AGRONOMIC APPLICATION OF GENETIC RESOURCES

Selection of Aluminum-Resistant Wheat Genotypes Using Multienvironment and Multivariate Indices

Jorge González Aguilera, Paulo Eduardo Teodoro,*
José Pereira da Silva Junior, Jorge Fernando Pereira, Alan Mario Zuffo, and Luciano Consoli

ABSTRACT

A gain in association was obtained by using a new multivariate index with alleles of *TaALMT1* and *TaMATE1B* genes compared with a previously published index. Wheat is among the major grain crops. Its growth can be affected by aluminum toxicity, an important stress factor that limits plant productivity. Thus, an efficient means to select wheat genotypes that are resistant to this stress is required. Here, 172 wheat cultivars and 23 synthetic hexaploid wheat plants showing different alleles for *TaALMT1* and *TaMATE1B* and different levels of Al resistance in field conditions (phenotyped in two crop seasons) were studied. The modified Lin and Binns method was applied to obtain the phenotype data in a multivariate manner aiming at a general identification of genotypes based on the multiple traits evaluated. The genotype × year interaction was significant for all traits that were evaluated in three growth stages (tillering, silking, and maturation). The top 20 Al-resistant genotypes obtained by the multivariate method were highlighted as having the best combination of the main desirable traits. Correlation analysis showed a gain in the association of the multivariate index with alleles of *TaALMT1* and *TaMATE1B* genes compared with a previously published index. The superior *TaMATE1B* allele (having an insertion in the promoter region) contributed a greater Al resistance independent of the *TaALMT1* allele. The higher association with Al resistance qualifies the selection index described in this study to be used for wheat breeding programs with the aim of improving the performance of wheat when grown under Al stress.

Core Ideas

- Wheat is among the major grain crops.
- Its growth can be affected by aluminum toxicity.
- Genotypes with high adaptability and stability were identified.
- A new multivariate index allowed greater association with Al resistance.

BRAZIL IS one of the major importers of wheat (*Triticum aestivum* L.) in the world. To increase food security, Brazil needs to increase its wheat production. However, the area dedicated to wheat cultivation in Brazil has remained virtually the same (around 2500 ha) for the past 10 yr (CONAB, 2018). Based on this, greater wheat production will have to come from increased productivity and not by increasing the cultivated area. This is the same globally because the primary mechanism of increasing food production in the world in the next 30 yr will need to rely on yield increase (Fischer et al., 2014).

Greater wheat production can be achieved by better cultivars and management practices. For instance, in the Cerrado region of Brazil, the increase of 37 kg of wheat produced per hectare per year is 47.4% due to improvements in management practices and 52.6% due to better cultivars (Cargnin et al., 2009). Nevertheless, most of the significant future improvements in wheat production will probably come from better cultivars. When focusing on breeding programs, the identification of lineages showing superior performance is one of the biggest challenges. The selection process is often based on the performance of the genotypes in different environments (years and/or locations). However, the decision to launch new cultivars is usually hampered by the frequent occurrence of genotype and its interactions with the environment (Cargnin et al., 2006). For this purpose, adaptability and stability analyses are performed, through which it is possible to identify cultivars that are adaptable to specific environments or that exhibit broad adaptability. Several methods of adaptability and stability analyses have been described for the evaluation of genetic materials (Cruz and Carneiro, 2003). Among these, we highlighted the methodology of Lin and Binns (1988), where the P_i parameter is estimated based on the mean square of the distance between the genotype mean and the maximum average response obtained in the environment. This parameter has variance property and measures the response of a genotype to the best genotype in each environment. This method has been used by several researchers because it is based on nonparametric statistics. Therefore, it can be applied to various environments and genotypes. In addition, the recommendation of this method is based on a single-step

J.G. Aguilera, P.E. Teodoro, A.M. Zuffo, Univ. Federal de Mato Grosso do Sul- Campus de Chapadão do Sul, Rodovia MS 306 km 105, Chapadão do Sul, MS, CEP 79560-000, Brazil; J.P. da Silva Junior, L. Consoli, Embrapa Trigo, Rodovia BR 285 km 294, Passo Fundo, RS, CEP 99001-970, Brazil; J.F. Pereira, Embrapa Gado de Leite, Rua Eugênio do Nascimento 610, Juiz de Fora, MG, CEP 36038-330, Brazil. Received 21 June 2019. Accepted 14 July 2019. *Corresponding author (eduteodoro@hotmail.com).

Abbreviations: CIATF, classification index of aluminum toxicity in the field; OA, organic acid; SHW, synthetic hexaploid wheat.

analysis that is not obtained in other methods, such as the method in Eberhart and Russell (1966), which uses an environmental index and parameters linked to the regression equation.

Although the P_i parameter selection index has been used for multiple traits in multiple environments to select wheat genotypes (Albrecht et al., 2007; Biudes et al., 2009; Cargnin et al., 2008; Caierão et al., 2006; Franceschi et al., 2010; Murakami et al., 2004), it has not been used to select wheat materials that show superior resistance to aluminum (Al). There is interest in applying this index in the selection of Al-resistant wheat genotypes because Al toxicity is found in acidic soils, which is one of the major factors limiting wheat production worldwide. In acidic soils, the toxic trivalent Al cation becomes prevalent, impacting elongation and division of the root cells, which results in plants with lower root growth (Kochian et al., 2015). Shorter roots may affect plant development and enhance the probability of nutritional, drought, and heat stresses. The chances of wheat plants facing Al toxicity are high because acidic soils constitute ~60% of the area in the tropical and subtropical regions (von Uexküll and Mutert, 1995) and constitute a similar proportion in the Brazilian territory (FAO, 2000).

