em delineamento em látice quadrado. Foram conduzidos dois experimentos: um com aplicação de nitrogênio em cobertura e outro sem aplicação. A produtividade de grãos sem aplicação de nitrogênio e a produtividade de grãos com aplicação de nitrogênio foram avaliadas e, a partir dessas estimativas, obteve-se a eficiência agronômica com baixa disponibilidade de N e a média harmônica do desempenho relativo (HMRP). Foram realizadas análises de variância, teste de Scott-Knott e caracterização da eficiência e responsividade dos híbridos. Os híbridos com maior estabilidade foram identificados pelo HMRP, e 23% dos híbridos foram classificados como eficientes e responsivos. Dos híbridos avaliados, 67% podem ser utilizados para aumentar a eficiência do uso de nitrogênio em programas de melhoramento.

**Palavras-chave adicionais:** Zea mays L; eficiência agronômica do uso de nitrogênio; interação genótipo por ambiente; média harmônica de desempenho relativo.

#### Introduction

Maize is a cereal crop of great importance, due to the diverse purposes for which it is used, such as for food, fuel and fiber. Brazil is one of the world's largest exporters, since the crop has high adaptability to climate and high genetic variability (Miranda et al., 2021). Brazil is the third largest maize producer in the world, with total production of 115.6 million tons in the 2021/22 crop year; estimates are that 77.2 million tons are consumed domestically and 37.5 million tons are exported (Conab, 2022).

In maize production, one of the nutrients of greatest demand is nitrogen (N), which is supplied by nitrogen fertilizers such as urea, ammonium sulfate, and others. These forms may require transformation to be taken up by plants and there are often N losses (De Laporte et al., 2021). In 2020, approximately 139 million tons of nitrogen fertilizers were used in world agricultural production (FAO, 2020).

The high amounts of nitrogen fertilizers often undergo losses through mediation of processes such as leaching, volatilization, nitrification, denitrification, mineralization, immobilization and mobilization (Wu et al., 2021). There are some ways of decreasing N losses as in biological N fixation, use of fertilizers with slow-release technology, and use of hybrids with greater N use efficiency in their physiological and metabolic processes, decreasing the quantity of inputs and maintaining or increasing yield (Sheoran et al., 2021).

Nutritional efficiency of a genotype is high ability in taking up necessary nutrients in smaller amounts and distributing them more efficiently in the plant (Nasar et al., 2021). Thus, through plant breeding, it is possible to obtain hybrids that differ in nutritional requirements. In characterization of hybrids, evaluated in environments with high and low N content, it is possible to differentiate them regarding efficiency and responsiveness in relation to N. Efficient hybrids have better nutritional efficiency under low N content and responsive hybrids have better nutritional efficiency under high N content (Prado, 2020).

Among the nutritional efficiencies is agronomic efficiency of nitrogen, in which the grain yield capacity is estimated under conditions of high and low N availability. Therefore, the aim of this study was to characterize single cross maize hybrids under conditions of low and high nitrogen availability to identify them in regard to efficiency, responsiveness, and stability in relation to N.

#### Material and methods

The experiments were conducted at Fazenda de Ensino, Pesquisa e Extensão – FEPE at UNESP-Jaboticabal, SP, Brazil - Lat. 21°14'58"S Long. 48°17'11"W - and at the São João da Montanha Farm belonging to ESALQ-USP in Piracicaba, SP, Brazil - Lat.22°42' S Long. 47°38' W- at the Department of Genetics and at Sertãozinho during the 2013/2014 and 2014/2015 1st crop seasons and, in the 2013/2014 crop year, the experiment was also conducted in the second crop season at FEPE. Through the combination of locations, crop seasons, and crop years, seven evaluation environments were considered (Table 1).

Environment	Acronym	Crop Year	Crop Season	Experimental Station
1	1E1314	2013/2014	1st	1
2	1S1314	2013/2014	1st	2
3	1U1314	2013/2014	1st	3
4	2U1314	2013/2014	2nd	3
5	1E1415	2014/2015	1st	1
6	1S1415	2014/2015	1st	2
7	1U1415	2014/2015	1st	3

Table 1. Descriptions of environments of the experiments.

This study involved 49 maize hybrids, consisting of 48 single cross hybrids developed through partial diallel crosses by the maize breeding program of the Department of Genetics of ESALQ/USP (Souza Júnior et al., 1993; Rezende & Souza Júnior, 2000) and one commercial hybrid (DKB 390).

Two experiments were set up in each environment: one with (HN) and the other without application of nitrogen in top dressing (LN). The two experiments were set up simultaneously and followed the same crop practices with the conventional soil fertilization tillage and base 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 - V4 stages of plant development. Supplemental irrigation was used only in the Experimental Station 1 (environment 1 and 5).

