ARTICLE

Stage-wise selection of tropical wheat populations using univariate and multivariate BLUP models

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Abstract: *Plant breeding programs often involve several segregating populations that must be selected for multiple traits. This study aimed to identify tropical wheat populations combining earliness and high grain yield (GY) using univariate and multivariate best linear unbiased prediction (BLUP)-based models within a stage-wise approach. Fifty-six F_2 and F_3 populations were evaluated in two environments for days to heading (DH) and GY. In the first stage, two modeling strategies were used: a univariate and multivariate model per generation. Genetic parameters and empirical genotypic values were estimated and used in the second stage for combined selection across generations. Both strategies yielded similar results in terms of genetic gains, genotype selection, and ranking, likely due to the low correlation between the traits. Populations 4H, 2F, 2D, 2A, 2E, 3E, 1G, 3A, 3B, 2G, 3F, 1D, and 1B were selected for earliness and yield and will be advanced to derive superior inbred lines.*

Keywords: *BLUP, grain yield, multivariate,* Triticum aestivum *L*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a staple crop in many countries worldwide, primarily because of its high nutritional value and broad versatility (Hazard et al. 2020). Plant breeding plays an important role in the development of cultivars adapted to target environments and features desired by growers and consumers. To develop the best cultivars, wheat breeding programs must obtain a large number of segregating populations with high genetic variability and a high frequency of favorable alleles (Fasahat et al. 2016). Therefore, many populations are generated during each cycle, resulting in laborious evaluation and selection processes, in which the best candidates are selected after evaluating a set of traits to define their superiority (Machado e Silva et al. 2023).

Ordinary least squares-based inferences impose challenges in the selection of the best populations because each trait is analyzed individually and considered independent. Nevertheless, these drawbacks can be overcome using linear mixed models (Patterson and Thompson 1971, Henderson 1975). Mixed models can handle unbalanced data and model covariance structures. In addition, selection decisions are based on genotypic values predicted via

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(best linear unbiased prediction (BLUP) using variance components estimated by restricted maximum likelihood (REML) (Silva et al. 2024). A key feature of BLUP is that it produces predictions that shrink toward the mean, which maximizes the correlation between the true and predicted genetic values (Piepho et al. 2008). To avoid selection bias and obtain empirical BLUPs, the multivariate BLUP methodology proposed by Henderson and Quaas (1976) is often used. This type of analysis tends to be more accurate because it simultaneously considers a greater amount of data and uses genetic and residual correlations between traits.

In addition to multivariate BLUP, several simultaneous selection indices have been developed to address the need for multi-trait selection. The multi-trait genotype–ideotype distance index (MGIDI) has emerged as a powerful strategy for selecting superior genotypes across multiple traits by simultaneously considering their desirable directions (Olivoto and Nardino 2021). Recent applications have demonstrated its effectiveness in identifying superior genotypes (Salami 2025) and improving selection efficiency using BLUP and BLUE (Archangi et al. 2022).

Multivariate BLUP-based methods have been reported for perennial crops (Viana et al. 2010), corn (Viana et al. 2011), grain sorghum (Souza et al. 2019) and soybean (Volpato et al. 2019). Nevertheless, its combination with the MGIDI for selection candidate analysis has often been overlooked, particularly for wheat. In this study, we hypothesized that employing a multivariate BLUP model could leverage the selection of segregating populations. Thus, we compared the genetic parameters obtained by fitting both univariate and multivariate models. The variance components and empirical BLUPs obtained during the first stage were used in the second stage as inputs in a combined selection strategy to evaluate genetic gains and genotype ranking. Finally, we selected the best performing tropical wheat population for grain yield (GY) and earliness.

MATERIAL AND METHODS

Genetic material and experimental design

A panel of 56 wheat segregating genotypes belonging to the Wheat Breeding Program of the Federal University of Viçosa (UFV) were obtained from crosses involving eight parents in a complete diallel scheme (Table 1), with the aim of earliness and GY. The F_1 populations were obtained in 2019 and advanced in a greenhouse during summer 2020. The F_2 populations were evaluated in the field during winter 2020. Subsequently, the populations in the F_3 generation were evaluated in the field during summer 2021.

