

## Strategy for grouping wheat genotypes according to environmental responses in multi-location trials

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### Abstract

A better understanding of genotype x environment interactions (GEIs) can improve the selection and recommendation of wheat genotypes that are widely adapted and stable in different environments. The objective of this study was to evaluate a strategy to perform a joint analysis in wheat yield trials with genotypes classified according to their interactions with the environment. Measurements of the adaptability and stability estimates were used in the proposed strategy. We analyzed grain yield data from 367 wheat genotypes in 348 yield trials classified into 58 groups. In each group, 25 genotypes at different numbers of locations were evaluated using data from 2010, 2011 and 2012. Joint analysis of variance was performed for each group to determine the genotype contribution to the GEI. The genotypes were grouped into two classes: genotypes that interacted with the environment and genotypes with no interaction with the environment. An additional joint analysis of variance was performed for each class. Grouping genotypes into different classes provided valuable information concerning the dynamics of the GEI, which could enable plant breeders to maximize the efficiency of selection and offer recommendations for stable and widely adapted genotypes. The highest selective accuracy for the genotype effects was obtained for the joint analysis when the GEI was insignificant. The joint analysis of trials for wheat genotypes that interacted with the environment presented the highest selective accuracy for the GEI effect and a reduced selective accuracy for the genotype effect.

**Keywords:** AMMI; interactions; joint analysis; multi-environment trial; REML; *Triticum aestivum* L.

**Abbreviations:**  $h_{mg}^2$  genotype mean heritability;  $F_{GEI}$  F-test value for genotype x environment interaction;  $V_{GEI}$  variance of GEI; VCU\_value for cultivation and use; AMMI1\_main effects and multiplicative interaction;  $F_G$  F-test value for genotype; GEI\_genotype x environment interactions; MET\_multi-environment trial; MSE\_mean square error; NI class\_genotypes without interaction with the environment; SAG\_selective accuracy of genotypes; WI class\_genotypes with interaction with the environment

### Introduction

Identifying adapted and stable wheat genotypes (*Triticum aestivum* L.) requires the maintenance of multi-environment trials (MET) through breeding programs. The response of each genotype to environmental variation results in different patterns of genotype x environment interactions (GEIs), which makes finding a single best variety for all environments unlikely. Thus, the selection and recommendation of adapted and stable wheat genotypes represents one of the limiting factors of breeding programs because the cost of developing a MET also needs to be taken into account. Improving the accuracy of genotype selection in wheat breeding programs is essential in Brazil due to the decrease in genetic gain over the last decade (Beche et al., 2014).

In Brazil, wheat cultivation is divided into homogeneous adaptation regions and value for cultivation and use regions (VCU regions 1, 2, 3 and 4) (Brasil, 2008). The edaphoclimatic differences among the different VCU regions influence the potential crop yield (Benin et al., 2013a; Munaro et al., 2014) and change the rank orders of genotypes among the tested environments (Silva et al., 2011). In a joint

analysis with GEI, some genotypes contributed more to the GEI than others (Yan et al., 2007; Hristov et al., 2010; De Vita et al., 2010; Mohammadi et al., 2011; Araújo et al., 2012). In this context, the stratification of genotypes according to their response patterns to the environment represents a feasible strategy to reduce the GEI, thereby facilitating the analysis and recommendation of adapted and stable genotypes. Francis and Kannenberg (1978) proposed grouping genotypes using the mean and coefficient of variation among the environments of each genotype. Lin (1982) proposed a method of grouping genotypes according to their responses to the environment; this method estimates the dissimilarity index between pairs of genotypes in terms of the distance adjusted for the average effects of the genotypes. Subsequently, this method was modified by Ramey and Rosielle (1983) and named the hierarchical agglomerative method.

A methodology to enable the identification of genotypes that contribute to the GEI was proposed by Araújo et al. (2012). This methodology utilizes the decomposition of the sum of squares of the interactions ( $SQ_{GE}$ ) resulting from each

genotype ( $W_i$ ); thus, the sum of the  $W_i$  values is equal to  $SQ_{GE}$ . This method enables the determination of the exact F-test for the hypothesis  $H_0: W_i=0$  (i.e., the contribution of genotype  $i$  to the interaction is null). Thus, the different classes of genotypes (i.e., those that do not interact with the environment (NI genotypes) and those that interact with the environment (WI genotypes)) can be statistically distinguished using a different strategy analysis. For the genotypes of the NI class, a joint analysis of variance and a test of multiple comparisons of means are often performed to identify the best genotypes for the region covered by the trials. For the genotypes of the WI class, a joint analysis of variance and adaptability and stability analyses can be performed (Cruz, 2013; Yan et al., 2007; Cargnelutti Filho et al., 2009; Miranda et al., 2009). However, how the parameter estimates obtained with these joint analyses differ from those obtained with analyses performed separately for each genotype class as a new strategy is unknown.

Accordingly, our objective was to evaluate the performance of a joint analysis of wheat yield trials with genotypes classified according to their interactions with the environment.

## Results and Discussion

### Joint analysis of variance by genotype class

The joint analysis of variance for the grain yield indicated the presence of GEI ( $\alpha < 0.05$ ) in all 58 groups of yield trials (Table 1) and a high GEI ( $\alpha < 0.01$ ) in 56 of them. The occurrence of GEIs in multi-environment wheat yield trials is common in Brazil (Silva et al., 2011) and other countries (Sabaghnia et al., 2012; Graybosch et al., 2012; Tsenov and Atanasova, 2013; Cormier et al., 2013; Malik et al., 2013; Roostaei et al., 2014). In similar cases where the GEI is present in the joint analysis, the characterization of genotypes using adaptability and stability analyses is indicated (Yan et al., 2007; Miranda et al., 2009; Araújo et al., 2012; Pereira et al., 2012; Colombari Filho et al., 2013).

