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Accuracy and simultaneous selection gains for N-stress tolerance and N-use efficiency in maize tropical lines

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ABSTRACT: Maize plants can be N-use efficient or N-stress tolerant. The first have high yields in favorable environments but is drastically affected under stress conditions; whereas the second show satisfactory yields in stressful environments but only moderate ones under optimal conditions. In this context, our aim was to assess the possibility of selecting tropical maize lines that are simultaneously N-stress tolerant and N-use efficient and check for differences between simultaneous selection statistical methods. Sixty-four tropical maize lines were evaluated for Nitrogen Agronomic Efficiency (NAE) and Low Nitrogen Tolerance (LNTI) response indices and two *per se* selection indices, Low Nitrogen Agronomic Efficiency (LNAE) and Harmonic Mean of Relative Performance (HMRP). We performed eight selection scenarios: LNAE; HMRP; Additive index; Mulamba-Mock index; and Independent culling levels. The last three was predicted by REML/BLUP single-trait and multi-trait using genotypic values of NAE and LNTI. The REML/BLUP multi-trait analysis was superior to the single-trait analysis due to high unfavorable correlation between NAE and LNTI. However, the accuracy and genotypic determination coefficient of NAE and LNTI were too low. Thus, neither single- nor multi-trait analysis achieved a good result for simultaneous selection nor N-use efficiency nor N-stress tolerance. LNAE obtained satisfactorily accurate values and genotypic determination coefficient, but its performance in selection gain was worse than HMRP, particularly in terms of N-use efficiency. Therefore, because of the superior performance in accuracy, genotypic determination coefficient and selection, HMRP was considered the best simultaneous selection methodology of the scenarios tested for N-use efficiency and N-stress tolerance.
Keywords: abiotic stress, correlation between traits, winter maize, mixed models

Introduction

The achievement of high yields in maize demands high levels of investment in N fertilization (Walsh et al., 2012). With this in mind, breeding programs focus on two research areas, N-stress tolerance and N-use efficiency (Maia et al., 2011). Plants that are N-stress tolerant tend to have satisfactory yields despite stressful environments. However, under optimal conditions, the yields are moderate or low. On the other hand, N-use efficient plants have high yields in favorable environments but suffer a significant reduction in yields under conditions of stress (Maia et al., 2011).

We can use plant response indices to evaluate these traits. These are mathematical expressions that meet the performance of materials under both favorable and stressful growing conditions. To evaluate N-use efficiency, Craswell and Godwin (1984) configured the N-agronomic efficiency (NAE) equation and Miti et al. (2010), proposed the low-N tolerance index (LNTI) to evaluate N-stress tolerance. However, according to Wu et al. (2011), selection based on these indices could present problems. Following the mathematical formulae, NAE may be selected for plants with low yield under stress conditions and LNTI for plants with low yield under both conditions. It is, therefore, important to understand the relationship between N-use efficiency and N-stress tolerance. The possibility of simultaneous gains would provide plants with a high yield under both optimal and stressful conditions. Simultaneous selection for N-use efficiency and N-stress tolerance can be performed by two strategies: *per se* selection indices and simultaneous selection methods.

Per se selection indices are mathematical expressions that take into account the performance of plants under both favorable and stressful conditions. However, the rankings lean towards selecting plants with high yields under both conditions. Some examples of these indices are the low-N agronomy efficiency (LNAE), proposed by Wu et al. (2011), and the Harmonic Mean of Relative Performance (HMRP), developed by Resende (2004). The simultaneous selection methods are linear functions that group important traits and estimate a value to represent the genotype's performance in two or more traits (Bernardo, 2010). By these methods, it is possible to use the plant response indices (NAE and LNTI) as they simultaneously gain traits in N-use efficiency and low-N stress tolerance. From the numerous simultaneous selection methods proposed in the literature, we selected the Additive Index (ADI) (Resende, 2007), the Mulamba and Mock (1978) index (MMI), and the Independent Culling Levels (ICL) (Bernardo, 2010).

Thus, our aim was to verify whether it is possible to simultaneously select tropical maize lines that have N-use efficiency and N-stress tolerance and to identify the best simultaneous selection method for these conditions.