Among the mechanisms by which plants cope with Al toxicity, organic acid (OA) efflux is highlighted in many crop species (Yang et al., 2019). In wheat, the genes *TaALMT1* and *TaMATE1B*, which code malate and citrate transporters, respectively, are responsible for OA efflux at the root tips (Sasaki et al., 2004; Tovkach et al., 2013). The alleles of *TaALMT1* and *TaMATE1B* differ in the promoter regions. Alleles I to VII contrasts in number of tandem repeats in the *TaALMT1*, and alleles (+) or (-) vary with the presence or absence of an upstream insertion in *TaMATE1B* (Garcia-Oliveira et al., 2014; Raman et al., 2008; Sasaki et al., 2006; Tovkach et al., 2013). Wheat haplotypes with superior alleles (V+ or VI+) usually show higher expression level of those genes, which are associated with greater OA efflux and, consequently, improved root growth. The impact of *TaALMT1* and *TaMATE1B* alleles has been studied in hydroponics (Garcia-Oliveira et al., 2014; Han et al., 2013, 2016; Raman et al., 2008; Sasaki et al., 2006) and in field conditions (Aguilera et al., 2016).

Studies evaluating Al resistance in field conditions are more realistic than those in controlled environments, and the analysis of field data results in the possibility of selecting Al-resistant genotypes in a more precise way. de Sousa et al. (1984) proposed a method of classification index of aluminum toxicity in the field (CIATF), where visual scores are given to wheat plants in three growth stages (tillering, silking, and maturation) based on the comparison with Al-resistant and Al-sensitive controls. This method was modified by de Sousa (1998) and based on the CIATF, wheat genotypes can be discriminated as Al-resistant, Al-moderately resistant, Al-moderately susceptible, and Al-susceptible. This method has previously been used by our group (Aguilera et al., 2016); however, it is unknown if other selection indexes, such as a P_i parameter–based selection index, can be more effective in discriminating Al resistance in field conditions. In this context, this work aims at using a selection index based on the P_i parameter to improve the selection of wheat cultivars that are resistant to Al and to evaluate the correlation between the allelic variability of *TaALMT1* and *TaMATE1B* genes with the Al resistance phenotype in field conditions.

MATERIALS AND METHODS
Plant Material

The phenotype and genotype information of wheat (including commercial cultivars, cultivars, synthetic wheat, and lineages) with contrasting levels of Al resistance (Supplementary Table S1), previously published by Aguilera et al. (2016), were used in the present study. We focused on a set of 172 genotypes that were selected to represent both Brazilian and foreign genotypes in approximately the same proportion (90 Brazilian genotypes and 82 genotypes from other countries), and we analyzed 23 synthetic hexaploid wheat (SHW) genotypes. All genotypes were evaluated in field conditions with three replicates. Among these genotypes, the cultivars IAC 5-Maringá and Anahuac 75 were considered Al-resistant and Al-sensitive controls, respectively.

Al Resistance in the Field

The Al resistance of 195 wheat genotypes, which was evaluated in field conditions at Embrapa Trigo's experimental field (28°15′ S, 52°24′ W) during two growing seasons (2012 and 2013), was obtained by Aguilera et al. (2016). Phenotyping was performed in the soil classified as Haplorthox, containing ~45% clay. The soil pH ranged from 4.3 to 4.7, and the soil contained 29 to 54 mmol dm^{-3} Al^{3+} throughout the 2-yr duration of the test. A randomized complete block design was performed where plots consisted of six rows (containing five different genotypes and one of the controls placed alternately) of 3 m length with three replications. The plants were evaluated at three developmental stages (tillering, silking, and maturation), and a CIATF was calculated based on the scores (Aguilera et al., 2016). The CIATF states that genotypes with the score of 0.05 to 1.25 are resistant, 1.26 to 2.5 are moderately resistant, 2.51 to 3.75 are moderately susceptible, and 3.76 to 5.00 are susceptible.

Alleles of the *TaAMLT1* and *TaMATE1B* Genes

Information about the *TaALMT1* and *TaMATE1B* promoter alleles was based on previous results (Aguilera et al., 2016) and is presented in Supplementary Table S1. *TaALMT1* alleles can be detected using primers LPF-F (CCTGGTTTTCTTGATGGGGGCACA), LPF-R (TGCCCACCATCTCGCCGTCGCTCTCT), SPF-F (GCTCCTACCACTATGGTTGCG), and SPF-R (CCAGGCCGACTTTGAGCGAG) according to amplification protocols described previously (Raman et al., 2008; Sasaki et al., 2006). *TaMATE1B* alleles can be discriminated by the presence or absence of the polymerase chain reaction product obtained from primers TaMATE1–4B-SLT-F (ATCCAT-CCTCCTTCCCTCAC) and TaMATE1–4B-SLT-R (ATGAATGCTGTGTCCACCAA) and by the amplification protocol described previously (Garcia-Oliveira et al., 2014).

Statistical Analyses

We performed individual ANOVA for each environment (year), followed by analysis of joint variance according to the statistical model described in Eq. [1]:

$$Y_{ijk} = m + B/E_{jk} + G_i + E_j + (G \times E_{ij}) + \varepsilon_{ijk} \qquad [1]$$

Table 1. Analysis of joint variance for the variable aluminum resistance observed in tillering, silking, and maturation among 195 wheat genotypes.