Initially, the F_2 generation was evaluated in the Diogo Alves de Melo experimental field, which belongs to the Department of Agronomy of UFV, Viçosa, MG, Brazil. The 56 F_2 combinations and eight parents were evaluated using a square lattice (8 × 8) experimental design with two replicates. The experimental plots consisted of three 3 m rows with 0.2 m row spacing and a sowing density of 10 seeds m^{-1}. The soil was conventionally prepared and the experiment was conducted under sprinkler irrigation.

The F_3 seeds harvested from the previous experiment were evaluated in a field experiment during summer 2021 in São Gotardo, MG, in an experimental area of Cooperativa Agropecuária do Alto Paranaíba. The 56 F_3 combinations and eight parents were evaluated using a square lattice (8 × 8) experimental design with two replicates. The experimental plots consisted of five 5 m rows with 0.2 m row spacing and 350 seed m^{-2} sowing density. Sowing was conducted in a

Table 1. Description of the parents used in an 8 × 8 diallel scheme

Code		Cultivar	Breeder	Pedigree	Release
♀	♂				
1	A	CD 1303	Coodetec	CD 150// BRS 177	2016
2	B	BRS 254	Embrapa	Buck Buck/Chiroca//Tui	2005
3	C	BRS 264	Embrapa	Embrapa 22*3/Anahac 75	2005
4	D	BRS 394	Embrapa	Embrapa 22//CM 106793	2014
5	E	TBIO Aton	Biotrigo	TBIO Mestre/Fuste//TBIO Mestre	2018
6	F	TBIO Duque	Biotrigo	Toruk#3/Celebra//Noble	2018
7	G	TBIO Ponteiro	Biotrigo	Fuste/TBIO Mestre	2017
8	H	TBIO Sossego	Biotrigo	BIO 08400 ‘S’/Quartzo//Quartzo	2015

direct sowing system, and the experiment was conducted without irrigation. For both experiments, fertilization and agrochemical application followed the recommendations for wheat cultivation in Brazil (Embrapa 2020).

Traits evaluated

Days to heading (DH) were evaluated when at least 50% of the plants in the plots had fully exposed ears, and GY (kg ha^{-1}) was evaluated after all plants in the plot were harvested, tracked, and weighed on a precision scale.

Statistical modeling

During the first stage, the REML/BLUP procedure was adopted to estimate the genetic parameters and predict empirical genotypic values using two different strategies: a univariate (Strategy I) and multivariate (Strategy II) model for each generation. The different modeling strategies are described as follows:

Strategy I - Univariate model

$$y = X\beta + Zg + Wb + e$$

where **y** is the phenotypic value vector; β is the replication fixed effects vector, added to the overall mean; g is the genotypic effects vector (assumed to be random), where $g \sim N(0, I\sigma^2_g)$; b is the incomplete blocks effects vector (assumed to be random), where $b \sim N(0, I\sigma^2_b)$; and e is the error vector, where $e \sim N(0, I\sigma^2_e)$. **X, Z,** and **W** are the incidence matrices for effects β, g, and b.

Strategy II - Multivariate model

$$y_1 = X\beta_1 + Zg_1 + Wb_1 + e_1$$

where y_1 is the multivariate response vector associated with each genotype; β_1 is the replication fixed effects vector, added to the overall mean; g_1 is the genotypic effects vector associated with each trait (assumed to be random), $g_1 \sim N(0, I\sigma^2_{g1})$; b_1 is the incomplete block effects vector (assumed to be random), $b_1 \sim N(0, I\sigma^2_{b1})$ and e_1 is the error vector, where $e \sim N(0, I\sigma^2_{e1})$. **X**, **Z** and **W** are the incidence matrices for effects β_1, g_1 and b_1. In both the univariate and multivariate models, blocks were considered random effects to allow for the generalization of inferences beyond the specific blocks used in the experiment.