Materials and Methods

Experimental field

We used sixty four (64) tropical maize lines, obtained from the germplasm from Viçosa, MG, Brazil, to generate a population with high genetic variability to assess N-use efficiency and N-stress tolerance (Dovale et al., 2013). The population structure analysis revealed three tropical heterotic pools, which have been used worldwide and by Brazilian maize seed companies. More details about pedigree and heterotic groups of these lines were described by Lanes et al. (2014). It was decided to use lines due to the predominance of the additive effects of N-stress tolerance and N-use efficiency (Dovale et al., 2012).

The lines were cultivated on two fields, side by side, one with high-N availability (HN) and the other with low-N availability (LN). An 8 × 8 lattice with two repetitions was planted in each field. This experiment was repeated three times, as follows: in Anhembi, SP, Brazil (22°50′51″ S, 48°01′06″ W, 466 m) sown in the winter seasons of 2014 and 2015; and in Piracicaba, SP, Brazil (22°42′23″ S, 47°38′14″ W, 535 m) sown in the winter season of 2015. Each combination of locale and year was considered a site; thus, there were three sites to be evaluated.

The experimental units consisted of a five-meter line with 0.80 meters between lines and 0.20 meters between plants. Fertilization was administered comprising 300 kg ha^{-1} of NPK 4-14-8. The 50 % reduction in maize yield due to N effects was used to determine the amount of N to be used in HN and LN fields (Dovale et al., 2011). Consequently, 35 kg ha^{-1} of N and 125 kg ha^{-1} of N were used in the LN and HN fields, respectively. In both fields, 12 kg ha^{-1} of N was applied at sowing, and the remaining N were split into two sections, one at 30 days (V6) and the other at 50 days (VT) after sowing.

Traits evaluated

From each experimental unit, the grain yield (GY), in kg ha^{-1}, and grain moisture (%) were collected. Using these data, the GY was corrected by 13 % of the grain moisture. After correction of the GY of each line in each N-availability field we estimated the plant response indices (NAE and LNTI) and the *per se* selection indices (LNAE and HMRP). For this step in the experimental design, the two lattices were merged into one lattice because the GY in both the LN and HN availability fields were used to estimate the indices.

Plant response indices

To assess the N-use efficiency, the Nitrogen Agronomic Efficiency (NAE) equation described by Craswell and Godwin (1984), was used as follows:

$$NAE_{ij} = \frac{GY_{(HN)_{ij}} - GY_{(LN)_{ij}}}{N_{(HN)} - N_{(LN)}}$$

where NAE_{ij} is the Nitrogen Agronomic Efficiency of line i in repetition j; $GY_{(HN)ij}$ the grain yield in the HN field (phenotypic value) of line i in repetition j; $GY_{(LN)ij}$ the grain yield in the LN field (phenotypic value) of line i in repetition j; $N_{(HN)}$ the amount of nitrogen applied in the HN field; and $N_{(LN)}$ the amount of nitrogen applied in the LN field.

In addition, to evaluate N-stress tolerance, the Low-N tolerance index (LNTI), described by Miti et al. (2010), was used as follows:

$$LNTI_{ij} = \left(1 - \frac{GY_{(LN)_{ij}}}{GY_{(HN)_{ij}}}\right) \cdot 100$$

where $LNTI_{ij}$ is the Low-N tolerance index of line i in repetition j; $GY_{(HN)ij}$ the grain yield in the HN field (phenotypic value) of line i in repetition j; and $GY_{(LN)ij}$ the grain yield in the LN field (phenotypic value) of line i in repetition j.

***Per se* selection indices**

The *per se* selection indices do not prioritize efficiency or tolerance due to their mathematical arrangement, but they do provide a balance between these traits. One of these is the Low-N agronomic efficiency (LNAE), introduced by Wu et al. (2011), as follows:

$$LNAE_{ij} = \frac{GY_{(LN)_{ij}}}{GY_{(HN)_{ij}}} \cdot GY_{(LN)_{ij}}$$

where $LNAE_{ij}$ is the Low-N agronomic efficiency of line i in repetition j; $GY_{(HN)ij}$ the grain yield in the HN field (phenotypic value) of line i in repetition j; and $GY_{(LN)ij}$ the grain yield in the LN field (phenotypic value) of line i in repetition j.