Source of variation	Degrees of freedom	Mean square		
		Tillering	Silking	Maturation
Blocks/years	4	2.79	3.69	2.88
Genotype (G)	194	3.00*	6.26*	9.43*
Year (Y)	1	7018*	264.41*	137.26*
G × Y	194	0.43*	0.72*	0.91*
Residue	776	0.35	0.30	0.47
CV, %		18.82	16.35	21.47
Overall average		3.14	3.34	3.21

* Significant at the 0.05 probability level.

Table 2. Estimates of the original mean (X_o), mean of the selected (X_s) genotype, selection differential, broad heritability, and selection gain based on the multivariate P_i (P_{im}) statistics of the genotypes with the highest Al resistance (lower grades).

Phases	Year	X_o	X_s	Selection differential	Heritability	Selection gain
					——— % ———	
Tillering	1	3.39	2.27	−1.12	81.54	−26.94
	2	2.90	1.69	−1.21	77.95	−32.52
Silking	1	3.82	1.53	−2.29	91.41	−54.80
	2	2.87	1.41	−1.46	91.50	−46.55
Maturation	1	3.55	1.21	−2.34	89.50	−58.99
	2	2.86	0.97	−1.89	92.52	−61.14

where Y_{ijk} is the observation of the kth block evaluated in the ith genotype and jth year, μ is the overall mean of the experiments, B/E_{jk} is the effect of block k within year j, G_i is the effect of the ith genotype considered as random, E_j is the effect of the jth year considered as fixed, $G \times E_{ij}$ is the random effect of the interaction between genotype i and environment j, and $ε_{ijk}$ is the random error associated with Y_{ijk} observation. After that, the estimate of adaptability and stability (P_i) for each character proposed by Lin and Binns (1988) was calculated according to Eq. [2]:

$$P_i = \frac{\sum_{j=1}^{e}\left(Y_{ij} - Y_{mj}\right)^2}{2e} \quad [2]$$

where Y_{ij} is the score of the ith genotype in the jth year, Y_{mj} is the estimate of the ideal hypothetical genotype in year j, and e is the number of years. For the selection of genotypes with higher Al resistance considering the scores at the different growth stages (tillering, silking, and maturation) and the 2 yr of evaluation, the multivariate statistic P_{im} was used, according to Eq. [3]:

$$P_{im} = \sum_{k=1}^{v}\left[\frac{P_{ik}}{\hat{\sigma}_{P_{ik}}}\right] \quad [3]$$

where P_{ik} is the univariate estimator of the adaptability and stability of the ith genotype associated with the kth variable, and $\hat{\sigma}_{P_{ik}}$ is the standard deviation of P_{ik}. After the selection of the genotypes with lower values of P_{im}, the estimates of the genetic gains (GS) for each character and year were obtained, according to Eq. [4]:

$$GS_{(\%)} = \frac{(X_s - X_o) \times h^2}{X_o} \quad [4]$$

where X_s is the 20 genotypes selected, X_o is the original character average, and h^2 is the broad heritability of character. Data of P_{im}, CIATF, and the different *TaALMT1* and *TaMATE1B* alleles and the combinations of alleles (haplotypes) were used in the correlation analysis using the GENES program (Cruz, 2013). The selection of the best genotypes was performed using the multivariate index P_{im}. All analyses were performed using Genes software (Cruz, 2013).

RESULTS

Variance for Al Resistance in Field Conditions

The Al resistance of the wheat genotypes studied here was evaluated previously (Aguilera et al., 2016) based on visual characteristics observed in the three different growth stages (tillering, silking, and maturation) of the wheat plants grown in acidic soil in two successive growing seasons. The mean scores for each growth stage of the 195 wheat genotypes are shown in the Supplementary Table S1. Based on these scores, an ANOVA was performed, and significant differences ($P < 0.05$) were obtained for the genotypes, years, and their interaction (genotype × year). This shows high diversity of responses among the genotypes (Table 1). The coefficients of phenotypic variation, which are indicative of the experimental precision, exhibited values <22%, showing high homogeneity of the data obtained in the experimental conditions (Table 1).

Genetic Estimators Associated with P_{im} in the Selection of Al-Resistant Genotypes

The average of the scores for Al resistance allowed the estimation of the P_i index, as proposed by Lin and Binns (1988), for the wheat genotypes. With these values in each of the growth stages, P_{im} was obtained, which allowed the estimation of the original mean, the mean of the selected genotype, selection differential, heritability, and selection gain for both years of evaluation (Table 2). Average reductions of more than 34, 52, and 67% for tillering, silking, and maturation, respectively, were observed. The observation was independent of the phase and the year sampled when applying a selection intensity of 9.5% (the best 20 genotypes). These results showed that Al resistance becomes more evident as the plants grow. The selection differential also showed this behavior (Table 2). For the traits evaluated, the heritability values were >78%, which indicate the possibility of gains, with the selection of this characteristic being of 27 to 61%, depending on the stage at which the selection was performed, being superior in maturation stage.

The P_{im} index allowed the selection of the top best 20 genotypes considering their performance in both years and in all different growth stages (Table 3). The ranking of P_{im} values confirmed the genotypes that were widely recognized as Al resistant (Toropi, Trintecinco, BH 1146, IAC 5-Maringá, and Giza 121) and as the best five genotypes among the top 20 genotypes, with values from 0.00 to 0.29. This shows their superiority in Al resistance in acidic soil in field conditions. Of the 20 selected genotypes, 15 were obtained from Brazil, which shows the great potential of the Brazilian wheat germplasm to resist Al if we consider the balance between the number of genotypes selected from Brazil (90 genotypes) and that from other parts of the world (82 genotypes). Most Brazilian genotypes have both Pollyssú and Alfredo Chaves lines in their genealogy, with the exception of Trintecinco and PG 1, which have only

Table 3. Evaluation of aluminum resistance of the top 20 wheat genotypes.