According to the hypothesis testing proposed by Araújo et al. (2012), a mean of 13 out of 25 genotypes in each group of yield trials (52%) does not contribute to the GEI (NI genotypes). In our study, this number ranged from four to 23 (Table 2), whereas the other genotypes (48%) interacted with the environment ( $\alpha < 0.05$ ). The number of genotypes in the NI class in the 58 groups of yield trials ranged from four to eight in seven groups (12%), from eight to 12 in 21 groups (36%), from 12 to 16 in 11 groups (19%), from 16 to 20 in 14 groups (24%) and from 20 to 24 in five groups (9%). The proportion of NI genotypes depended on the set of tested genotypes in each group of yield trials and the number of interactions that occurred within the tested environments. The factor that most strongly determined the magnitude of the GEI was the genetic composition of the genotype list rather than the number of genotypes that interacted with the environment (Cooper et al., 2001).

The Pearson correlation coefficient, which was related to the mean yield and the F-test value for the interaction ( $F_{GEI}$ ) ( $r = -0.64$ ;  $\alpha < 0.01$ ;  $n = 58$ ), confirmed that a higher GEI was associated with a lower grain yield. This effect is explained by the fact that the occurrence of biotic stresses (e.g., pests and diseases) and abiotic stresses (e.g., radiation, temperature, humidity and water stress) in marginal environments for crop development causes greater instability of the genotypic performance and consequently a lower mean yield. The experimental error (MSE) was also correlated with the grain yield ( $r = 0.67$ ;  $\alpha < 0.01$ ) and the  $F_{GEI}$  of the

interaction ( $r = -0.61$ ;  $\alpha < 0.01$ ). These data suggest that GEIs under conditions of reduced error variance have lower  $\alpha$ -values when the MSE is the denominator of the  $F_{GEI}$  and the yields decrease due to stresses that promote instability (environmental variation).

Farmers are interested in genotypes without GEIs (NI class) as long as the average production of the crop remains high because more predictable behavior is guaranteed and the genotypes can be used in a wider cultivation area. Genotypes that interact with the environment (WI class) complicate the interpretation of the results from the multi-environment yield trials and thus the cultivar recommendation. The analyses of sets of genotypes including those that interact with the environment can lead to inaccurate estimates of phenotypic stability. Hu et al. (2014) reported that the heterogeneity of the GEI must be considered in data analyses to obtain more precise conclusions regarding genotypes in multi-environment yield trials. The presence of GEIs compromises the reliability of estimates of heritability and makes it difficult to accurately predict the genetic progress of a given trait (Alake and Ariyo, 2012). Consequently, genotype classes (WI and NI) should be analyzed separately because the adaptability and stability of a given genotype are associated with the tested genotypes. The contribution of NI genotypes to the GEI may compromise the ability to describe WI genotypes using the stability analysis.

The results of the analyses performed separately for the NI and WI genotypes are shown in table 2. In the NI class, the mean F-test value for the GEI ( $F_{GEI}$ ) was 1.44 and the mean  $\alpha$ -value was 0.175. However, GEI was present for 23 of the 58 groups (40%) in this class; thus, the mean MSE obtained in these 23 groups with an interaction (0.12534) was lower than the mean MSE obtained in the remaining 35 groups (0.18234). The  $F_{GEI}$  value obtained for the different genotypes could not be compared with the  $F_{GEI}$  value obtained using all genotypes (NI+WI class, Table 2) because the GEI was present for all groups. Thus, because the MSE of the different genotype classes did not differ for the 58 groups, the recurrence of an interaction in the genotypes of the NI class occurred in the groups of trials with a lower MSE. Additionally, the path analysis (data not shown) indicated that the number of genotypes in the NI class was directly or indirectly associated with the magnitude of the MSE, inversely associated with the magnitude of the  $F_{GEI}$ , and not associated with the mean yield. This finding suggests that the yield is independent of the magnitudes of the GEI and MSE.

Based on the joint analysis of variance for genotypes of the WI class, a GEI ( $\alpha < 0.05$ ) occurred in 57 of the 58 groups. The mean  $F_{GEI}$  value was 5.448, which was higher than the mean  $F_{GEI}$  obtained when the genotypes of the NI class were included (mean  $F_{GEI} = 1.440$ ) and when all genotypes were used in the joint analysis (Table 2, mean  $F_{GEI} = 3.352$ ). Removal of the NI genotypes increased the magnitude of the GEI by 54%, which might improve the parameter estimates for the stability and adaptability analyses used for the WI genotypes.

It is important to note that the mean  $F_G$  values (genotype effect) were higher for the NI genotypes (Table 2) than for the WI genotypes, which could be explained by the crossover of an interaction between genotypes in the WI class. A crossover interaction occurs when the ranking of two genotypes are not identical throughout the environments (Truberg and Huhn, 2000). Schulthess et al. (2013) reported that the occurrence of a crossover-type interaction was important in breeding because it prevented the breeder from making a decision based solely on the mean yield of a single

