

Adaptability and stability of wheat genotypes for the expression of amino acids in their grains

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Abstract – This study aimed to select wheat genotypes based on the adaptability and stability of their amino acid expression in their grains. Our experiment was conducted during the 2019 harvest in five environments in the state of Rio Grande do Sul (RS). An experimental design was used with randomized blocks which were organized in a factorial scheme with five wheat genotypes cultivated in five environments, with three replicates per environment. The BRS Parrudo genotype potentiates the expression of amino acids in its grains when cultivated in Cachoeira do Sul-RS. By the BLUP methodology, it also best expresses all assessed amino acids in its grains. Research can identify and position wheat genotypes to obtain higher amino acid concentration in their grains. The amino acids aspartate, proline, and tryptophan show the highest broad-sense heritability, favoring the selection of genotypes to increase these characteristics.

Index terms: *Triticum aestivum*; REML/BLUP; AMMI; GGE.

Adaptabilidade e estabilidade de genótipos de trigo para a expressão de aminoácidos em seus grãos

Resumo – Este trabalho teve como objetivo selecionar genótipos de trigo com base na adaptabilidade e estabilidade da expressão de aminoácidos em seus grãos. Nosso experimento foi conduzido durante a safra 2019 em cinco ambientes no estado do Rio Grande do Sul (RS). Utilizou-se o delineamento experimental em blocos ao acaso organizados em esquema fatorial com cinco genótipos de trigo cultivados em cinco ambientes, com três repetições por ambiente. O genótipo BRS Parrudo potencializa a expressão de aminoácidos em seus grãos quando cultivado em Cachoeira do Sul-RS. Pela metodologia BLUP, também expressa melhor todos os aminoácidos avaliados em seus grãos. A pesquisa pode identificar e posicionar genótipos de trigo para obter maior concentração de aminoácidos em seus grãos. Os aminoácidos aspartato, prolina e triptofano apresentam as maiores herdabilidades no sentido amplo, favorecendo a seleção de genótipos para aumentar essas características.

Termos para indexação: *Triticum aestivum*; REML/BLUP; AMMI; GGE.

Introduction

Wheat is one of the main winter cereals in southern Brazil, a region which concentrates about 91% of the total wheat produced in the country (COMPANHIA NACIONAL DE ABASTECIMENTO, 2021). The insufficient supply of wheat grains to meet the domestic Brazilian demand reflects that its production has greater magnitude only in the Brazilian South region. Thus, research aimed to develop superior genotypes and expand wheat cultivation (especially to the Brazilian Midwest) to increase national grain production (PASINATO et al., 2018).

However, grain yield must be associated with good quality (i.e., its chemical composition and content of reserve proteins such as gluten), which defines the quality of its flour and how the dough behaves during baking (SCHEUER et al., 2011). Cultivar characteristics, management practices (such as nitrogen fertilization), and environmental conditions (such as varying temperatures and atmospheric CO₂ concentration) especially affect these parameters.

Wheat proteins have a low amount of some essential amino acids (especially lysine and threonine) but show large quantities of glutamine and

proline, which can change according to the interaction between genotypes and the environment. This can modify the levels of proteins such as threonine, lysine, valine, methionine, and histidine, an aspect which may potentiate genetic improvement. If the phenotypic expression of a genotype for a given trait depends on environmental conditions, measures of its heritability may vary according to environmental conditions (SANTOS et al., 2018). To explore this potential, we can find which amino acids the environment will promote and which of the latter will form a specific amino acid. Thus, research can complement diets and nutritional

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deficiencies by, for example, elaborating lysine- and threonine- rich flours, amino acids lacking in commercial flours found on the market nowadays.

Several cultivars are available for commercialization. They show different flour colors, gluten strength, stability, and absorption. For example, pasta requires stronger flour, whereas Bread, protein balance. Thus, industries, to meet the demand for adequate rheological characteristics, provide bonuses to certain genotypes with superior rheological quality, developing then a niche market. Therefore, even at smaller magnitudes, the development of wheat genotypes biofortified with amino acids can easily be expanded. Industries already have specialized structures to produce flour according to genotypes.

Research still greatly lacks bibliographic references on this subject and on zones which better form gluten strength (W) and greater potential to produce specific and essential amino acids. In this context, this study aimed to select wheat genotypes based on the adaptability and stability of the amino acids in their grains.

Materials and methods

Our experiment was conducted during the 2019 harvest in five wheat-growing environments in the state of Rio Grande do Sul, Brazil. A randomized block design was used with five culture environments and five cultivars with three replicates. The fields used as cultivation environments were located in the following municipalities: Cachoeira do Sul (A_1), Passo Fundo (A_2), Santo Augusto (A_3), São Gabriel (A_4), and São Luiz Gonzaga (A_5), which were chosen due to their varied locations within Rio Grande do Sul. According to Köppen, their climate is classified as Cfa, humid subtropical (KÖPPEN, 1936). The wheat cultivars used were BRS Parrudo (C1), TBIO Marfim (C2), Quartzo (C3), TBIO Mestre (C4), and TBIO Sinuelo (C5).

