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Meier, Carine; Marchioro, Volmir Sergio; Meira, Daniela; Olivoto, Tiago; Klein, Luís Antônio

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Genetic parameters and multiple-trait selection in wheat genotypes¹

Carine Meier², Volmir Sergio Marchioro³,
Daniela Meira⁴, Tiago Olivoto⁵, Luís Antônio Klein³

ABSTRACT

Mixed models and multivariate analysis are powerful tools for selecting superior genotypes in plant breeding programs. The BLUP (best linear unbiased prediction) method has been used to predict genetic values without environmental effects. Furthermore, the FAI-BLUP (ideotype-design index) procedure is especially valuable for plant breeding because of multiple-trait selection. This study aimed to determine the genetic potential of advanced wheat generations using REML/BLUP in combination with multivariate techniques for the selection of superior genotypes. The experiment consisted of eleven wheat (*Triticum aestivum* L.) genotypes. The experimental design was randomized blocks, with three replications. Plant height, spike insertion height, number of tillers, number of spikelets, kernel width, hectoliter weight and kernel weight per plant were determined. The genetic parameters were estimated using the REML/BLUP methodology, and the FAI-BLUP index was calculated using predicted genetic values. The genotypes UFSMF 1-02, UFSMF 1-05 and UFSMF 1-04 show potential to increase the grain yield. The selection gains for number of tillers (14.63 %) and kernel weight per plant (22.35 %) indicate the potential to select superior genotypes.

KEYWORDS: *Triticum aestivum* L., plant breeding, mixed models.

RESUMO

Parâmetros genéticos e seleção de múltiplas características em genótipos de trigo

Modelos mistos e análises multivariadas são ferramentas poderosas para selecionar genótipos superiores em programas de melhoramento de plantas. O método BLUP (best linear unbiased prediction) tem sido usado para prever valores genéticos, eliminando efeitos ambientais. Além disso, o método FAI-BLUP (ideotype-design index) é importante para o melhoramento genético de plantas, devido à seleção de múltiplas características. Objetivou-se determinar o potencial genético de gerações avançadas de trigo, utilizando-se REML/BLUP em combinação com técnicas multivariadas, para a seleção de genótipos superiores. O experimento foi constituído de onze genótipos de trigo (*Triticum aestivum* L.). O delineamento experimental foi o de blocos casualizados, com três repetições. Foram avaliadas as variáveis altura da planta, altura de inserção da espiga, número de afilhos, número de espiguetas, largura do grão, peso hectolitro e peso de grãos por planta. Os parâmetros genéticos foram estimados utilizando-se a metodologia REML/BLUP, e o índice FAI-BLUP foi calculado por meio dos valores genéticos preditos. Os genótipos UFSMF 1-02, UFSMF 1-05 e UFSMF 1-04 apresentam potencial de incremento para produtividade de grãos. Os ganhos de seleção para número de afilhos (14,63 %) e massa de grãos por planta (22,35 %) são potenciais para a seleção de genótipos superiores.

PALAVRAS-CHAVE: *Triticum aestivum* L.; melhoramento de plantas, modelos mistos.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a staple food worldwide and, as such, an important part of the daily diet and a carbohydrate source of millions of

people (Litoriya et al. 2018). Also, wheat is widely used in Brazil, due to the development of high-yield cultivars and a high market consumption of food with a better industrial quality and high nutritional content (Battenfield et al. 2016).

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² Escola Estadual Técnica Celeste Gobbato, Palmeira das Missões, RS, Brasil.

E-mail/ORCID: meiercarine5@gmail.com/0000-0003-0803-7134.

³ Universidade Federal de Santa Maria, Frederico Westphalen, RS, Brasil.

E-mail/ORCID: volmir@marchioro.eng.br/0000-0003-3873-9567; luis.antonio.klein@hotmail.com/0000-0001-6978-3988.