Genotype	Origin	$TaALMT1$ allele	$TaMATE1B$ allele	Tillering			Silking			Maturation			P_{im}	CIATF‡
				Year 1	Year 2	P_i†	Year 1	Year 2	P_i	Year 1	Year 2	P_i		
Toropi	Brazil	VI	+	1.47	0.82	0.00	0.50	1.00	0.00	0.87	0.50	0.01	0.00	R
Trintecinco	Brazil	VI	+	1.65	0.99	0.03	0.48	1.03	0.00	1.05	0.50	0.02	0.03	R
BH 1146	Brazil	V	-	1.54	0.79	0.01	1.95	1.26	0.56	1.17	0.65	0.06	0.25	R
IAC 5- Maringá	Brazil	VI	+	2.10	0.93	0.14	1.56	1.21	0.30	1.34	0.94	0.17	0.27	MR
Giza 121	Egypt	V	–	1.38	1.35	0.08	1.71	1.21	0.39	1.35	1.13	0.24	0.29	MR
PG 1	Brazil	VI	+	1.88	1.82	0.33	0.78	1.11	0.03	0.84	1.08	0.16	0.30	R
Jesuita	Brazil	VI	+	1.48	2.00	0.37	1.28	1.05	0.16	1.00	0.75	0.06	0.35	MR
Klein Lucero	Argentina	VI	+	2.04	2.21	0.61	0.77	1.21	0.03	1.00	0.91	0.10	0.48	MR
Trigo BR 35	Brazil	VI	–	2.54	1.37	0.42	2.28	1.34	0.84	1.00	1.10	0.17	0.69	MR
BRS 49	Brazil	V	–	2.38	1.99	0.61	1.78	1.75	0.57	1.55	1.12	0.30	0.76	MR
Menceki	Turkey	V	–	2.71	1.82	0.71	2.07	1.23	0.65	1.34	0.29	0.06	0.78	MR
Trigo BR 32	Brazil	V	–	2.71	1.90	0.75	1.95	1.37	0.58	1.00	0.95	0.12	0.80	MR
Colonista	Brazil	VI	+	2.31	2.32	0.81	1.39	1.99	0.45	1.14	1.19	0.22	0.83	MR
IAS 20-Iassul	Brazil	V	-	2.95	1.12	0.65	2.21	1.05	0.75	1.50	0.98	0.23	0.84	MR
Bet Dagan 131	Israel	V	+	3.04	1.66	0.88	1.78	1.46	0.48	1.34	1.00	0.19	0.88	MR
Colonias	Brazil	VI	+	2.80	2.16	0.97	1.38	1.98	0.44	1.13	0.87	0.10	0.90	MR
Emb_92102	Brazil	VI	-	2.54	1.82	0.61	2.44	1.45	1.01	1.50	1.01	0.24	0.91	MR
Maya 74	Guatemala	V	+	2.71	2.16	0.91	1.77	1.55	0.49	1.00	1.41	0.32	0.95	MR
Emb_90101	Brazil	V	–	3.04	1.99	1.05	1.45	1.57	0.32	1.67	1.23	0.39	1.00	MR
Frondoso	Brazil	V	–	2.04	2.58	0.91	1.01	2.33	0.52	1.50	1.80	0.68	1.08	MR

† Averages of the notes and estimates of Pi are shown for each of the growth stages (tillering, silking, and maturation). The lowest estimates of multivariate P_i (P_{im}) indicate greater Al resistance.

‡ Classification index of aluminum toxicity in the field (MR, moderately resistant; R, resistant).

one resistance genotype (Alfredo Chaves or Pollyssú lines) as an ancestor. However, non-Brazilian genotypes (Klein Lucero and Menceki) have different sources of resistance and do not share common ancestors (Supplementary Table S2). The P_{im} index values for all the wheat genotypes in this study are shown in Supplementary Table S1. Among the evaluated genotypes, Zambezi (Zimbabwe) and Angas (Australia) genotypes matched the behavior of the susceptible control Anhauac 75 (Mexico) and could be used as susceptible controls in future experiments.

Correlations Associated with P_{im} in the Selection of Al-Resistant Genotypes

The most important mechanisms of Al resistance in hexaploid wheat are based on the efflux of malate and citrate anions from the root apices, which is controlled by the $TaALMT1$ and $TaMATE1B$ genes, respectively (Sasaki et al., 2004; Tovkach et al., 2013). The correlation of the P_{im} index with malate and citrate transporters associated with the $TaALMT1$ and

Table 4. Pearson correlation, obtained by multivariate P_i (P_{im}) index, with the promoters $TaALMT1$ and $TaMATE1B$ and the Al resistance of 195 wheat genotypes cultivated in acidic soil in field conditions. The correlation of the classification index of aluminum toxicity in the field (CIATF), obtained previously (Aguilera et al. 2016), is shown.

Index†	$TaAMLT1$	$TaMATE1B$	Haplotype
P_{im}	−0.79**	−0.41**	−0.82**
CIATF (338)	0.69**	0.37**	0.71**
CIATF (195)	−0.46**	0.02	−0.44**
Index gain (338)	0.10	0.04	0.11
Index gain (195)	0.33	0.37	0.38

** Significant at the 0.01 probability level.

† Number in parentheses refers to the dataset used: 338 wheat genotypes used by Aguilera et al. (2016) and 195 wheat genotypes used in the present work.

