South American Sciences

ISSN: 2675 – 7222 - Original Article – Agricultural Sciences

Environmental stratification for wheat in South America

Subject - 06/27/2021 **Approved - 08/05/2021** **Published - 09/03/2021**

http://dx.doi.org/10.52755/sas.v2i2.122

Rodrigo Gomes Branquinho
Agronomist, PhD in Genetics and Plant Freeding, Faculdade Quirinópolis – FAQUI, e-mail: rodrigogobr@gmail.com.

Cricya Naquielle Freitas Martins
Agronomist, Faculdade Quirinópolis – FAQUI, e-mail: crisnaquielle@gmail.com.

Ítalo Brenno Castro Resende
Agronomist, Faculdade Quirinópolis – FAQUI, e-mail: Italo.agro20@gmail.com.

Luciana Claricinda da Silva
Agronomist, Faculdade Quirinópolis – FAQUI, e-mail: lucianaclaricinda@gmail.com.

Felipe Corrêa Veloso dos Santos
Irrigation Technologist, PhD in Agronomy with a concentration in Soil and Water, Pontifical Catholic University of Goiás – PUC/GO, e-mail: felipesantos@pucgoias.edu.br.

ABSTRACT

In South America, wheat is cultivated in an extensive territorial area, which includes very heterogeneous cultivation environments. Grain yield ends up influenced by the interaction of genotypes (G) with environments (A). The aim of the present study was to investigate the possibility of stratification of this important cultivated region, in order to minimize the negative effects of the GA interaction. Fifty wheat lines were evaluated for grain yield character, in eight locations in South America, during spring/summer and summer/autumn 2011. Data were provided by CIMMYT and submitted to joint analysis of variance by means of linear model with random effects. The genetic correlation between pairs of sites was estimated by obtaining a matrix of Euclidean distances, on which cluster analysis was applied by the Ward method. Three environmental strata were identified, formed by the grouping of Quilamapu-CL, Santa Catalina-EC, Marcos Juarez-AR (sub-region 1), Molle-Molle-BO and San Pedro-BO (sub-region 2), and Criadero- AR, INIAF-BO, El Montero-PE (sub-region 3). The evaluation of wheat lines in INIAF and El Montero should be prioritized, given the greater power of discrimination of genotypes in these locations.

Keywords: ***Triticum aestivum; genotype x environment interaction; VCU tests; regionalization; genetical enhancement.***

Estratificação ambiental para trigo na América do Sul

RESUMO

Na América do Sul, o trigo é cultivado em extensa área territorial, a qual inclui ambientes de cultivo muito heterogêneos. O rendimento de grãos acaba influenciado pela interação dos genótipos (G) com os ambientes (A). O objetivo com o presente estudo foi investigar a possibilidade de estratificação dessa importante região cultivada, de forma a minimizar os efeitos negativos da interação GA. Cinquenta linhagens de trigo foram avaliadas quanto ao carácter produtividade dos grãos, em oito locais da América do Sul, durante a primavera/verão e verão/outono de 2011. Os dados foram disponibilizados pelo CIMMYT e submetidos à análise conjunta de variância, por meio de modelo linear com efeitos aleatórios. A correlação genética entre pares de locais foi estimada obtendo-se uma matriz de distâncias euclidianas, sobre a qual foi aplicada análise de agrupamento pelo método Ward. Foram identificados três estratos

ambientais, formados pelo agrupamento de Quilamapu-CL, Santa Catalina-EC, Marcos Juarez-AR (sub-região 1), Molle-Molle-BO e San Pedro-BO (sub-região 2), e Criadero-AR, INIAF-BO, El Montero-PE (sub-região 3). A avaliação de linhagens de trigo em INIAF e El Montero deve ser priorizada, haja vista o maior poder de discriminação dos genótipos nesses locais.

Palavras-chave: ***Triticum aestivum; interação genótipo x ambiente; ensaios de VCU; regionalização; melhoramento genético.***