In the univariate approach, the response vector includes only one trait at a time. In contrast, in the multivariate approach, the response vector simultaneously includes two traits (GY and DH, in this study). This allows the prediction of genotypic values to account not only for the performance of each trait individually but also for the correlation structure between traits, thereby providing a more comprehensive basis for selection.

The significance of random effects was tested using the likelihood ratio test (LRT) (Wilks 1938), as follows:

$$LRT = -2\,(LogL_F - LogL_R)$$

where $LogL_F$ and $LogL_R$ are the logarithm of the restricted likelihood function of the full and reduced models, respectively. The significance of random effects was tested using at chi-square distribution with a 5% probability.

The variance components were used to estimate broad-sense heritability (h^2) as follows:

$$h^2 = \frac{\sigma^2_g}{\sigma^2_p}$$

where σ^2_g and σ^2_p are the genotypic and phenotypic variances, respectively. The selection accuracy (h) was calculated as $h = \sqrt{h^2}$.

Population selection

The variance components and empirical genotypic values obtained in the first stage were used as inputs in the second stage for a combined selection of the best-performing populations. The MGIDI, proposed by Olivoto and Nardino (2021), which calculates genotype scores based on factor analysis, was used to rank genotypes based on information from multiple traits, in this case, the DH and GY BLUPs in each generation. The MGIDI was fitted as follows:

$$MGIDI_i = [\sum_{j=1}^{f} (\gamma_{ij} - \gamma_j)^2]^{0.5}$$

where $MGIDI_i$ is the *MGIDI* for the *i-th* genotype; Y_{ij} is the score of the *i-th* genotype in the *j-th* factor (i = 1, 2, …, g; j = 1, 2, …, f); g and f are the number of genotypes and factors, respectively; and Y_j is the jth ideotype score.

The percentage selection gain (SG) was calculated for each trait considering a selection proportion of 25%, as follows:

$$SG_{\%} = \frac{\mu_s - \mu_o}{\mu_o} \times 100$$

where μ_s is the mean of the selected populations, and μ_o is the original mean.

The agreement between the strategies on population rankings was evaluated using the kappa coefficient (K) proposed by Cohen (1960) as follows:

$$K = \frac{p_o - p_c}{1 - p_c}$$

where p_o is the proportion of matching selected genotypes and p_c is the proportion of matching selected genotypes expected by chance.

Softwares

The ASReml-R version 4.1 software (Butler et al. 2018) was used for deviance analysis and prediction of the empirical BLUPs. Using R software (R Core Team 2020), the MGIDI selection index was adjusted according to the protocol provided by Olivoto and Nardino (2021) in the metan package (Olivoto and Lúcio 2020). The kappa index was calculated using the IRR package (Gameret al. 2022). Finally, figures were obtained using the ggplot2 package (Wickham 2016).

RESULTS AND DISCUSSION

Population performance across generations is shown in Figure 1. On average, F_2 populations exhibited higher GY (2,729.31 kg ha^{-1}), but longer cycles (68.35 DH) compared to F_3 populations (1,747.32 kg ha^{-1} and 57.45 DH). This inverse relationship between yield and earliness reflects a known trade-off in wheat breeding, where late-flowering genotypes often have extended grain filling periods and greater biomass (Wang et al. 2019). In addition, the differences in GY and DH between generations can be explained by the distinct environmental conditions under which the experiments were conducted. The F_2 generation was cultivated under irrigated conditions to ensure consistent water availability throughout the cycle. In contrast, the F_3 generation was grown under rainfed conditions and was exposed to variable precipitation and potential water stress. This shift in the water regime likely contributed to the observed reduction in GY and a shortened cycle length in the F_3 generation. The Cerrado region is characterized by a pronounced dry season and erratic rainfall distribution during the wet season (Nóia Júnior et al. 2024). Rainfed cultivation in this environment often leads to suboptimal water availability during critical phenological stages, such as flowering and grain filling, which are highly sensitive to drought stress (Nardino et al. 2022). These findings highlight how environmental regimes strongly influence trait expression in segregating generations and reinforce the importance of considering irrigation versus rainfed management when interpreting results and making breeding decisions for Cerrado growing conditions.

