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Selection strategy for grain quality traits in upland rice breeding programs

Estratégia de seleção para características de qualidade de grãos em programas de melhoramento de arroz de terras altas

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ABSTRACT

The evaluation of grain quality traits is essential in rice breeding programs. However, their complexity and multitude of components make the process challenging. The aim was to evaluate the industrial, physical, chemical, and cooking quality traits of upland rice grains and to identify a reduced set of factors that accurately represent these traits, facilitating the indirect selection of desirable genotypes in the breeding programs. A total of 27 genotypes from the Value of Cultivation and Use (VCU) trial of the Upland Rice Breeding Program were evaluated in four environments over two years. The traits evaluated were total yield, milling yield, chalkiness, grain size, apparent amylose content, gelatinization temperature, minimum cooking time, water absorption index, volume expansion coefficient, hardness, and stickiness. The measured values were subjected to joint analysis of variance and principal component analysis (PCA). Subsequently, phenotypic correlation coefficients and path analyses of the traits that emerged prominently in the PCA were conducted. Grain size, water absorption index, and chalkiness are independent, indicating the importance of direct selection to achieve genetic gains. Apparent amylose content and gelatinization temperature are strongly associated, allowing for indirect selection between them.

Index terms: *Oryza sativa* L.; indirect selection; principal component; path analysis.

RESUMO

A avaliação das características de qualidade dos grãos é fundamental em programas de melhoramento de arroz. No entanto, sua complexidade e multiplicidade de componentes tornam o processo desafiador. O objetivo foi avaliar as características de qualidade industrial, física, química e de cozimento dos grãos de arroz de terras altas e identificar um conjunto reduzido de fatores que as representem com precisão, facilitando a seleção indireta de genótipos desejáveis nas fases iniciais dos programas de melhoramento. Foram avaliados 27 genótipos do ensaio de Valor de Cultivo e Uso (VCU) do Programa de Melhoramento de Arroz de Terras Altas em quatro ambientes e duas safras. As características mensuradas foram renda, rendimento, gessamento, dimensão do grão, teor de amilopectina aparente, temperatura de gelatinização, tempo mínimo de cozimento, índice de absorção de água, coeficiente de expansão em volume, dureza e pegajosidade. Os valores mensurados foram submetidos à análise conjunta de variância e análise de componentes principais (PCA). Posteriormente, foram obtidos coeficientes de correlação fenotípica e análises de trilha das características que se destacaram na PCA. Dimensão do grão, índice de absorção de água e gessamento são independentes, indicando a seleção direta para obtenção de ganhos genéticos. Já teor de amilopectina aparente e temperatura de gelatinização estão fortemente associados, permitindo seleção indireta entre elas.

Termos para indexação: *Oryza sativa* L.; seleção indireta; componentes principais; análise de trilha.

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Introduction

Rice (*Oryza sativa* L.) stands as one of the most widely cultivated species globally, playing a pivotal role in human nutrition. It constitutes 20% of the daily calorie intake required by humans and serves as a rich source of proteins, minerals, and vitamins. The Asian continent accounts for 50% of the global rice consumption, with rice being a dietary staple in many developing countries. Consequently, it serves as a primary food source for over half of the world's population (Rezvi et al., 2023).

This cereal is predominantly marketed in its grain form, and ensuring the superior quality of this product not only enhances its market value but also fosters better consumer acceptance. Nevertheless, there lacks a definitive definition of rice grain quality due to the variations in preferences for grain type and appearance both before and after cooking across different countries. Each market adheres to specific quality standards, contributing to the diverse criteria for assessing rice quality (Gong et al., 2024).

In Brazil, consumer demand is predominantly for long thin rice grains, known as the "agulhinha" type, with high translucency and minimal chalkiness, as well as the absence of imperfections

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such as insect damage or disease-related blemishes, which reduce product value (Brasil, 2009). In terms of cooking properties, rice with intermediate amylose content (21–25%) is preferred, as it results in dry, non-sticky grains after cooking. Additionally, a gelatinization temperature below 73°C is desirable, as it requires less water and a shorter cooking time (Kumar, Maruyama, & Moon Kumar, 1994). The non-glutinous nature of these grains is primarily determined by the amylose-to-amylopectin ratio, with amylose content ranging from 8% to 37% (Ferreira et al., 2005; Souza & Ferreira, 2021).

While rice crop management exerts a significant influence on grain quality, it is crucial to acknowledge the impact of genetic composition on this aspect. Hence, selecting cultivars that cater to both the rice production chain and the consumer becomes paramount. The assessment of all traits related to grain quality in a plant breeding program holds fundamental significance for elite lines selection. Nevertheless, breeding endeavors targeting genotypes with superior grain quality encounter challenges due to the complexity of these traits, the genotypes and environments interaction, and the multitude of traits requiring consideration (Golam & Podhan, 2013; Jin et al., 2023).

Consequently, in breeding programs with a substantial number of genotypes, conducting multiple analyses for grain quality traits proves costly, demands specialized labor, and may render laboratory operations unfeasible. Streamlining the evaluation process by identifying genetic correlations among traits can facilitate more efficient genotype selection during the initial phases of breeding programs.

