

Border effect on the agronomic evaluation of wheat cultivars

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Abstract: The aim of this study was to assess the border effect in wheat agronomic trials in Minas Gerais, Brazil. Experiments were conducted in three environments using 16 cultivars in plots of five rows (two outer rows as the border area). The traits evaluated were plant height (PH), grain yield (GY), hectoliter weight (HW), and thousand-kernel weight (TKW). Data from the useful, border, and total plot areas were analyzed separately and, in a split-plot-in-space design using a mixed-model approach. The experimental precision and reliability in selection were similar among the areas. Differences were found among cultivars for all traits, except GY. A border effect was observed only for PH and GY traits. The use of a border area is recommended in wheat trials. However, the total plot area may also be used for evaluation, as the experimental accuracy and reliability of selection are comparable to evaluation using the useful plot area.

Keywords: *Triticum aestivum L.*, experimental precision, selective accuracy, plant breeding

INTRODUCTION

In breeding programs, an important step prior to commercial release of a cultivar is its registration and legal protection. The aim of this process is to ensure the breeder's intellectual property rights over a new cultivar, allowing financial return on the investments made in the breeding process (Carvalho et al. 2009, Faleiro et al. 2021). In Brazil, for registration and protection of wheat cultivars, multiple field evaluation trials, such as Value for Cultivation and Use (VCU) trials, must be conducted, following the guidelines established by Normative Instruction No. 58 of the Ministério da Agricultura e Pecuária (MAPA 2008).

Field trials constitute an essential stage in plant breeding programs. Proper agronomic management of these trials minimizes the variation attributable to random environmental factors among plots, enabling identification of genotypes with superior performance (Honsdorf et al. 2022). Furthermore, high experimental precision allows for accurate estimation of genetic effects,

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increases experimental efficiency, ensures the validity and generalizability of the results, and avoids biased estimates and incorrect interpretations (Ribeiro Santos and Sermarini 2025).

Among the experimental practices adopted to improve precision is the use of border rows, i.e., rows located along the outside edges of the useful area of the plot, intended to prevent mutual interference between adjacent plots and reduce interplot competition (Costa and Zimmermann 1998). The difference in performance observed between the useful area and the border is referred to as the border effect (Thomas 2006). Moreover, the use of border rows is required by the aforementioned Normative Instruction for the establishment of characterization trials for new wheat cultivars to be released.

Quantifying the border effect is crucial to determine whether its use is necessary, as the inclusion of border rows increases plot size and, consequently, the demand for inputs, labor, and land area to conduct the experiment (Schierolt et al. 2020), which, in turn, may increase experimental error (Honsdorf et al. 2022). Thus, the use of border rows is recommended only when they demonstrably improve experimental precision by reducing interplot competition (Cargnelutti Filho et al. 2003). Otherwise, according to Storck et al. (2000), researchers may collect data from the entire plot area, allowing the use of smaller plots and a greater number of replications within the same experimental area, which may result in higher precision.

Several studies have evaluated the border effect in wheat (May and Morrison 1986, Romani et al. 1993, Bulinski and Niemczyk 2010, Bulinski and Niemczyk 2015), yet they have had divergent conclusions regarding its effectiveness. Therefore, further studies are needed that consider current management practices, modern genotypes, and, particularly, the cropping systems used in the Brazilian Cerrado region, where wheat cultivation has expanded and is characterized by specific agronomic and environmental conditions. In this context, the objective of the present study was to evaluate the border effect in agronomic performance trials of wheat cultivars in Minas Gerais, Brazil.

MATERIAL AND METHODS

Site description

Three experiments were conducted in the municipality of Lavras, located in the Campo das Vertentes mesoregion of the state of Minas Gerais, Brazil. In the 2022 growing season, one experiment was carried out at the Experimental Field of the Colégio Adventista de Minas Gerais (FADMINAS), Campus I, Distrito de Itirapuã (lat 21° 18' 03" S, long 44° 56' 20" W, alt 920 m asl) and another at the Crop and Livestock Scientific and Technological Development Center (CDCTA), Muquém Farm, of the Universidade Federal de Lavras (lat 21° 14' 00" S, long 45° 00' 00" W, alt 918 m asl). In the 2023 growing season, another experiment was conducted at CDCTA. Thus, each experiment was carried out under a distinct environmental condition (location × season).