**Table 1.** Characteristics of the test environments in Brazil evaluated in this study.

Environment (City, State*)	Years			VCU region <sup>(1)</sup>	Coordinates		Elevation (m)
	2010	2011	2012		Latitude	Longitude	
Campos Novos, SC	5 <sup>(2)</sup>	5		1	27°24'S	51°13'W	934
Castro, PR	8			1	24°47'S	50°00'W	988
Cruz Alta, RS	2	2	2	1	28°38'S	53°36'W	452
Guarapuava, PR	5	13	8	1	25°23'S	51°27'W	1120
Não-Me-Toque, RS	10	10	8	1	28°27'S	52°49'W	514
Passo Fundo, RS			2	1	28°15'S	52°24'W	687
Vacaria, RS			2	1	28°30'S	50°56'W	971
Abelardo Luz, SC	10	10	8	2	26°33'S	52°19'W	760
Cachoeira Do Sul, RS			3	2	30°02'S	52°53'W	68
Campo Mourão, PR	8	6	8	2	24°02'S	52°22'W	585
Cascavel, PR	39	14	30	2	24°57'S	53°27'W	781
Itaberá, SP			2	2	23°51'S	49°08'W	651
Santa Rosa, RS	3			2	27°52'S	54°28'W	277
Santo Augusto, RS	5			2	27°51'S	53°46'W	528
São Luiz Gonzaga, RS	2	2	2	2	28°24'S	54°57'W	231
Taquarivaí, SP	5			2	23°55'S	48°41'W	555
Arapongas, PR	2	2	1	3	23°25'S	51°25'W	729
Dourados, MS	5		6	3	22°13'S	54°48'W	430
Goioerê, PR	3			3	24°11'S	53°01'W	505
Manduri, SP			1	3	23°00'S	49°19'W	710
Palmital, PR	5			3	24°53'S	52°12'W	840
Palotina, PR	28	28	20	3	24°17'S	53°50'W	333
Ponta Porã, MS	3			3	22°32'S	55°43'W	655
Rolândia, PR	5			3	23°18'S	51°22'W	730

<sup>(1)</sup>VCU region: Value for cultivation and use regions based on Brasil (2008); <sup>(2)</sup> Number of yield trials used to form one or more groups with different genotypes (total of 348 trials). \* RS - Rio Grande do Sul, SC - Santa Catarina, PR - Paraná, SP - São Paulo, MS - Mato Grosso do Sul.

**Table 2.** Number of trials per group (J), mean grain yield (Mg ha<sup>-1</sup>), mean MSE, F-test value for the interaction ( $F_{GEL}$ ) and its  $\alpha$ -value ( $\alpha_{GEL}$ ), F-test value for the genotype ( $F_G$ ) and its  $\alpha$ -value ( $\alpha_G$ ) and the number of genotypes without an interaction with the environment per trial group (nNI) with mean, minimum and maximum values.

Statistics	J	Yield	MSE	$F_{GEL}$	$\alpha_{GEL}$	$F_G$	$\alpha_G$	nNI
25 genotypes (NI+WI)								
Mean	6.0	3.525	0.16420	3.352	<0.001	3.730	0.001	12.9
Minimum	4	2.488	0.04350	1.383	<0.001	1.024	0.020	4
Maximum	11	4.818	0.33270	9.470	0.015	12.676	0.031	23
Classes of genotypes without interaction (NI Class) with the environment								
Mean		3.569 a*	0.15979	1.440	0.175	5.625	0.006	
Minimum		2.441	0.03670	0.811	<0.001	1.2	<0.001	
Maximum		4.895	0.36020	2.857	0.746	14.2	0.245	
Classes of genotypes with interaction (WI Class) with the environment								
Mean		3.478 a	0.16967	5.448	0.005	2.993	0.145	
Minimum		2.506	0.04391	1.385	0.014	0.482	<0.001	
Maximum		4.621	0.57779	12.705	0.127	13.289	0.925	

\* Means not connected by the same letter were different by the paired t-test ( $\alpha < 0.05$ ).

**Table 3.** Proportion of trials with non-normal error distributions (No, Shapiro-Wilk test,  $\alpha < 0.05$ ), the mean of the Spearman correlation coefficient ( $r_s$ ) between pairs of environments and proportion of significant tests of  $r_s$  (pH1,  $\alpha < 0.05$ ) within each group of wheat trials, and the mean squared error (MSE) and proportion of groups in which the MSEs between the environments are heterogeneous (Het, Bartlett test,  $\alpha < 0.05$ ) by the range of the number of genotypes in class NI (nSI).

Range	N groups	No	$r_s$	pH1	MSE	Het
25 genotypes (NI+WI)						
8 < nNI < 18	37	0.221	0.259	0.253	0.1599	0.919
nNI ≤ 8	9	0.264	0.248	0.255	0.1245	0.778
nNI ≥ 18	12	0.289	0.293	0.261	0.2074	0.833
Mean	58	0.242	0.264	0.255	0.1642	0.879
Classes of genotypes without interaction (NI Class) with the environment						
8 < nNI < 18	37	0.071	0.374	0.152	0.1552	0.676
nNI ≤ 8	9	0.088	0.428	0.105	0.1172	0.444
nNI ≥ 18	12	0.280	0.343	0.257	0.2059	0.750
Mean	58	0.117	0.376	0.166	0.1598	0.655
Classes of genotypes with interaction (WI Class) with the environment						
8 < nNI < 18	37	0.120	0.180	0.077	0.1633	0.811
nNI ≤ 8	9	0.162	0.211	0.204	0.1286	0.667
nNI ≥ 18	12	0.029	0.118	0.026	0.2201	0.417
Mean	58	0.108	0.172	0.086	0.1697	0.707

environment. This situation may attenuate the discrepancies in the means and F-test values for the different lists of genotypes. Because the genotypes of the WI class interact with the environment, they are more sensitive to environmental variation (i.e., they present greater adaptability); therefore, they may have a lower overall mean yield in stressful environments. The yields were different among the genotypes in all groups with NI genotypes, with the exception of one group.