Statistical methodologies like principal component analysis (PCA) and path analysis serve as tools for decision-making in research. They enable researchers to explore both direct and indirect effects influencing traits, thereby facilitating comprehensive observations of trait behavior and their associations (Hair Júnior et al., 2009; Johnson & Wichern, 2007; Li et al., 2013; Prakash et al., 2024).

Therefore, this study aimed to assess the industrial, physical, chemical, and cooking quality attributes of upland rice grains, with the goal of identifying a reduced set of factors representing grain quality traits. Subsequently, the study sought to identify standout traits that could facilitate the indirect selection of genotypes, particularly in the early phases of breeding programs.

Material and Methods

The experiments were carried out over two years in four environments. The experiments were conducted in Lavras, Minas Gerais state, Brazil, at the Center for Scientific and Technological Development in Agriculture of the Federal University of Lavras (E1) and at experimental field of the Agricultural Research Company of Minas Gerais (E3) located at a latitude of 21°14’ 00” S, a longitude of 45°00’00” W and an altitude of 910 m. Also, in the state of Minas Gerais, at the experimental field of the Agricultural Research Company of Minas Gerais (E2) in Lambari (latitude -21°50’32”, longitude -45°21’01”, 887 m). In São Paulo state, in municipality of Registro (E4) at a latitude of 24°29’15” S, a longitude of 47°50’37” W and an altitude of 25 m. Table 1 presents the soil chemical property data obtained from the experimental areas.

Table 1: Soil chemical composition from experimental sites conducted in four environments (E) for two years.

Chemical properties		First year		Second year			
		E1	E2	E1	E2	E3	E4
pH	H_2O	5.8	5.7	5.9	6.1	6.2	5
Ca^{2+}		3.01	2.42	4.81	2.84	4.93	34
Mg^{2+}			0.82	1.19	0.96	0.9	15
Al^{3+}	Cmolc/dm³	0.11	0.1	0.06	0.1	0.04	0
$H^+ Al^{3+}$		3.82	4.88	3.24	4.34	1.98	33
SB		4.25	3.43	6.28	4	6.09	51.3
P - Rem	mg/L	42.62	34.77	26.84	37.2	13.58	-
K		155.62	72.48	107.78	78.37	102	2.3
Zn^{2+}		4.88	7.31	5.43	7.6	7.3	0.7
Mn^{2+}		14.4	25.1	45.56	24.8	92.5	0.2
Cu^{2+}	mg/dm³	0.25	1.24	0.9	1.19	2	0.2
B		0.03	0.21	0.14	0.28	0.43	0.34
Fe^{2+}		53.52	53.83	44.78	56.22	61.5	95
S		7.19	12.69	14.4	12.93	1.85	-
MO	dag/kg	2.37	3.21	3.25	3.34	3.15	-
V	%	52.65	41.23	65.93	47.96	75.46	60.9

SB: sum of bases; MO: organic matter; V: base saturation.

In the initial year, 16 lines were evaluated alongside three commercial cultivars, BRS Esmeralda, BRSMG Caçula, and BRSMG Caravera, as well as one Multiline composed of a mixture of five isogenic lines (Castro et al., 2022). In the subsequent year, 17 lines were evaluated, 11 of which were common to the first year, with the cultivars BRS Esmeralda and BRSMG Caçula and the Multiline (Table 2). All these genotypes were part of the Value for Cultivation and Use trail from the Upland Rice Breeding Program. This program is a collaborative effort involving the Federal University of Lavras (UFLA), the Brazilian Agricultural Research Corporation (EMBRAPA), and the Agricultural Research Corporation of Minas Gerais (EPAMIG).

Table 2: Genotypes from the Upland Rice Breeding Program of the Federal University of Lavras evaluated in two harvest seasons.

	First year		Second year
1	CMG 2162	21	CMG F6 LAM 20-2
2	CMG 2168	22	CMG ERF 85-14
3	BRS Esmeralda	3	BRS Esmeralda
4	CMG 2170	24	CMG ERF 221-16
5	CMG 2119	5	CMG 2119
6	BRSMG Caçula	6	BRSMG Caçula
7	CMG 2185	25	CMG ERF 85-6
8	CMG 2187	8	CMG 2187
9	CMG 2188	9	CMG 2188
10	CMG 2085	10	CMG 2085
11	BRSMG Caravera	26	CMG ERF 85-15
12	CMG ERF 221-4	12	CMG ERF 221-4
13	CMG 1511	27	CMG F6 LAV 1-7
14	CMG ERF 221-7	14	CMG ERF 221-7
15	CMG 1896	15	CMG 1896
16	CMG ERF 221-9	16	CMG ERF 221-9
17	CMG ERF 221-19	17	CMG ERF 221-19
18	CMG ERF 221-29	18	CMG ERF 221-29
19	CMG 1590	19	CMG 1590
20	Multiline	20	Multiline

The experiments were conducted in a randomized block design with three replications and plots consisted of five 4-meter rows with a spacing of 0.35 meters. The two outer rows of each plot were discarded at harvest to prevent varietal mixing. Following harvest, the rice grains were dried to approximately 13% moisture content. Subsequently, the traits were measured:

Milled Rice Yield (MRY, %): is the weight percentage of paddy rice that remains as milled rice, which includes the sum of head rice and broken (less than three-fourths kernel). This measurement was conducted using a sample of 100 grams of paddy rice.