The mean annual temperature in Lavras, MG, is approximately 19.4 °C, and the mean annual rainfall is 1461.8 mm. The climate is classified as highland tropical (Cwa) according to the Köppen classification, and the soil type in the experimental areas is a Latossolo Vermelho-Amarelo. Monthly climatic data for rainfall and minimum, mean, and maximum temperatures for the 2022 and 2023 growing seasons (from sowing to harvest), based on INMET records, are shown in Figure 1. At FADMINAS, wheat was sown on 5 April 2022 and harvested on 2 August 2022. At CDCTA, sowing and harvest were on 21 March 2022 and 4 August 2022 (2022 season) and on 31 March 2023 and 1 August 2023 (2023 season), respectively.

Cultivars, experimental design, and crop management

Sixteen wheat cultivars from different breeding institutions or programs in Brazil were evaluated. The cultivars were from GDM/Biotrigo Genética ('TBIO Audaz', 'TBIO Aton', 'TBIO Duque', 'TBIO Mestre', and 'TBIO Sintonia'), Embrapa ('BR 18-Terena', 'BRS 264', and 'BRS 404'), EPAMIG ('MGS Brilhante'), and OR Genética de Sementes ('ORS 1403', 'ORS Absoluto', 'ORS Feroz', 'ORS Guardião', 'ORS Premium', 'ORS Senna', and 'ORS Soberano').

The experiments were arranged in a 4 × 4 alpha-lattice design with three replications. Each plot consisted of five 5.0-m-length rows, with a row spacing of 20 cm. The sowing density was 300 viable seeds per square meter. The experimental areas were prepared by chemical desiccation of weeds with a glyphosate-based herbicide prior to sowing,

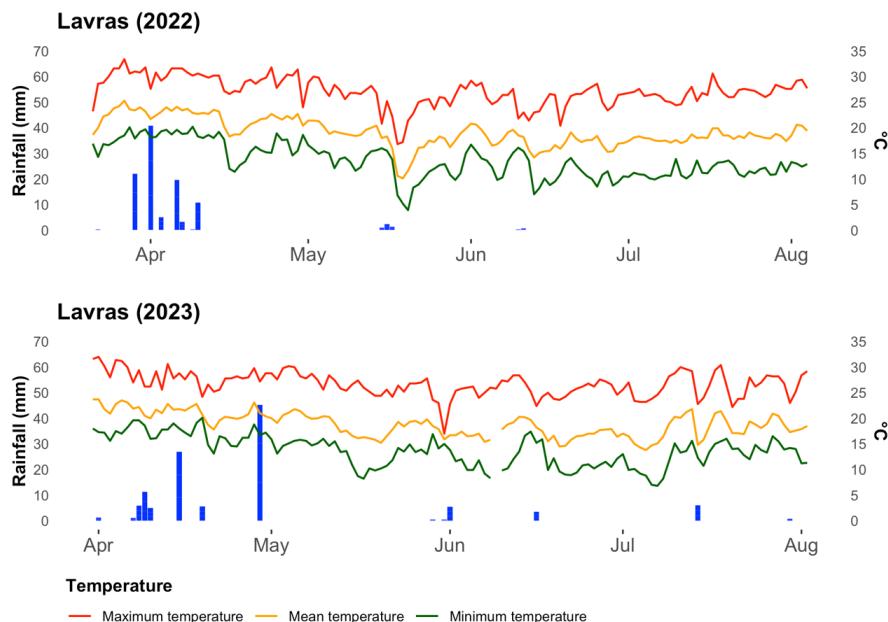


Figure 1. Maximum, mean, and minimum temperatures and rainfall during the experimental period in Lavras, Minas Gerais, Brazil, in the 2022 and 2023 growing seasons.

followed by mowing. Furrowing, basal fertilization and sowing were performed using a Semina II five-row plot seeder-fertilizer. It was applied 200 kg ha⁻¹ of an NPK 8-28-16 formulation.

The experiments were conducted under rainfed conditions. Topdressed fertilizer was applied 15 days after seedling emergence at a rate 45 kg N ha⁻¹. Weed control was carried out using herbicides based on clodinafop-propargyl 240 EC (150 mL ha⁻¹) and metsulfuron-methyl (3.0 g ha⁻¹), supplemented by mechanical weeding. No plant protection treatments were applied for disease or pest control. Harvesting was performed manually, considering the two outer rows of each plot as the border area and the three central rows as the useful area. Data for the total plot area were estimated for each trait based on the mean or total of the measurements from the border and useful areas.