Experimental units consisted of five five-meter-long sowing rows spaced 0.20 meters apart. Our population density was composed of 330 viable seeds per square meter. Sowing was carried out on the same day for all genotypes in each environment, considering the

recommended agricultural zoning for each region. Nutritional management consisted of 250kg ha⁻¹ of 08-25-20 NPK at the base of sowing and 50kg ha⁻¹ of nitrogen was used per cover in the form of urea (46% of N) in a single application in the full tillering stage. Crops were agronomically managed to avoid biotic damage by weeds, insect pests, and diseases. Management was standardized for all environments and genotypes.

Each experimental unit was individually harvested and the collected seeds had their mass adjusted to 13% moisture. Production was estimated in kg ha⁻¹. The harvested seeds were milled into flour and 500-gram samples from each treatment, separated. Then, LC-ESI-qToF-MS mass spectrometry was used to quantify amino acid levels in the seeds of each experimental unit. The amino acids leucine (LEU, pmol), isoleucine (ISOL, pmol), glycine (GLY, pmol), serine (SER, pmol), threonine (THRE, pmol), proline (PRO, pmol), aspartate (ASPA, pmol), phenylalanine (PHEN, pmol), glutamine (GLU, pmol), asparagine (ASPG, pmol), and tryptophan (TRY, pmol) were evaluated.

Model assumptions, residual normality, and variance homogeneity were assessed (RAMALHO et al., 2012) and interactions between growing environments (E) and wheat genotypes (G) at 5% probability were described. When a significant G × E interaction was found, additive main effect and multiplicative interaction (AMMI) was used, combining the variances of the additive effects of genotypes and cultivation environments with the multiplicative effects of G × E interactions. Best linear unbiased prediction (BLUP), genotype main effects + genotype × environment interaction biplot method (GGE), and the simultaneous selection for mean performance and stability (MTSI) (OLIVOTO et al., 2019) were also applied.

A restricted maximum likelihood (REML) method was used to estimate variance components and genetic parameters, whose significance was obtained via deviance analysis at 5% probability by the Chi-squared test (RESENDE, 2007). Thus, genotypic variance (σ^2G), genotypic variance × environmental interaction (σ^2INT),

phenotypic variance (σ^2PV), residual variance (RVe), broad-sense heritability (H^2), broad-sense heritability of the mean of the genotype (\hat{h}^2mg), genotype selection accuracy ($Acgen$), coefficient of determination of genotypic effects × environmental interaction (C^2INT), genotypic correlation between the performance of environments ($rgloc$), the coefficient of genotypic variation (CVg), and the coefficient of residual variation (CVe) were estimated. R was used (R CORE TEAM, 2021) for statistical analyses.

Results and discussion

We assessed likelihood ratio test values at 5% for all evaluated characters to find the possible significance of our results. We found statistical significances for all aspects. We observed a high significance for the interaction between genotypes and environment for the evaluated factors, associated to the interaction between genotypes and the environment in amino acid expression, indicating a complex interrelation.

We found that the BRS Parrudo genotype showed the highest average expression of leucine, aspartate, phenylalanine, glutamine, asparagine, threonine, proline, and tryptophan (Figure 1). Although it failed to show the highest expression for the remaining amino acids, their concentration was higher than the general genotypic average for the remaining genotypes. TBIO Mestre, TBIO Marfim, and TBIO Sinuelo expressed higher mean isoleucine, glycine, and serine levels, respectively, indicating that genetic improvement programs may use these genotypes as parents to develop genotypes which better express these traits.

Due to the ease of viewing the results, research widely uses AMMI to position soybean (CARVALHO et al., 2016) and wheat genotypes (SZARESKI et al., 2021; SCHNEIDER et al., 2021). The first two main components explained more than 80% of our total data amino acid variability, except for asparagine (77.3%) (Figure 2). All genotypes showed good leucine, aspartate, glutamine, and tryptophan expression since they lie at the vertices of the polygon.

