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Wheat grain biofortification for essential amino acids

Abstract – The objective of this work was to select wheat genotypes aiming to increase the essential amino acids in their grains. The study was carried out in the 2019 crop year, in a randomized complete block design, organized in a 5x5 factorial arrangement – five environments in the state of Rio Grande do Sul, Brazil (Cachoeira do Sul, Cruz Alta, Santo Augusto, São Gabriel, and Vacaria), and five wheat genotypes ('BRS Parrudo', 'Marfim', 'Quartzo', 'TBIO Mestre', and 'TBIO Sinuelo') –, with two replicates. Polar metabolites were extracted from the flour of the ground wheat grains, derivatized, and evaluated by gas chromatography-mass spectrometry. Both variance components and genetic parameters were estimated for the metabolites. To select the genotypes for the traits of interest, the multi-trait index based on factor analysis and ideotype design, the multi-trait genotype-ideotype distance index, and the multi-trait stability index were applied. The wheat genotypes express a high genetic variability and selection possibility for gentiobiose, butyric acid, galactopyranosyl, phenylalanine, tryptophan, leucine, and isoleucine. The 'Marfim' genotype remains stable for essential amino acid levels in the studied environments. The 'Quartzo' genotype stands out in the expression of leucine, isoleucine, phenylalanine, and tryptophan in its grains.

Index terms: *Triticum aestivum*, genetic parameters, metabolites, plant breeding, selection index.

Biofortificação do grão de trigo para aminoácidos essenciais

Resumo – O objetivo deste trabalho foi selecionar genótipos de trigo com vista ao aumento de aminoácidos essenciais nos seus grãos. O estudo foi realizado no ano agrícola de 2019, em delineamento de blocos ao acaso, organizados em arranjo fatorial 5x5 – cinco ambientes no estado do Rio Grande do Sul, Brasil (Cachoeira do Sul, Cruz Alta, Santo Augusto, São Gabriel e Vacaria), e cinco genótipos de trigo ('BRS Parrudo', 'Marfim', 'Quartzo', 'TBIO Mestre' e 'TBIO Sinuelo') –, com duas repetições. Os metabólitos polares foram extraídos da farinha dos grãos de trigo moídos, derivatizados e avaliados por cromatografia gasosa-espectrometria de massa. Tanto os componentes de variância como os parâmetros genéticos foram estimados para os metabólitos. Para selecionar os genótipos para as características de interesse, aplicaram-se o índice multicaracterística baseado em análise fatorial e desenho de ideótipos, o índice de distância genótipo-ideótipo multicaracterística e o índice de estabilidade multicaracterística. Os genótipos de trigo expressam alta variabilidade genética e possibilidade de seleção para gentiobiose, ácido butírico, galactopiranosil, fenilalanina, triptofano, leucina e isoleucina. O genótipo 'Marfim' mantém-se estável para os níveis de aminoácidos essenciais nos ambientes estudados. O genótipo 'Quartzo' se destaca na expressão de leucina, isoleucina, fenilalanina e triptofano em seus grãos.

Termos para indexação: *Triticum aestivum*, parâmetros genéticos, metabólitos, melhoramento de plantas, índice de seleção.

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Introduction

Cereals are the main source of food and feed globally, among which wheat (*Triticum aestivum* L.) stands out as one of the most widely cultivated and consumed, being considered a staple food in most parts of the world (Shewry & Hey, 2015; Zamaratskaia et al., 2021). Cereal grains are also important due to their biochemical composition, including compounds that act as a natural protection for plants and as source of nutrients and of prophylactic and curative effects for human beings (Malaguti et al., 2014; Shewry & Hey, 2015; Zamaratskaia et al., 2021).

The study of the metabolism of plants is a natural procedure to improve their growth and yield and to enhance secondary metabolic processes (Feduraev et al., 2020). Plant species produce several structural metabolites that are essential for their own growth and development, cell replacement, resource allocation, and stress responses (Wen et al., 2014). Therefore, in plant breeding programs, metabolomics is an efficient strategy to analyze plant responses to biotic or abiotic events, allowing for a thorough quantification of a wide range of metabolites by means of molecular markers, hybridization, heritability, and the selection of transgressive genotypes towards an agronomic ideotype (Păucean et al., 2021). According to these same authors, in the case of wheat, this approach can be used to select genotypes with specific traits that improve bakery (Păucean et al., 2021).

In bakery, the current trend due to the healthier diet sought by the consumer market is the development of products with functional ingredients (Duarte et al., 2021), such as essential amino acids, bioactive compounds, carotenoids, flavonoids, and antioxidant compounds. The search for nutritionally superior genotypes is also justified by the reduction in food and nutritional deficiencies and the increment in disease prevention (Liu et al., 2020).

In order to meet the expectations of breeders and the consumer market, it is important to use new methods to estimate and predict the environments that are the most favorable for genotypes to express nutraceutical compounds. A selection process combining multi-environment and multi-trait analyses increases the efficiency of breeding programs and reduces the involved costs (Olivoto et al., 2019). Examples of these methods include: the multi-trait genotype-ideotype distance index (MGIDI), which is easy to interpret, does not require coefficient weighting, and can handle multicollinearity (Olivoto & Nardino, 2021); and the multi-trait index based on factor analysis and ideotype design (FAI-BLUP), which uses the correlation structure for selection close to the trait sought by the breeder (Rocha et al., 2018). These methodologies make it is possible to select genotypes with high-performance traits, contributing to the best decision-making regarding the selection of genotypes and the recommendation of environments.

The objective of this work was to select wheat genotypes aiming to increase the essential amino acids in their grains.

Materials and Methods

The study was carried out in the 2019 crop year, in the five following cultivation environments (municipalities), located in the state of Rio Grande do Sul, Brazil: Cachoeira do Sul (27°52'15"S, 54°28'53"W, at 277 m), Cruz Alta (27°22'16"S, 53°45'30"W, at 390 m), Santo Augusto (27°56'38"S, 52°55'23'W, at 503 m), São Gabriel (28°53'10"S, 52°59'55"W, at 513 m), and Vacaria (28°53'10"S, 52°59'55"W, at 513 m). In each environment, the five following wheat genotypes were sown: 'BRS Parrudo', 'Marfim', 'Quartzo', 'TBIO Mestre', and 'TBIO Sinuelo'. The experimental design was randomized complete blocks in a 5x5 factorial arrangement with only two replicates due to the high cost of analyzing the grains to determine the metabolite concentrations. The experimental unit consisted of seven rows, with 5.0 m of length, spaced at 0.17 m. The population density of the genotypes was 300 plants per square meter under a no-tillage system. The base fertilization was 12.5 kg ha^{-1} N, 50 kg ha^{-1} P_2O_5, and 50 kg ha^{-1} K_2O, followed by a subsequent broadcast application of 67.5 kg ha^{-1} N. The control of weeds, diseases, and pests was preventive.