The demand for early and high-yielding genotypes of wheat has increased due to the need for land-use intensification and reduced exposure to climatic and biotic stresses (Wang et al. 2019, Hazard et al. 2020). Historically, yield improvements have been driven by genetic gains and multidisciplinary efforts in breeding programs (Beche et al. 2014). However, these gains have slowed in recent decades, likely because of the increasing impact of climate change, environmental unpredictability, and the complex genetic control of yield (Ray et al. 2012, Van Roekel et al. 2015, Lo Valvo et al. 2018).

Genotypic variance estimates were significant for most trait-generation combinations in both univariate and multivariate models, indicating sufficient genetic variability for selection (Table 2). An exception occurred for GY in the F_2 generation under the univariate model (Strategy I), suggesting environmental noise or early-stage instability in expression. These results reinforce the importance of evaluating populations across multiple generations and environments, which is commonly practiced in wheat breeding programs (Scheeren and Caierão 2015). These evaluations are typically performed in different environments (seasons, locations, and years), which allow for the accumulation of a considerable amount of information that needs to be explored to guide data-driven decisions. Therefore, the need to identify techniques

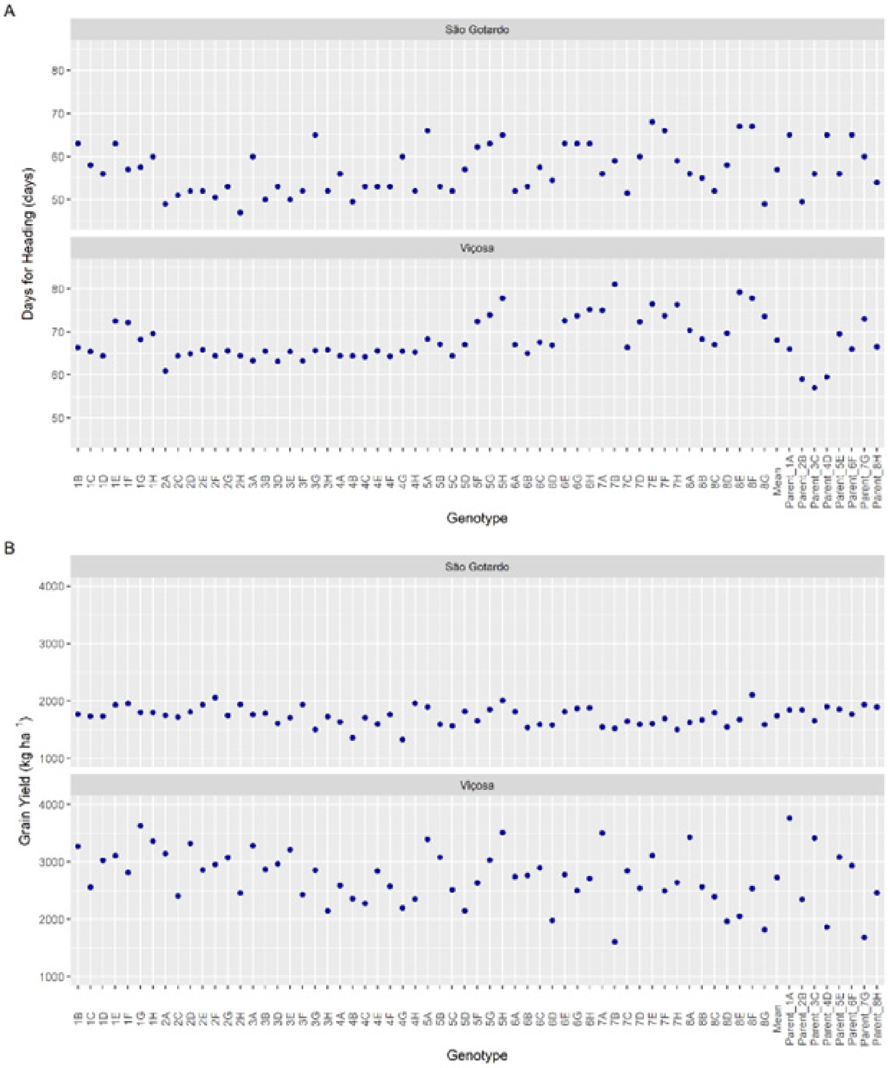

Figure 1. *Average values of 56 F_2 (Viçosa, MG) and F_3 (São Gotardo, MG) populations and eight parents for the DH (A) and GY (B) traits.*

that ensure the existing amount of information, obtained from accurate prediction of empirical genotypic values of individuals or populations under selection, is desirable in any breeding program (Henderson 1974).

Broad-sense heritability and selection accuracy were higher for DH than for GY, particularly under Strategy I. For example, heritability for DH reached 0.76 and 0.91 in F_2 and F_3, respectively, compared to 0.30 and 0.33 for GY. This disparity reflects both higher environmental sensitivity and the complex polygenic architecture of yield (Van Roekel et al. 2015).