Estratificación ambiental para el trigo en Sudamérica

RESUMEN

En América del Sur, el trigo se cultiva en un área territorial extensa, que incluye ambientes de cultivo muy heterogéneos. El rendimiento de grano termina influenciado por la interacción de los genotipos (G) con los ambientes (A). El objetivo de este estudio fue investigar la posibilidad de estratificación de esta importante región cultivada, con el fin de minimizar los efectos negativos de la interacción GA. Se evaluaron cincuenta líneas de trigo para determinar el carácter de rendimiento de grano, en ocho localidades de América del Sur, durante la primavera / verano y verano / otoño de 2011. Los datos fueron proporcionados por el CIMMYT y sometidos a análisis conjunto de varianza mediante un modelo lineal con efectos aleatorios. La correlación genética entre pares de sitios se estimó mediante la obtención de una matriz de distancias euclidianas, sobre la cual se aplicó el análisis de conglomerados por el método de Ward. Se identificaron tres estratos ambientales, conformados por la agrupación de Quilamapu-CL, Santa Catalina-EC, Marcos Juárez-AR (subregión 1), Molle-Molle-BO y San Pedro-BO (subregión 2), y Criadero - AR, INIAF-BO, El Montero-PE (subregión 3). Se debe priorizar la evaluación de líneas de trigo en INIAF y El Montero, dado el mayor poder de discriminación de genotipos en estas localidades.

Palabras clave: ***Triticum aestivum; interacción genotipo x ambiente; Ensayos VCU; regionalización; mejoramiento genético.***

Introduction

Wheat is cultivated in a wide range of environments, in regions that extend between the parallels of latitude 65º North and 45º South (FEDEREZZI *et al.*, 2005). Its cultivation is exposed to often limiting environmental conditions, which include water scarcity, inadequate ambient temperature, and unpredictable environmental fluctuations within and between growing seasons. As a result, the response of the genotypes ends up heavily influenced by the interaction with the cultivation environments. The differential response of genotypes to environmental factors (GA interaction) results in changes in the ordering of genotypes, with a consequent change in selection. This ordering change is due to the interaction of a cross nature. Interaction partitioning has shown that much of this source of variation is cross-in nature (MOHAMMADI *et al.*, 2017).

The GA interaction has been investigated for different purposes, including the study of stability and adaptability of wheat genotypes (MOHAMMADI, 2016; KIZILGECI *et al.*, 2019); the identification of homogeneous sub-regions, within which the GA interaction is not significant (NAVABI *et al.*, 2006; SILVA *et al.*, 2016), and with both purposes (KIZILGECI *et al.*, 2019).

The study of the GA interaction, with an emphasis on environmental stratification, can guide plant breeders regarding the planning of the experimental network. The experimental network consists of sites that interact, similarly, with genotypes, and that, for this reason, can be eliminated. The elimination of redundant sites contributes to the reduction of budgetary resources spent on this important phase of wheat improvement. Overall, the analysis of the GA interaction in wheat has shown the possibility of considerable reduction in the experimental network, with only one third of the test sites remaining, as seen in the CIMMYT breeding program in Turkey (KAYA *et al.*, 2006) and in Brazil (SILVA *et al.*, 2016).

Studies addressing the genotype x environment interaction in wheat have been conducted in different regions of the world, especially in Asia (MOHAMMADI *et al.*, 2017; SAXESENA *et al.*, 2017; MOHAMMADI *et al.*, 2020), in North America (NAVABI *et al.*, 2006; KUCEK *et al.*, 2019; HERNÁNDEZ *et al.*, 2019; SJOBERG *et al.*, 2020), in Africa (HADDAD *et al.*, 2016; MATLALA *et al.*, 2019; ABRAHA *et al.*, 2020) and in Europe (BAXEVANOS *et al.*, 2017; KIZILGECI *et al.*, 2019).

However, the subject seems neglected in South America, where recent studies, applied to environmental stratification, are rarely found. This situation does not reflect the great importance of wheat in South America, given that there are large producing countries, such as Argentina, responsible for a significant portion of the wheat produced worldwide. In this sense, the objective of this study was to investigate the heterogeneity of this wide region cultivated with wheat in Latin America, seeking to propose an environmental stratification capable of attenuating the negative effects of the

GA interaction, thus enhancing the grain yield in this important wheat-producing region.

Material and methods

Experimental material and Multi-Environment Trials

The experimental data analyzed in the study come from the international wheat cultivar development program (Triticum aestivum), organized and managed by the International Maize and Wheat Improvement Center (SINGH; PAYNE, 2017). Yield data from 50 inbred lines of wheat evaluated at eight sites in South America during the commercial growing season in 2011 were considered (**Table 1**).

Table 1. Locations for conducting experiments to evaluate wheat lines and cultivars, geographic location and average grain yield.