Polar metabolites were extracted from the flour of the collected and milled wheat grains, derivatized, and evaluated by gas chromatography-mass spectrometry according to Lisec et al. (2006). For their identification, the metabolites (Table 1) were compared with those in the database of National Institute of Standards and Technology (Gaithersburg, MD, USA). The peak area of each metabolite was used in the statistical analysis.

The obtained data were subjected to the normality and homogeneity tests of Shapiro-Wilk and Bartlett, and the independence of errors was checked.

Table 1. Metabolites identified in wheat (*Triticum aestivum*) genotypes by gas chromatography-mass spectrometry.

Compound	Retention time (min)
Aspartate	0.68
Glutamine	1.53
Proline	1.91
Phenylalanine	6.41
Butanoic acid	7.17
Isoleucine	8.03
Asparagine	8.06
Glycerol	8.19
Leucine	8.65
2-butenedioic acid	8.82
Nonanoic acid	8.93
Butanedioic acid	0.52
Glycine	11.54
Serine	11.54
Octanedioic acid	12.17
Threonine	12.45
D-fructofuranose	13.38
Tryptophan	15.00
Myo-inositol	15.32
Xylitol	12.67
Phosphoric acid	12.86
Azelaic acid	12.92
α-D-lyxopyranose	12.98
Ribonic acid	13.18
1,2,3-propanetricarboxylic acid	13.29
d-Gluconic acid	13.76
Galactonic acid	13.87
Galactopyranose	13.95
Talose	14.05
Trimethylsilyl ether of glucitol	14.34
β-D-galactofuranose	14.66
D-gluconic acid	14.75
Melibiose	15.87
Glyceryl-glycoside	16.59
Myristic acid	16.73
β-D-glucopyranuronic	16.83
D-(+)-cellobiose	17.65
Adenosine	17.98
Octadecanoic acid	18.68
D-(+)-trehalose	18.90
D-fructose	18.98
D-glucopyranose	19.20
D-glucose	19.35
β-gentiobiose	19.39
4-O-β-galactopyranosyl-D-mannopyranose	19.53
D-lactitol	19.88
Galactinol	20.09
α-D-glucopyranoside	21.19
Sucrose	21.23

Multicollinearity was measured by the variance inflation factor. Subsequently, the deviance analysis, at 5% probability, was performed using the chi-square test in order to identify the significance of the variance components and genetic parameters estimated for the following metabolites: butyric acid, inositol, glyceryl-glycoside, sucrose 1, myristic acid, glucuronic acid, adenosine, D-glucopyranose, sucrose 2, D-glucose, gentiobiose, galactopyranosyl, gentiobiose 1, galactopiranoside, sucrose 3, leucine, isoleucine, galactopyranosyl 1, glycine, serine, galactopiranoside 1, threonine, proline, sucrose 4, aspartate, phenylalanine, glutamine, asparagine, and tryptophan.

The components of variance and the genetic parameters of the metabolites were estimated through the restricted maximum likelihood (REML), using the statistical model: $y = Xr + Za + Wp + e$, where y is a data vector; r are the effects of replicates (fixed); a is the individual additive genetic effects 5x5 (random); p is the effect attributed to the genotype x environment interaction; e are the effects of residues (random); and X, Z, and W represent the incidence matrices for these effects (Resende, 2007).

Using this approach, it was possible to estimate: genotypic variance, phenotypic variance, environmental variance, broad-sense heritability with the effects of the genotype x environment interaction, broad-sense heritability without the effects of the genotype x environment interaction, coefficient of determination of the effects of the genotype x environment interaction, accuracy of genotype selection, genotypic correlation of genotype performance between environments, genotypic coefficient of variation, residual coefficient of variation, and the ratio between the genetic and residual coefficient. The variables that showed significant deviance at 5% probability using the chi-square test were subjected to best linear unbiased predictions (BLUPs) with a high heritability criteria, high genetic coefficient of variation, and low residual coefficient of variation.

Based on grain biofortification needs, the studied genotypes were selected for an ideotype that increases essential amino acids – such as leucine, isoleucine, phenylalanine, and tryptophan – in their grains. The FAI-BLUP, MGIDI, and the multi-trait stability selection index (MTSI) were used to select the genotypes for the traits of interest (Rocha et al., 2018)

through the weighted average absolute score of BLUPs (WAASB), considering only genotype stability.

For the application of the multi-trait indices, the criteria for the inclusion of independent and uncorrelated variables were: vital biological function (amino acids essential to the human organism), significance of deviance, REML (reliable), and BLUPs (informative).

To estimate the BLUPs, the variables included in the routine of the analysis for a favorable selection were leucine, isoleucine, phenylalanine, and tryptophan, aiming to increase their levels in the wheat grains. To design the ideotype in the FAI-BLUP for the desired gains in these four amino acids, the maximum standard genetic values were applied (Rocha et al., 2018). The MGIDI index was used to select genotypes with maximum values (positive gains) for leucine, isoleucine, phenylalanine, and tryptophan, which was done by adopting maximum and minimum values of 100 and 0, respectively, after rescheduling to obtain positive gains, as proposed by Olivoto & Nardino (2021). The MTSI, calculated considering the WAASB index, was used to characterize the ideotype by means of maximum stability for leucine, isoleucine, phenylalanine, and tryptophan, with a maximum value of 100 (Olivoto et al., 2019).

The analyses were performed using the following R packages: agricolae, version 1.3-5 (Mendiburu, 2021), to verify the assumptions of the analyses; metan, version 1.16.0 (Olivoto & Lucio, 2020), to apply deviance analyses, BLUP, and multi-trait selection indices; and ggplot2, version 3.3.6 (Wickham, 2016), to develop the graphic performances of the genotypes. All statistical analyses were carried out with the R, version 3.5.6, software (R Core Team, 2022).

Results and Discussion

The deviance analysis for the genotype x environment interaction was significant for all metabolites using the chi-square test, at 5% probability (Table 2). This is indicative that the expression of the metabolites was different in the environments, i.e., the factors genotype and environments are dependent. Moreover, the variance components and genetic parameters estimated by REML show the existence of genetic variability for the traits evaluated for the five wheat genotypes grown in the five environments (Table 2 and Figure 1).

The effects of environment, genetic variation, and the interaction of genotypes with the environments are related to phenotypic magnitude (Carvalho et al., 2018). Therefore, the ratio between genetic variance and phenotypic variance evidences the broad-sense heritability (H^2) of the studied traits, also indicating how much of phenotypic variation is of genetic origin, which makes it possible to determine the reliability of experimental precision for a phenotype.

Higher magnitudes of H^2 of 58.2, 47.3, and 44.8% were found for gentiobiose, butyric acid, and galactopyranosyl, respectively, which is indicative that these metabolites showed marked genetic variability and experimental precision, suggesting a favorable condition for selection. The H^2 without environmental effect was also considered very high for gentiobiose, butyric acid, and galactopyranosyl, with values of 88.1, 83.3, and 80.8%, respectively, which is an important parameter for the prediction of success in improvement because it minimizes residual deviations from experimental causes (Cargnelutti Filho & Storck, 2009).