Head Rice Yield (HRY, %): represents the weight percentage of paddy rice that remains as whole rice (three-fourths kernel or greater) after complete milling. A sample of 100 grams of paddy rice was utilized for this measurement.

Chalkiness (CH, %): estimated as the percentage of grains exhibiting opacity instead of translucency in the milled rice grain. When this condition occurs, the grain is classified as chalky. Chalkiness assessment was conducted using a 100 g sample of whole and polished grains, following the instructions and protocols outlined in Normative Instruction No. 6 (Grain Classification) issued by the Brazilian Ministry of Agriculture, Livestock, and Food Supply (Brasil, 2009).

Grain size (GS, mm): determined through image analysis using a subsample of 100 polished grains from each plot. Seed imaging was conducted using the GroundEye® system, employing high-resolution cameras for accurate capture. Following this, biometric analyses were carried out on the grains to measure their length (L) and thickness. The grains were classified as: long-thin ($L \geq 6$ mm, $T \leq 2.17$ mm, and $L/T > 2.75$), long ($L \geq 6$ mm), medium ($L < 6$ and ≥ 5 mm) and short ($L < 5$ mm).

Apparent amylose content (AC, %): calculated using a calibration curve prepared with preselected standard rice cultivars. These cultivars had known amylose contents, which were previously determined by size-exclusion chromatography/gel permeation chromatography (SEC/GPC) by the International Rice Research Institute (IRRI) (Fitzgerald, McCouch & Hall, 2009). Following this methodology, the samples underwent analysis and classification using a scoring scale (Table 3) adapted from Juliano (2003).

Gelatinization temperature (GT, °C): obtained through the alkali spreading test, which involves estimating the degree of alkali dispersion and clarification of raw rice grains when in contact with an alkaline solution. The methodology was developed by Martinéz and Cuevas (1989) and adapted by the authors. For the analysis, ten grains (whole and polished) from each sample were evenly distributed on a plastic plate with a diameter of 4.8 cm, containing 10 mL of 1.7% potassium hydroxide (KOH) solution. Subsequently, the plates were sealed and incubated in a FISHER brand oven, model 255G, at 30 °C for 23 hours. After this period, visual evaluation of the grains was performed, and based on the dissolution of each grain, it was classified on a numerical scale from 1 to 7, as described by Martinéz and Cuevas (1989) (Table 4), to determine the degree of alkali dispersion. The average value of GT for each sample was obtained by multiplying the number of rice grains by the corresponding degree of dispersion value, which was then summed and divided by ten. The standard varieties Colombia 1 (high GT), Bluebonnet 50 (intermediate GT), and IR 8 (low GT) were used as references. Analyses were performed in duplicate per sample.

Table 3: Classification of rice amylose content and corresponding culinary characteristics.

Classification	Apparent amylose content (%)	Expected characteristics after cooking
High amylose	25 – 33	Dry, loose, and firm grain
Intermediate amylose	20 – 25	Dry, loose, and soft grain
Low amylose	12 – 20	Sticky and soft grain
Very low amylose	5 – 12	Very sticky and soft grain
Waxy	0 – 4	Very soft grain

Table 4: Scale of rice degrees of dispersion and gelatinization temperature.

Degree of dispersion	Characteristics	Gelatinization temperature
1, 2 and 3	Grains unaffected by the alkaline solution	High (74 – 80 °C)
4 and 5	Grains partially disintegrate	Intermediate (69 – 73 °C)
6 and 7	Grains dissolve completely	Low (63 – 68 °C)

Minimum cooking time (MCT, min): A total of 5 grams of whole rice were weighed and added to 135 mL of boiling distilled water. After 15 minutes, three grains were placed between two glass slides and compressed. This procedure was repeated at specific time intervals until the rice was fully cooked, indicating complete gelatinization of the starch (Ciacco & Chang, 1986; Hummel, 1966; Rosário Neto et al., 2019).

Water absorption index (WAI, g): 5 g of rice grains (polished and whole) from each plot were weighed and cooked for the minimum time established by MCT. Subsequently, each sample was drained in a colander and placed on absorbent paper for five minutes. After this process, the cooked grains of each sample were weighed, and the water absorption index was calculated using the equation adapted from Hummel (1966) and Donelly (1979), $WAI = \frac{Cooked\ Rice\ Sample\ Weight\left(g\right)}{Raw\ Rice\ Sample\ Weight\left(g\right)}$.

Volume expansion coefficient (VEC, ml): two samples of 5 g of rice grains (polished and whole) from each plot were weighed, one with raw rice grains and the other cooked for the MCT. Subsequently, the volume of 50 mL of kerosene was measured in a graduated cylinder, and then the raw rice sample was placed inside the cylinder, allowing for visualization of the volume displaced by the rice grains. This procedure was repeated for the cooked rice grains. The calculation of the volume expansion coefficient was performed using the equation adapted from Ciacco and Chang (1986) and Donnelly (1979), $VEC = \frac{Volume\ displaced\ by\ the\ cooked\ rice\left(ml\right)}{Volume\ displaced\ by\ the\ raw\ rice\left(ml\right)}$.