A 7 × 7 square lattice experimental design was used, with two replications. Each plot consisted of one 4-m length row, with a row spacing of 0.8 m and 0.20 m between plants. With, 20 plants remained in the plot after thinning, representing a population of 62.500 plants ha<sup>-1</sup>.

Grain yield was assessed by weighing the grain shelled from the ears harvested in each plot. Also, plant stand was evaluated by counting the number of plants in the each plot at the harvest time, as well as grain moisture at harvest, measured in a grain sample from each plot. Grain yield was corrected to 13% moisture, adjusting it to the mean stand by covariance (Zuber, 1942) and converting it to tha<sup>-1</sup>.

The agronomic efficiency at low N availability (AELN) was computed by the following expression, adapted from Santos et al. (2019) - Eq. 1:

 $AELN = GYLN ] ^{2}((GYHN*GYLN)) (1)$ 

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 yield of the hybrids (Resende, 2007) and was estimated by the following expression (Santos et al.,2019 apud Resende, 2007) – Eq. 2:

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

where X\_HN and X\_LN is the overall mean of grain yield of the hybrids assessed with and without application of nitrogen in top dressing, respectively.

Grain yield without application of nitrogen in top dressing (GYLN), grain yield with application of nitrogen in top dressing (GYHN), agronomic efficiency at low N availability (AELN), and the harmonic mean of the relative performance (HMRP) were submitted to joint analysis of variance in the R software using the "metan" package (Olivoto & Lúcio, 2020) (R Core Team, 2019).

The adjusted mean values were clustered by the Scott-Knott grouping test (5% probability). The methodology adapted from Fageria & Kluthcouski (1980) was applied using the adjusted mean values of AELN and GYLN to classify the hybrids regarding responsiveness and efficiency in regard to nitrogen. Figures were created by the "ggplot2" package (Wickham, 2011).

#### **Results and discussion**

There was significance (p < 0.01) for the effect of the hybrids, of the Hybrid by environment interaction (Hybrid\*Environment), and of the environments for all the variables. The experimental coefficient of variation was within the acceptable range for GYLN, GYHN, and HMRP (Gurgel et al., 2013) and a little above 20% for AELN (Table 2).

Table 2. Summary of combined analysis of variance of the hybrids, of environment, and of their interaction (Hybrid\*Environment), their respective mean squares for the of grain yield without in t ha <sup>-1</sup> (GYLN) and with application of nitrogen in top dressing in t ha <sup>-1</sup> (GYHN), agronomic efficiency at low N availability (AELN), and harmonic mean of the relative performance (HMRP).

Source of Variation	Degrees of Freedom	GYLN	GYHN	AELN	HMRP
Hybrid	48	4.45**	6.73**	0.089**	0.098**
Hybrid*Environment	288	2.53**	1.52**	0.078**	0.033**
Environment	6	468.03**	501.58**	5.026**	9.432**
Rep (Environment)	7	20.07**	0.87 <sup>ns</sup>	0.413**	0.156**
Block(Rep* Environment)	84	1.96**	1.87**	0.065**	0.024**
Residual	252	0.99	0.89	0.039	0.012
CV(%)		15.63	12.65	22.02	11.34
Mean		6.36	7.46	0.90	0.97

\*\*, and <sup>ns</sup> mean significant at 5% and not significant by the F test, respectively.

The results obtained show that at least one Hybrid differs from the others and that interaction of hybrids by environment significant, the hybrids behave differently to environmental variations (Table 2). Therefore, it can be seen that there are hybrids that have greater agronomic efficiency in nitrogen use, with better stability and adaptability that is measured through the HMRP. Various studies show the importance of selection of hybrids with high efficiency in N use (Santos et al., 2017, Santos et al., 2019, Ullah et al., 2019, Zhai et al., 2019, Dong & Lin, 2020).

The hybrids were able to be clustered in three groups for GYLN; the first group being composed of only hybrid 49, with a mean value of 8.48 t ha-1; and in groups b and c, the mean values were 7.18 and 6.17 t ha-1, respectively. For GYHN, the hybrids formed four groups: in group a, it had the mean of the hybrids of 8.51 t ha-1; in group b, the mean was 7.81 t ha 1; in group c, it was 7.17 t ha-1; and in group d, characterized by the lowest mean yield, it was 6.41 t ha-1. These yields are considered high when compared to maize yield in the 1st harvest of 22/23 in Brazil, which was 6.18 t ha-1 (Conab, 2023). Two groups were formed for the AELN trait: group a, with the hybrids of higher mean values (0.98); and group b, those of lower mean values (0.84). The HMRP trait exhibited four groups: the one with the highest mean value with 1.12 (a) and the lowest mean value with 0.87 (d); group b had a mean of 1.02 and group c of 0.95 (Figure 1) (Table 3).