The following agronomic traits were evaluated: Plant height (PH, cm) – measured from the soil surface to the tip of the spike, using a ruler, after all plants had reached anthesis. Measurements were taken at two points in both the border and useful areas of each plot, and the mean was calculated. Thousand-kernel weight (TKW, g) – determined by counting 1,000 dehulled kernels using an ESC 2011 Comp electronic seed counter (Sanik®), which were then weighed on a bench scale. Hectoliter weight (HW, kg hL⁻¹) – determined using a Gehaka® G650 grain moisture and impurity analyzer, based on a sample of dehulled kernels with moisture content of approximately 13%. Grain yield (GY, kg ha⁻¹) – determined by weighing dehulled kernels on a scale, with grain weight corrected to 13% moisture.

Statistical analyses

Data from the useful area, border area, and total plot area of each experiment in the alpha-lattice design were analyzed according to the following model:

$$y_{ijk} = \mu + r_k + b_{j(k)} + g_i + e_{ijk},$$

where y_{ijk} is the observation of the plot in the j -th block of the k -th replication that received the i -th cultivar; μ is a constant associated with all observations; r_k is the effect of the k -th replication (complete block); $b_{j(k)}$ is the effect of the j -th block within the k -th replication, with $b_{j(k)} \sim N(0, \sigma_b^2)$, where σ_b^2 is the variance among blocks within replications; g_i is the effect of the i -th cultivar; and e_{ijk} is the residual error associated with each observation y_{ijk} , with $e_{ijk} \sim N(0, \sigma_e^2)$, where σ_e^2 is the residual variance.

Data from the border and useful areas of each experiment were analyzed using the following split-plot-in-space model (main-plot factor: cultivar; subplot factor: position—border or useful area):

$$y_{ijkl} = \mu + r_k + b_{j(k)} + g_i + e_{ik} + t_l + gt_{il} + \varepsilon_{ijkl}$$

where y_{ijkl} is the observation of the i -th cultivar in the j -th block of the k -th replication at the l -th position; ε_{ijkl} is the residual associated with the plot, with $\varepsilon_{ijkl} \sim N(0, \sigma_e^2)$, where σ_e^2 is the plot-level residual variance; t_l is the effect of the l -th position in the plot (border or useful area); gt_{il} is the effect of the interaction between the i -th cultivar and the l -th position; and ε_{ijkl} is the residual associated with the observation y_{ijkl} , with $\varepsilon_{ijkl} \sim N(0, \sigma_e^2)$, where σ_e^2 is the subplot-level residual variance.

Multi-environment analyses of border and useful area data were performed using the following model:

$$y_{ijklm} = \mu + a_m + r_{k(m)} + b_{j(km)} + g_i + ga_{im} + e_{ijkm} + t_l + gt_{il} + ta_{lm} + gta_{ilm} + \varepsilon_{ijklm}$$

where y_{ijklm} is the observation of the i -th cultivar in the j -th block of the k -th replication at the l -th position (useful area or border) in the m -th environment; μ is a constant associated with all observations; $r_{k(m)}$ is the effect of the k -th replication in the m -th environment; $b_{j(km)}$ is the effect of the j -th block within the k -th replication in the m -th environment, with $b_{j(km)} \sim N(0, \sigma_b^2)$; ga_{im} is the effect of the interaction between the i -th cultivar and the m -th environment; e_{ijkm} is the plot-level residual in the m -th environment, with $e_{ijkm} \sim N(0, \sigma_e^2)$; ta_{lm} is the effect of the interaction between the l -th position and the m -th environment; gta_{ilm} is the effect of the interaction between the i -th cultivar at the l -th position in the m -th environment; and ε_{ijklm} is the subplot-level residual associated with the observation y_{ijklm} , with $\varepsilon_{ijklm} \sim N(0, \sigma_e^2)$.

Data were analyzed using the mixed linear model approach proposed by Henderson (Resende 2002), implemented in the lme4 package (Bates et al. 2015) in R (R Core Team 2025). Variance components were estimated by the restricted maximum likelihood (REML) method, and their significance was assessed using the likelihood ratio test at a 5% probability level through the lmerTest package in R (Kuznetsova et al. 2017). The significance of fixed effects was evaluated using the F-Snedecor test at a 5% probability level using the car package in R (Fox and Weisberg 2019).

The quality metrics experimental coefficient of variation (CV_e) and selective accuracy (r_{gg}) (Resende and Duarte 2007) were estimated for each experiment using the following estimators:

$$CV_e = \sqrt{\frac{\sigma_e^2}{y}} ; r_{gg} = \sqrt{1 - \frac{1}{F}}$$

where σ_e is the standard deviation of the experimental error; y is the overall mean of the experiment; and F is the value of the F-Snedecor test for the cultivar effect.