#### **Number of genotypes by class**

The number of genotypes in each class (NI and WI) was variable in the 58 analyzed yield trial groups. Some groups contained few genotypes in the NI class and consequently many genotypes in the WI class. The separation of groups into ranges due to the number of genotypes in the NI class (nNI) is shown for three ranges (Table 3). In these ranges, there were limited cases with genotypes of the NI class (nNI  $\leq$  8; 9 groups) or a few WI class genotypes (nNI  $\geq$  18; 12 groups), whereas the other two-thirds of the groups (37/58 = 64%) represented the majority. In this manner, we attempted to show the consequences of the nNI ranges on important statistics. The proportion of yield trials with a non-normal distribution of errors (No) was lower when the groups were analyzed by class (WI class or NI class) versus the class NI+WI = 25 genotypes. The absence of normality was also smaller when there were fewer WI genotypes in the WI class (No = 0.029 = 2.9%) or fewer NI genotypes in the NI class (No = 0.088 = 8.8%). We found that 24.2% of the 348 yield trials for which all genotypes were considered in the analysis did not fit a normal distribution. In an analysis of 572 wheat yield trials, Benin et al. (2013b) also found that 12% of the trials did not have normality of errors. Therefore, the lack of error normality occurs in yield trials with low frequencies and can be reduced by stratifying the genotypes based on their ability to interact with the environment. As a result of the analysis by genotype class, the presuppositions of error normality are more frequent and hypothesis tests are favored. The nNI ranges from all pairs of environments did not affect the average estimate of the Spearman's correlation coefficients ( $r_s$ ) (Table 3). However, the  $r_s$  estimates were lower in the WI genotype class ( $r_s = 0.172$ ) and greater in the NI genotype class ( $r_s = 0.376$ ). This result was expected because the order of genotypes for different environments remained the same in the absence of GEI. As expected, the proportion of significant  $r_s$  estimates (pH1) was lower in the WI genotypes class and even lower when nNI  $\geq$  18. These results showed that although the separation of genotypes into the WI and NI classes was not perfect, the test proposed by Araújo et al. (2012) might be suitable to improve the analysis of each class separately. Estimates of the MSE in the three classes of genotypes (WI, NI and WI+NI) were similar (Table 3). The average MSE was lower in the range of nNI  $\leq$  8 (few genotypes in the NI class) in class WI (MSE = 0.1286) and in the range nNI  $\leq$  8 of the NI class (MSE = 0.1172). Hence, analysis of only a few genotypes and their interaction with the environment implied a lower MSE and a high precision on multiple comparisons of means using the NI class genotypes. The proportion of yield trial groups in which the homogeneity presupposition of the MSE between environments was not acceptable (Het) was high when all genotypes were considered (Het = 0.879). This proportion was lower when the genotypes were analyzed by their WI (Het = 0.707) and NI (Het = 0.655) interaction classes. Moreover, the Het was lower in the range nNI  $\geq$  18 class WI (Het = 0.417) and in the range nNI  $\leq$  8 class NI (Het =

0.444). Similar behavior was also observed for the normal presupposition test. The high proportion of groups with MSE heterogeneity between environments was in response to the data used in this application. In this case, the heterogeneity should be lower in groups with yield trial MSEs that do not exceed a ratio of approximately 7:1. Alternatively, Hu et al. (2013) showed that the consequences of MSE heterogeneity could be minimized by the use of a mixed model procedure with restricted maximum likelihood (REML) estimation. Modeling the between-yield trial residual heterogeneity can greatly improve the combined analyses of multi-location yield trials.

#### **Additive main effects and multiplicative interaction (AMMI) analysis**

According to the AMMI method, the variability in grain yield (mean 56%) explained by the environment (E) when all 25 genotypes of each yield trial were used did not differ from the estimated variability obtained when only the genotypes of the WI class were used (mean 51%) (Table 4). However, the variability was different ( $\alpha < 0.05$ ) when the genotypes of the NI class were used (mean 68%). The genotypes of the NI class were less sensitive to environmental variation than the genotypes of the WI class. The variability explained by the genotype (G) and by the interaction (GEI) was lower than the variability explained by the environment (E). The variability due to the genotypes was not different among the classes of genotypes (NI+WI, NI and WI). Large proportions of the variability in the grain yield in wheat crops were previously demonstrated to be due to the environment (Silva et al., 2011; Zhang et al., 2013). The average variability explained by the GEI interaction (multiplicative effect) was higher for the WI genotypes than for the NI genotypes, whereas the joint class (NI+WI) demonstrated an intermediate variability. Similar results were obtained when the classes of genotypes were compared using statistical analyses that included the variance of GEI ( $V_{GEI}$ ), genotype mean heritability ( $h_{mg}^2$ ) and selective accuracy of genotypes (SAG) of the REML method. These results demonstrate that there is a real advantage in separating the genotypes into two classes and analyzing each class separately.

#### **Correlations among statistics**

The proportion of variation due to the environment, the genotype and both statistics together did not differ among the classes (all genotypes, genotypes of the WI class and genotypes of the NI class). Furthermore, a positive linear correlation (Table 5) was observed between the WI+NI and WI classes, indicating that the removal of the genotypes in the NI class did not change the way that the environment affected the performance of the genotypes and/or the way that they interacted with the environment. The main advantage of analyzing the NI genotype separately was to maximize the ability to successfully identify genotypes with superior performances that were characterized by a heterogeneous response to the environment within groups; this ability is particularly important because adaptability and stability are related to the total set of genotypes included in the experiment. Furthermore, separate analysis of the genotypes is important because the effect of the residue on the selection of genotypes becomes non-significant.

The proportion of variation explained by the environment (E) for the 58 groups was similar for all classes of genotypes, regardless of whether they interacted with the environment ( $r = 0.89$ ,  $\alpha < 0.05$ ) (Table 5). However, the variation explained

**Table 4.** The mean proportion of variation (AMMI model) explained by the effects of environment (E), genotype (G), their interaction (GEI), variance of GEI ( $V_{GEI}$ ), genotype mean heritability ( $h_{mg}^2$ ), and accuracy of the selection of genotypes (SAG) in the analysis of the grain yield of 25 wheat genotypes using all genotypes (NI + WI classes), the genotypes in the NI class and the genotypes in the WI class; mean, minimum and maximum.