The coefficient of determination of the effects of the genotype x environment interaction (C^2_{INT}) allows of quantifying the effects of this interaction on a variable. According to the obtained results, the metabolites inositol, glyceryl-glycoside, sucrose 1, sucrose 2, sucrose 3, isoleucine, and asparagine were the ones that contributed the most to such interaction ($C^2_{INT} > 0.93$).

High accuracies promote a greater experimental precision and effectiveness in selection strategies, as well as in genetic gain (Cargnelutti Filho & Storck, 2009). In the present study, the class limits of experimental precision were based on selective accuracy (SA) according to Resende (2007). High accuracies (SA > 0.70 and < 0.90) were found for butyric acid, glucuronic acid, D-glucopyranose, D-glucose, gentiobiose, galactopiranoside, galactopiranoside, galactopiranoside, leucine, threonine, proline, aspartate, phenylalanine, and glutamine. Moderate accuracies (SA > 0.50 and < 0.70) were obtained for sucrose 4 and glycine, whereas a low accuracy (SA < 0.50) was observed for glyceryl-glycoside due to the effects of the genotype x environment interaction that strongly acts on these compounds (Resende, 2007; Cargnelutti Filho & Storck, 2009).

Table 2. Probabilities for the restricted likelihood ratio test, variance components, and genetic parameters of the traits evaluated for wheat (*Triticum aestivum*) genotypes grown in different environments in the state of Rio Grande do Sul, Brazil[1].

Model	Butyric acid	Inositol	Glyceryl-glycoside	Myristic acid	Glucuronic acid	Adenosine	D-gluco-pyranose	D-glucose	Gentio-biose	Gentio-biose
Gen	0.00973	1.00000	0.92600	0.37700	0.14600	0.44000	0.04580	0.12000	1.00000	0.00187
GxE	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.03420	0.00001
Model	Galacto-pyranosyl	Galacto-pyranosyl	Galacto-pyranoside	Sucrose 1	Galacto-pyranoside	Sucrose 2	Sucrose 3	Sucrose 4	Leucine	Isoleucine
Gen	0.01770	0.37900	0.11000	1.00000	0.35674	1.00000	1.00000	0.52109	0.24494	0.71000
GxE	0.00001	0.00001	0.00001	0.00001	0.00821	0.00001	0.00001	0.00587	0.00428	0.00001
Model	Glycine	Serine	Threonine	Proline	Aspartate	Phenyl-alanine	Glutamine	Aspara-gine	Tryptophan	
Gen	0.51900	1.00000	0.10600	0.35600	0.11400	0.13100	0.12500	1.00000	0.39700	
GxE	0.00001	0.00023	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	

Parameter[2]	Characters									
	Butyric acid	Inositol	Glyceryl-glycoside	Myristic acid	Glucuronic acid	Adenosine	D-gluco-pyranose	D-glucose	Gentio-biose	Gentio-biose1
σ^2_P	0.00008	0.00021	0.00225	0.00009	0.00026	0.00020	0.00061	0.00210	0.00038	0.00080
H^2	0.47358	0.00001	0.01476	0.15604	0.26882	0.13450	0.37748	0.28702	0.00000	0.58205
C^2_{INT}	0.42240	0.98585	0.97741	0.83785	0.71924	0.85073	0.59924	0.68567	0.45556	0.36713
H^2_{mg}	0.83309	0.00001	0.06995	0.48128	0.64954	0.43937	0.75548	0.67236	0.00001	0.88115
SA	0.91274	0.00001	0.26447	0.69374	0.80594	0.66285	0.86918	0.81997	0.00001	0.93870
$\check{r}g_{loc}$	0.80241	0.98585	0.99206	0.99276	0.98367	0.98294	0.96261	0.96170	0.45556	0.87843
CVg	56.16937	0.00001	4.86940	21.79915	21.34950	16.13517	23.87027	24.10590	0.00000	60.65425
CVr	26.32365	4.43847	3.54568	4.31408	4.49899	5.34573	5.92722	7.43568	41.31878	17.92092
CVg/CVe	2.13380	0.00001	1.37333	5.05302	4.74540	3.01833	4.02723	3.24192	0.00001	3.38455

Parameter	Characters									
	Galactopyra-nosyl	Galactopyra-nosyl	Galactopyra-noside	Sucrose 1	Galactopyra-noside	Sucrose 2	Sucrose 3	Sucrose 4	Leucine	Isoleucine
σ^2_P	0.00045	0.00023	0.00060	0.00304	0.00114	0.00085	0.00089	0.00119	0.00317	0.00061
H^2	0.44865	0.15428	0.29846	0.00000	0.13278	0.00001	0.00001	0.08958	0.17643	0.06201
C^2_{INT}	0.51212	0.82346	0.68998	0.98999	0.48549	0.96994	0.98751	0.52689	0.49043	0.93231
H^2_{mg}	0.80838	0.48033	0.68202	0.00001	0.49535	0.00001	0.00000	0.38396	0.57313	0.24899
SA	0.89910	0.69305	0.82584	0.00001	0.70381	0.00003	0.00001	0.61964	0.75706	0.49899
$\check{r}g_{loc}$	0.92885	0.97367	0.98352	0.98999	0.55982	0.96994	0.98751	0.57873	0.59549	0.99394
CVg	28.87227	17.12830	27.59495	0.00001	18.69280	0.00053	0.00001	15.06567	22.70899	24.25049
CVr	8.53736	6.50692	5.43139	4.07532	31.69476	7.96616	5.96111	31.17305	31.20563	7.34031
CVg/CVe	3.38187	2.63232	5.08064	0.00001	0.58978	0.00007	0.00001	0.48329	0.72772	3.30374

Parameter	Characters								
	Glycine	Serine	Threonine	Proline	Aspartate	Phenyl-alanine	Glutamine	Aspara-gine	Tryptophan
σ^2_P	0.00013	0.00299	0.00205	0.00707	0.02242	0.00238	0.05979	0.08444	0.21714
H^2	0.10980	0.00001	0.30268	0.16392	0.29566	0.28150	0.28618	0.00001	0.14823
C^2_{INT}	0.86138	0.70978	0.69095	0.82933	0.70013	0.71315	0.70631	0.98196	0.83214
H^2_{mg}	0.38531	0.00001	0.68556	0.49604	0.67795	0.66288	0.66835	0.00001	0.46816
SA	0.62074	0.00001	0.82799	0.70430	0.82338	0.81417	0.81752	0.00001	0.68422
$\check{r}g_{loc}$	0.96762	0.70978	0.99086	0.99193	0.99402	0.99255	0.98948	0.98196	0.97696
CVg	11.26566	0.00001	25.98529	42.88277	43.01461	42.45081	39.18242	0.00001	16.65937
CVe	5.77208	39.16018	3.77001	8.69905	5.13224	5.85363	6.34776	6.75395	6.06187
CVg/CVe	1.95175	0.00001	6.89264	4.92959	8.38125	7.25205	6.17264	0.00001	2.74822

[1]All variables with a significant genotype x environment (GxE) interaction ($p<0.05$). [2]σ^2_P, phenotypic variance; H^2, broad-sense heritability with the effects of the GxE interaction; H^2_{mg}, broad-sense heritability without the effects of the GxE interaction; C^2_{INT}, coefficient of determination of the effects of the GxE interaction; SA, accuracy for genotype selection; $\check{r}g_{loc}$, genotypic correlation of genotype performance between environments; CVg, genotypic coefficient of variation; CVr, relative coefficient of variation; CVe, residual coefficient of variation; and CVg/CVe, ratio between the genetic and residual coefficient.