Adjusted phenotypic mean values for cultivar, position, and cultivar within each position were estimated using the emmeans package in R (Lenth 2025). The Scott–Knott clustering test was performed via a customized script from the sk function in the ExpDes package in R (Ferreira et al. 2021). Spearman rank correlations were calculated for adjusted means in the border, useful area, and total plot area, and results were plotted using the ggplot2 package in R (Wickham 2016).

RESULTS AND DISCUSSION

The traits PH, HW, and TKW proved to be more stable, exhibiting low experimental coefficients of variation ($CV_e \leq 10\%$), whereas GY was notably unstable, with higher CV_e values, ranging from 13% to 30% (Table 1). These CV_e values are consistent with the ranges reported by Nardino et al. (2023) for wheat.

Selective accuracy (r_{gg}) values showed wide variation, from 0 to 0.98, with particularly high (> 0.70) to very high (> 0.90) values for PH, TKW, and HW, and low or null values for GY (Table 1). Selective accuracy is not only influenced by experimental error, but also directly related to the existing genetic variability that effectively translates into phenotypic differences (Resende and Duarte 2007). Cultivars differed significantly for all traits except GY (Table 1), which explains the null or low accuracy values for this trait.

Experimental precision is an essential condition for drawing valid conclusions from data. Several strategies have been used to increase experimental precision, such as selecting an appropriate experimental design, increasing the number of replications, adjusting plot size, and including border rows (Ramalho et al. 2012). In wheat, Normative Instruction No. 58 requires the use of border rows in VCU trials to minimize end-plant effects, reduce interference between adjacent

Table 1. Estimates of *P*-values from the F-Snedecor test for cultivars, experimental coefficient of variation (CV_e , %), selective accuracy (r_{gg}), and mean values (\bar{Y}) of plant height (PH, cm), grain yield (GY, kg ha⁻¹), hectoliter weight (HW, kg hL⁻¹), and thousand-kernel weight (TKW, g) from wheat cultivar evaluation trials, considering different positions within the plot, in three environments during the 2022 and 2023 growing seasons

Traits	Position	FADMINAS 2022			FADMINAS 2023			CDCTA 2023					
		P-value	CV_e (%)	r_{gg}	\bar{Y}	P-value	CV_e (%)	r_{gg}	\bar{Y}	P-value	CV_e (%)	r_{gg}	\bar{Y}
PH	Useful	0.0001	10	0.89	62.89a ¹	4.16e ⁻¹⁰	5	0.97	83.28a	0.0002	6	0.89	65.34b
	Border	7.10e ⁻⁰⁵	10	0.90	61.45b	5.1e ⁻¹¹	4	0.98	77.56b	4.11e ⁻⁰⁹	4	0.97	68.48a
	Total	5.23e ⁻⁰⁵	10	0.91	62.17	1.11e ⁻¹³	3	0.99	79.47	8.81e ⁻⁰⁹	4	0.96	67.42
GY	Useful	0.6727	30	0.00	1588.23b	0.0899	13	0.67	3286.66b	0.4635	13	0.16	2370.33b
	Border	0.2769	30	0.47	2135.44a	0.2513	20	0.50	3681.65a	0.2516	14	0.50	3406.08a
	Total	0.3107	26	0.43	1807.08	0.2177	15	0.54	3442.13	0.4780	13	0.08	2784.64
HW	Useful	0.1436	2	0.62	81.59	0.0004	2	0.88	78.16a	0.0561	3	0.71	77.96b
	Border	0.1436	2	0.62	81.59	0.1711	3	0.59	77.15b	0.0001	1	0.90	78.42a
	Total	0.7586	3	0.00	81.69	0.0006	2	0.87	77.58	2.036e ⁻⁰⁵	1	0.92	78.19
TKW	Useful	-	-	-	-	1.423e ⁻⁰⁹	5	0.97	40.67a	4.951e ⁻¹²	4	0.98	36.17b
	Border	-	-	-	-	1.87e ⁻⁰⁹	5	0.96	39.89b	9.54e ⁻⁰⁹	6	0.96	36.21a
	Total	-	-	-	-	2.18e ⁻¹²	4	0.98	40.28	2.21e ⁻¹³	4	0.98	36.19

¹Mean values for each position followed by different letters within a trait differ according to the F-Snedecor test at 5% probability.

genotypes, and improve experimental precision. However, the use of border rows is debatable, as it increases the experimental area, which may result in a loss of precision (Mattos et al 2019). Based on the CV_e and r_{gg} values from the wheat trials, experimental precision was generally similar when considering data from the border area, useful area, or total plot area for all traits (Table 1). For GY, CV_e values from border data were slightly higher than values from the useful and total plot areas (Table 1).