Region	Ng	E	G	GEI	Sum	$V_{GEI}$	$h_{mg}^2$	SAG
25 genotypes (NI+WI Class)								
Mean	-	56.1	18.7	13.1	87.9	0.11	0.63	0.79
Minimum	-	4.7	1.4	2.2	73.4	0.10	0.62	0.77
Maximum	-	91.9	58.7	36.6	98.0	0.13	0.66	0.81
Classes of genotypes without interaction (NI Class) with the environment								
Mean	-	67.5	18.1	6.8	92.4	0.02	0.77	0.87
Minimum	-	57.5	9.6	4.0	90.2	0.02	0.71	0.83
Maximum	-	80.5	23.0	9.7	94.1	0.02	0.81	0.90
Classes of genotypes with interaction (WI Class) with the environment								
Mean	-	50.8	17.5	18.9	87.2	0.22	0.48	0.65
Minimum	-	4.2	0.7	3.2	67.7	0.17	0.47	0.64
Maximum	-	89.9	63.7	51.5	98.6	0.28	0.49	0.67

**Table 5.** Estimates of Pearson correlation coefficients for the mean yield (Yield), mean squared error (MSE), F-test value for the interaction ( $F_{GEI}$ ), the proportion of variation explained by the effects of the environment (E), the genotype (G), and their interaction (GEI), component of variance of GEI ( $V_{GEI}$ ), genotype mean heritability ( $h_{mg}^2$ ) and selective accuracy of genotypes (SAG) between the classes of genotypes that interact with the environment (WI class), the classes of genotypes that do not interact with the environment (NI class) and the total (WI+NI class).

Class	Yield		MSE		$F_{GEI}$	
	WI+NI	WI	WI+NI	WI	WI+NI	WI
WI	0.98*		0.73*		0.74*	
NI	0.99*	0.96*	0.89*	0.47*	0.39*	0.05
E						
WI	0.98*		0.90*		0.90*	
NI	0.92*	0.891*	0.78*	0.47*	0.75*	0.76*
G						
WI	0.98*		0.90*		0.90*	
NI	0.92*	0.891*	0.78*	0.47*	0.75*	0.76*
GE						
WI	0.98*		0.90*		0.90*	
NI	0.92*	0.891*	0.78*	0.47*	0.75*	0.76*
GEI						
WI	0.55*		0.75*		0.75*	
NI	0.34*	0.05	0.35*	0.11	0.29*	0.06

\* Significant by t-test ( $\alpha < 0.05$ ); n = 58 groups.

by the genotype (G) was weakly correlated for the different classes of genotypes ( $r = 0.47$ ,  $\alpha < 0.05$ ), which demonstrated that the NI and WI classes responded differently. The variation of the interaction ( $V_{GEI}$ ,  $r = 0.05$ ), which was related to the magnitude of the GEI, and the values for  $h_{mg}^2$  ( $r = 0.11$ ) and SAG ( $r = 0.06$ ) differed by genotype class. Thus, the classification of genotypes into classes according to the occurrence of GEIs is reasonable because these estimators do not behave similarly. Karimizadeh et al. (2012) reported that genotype grouping was important because it allowed plant breeders to group a set of data in homogeneous subsets that facilitated the study of the GEI structure. Moreover, grouping the genotypes improved the identification of variation among the genotypes. Higher F values ( $F_{GEI}$  and  $F_G$ ) indicate greater selective accuracy (SA) where  $SA = (1 - 1/F)^{1/2}$  (Resende and Duarte, 2007). The NI class demonstrated a higher value for  $F_G$  and therefore was characterized by a greater SA or a greater efficiency in identifying genetic values and performing multiple comparisons of the means of the genotypes. The WI class demonstrated a higher value for  $F_{GEI}$ , indicating a higher accuracy for the estimation of the effects of the interaction and for the various statistics used to analyze the adaptability and stability. According to Resende and Duarte (2007), the accuracy of an experiment is very high when the  $SA > 0.90$  (i.e., when  $F > 5.26$ ). The mean  $F_{GEI}$  in the WI class and the mean  $F_G$  in the NI class were higher than this threshold, which indicated that the interaction in the WI class and the genotype effect in the NI class were estimated with great accuracy. In summary, the results and interpretations presented here indicate that the analysis of the experimental network to assess wheat genotypes should be performed separately for each genotype class. The conduction of hypothesis testing using the error (MSE)

variances of each genotype class will improve the data quality of the yield trials. The occurrence of heterogeneity in error variance could be eliminated using this approach, leading to greater accuracy for the tests in the joint analysis of multi-environment yield trials (Hu et al., 2013). A single test to compare the means could be sufficient for the genotypes in the NI class, and the best genotypes for a given environment (i.e., one in which the genotype was evaluated or a similarly representative environment) could be quickly identified. In contrast, for the WI class the analysis should be used to classify genotypes in terms of their adaptability and stability and to decide which genotype should be used in environments with specific characteristics (e.g., superior, medium, inferior or overall). The joint analysis of yield trials in the absence of GEIs has the highest selective accuracy for the genotype effect. Furthermore, the joint analysis of yield trials for wheat genotypes that interact with the environment presents the highest selective accuracy for the GEI effect and a reduced selective accuracy for the genotype effect. This strategy also enables the selection of wheat genotypes for specific environments that is not confounded by genotypes without GEIs.

