

## Investigating suitable test locations and mega-environments for evaluating spring wheat in Brazil

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### Abstract

Wheat breeders aim to identify environments that will allow greater discrimination among wheat genotypes. This study assessed the effects of genotype, environmental factors and genotype-environment interactions on the identification and validation of mega-environments and essential test locations in various wheat-growing regions. The grain yield of wheat genotypes was evaluated at 14 locations in Brazil in 2009, 2010, and 2011. GGE biplot analysis was used to visualize the genotype x location patterns and interrelationships between test locations. The results showed that complex genotype-environment interactions occurred, suggesting that the response over three years was not well defined relative to the possible formation of distinct mega-environments. Several test sites were highly correlated, indicating that some sites could be discarded. Wheat breeders in Brazil require an assessment strategy for the development of cultivars that are stable over a wide range of environments, although this requirement may limit the gains in productivity expected from the breeding process. Given limited resources, multi-environment trials should include the use of discriminant-effective and representative locations or essential test sites rather than extensive trials in locations with similar characteristics that are related to one or more aspects relevant to the aims of the breeding initiative and enterprise.

**Keywords:** GGE biplot; genotype-environment interaction; mega-environments; multiple-environment trial; *Triticum aestivum* L.

**Abbreviations:** VCU\_value for cultivation and use; GEI\_genotype-environment interaction; MET\_multi-environment trial; ANOVA\_analysis of variance; L\_location; G\_genotype; GEI\_genotype-location interaction; GY\_grain yield; GL\_genotype-location interaction.

### Introduction

Wheat (*Triticum aestivum* L.) is grown in three principal regions of Brazil: Central, South Central, and South. To better evaluate wheat genotypes within a geographical-climatological context and to achieve the breeding objectives, these wheat-growing regions may be subdivided according to water regime, air temperature and elevation above sea level. The categorization of these regional subdivisions is based on value for cultivation and use (VCU), as follows: wet, cold, and high elevation (VCU 1); wet, moderately warm, and low elevation (VCU 2); moderately dry, warm, and low elevation (VCU 3) and dry, warm, and Cerrado (VCU 4) (Brasil, 2008). Genotypes frequently show fluctuations in grain yield (GY) performance in different environments as a result of genotype-environment interactions (GEI). Because GEI is expected, the selection of genotypes with superior GY requires a multi-environment trial (MET) (Qiao et al., 2004; Roozeboom et al., 2008). Genotype evaluation by MET has several benefits, including i) the ability to select genotypes that are widely adapted based on the average yield performance in various environments, ii) the ability to select genotypes that are specifically adapted to certain environments, and iii) the ability to identify redundant test locations or environments such that information about wheat genotypes is obtained with minimal duplication (Yan et al. 2010).

In METs, the evaluation of the GY performance of selected genotypes is commonly performed over multiple years and in multiple locations. When designing an MET, it is important to be aware of both the predictable and unpredictable aspects of GEI. This knowledge affords greater confidence in the selection of superior genotypes (Mirzawan et al., 1994) and the identification of mega-environments. However, due to limited financial resources, it is often necessary to establish the minimum number of test sites necessary to accurately represent the range of environmental conditions in different regions. Indeed, cultivars may require testing at only a few selected sites, and the use of several test sites may result in redundant information and waste financial resources (Fan et al., 2001). The accurate identification of test sites ensures that the selected genotypes show superior performance across several environments (Blanche and Myers, 2006) and improves the efficiency of the breeding process and the use of financial resources. The selection of appropriate test environments is critical for the characterization and identification of key locations and involves the selection of representative locations for each sub region. A number of techniques have been used to interpret data from multi-environment trials (Eberhart and Russel 1966, Gauch and Zobel 1997, Yan et al. 2000), including GGE biplot analysis for effectively identifying mega-environments (Yan and

Rajcan, 2002; Naroui Rad et al., 2013; Munaro et al., 2014) and select genotypes (Silva et al., 2011; Naroui Rad et al., 2013). GGE biplot analyses provide ample information and enable comprehensive visualization of patterns among test sites. The results of these analyses can improve discrimination between genotypes and enhance the precision of the identification of genotypic variance among environments. Moreover, GGE biplot analysis have been successfully used to identify superior genotypes when large numbers of genotypes are tested in multi-environment trials (Alwala et al., 2010).