⁴ Universidade Tecnológica Federal do Paraná, Pato Branco, PR, Brasil.

E-mail/ORCID: dmdanielameira94@gmail.com/0000-0001-7843-4472.

⁵ Instituto de Desenvolvimento Educacional do Alto Uruguai, Getúlio Vargas, RS, Brasil.

E-mail/ORCID: tiagoolivoto@gmail.com/0000-0002-0241-9636.

The selection of superior genotypes is usually based on phenotypic expression, which involves genetic and environmental effects. However, the use of mixed models facilitates the prediction of genotypic values and improves selection efficiency (Pimentel et al. 2014). In this context, one of the most appropriate selection procedures involves the estimation of variance components (i.e., restricted maximum likelihood - REML) and the prediction of genotypic values (i.e., best linear unbiased prediction - BLUP) (Resende 2007). Accordingly, plant breeding studies use these methods, such as for white oat (*Avena sativa* L.; Olivoto et al. 2019), soybean (*Glycine max* L.; Follmann et al. 2019), bean (*Phaseolus vulgaris* L.; Santos et al. 2019) and corn (*Zea mays* L.; Olivoto et al. 2017), as well as in early-segregating generations of wheat (Pimentel et al. 2014, Woyann et al. 2019b).

The use of mixed models is a strategy to improve selection efficiency in the improvement of autogamous plants, when using plant breeding methods for selection with progeny testing (Ramalho & Araújo 2011). To obtain cultivars with desirable agronomic traits, studies must evaluate a vast number of characters. However, at the selection time, it is difficult to identify individuals with traits of interest. Thus, multivariate techniques have been used to discriminate soybean (Tobar-Tosse et al. 2015) and wheat (Costa et al. 2013) lines. One of the most used techniques is principal component analysis (PC), which condenses numerous variables into a limited number of new variables (PCs). Each PC is a linear combination of original, independent and uncorrelated variables, where the former is the most important, because it retains most of the total variation in the original data (Cruz et al. 2014).

To select potentially high-yield wheat lines in breeding programs, superior genotypes must be selected with great accuracy. Thus, among the recently released selection indexes, Rocha et al. (2018) proposed a selection index based on factor analysis and genotype-ideotype distance (FAI-BLUP index). The calculation of such a multi-trait index predicts genetic effects with mixed models, and the calculation of genetic values using REML/BLUP (Resende et al. 2014) is a good strategy for selecting genotypes. Silva et al. (2018), Oliveira et al. (2019), Rocha et al. (2019) and Woyann et al. (2019a) also highlighted the efficiency of this selection index for crop breeding.

This study aimed to determine the genetic potential of advanced wheat genotypes using REML/BLUP combined with multivariate techniques to select superior genotypes.

MATERIAL AND METHODS

The study was conducted in the 2018 crop season, in Frederico Westphalen, Rio Grande do Sul state, Brazil (27°23'26"S, 53°25'43"W and altitude of 481 m). The soil is classified as a typical Dystrophic Red Latosol (Santos et al. 2013) or a Typic Hapludox (USDA 2014). The climate is classified as Cfa, according to the Köppen classification (Alvares et al. 2013).

F7 generation wheat genotypes were selected from hundreds of lines obtained from populations generated in 2011 from crosses between productive cultivars grown in Rio Grande do Sul at that time. The genotypes derived from single crosses: Mirante/Quartzo (UFSMFW 1-01), Mirante/Fundacep Horizonte (UFSMFW 1-02), Supera/Quartzo (UFSMFW 1-03), Supera/Quartzo (UFSMFW 1-04), BRS 327/Fundacep Horizonte (UFSMFW 1-05), Supera/Abalone (UFSMFW 1-06), BRS 327/Fundacep Horizonte (UFSMFW 1-07), Supera/Abalone (UFSMFW 1-08) and BRS 327/Fundacep Horizonte (UFSMFW 1-09). The cultivars TBIO Toruk and Quartzo were used as controls.