The genotypic correlation of the genotypic performance between environments ($\check{r}g_{loc}$) was high (> 0.70) for butyric acid, inositol, glyceryl-glycoside, myristic acid, glucuronic acid, adenosine, D-glucopyranose, D-glucose, gentiobiose, galactopyranosyl, galactopyranosy l, galactopyranoside, sucrose 1, sucrose 2, sucrose 3, isoleucine, glycine, serine, threonine, proline, aspartate, phenylalanine, glutamine, asparagine, and tryptophan, which shows the predominance of a single interaction in these compounds. Low coefficients ($\check{r}g_{loc} < 0.50$) were found for gentiobiose, which causes a complex phenomenon resulting in genotypes with a lower phenotypic stability.

The genotypic coefficient of variation has been used to describe genetic variability (Resende, 2007). According to this parameter, butyric acid (56.16%) and gentiobiose (60.65%) showed a greater genetic variability among genotypes. Contrastingly, inositol, gentiobiose, sucrose 1, sucrose 2, sucrose 3, serine, and asparagine presented the lowest genetic variations. The residual coefficient of variation was high – above 25% – for butyric acid, gentiobiose, galactopiranoside, sucrose 4, leucine, and serine. Therefore, it can be inferred that, for these compounds, environmental variance, which is the variation between replicates of the same treatment, is higher than genetic variance, which represents the variation between genotypes. The overall mean of the experiment plus genetic variance, without residual and environmental effects, represents the genotypic effect obtained via BLUP.

The 'TBIO Mestre', 'TBIO Sinuelo', and 'Marfim' genotypes showed an increase in butyric acid in their grains (Figure 2). This is of great interest since, at high levels, this metabolite benefits human health by acting as an anti-inflammatory agent – reducing pathogenic microorganisms such as *Escherichia coli*, *Campylobacter* spp., *Salmonella* spp., and *Shigella* spp. –, preventing colon cancer, and maintaining gut integrity (Chen & Walker, 2005).

In addition, 'TBIO Sinuelo' and 'TBIO Mestre' showed an increased concentration of glycerol-

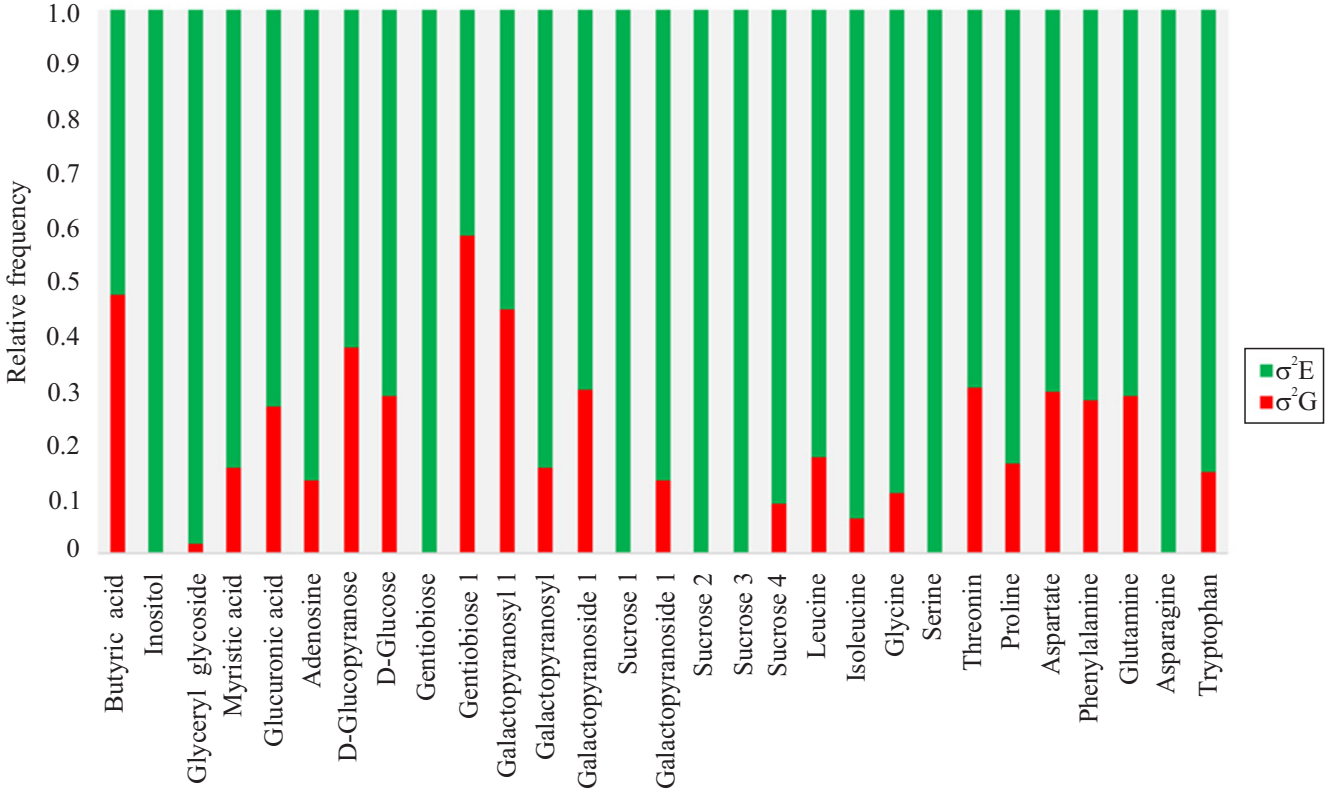

Figure 1. Proportion of genetic variance components of the metabolites evaluated in wheat (*Triticum aestivum*) genotypes cultivated in different environments in the state of Rio Grande do Sul, Brazil. σ^2G, genetic variance; and σ^2E, environmental variance.

glycoside (floridoside), which is the main reservoir of soluble carbon fixed by photosynthesis, being a precursor of cell wall polysaccharides (Ryu et al., 2015), as well as a potential therapeutic agent with the ability to increase immunity (Kim et al., 2013) and promote bone formation (Ryu et al., 2015). Therefore, both genotypes are possible sources of alleles and genes for that trait, which, when enhanced, can contribute to the industrialization of raw materials.