The aim of this study was to assess the effects of plant genotype and environment on the identification and validation of mega-environments and essential test locations in different wheat-growing regions of Brazil.

## Results

### *Mega-environment identification*

An ANOVA showed that the location (L), genotype (G) and genotype-location interaction (GL) were all highly significant ( $P < 0.001$ ) for GY in wheat (Table 1). The location explained the greatest amount of variation in GY, explaining more than 60% of the total variation in GY in the three study years. Moreover, the ratio of (G) to (G+GL) was approximately 30% across the three study years, suggesting that multiple wheat mega-environments exist in Brazil.

Although the ANOVA results suggest that several wheat mega-environments exist in Brazil, the specific mega-environments were not uniquely identifiable because the distinct mega-environments were not entirely consistent between years (Fig. 1). In 2009 and 2010, the test locations were clearly subdivided into four mega-environments; in 2010 and 2011, however, we identified only two mega-environments. The associations between the locations defining the mega-environments were not consistent with the geographical proximity between the environments or the VCU region to which the location belonged. In addition, the associations between environments were not consistent across different crop seasons, and most of the significant associations occurred only in a single year. In all years of the assessment, obtuse angles were observed between the evaluated environments in each plot; these angles are indicative of negative associations between the environments and unpredictable GL values. However, these inter-location associations were not consistent over the three study years. For example, the association between the Londrina and Cambará locations (VCU 3) was negative in 2009 and positive in 2010.

### *Visualization of the representativeness and discriminating ability of environments*

GGE biplot analysis is used to assess the representativeness and discriminating ability of environments using environment-focused singular-value partitioning. The cosine of the angle between two environments approximates the genetic correlation between them. This approximation indicates that the two environments are negatively correlated if the cosine is negative and positively correlated if the cosine is positive. The small circle in the plot represents the average environment, and the thick arrow-shaped line is the average environment vector. A test environment having a smaller angle with the horizontal axis is more representative of other environments, and the length of the environment vector is a

measure of the discriminating ability of the environment (Yan and Tinker, 2006).

The biplots shown in Figure 2 indicate the representativeness and discriminating ability of the tested environments. Based on these results, Campo Mourão (2009, 2010 and 2011), Mauá da Serra (2010), Cascavel (2010), Abelardo Luz (2010) and Cruzmaltina (2009 and 2011) were the most representative locations, and Londrina (2009), Cruzmaltina (2009 and 2011), Pato Branco (2009 and 2010), Campos Novos (2009 and 2010), and Ponta Grossa (2010 and 2011) had the greatest discriminating ability. These locations would be ideal for selecting wheat genotypes with high GY. Cruzmaltina (2009 and 2011), Pato Branco (2009) and Campo Mourão (2009) have an effective discriminating ability and are highly representative; therefore, these locations would be most useful for selecting generally adapted plant genotypes.

### *Identification of essential test locations*

A close association between test environments implies that the same information regarding the genotypes can be obtained in both environments (group environments). If each group environment can be represented by a single test environment, a group of critical test sites can be identified (Yan et al., 2010). In 2009, Ibirarema and Londrina appeared in the same group environment (Table 4); this group environment can be represented by Londrina, the site of the IAPAR breeding program. Similarly, the close association between Pato Branco and Abelardo Luz allows Abelardo Luz to be omitted in favor of the IAPAR research unit in Pato Branco. Therefore, Londrina, Cruzmaltina, Pato Branco, Ponta Grossa and Campo Mourão were identified as essential test locations. Eliminating highly correlated locations can reduce costs and optimize MET (Munaro et al., 2014).

In the biplot, genotypes positioned at the vertices of the polygon show either the best or worst performance in one or more environments; the polygon is formed by connecting these genotype vertices. Perpendicular lines drawn from the center of the biplot to the sides divide the plot into two sectors: the genotypes associated with lower levels of grain production appear on the left side of the plot, whereas the genotypes associated with higher levels of grain production appear on the right side (Yan and Tinker, 2006).