Strategy II, which applied multivariate BLUP modeling (Henderson and Quaas 1976), produced lower or equivalent predictive accuracy values (h ≈ 0.57 across traits and generations). The weak correlations between DH and GY ($r = 0.07$ in F_2; $r = 0.18$ in F_3) limited the advantage of multivariate modeling. Although multivariate approaches are theoretically more accurate when leveraging genetic and residual correlations (Piepho et al. 2008), their benefits are reduced when the traits are poorly correlated (Volpato et al. 2019).

Nonetheless, the MGIDI applied during the second stage led to promising SGs under both strategies (Figure 2). Univariate modeling achieved the highest overall gain (18.07%) compared to 15.26% for the multivariate approach, particularly for DH in F_3 (5.90%) and GY in F_2 (6.51%). This demonstrates the effectiveness of the MGIDI in capturing multi-trait performance, even when traits show a weak correlation (Olivoto and Nardino 2021).

Population ranking and selection were largely consistent between the models. Twelve populations were selected using both strategies, including 4H, 2F, 2D, and 1G, with population 2F ranking first under both approaches (Figures 3 and 4). A K of 0.83 (Figure 5) confirms a strong agreement and validates the robustness of the selection outcomes

Table 2. *Parameters estimated from the univariate and multivariate models for the DH and GY (kg ha^{-1}) traits considering the F_2 and F_3 populations and their parents*

Parameter	Strategy I			
	F_2		F_3	
	DH	GY	DH	GY
$\hat{\sigma}^2_b$	0.31	12,762.68	8.74×10^{-7}	1,206.97
$\hat{\sigma}^2_g$	21.70*	104,267.57	30.01*	13,019.84*
$\hat{\sigma}^2_e$	6.65	227,483.60	3.09	25,503.31
h^2	0.76	0.30	0.91	0.33
h	0.87	0.55	0.95	0.57

Parameter	Strategy II	
	F_2	F_3
	DH:GY	
$\hat{\sigma}^2_b$	6.00×10^{-7}	6.00×10^{-7}
$\hat{\sigma}^2_{gDH}$	21.00*	30.00*
$\hat{\sigma}^2_{gGY}$	110,000.00*	1,217.67*
$\hat{\sigma}^2_e$	1.00	1.00
$\hat{\sigma}^2_{eDH:DH}$	6.60	3.09
$\hat{\sigma}^2_{eDH:GY}$	200.00	1.07
$\hat{\sigma}^2_{eGY:GY}$	230,000.00	26,778.37
$\rho_{DH:GY}$	-0.07	0.18
h^2	0.32	0.32
h	0.57	0.57