The trials were conducted in a randomized blocks design with 11 wheat genotypes and six replications. The plots were composed by four 4 m long rows, with row spacing of 0.17 m and plant density of 350 plants m⁻². Sowing was conducted on May 26, 2018, with a base fertilization rate of 200 kg ha⁻¹ of N-P₂O₅-K₂O (08-24-12). Supplementary fertilization of 90 kg ha⁻¹ of N (urea; 45 % of N) was applied twice, once during tillering and once at the elongation stage. Weeds, pests and diseases were controlled according to the technical recommendations for wheat.

The evaluated agronomic traits were plant height (cm), measured as the distance from the soil to the spike of the main tiller; number of fertile tillers, obtained by counting fertile tillers; spike insertion height (cm), measured from the soil level to the spike insertion; number of spikelets; hectoliter weight (kg hL⁻¹), measured by the volume of grain per unit; kernel width (mm); and kernel weight per plant (g).

Each trait was analyzed using a mixed-effect model based on the following equation: $y = Xb + Zu + e$, where y is an $n[=\sum_{j=1}^r(gr)] \times 1$ vector of response variable, i.e., the response of the i th genotype in the j th block ($i = 1, 2, \dots, g; j = 1, 2, \dots, r$); $y = [y_{11}, y_{12}, \dots, y_{gr}]'$; b a $1 \times r$ vector of unknown and unobservable block fixed effects ($b = [y_1, y_2, \dots, y_r]'$); u an $m[= 1 \times g]$ vector of unknown and unobservable genotype random effects ($u = [\alpha_1, \alpha_2, \dots, \alpha_g]'$); X an $n \times r$ design matrix of 0s and 1s relating y to b ; Z represents an $n \times r$ design matrix of 0s and 1s relating y to u ; e is an $n \times r$ vector of random errors ($e = [y_{11}, y_{12}, \dots, y_{gr}]'$); and the prime symbol ($'$) is the vector transposition. The random vectors u and e are assumed as normal and independently distributed with a zero mean and variance-covariance matrices G and R , respectively, so that

$$\begin{bmatrix} u \\ e \end{bmatrix} \sim N \left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} G & 0 \\ 0 & R \end{bmatrix} \right)$$

where the simplest forms of G and R , namely $G = \hat{\sigma}_a^2 I_g$ and $R = \hat{\sigma}_e^2 I_n$, will be used, where $\hat{\sigma}_a^2$ and $\hat{\sigma}_e^2$ are variances for genotypes and random errors, respectively, and I_g and I_n are identity matrices of order g and n , respectively. The vectors b and u were then estimated using the well-known mixed model equation (Henderson 1975):

$$\begin{bmatrix} \hat{b} \\ \hat{u} \end{bmatrix} = \begin{bmatrix} X'R^{-1}X & X'R^{-1}Z \\ Z'R^{-1}X & Z'R^{-1}Z + G^{-1} \end{bmatrix}^{-1} \begin{bmatrix} X'R^{-1}y \\ Z'R^{-1}y \end{bmatrix}$$

where the superscripts “ -1 ” and “ $-$ ” are the inverse and generalized inverse of the matrices, respectively. The variance components \hat{G} and \hat{R} were obtained by Restricted Maximum Likelihood (REML), using the expectation-maximization algorithm (Dempster et al. 1977). The significance of random effects was evaluated using a likelihood ratio (LR) test, and the probability was then obtained using a two-tailed Chi-square test with one degree of freedom (χ_1^2). Since data were balanced, the effect of the i th genotype (\hat{g}_i) within u was given in standard Anova notation, as it follows: $g_i = h_g^2 (\bar{y}_i - \bar{y})$, where

$$h_g^2 = \frac{r\hat{\sigma}_a^2}{r\hat{\sigma}_e^2 + r\hat{\sigma}_a^2}$$

is the shrinkage effect for the genotype effect. The BLUP of the i th genotype was given by $BLUP = \mu + \hat{g}_i$, where μ is the overall mean. Confidence intervals for the BLUPs were estimated according to the following equation (Resende 2002): $CI = Gv \pm t[(1 - r_{aa}^2)\alpha_g^2]^{0.5}$,

where CI is the confidence interval, Gv the genotypic value, t the Student t distribution value associated with a given significance level in a two-tailed test ($t = 2.57$, considering $\alpha = 0.05$), r_{aa}^2 the selective accuracy and α_g^2 the genotypic variance.