GGE biplots showing only the essential test locations were constructed (Fig. 3). For 2009, genotypes BRS Tangará and Safira would be selected for Ponta Grossa, genotypes BRS Gaivota and BRS Tangará for Pato Branco, genotype PF014384 for Cruzmaltina, genotype IPR 128 for Londrina and genotypes IPR 130 and LD 072212 for Campo Mourão. In 2010, genotype BRS Gralha Azul would be selected for Ponta Grossa and Cruzmaltina, genotype Quartzo for Londrina and Campo Mourão and genotype WT 07106 for Pato Branco. For 2011, genotype BRS Gralha Azul would be selected for Ponta Grossa, genotype WT 08014 for Cruzmaltina and Pato Branco, genotype Quartzo for Campo Mourão and genotypes Abalone and IPR 128 for Londrina.

## Discussion

The test location had a substantial effect on GY (Table 3). Similar trends have been reported in other multilocation or multi-environment field experiments (Yan et al., 2010; Badu-Apraku, 2012; Rakshit et al., 2012). However, the magnitude of environmental effects is irrelevant because the adaptive capacity of a genotype is more important for genotype selection than specific environmental conditions (Camargo-

**Table 1.** Analysis of variance (ANOVA) for grain yield of wheat genotypes in different locations in each year.

Source	2009		2010		2011	
	DF	MS	DF	MS	DF	MS
Block (Location)	28	102,651*	28	173,853*	28	80,408*
Location (L)	13	26,124,109 <sup>†</sup>	13	100,597,065 <sup>†</sup>	13	57,499,480 <sup>†</sup>
Genotype (G)	18	3,801,973 <sup>†</sup>	16	3,045,041 <sup>†</sup>	13	4,529,825 <sup>†</sup>
GL	234	719,450 <sup>†</sup>	192	530,522 <sup>†</sup>	169	894,727 <sup>†</sup>
Error	504	71,316	491	81,542	390	91,438
Grand mean (kg ha <sup>-1</sup> )		3512		4361		4230
CV (%)		7.6		6.5		7.1
G/(G+GL) (%)		29		30		28

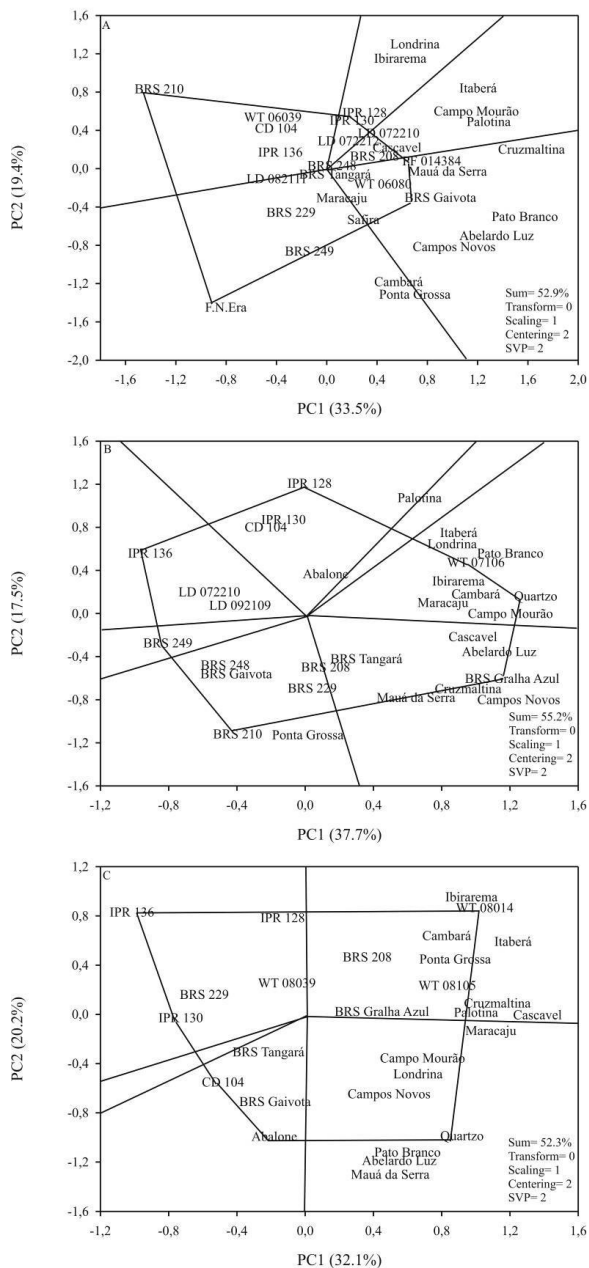
\* P > 0.05; <sup>†</sup> P < 0.001**Table 2.** List of wheat genotypes tested in each of the 3 years.