However, the Quartzo genotype had

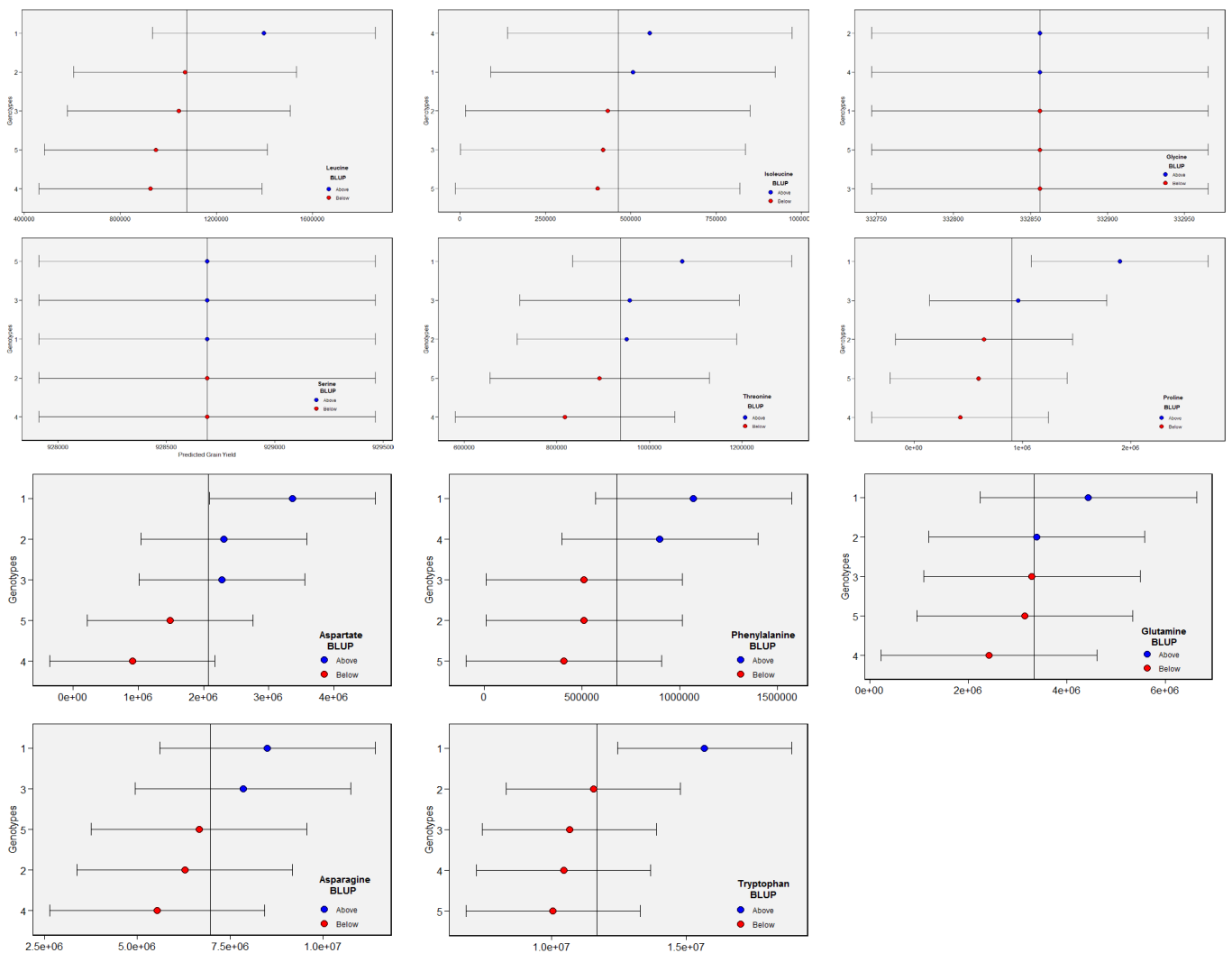


Figure 1. Representation of estimates obtained by the restricted maximum likelihood analysis method/best unbiased linear predictor (REML/BLUP) for the amino acids measured in five wheat genotypes: BRS Parrudo (C1), TBIO Marfim (C2), Quartzo (C3), TBIO Mestre (C4), and TBIO Sinuelo (C5)

Figura 1. Representação das estimativas obtidas pelo método de análise de máxima verossimilhança restrita/melhor preditor linear imparcial (REML/BLUP) para os aminoácidos medidos em cinco genótipos de trigo: BRS Parrudo (C1), TBIO Marfim (C2), Quartzo (C3), TBIO Mestre (C4), e TBIO Sinuelo (C5)

the lowest isoleucine, phenylalanine, serine, and threonine concentrations. Similarly, TBIO Marfim also expressed lower isoleucine, glycine, and serine concentrations. We found lower phenylalanine and proline levels in TBIO Mestre and TBIO Sinuelo, respectively. Both genotypes also expressed less asparagine. All other unmentioned genotypes performed better for specific amino acids.

São Luiz Gonzaga and Cachoeira do Sul showed favorable environmental conditions for the highest genotypic performance for all evaluated amino acids. Passo Fundo showed conditions resembling those two but its conditions reduced the expression of tryptophan

and aspartate by the genotypes. Santo Augusto promoted favorable conditions for the high expression of only glycine, threonine, proline, and leucine. On the other hand, São Gabriel provided unfavorable conditions for the expression of all amino acids.

We found that some environments promoted the greater expression of amino acids in the grains of some genotypes. However, positioning a specific genotype for each environment is an unfeasible practice for companies. Thus, it is essential to identify the formation of megaenvironments to position genotypes.