The principal component (PC) analysis of agronomic traits of the wheat genotypes was performed using the *stats* and *factoextra* packages (Kassambara & Mundt 2017) in R (version 3.5.3; R Core Team 2019).

Genetic values were used to calculate a multi-trait index based on the FAI-BLUP index (Rocha et al. 2018). The factor analysis and ideotype-design (FAI-BLUP) index (Rocha et al. 2018) were computed aiming to rank the genotypes based on multitrait, free from multicollinearity. A selection intensity of ~18 % was considered (two genotypes selected). The maximum values for kernel width, number of spikelets, hectoliter weight and kernel weight per plant, mean values for fertile tillers and minimum values for plant height and spike height insertion were used to identify ideal traits. To compute the index, the FAI-BLUP function of the *metan* package was used (Olivoto & Lúcio 2020). A radar chart was generated using the *radarchart* function of the *fmsb* package (Nakazawa 2018).

RESULTS AND DISCUSSION

There was a significant difference by the likelihood-ratio test at 1 % of error probability (Table 1) for the variables plant height, number of fertile tillers, spike height insertion, kernel weight per plant, hectoliter weight and kernel width. For kernel weight per plant and hectoliter weight, there was also a significant difference at 1 % of error probability. Only for the variable number of spikelets there was no significant difference. These results reveal the existence of an important genetic variability among the wheat genotypes.

There was a higher phenotypic variance for kernel weight per plant than for the other traits, due to environmental variation (Table 1). The highest individual heritability occurred for plant height (0.73) and fertile tillers (0.51). These results reveal a favorable condition for genetic gains with selection, mainly for number of tillers, which is related to increases in grain quantity, consequently increasing yield. Valério et al. (2009) studied dozens of wheat populations and found extremely low values ranging

Table 1. Estimates of variance components and genetic parameters for plant height (PH; cm), number of fertile tillers (NT), spike height insertion (SHI; cm), number of spikelets (NS), kernel weight per plant (KWP; g), hectoliter weight (HW; kg hL⁻¹) and kernel width (KW; mm) for 11 wheat genotypes.

Parameters	Traits						
	PH	NT	SHI	NS	KWP	HW	KW
Vg	6.95	0.13	3.66	0.05	63.83	0.01	0.02
Ve	2.56	0.12	7.95	0.42	150.58	0.03	0.06
Vf	9.51	0.25	11.60	0.48	214.42	0.03	0.08
h2g	0.73	0.51	0.32	0.11	0.30	0.27	0.27
h2ml	0.94	0.86	0.73	0.43	0.72	0.69	0.69
Acgen	0.97	0.93	0.86	0.66	0.85	0.83	0.83
CVgi%	3.63	10.37	2.98	1.58	16.61	0.14	5.34
CVe%	2.20	10.10	4.39	4.46	25.51	0.24	8.76
Mean	72.69	3.44	64.26	14.58	9.61	79.21	2.76
LRT	40.71**	23.24**	9.47**	1.48 ^{ns}	8.55**	7.06*	7.20*

Vg: genotypic variance; Ve: environmental variance; Vf: phenotypic variance; h2g = h2: heritability of individual plots in the broad sense; h2ml: heritability of genotype mean, assuming complete survival; Acgen: selective accuracy assuming no loss of plots; CVgi%: genotypic coefficient of variation; CVe%: residual coefficient of variation; Mean: overall experiment mean; LRT: likelihood-ratio test (** p < 0.01; * p < 0.05; ^{ns} not significant).

up to a maximum of 0.48 for heritability in the broad sense, reinforcing the importance of the results found in this study.