Genotype	2009	2010	2011	Genotype	2009	2010	2011
BRS 208	x	x	x	Quartzo		x	x
BRS 210	x	x		LD 072210	x	x	
BRS 229	x	x	x	LD 092109		x	
BRS 248	x	x		LD 072212	x		
BRS 249	x	x		LD 082111	x		
BRS Gaivota	x	x	x	PF 014384	x		
BRS Gralha Azul		x	x	WT 08014			x
BRS Tangará	x	x	x	WT 08039			x
CD 104	x	x	x	WT 08105			x
IPR 128	x	x	x	WT 07106		x	
IPR 130	x	x	x	WT 06039	x		
IPR 136	x	x	x	WT 06080	x		
Safira	x			Fundacep Nova Era	x		
Abalone		x	x				

Buitrago et al., 2011). The test locations did not correspond to different mega-environments owing to the presence of complex GEIs. This result implies that the breeding program for spring wheat in Brazil will be problematic and experience certain difficulties. Similarly, Yan and Rajcan (2002) observed that evidence for different mega-environments was insufficient among test sites for the selection of soybeans in Ontario. Moreover, Ramburan et al. (2012) reported that different mega-environments did not exist among test sites for the selection of sugarcane in South Africa. If the GEI is unpredictable, the best genotype in one environment may be the worst in another; however, if the GEI is predictable, no change occurs in the classification of a genotype between environments (Rakshit et al., 2012). The results of this study indicate an unpredictable GEI, and thus, it is necessary to select genotypes based on their average GY and stability (Yan and Tinker, 2006). In addition to a complex GEI, changes in the genotype ranking may also interfere with the formation of distinct mega-environments for wheat cultivation in Brazil. This study found changes in the genotype rankings, which might be explained by variations in the climate (Ramburan et al., 2012). For example, genotype BRS Gralha Azul showed the poorest GY in 2009 and a better GY in 2011 in Maracaju. Thus, given that Brazil is a large country with diverse agro-climatic conditions in wheat-growing areas, it is possible that mega-environments do not exist for this crop.