$TaMATE1B$ promoter alleles, respectively, was calculated (Table 4). Among the 195 wheat genotypes evaluated, five $TaALMT1$ alleles and two $TaMATE1B$ alleles were detected. For $TaALMT1$, the alleles V (47%) and I (30%) were the most frequent, whereas for $TaMATE1B$, the absence (90%) of the insertion was detected in more genotypes than its presence (10%) (Supplemental Table S1). All the top 20 most Al-resistant genotypes showed $TaALMT1$ alleles VI or V in equal proportion. The insertion in the $TaMATE1B$ promoter was detected in 10 genotypes that are among the top 20. When combining the alleles from both genes, nine haplotypes were detected with the allele $TaMATE1B$ (+) not being detected along with $TaALMT1$ alleles II, III, and IV (Fig. 1a, b). In Fig. 1a, a positive effect of $TaMATE1B$ (+) allele is observed for higher alleles V and VI, in addition to allele I (even though it is represented by only one genotype). On average, the presence of $TaMATE1B$ superior allele (containing the insertion in the promoter region) decreased the P_{im} (increased Al resistance) by 105, 138, and 259% in the presence of $TaALMT1$ alleles I, V, and VI, respectively (Fig. 1a). All 23 SHW lines analyzed showed only three haplotypes [I(-), V(-), and VI(-)]. The favorable allele of the $TaMATE1B$ gene was not observed in any of these lines, indicating the absence of the tetraploid ancestors (Fig. 1b). The correlations showed highly significant differences ($P < 0.001$) across all data while showing the efficiency and the gain obtained by comparing the P_{im} with the CIATF index obtained by Aguilera et al. (2016) (Table 4). The highest correlations were obtained with haplotypes > $TaALMT1$ > $TaMATE1B$. This behavior indicates that the new index did not alter the results of the correlations between CIATF and the alleles but altered the magnitude of the correlations (Table 4). Nevertheless, the new index improved the prediction of the behavior when selecting aluminum-resistant wheat genotypes.
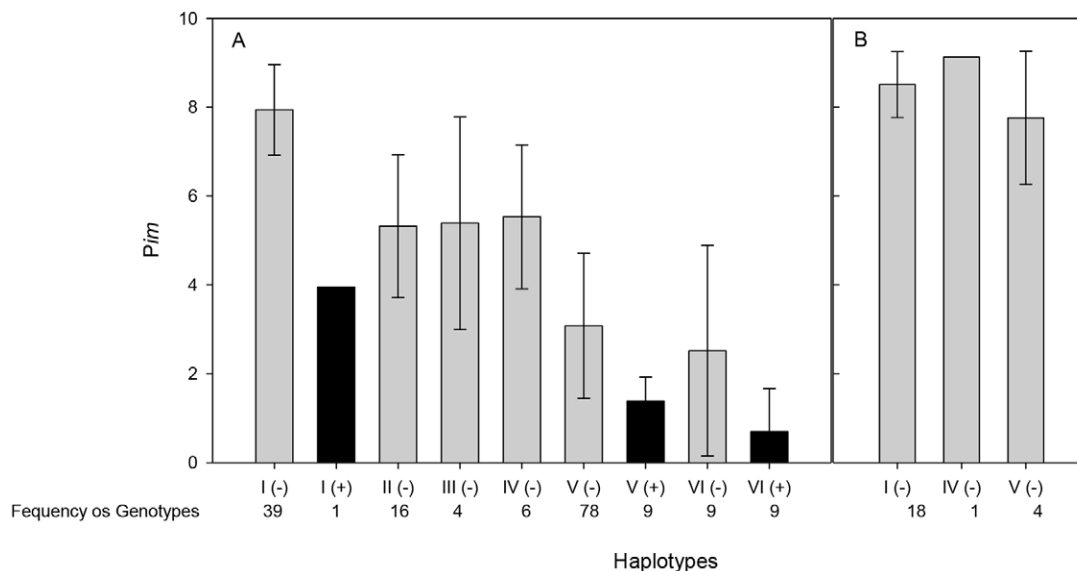
Fig. 1. Effect of the *TaMATE1B* alleles on the P_{im} index of 172 genotypes of *Triticum aestivum* (a) and 23 genotypes synthetic hexaploid wheat (b). Bars indicate the mean and SD of P_{im} for the different haplotypes of *TaAMLT1* and *TaMATE1B* promoters (a lower P_{im} value indicates greater Al resistance). The absence of the insertion in the *TaMATE1B* promoter (inferior allele, -) is represented by gray bars; the presence of that insertion (superior allele, +) is indicated by black bars. I to VI indicate the type of the *TaALMT1* alleles. The frequency of genotypes in each haplotype is also shown.

DISCUSSION

The identification of the resistant genotypes and the molecular markers linked to Al resistance are key assets to increasing wheat performance in acidic soil. In this work, we used the information of field tests, where Al resistance of wheat genotypes was measured in two growing seasons and in different growth stages (Aguilera et al., 2016), to create a new selection index (P_{im}) by modifying the method of Lin and Binns (1988). This new index revealed a wide diversity in plant performance in acidic soil. Additionally, the correlation of P_{im} with *TaALMT1* and *TaMATE1B* alleles improved the selection capacity of the genotypes in relation to the CIATF described previously (Aguilera et al., 2016; de Sousa, 1998; de Sousa et al., 1984).