Heritability estimates based on genotype average (h2ml) were high for all traits, ranging from 0.43 to 0.94 for number of spikelets and plant height, respectively. These values were generally greater than heritability estimates at the individual level (h2g), what suggests that the evaluated traits can be used to evaluate genetic variation and predict the set of genotypes.

The selective accuracy assuming no loss of plots (Acgen) used to predict genetic values reflects the results quality. This parameter is associated with selection accuracy and refers to the correlation between predicted genetic values and genetic values

of individuals (Pimentel et al. 2014). The selective precision was 0.66 for number of spikelets and 0.97 for plant height, which are considered high values.

The coefficients of genetic variance (CVgi) for kernel weight per plant and fertile tillers were 16.61 and 10.37, respectively. High values are desirable in searching for high genetic constitutions, since this variable quantifies the magnitude of genetic variation available for selection.

Positive genotypic effects occurred for plant height in the genotypes UFSMFW 1-01, UFSMFW 1-05, UFSMFW 1-06, TBIO Toruk, UFSMFW 1-04 and UFSMFW 1-03 (Figure 1a). On the other hand, there were negative genotypic effects for plant height in UFSMFW 1-08, UFSMFW 1-07, Quartzo, UFSMFW 1-02 and UFSMFW 1-09. It should be

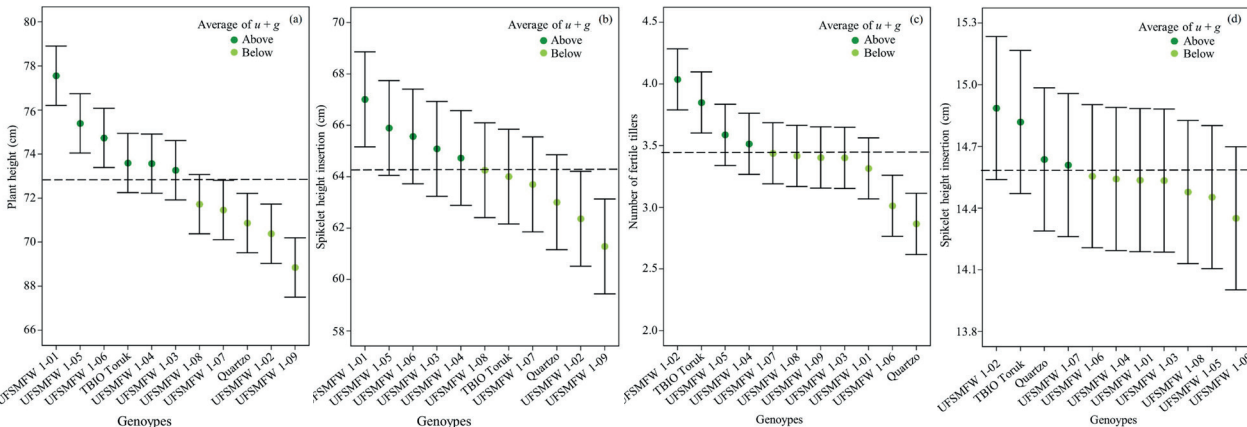


Figure 1. Predicted genetic values for plant height (a), spike height insertion (b), number of fertile tillers (c) and number of spikelets (d) for 11 wheat genotypes.

emphasized that genotypic effect is the difference between each point and the horizontal dashed line, which represents the overall mean of the trait. UFSMFW 1-01 presented the greatest predicted mean plant height (77.55 cm), but was not statistically different from that of the genotype UFSMFW 1-05 (75.39 cm) due to overlapping confidence intervals. UFSMFW 1-09, UFSMFW 1-02, Quartzo and UFSMFW 1-07 represented a minimum predicted mean, indicating that there is no difference among these genotypes for the evaluated trait. Such height reduction is important for breeding programs and is an essential and desirable trait associated with the *Rht1* gene (Keser et al. 2017, Yao et al. 2018).