## Materials and Methods

### Description of data

A total of 367 genotypes (34 cultivars and 333 inbred lines) were evaluated in 348 yield trials from 2010-2012. Each yield trial was formed by 25 genotypes that were grouped according to the year, test location and growing season to generate 58 groups of experiments. Table 1 shows the experiments conducted at 24 selected test locations. These sites are representative of the main wheat-growing regions in

Brazil. Some genotypes were exchanged during the three test years and sites due to the usual practice of discarding inferior genotypes and including new breeding lines. A mean of 14.7 coincident genotypes (59%) was identified in the different yield trial groups. The most common genotypes in the yield trials were the following cultivars: BRS Guamirim (52 groups); Quartzo and Mirante (36 groups); CD 114 (30 groups); CD 150 (20 groups); and Fundacep Raízes, Onix and BRS Pardela (18 groups). Additionally, 196 genotypes were tested in one or two groups, 114 genotypes were tested in three to five groups, 32 genotypes were tested in six to eight groups, and 17 genotypes were tested in nine to twelve groups.

All yield trials were conducted using a completely randomized block design with three replications. The plots consisted of six rows 5 m in length spaced 0.20 m apart for a total area of 6.0 m<sup>2</sup>. The grain yield was measured by harvesting the whole area of each plot and then corrected to 13% moisture (on a wet basis) and converted into kg ha<sup>-1</sup>.

### Genotype class identification

A joint analysis of variance was performed (block within the environment, genotype, environment and GEI) for each yield trial group (25 genotypes in J environments). Genotype effects were assumed to be fixed, whereas the blocks, environment and GEI were assumed to be random effects. This analysis was performed using the Genes software (Cruz, 2013). The overall mean, mean squared error (MSE), F-test for the genotype ( $F_G$ ) and its  $\alpha$ -value ( $\alpha_G$ ) and F-test for the GEI ( $F_{GEI}$ ) and its  $\alpha$ -value ( $\alpha_{GEI}$ ) were recorded in a worksheet.

The analyses of stability and adaptability were performed according to the Wricke method using the Genes software (Cruz, 2013). The known ecovalence ( $W_i$ ) values for the genotype  $i = 1, 2, \dots, I=25$  were recorded from this analysis. The estimate  $W_i$  corresponded to the sum of squares of the environment effect of the estimates of the GEI effects for the genotype  $i$  (i.e.,  $W_i = K \sum_{j=1}^J \hat{\tau}_{ij}^2$ ), where  $\hat{\tau}_{ij}$  was the estimate of the GEI effect obtained in  $k$  replications (i.e.,  $\hat{\tau}_{ij} = \bar{Y}_{ij} - \bar{Y}_{i.} - \bar{Y}_{.j} + \bar{Y}_{...}$ ),  $\bar{Y}_{ij}$  was the estimated mean of the  $i$ -th genotype in the  $j$ -th environment,  $\bar{Y}_{i.}$  was the estimated mean of the  $i$ -th genotype,  $\bar{Y}_{.j}$  was the estimated mean of the  $j$ -th environment and  $\bar{Y}_{...}$  was the estimate of the overall mean. The significance of the  $W_i$  values (Ho:  $W_i = 0$ ) was tested with the F-test ( $\alpha < 0.05$ ) using the F-test proposed by Araújo et al. (2012) (i.e.,  $Fw_i = (W_i/DFw_i)/MSE$ ) with the degrees of freedom (DF) for the numerator and denominator equal to  $DFw_i = (I-1)(J-1)/I$  and  $DFe = J(I-1)(K-1)$ , respectively, and the MSE representing the mean square error of the joint analysis of variance. The hypothesis testing was performed in Excel using the  $W_i$ , MSE,  $DFw_i$  and  $DFe$  values. The genotypes that did not contribute to the GEI (class NI genotypes) (i.e., the genotypes for which the hypothesis Ho:  $W_i = 0$  was accepted with  $\alpha > 0.05$ ) were identified. Two classes of genotypes (i.e., NI and WI genotypes) were distinguished. Thus, we identified three classes of genotypes: NI class, WI class and NI+WI class.

### Statistical methods for adaptability and stability

The joint analysis of variance was performed using the same model used for all genotypes (NI+WI) through the Genes software for each class of genotype (NI and WI). The estimated mean, MSE, F-test value for the effect of genotype ( $F_G$ ) and its  $\alpha$ -value ( $\alpha_G$ ), and the F-test value for the GEI

( $F_{GEI}$ ) and its  $\alpha$ -value ( $\alpha_{GEI}$ ) were recorded from these analyses. The results for the 58 groups were recorded in Excel to examine the frequencies and means of the statistics in general. The efficiency of separating the genotypes into two classes (WI and NI) within each yield trial group was estimated by averaging the Spearman correlation coefficients ( $r_s$ ) from the yield rank of the genotypes between all pairs of environments. The ratio of significant correlations ( $\alpha < 0.05$ ; two-sided test) was also determined between all pairs of environment types for each genotype class. We calculated the proportion of yield trials with non-normal error distributions (Shapiro-Wilk test,  $\alpha < 0.05$ ) within each group and for each genotype class. The proportion of groups of yield trials in which the MSE between the environments was heterogeneous was also calculated (Bartlett test,  $\alpha < 0.05$ ) for each genotype class. Features available in the R software (R Development Core Team, 2014) were used to calculate these statistics. The adaptability and stability analyses were performed for all genotypes and classes (WI and NI) with the additive main effects and multiplicative interaction (AMMI) model, which combined an analysis of variance with principal component analysis to adjust for the main effects (genotypes and environments) and the GEI effects, respectively (Zobel et al., 1988). The AMMI model was represented by the equation  $Y_{ij} = \mu + g_i + e_j + \sum_{k=1}^n \lambda_k \gamma_{ik} \alpha_{jk} + \rho_{ij} \varepsilon_{ij}$ , where  $Y_{ij}$  was the mean response of the  $i$ -th genotype ( $i = 1, 2, \dots, I$  genotypes) in the  $j$ -th environment ( $j = 1, 2, \dots, J$  environments),  $\mu$  was the overall mean of the yield trial,  $g_i$  was the fixed effect of the  $i$ -th genotype,  $e_j$  was the fixed effect of the  $j$ -th environment,  $\lambda_k$  was the  $k$ -th singular value (scalar) of the original matrix interactions (denoted by GEI),  $\gamma_{ik}$  was the element that corresponded to the  $i$ -th genotype in the  $k$ -th singular vector of the GEI matrix column,  $\alpha_{jk}$  was the element that corresponded to the  $j$ -th environment in the  $k$ -th singular vector of the GEI matrix row,  $\rho_{ij}$  was the noise associated with the expression  $(ge)_{ij}$  of the classical interaction between the  $i$ -th genotype and the  $j$ -th environment, and  $\varepsilon_{ij}$  was the mean experimental error. The percentage of explanation (contribution) captured by the main effects of the environment, genotype and the first main component of the GEI (Zobel et al., 1988) were determined. This analysis was performed using the GGE biplot software (Yan, 2001).