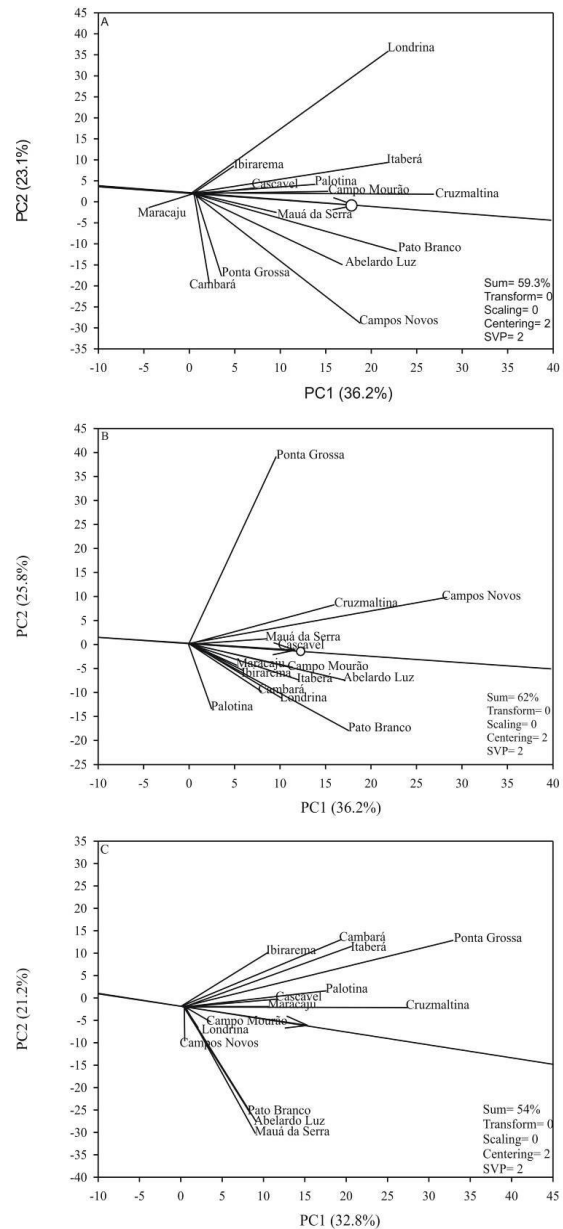
Null associations between two environments that are located in geographical proximity or show similar climatic conditions have also been observed in previous studies (Yan et al., 2000; Putto et al. 2009; Rakshit et al. 2012). In addition, in this study, locations that differ climatically were found in the same cluster, e.g., Itaberá and Ibirarema. This type of grouping can occur in response to the growing system, soil type, and planting date (Atlin et al., 2000). Ibirarema and Itaberá both had supplemental irrigation, which may have

mitigated the effect of climatic variations on GY and, hence, influenced genotype selection. Similarly, Abelardo Luz and Pato Branco showed a strong association with each other, which may have been due to their geographical proximity and similar climatic conditions. In the SD-scaled biplot, the location vectors should be of equal or similar length if the plot adequately displays the patterns in the data; however, if the vector of a given location is much shorter than the other location vectors, this difference indicates that the biplot does not adequately display the patterns in the data (Yan and Tinker, 2006). In 2009, Maracaju, Mauá da Serra, and Cascavel showed shorter location vectors than the other locations, suggesting that these locations can be considered independent test environments (Yan et al., 2010). Although locations that are both representative and discriminating are the most desirable as test locations (Yan and Tinker, 2006; Yan et al., 2011), such ideal environments are difficult to identify. Our results demonstrated that Cruzmaltina (2009 and 2011), Abelardo Luz (2009) and Campo Mourão (2009) were the best test locations for wheat. It is likely that the observed genotypic differences among the representative environments (Figure 2) are repeated over time (Yan et al., 2011). The identification of representative locations is essential for selecting test sites that are well suited for breeding programs and the selection of cultivars with wide adaptability (Malla et al., 2010). Furthermore, representative environments may be important if there is interest in the early selection and subsequent testing of METs (Yan et al., 2011). The identification of test sites based on representativeness, discriminating ability, and local accessibility allows greater precision in genotype evaluation. Using these test sites, it is possible to discard undesirable breeding lines in the preliminary stages of evaluation (Yan et al., 2011). Our results show that the early selection of genotypes with high GY potential should be conducted in Ponta Grossa (VCU 1), Cascavel and Pato Branco (VCU 2), and Londrina (VCU 3).



**Fig 1.** Genotype + genotype × environment biplots including all locations during the years (A) 2009, (B) 2010, and (C) 2011.

In Brazil, current law requires that superior genotypes be tested in at least one location in each VCU region in each state for three years or in two locations in each VCU region in each state for one year. However, our results demonstrated that the best genotypes should be tested in Ponta Grossa (VCU 1), Pato Branco and Campo Mourão (VCU 2) and Londrina and Cruzmaltina (VCU 3). In a result that is not consistent with current law, we observed that only one test location in the VCU 1 region is sufficient for the state of Paraná, whereas two locations are required in VCU 2 and 3 regions. The primary cause of this result is the wider climatic variability in VCU regions 2 and 3 compared to VCU 1. Because of its climatic variability, all essential test environments could be located in Paraná State. In addition, Paraná State includes sites belonging to three of the four VCU regions.