There were negative genotypic effects for spike height insertion in UFSMFW 1-08, TBIO Toruk, UFSMFW 1-07, Quartzo, UFSMFW 1-02 and UFSMFW 1-09 (Figure 1b). These genotypes have desirable traits for plant breeding, since a greater spike height insertion is not always desired in cultivars with a low stem resistance. According to Souza et al. (2013), a greater spike height insertion in wheat exerts a greater force for tipping, leading to lodging, crop losses and less yield.

When considering confidence intervals, the mean fertile tillers values for UFSMFW 1-02 (NT = 4.04), TBIO Toruk (NT = 3.84) and UFSMFW 1-05 (NT = 3.58) were statistically similar (Figure 1c), indicating that fertile tillers can be used for an indirect selection of grain yield. In wheat, the number of fertile tillers is positively correlated with kernel weight per plant (Meier et al. 2019). Thus, genotypes with mean fertile tillers values higher than 3.44

have additive genetic effects and may be positively correlated with grain yield.

However, there were no significant differences in the number of spikelets for the genotypes included in the present study (Figure 1d). The mean number of spikelets of the present study (14.60) was similar to that reported for wheat by Silva et al. (2015). Inflorescence yield components may contribute to yield, because increases in the number of spikelets may increase the number of grains per spike, which is considered an important component of wheat yield.

The genotypes with the most favorable BLUPs for hectoliter weight (Figure 2a) were those between UFSMFW 1-03 (79.25 kg hL⁻¹) and UFSMFW 1-09 (79.05 kg hL⁻¹). The predicted values were lower than those that Martin et al. (2017) observed. The authors studied wheat genotypes with the same genetic basis as those aforementioned, but that difference may be attributed to environmental conditions, especially rainfall (Tavares et al. 2014). Therefore, decreases in hectoliter weight may be associated to successive changes in grain moisture due to the sequence of rain before harvest.

For kernel width (Figure 2b), there were positive genotypic effects for UFSMFW 1-01, UFSMFW 1-06, UFSMFW 1-02, UFSMFW 1-03 and UFSMFW 1-04. However, there were no statistical differences in the mean kernel width of UFSMFW 1-01 and UFSMFW 1-09. Kernel width is an important agronomic trait, since seed size is positively correlated with seed quality, and larger seeds correspond to high quality seedlings in black oat (Meira et al. 2017).