Country	Location	Code	Lat.	Long.	Alt.	Irrigated	Cycle	Precipitation (mm)	Averages kg ha^{-1}
AR	Marcos Juarez	MJ	32°42'S	62°7'W	110	Yes	Spring/Summer	190	5,48
AR	Criadero	CO	33°32'S	60°39'W	72	No	Spring/Summer	180	2,63
CL	Quilamapu	QU	36°31'S	71°55'W	217	Yes	Spring/Summer	100	9,58
PY	Cria	CA	27°17'S	55°49'W	200	No	-	-	2,24
BO	San Pedro	SP	16°50'S	63°30'W	300	No	Summer/Autumn	-	4,03
BO	INIAF	IN	17°19'S	63°15'W	292	No	Spring/Summer	70	0,94
BO	Molle Molle	MM	17°40'S	65°18'W	2496	No	Summer/Autumn	-	4,33
EC	ST Catalina	SCP	0°22'S	78°33'W	3200	No	Summer/Autumn	1119	2,97
PE	El Montero	EM	11°45'S	75°20'W	3316	No	Spring/Summer	617	6,28

The evaluated lines are adapted to low rainfall conditions, with or without irrigation supply. The evaluated wheat germplasm was considered a representative sample of the population of genotypes developed and evaluated in the final phase, by the CIMMYT breeding program. The experiments were carried out in irrigated and dryland areas. In each experiment, $g = 50$ genotypes were evaluated in a lattice design with $b = 2$ blocks, $sb = 5$ sub-blocks with $k = 10$ plots, totaling $g \times b = b \times sb \times k = 100$ experimental units. Each genotype was cultivated in a plot with six rows of six meters, spaced 0.20 m apart. Recommended management practices in commercial crops were followed. Grain yield data were recorded as grams per harvested plot, being extrapolated to kg ha^{-1}.

Statistical Analysis

Productivity data were subjected to joint analysis of variance using a linear random effects model, obtaining variance components. The genetic correlation between pairs of sites was estimated. From this, a matrix of distances between locations was obtained, and the grouping graph was produced by the Ward method.

Analysis of variance

Initially, individual analyzes of variance were carried out for each experiment, considering, for this, the plot design in the lattice scheme. Productivity data were subjected to variation analysis using the mathematical/statistical model with random effects (**Equation 1**):

$$y_{ijk} = \mu + \text{bloco}_i + \text{subbloco}_j(\text{bloco}_i) + \text{gen}_k + e_{ijk}$$

where y_{ijk} is the grain yield, in kg ha^{-1}, is the general average common to all observations, bloco_i is the random effect of the “i” block (i = 1,2), sub-$\text{bloco}_j(\text{bloco}_i)$ is the random effect of the “j” sub-block within the “i” block, gen_k is the random effect of the “k” genotype (k = 1, 2, ..., 50) and e_{ijk} is the random effect of the experimental error associated with block “j”, of genotype “k”.

In the joint analysis of the experiments, the productivity data were modeled by equation 2:

$$y_{ijk} = \mu + \text{loc}_l + \text{bloco}_i(\text{loc}_l) + \text{subbloco}_j(\text{bloco}_i \text{loc}_l) + \text{gen}_k + \text{gen}_k \times \text{loc}_l + e_{lkij}$$

where, in addition to the terms already defined, loc_L is the random effect of location “L” (L=1, 2, ..., 8), gen_k x loc_L is the effect of the interaction of genotype “k” with location “L”, and e_{Lkij} is the experimental error associated with each experimental unit. In both models, the effects were assumed to be of a random nature, being normally and independently distributed, with zero mean and specific variance.

Genetic Correlation between Pairs of Locations

The genetic correlation matrix between pairs of sites was estimated, considering only the grain yield character, from which a distance matrix was calculated as the identity matrix minus the genetic correlation matrix. The distance matrix was used to perform the cluster analysis by the Ward method, as well as to create the cluster dendrogram. A biplot plot was produced from the analysis of the first two main components of the mentioned distance matrix.

The genetic correlation between locations was calculated using Cooper's (1994) equation:

$$r_{Gij} = \frac{r_{Pij}}{h_i h_j}$$

where r_{Pij} is the phenotypic correlation between places “i” and “j”, and h_i and h_j are the square roots of heritability in environments “i” and “j”, respectively.

In turn, the heritability "h" in a given location was obtained by the expression:

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_E^2 / R}$$

where σ_G^2 e σ_E^2 are, respectively, the components of genotype variance and experimental error, respectively.

Computational implementation of analysis

The data analysis procedures were computationally implemented in the statistical program R, version “4.0.2” (R Development Core Team, 2009), with support in the auxiliary application GNU-Emacs. The main analyses, implemented to estimate the genetic correlation as well as the distance matrix used in the cluster analysis, were performed in the META-R application, which operates on the R platform, but in a Java computational environment. The Microsoft Excel 2010 application was also used for data tabulation and to support some analysis procedures.