*Strategy I: univariate model for each generation; Strategy II: multivariate model for each generation; $\hat{\sigma}^2_b$: block variance; $\hat{\sigma}^2_g$: genotypic variance; $\hat{\sigma}^2_e$: residual variance; $\hat{\sigma}^2_{b:ger}$: block variance within generation; $\hat{\sigma}^2_g$: genotypic variance; $\hat{\sigma}^2_{g:ger}$: genotypic variance within generation; $\hat{\sigma}^2_{gDH}$: variance of DH; $\hat{\sigma}^2_{gGY}$: variance of GY; $\hat{\sigma}^2_{eDH:DH}$: residual variance of DH within DH; $\hat{\sigma}^2_{eDH:GY}$: variance of DH within GY; $\hat{\sigma}^2_{eGY:GY}$: variance of GY within GY; $\rho_{DH:GY}$: correlation between DH and GY; h^2: heritability; h: selective accuracy; *: significant at 5% probability by the Chi-square test.*

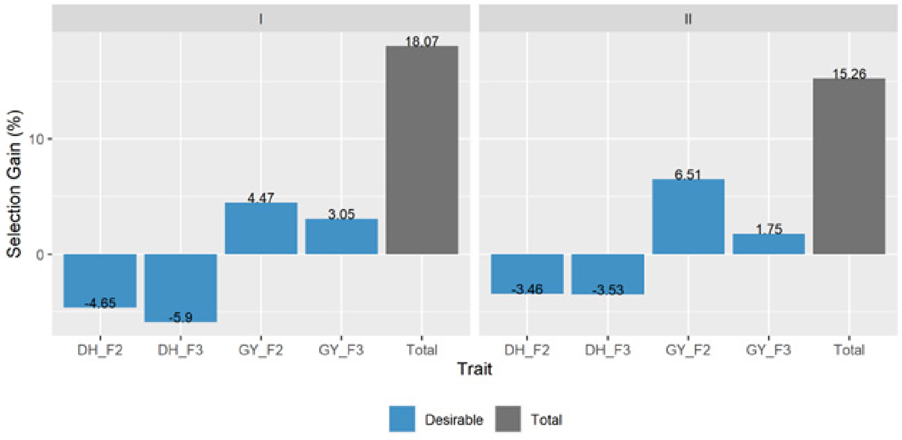

Figure 2. *Percentual Selection Gain (SG) of the best performing segregating populations selected in the second stage by MGIDI using the genetic parameters and empirical genotypic values obtained in the first stage, considering two distinct modeling strategies. Strategy I: univariate model for each generation; Strategy II: multivariate model for each generation; DH_F_2: DH in generation F_2; DH _F_3: DH in generation F_3; GY_F_2: GY in generation F_2; GY _F_3: grain yield in generation F_3.*