The genetic parameters, including the variance of the GEI ( $V_{GEI}$ ), the genotype mean heritability ( $h_{mg}^2$ ) and the selective accuracy of genotypes (SAG), were determined using REML analysis. This statistical model was described by Resende (2007) and was expressed by the equation  $Y = X_r + Z_g + W_i + \varepsilon$ , where  $Y$  was the data vector,  $r$  was the vector of the effects of replication (fixed effects) summed to the overall mean,  $g$  was the vector of the genotypic effects (random effects),  $i$  was a vector of the GEI (random effects) and  $\varepsilon$  was the vector of the errors or residues (random effects).  $X$ ,  $Z$  and  $W$  represented the matrices of the incidence of the respective effects. The genotype mean heritability ( $h_{mg}^2$ ) was estimated through the inverse of the coefficients of the mixed model equation (MME) matrix (Resende, 2007). The accuracy of the genotype selection (SAG) was estimated by the equation  $SAG = (h_{mg}^2)^{0.5}$ . Using the AMMI model, the proportion of variance explained by the environment (E), genotype (G) and GEI (GE), the sum of these variables (E+G+GE), the REML,

the variance of the GEI ( $V_{GEI}$ ), the genotype mean heritability ( $h_{mg}^2$ ) and the selective accuracy of genotypes (SAG) were used to determine the minimum, maximum and the overall means. Pearson's correlation coefficients were calculated for the estimated grain yields and the statistics (MSE,  $F_{GEI}$ , E, G, EG, sum,  $V_{GEI}$ ,  $h_{mg}^2$  and SAG) between each genotype class (NI + WI class, WI class and NI class).

## Conclusion

The grouping of genotypes into different classes provided valuable information on the dynamics of the GEI, which could enable plant breeders to maximize the efficiency of the selection and the recommendation of stable, widely adapted genotypes. The highest selective accuracy for the genotype effects was obtained for the joint analysis when there the GEI was not present. The joint analysis of trials for wheat genotypes that interacted with the environment presented the highest selective accuracy for the GEI effect and a reduced selective accuracy for the genotype effect.