Instrumental texture analysis - hardness (HAR) or stickiness (STI): conducted on cooked rice grains with three readings for each plot. Approximately 10 g of rice grains (polished and whole) and 17 g of distilled water were placed in a Petri dish and cooked in an electric cooker (Cadence®, 2 L capacity) with 350 mL of water for 30 minutes. Subsequently, ten whole grains were evenly distributed on the base of the texture analyzer for the evaluation of mechanical properties, hardness and stickiness, using the Texture Analyser Stale Microsystems (TA-XT Plus, Surrey, England), equipped with a 50 kg load cell, a cylindrical probe of 40 mm, pre-test speed of 0.5 mm.s^{-1}, test speed of 0.5 mm.s^{-1}, post-test speed of 0.5 mm.s^{-1}, a return distance of 5 mm, and a Trigger of 3gf (0.04903 N). The uniaxial compression method was employed, using 10 intact rice grains per reading (Carvalho et al., 2015; Sesmat & Meullenet, 2001).

Data was analyzed using mixed model approach with R software (R Core Team, 2019). The variance components were estimated by restricted maximum likelihood (REML), and genotypic values were predicted by best linear and unbiased predictor (BLUP) from joint analyses according to the mixed linear model Equation (1):

$$y = X_b + Z_g + W_c + V_d + e\ \#1 \tag{1}$$

Where *y*: data vector; *b*: vector of the fixed effects of environment-year combinations; *g*: vector of the effects of genotypes, where $g \sim \text{NMV}\left(0, \sigma_g^2\right)$ and σ_g^2 is the genetic variance of genotypes; *c*: vector of the genotypes x environment-year interaction effects, where $c \sim \text{NMV}(0, \sigma_c^2)$ and σ_c^2 is the variance of the effects genotypes x environment-year interaction; *d*: is the vector of the block within the environment-year effects, where $d \sim \text{NMV}(0, \sigma_d^2)$ and σ_d^2 is the variance of the effects of the block within the environment-year combination; *e*: vector of error effects, where $e \sim \text{NMV}(0, \sigma_e^2)$ and σ_e^2 is the error variance; *X*, *Z*, *W* and *V*: incidence matrices for the effects *b*, *g*, *c* and *d* respectively.

Before fitting the mixed models, statistical assumptions were verified. Residual diagnostics were performed to evaluate the normality and homoscedasticity of residuals. The visual assessments indicated no major deviations from normality or heteroscedasticity. These results validate the use of REML for variance component estimation and BLUP for genotypic predictions, as also supported by Moura et al. (2021).

The significance of the variance components was checked by the likelihood-ratio test (LRT) at 1% probability associated with the deviance analysis. Selective accuracies ($r_{\hat{g}g}$) associated with predicted genetic values were estimated from the following expression: $r_{\hat{g}g} = \sqrt{1 - \frac{\overline{PEV}_g}{\sigma_g^2}}$, in which PEV is the mean variance of the prediction error of the genetic values (Resende & Alves, 2022; Resende & Duarte, 2007). The experimental precision was estimated based on the coefficient of variation ($CV\%$), by the equation, $CV\% = \frac{\sqrt{\sigma_e^2}}{\bar{x}} \times 100$, where σ_e^2 is the error variance and $\bar{x}$ is the overall mean.

To investigate the relationship between the evaluated traits, Principal Component Analysis (PCA) was conducted, following these procedures:

a) Heritability and coheritability matrix;

b) Spectral decomposition: square matrix factorization: $h^2 = V\Lambda V'$, where V is an eigenvector and Λ is an eigenvalue;

c) The principal components were obtained by multiplying each eigenvector by the root of each corresponding eigenvalue: $PC = (V\Lambda^{1/2})$, where V is an eigenvector and Λ is an eigenvalue.

Based on the PCA results, path analyses were performed. These analyses were carried out using Genes software (Cruz, 2006). The effects of the path coefficients were interpreted based on the phenotypic correlation coefficient (r_{xy}) according to Dhurai, Reddy and Ravi (2016) and Priya et al. (2017), using the expression: $r_{xy} = \frac{COV_{xy}}{\sqrt{\sigma_x^2 \times \sigma_y^2}}$, in which r_{xy} is the phenotypic correlation between x and y traits, COV_{xy} is the phenotypic covariance between x and y traits and σ_x^2 and σ_y^2 are the phenotypic variance of the trait x and y, respectively.

Results and Discussion

The variance among genotypes was significant ($p \leq 0.01$) by LRT for CH, GS, AC, GT, MCT and HAR traits, indicating the possibility of selecting superior upland rice lines for these traits (Table 5). It was not possible to observe genetic variability among the evaluated lines for the traits MRY, HRY, WAI, VEC, and STI. This is expected for some traits since the lines are derived from the final phase of the breeding program, the Value of Cultivation and Use (VCU) trial, and some materials originated from the same population of biparental crosses.