The 'BRS Parrudo', 'TBIO Sinuelo', and 'Marfim' genotypes had lower levels of myristic acid, a fatty acid widely distributed in vegetable and animal fats that is undesirable at high levels in food. The compound is considered atherogenic because it forms an atheromatous plaque that can increase the content of low-density lipoprotein in the blood, increasing the risk of cardiovascular disease (Lottenberg et al., 2009). Although genotypes 'Quartzo' and 'TBIO Mestre'

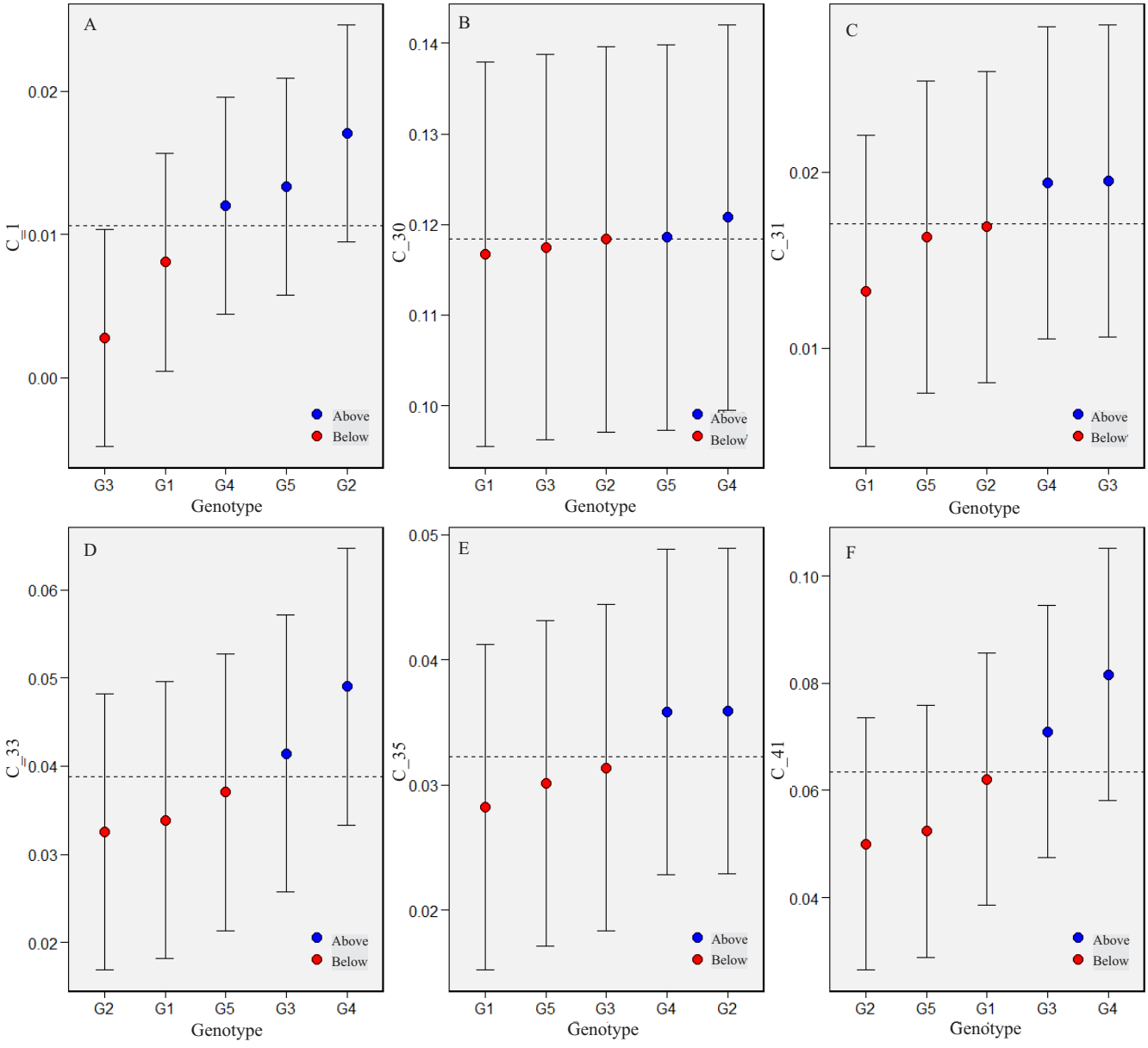

Figure 2. Predictions for the best linear unbiased predictor for the following metabolites in wheat (*Triticum aestivum*) genotypes: A, butyric acid; B, glycerol-glycoside; C, myristic acid; D, glucuronic acid; E, adenosine; and F, D-glucopyranose. G1, 'BRS Parrudo'; G2, 'Marfim'; G3; 'Quartzo'; G4, 'TBIO Mestre'; and G5, 'TBIO Sinuelo'.

showed higher myristic acid levels, they also promoted higher concentrations of glucuronic acid, being selected for the benefits of this compound to human nutrition and plant protection. Fujiwara et al. (2018) found that this metabolite from glucose is involved in the detoxification of foreign compounds in the human body, whereas Lorence et al. (2004) concluded that it acts in the synthesis of pectic substances in the cell wall of plants, being responsible for their biosynthesis of ascorbic acid and protection against oxidative stress.

The 'TBIO Mestre' and 'Marfim' genotypes also maximized the concentrations of adenosine, a metabolite expressed as a nucleoside responsible for numerous physiological functions, such as energy transfer via adenosine tri-phosphate (Löfgren et al., 2018) and adenosine diphosphate, being an essential storage for the energy metabolism of animals and plants (Antonioli et al., 2013).

Genotypes 'Quartzo' and 'TBIO Mestre' showed higher concentrations of D-glucose (Figure 3). This compound presents itself in the form of a cyclic hemiacetal analogous to glucose that synthesizes L-ascorbic acid (a precursor of vitamin C), has an energy reserve function in vegetables (starch) and animals (glycogen), and is commercially produced by acid hydrolysis via potato starch (Silva et al., 2018). Considering the high demand for this metabolite, mainly by the pharmaceutical industry, priority was given to the selection of both of these genotypes, which genetically enhance the nutraceutical quality of the obtained grains.

'TBIO Mestre' and 'Quartzo' incremented gentiobiose, a disaccharide composed of two D-glucose units joined by a β bond – a white crystalline solid soluble in water –, with an osmoprotective function, stabilizing cell membranes under water deficit conditions (Lokhande et al., 2012). Therefore, the genetic contribution of these two genotypes can promote better plant performance under osmotic stress. These same genotypes can also be selected to increase the concentrations of epilactose, a disaccharide that differs in the carbon 2 configuration of the D-glucose residue, being a natural sugar that may have clinical benefits despite the limited data on it. The increase in the contents of this disaccharide through selection, aiming at its commercial production on a large scale worldwide, is of interest among various industrial sectors due to its valuable properties and consequent wide range of applications.