Results and discussion

Analysis of variance

The magnitude of the sources of variation related to genotype (G), location (L) and GL interaction is often approximately 10:80:10. These percentages have been observed in the wheat crop, particularly for the grain yield character (YAN *et al.*, 2000; KAYA *et al.*, 2006). In the present study, heritable variation, related to genotypic effects, and variation in the GL interaction, contributed 32% of the variation G:L:GL (08:67:25). This is an important finding, which, in principle, would justify the effort to stratify the target region of this research. The sites participated preponderantly in the G:L:GL variation, but, although this happened, this source of variation is irrelevant for the purposes of environmental stratification (FOX; ROSIELLE, 1982).

In the dataset involving all locations, the effects of the GL interaction were significant, with the variance component of the GL interaction being greater than the genetic component. Despite this, the heritability for the grain yield character was high (h^2=0.72), indicating that the selection of more productive wheat lines has been successful even without the use of any regionalization. Stratification of the target region has the potential to further increase heritability, which, however, was verified only in stratum E2 (h^2=0.83). The Molle-Molle and San Pedro sites, grouped in this stratum, interact similarly with the wheat genotypes, as demonstrated by the magnitude of the GL interaction $(\sigma^2_{\mathrm{GL}} = 0)$ (**Table 2**).

The identification of environmental covariates, capable of explaining a significant portion of the GA interaction, allows the use of strategies that minimize or even capitalize on the effects of the interaction. Among the environmental covariates, altitude is one of the most important. Oliveira *et al.*

(2006) found that 24% of the interaction of soybean genotypes is explained by the altitude of the assessment sites. In the present study, altitude does not seem to influence the GA interaction. This can be demonstrated by the absence of GA interaction between locations with very different altitudes, such as Molle-Molle (2496 m) and San Pedro (300 m) (**Table 1**), which, as already mentioned, were grouped into a stratum with GL interaction null. The two growing seasons (spring/summer and summer/autumn) constitute another important environmental covariate. Haddad *et al.* (2016) found that the sowing season (thus the growing season) significantly contributes to the GA interaction. However, in the present study, the clustering of sites did not follow any pattern related to the growing season. The same was not observed for latitude and longitude. Thus, a more assertive conclusion can only be produced with additional analyses, unfolding the GA interaction into sources of variation for each of these environmental covariates.

Table 2. Variance components for wheat grain yield data (kg ha^{-1}) in the CIMMYT experimental evaluation network, in 2011.

Statistic	ŦExperimental Sets			
	All	E1	E2	E3
Average Prod. (t ha^{-1})	4,54	4,18	3,29	6,01
Number of locations	8	3	2	3
Loc	2,628±1,404	10,97±3,332	0,021±0,344	7,0410±2,702
Gen x Loc (σ^2_{GL})	0,271±0,105	0,076±0,209	0,000±0,080	0,0564±0,107
Genotype (σ^2_{G})	0,190±0,079	0,057±0,166	0,416±0,174	0,0156±0,115
Residual (σ^2_{E})	0,628±0,047	0,350±0,072	0,528±0,088	0,1718±0,051
heritability (h^2)	0,72	0,31	0,83	0,35

Ŧ Experimental sets comprising all the experiments, and reduced experimental groups, related to strata E1, E2 and E3.

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Test site evaluation

The dendrogram **(Figure 1a**) and the scatter plot (**Figure 1b**) were constructed from the genetic correlation matrix, identifying three environmental strata: stratum 1 (MM, SP); stratum 2 (CO, IN, MS); and stratum 3 (SCP, MJEI, QU). This grouping was identified in the dendrogram, using a cutoff point that would reduce the dissimilarity between the locations within each stratum. The same grouping could be identified in the scatter plot containing the first two axes of the PCA applied to the GA interaction. The sites gathered in the same group were genetically correlated, offering the opportunity to select and recommend a small group of genotypes. In theory, just three genotypes are sufficient to meet demand across the target region.

By the identified clusters, no causal relationship was identified involving environmental covariates such as altitude, latitude, growing season and rainfall. The E1 stratum is formed by places with approximate latitudes and the same growing season, but with a great difference in altitude. In stratum E3, although the clustered sites are from the same growing season, there is a big difference in altitude and latitude. In stratum E2, there is an even greater discrepancy, with groups of places with very different latitudes and altitudes. This lack of relationship with the mentioned environmental covariates should be better studied in a later study, analyzing the interaction

AG as a function of these covariates, using the methodology proposed by Van Eeuwijk et al. (1996), and used in many studies (OLIVEIRA *et al.*, 2006; HADDAD *et al.*, 2016; MOHAMMADI *et al.*, 2017).