Another estimated *per se* selection index was the Harmony Mean of Relative Performance (HMRP), proposed by Resende (2004), as follows:

$$HMRP_{ij} = \frac{2}{\left(\frac{GY_{(HN)_{ij}}}{\bar{X}_{(HN)}}\right)^{-1} + \left(\frac{GY_{(LN)_{ij}}}{\bar{X}_{(LN)}}\right)^{-1}}$$

where $HMRP_{ij}$ is the a Harmony Mean of Relative Performance of line i on repetition j; $GY_{(HN)ij}$ the grain yield in the HN field (phenotypic value) of line i in repetition j; $GY_{(LN)ij}$ the grain yield in the LN field (phenotypic value) of line i in repetition j; $\bar{X}_{(HN)}$ the mean yield of the HN field; and $\bar{X}_{(LN)}$ the mean yield of the LN field.

Deviance analysis and prediction of genotypic values

After obtaining the values of the plant response indices and *per se* selection indices for each repetition of each line, we carried out a deviance analysis (ANADEV). Additionally, the variance components and genotypic values of the lines were estimated in each index by a Restricted Maximum Likelihood/Best Linear Unbiased

Predictor (REML/BLUP) single-trait (univariate) analysis, as follows:

$$y = Xr + Zg + Wb + Ti + \varepsilon$$

where y is the vector of the plant response indices (NAE and LNTI) and the *per se* selection indices (LNAE and HMRP); r the site and repetition within site effect vector plus the mean, considered fixed and $r \sim N(r,\Phi r)$; g the line effect vector and was considered random where $g \sim N(0, G)$; b the vector of the block within repetition effect and was considered random where $b \sim N(0,\sigma 2b)$; i the line × site interaction effect vector and was considered random $i \sim N(0, \sigma 2i)$; $\mathcal{E}$ the experimental error where $\mathcal{E} \sim N(0, R)$. X, Z, W and T are incidence matrices that relate the independent vector effects from each matrix to the dependent y vector. Thus, for the single-trait model, the following equation was used:

$$R = I_{(n1)}\sigma_e^2;\ G_1 = I_{(n2)}\sigma_g^2;\ G_2 = I_{(n3)}\sigma_b^2;\ G_3 = I_{(n4)}\sigma_i^2$$

where I is the incidence matrix with the dimension n_1 (repetition × site × line), n_2 (line), n_3 (block × repetition × site) and n_4 (line × site); and σ_e^2, σ_g^2, σ_b^2 and σ_i^2 are the residual, genetic, block and line × site interaction variance components, respectively.

Due to an unfortunate genotypic correlation between N-use efficiency and N-stress tolerance (Maia et al., 2011), the genotypic values of NAE and LNTI were also predicted by REML/BLUP multi-trait (multivariate), in which the genotypic values are corrected by the covariance between the traits (Henderson and Quaas, 1976). The equation is as follows:

$$R = I_{(n1)} \otimes Cov_e;\ G_1 = I_{(n2)} \otimes Cov_g;\ G_2 = I_{(n3)} \otimes Cov_b;\ G_3 = I_{(n4)} \otimes Cov_i$$

where I is the incidence matrix with the dimensions n_1 (repetition × site × line), n_2 (line), n_3 (block × repetition × site) and n_4 (line × site); Cov_e, Cov_g, Cov_b and Cov_i are the residual, genetic, block and line × site interaction variance-covariance matrixes, respectively, between NAE and LNTI; and ⊗ indicates the Kronecker product.

All of the analyses were done by ASReml-R® (Gilmour et al., 2009) using the R software (version 3.3.1).

Scenarios of simultaneous selection

After obtaining the genotypic values of the plant response indices and *per se* selection indices, we applied the scenarios of simultaneous selection. The primary difference between the *per se* selection indices and the plant response indices is that the first rankings based on the genotypic values must lead to a simultaneous selection for efficiency and tolerance. On the other hand, to obtain a simultaneous selection for efficiency and tolerance using plant response indices, the genotypic values cannot be directly used, and the genotypic values of both indices (NAE end LNTI) are needed for composing the simultaneous selection methods (ADI, MMI and ICL).