We managed to form high performance megaenvironments for

some amino acids. Those in Santo Augusto and Cachoeira do Sul formed a megaenvironment which greatly expressed glycine. Moreover, BRS Parrudo performed the best in this megaenvironment. On the other hand, Cachoeira do Sul and Passo Fundo show similar environmental conditions to favorably express serine via TBIO Sinuelo. Similarly, Santo Augusto and Passo Fundo form a megaenvironment with similar conditions and high threonine production via TBIO Sinuelo.

When we evaluated proline and isoleucine, we found that São Luiz Gonzaga and Cachoeira do Sul could form a megaenvironment, in which TBIO Sinuelo and BRS Parrudo expressed the

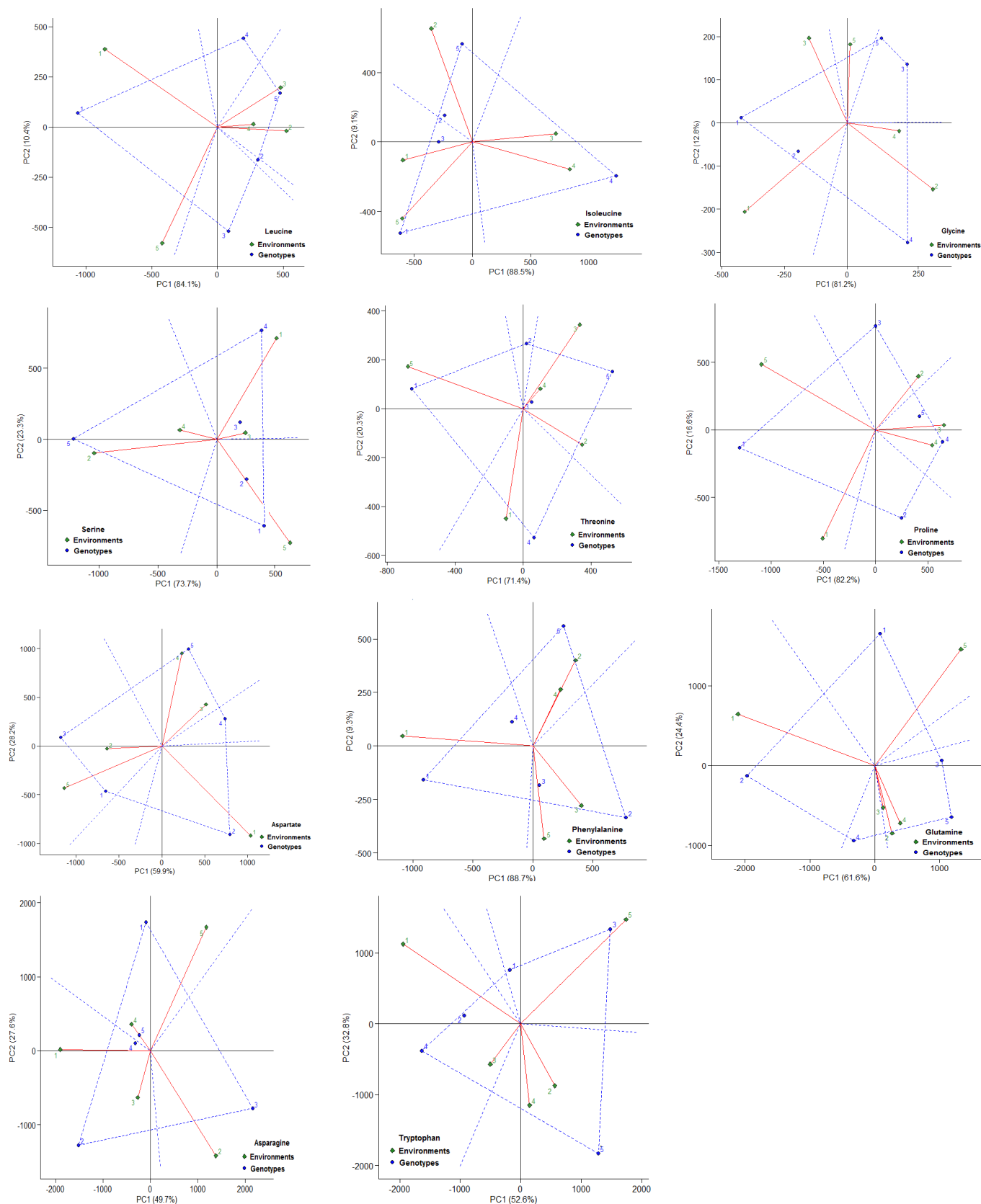


Figure 2. AMMI Biplot for the amino acids evaluated in five wheat genotypes and five growing environments: Cachoeira do Sul (A1), Passo Fundo (A2), Santo Augusto (A3), São Gabriel (A4), and São Luiz Gonzaga (A5). Genotypes: BRS Parrudo (C1), TBIO Marfim (C2), Quartzo (C3), TBIO Mestre (C4), and TBIO Sinuelo (C5)