Genotypes 'TBIO Mestre' and 'Marfim' were selected due to their higher expression of sucrose (glucose + fructose), which is the final product of photosynthesis, an energy vector for plant organs incapable of carrying out this process, a source of carbon skeletons, and the primary sugar transported in the phloem of most plants (Liu et al., 2017). Therefore, the increase in sucrose in wheat grains may be associated with genotypes with a greater efficiency in photosynthesis.

Genotypes 'BRS Parrudo', 'TBIO Sinuelo', and 'Marfim' were selected to promote the increase of essential amino acids, such as leucine, which is important for protein and adenosine triphosphate synthesis, promoting wound signaling in vegetables, as well as meiotic recombination, cell cycle progression, and embryonic and seedling development (Fowler et al., 2009). Wan et al. (2021) highlighted that amino acids play an important role in nitrogen transport, in the synthesis of reserve proteins in the starchy endosperm, and in embryonic development.

The 'BRS Parrudo', 'Marfim', and 'Quartzo' genotypes showed the highest levels of tryptophan (Figure 4). This essential amino acid is considered a precursor either of the 5-HT produced in the central nervous system, acting in the formation of proteins and metabolites (Sánchez et al., 2015), or of auxin, a hormone that promotes plant growth, functioning as a connector between the melatonin and auxin biosynthetic pathways (Woodward & Bartel, 2005).

Both 'BRS Parrudo' and 'Marfim' also potentiated glutamine, which makes their selection interesting. This metabolite, although non-essential for humans, is determinant for the synthesis of body tissues (Kim & Kim, 2017). In plants, glutamine contributes to the synthesis of chlorophyll, enhancing photosynthetic relationships, which culminates in a greater biosynthesis of assimilates and nitrogen and cytokinin compounds (Kamada-Nobusada et al., 2013).

Genotypes 'BRS Parrudo', 'Marfim', and 'Quartzo' were prioritized for selection because they maximized aspartate, a non-essential amino acid that synthesizes lysine, methionine, and isoleucine in plants (Alfosea-Simón et al., 2021), indirectly enhancing plant metabolism and leading to superior quality products. 'BRS Parrudo' and 'Quartzo' also stood out for the accumulation of proline, which plays a role in the

regulation of energy production and intracellular signaling under stressful conditions, being used by cells as a source of energy and for survival under stress (Olivares et al., 2017); therefore, these genotypes possibly show a greater resilience to water deficit.

'BRS Parrudo' presented higher levels of proline and glutamine, which are amino acids whose contents make up most part of the glutenin and gliadin proteins (Tian et al., 2015) responsible for the so-called gluten strength (W value) that gives bread dough desirable characteristics, such as viscosity, elasticity, and extensibility (Wan et al., 2021). For this reason, it can be inferred that this genotype may have a superior bread-making quality.

Furthermore, the 'BRS Parrudo' and 'Quartzo' genotypes enhanced threonine levels. This is important since a high concentration of this metabolite in grains is crucial for humans and animals – especially for

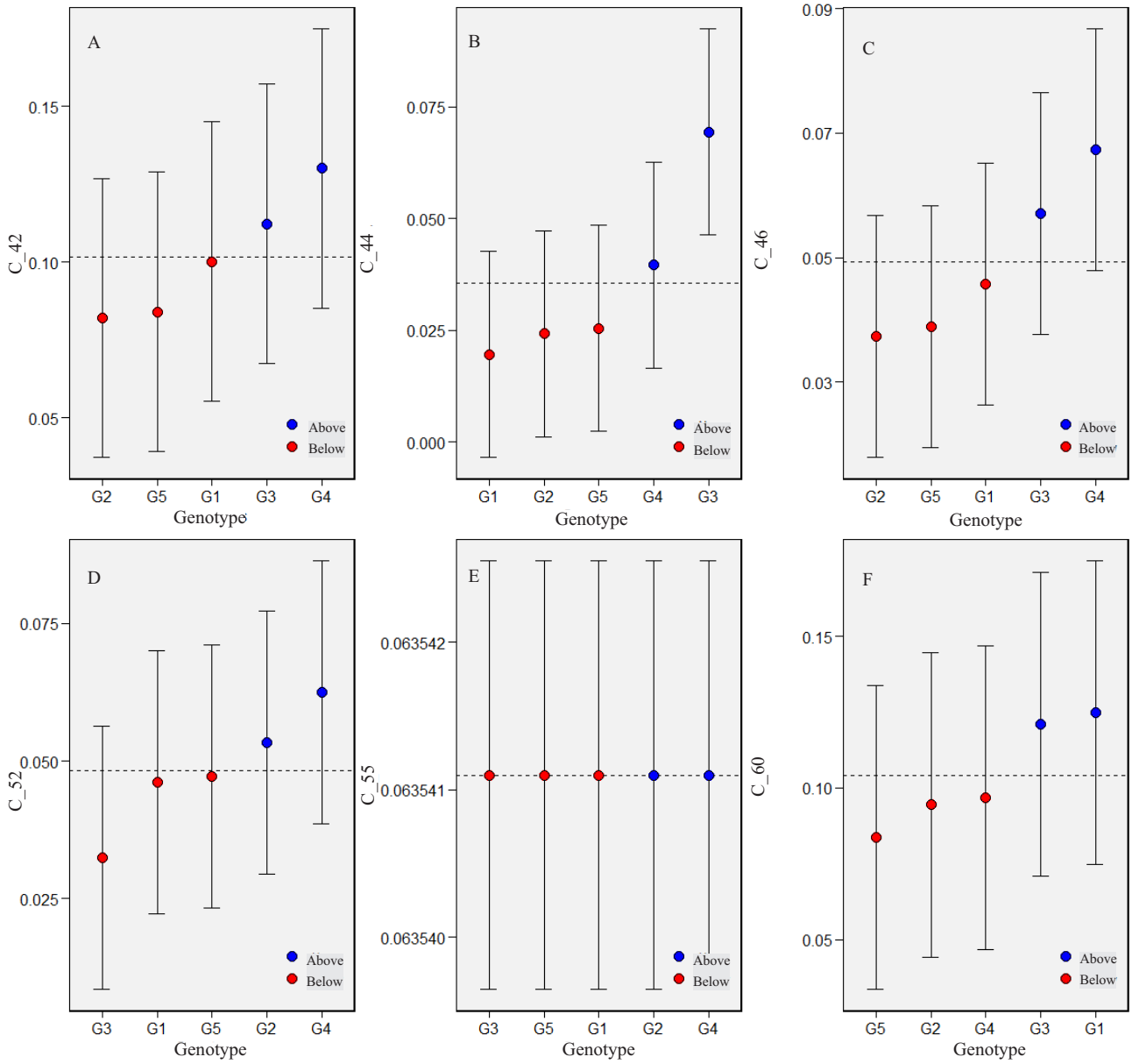

Figure 3. Predictions for the best linear unbiased predictor for the following metabolites in wheat (*Triticum aestivum*) genotypes: A, D-glucose; B, gentiobiose; C, epilactose; D, galactopiranoside; E, sucrose 2; and F, leucine. G1, 'BRS Parrudo'; G2, 'Marfim'; G3; 'Quartzo'; G4, 'TBIO Mestre'; and G5, 'TBIO Sinuelo'.