Experimental precision, measured by the coefficient of variation (CV%), which depends solely on the residual variation as a proportion of the experiment mean, showed satisfactory values (~ 20%) for most traits, indicating good experimental precision (Pimentel-Gomes, 2022).

Accuracies from 0.38 (WAI) to 0.98 (GS) were observed in this experiment. In plant breeding experiments, the precision of genetic predictions depends on accuracy, a crucial indicator of selection reliability. Notably, this parameter is directly related to genetic variability. Considering the traits where this parameter had a significant value ($p \leq 0.01$), accuracy ranged from 0.76 (MCT) to 0.94 (GS), indicating, according to Resende and Duarte (2007), high precision in genotypic evaluation. Moreover, for these traits, heritability also displayed higher magnitude values (≥ 57,40%). GS exhibited high heritability, at 96.09%. Indeed, high heritability for GS has been found in other studies (Adjah et al., 2020; Nirmaladevi et al., 2015; Singh et al., 2021). Heritability is a measure of the inheritance of a trait (Ramalho et al., 2024), and the higher its value, the greater the influence of genetic variance and lower is the environmental influence.

Significant effects ($p \leq 0.01$) were found in the genotype x environment-year interaction for most traits, MRY, HRY, CH, GS, AC, GT, MCT, meaning that the lines exhibited different behaviors in different environments. It is worth noting that, in addition to the variation within each evaluation site, there was also an intrinsic effect of the different years in which the experiment was conducted. The genotype x environment interaction is a challenge for the release of cultivars adapted to a wide region (Ramalho et al., 2024). Therefore, the breeder should use strategies to select lines targeted for a specific environment or expand studies on adaptability and stability.

When evaluating many genotypes, measuring various traits that encompass physical, chemical, industrial, and cooking qualities for rice grain selection becomes challenging and expensive. Therefore, to streamline the trait evaluation process in a rice breeding program, Principal Component Analysis (PCA) was conducted using the heritability and coheritability matrix, aiming the reduction of evaluated traits by transforming them into a set of correlated traits.

The contributions of the first two PCs to the total variance were 19% and 32.1%, respectively, for a cumulative contribution of 51.1% (Figure 1). From the PCA biplot, the traits AC, GS, GT, CH, and WAI are positioned far from the origin of the plot, where PC1 and PC2 intersect. This spatial distribution indicates that these traits exhibit distinct and independent selection gains compared to the other traits, which are grouped together. Consequently, breeders should ideally conduct separate selection processes for AC, GS, WAI, CH, and GT.

Moreover, these traits are located farther from the origin, indicating a greater contribution to the total variation among genotypes. Their positioning in different directions further reflects their relative independence. AC and GS display distinct patterns, forming an approximate 90° angle with each other, suggesting that gains from selecting these traits occur independently. Conversely, AC and WAI, exhibit inverse selection gains with GT and CH, as they form an angle of approximately 180° between them. AC and WAI, being in the same direction relative to the origin, demonstrate similar behaviors; thus, gains in AC correspond to gains in WAI. This relationship holds true for GT and CH as well. It is worth noting that all the traits that stood out in the PCA, except for WAI, have high heritability, favoring the use of these traits in the selection of genotypes with good grain quality.

Traits such as MRY, HRY, HAR, VEC, MCT, and STI are grouped close to the origin and clustered together, indicating a limited contribution to variability. This suggests that these traits are genetically similar and may share a common background, possibly due to prior selection and shared ancestry. Their proximity also indicates high redundancy, meaning they provide overlapping information for genotype differentiation. For plant breeders, these spatial relationships are valuable for indirect selection. Traits that are well-separated and contribute strongly to principal components should be prioritized when selecting genotypes with specific quality profiles. Similar findings were reported by Bao (2014) and Hori and Sun (2022), who demonstrated that traits related to grain milling and post-harvest behavior often exhibit strong correlations due to their shared dependence on grain integrity and starch structure. Fitzgerald McCouch and Hall (2009) also highlighted that traits such as head rice yield and milling efficiency are influenced by physical grain traits established early in grain development. Therefore, the grouping of these traits in PCA supports the decision to exclude them from subsequent analyses, allowing breeders to focus on traits with greater discriminative power and strategic value.

The correlation coefficient measures the statistical linear relationship between two traits. For the traits that stood out in the PCA, their relationships were evaluated using the phenotypic correlation coefficient. The magnitudes of the significant correlations ranged from -0.51 to 0.57 (Table 6). CH was positively correlated with AC and GT, and since they are also positively correlated, this indicates that, in principle, selecting for any of these traits could lead to gains in the others through indirect selection. It is worth mentioning that, to achieve higher grain quality, lower values are necessary for all three traits.

A negative correlation can be observed between CH and WAI, as well as between GS and both AC and GT. This type of correlation has been reported in other studies and suggests that the genes controlling these traits can favor one over the other (Cruz, Regazzi & Carneiro, 2012; Hori & Sun, 2022; Kumar, 2015).