This way, for *per se* selection indices, the following scenarios were analyzed: LNAE, which according to Wu et al. (2011), promotes the selection of plants with satisfactory performance under optimal and stressful growing conditions, and HMRP, which selects productive and stable plants over a range of N-availability fields (Resende, 2004). In addition, to compose the scenarios of simultaneous selection using the plant response indices (NAE and LNTI), a factorial (3 × 2) with three simultaneous selection methods (ADI, MMI and ICL) and two prediction methods of genotypic values (REML/BLUP single-trait and multi-trait) was generated. These three simultaneous selection methods were chosen to represent three distinct ways to gather the traits. The equations for these methods were:

$$ADI_i = b_1X_{1i} + b_2X_{2i}$$

where ADI_i is the value of the additive index to line i; b_1 the weight for NAE (0.5); b_2 the weight for LNTI (0.5); X_{1i} the standardized genotypic value of NAE to line i; and X_{2i} the standardized genotypic value of LNTI to line i; and:

$$MMI_i = \frac{P_{1i} + P_{2i}}{2}$$

where MMI_i is the value of the Mulamba-Mock index to line i; P_{1i} the positon of line i on NAE's rank; and P_{2i} the positon of line i on LNTI's rank.

Selection by independent culling levels (ICL) assigned each axis of a graph with NAE and LNTI genotypic values. Then, lines were plotted according to their genotypic values on each trait, and the lines that have both favorable values were selected (Bernardo, 2010).

Therefore, the total number of scenarios of simultaneous selection were eight: two by *per se* selection indices (LNAE and HMRP) and six by simultaneous selection methods using NAE and LNTI values (ADI, MMI and ICL, single-trait and multi-trait).

Comparison of the scenarios of simultaneous selection

We simulated 10 % selection intensity in all of the scenarios. The comparisons were based on the expected selection gains (SG %), accuracy ($\hat{r}$) and genotypic determination coefficient (h^2). Accuracy, according to Resende and Duarte (2007), is the most appropriate criterion for assessing predictive quality because the accuracy takes into account the genetic and residual coefficients of variation and the number of repetitions. The average of all of the sites was used to obtain the following estimations:

$$SG(\%) = \frac{\left(\frac{\sum_i^n GV_{ij}}{n}\right)}{\bar{x}_j} \times 100$$

where $SG(\%)$ is the expected selection gain in percentage; GV_{ij} the genotypic value of selected line i to trait j; n the number of lines selected; and $\bar{x}_j$ the mean of trait j;

$$\hat{r} = \left[1 - \frac{1}{1 + b.\left(\frac{CVg}{CVe} \right)^2} \right]^{1/2}$$

where $\hat{r}$ is the accuracy; b the number of repetitions; CVg the genetic coefficient of variation; and CVe the residual coefficient of variation;

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 \frac{\sigma_i^2}{s} + \frac{\sigma_e^2}{r.s}}$$

where h^2 is the genotypic determination coefficient; σ_g^2, σ_i^2, σ_e^2, are genetic, interaction line × site and residual variance, respectively; s is the number of sites; and r the number of repetition on each site.

Results

ANADEV and the parameters of *per se* selection indices and plant response indices

According to ANADEV, the line effect was significant for LNAE, HMRP and LNTI (Table 1), indicating genetic variability among lines for these indices. Only the *per se* selection indices showed significance for line × site interaction. For these, the ANADEV was performed for each site, and the line effect was significant in two of the three sites for LNAE and in all of the sites for HMRP (data not shown).

The line × site interaction was significant by the *per se* selection indices, but the average of all of the sites was used to obtain the expected selection gains. This study was conducted at different sites to identify materials with high yields that are stable across sites, and not just specific selections at each site.

HMRP achieved the highest genotypic determination coefficient, accuracy and the lowest coefficient of variation. For NAE and LNTI, multi-trait analysis yielded superior accuracy and genotypic determination coefficient compared with single-trait analysis. However, the values were much lower than LNAE and HMRP (Table 2).