birds fed on concentrate-based diets –, promotes the sustainability of production systems, and minimizes fortification expenses and the biofortification of food and derivatives (Shewry & Hey, 2015). 'BRS Parrudo' and 'Quartzo', as well a 'Marfim', also presented higher concentrations of glycine, a non-essential amino acid generated through the catabolism of threonine (Wang et al., 2013). Although Rezaei et al. (2013) concluded that the amount of glycine synthesized is insufficient to meet the metabolic reactions of cells in animals, the accumulation of this amino acid can be enhanced in wheat through selection. This means that genotypes can act as suppliers of grains biofortified for this trait or even as a source of genes and alleles for new superior genetic constitutions in future cultivars.

Genotypes 'BRS Parrudo', 'Quartzo', and 'Marfim' are also effective in maximizing isoleucine, an essential amino acid that acts in human physiological functions,

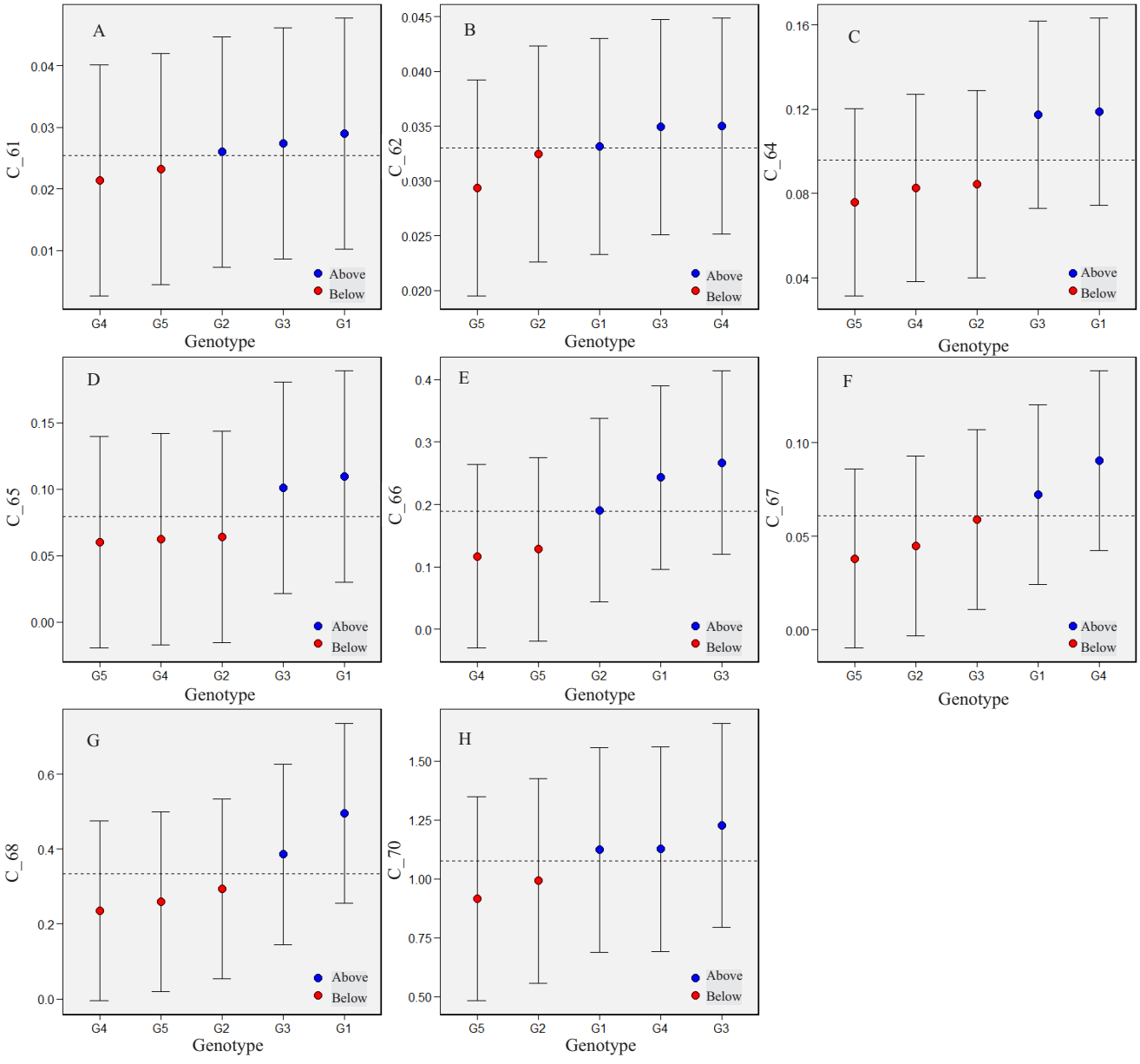

Figure 4. Predictions for the best linear unbiased predictor for the following metabolites in wheat (*Triticum aestivum*) genotypes: A, isoleucine; B, glycine; C, threonine; D, proline; E, aspartate; F, phenylalanine; G, glutamine; and H, tryptophan. G1, 'BRS Parrudo'; G2, 'Marfim'; G3; 'Quartzo'; G4, 'TBIO Mestre'; and G5, 'TBIO Sinuelo'.

including growth, immunity, protein metabolism, fatty acids, and glucose transport (Zhang et al., 2016).

The genotypes with a greater stability in essential amino acids in their grains, regardless of the environment, were also selected using different indices. The MTSI proposed by Olivoto et al. (2019) was used to select genotypes based on the stability of the expression of essential amino acids such as leucine, phenylalanine, tryptophan, and isoleucine (Figure 5). The first main component represented more than 83% of total variability and was used for this index, through which 'Marfim' was selected, making it possible to infer that the levels of essential amino acids in its grains showed the highest predictability