Figura 2. AMMI Biplot para os aminoácidos avaliados em cinco genótipos de trigo e cinco ambientes de cultivo: Cachoeira do Sul (A1), Passo Fundo (A2), Santo Augusto (A3), São Gabriel (A4) e São Luiz Gonzaga (A5). Genótipos: BRS Parrudo (C1), TBIO Marfim (C2), Quartzo (C3), TBIO Mestre (C4) e TBIO Sinuelo (C5)

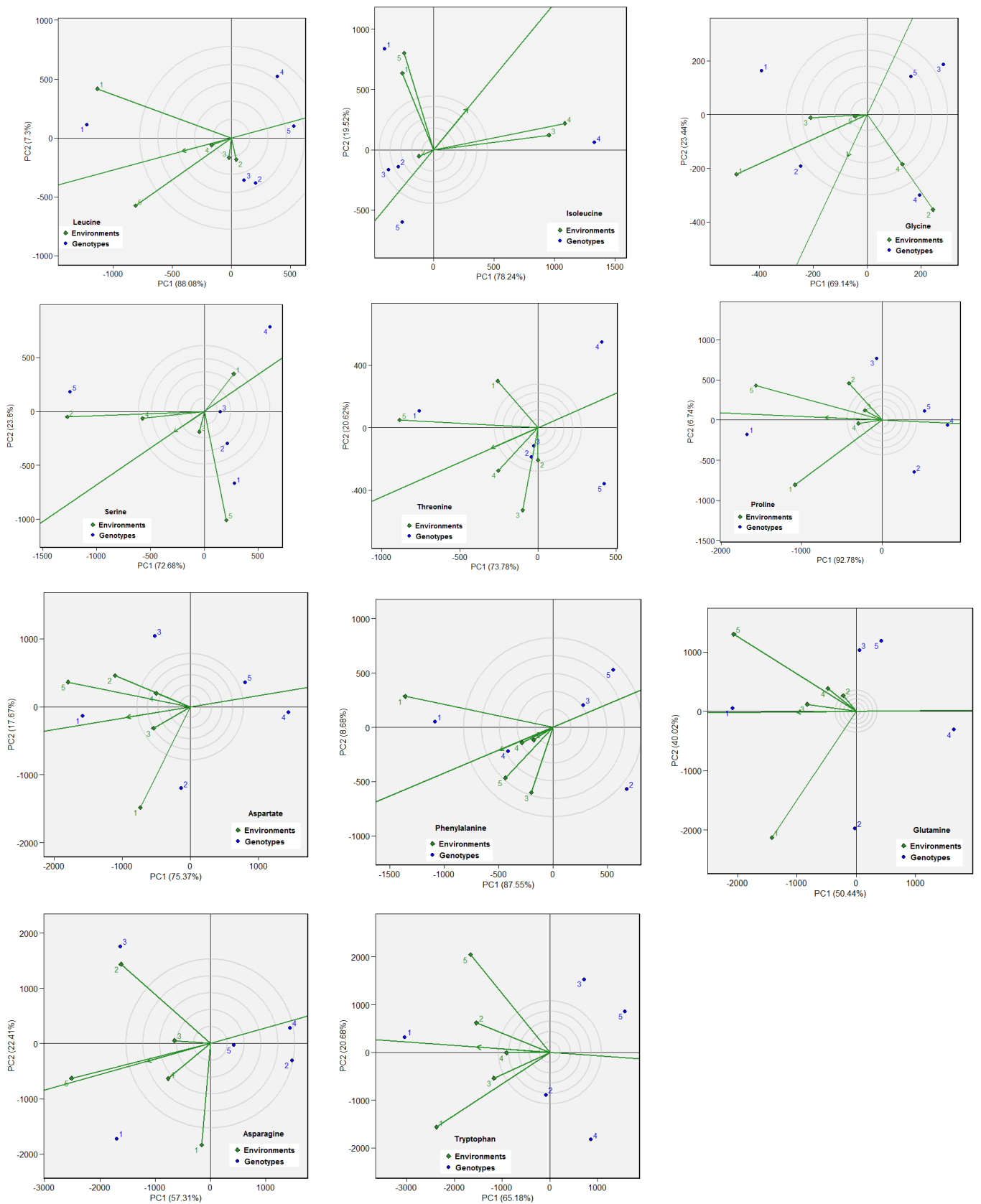


Figure 3. GGE Biplot for the amino acids evaluated in five wheat genotypes and five growing environments: Cachoeira do Sul (A1), Passo Fundo (A2), Santo Augusto (A3), São Gabriel (A4), and São Luiz Gonzaga (A5). Genotypes: BRS Parrudo (C1), TBIO Marfim (C2), Quartzo (C3), TBIO Mestre (C4), and TBIO Sinuelo (C5)