Analyses for each environment showed a border effect for all traits (Table 1). However, in the multi-environment analysis, the border effect was significant only for PH and GY (Table 2). This result contrasts with that reported by Zhu et al. (2016), who found higher TKW values in the useful area. As shown by the mean values in Table 1, plants in the border rows were higher yielding in all evaluated environments. Similar results were reported by Rajab et al. (2021) and Fan et al. (2023). These results may be a consequence of border rows intercepting more light than internal rows (Wang et al. 2015), which can influence chlorophyll content, stomatal conductance, and photosynthetic rates, ultimately increasing yield (Li et al. 2020). When an aisle separates the plots, the border effect likely reflects competition between the outer rows of adjacent plots. In this study, the sowing machinery was adjusted to maintain consistent row spacing. Consequently, analysis allowed the hypothesis of intragenotypic competition among the inner (useful) rows within each plot and intergenotypic competition between the border rows of neighboring plots to be tested. For PH, the border effect was not consistent across environments: border plants were taller in CDCTA 2023 but shorter in FADMINAS 2022 and 2023 compared with plants in the useful area (Table 1).

The environment effect was significant for all traits, indicating macro-environmental differences (Table 2). Notably, the lowest mean values for PH (62.17 cm) and GY (1,807.08 kg ha⁻¹) were observed in FADMINAS 2022 (Table 1), which may reflect differences in resource availability, such as water (Figure 1) and nutrients, directly affecting vegetative growth (Wang et al. 2014). For hectoliter weight (HW), the highest mean was recorded in FADMINAS 2022 (81.69 kg hL⁻¹); however, HW values likewise exceeded 78 kg hL⁻¹ in the other environments, indicating grain technological quality suitable for flour production (MAPA 2010).

The evaluated cultivars showed considerable variation for PH, HW, and TKW, but did not differ for GY (Table 2). Among the cultivars, 'MGS Brilhante' stood out for its greater height; 'BR 18-Terena' and 'ORS Guardião' for larger kernels; and 'BR 18-Terena' also for higher HW. The genetic variability observed in wheat in Minas Gerais for various traits, along with the inconsistent relative responses across environments observed for PH, reflects the commonly reported genotype \times environment interaction in this crop (Teixeira et al. 2023).

Table 2. Estimates of *P*-values from the F-Snedecor test for plant height (PH, cm), grain yield (GY, kg ha⁻¹), hectoliter weight (HW, kg hL⁻¹), and thousand-kernel weight (TKW, g) from wheat cultivar evaluation trials, considering different positions within the plot (border or useful area) in different environments during the 2022 and 2023 growing seasons

Source of variation	PH	GY	HW	TKW
Environments	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cultivars	< 0.0001	0.1115	< 0.0001	< 0.0001
Cultivars × Environments	0.0045	0.0766	0.5805	0.0807
Positions	0.0004	< 0.0001	0.5637	0.1288
Environments × Positions	< 0.0001	< 0.0001	0.0389	0.0950
Cultivars × Positions	0.2719	0.0189	0.9894	0.9939
Cultivars × Environments × Positions	0.2453	0.5932	0.9765	0.7200