Group	GYLN	GYHN	AELN	HMRP
А	49	1, 5, 6, 11, 12, 19, 23, 41, 49	3, 4, 7, 15, 16, 17, 22, 23, 24, 26, 27, 33, 34, 39, 43, 45, 47, 49	5, 7, 23, 41, 47, 49
В	3, 5, 7, 10, 23, 41, 47	7, 9, 10, 18, 20, 24, 36, 37, 40, 42, 47, 48	1, 2, 5, 6, 8, 9, 10, 11, 12, 13, 14, 18, 19, 20, 21, 25, 28, 29, 30, 31, 32, 35, 36, 37, 38, 40, 41, 42, 44, 46, 48	1, 3, 6, 9, 10, 11, 18, 19, 20, 24, 29, 37, 40, 42
С	1, 2, 4, 6, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 43, 44, 45, 46, 48	2, 3, 4, 8, 13, 14, 17, 21, 25, 27, 28, 29, 30, 31, 32, 35, 38, 39, 43, 44, 46	-	4, 8, 12, 13, 14, 17, 25, 27, 30, 31, 35, 36, 39, 43, 44, 48
D	-	15, 16, 22, 26, 33, 34, 45	-	2, 15, 16, 21, 22, 26, 28, 32, 33, 34, 38, 45, 46

Table 3. Hybrids contained in each group by the Scott-Knott test for the traits of grain yield without in t ha <sup>-1</sup> (GYLN) and with application of nitrogen in top dressing in t ha <sup>-1</sup> (GYHN), agronomic efficiency at low N availability (AELN), and harmonic mean of the relative performance (HMRP).

Hybrid 49 (DKB 390) is a commercial hybrid and its mean values in the traits were important for comparison with the other hybrids, which are experimental. This is a responsive, efficient, and stable hybrid. Hybrids with efficiency equal to that of the control could be identified in the crosses studied, as for example, hybrid 23 (Table 3).

Exploiting stability and adaptability through the harmonic mean of the relative performance (HMRP) variable proposed by Resende (2007) is a simple strategy. Adaptability is the capacity of a Hybrid to take better advantage of the variations in the environment, and stability is the capacity of being highly predictable to environmental changes (Borém et al., 2021). Hybrids 5, 7, 23, 41, 47, and 49 are in the same group with the highest mean value (1.12). Thus, they are highly stable and adaptable and important for selection of yield and relation to agronomic efficiency of nitrogen use. With the aim of increasing yield, maize breeding has been carried out under optimal conditions for yield and high plant nutrition, such the hybrids to not be selected for the characteristic of using the nitrogen and often genetic variability for efficiency in nitrogen use declines (DoVale et al., 2012).

By the methodology of Fageria & Kluthcouski (1980), the maize hybrids were classified as efficient and/or responsive to increase in nitrogen levels, and hybrids 3, 4, 7, 17, 23, 24, 25, 29, 31, 45, and 47, that is, 23% of them were characterized as ideal, being efficient and responsive. The efficient and unresponsive hybrids represented 21%, the inefficient and responsive constitute 23%, and the inefficient and unresponsive were 33% (Figure 2).



Figure 2. Classification of maize hybrids regarding efficiency and responsiveness to nitrogen use using the traits GYLN (grain yield without the application of nitrogen in top dressing) and AELN (agronomic efficiency of nitrogen use).

The efficiency and responsiveness of the hybrids allow in more accurate selection and, that way, the hybrids can be classified as efficient and responsive when they have high yield with low N content and high response to increase in this nutrient (Fidelis et al., 2014). Efficient and unresponsive hybrids have high yield under low N content, but do not respond to increase in this nutrient. In contrast are hybrids characterized as inefficient and responsive, which produce little under low N content but have a high response to increase in this nutrient, and finally, the inefficient and unresponsive hybrids, which produce little under high or low N content. Thus, 67% of the hybrids evaluated are desirable for the aim of the program, because they have efficiency and/or responsiveness, and may result in a viable selection for a plant breeding program for N use efficiency. The 33% of the hybrids that were characterized as inefficient and unresponsive could be removed from the breeding program with the aim of N use efficiency.

Maize hybrids that have high agronomic efficiency in nitrogen use are ideal for production conditions, because applying a smaller amount of nitrogen and obtaining high yield reduces costs and contributes to lower environmental contamination. Agronomic efficiency in nitrogen use is a complex component for it is dependent on genetic control, the effect of the environment, and knowledge of all the metabolic processes of the plants for making better use of this nutrient in an efficient manner (Anas et al., 2020).

#### Conclusions

The hybrids evaluated, 67% of them can be used to increase the efficiency of nitrogen used, as they exhibited agronomic efficiency in the use of this nutrient and responsiveness, especially hybrids 5, 7, 17, 23, 24, 25, 29, 31, 41, 45, 47, and 49.

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9