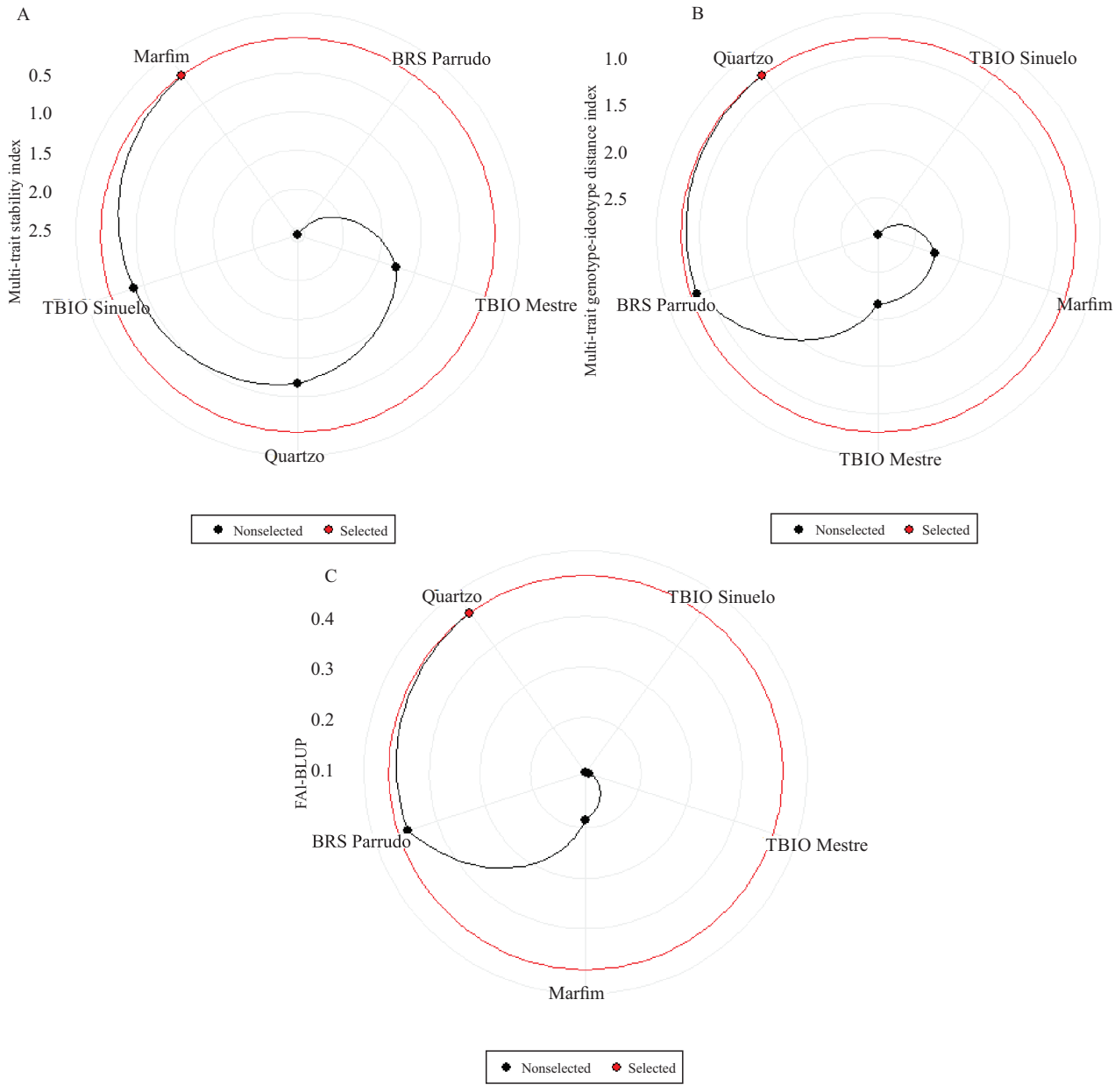

Figure 5. Classification of five wheat (*Triticum aestivum*) genotypes selected for essential amino acid levels by: A, the multi-trait stability index; B, the multi-trait genotype-ideotype distance index; and C, the multi-trait index based on factor analysis and ideotype-design (FAI-BLUP).

in the evaluated environments. The highest selection gain was 2.31% for isoleucine, whereas heritability values were 25% for isoleucine and above 45% for leucine, phenylalanine, and tryptophan, reflecting the successful selection of superior genotypes through the multi-trait approach.

The MGIDI (Olivoto & Nardino, 2021) and FAI-BLUP (Rocha et al., 2018) revealed that the first two principal components showed significantly higher eigenvalues, explaining more than 95% of variability, which is indicative of the suitability of these two indices. Both of them adopt the agronomic ideotype prioritized by the breeder, as well as the maximum standard genetic values of the leucine, isoleucine, phenylalanine, and tryptophan amino acids. The genotypes selected by these methods are characterized by stability and a high average amino acid performance in their grains.

The superior genotypes led to an average increase in leucine, isoleucine, and tryptophan. A 15% selection pressure was used to identify accurately the genotypes suitable for multi-trait selection. In general, the amino acids were successfully selected by the MGDI and FAI-BLUP; however, phenylalanine showed an unwanted selection differential of -3.94, with a higher heritability of 56% when compared with those of tryptophan, leucine, and isoleucine, which were 30.5, 30.2, and 25.4%, respectively. Both indices expressed gains of 6.24% for tryptophan, 5.83% for leucine, and 5.04% for isoleucine regardless of the cultivation environment. The obtained results are indicative of a unique and easy selection process for genotypes with a high concentration of essential amino acids in their grains. The 'Quartzo' genotype was selected due to its superior values for essential amino acids in all environments, whereas 'Marfim' showed good stability and average levels of essential amino acids.

In Brazilian subtropical wheat farming, the available genotypes provide superior genetic constitutions for grain yield and disease tolerance (Torres et al., 2022) and technological quality (Vancini et al., 2019); however, their nutritional and metabolic potential for consumer markets still needs to be better understood. This knowledge could be used to improve human diets through biofortification, especially with essential amino acids, as well as to enrich animal formulas and feeds with nutrients from the obtained grains. In the present study, metabolite accumulation in wheat grains was intrinsic to the genotype, which is attributed to changes in the composition of specific cell membrane structures and also to the metabolic processes occurring in the cytoplasm and its membranes. Therefore, each genotype has a gene expression profile that encodes proteins and enzymes that promote modifications in membrane amino acid transporters. Some genotypes show superiority in terms of the accumulation of metabolites in the endosperm of the produced grains due to the accumulation of specialized cells and, consequently, of protein in the vacuole (Wan et al., 2021).

The genotypes that were selected here for essential amino acids and metabolites of interest may promote genetic gains in breeding programs and be used to build new genetic constitutions. This finding was possible by estimating the components of variation and genetic parameters of the main genotypes used for the food industry across different cultivation environments representative of wheat farming in Brazil.

Conclusions

1. The studied wheat (*Triticum aestivum*) genotypes express a high genetic variability and selection possibility for gentiobiose, butyric acid, galactopyranosyl, phenylalanine, tryptophan, leucine, and isoleucine.

2. According to the multi-trait stability selection index, the 'Marfim' genotype remains stable for essential amino acid levels in the studied environments.

3. The 'Quartzo' genotype stands out in the expression of leucine, isoleucine, phenylalanine, and tryptophan in its grains, being selected by the multi-trait index based on the factor analysis and ideotype design and the multi-trait genotype-ideotype distance index.

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