The method initially proposed by Lin and Binns (1988) considers the developmental deviations of the cultivars in the environments and takes into account the main characteristic (many yields) and the relative response of the genotype. Its main purpose is to identify cultivars that can maintain their performance in a wide range of environments, through a statistic called P_i, and to identify genotypes for favorable and unfavorable environments. This type of analysis of adaptability and stability of wheat genotypes assists the breeders in identifying new cultivars, indicating lines of wide or specific adaptation in different environments. This procedure is particularly important for wheat cultivation, especially considering the great diversity of environments to which wheat is subjected in Brazil. Also, several studies have been reported using this approach (Albrecht et al., 2007; Biudes et al., 2009; Cargnin et al., 2008; Caierão et al., 2006; Franceschi et al., 2010; Murakami et al., 2004), and most of them recommend the method of Lin and Binns (1988) as the most suitable for the species. However, no work has reported the use of this method in the selection of Al-resistant wheat. The new multivariate index, P_{im}, proposed in this study takes into account the response of each genotype

to different environments (years) and its evaluation in different stages of development, thus modifying the method proposed by Lin and Binns (1988). We observed a gain in selection using the P_{im} index. This is an advancement in our studies because, until recently, our group has used the index proposed by de Sousa et al. (1984) as modified by de Sousa (1998). This latter index is the average observation calculated from field evaluations in several stages of development of the wheat plant. The method proposed in this current study (P_{im} index) uses a more complex statistical tool to analyze the different variables observed in relation to reaction to Al toxicity. We found a higher correlation ($r = -0.82$; $P < 0.001$) between the P_{im} index and *TaALMT1* and *TaMATE1B* promoter alleles and the Al resistance in the field. That correlation was previously $r = 0.71$ ($P < 0.001$) when a CIATF was used (Aguilera et al., 2016).

Considering that 15 of the top 20 Al-resistant genotypes selected by the P_{im} index were obtained in Brazil, and the superiority of the Brazilian materials continued to manifest as previously reported (Aguilera et al., 2016; de Sousa, 1998; Raman et al., 2008). Among the top 20 genotypes, only *TaALMT1* alleles V and VI were observed, supporting previous results that these alleles are the best for wheat growth under Al toxicity (Raman et al., 2008; Sasaki et al., 2006). Half of the top 20 genotypes have the insertion in the *TaMATE1B* promoter (allele +) (Fig. 1a). High P_{im} index values (low Al resistance) were observed in SHW lines even with the presence of favorable V allele of the *TaALMT*1 gene (Fig. 1b). These SHWs are amphiploids obtained by crossing *Triticum turgidum* (AABB) and *Aegilops tauschii* (DD). These results agree with the Al sensitivity previously observed in durum wheat and SHW lines (Han et al., 2016; Ryan et al., 2010; Zhou et al., 2007). Although the *TaMATE1B* (+) allele has been shown to be more advantageous when combined with the inferior *TaALMT1* alleles (Han et al., 2016), our results from the 172 *T. aestivum* genotypes indicate that, on average, *TaMATE1B* (+) has an additive effect

independently if the genotype contains the *TaALMT1* alleles I, V, or VI (Fig. 1a). Thus, the selection of Al-resistant wheat genotypes should be based on the superior alleles of *TaALMT1* and *TaMATE1B* (Fig. 1a). This strategy (pyramiding Al-resistance loci) has been listed as one of the potential strategies for improving the Al resistance of wheat (Ryan, 2018).

There were non-Brazilian genotypes among the top 20 observed here: Giza [from Egypt with haplotype V(-)], Klein Lucero [from Argentina with haplotype VI(+)], Menceki [from Turkey with haplotype V(-)], Bet Dagan 131 [from Israel with haplotype V(+)], and Maya 74 [from Guatemala with haplotype V(+)]. The genealogy of these genotypes (Supplementary Table S2) showed that only the sources of resistance for Klein Lucero and Menceki were different from those of Brazil because they do not share common ancestors with Alfredo Chaves and Polyssú lines. In that case, it would be interesting to evaluate if Al resistance of these genotypes can be associated with genes other than *TaALMT1* and *TaMATE1B*. These other genes could be introduced into Brazilian germplasm to increase Al resistance even further. There are several other genes associated with Al resistance that do not rely on the efflux of organic acid at the roots, and several genomic regions in wheat (different from chromosomes 4B and 4D where *TaALMT1* and *TaMATE1B* reside) have been associated with greater performance with Al toxicity (Froese and Carter, 2016; Navakode et al., 2014; Raman et al., 2010; Ryan et al., 2011). The introduction of new genes (different from *TaALMT1* and *TaMATE1B*) can help to increase wheat yield, which is important to reduce Brazil's dependency on wheat importation. This is an important issue because projections by 2050 show that wheat import needs in Brazil will be higher than the current level (Weigand, 2011).

Among the genotypes selected by P_{im} index as superior (top 20 among the 195 genotypes analyzed here), only three showed inferior P_{im} (greater Al resistance) compared with Al-resistant control (IAC 5-Maringá). These genotypes were released a long time ago (Toropi in 1965, Trintecinco in 1936, BH 1146 in 1955, and IAC 5-Maringá in 1966), and they are not currently recommended for cultivation in Brazil. This means that Al resistance in wheat is not the major focus in the selection of new cultivars in Brazil. When considering "modern" genotypes, there are 16 cultivars in common with the 195 studied here and the 124 recommended for cultivation in Brazil in the crop season of 2018 (Franco and Evangelista, 2018) (the cultivars in common are highlighted in bold in Supplementary Table S1). Among them, the most Al-resistant cultivar is MGS1 Aliança (22nd in the ranking), showing good performance under rain-fed growth at the Brazilian Cerrado. BRS Umbu (position 38th) is the second most Al-resistant cultivar, and the remaining 14 genotypes are placed between the 49th and 167th positions. The fact that most of these "modern" cultivars do not show great levels of Al resistance is not an impediment for them to show good yield. Undoubtedly, the performance of these cultivars requires good management practices (e.g., lime application), although long periods are needed to ameliorate the subsoil acidity, and these practices might be costly for farmers in several countries. Thus, wheat cultivars with Al resistance are still an important option for farmers (Ryan, 2018).