Figura 3. GGE biplot para os aminoácidos avaliados em cinco genótipos de trigo e cinco ambientes de cultivo: Cachoeira do Sul (A1), Passo Fundo (A2), Santo Augusto (A3), São Gabriel (A4) e São Luiz Gonzaga (A5). Genótipos: BRS Parrudo (C1), TBIO Marfim (C2), Quartzo (C3), TBIO Mestre (C4) e TBIO Sinuelo (C5)

highest levels of these amino acids. We also managed to form another megaenvironment for leucine, consisting of Santo Augusto and Passo Fundo, indicating it for TBIO Sinuelo. It was impossible to form megaenvironments for the other evaluated amino acids. This indicates that it was impossible to show similarities between the evaluated environments in the expression of these amino acids.

The first two main components by the GGE method, showed an explaining factor greater than 80% of our total

data variability for all evaluated amino acids (Figure 3). We observed that TBIO Marfim and Quartzo expressed similar leucine contents in their grains. Passo Fundo, Santo Augusto, and São Gabriel constituted similar environments. Cachoeira do Sul and São Luiz Gonzaga performed the best. Moreover, the simultaneous selection for mean performance and stability model (Figure 4) showed that Quartzo, TBIO Marfim, and BRS Parrudo showed greater phenotypic stability for leucine.

Isoleucine failed to show similarities

among genotypes. Cachoeira do Sul and São Luiz Gonzaga resemble each other, as do Santo Augusto and São Gabriel. According to the results obtained in the simultaneous selection for mean performance and stability model (figure 4), BRS Parrudo and TBIO Marfim showed a greater phenotypic stability for isoleucine.

For the other amino acids, we found no similarity in their expression among the evaluated genotypes. Santo Augusto and São Gabriel showed similar glycine expressions. Moreover, Cachoeira do