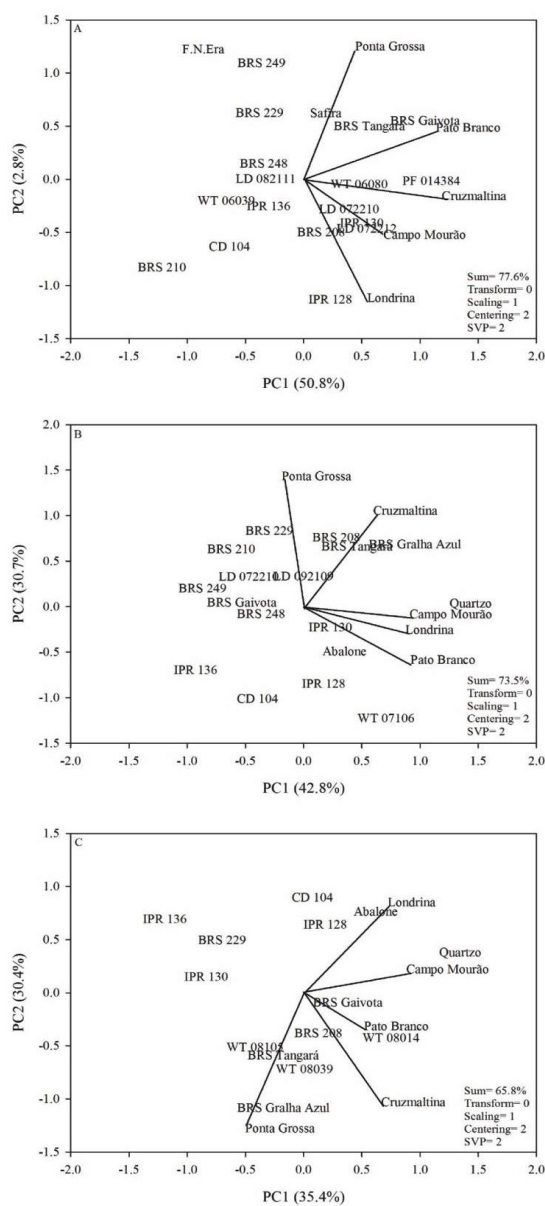
**Fig 2.** Discriminant and representativeness ability by Genotype + genotype × environment biplots including all locations during the years (A) 2009, (B) 2010, and (C) 2011.

Unpredictable GEIs were identified in this study. These interactions suggest that changes must be implemented in breeding programs. If predictable genotype-environment interactions occur, it is possible to release cultivars that are widely adaptable or that are specific to each environment. However, wheat breeders in Brazil need to develop cultivars that are stable over a wide variety of environments, even though this may limit gains in productivity expected from the breeding process. The approach discussed in this paper may significantly contribute to a reduction of costs and an increase in the efficiency of wheat breeding programs.

**Table 3.** The test locations in Brazil used in this study.

Location	Latitude	Longitude	Elevation (m)	VCU*	Sowing† date <sup>b</sup>
Ponta Grossa	25° 05' S	51° 30' W	1098	1	Jun
Campos Novos	27° 24' S	51° 12' W	962	1	Jun
Mauá da Serra	23° 53' S	51° 15' W	948	2	May
Abelardo Luz	26° 34' S	52° 21' W	850	2	Jul
Pato Branco	26° 08' S	52° 39' W	736	2	Jun
Itaberá‡	23° 49' S	49° 03' W	675	2	May
Campo Mourão	24° 03' S	52° 22' W	616	2	May
Cascavel	24° 57' S	53° 28' W	613	2	May
Cruzmalina	24° 00' S	51° 24' W	752	3	Apr
Londrina	23° 22' S	51° 10' W	543	3	Apr
Ibirarema‡	22° 42' S	49° 58' W	487	3	May
Cambará	23° 00' S	50° 01' W	460	3	Mar
Maracaju	22° 08' S	55° 08' W	390	3	Apr
Palotina	24° 20' S	53° 50' W	355	3	Apr

\* Value for Cultivation and Use: VCU 1 (wet, cold and high elevation), VCU 2 (wet, moderately warm and low elevation and VCU 3 (moderately dry, warm and low elevation); † The sowing date was fixed as the fifth day of the month; ‡ when necessary, supplementary irrigation was used.