In conclusion, the strategy proposed with P_{im} index allowed the selection of superior genotypes for resistance to aluminum

and confirmed that the Brazilian genotypes are main sources of resistance when compared with other genotypes from other countries. The P_{im} index selected genotypes associated with the superior alleles for the genes *TaALMT1* and *TaMATE1B*. The genotypes used as control in this work were accurately evaluated by the P_{im} index. The results showed that the proposed multivariate index is a tool to be used for breeding programs aiming to select superior genotypes in environments where Al resistance can be advantageous, such as areas with subsoil acidity. Another great advantage of the proposed procedure is that it is based on nonparametric statistics. Therefore, the proposed index can be applied to any number of environments, traits, and genotypes without statistical assumption.

REFERENCES

Aguilera, J.G., J.A.D. Minozzo, D. Barichello, C.M. Fogaça, J.P. Silva, L. Consoli, and J.F. Pereira. 2016. Alleles of organic acid transporter genes are highly correlated with wheat resistance to acidic soil in field conditions. Theor. Appl. Genet. 129:1317–1331. doi:10.1007/s00122-016-2705-3

Albrecht, J.C., E.A. Vieira, M.S. Silva, J.M.V. Andrade, P.L. Scheeren, M.G. Trindade, et al. 2007. Adaptabilidade e estabilidade de genótipos de trigo irrigado no Cerrado do Brasil Central. Pesqi. Agropecu. Bras. 42:1727–1734. doi:10.1590/S0100-204X2007001200009

Biudes, G.B., C.E. Camargo, A.W.P. Ferreira Filho, A. Pettinelli Júnior, D.E. Foltran, J.L. Castro, and J.A. Azevedo Filho. 2009. Adaptabilidade e estabilidade de linhagens diaplóides de trigo. Bragantia 68:63–74. doi:10.1590/S0006-87052009000100008

Caierão, E., M.S. Silva, P.L. Scheeren, L.J.A. del Duca, A. Nascimento, Junior., and J.L. Pires. 2006. Análise da adaptabilidade e da estabilidade de genótipos de trigo como ferramenta auxiliar na recomendação de novas cultivares. Cienc. Rural 36:1112–1117. doi:10.1590/S0103-84782006000400011

Cargnin, A., M.A. de Souza, P.C.S. Carneiro, and V. Sofiatti. 2006. Interação entre genótipos e ambientes e implicações em ganhos com seleção em trigo. Pesqi. Agropecu. Bras. 41:987–993. doi:10.1590/S0100-204X2006000600014

Cargnin, A., M.A. Souza, and C.M. Fogaça. 2008. Comparação de métodos de análise de adaptabilidade e estabilidade em trigo. Ceres 55:243–250.

Cargnin, A., M.A.D. Souza, V. Fronza, and C.M. Fogaça. 2009. Genetic and environmental contributions to increased wheat yield in Minas Gerais, Brazil. Sci. Agric. 66:317–322. doi:10.1590/S0103-90162009000300006

Companhia Nacional do Abastecimento. 2018. A cultura do trigo: Análise dos custos de produção e da rentabilidade nos anos-safra 2009 a 2017. CONAB, Brasília, Brazil.

Cruz, C.D., and P.C.S. Carneiro. 2003. Modelos biométricos aplicados ao melhoramento genético II. Editora UVF, Viçosa, Brazil.

Cruz, C.D. 2013. GENES: A software package for analysis in experimental statistics and quantitative genetics. Acta Sci. Agron. 35:271–276. doi:10.4025/actasciagron.v35i3.21251

de Sousa, C.N.A. 1998. Classification of Brazilian wheat cultivars for aluminium toxicity in acid soils. Plant Breed. 117:217–221. doi:10.1111/j.1439-0523.1998.tb01929.x

de Sousa, C.N.A., J.C.S. Moreira, L.J.A. Del Ducca, and P.L. Scheeren. 1984. Reação de cultivares estrangeiras de trigo ao crestamento em condições de campo. In: Resultados de Pesquisa do Centro Nacional de Pesquisa de Trigo. XIII Reunião Nacional de Pesquisa de Trigo, Embrapa-CNPT, Passo Fundo. p. 212–214.

Eberhart, S.A., and W.A. Russell. 1966. Stability parameters for comparing varieties. Crop Sci. 6:36–40. doi:10.2135/cropsci1966.0011183X000600010011x

FAO. 2000. Land resource potential and constraints at regional and country levels. Land and Water Development Division, FAO, Rome, Italy.

Fischer, T., D. Byerlee, and G. Edmeades. 2014. Crop yields and global food security: Will yield increase continue to feed the world? ACIAR Monograph 158. Australian Centre for International Agricultural Research: Canberra, Australia.

Franceschi, L., G. Benin, V.S. Marchioro, T.N. Martin, R.R. Silva, and C.L. Silva. 2010. Métodos para análise de adaptabilidade e estabilidade em cultivares de trigo no estado do Paraná. Bragantia 69:797–805. doi:10.1590/S0006-87052010000400004

Franco, F.A., and A. Evangelista. 2018. Informações técnicas para trigo e triticale: Safra 2018. Coodetec, Cascavel, Brazil.