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## References

- Alake CO, Ariyo OJ (2012) Comparative Analysis of Genotype  $\times$  Environment Interaction Techniques in West African Okra, (*Abelmoschus caillei*, A. Chev Stevels). *J Agr Sci*. 4:135-150.
- Araújo MFC, Araújo LB, Faria PN, Dias CTS (2012) Statistical test for genotype and environment contribution in the genotype  $\times$  environments interaction matrix. *Interciencia (Caracas)*. 7:515-519.
- Beche E, Benin G, Silva CL, Munaro LB, Marchese JA (2014) Genetic gain in yield and changes associated with physiological traits in Brazilian wheat during the 20th century. *Eur J Agron*. 69:49-59.
- Benin G, Storck L, Marchioro VS, Franco FA, Schuster I (2013a) Precisão experimental de ensaios de trigo em regiões homogêneas de adaptação. *Pesqui Agropecu Bras*. 48: 365-372.
- Benin G, Storck L, Marchioro VS, Franco FA, Schuster I, Trevizan DM (2013b) Improving the precision of genotype selection in wheat performance trials. *Crop Breed Appl Biot*. 13:234-240.
- Brasil (2008) Ministério da Agricultura, Pecuária e Abastecimento. Instrução normativa nº 58, de 19 de novembro de 2008. Valor de cultivo e uso de trigo. Diário Oficial da República Federativa do Brasil, Nov 25, 2008. Seção 1.
- Cargnelutti Filho A, Storck L, Riboldi J, Guadagnin JP (2009) Associação entre métodos de adaptabilidade e estabilidade em milho. *Cienc Rural*. 39:340-347.
- Colombari Filho JM, Resende MDV, Morais OP, Castro AP, Guimarães EP, Pereira JA, Utumi MM, Breseghello F (2013) Upland rice breeding in Brazil: a simultaneous genotypic evaluation of stability, adaptability and grain yield. *Euphytica*. 192:117-129.
- Cooper M, Woodruff DR, Phillips IG, Basford KE, Gilmour AR (2001) Genotype-by-management interactions for grain yield and grain protein concentration of wheat. *Field Crop Res*. 69:47-67.
- Cormier F, Faure S, Dubreuil P, Heumez E, Beauchêne K, Lafarge S, Praud S, Le Gouis J (2013) A multi-environmental study of recent breeding progress on nitrogen use efficiency in wheat (*Triticum aestivum* L.). *Theor Appl Genet*. 126: 3035-3048.
- Cruz CD (2013) Genes – a software package for analysis in experimental statistics and quantitative genetics. *Acta Sci-Agron*. 35:271-276.
- De Vita P, Mastrangelo AM, Matteu L, Mazzucotelli E, Virzi N, Palumbo M, Storto MLO, Rizza F, Cattivelli L (2010) Genetic improvement effects on yield stability in durum wheat genotypes grown in Italy. *Field Crop Res*. 119:68–77.
- Francis TR, Kannenberg LW (1978) Yield stability studies in short-season maize. I. A descriptive method for grouping genotypes. *Can J Plant Sci*. 58:1029-1034.
- Graybosch R, Peterson C (2012) Specific adaptation and genetic progress for grain yield in Great Plains hard winter wheats from 1987 to 2010. *Crop Sci*. 52:631–643.
- Hristov N, Mladenov N, Djuric V, Kondic-Spika A, Marjanovic-Jeromela A, Simic D (2010) Genotype by environment interactions in wheat quality breeding programs in southeast Europe. *Euphytica*. 174:315-324.
- Hu X, Yan S, Li S (2014) The influence of error variance variation on analysis of genotype stability in multi-environmental trials. *Field Crop Res*. 156:84-90.
- Hu X, Yan S, Shen K (2013) Heterogeneity of error variance and its influence on genotype comparison in multi-location trials. *Field Crop Res*. 149:322-328.
- Karimizadeh R, Mohammadi M, Sabaghnia N, Hosseinpour T, Shafazadeh MK (2012) Analysis of genotype and genotype  $\times$  environment interaction in durum wheat in warm rainfed areas of Iran. *Crop Breed J*. 2:71-78.
- Lin CS (1982) Grouping genotypes by a cluster method directly related to genotype-environment interaction mean square. *Theor Appl Genet*. 62:277-280.
- Malik AH, Kuktaite R, Johansson E (2013) Combined effect of genetic and environmental factors on the accumulation of proteins in the wheat grain and their relationship to bread-making quality. *J Cereal Sci*. 57:170-174.
- Miranda GV, Souza LV, Guimarães LJM, Namorato H, Oliveira LR, Soares MO (2009) Multivariate analyses of genotype  $\times$  environment interaction of popcorn. *Pesqui Agropecu Bras*. 44:45-50.
- Mohammadi R, Sadeghzadeh D, Armion M, Ahmadi MM (2011) Analysis of stability and adaptability of grain yield in durum wheat genotypes. *Agron J*. 24:70-78.
- Munaro LB, Benin G, Marchioro VS, Franco FD, Silva RR, Silva CL, Beche E (2014) Brazilian Spring Wheat Homogeneous Adaptation Regions can be Dissected in Major Megaenvironments. *Crop Sci*. 54:1374-1383.
- Pereira DGS, Rodrigues PC, Mejza I, Mejza S, Mexia JT (2012) Analyzing genotype-by-environment interaction using curvilinear regression. *Sci Agr*. 69:357-363.
- R Development Core Team (2014) R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available at <http://www.R-project.org>.
- Ramey TB, Rosielle AA (1983) HASS cluster analysis: a new method of grouping genotypes or environments in plant breeding. *Theor Appl Genet*. 66:131-133.

- Resende MDV (2007) Selegen REML/BLUP: sistema estatístico e seleção genética computadorizada via modelos lineares mistos. Embrapa Florestas, Colombo, Brazil.
- Resende MDV, Duarte JB (2007) Precisão e controle de qualidade em experimentos de avaliação de cultivares. *Pesqui Agropecu Tropical*. 37:182-194.
- Roostaei M, Mohammadib R, Amri A (2014) Rank correlation among different statistical models in ranking of winter wheat genotypes. *The Crop J*. 2:1154-163.
- Sabaghnia N, Karimizadeh R, Mohammadi M (2012) The use of corrected and uncorrected nonparametric stability measurements in durum wheat multi-environmental trials. *Span J Agric Res*. 10:722-730.
- Schulthess A, Matus I, Schwember AR (2013) Genotypic and environmental factors and their interactions determine semolina color of elite genotypes of durum wheat (*Triticum turgidum* L. var. *durum*) grown in different environments of Chile. *Field Crop Res*. 149:234-244.
- Silva RR, Benin G, Silva GO, Marchioro VS, Almeida JL, Matei G (2011) Adaptabilidade e estabilidade de cultivares de trigo em diferentes épocas de semeadura, no Paraná. *Pesqui Agropecu Bras*. 46:1439-1447.
- Truberg B, Hühn M (2000) Contributions to the analysis of genotype x environment interactions: comparison of different parametric and non-parametric tests for interactions with emphasis on crossover interactions. *J Agr Crop Sci*. 185:267-274.
- Tsenov N, Atanasova D (2013) Influence of environments on the amount and stability of grain yield in modern winter wheat cultivars. *Agric Sci Technol*. 5:153-159.
- Yan W (2001) GGEbiplot – A Windows application for graphical analysis of multi-environment trial data and other types of two-way data. *Agron J*. 93:1111-1118.
- Yan W, Kang MS, Ma B, Woods S, Cornelius PL (2007) GGE biplot vs. AMMI analysis of genotype-by-environment data. *Crop Sci*. 47:643–653.
- Zhang H, Berger JD, Milroy SP (2013) Genotype × environment interaction studies highlight the role of phenology inspecific adaptation of canola (*Brassica napus*) to contrasting Mediterranean climates. *Field Crop Res*. 144:77–88.
- Zobel RW, Wright MJ, Gauch H (1988) Statistical analysis of a yield trial. *Agron J*. 80:388-393.