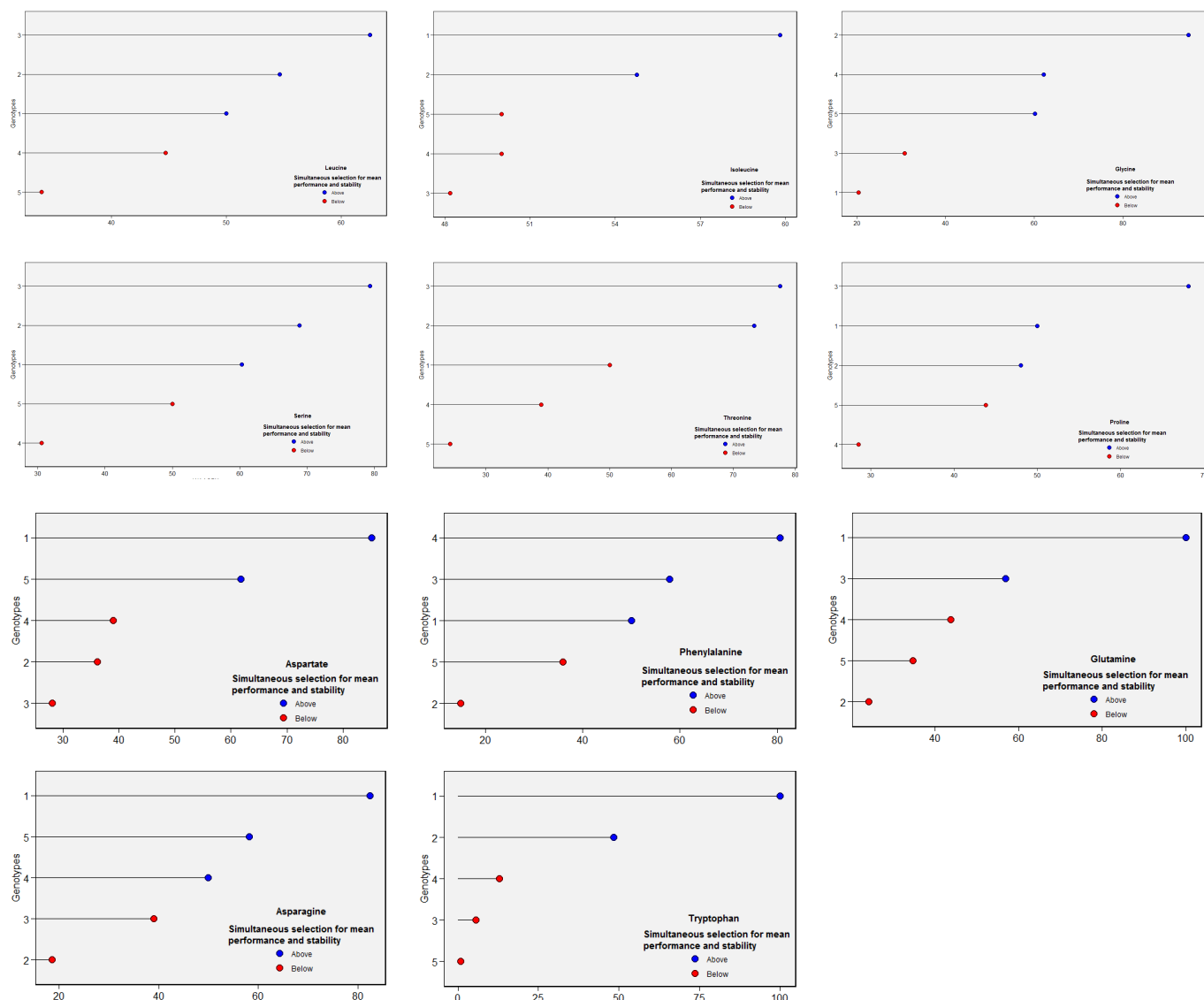


Figure 4. Representation of estimates obtained by the simultaneous selection for mean performance and stability model for the character of aspartate (ASPA), asparagine (ASPG), phenylalanine (PHEN), threonine (THRE), glutamine (GLU), serine (SER), tryptophan (TRY), leucine (LEU), proline (PRO), isoleucine (ISOL), and glycine (GLY), measured for five wheat genotypes: BRS Parrudo (C1), TBIO Marfim (C2), Quartzo (C3), TBIO Mestre (C4), and TBIO Sinuelo (C5)

Figura 4. Representação das estimativas obtidas pelo modelo de seleção simultânea para desempenho médio e estabilidade, para o caráter dos aminoácidos aspartato (ASPA), asparagina (ASPG), fenilalanina (PHEN), treonina (THRE) e glutamina (GLU), serina (SER), triptofano (TRY), leucina (LEU), prolina (PRO), isoleucina (ISOL) e glicina (GLY) medidos para os cinco genótipos de trigo: BRS Parrudo (C1), TBIO Marfim (C2), Quartzo (C3), TBIO Mestre (C4) e TBIO Sinuelo (C5)

Sul and Passo Fundo showed great amino acid expressions. By the MTSI index, TBIO Marfim, Tbio Mestre, and Tbio Sinuelo showed stable phenotypic expressions of glycine.

We found no ideal genotypes for serine expression. Passo Fundo and São Luiz Gonzaga expressed similar conditions. The MTSI index indicated the great stability of serine expression in BRS Parrudo, TBIO Marfim, and Quartzo. São Luiz Gonzaga expressed the most threonine. TBIO Marfim and Quartzo showed greater stability and average performance of threonine (Figure 4).

Cachoeira do Sul (A1), Passo Fundo, and São Luiz Gonzaga showed high performance but without similarity for proline. According to simultaneous selection for average performance and stability, BRS Parrudo, TBIO Marfim and Quartzo showed similarly stable proline phenotypic expression.

Santo Augusto and São Gabriel expressed similar aspartate levels. Moreover, Cachoeira do Sul, Passo Fundo, and São Luiz Gonzaga showed high expression of that amino acid. Still according to simultaneous selection for average performance and stability, BRS Parrudo and Tbio Sinuelo showed phenotypic stability for aspartate expression.

Tbio Mestre came closest to the ideal for phenylalanine. Cachoeira do Sul promoted favorable conditions to potentiate the levels of this amino acid. We observed that BRS Parrudo, Quartzo, and Tbio Mestre showed stable phenylalanine expression by the MTSI method.

It was impossible to identify the ideal genotype for glutamine. While Cachoeira do Sul and São Luiz Gonzaga performed well no environment showed similar phenotypic expressions. According to the simultaneous selection model for average performance and stability, BRS Parrudo and Quartzo showed stable glutamine phenotypic expression.

We found no occurrence of an ideotype for asparagine. Cachoeira do Sul, Passo Fundo, and São Luiz Gonzaga promoted the best performance of this amino acid. According to the simultaneous selection method for average performance and stability, BRS Parrudo, Tbio Mestre, and Tbio Sinuelo

show stable asparagine phenotypic expression (Figure 4).

We found no genotype close to the ideal tryptophan expression. Environments showed no similarities and those in Cachoeira do Sul and São Luiz Gonzaga showed conditions to potentiate this amino acid. According to the evaluation of the simultaneous selection method for average performance and stability, we observed that BRS Parrudo and TBIO Marfim showed stable tryptophan phenotypic expression.

Heritability shows the fraction of genetic variance in phenotypic variance, which may indicate reliability and experimental precision for a phenotype (RAMALHO et al., 2012). We found that proline, aspartate, phenylalanine, and tryptophan obtained a heritability above 39%. This indicates that these amino acids suffer greater influence from the environment, since their characteristics are associated with environmental manifestations (Table 1).

We also evaluated the broad-sense heritability of genotype means without these environmental effects (H^2_{mg}), assessing how their absence affected phenotypic expression. We considered these as high for proline (83%), aspartate (84%), and tryptophan (83%).