To indirect selection of traits, the combination of correlation coefficients and path analysis seems to be more effective. In this context, path analysis helps to confirm the estimated direct and indirect effects of the explanatory traits on the primary response trait. Five path analyses were conducted using the traits that showed distinct selection gains in the PCA (AC, GT, CH, GS, and WAI). The coefficients of determination (R^2) for the path analysis models ranged from 0.27 to 0.45, indicating that 26.92% to 44.68% of the variation in the main trait was explained by the explanatory traits (Figure 2A-E).

CH had a strong direct influence on AC (0.4572, Figure 2A) and AC had a positive direct effect (0.4650, Figure 2E) on CH, with an estimated phenotypic correlation of 0.57 (Table 6). The two traits also had a positive effect on GT, with values of 0.0303 (CH) and 0.2881 (AC) (Figure 2C).

The trait WAI (-0.3203) had opposite effect on CH (Figure 2C), resulting in estimated phenotypic correlation value of -0.44 (Table 6). The same trend was observed for WAI, where CH and GT exhibited negative direct effects, with values of -0.4482 and -0.3707, respectively (Figure 2B). These contributed to negative estimates of the phenotypic correlation coefficient, -0.5087 for GT and -0.4448 for CH (Table 6).

Table 5: Estimates of genetic and environmental parameters for grain quality traits as milled rice yield (MRY, %) head rice yield (HRY, %), chalkiness (CH, %), grain size (GS, mm), apparent amylose content (AC, %), gelatinization temperature (GT, °C), minimum cooking time (MCT, min), water absorption index (WAI, g), volume expansion coefficient (VEC, ml), hardness (HAR) and stickiness (STI), evaluated in 27 upland rice lines in six environment-year combination.

	MRY	HRY	CH	GS	AC	GT	MCT	WAI	VEC	HAR	STI
σ_g^2	0.524	7.010	54.410*	0.040*	0.373*	0.038*	0.505*	3.639	91.470	33.818*	0.414
σ_{ga}^2	1.943*	39.180*	48.890*	0.004*	0.126*	0.027*	0.507*	23.935	433.140	1.175	0.000
σ_e^2	4.086	52.760	40.310	0.005	0.708	0.098	2.676	197.753	4227.100	160.051	5.737
$r_{\hat{g}g}$	0.62	0.57	0.87	0.98	0.87	0.82	0.76	0.38	0.42	0.94	0.67
CV(%)	2.90	21.21	42.75	2.08	3.87	9.77	2.85	4.85	16.86	53.54	41.23
h^2(%)	38.49	32.88	76.29	96.09	76.28	66.76	57.40	14.34	17.45	88.14	44.60

*Significant by the likelihood-ratio test (LRT) at 1%; σ_g^2: genetic variance; σ_{ga}^2: variance of genotypes x environment-year interaction; σ_e^2: error variance; $r_{\hat{g}g}$: selective accuracy; h_a^2: broad sense heritability.

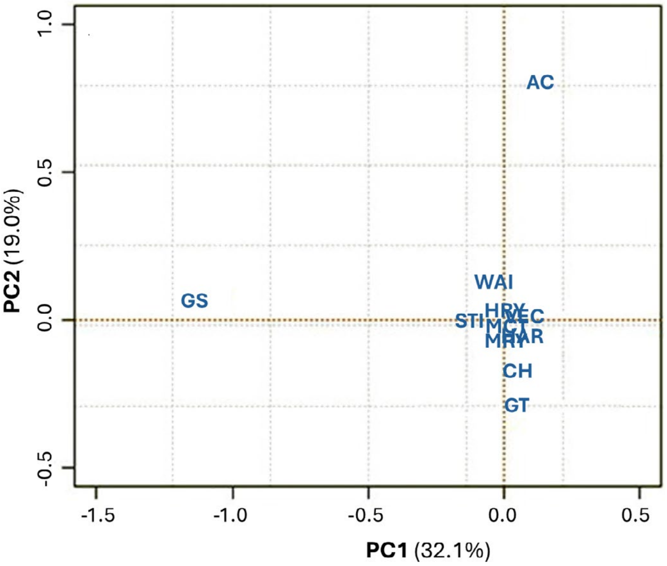

Figure 1: Biplot of the two principal components (PC1 and PC2) for the Analysis of the Principal Component of the rice grain quality traits, milled rice yield (MRY, %), head rice yield (HRY, %), chalkiness (CH, %), grain size (GS, mm), apparent amylose content (AC, %), gelatinization temperature (GT, °C), minimum cooking time (MCT, min), water absorption index (WAI, g), volume expansion coefficient (VEC, ml), hardness (HAR) and stickiness (STI) based on the evaluation of 27 lines over two years, in three locations.

Table 6: Phenotypic correlation coefficient for the evaluation of 27 lines in six environment-year based on BLUP predictions for chalkiness (CH), grain size (GS), apparent amylose content (AC), gelatinization temperature (GT) and water absorption index (WAI).

	GS	AC	GT	WAI
CH	-0.3456	0.5685*	0.4395*	-0.4448*
GS		-0.4413*	-0.4465*	0.2399
AC			0.4809*	-0.2084
GT				-0.5087*

Significant at 5% probability by Student's t-test.