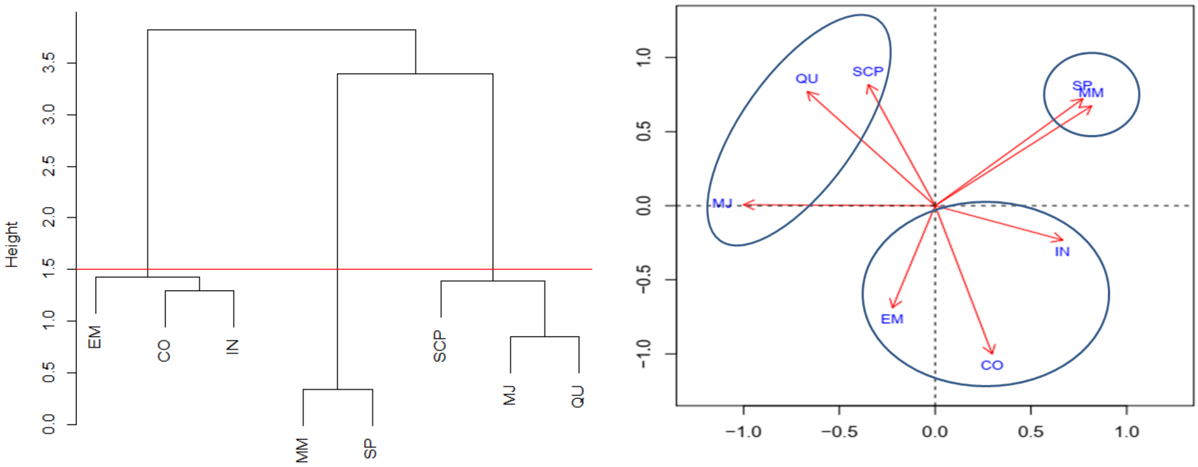

Figure 1.(a) Dendrogram with hierarchical grouping (Ward's method) of wheat genotype evaluation sites in South America. (b) Scatter plot of sites with the first two axes of principal component analysis, applied to the correlation matrix genetics. The nominal identification of the places is abbreviated: Santa Catalina (SCP), Marcos Juarez (MJ), Quilamapu (QU), Molle-Molle (MM), San Pedro (SP), Criadero (CO), INIAF (IN), El Montero (EM).

In a stratification like this, based on the genetic correlation matrix, the sites gathered in the same stratum should have the same winning genotype. The winning genotype, that is, the one with the highest productivity, is often used for grouping similar locations. By this method, the places that have, in common, the same winner, must be grouped, since they lead to the same genotypic selection (GAUCH; ZOBEL, 1997). The same genotype (L40) was selected only in the MM and SP locations, from stratum 1. As they presented different winners, the locations in strata 2 and 3 should not be grouped (**Table 3**). However, it is worth mentioning an important flaw in the winning genotype method, related to the non-application of a statistical test to compare genotypic means. Therefore, the aforementioned divergence in the winning genotypes does not invalidate the grouping of locations that resulted in strata 2 and 3.

Table 3. Higher strains with productive performance (winner genotypes) in the CIMMYT experimental network sites in South America.

Stratum	Location	Winning Genotype	Productivity t ha^{-1}
E1	Quilamapu (QU)	L23	11,43
E1	Santa Catalina (SCP)	L48	4,4
E1	Marcos Juarez (MJ)	L7	7,33
E2	Molle Molle (MM)	L40	7,39
E2	San Pedro (SP)	L40	6,96
E3	Criadero (CO)	L13	3,71
E3	INIAF (IN)	L37	1,46
E3	El Montero (EM)	L41	8,66

The length of the environmental vectors (straight line between the origin and the location marker), represented in a flat figure, whose axes are the first two main components of the GA interaction, provides a measure of the ability of locations to discriminate genotypes (YAN, 2001). In this sense, the IN and EM sites are less able to discriminate the most productive genotypes from those with the lowest grain yield (**Figure 1b**). From this point of view, the permanence of these locations in the experimental network in the coming years should be passed over in favor of other locations, with greater power of discrimination.

Conclusion

The target region of the present study, represented by eight locations in the region cultivated with wheat in South America, can be divided into three sub-regions: sub-region 1 (Quilamapu, Santa Catalina, Marcos Juarez), sub-region 2 (Molle- Molle, San Pedro), sub region 3 (Criadero, INIAF, El Montero). Heritability for the grain yield character is relatively high in the non-stratified region. With the division of the target region, the heritability in subregion 2 can increase, the same not happening in subregions 1 and 3.

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