According to Resende & Duarte (2007), accuracy refers to a correlation between the real genotypic value of genetic treatments and that which experimental information estimates or predicts. We found an accuracy greater than 75 for all evaluated amino acids. This indicates the high experimental precision of our research. The coefficient for determining the effects of genotype \times environmental interaction (C^2_{int}) indicates a participation of the effects of total interaction in character variation, in which parameters showed a great intensity, especially for isoleucine, glycine, serine, and glutamine, which showed 0.99, 0.98, 0.99, and 0.81 values, respectively.

The genotypic correlation of genotype performance between cultivation environments (RGE) classifies the nature of the interactions, in which the higher the magnitude of this parameter, the simpler its characterization and the less distortions in the classification of genotypes

(PUPIN et al., 2015). Thus, we found the following mean RGE values: proline (0.50), aspartate (0.51), and phenylalanine (0.40), whereas we observed low RGE leucine (0.26), isoleucine (0.004), glycine (0.004), serine (0.0007), threonine (0.24), glutamine (0.19), asparagine (0.26), and tryptophan (0.49) coefficients. This shows the complex genotype \times environment interactions for characters with low RGE coefficients. In other words, environmental conditions strongly influence the expression of these characters.

Thus, we can infer that proline, aspartate, and phenylalanine tend to be expressed in similar magnitudes among environments. This favors the selection of genotypes for these characters. However, the other amino acids with genotypic performance correlations in low-magnitude environments tend to hinder the selection of ideal genotypes since the environment strongly influences the expression of these characters.

The coefficient of genotypic variation (CVg^*) is a parameter used to quantify the percentage of total variation from genetic effects. We observed values greater than 48% for proline, aspartate, and phenylalanine, indicating a high genotypic variation. The experimental variation coefficient ($CVr^*\%$) represents the precision obtained in conducting the experiment and has a statistical and non-genetic nature (ROSA et al., 2021). We observed that this coefficient was low for all parameters, below 10% in all evaluated parameters and above 5% only for proline (7.2%) and isoleucine (6.4%), showing good experimental precision.

Conclusions

- The Parrudo genotype potentiates the expression of amino acids in its grains if cultivated in Cachoeira do Sul.
- By the BLUP methodology, BRS Parrudo better expresses all amino acids in its grains.
- We managed to identify and position wheat genotypes for higher concentration of amino acids in their grains.
- Aspartate, proline, and

Table 1. Estimates of variance components and genetic parameters for the amino acids present in flour obtained from wheat grains grown in different environments in the state of Rio Grande do Sul, Brazil

Tabela 1. Estimativas de componentes de variância e parâmetros genéticos para os aminoácidos presentes em farinha obtida de grãos de trigo cultivados em diferentes ambientes no estado do Rio Grande do Sul, Brasil

Parameters	LEU	ISOL	GLY	SER	THRE	PRO
Heritability	0.256	0.004	0.005	0.001	0.242	0.500
H ² mg	0.632	0.020	0.022	0.003	0.616	0.834
Accuracy	0.795	0.141	0.149	0.059	0.785	0.913
C ² int	0.741	0.993	0.981	0.997	0.748	0.495
RGE	0.257	0.004	0.005	0.001	0.244	0.502
CVg*(%)	22.147	7.695	1.953	1.543	12.657	71.298
Cvr*(%)	2.734	6.419	3.493	2.966	2.648	7.233
LRT	Signif.	Signif.	Signif.	Signif.	Signif.	Signif.

Parameters	ASPA	PHEN	GLU	ASPG	TRY
Heritability	0.509	0.394	0.188	0.260	0.493
H ² mg	0.838	0.765	0.536	0.637	0.830
Accuracy	0.916	0.875	0.732	0.798	0.911
C ² int	0.490	0.605	0.809	0.736	0.500
RGE	0.509	0.395	0.188	0.261	0.496
CVg*(%)	49.148	48.492	29.576	21.539	21.649
Cvr*(%)	2.692	2.913	3.736	2.946	2.416
LRT	Signif.	Signif.	Signif.	Signif.	Signif.

H²mg: Mean genotype heritability of the; Ac gen: accuracy for genotype selection; C²int: coefficient to determine the effects of genotype x environment interaction; RGE: The genotypic correlation of genotype performance between cultivation environments; CVg*: Genotypic variation coefficient; Cvr*: Coefficient of residual variation; LRT: Likelihood Ratio Test. FV: Factor of variation, DF: Degree of freedom, LEU: Leucine, ISOL: Isoleucine, GLY: Glycine, SER: Serine, THRE: Threonine, PRO: Proline, ASPA: Aspartate, PHEN: Phenylalanine, GLU: Glutamine, ASPG: Asparagine, TRY: Tryptophan.

tryptophan showed the highest broad-sense heritability, favoring the selection of genotypes to increase these characters.

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