Froese, P.S., and A.H. Carter. 2016. Single nucleotide polymorphisms in the wheat genome associated with tolerance of acidic soils and aluminum toxicity. Crop Sci. 56:1662–1677. doi:10.2135/cropsci2015.10.0629

Garcia-Oliveira, A.L., P. Martins-Lopes, R. Tolrá, C. Poschenrieder, M. Tarquis, H. Guedes-Pinto, and C. Benito. 2014. Molecular characterization of the citrate transporter gene *TaMATE1* and expression analysis of upstream genes involved in organic acid transport under Al stress in bread wheat (*Triticum aestivum*). Physiol. Plant. 152:441–452. doi:10.1111/ppl.12179

Han, C., S.F. Dai, D.C. Liu, Z.J. Pu, Y.M. Wei, Y.L. Zheng, D.J. Wen, L. Zhao, and Z.H. Yan. 2013. *TaALMT1* promoter sequence compositions, acid tolerance, and Al tolerance in wheat cultivars and landraces from Sichuan in China. Genet. Mol. Res. 12:5602–5616. doi:10.4238/2013.November.18.9

Han, C., P. Zhang, P.R. Ryan, T.M. Rathjen, Z.H. Yan, and E. Delhaize. 2016. Introgression of genes from bread wheat enhances the aluminium tolerance of durum wheat. Theor. Appl. Genet. 129:729–739. doi:10.1007/s00122-015-2661-3

Kochian, L.V., M.A. Piñeros, J. Liu, and J.V. Magalhaes. 2015. Plant adaptation to acid soils: The molecular basis for crop aluminum resistance. Annu. Rev. Plant Biol. 66:571–598. doi:10.1146/annurev-arplant-043014-114822

Lin, C.S., and M.R. Binns. 1988. A superiority measure of cultivar performance for cultivar x location data. Can. J. Plant Sci. 68:193–198. doi:10.4141/cjps88-018

Murakami, D.M., A.A. Cardoso, C.D. Cruz, and N. Bizão. 2004. Considerações sobre duas metodologias de análise de estabilidade e adaptabilidade. Cienc. Rural 34:71–78. doi:10.1590/S0103-84782004000100011

Navakode, S., K. Neumann, B. Kobiljski, U. Lohwasser, and A. Börner. 2014. Genome wide association mapping to identify aluminium tolerance loci in bread wheat. Euphytica 198:401–411. doi:10.1007/s10681-014-1114-4

Raman, H., P.R. Ryan, R. Raman, B.J. Stodart, K. Zhang, P. Martin, et al. 2008. Analysis of *TaALMT1* traces the transmission of aluminum resistance in cultivated common wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 116:343–354. doi:10.1007/s00122-007-0672-4

Raman, H., B. Stodart, P.R. Ryan, E. Delhaize, L. Emebiri, R. Raman, N. Coombes, and A. Milgate. 2010. Genome-wide association analyses of common wheat (*Triticum aestivum* L.) germplasm identifies multiple loci for aluminium resistance. Genome 53:957–966. doi:10.1139/G10-058

Ryan, P.R., H. Raman, S. Gupta, T. Sasaki, Y. Yamamoto, and E. Delhaize. 2010. The multiple origins of aluminium resistance in hexaploid wheat include *Aegilops tauschii* and more recent cis mutations to *TaALMT1*. Plant J. 64:446–455. doi:10.1111/j.1365-313X.2010.04338.x

Ryan, P.R., S.D. Tyerman, T. Sasaki, T. Furuichi, Y. Yamamoto, W.H. Zhang, and E. Delhaize. 2011. The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils. J. Exp. Bot. 62:9–20. doi:10.1093/jxb/erq272

Ryan, P.R. 2018. Assessing the role of genetics for improving the yield of Australia's major grain crops on acid soils. Crop Pasture Sci. 69:242–264. doi:10.1071/CP17310

Sasaki, T., Y. Yamamoto, B. Ezaki, M. Katsuhara, S.J. Ahn, P.R. Ryan, E. Delhaize, and H. Matsumoto. 2004. A wheat gene encoding an aluminum-activated malate transporter. Plant J. 37:645–653. doi:10.1111/j.1365-313X.2003.01991.x

Sasaki, T., P.R. Ryan, E. Delhaize, D.M. Hebb, Y. Ogihara, K. Kawaura, et al. 2006. Sequence upstream of the wheat (*Triticum aestivum* L.) *ALMT1* gene and its relationship to aluminum resistance. Plant Cell Physiol. 47:1343–1354. doi:10.1093/pcp/pcl002

Tovkach, A., P.R. Ryan, A.E. Richardson, D.C. Lewis, T.M. Rathjen, S. Ramesh, et al. 2013. Transposon-mediated alteration of *TaMATE1B* expression in wheat confers constitutive citrate efflux from root apices. Plant Physiol. 161:880–892. doi:10.1104/pp.112.207142

von Uexküll, H.R., and E. Mutert. 1995. Global extent, development and economic impact of acid soils. Plant Soil 171:1–15. doi:10.1007/BF00009558

Weigand, C. 2011. Wheat import projections towards 2050. US Wheat Associates, Arlington, VA.

Yang, J.L., W. Fan, and S.J. Zheng. 2019. Mechanisms and regulation of aluminum-induced secretion of organic acid anions from plant roots. J. Zhej. Univ. 20:513–527. doi:10.1631/jzus.B1900188

Zhou, L.L., G.H. Bai, B. Carver, and D.D. Zhang. 2007. Identification of new sources of aluminum resistance in wheat. Plant Soil 297:105–118. doi:10.1007/s11104-007-9324-3