Expected selection gains

Both of the *per se* selection indices, LNAE and HMRP, achieved good direct expected selection gains. Likewise, LNTI found good direct expected selection gains in both analyses, but multi-trait was superior (Table 3). There was no selection gain found for NAE because of the absence of genetic variability that was previously shown (Table 1).

Table 1 – Wald test of fixed effects and the likelihood-ratio test (LRT) of random effects for *per se* selection indices Low Nitrogen Agronomic Efficiency (LNAE) and Harmonic Mean of Relative Performance (HMRP) and plant response indices Nitrogen Agronomic Efficiency (NAE) and Low Nitrogen Tolerance index (LNTI).

Variation factor	LNAE	HMRP	NAE	LNTI
Fixed effects				
Site	60.99**	0.680^{ns}	9.98**	3.96^{ns}
Repetition/Site	1.58^{ns}	9.20*	5.57^{ns}	0.59^{ns}
Random effects				
Block/Repetition	0.06 ^{ns}	4.10*	0.10^{ns}	0.01^{ns}
Line	27.61**	62.38**	0.26^{ns}	5.48*
Line × Site	5.07*	17.47**	0.10^{ns}	3.82^{ns}

ns = not significant $p > 0.05$; *$p < 0.05$ by LRT or F Wald; **$p < 0.01$ by LRT or Wald test.

Table 2 – Parameters of Low Nitrogen Agronomic Efficiency (LNAE), Harmonic Mean of Relative Performance (HMRP), Nitrogen Agronomic Efficiency (NAE) and Low Nitrogen Tolerance index (LNTI).

Parameters	Single-trait				Multi-trait	
	LNAE	HMRP	NAE	LNTI	NAE	LNTI
Mean ($\bar{x}$)	1568.89	1.01	7.39	0.26	7.41	0.26
Coe. Var. (CV %)	41.38	19.94	82.68	59.89	84.61	64.18
Genotypic det. coe. (h^2)	0.61	0.73	0.09	0.06	0.15	0.26
Accuracy ($\hat{r}$)	0.72	0.89	0.19	0.19	0.24	0.32

Table 3 – Expected selection gains (%) based on Low Nitrogen Agronomic Efficiency (LNAE), Harmonic Mean of Relative Performance (HMRP), Nitrogen Agronomic Efficiency (NAE) and Low Nitrogen Tolerance index (LNTI) and direct and indirect expected selection gains (%) for Additive index (ADI), Mulamba-Mock index (MMI) and Independent Culling Levels (ICL).

Selection method	Single-trait			Multi-trait		
	Direct	Indirect		Direct	Indirect	
		Efficiency (NAE)	Tolerance (LNTI)		Efficiency (NAE)	Tolerance (LNTI)
LNAE	46.51	-	-	-	-	-
HMRP	9.39	-	-	-	-	-
NAE+	0.00	-	-	0.00	-	-
LNTI	24.00	-	-	36.14	-	-
ADI	9.56	2.77	7.08	47.21	12.15	24.24
MMI	36.16	1.38	9.28	85.72	12.15	24.24
ICL	-	2.53	6.09	-	12.15	24.24

+Without genetic variability to be selected.

The three simultaneous selection methods (ADI, MMI and ICL) composed of NAE and LNTI values were used to estimate both direct and indirect expected selection gains. These gains were always higher in multi-trait analysis compared with single-trait analysis. Moreover, in multi-trait analysis, the indirect gains were equal among ADI, MMI and ICL (Table 3), probably because all these simultaneous selection methods selected the same lines.

Despite the performance of NAE and LNTI, our aim was simultaneous selection for N-use efficiency and N-stress tolerance. Towards this goal, the primary comparison was between the *per se* selection indices (LNAE and HMRP) and the simultaneous selection methods (ADI, MMI and ICL).

Discussion

Performance of *per se* selection indices

Both *per se* selection indices performed well in accuracy and expected selection gains. The accuracy, genotypic determination coefficient and coefficient of variation values were more favorable in HMRP. On the other hand, LNAE obtained a higher expected selection gain (Table 3). However, this gain should be cautiously analyzed because high values of selection gain for this index may not represent the gain in HN and LN yields. Thus, the yield in HN and LN of the selected lines by each index is more important than the expected selection gain values to determine which index is more efficient for simultaneous selection.