In contrast, for GT, CH and AC showed positive direct effects of 0.0303 and 0.2881, respectively (Figure 2C). Overall, the indirect effect was positive, contributing to positive estimates of phenotypic correlation, 0.4395 for CH and 0.4809 for AC (Table 6).

Regarding GS, the traits that resulted in negative direct effects were AC (-0.2651) and GT (-0.2849) (Figure 2D). These effects, combined with the negative direct effects of other traits, led to negative estimates of phenotypic correlation, with values of -0.4413 and -0.4465, respectively (Table 6).

The positive correlation observed between AC and GT, can be explained by the behavior of starch in the grains. AC can affect the physicochemical properties of rice; higher amylose content tends to result in harder grains after cooking (high GT), while lower amylose content leads to a softer texture after cooking (low/intermediate GT) (Mingotte, Hanashiro & Fornasieri Filho, 2012; Ferreira & Barrigossi, 2021). Additionally, the chain length of amylopectin also influences GT, showing a negative correlation with short-chain amylopectin, whereas a positive correlation is observed between GT and long-chain amylopectin (Chávez-Murillo et al., 2012; Wang et al., 2023). This association is desirable because both AC and GT are important traits for grain selection based on cooking quality. Brazilian breeding programs aim to maintain an intermediate amylose content (21–25%) and a lower/intermediate gelatinization temperature (Ferreira et al., 2005; Ferreira & Barrigossi, 2021).

In principle, if selecting for CH, it is possible to achieve gains for AC and GT through indirect selection. This relationship exists because chalky grains not only exhibit opaque areas in the endosperm but also contain starches with lower amylose content and higher amounts of short-chain amylopectin compared to translucent grains (Xi et al., 2016). Additionally, high temperatures during grain filling, a favorable condition for the emergence of chalky grains, can influence the biosynthetic enzymes of starch and alter its granules. Temperature can affect both the accumulation rate and properties of starch (Ashida, Iida, & Yasui, 2009; Beckles & Thitisaksakul, 2014; Gann et al., 2023; Lin et al., 2016; Zhou et al., 2015). These findings are consistent with the PCA results, where selecting for CH leads to a decrease in GT score and an increase in AC, representing positive gains for breeding programs for high-quality rice grains.

On the other hand, a decrease in WAI leads to a higher occurrence of chalky grains (CH) and higher GT scores, which is undesirable. The positive correlation between WAI and GT is consistent with previous findings that higher gelatinization temperatures are associated with slower water absorption and starch swelling, which often results in greater water uptake over prolonged cooking periods (Calingacion et al., 2014; Champagne et al., 2010; Hori & Sun, 2022). Rice with high GT requires more heat energy for starch granules to gelatinize, which enhances water absorption capacity under standard cooking conditions (Kennedy & Burlingame, 2003). As for the positive correlation between CH and GT, studies indicate that higher GT is often observed in genotypes with immature or loosely packed endosperms, which also tend to present higher chalkiness (Fitzgerald, McCouch & Hall, 2009; Hori & Sun, 2022). Chalkiness is linked to irregular starch granule packing, which can delay or alter gelatinization behavior, causing a shift toward higher gelatinization temperatures (Bhat & Riar, 2016). This relationship may also reflect the influence of environmental factors such as high temperatures during grain filling, which affect both CH and GT expression (Lur et al., 2022).

Moreover, chalky grains may exhibit uneven water penetration, requiring higher cooking temperatures to achieve uniform gelatinization across the endosperm (Custódio et al., 2023; Garcia et al., 2011). Consequently, the association between WAI, CH, and GT appears to be physiologically interconnected, influenced by starch structure, granule arrangement, and physical grain integrity.

In the concluding path analysis, where GS was utilized as the main variable, it was demonstrated that this trait lacked positive correlations with any other trait. This finding is consistent with the results obtained from PCA, suggesting that GS operates independently. As a result, it is recommended that this trait be assessed from the beginning of rice breeding programs. Due to its reliance on image processing and lack of necessity for specialized labor, GS analysis becomes feasible for evaluating many genotypes, providing rapid results with high precision.

Furthermore, the negative correlation observed between GS and AC/GT in our study suggests that selecting longer or larger grains (higher GS) tends to reduce AC and GT, leading to a softer texture (Fitzgerald, McCouch & Hall, 2009). This trait may be desirable in breeding programs targeting markets that prefer softer and more cohesive rice, such as Japan and parts of Asia. However, in regions like Brazil, where consumers strongly prefer firm and non-sticky grains, selection for increased GS must be carefully managed to prevent undesirable reductions in AC and GT (Calingacion et al., 2014; Ferreira & Barrigossi, 2021). Consequently, this negative correlation imposes constraints on the simultaneous selection of GS with AC and GT, emphasizing the need for balanced breeding strategies. Breeders should consider market-specific preferences and may adopt restricted selection models or weight selection indices to optimize the improvement of these antagonistic traits (Ramalho et al., 2024; Cruz, Ragazzi & Carneiro, 2012).