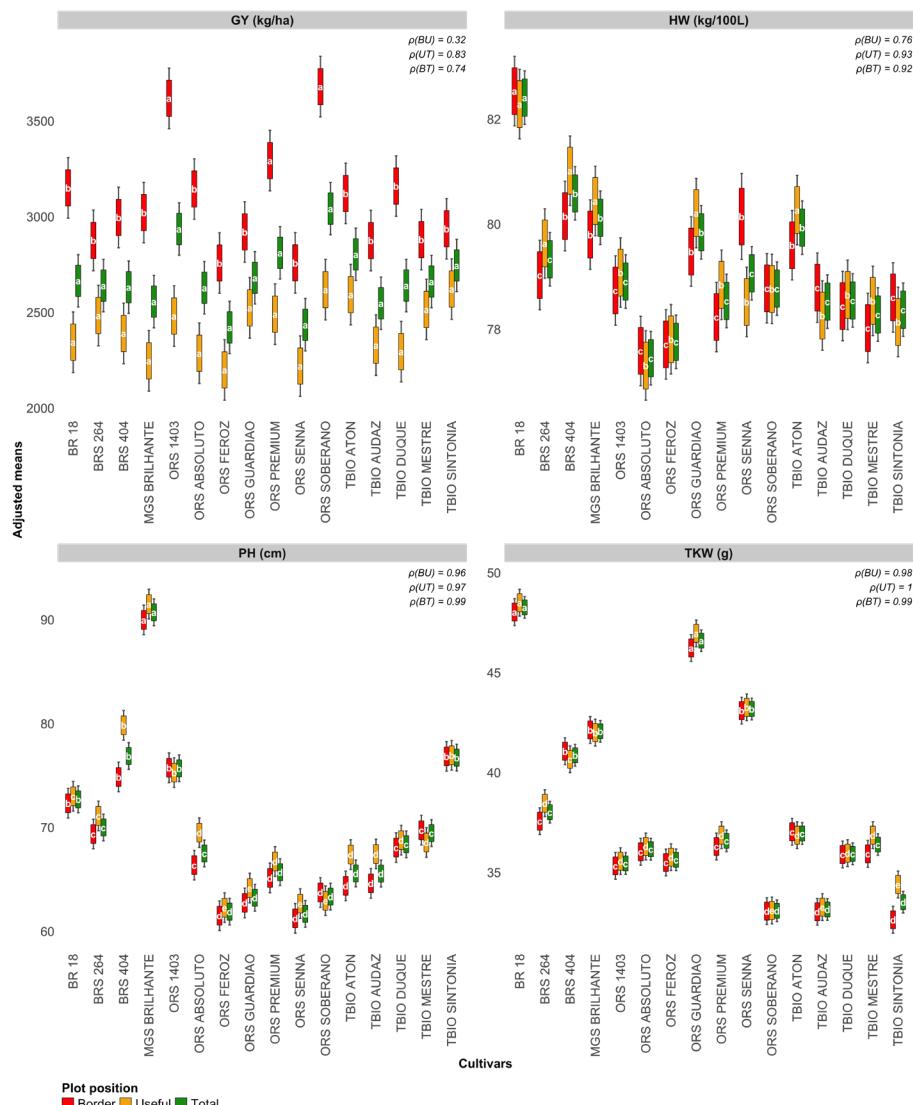


Figure 2. Adjusted phenotypic mean values of wheat cultivars and their respective confidence intervals, Scott–Knott test results, and Spearman correlations considering the border area, useful area, and total plot area for plant height (PH, cm), grain yield (GY, kg ha⁻¹), hectoliter weight (HW, kg hL⁻¹), and thousand-kernel weight (TKW, g) from experiments conducted in different environments during the 2022 and 2023 growing seasons.

The cultivars responded differently in the border compared with the useful area of the plot for GY (Table 2). This cultivar \times position interaction was not detected for the other traits. As shown in Figure 2, this interaction for GY was predominantly complex, as indicated by the low rank correlation between the adjusted phenotypic mean values of cultivars in the border and useful areas ($\rho_{UB} = 0.32$). The greater variation among cultivars in the border area than in the useful area is also evident. For the other traits (PH, TKW, and HW), this correlation was high ($\rho_{UB} > 0.75$), indicating a strong correspondence between the border and useful areas and suggesting that the border effect had little impact on these traits. These results differ from those reported by Sun et al. (2023), who found a significant border effect for TKW when evaluating the performance of wheat grain yield components under a hill-seeding method.

Overall, the results support the MAPA recommendation to use border rows in VCU trials. However, the Spearman rank correlations were high between the mean values of cultivars in the border and useful areas and those in the total plot area (Figure 2), indicating that when the entire plot is considered, the border effect is mitigated. The correlation between the useful area and total area for GY was high (0.83), showing that the cultivar \times position interaction is more associated with the border area. This finding is highly relevant for preliminary and intermediate trials in which a large number of genotypes are evaluated in smaller plots of only a few rows (e.g., three-row plots). In such cases, it is common to evaluate the entire plot, as the use of border rows is clearly impractical.

A border effect was observed for PH and GY, whereas no effect was found for HW and TKW. These findings reinforce the recommendation to use the useful area in wheat VCU trials but also show that evaluating the total area may maintain similar precision and reliability in selection.

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DATA AVAILABILITY

The datasets generated and/or analyzed during the current research are available from the corresponding author upon reasonable request.

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