In situations in which a trait has unexploited genetic variability, there is no selection gain. In this context, the HMRP values obtained in all of the sites showed a more stable expression of genetic variability. In addition, this index was less sensitive to environmental effects based on the genotypic determination coefficient and coefficient of variation, which contributed to more accurate selection (Table 2). The HMRP also showed a strong line × site interaction, which was not observed in LNAE. This interaction explains the low mean gain selection of HMRP compared with LNAE (Table 3).

According to Wu et al. (2011), the main characteristic of LNAE is the selection of materials that can maintain high productivity under stressful conditions. On the other hand, Resende (2004) argued that HMRP leads to simultaneous selection for stability and adaptability; in other words, plants that maintain their productivity under optimal and stressful conditions are selected. Conceptually, the selection by these two indices is similar and should lead to a high coincidence selection between LNAE and HMRP; however, this similarity was not verified. The coincidence in selection was only 57 %. Thus, even with similar proposals, there is a clear difference between selection by HMRP and by LNAE.

Performance of plant response indices (single-trait and multi-trait)

Plants that are N-stress tolerant have low LNTI values and plants that are N-use efficient have high NAE values (Craswell and Godwin, 1984; Miti et al., 2010). A positive correlation between NAE and LNTI is problematic for simultaneous selection because a line with high NAE likely hashigh LNTI or is efficient but not tolerant.

This problem was confirmed when we found a genotypic correlation of 0.69 between NAE and LNTI. This value confirms the results presented by Maia et al. (2011), which stated that N-use efficiency and N-stress tolerance are inversely proportional traits. Thus, stress tolerant plants tend to be N-use inefficient and vice versa. This way, the selection by single-trait analysis is impaired because it is difficult to find genotypes with favorable values of NAE and LNTI simultaneously. This difficulty is represented by the single-trait graph (Figure 1A), where there are only a few lines within quadrant IV (high NAE and low LNTI).

On the other hand, NAE and LNTI predicted by multi-trait analysis achieved a -0.96 Pearson correlation.

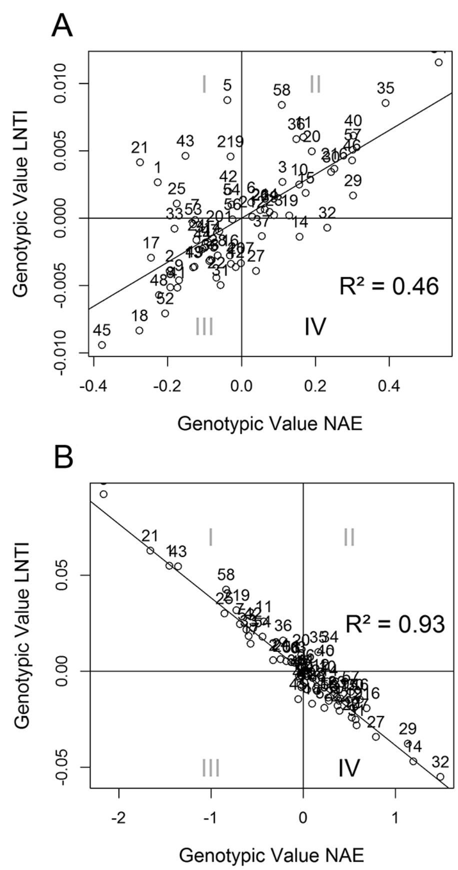

Figure 1 – Graphics of genotypic values for Nitrogen Agronomic Efficiency (NAE) and Low Nitrogen Tolerance index (LNTI) predicted by single-trait (A) and multi-trait (B).

This means that using covariance to adjust the genotypic values of the traits changed these values. Therefore, we were able to identify more lines with high NAE and low LNTI (quadrant IV). Comparing both graphs (Figure 1A and B), the impact of the genotypic values in each prediction method can be found.

The superiority of multi-trait predictions was also observed in the accuracy and genotypic determination coefficient values that are higher in multi-trait analysis (Table 2). Consequently, the expected selection gains were higher using multi-trait analysis (Table 3). The significant changes in the genotypic values of the lines not only increased the expected selection gains but also were responsible for selection coincidence between ADI, MMI and ICL (Table 4). This indicates the strong capacity of multi-trait analysis in demonstrating which materials need to be selected, regardless of the method.