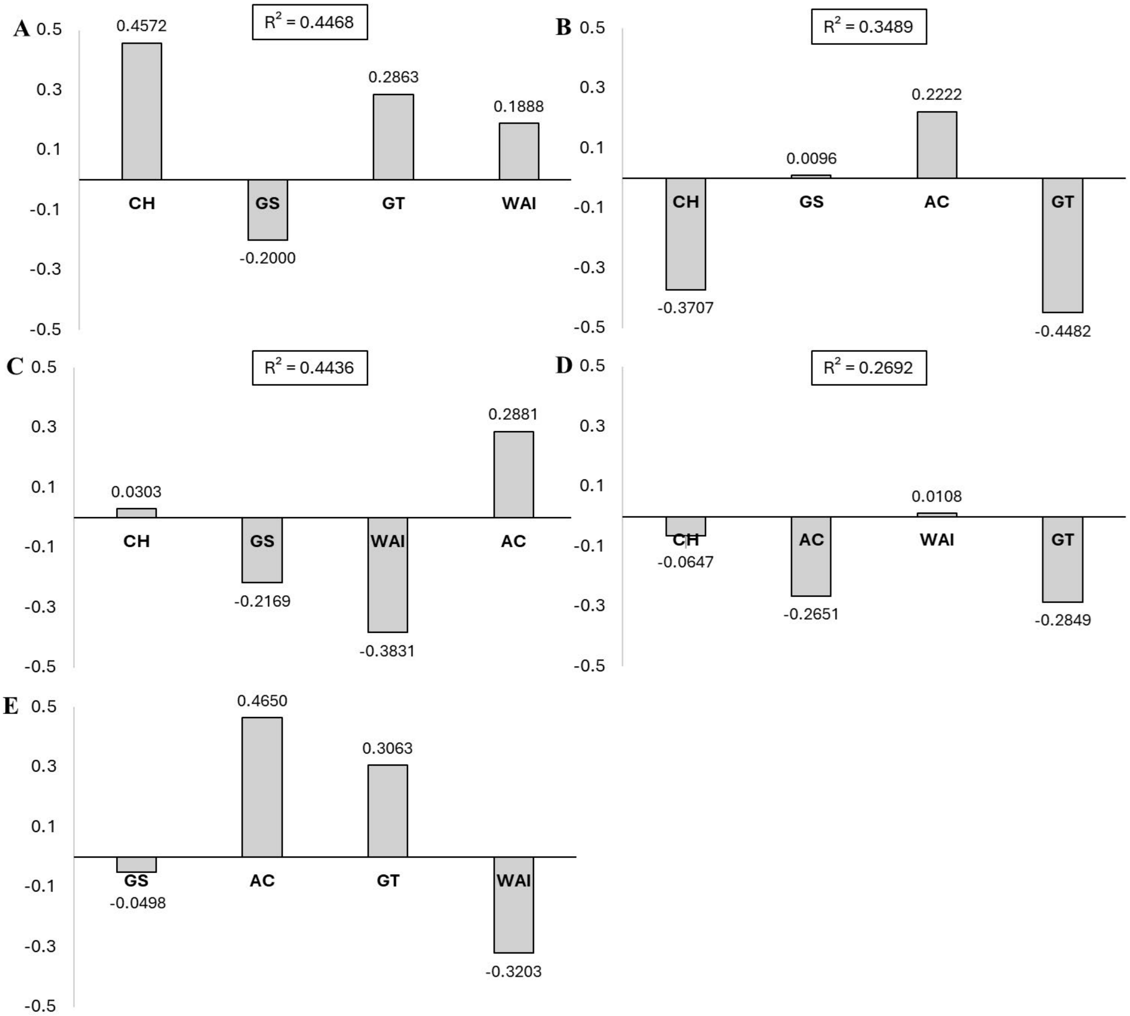

Figure 2: Estimates of the direct effects of path coefficients, derived from phenotypic correlations, for the (A) apparent amylose content (AC), (B) water absorption index (WAI), (C) gelatinization temperature (GT), (D) grain size (GS), and (E) chalkiness (CH) of upland rice grains based on the evaluation of 27 lines over two years, in three locations.

Conclusions

GS, WAI, and CH are independent traits, allowing direct selection. AC and GT are strongly associated, enabling indirect selection. Direct selection for GT reduces CH. Selecting AC and WAI directly enhances grain softness, water absorption, and expansion after cooking. An increase in GS corresponds to a reduction in TG and AC, highlighting the need for breeders to carefully balance consumer preferences with market demands. The results indicate the possibility of reducing the number of traits evaluated in rice breeding programs.

Authors' Contributions

Conceptual idea: Bassinello, PZ; Botelho, FBS. Methodology design: Silva, CSC da; Bassinello, PZ; Botelho, FBS. Data collection: Silva, CSC da; Berchembrock, YVB; Moura, AM de. Data analysis and interpretation: Silva, CSC da; Bassinello, PZ; Mendes, CTE; Botelho, FBS. Manuscript writing: Silva, CSC da; Berchembrock, YVB; Lima, GAS; Pereira, JS; Botelho, FBS. Manuscript editing: Silva, CSC da; Berchembrock, YVB; Lima, GAS; Pereira, JS; Botelho, FBS.

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