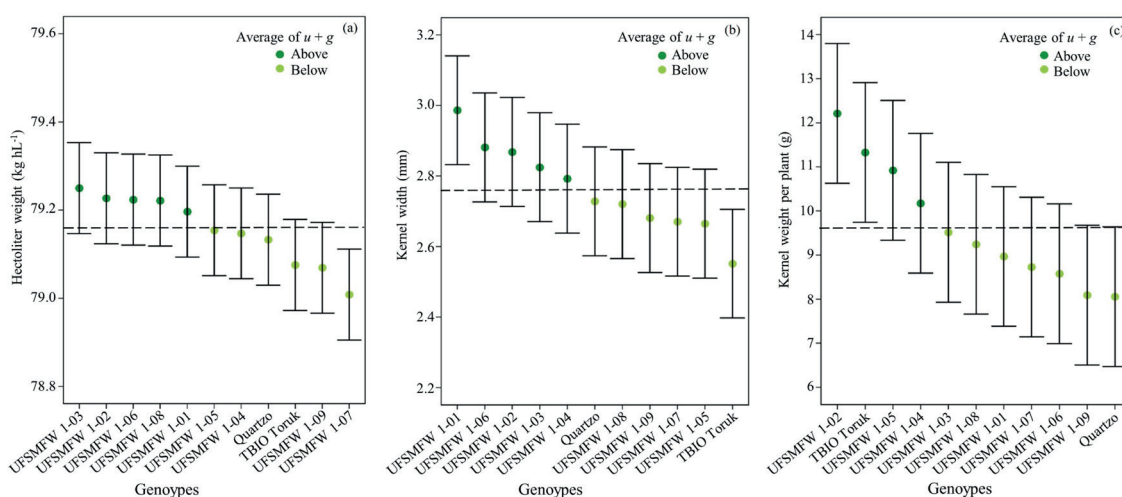


Figure 2. Predicted genetic values for hectoliter weight (a), kernel width (b) and kernel weight per plant (c) for 11 wheat genotypes.

Kernel weight per plant is the main trait associated with agronomic performance. The BLUPs for kernel weight per plant (Figure 2c) revealed that UFSMFW 1-02, TBIO Toruk, UFSMFW 1-05 and UFSMFW 1-04 showed positive genotypic effects, whereas UFSMFW 1-03, UFSMFW 1-08, UFSMFW 1-01, UFSMFW 1-07, UFSMFW 1-06, UFSMFW 1-09 and Quartzo had negative genotypic effects. Based on the confidence intervals, there were no significant differences in the mean kernel weight per plant for UFSMFW 1-02 to UFSMFW 1-08, likely due to the high contribution of the phenotypic and environmental variation. A greater residual variation is associated with greater retraction effects on predicted means. Because the components of the random effects are considered in the prediction of the response variable, these findings indicate that individuals with these genotypes could possess concentrated favorable alleles for grain yield.

The genotypes UFSMFW 1-02, UFSMFW 1-04 and UFSMFW 1-05 presented a high genetic potential, since the BLUP method relies on the estimating of genotypic values, with no environmental effects. Cortes et al. (2019) studied superior lines of papaya and argued that genetic values are more important for breeders because they represent true values in predicting and representing the genetic potential of individuals.

Principal component analysis aims to understand the genetic diversity among genotypes for the evaluated agronomic traits. The seven traits analyzed in the present study were low in two main components, which, together, accounted for 72 % of the phenotypic variation (Figure 3). Gonzalez-Navarro et al. (2016) reported similar results. The authors investigated the variation in the development patterns of 27 elite wheat lines and their relationships with yield and observed that 60 % of the total variance was explained by two PCs.

In the present study, the traits associated with grain yield are to the left of PC1 (Figure 3). The association between the set of traits and wheat genotypes revealed that TBIO Toruk and UFSMFW 1-02 were likely to have a greater kernel weight per plant, more fertile tillers and a higher number of spikelets. However, UFSMFW 1-01 showed a greater association to spike height insertion and plant height. Benin et al. (2012) also observed different behaviors for wheat genotypes, depending on the variables

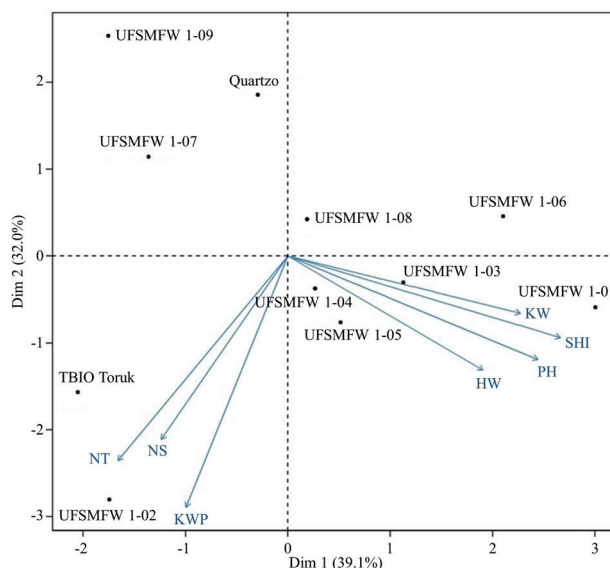


Figure 3. Plot of scores for principal components and association of wheat genotypes with agronomic traits. PH: plant height; NT: number of fertile tillers; SHI: spike height insertion; NS: number of spikelets; KWP: kernel weight per plant; HW: hectoliter weight; KW: kernel width.