The criteria between single-trait and multi-trait analyses were presented by Bauer and Leon (2008). According to these authors, inversely correlated traits present higher simultaneous selection gains using multi-trait analysis. The selection using single-trait analysis for these traits could result in gains in one trait and losses in the other trait. In addition, Viana et al. (2010) simultaneously selected for yield and expansion volume in popcorn and noted that in cases of traits with similar genotypic determination coefficient, the decision between single-trait and multi-trait analysis is the absolute difference between genetic and residual correlations. If both correlations are similar, there is no difference between these prediction methods.

Thus, NAE and LNTI meet the requirements that lead to greater accuracy and expected selection gains by multi-trait analysis. The correlation is unfavorable and strong (Pearson correlation of 0.69), and although the genotypic determination coefficient is lower, it is similar (Table 2). In addition, the genetic and residual correlations are 0.41 and 0.61, respectively, which indicates a considerable difference between them.

Differential selection between the scenarios

Despite the fact that multi-trait prediction was better than single-trait prediction, the performance of multi-trait analysis was lower compared with *per se* selection indices. This was due to the lower accuracy and genotypic determination coefficient of NAE and LNTI and the high coefficient of variation (Table 2). In fact, accuracy and the genotypic determination coefficient increased using multi-trait analysis, which corroborated the published literature, but the values were so low that this increase did not provide active values near LNAE and HMRP levels (Table 2). Thus, the low success level of simultaneous selection methods was caused by the low performance of NAE and LNTI. These plant response indices are not accurate and cannot select the higher yield lines in HN and LN as these lines were not selected by ADI, MMI and ICL (Figure 2).

The better performances of the *per se* selection indices were confirmed by checking the materials selected by each index (Figure 2) and the expected selection gains for each N-availability (Table 5). The productive lines were prioritized in HN and LN. However, there were verified differences between LNAE and HMRP. Although LNAE selected productive materials under both N-availability scenarios, there was a tendency to select lines with higher productivity in low-N. In the case of HMRP, the selection appeared more balanced, and the best lines were selected. Thus, HMRP showed the highest expected selection gain in HN and LN (Table 5).

In their article, Wu et al. (2011) developed LNAE as the LN yield divided by the HN yield and multiplied by the LN yield. In Figure 2, we noted that this multiplication by LN yield leads to a selection of materials with great performance in LN, instead of yielding materials under both conditions. The greater balance presented by HMRP better suits the aim of this study.

Table 4 – Selection coincidence among the scenarios of simultaneous selection.

Selection method	*per se* selection indices		Single-trait			Multi-trait		
	LNAE	HMRP	ADI	MMI	ICL	ADI	MMI	ICL
	%							
per se selection indices								
LNAE	-	57	57	43	29	57	57	57
HMRP		-	57	43	43	57	57	57
Single-trait								
ADI			-	86	57	100	100	100
MMI				-	57	86	86	86
ICL					-	57	57	57
Multi-trait								
ADI						-	100	100
MMI							-	100
ICL								-

LNAE = Low Nitrogen Agronomic Efficiency; HMRP = Harmonic Mean of Relative Performance; ADI = Additive index; MMI = Mulamba-Mock index; ICL = Independent Culling Levels.

Table 5 – Expected selection gains (SG %) to grain yield (GY) in high nitrogen (HN) and low nitrogen (LN).

Selection method	SG (%) HN	SG (%) LN
LNAE	24.01	39.94
HMRP	34.01	41.04
ADI (single-trait)	31.64	32.84
MMI (single-trait)	27.89	31.80
ICL (single-trait)	25.49	25.63
ADI (multi-trait)	31.64	32.84
MMI (multi-trait)	31.64	32.84
ICL (multi-trait)	31.64	32.84

LNAE = Low Nitrogen Agronomic Efficiency; HMRP = Harmonic Mean of Relative Performance; ADI = Additive index; MMI = Mulamba-Mock index; ICL = Independent Culling Levels.