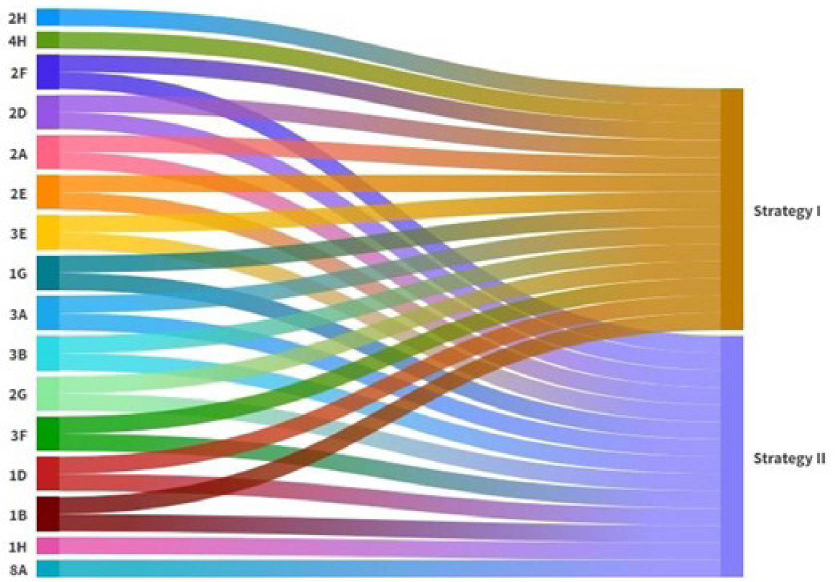

Figure 3. *Sankey plot of the best performing segregating populations selected in the second stage by MGIDI using the genetic parameters and empirical genotypic values obtained in the first stage, considering two distinct modeling strategies. Strategy I: univariate model for each generation; Strategy II: multivariate model for each generation.*

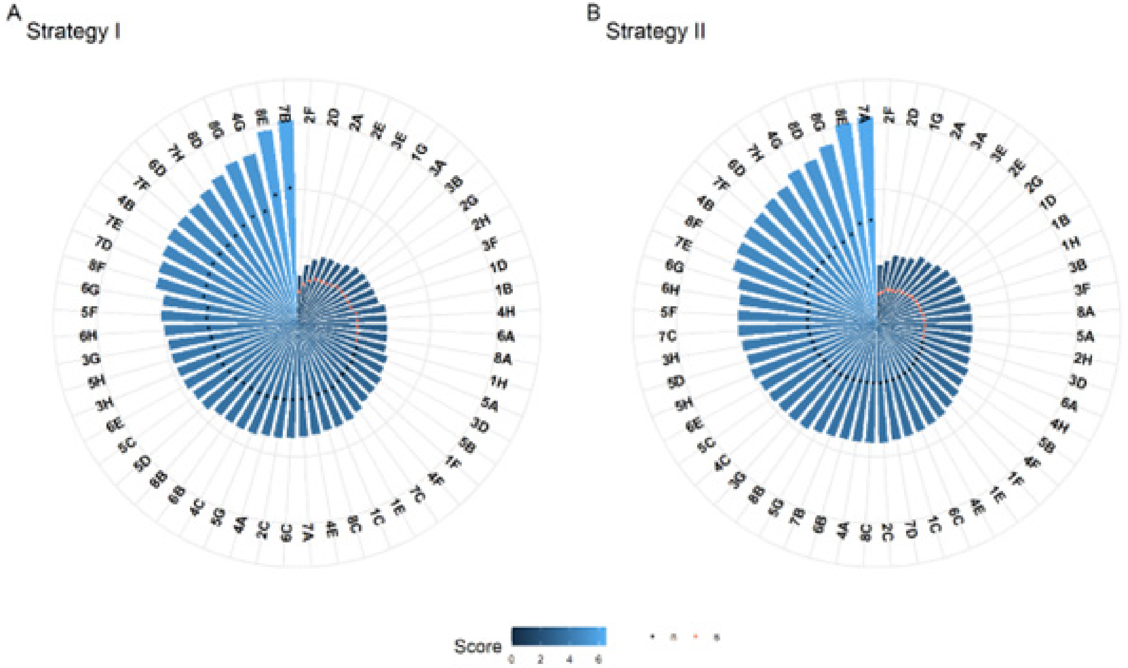

Figure 4. *Classification of 56 wheat populations (ascending order) in the second stage by MGIDI using the genetic parameters and empirical genotypic values obtained in the first stage, considering two distinct modeling strategies. Strategy I: univariate model for each generation; Strategy II: multivariate model for each generation. Black dots indicate non-selected genotypes, red dots indicate selected genotypes.*

Kappa coefficient = 0.83.

(Cohen 1960). These results highlight the reliability of both modeling approaches in the presence of a low trait correlation.