studied. To determine the ideotype, the FAI-BLUP index calculates the genetic correlation of each factor, thereby identifying three factors. Factor 1 had a high genetic correlation with fertile tillers and kernel weight per plant, whereas Factor 2 was correlated with plant height, spike height insertion and hectoliter weight, and Factor 3 was correlated with number of spikelets and kernel width (Table 2). The genetic gain observed for the evaluated traits revealed a possibility of reduction in plant height (-1.19 %) and spike height insertion (-1.69 %) (Table 2). In addition, fertile tillers and kernel weight per plant gains were 14.63 and 22.35 %, respectively. The evaluated genotypes included genetic constitutions with desirable characteristics as for grain yield. The mean communality (i.e., common variance) accounted for 86 % of all the genetic variability in the dataset (Table 2).

The FAI-BLUP index has the potential to improve multiple traits simultaneously, since it considers predicted genetic effects (Rocha et al. 2018). This multiple trait index identifies genotypes with traits closest to those of the ideotype (Figure 4). The FAI-BLUP index indicates that the genotypes UFSMFW 1-02 and TBIO Toruk show the greatest potential for wheat breeding programs, because of traits closer to the ideotype, a relatively low PH, relatively high fertile tillers and kernel weight per plant.

Table 2. Factorial loadings, communalities and predicted genetic gains based on the FAI-BLUP index.

Trait	Factor			Communality	Predicted genetic gain (%)
	Factor 1	Factor 2	Factor 3		
PH (cm)	0.00	-0.95	0.03	0.90	-1.19
SHI (cm)	0.09	-0.97	-0.04	0.95	-1.68
NT	-0.98	0.12	-0.01	0.98	14.63
NS	-0.51	0.15	0.78	0.90	1.86
HW (kg hL ⁻¹)	-0.05	-0.58	0.18	0.37	-0.01
KW (mm)	-0.01	0.26	-0.93	0.94	-3.19
KWP (g)	-0.93	-0.14	0.27	0.96	22.35
Communality mean				0.86	-

PH: plant height; SHI: spike height insertion; NT: number of fertile tillers; NS: number of spikelets; HW: hectoliter weight; KW: kernel width; KWP: kernel weight per plant.

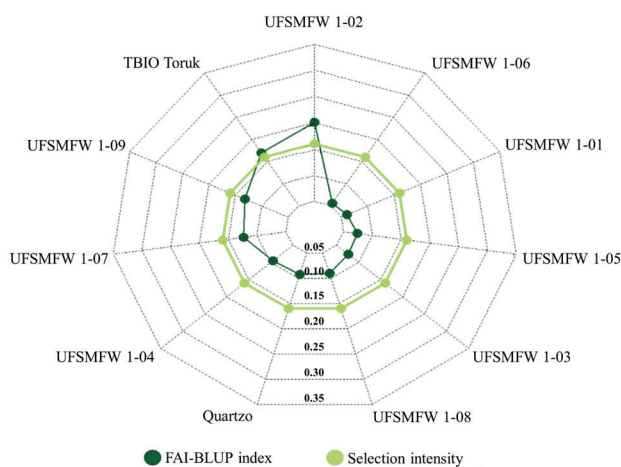


Figure 4. Wheat genotypes ranking and genotypes selected using the FAI-BLUP index.

CONCLUSIONS

1. The number of fertile tillers and kernel weight per plant provided the highest predicted genetic gains;
2. The FAI-BLUP index identified the genotypes UFSMFW 1-02 and TORUK as those to be used in future wheat breeding programs.

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