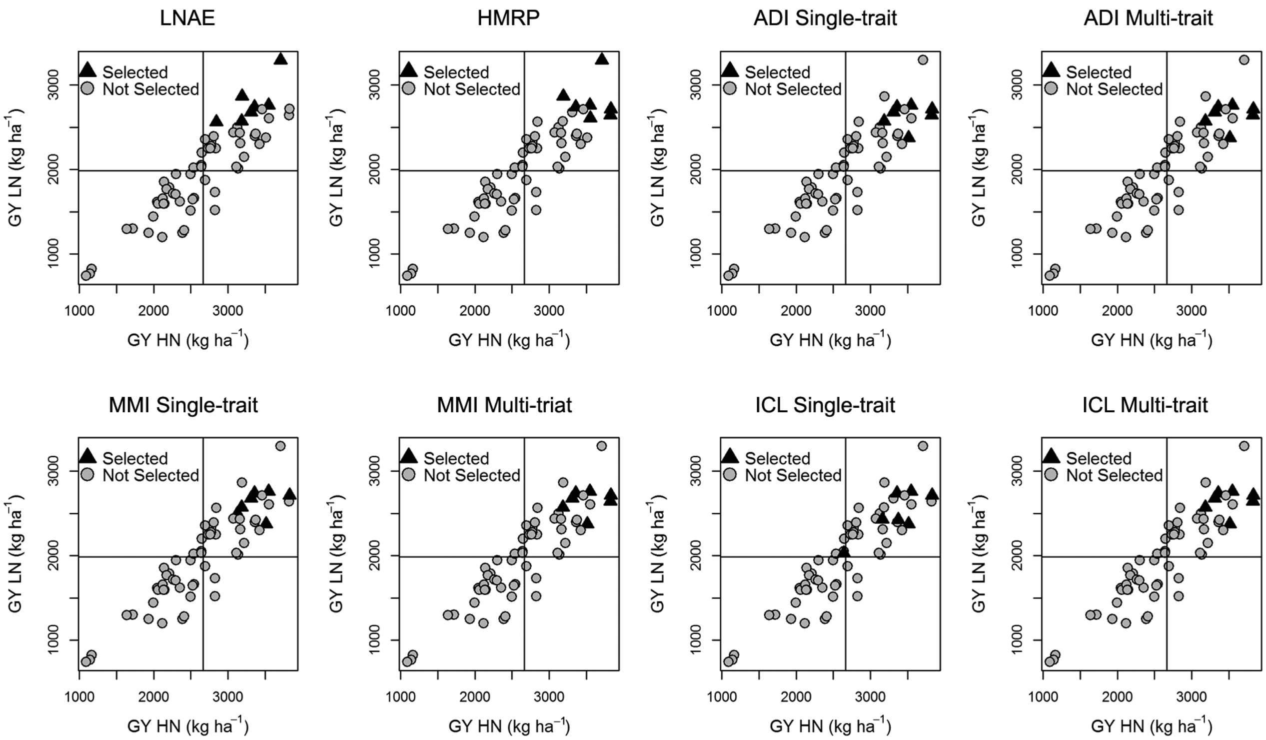

Figure 2 – Lines selected by each simultaneous selection scenario; LNAE = Low Nitrogen Agronomic Efficiency; HMRP = Harmonic Mean of Relative Performance; ADI = Additive index; MMI = Mulamba-Mock index; ICL = Independent Culling Levels.

Furthermore, HMRP surpassed LNAE in LN conditions, which seemed to be the strong point of selection by LNAE (Table 5).

Several articles reinforce the capacity of HMRP to select productive and stable genotypes. For example Carbonell et al. (2007) showed this in the common bean, Borges et al. (2010) examined this in rice, and Oliveira et al. (2005) analyzed this in sugarcane. Another advantage of HMRP is the possibility of considering more than two N-availability scenarios, making it possible to evaluate stress gradients (Resende, 2004).

These results and the aforementioned superior performance in accuracy and genotypic determination coefficient allow us to conclude that simultaneous selection of tropical maize lines for N-stress tolerance and N-use efficiency is possible. We also conclude that under the scenarios analyzed, HMRP is the most suitable method among the tested methods.

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