**Fig 3.** Genotype + genotype × environment biplots including five essential locations during the years (A) 2009, (B) 2010, and (C) 2011.

## Materials and Methods

### Experimental outline

The data set consisted of the mean GY values for 19, 17 and 14 Brazilian wheat genotypes harvested in 2009, 2010 and 2011 (Table2), respectively. The number of genotypes differed from year to year because new genotypes were introduced and inferior genotypes were discarded from the breeding program. These genotypes were evaluated in field experiments performed in 14 wheat-growing locations across Brazil and in different VCU regions (Table3). These test sites were chosen because they represented the major wheat-growing areas in Brazil, which are characterized by differences in soil and climate. The field experiments were performed in a completely randomized block design with three replications. The size of each plot was 5.10 m<sup>2</sup> (5 m×1.02 m). The GY was determined by harvesting each plot and was assessed as kg ha<sup>-1</sup>, assuming 13% moisture. The local management procedures were followed, and the GY was recorded at each test location.

### Statistical analysis

Because of the use of a different set of genotypes each year, an analysis of variance (ANOVA) was conducted separately for each year (Yan et al., 2010). In addition to a conventional ANOVA, which served to quantify the relative importance of genotype-environment interactions, GGE biplot analyses (Yan et al., 2000) were conducted to visualize the genotype x location patterns and the interrelationships between the test locations (discriminating ability and representativeness of test environments in mega-environment identification). These analyses are represented as follows:

$$(1) \quad Y_{ij} - \beta_j = \lambda_1 \rho_{i1} \eta_{j1} + \lambda_2 \rho_{i2} \eta_{j2} + \varepsilon_{ij},$$

where  $Y_{ij}$  is the mean yield of genotype  $i$  in location  $j$ ;  $\beta_j$  is the mean yield of all genotypes in location  $j$ ;  $\lambda_1$  and  $\lambda_2$  are the singular values for PC1 and PC2, respectively;  $\rho_{i1}$  and  $\rho_{i2}$  are the PC1 and PC2 eigenvectors, respectively, for genotype  $i$ ;  $\eta_{j1}$  and  $\eta_{j2}$  are the PC1 and PC2 eigenvectors, respectively, for location  $j$ ; and  $\varepsilon_{ij}$  is the residual of the model associated with the combination of genotype  $i$  in location  $j$  (Yan et al. 2000). GGE biplot software (Yan, 2001) was used to generate a polygon-view biplot to identify mega-environments. The stratification of the mega-environments was performed using the “entry tester relationship” GGE biplot tool. A standard deviation (SD)-scaled GGE biplot was used in the analysis (except for the visualization of the representativeness and discriminating ability of the environment). In the display of this standardized two-way table in a biplot, the location vectors should be of equal or similar length if the biplot effectively displays the patterns in the data. The SD-scaled GGE biplot was chosen because the purpose of the analysis was to assess the similarities among the test locations for genotype evaluation. If two test locations are very similar, then it may be possible to omit one location and risk only a small probability of a significant amount of information loss regarding the genotypes (Yan et al., 2010).

## Conclusions

The wheat-growing regions of Brazil represented by the localities included in this study can be considered a single complex mega-environment. Sites suitable as testing sites for the selection of genotypes with specific and wide adaptability to different environmental conditions in Brazil were identified. Test sites that could be discarded based on their close correlations with other sites in all years of the study were also identified. Finally, to specify suitable test locations, it was concluded that the successful completion of METs using limited resources should include only discriminating and representative locations or essential test sites instead of conducting more extensive trials across related locations.

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