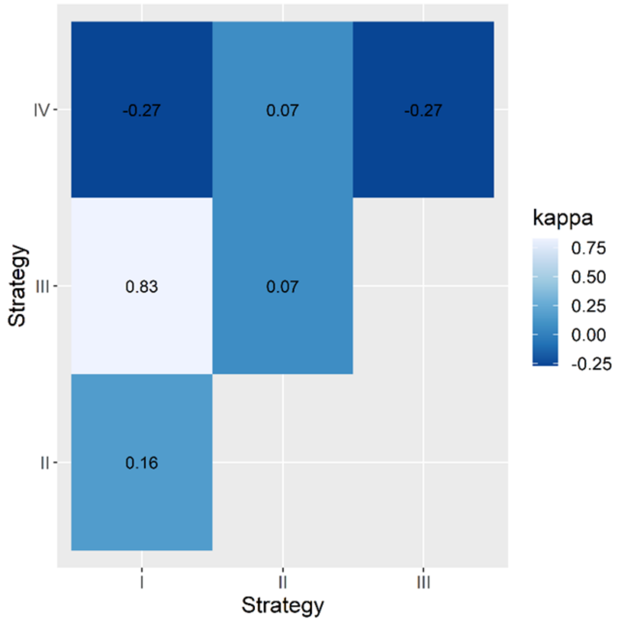

Figure 5. *Kappa coefficient among the different selection strategies. Strategy I: univariate model for each generation; Strategy II: univariate and multi-generational model; Strategy III: multivariate model for each generation; Strategy IV: multivariate and multi-generational model.*

As previously discussed by Henderson (1974), BLUP-based prediction allows for an unbiased estimation of genotypic values under selection, thereby improving breeding decisions. The current results confirm that even under weak trait correlation, stage-wise selection supported by BLUP and MGIDI can effectively guide the derivation of superior inbred lines.

Despite the fact that strategy I demonstrated a slightly higher genetic gain than strategy II, one could say that these models were equivalent in the current study, because the differences in genetic gains were negligible, and they agreed on the selection of the best candidates. As discussed previously, strategy II did not present any advantages over strategy I because the traits were poorly correlated. In cases where traits are highly correlated, genetic gains can benefit from multivariate approaches that model the covariance structures of the relationships between traits, as demonstrated in other studies (Volpato et al. 2019).

The patterns observed here are consistent with those of previous reports on wheat (Ghaed-Rahimi et al. 2014, Tahmasebi et al. 2014, 2017, Sobhanian et al. 2019). Other studies have also demonstrated that integrating genetic variance estimation with molecular or statistical tools enhances accuracy and accelerates genetic gains (Salarpour et al. 2021, Heidari 2024). Our results confirm that, even under contrasting water regimes and weak trait correlations, sufficient genetic variability exists for selection and effective gains can be realized. Compared to traditional selection approaches, the strategies tested here offer clear advantages. Strategy I (univariate BLUP) maximizes accuracy when traits are independent or weakly correlated, whereas Strategy II (multivariate BLUP) is most useful when exploiting strong correlations among traits. Use of the MGIDI in both strategies further improved multi-trait selection efficiency, demonstrating its value in identifying populations with superior overall performance. Collectively, these approaches provide breeders with flexible tools to optimize selection decisions in variable environments.

CONCLUSION

This study demonstrated that both univariate and multivariate BLUP-based models are effective tools for the stage-wise selection of segregating wheat populations for GY and earliness. Despite the theoretical advantages of multivariate models, particularly when traits are correlated, the low correlation between DH and GY in this study limited the benefits of the multivariate approach. Consequently, the univariate model yielded slightly higher predicted genetic gains, particularly for earliness.

Nonetheless, both strategies showed strong agreement in population ranking and selection, as evidenced by the high K. A set of 13 populations, 4H, 2F, 2D, 2A, 2E, 3E, 1G, 3A, 3B, 2G, 3F, 1D, and 1B, were consistently selected across the methods and represented promising candidates for the development of tropical wheat inbred lines that combine early flowering and high yield.

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DATA AVAILABILITY

The datasets generated and/or analyzed during the current research are available from the corresponding author upon reasonable request.

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