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Unraveling the genetic diversity of coffee processing traits in *Coffea canephora*

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ABSTRACT: With advancements in coffee cultivation, several traits may be considered in selection of plants, which must exhibit a set of favorable characteristics. The outturn index, defined as the relationship between the mass of mature fruit and processed beans, emerges as a key factor influencing productivity. This study characterized the outturn over two harvests of 57 clones marketed in the public domain and 10 registered cultivars. The analysis considers the effects of genotypes, measurements, and genetic progress achieved through plant selection. According to the maturation cycle of each clone, washed samples of cherry coffee were collected considering a completely randomized factorial design for characterization of the effects of genotypes, years, and the genotype × years (GY) interaction. The drying, peeling, and the outturn index were individually assessed. Despite the significant effects of the GY interaction, genotypes demonstrated minimal alterations in their ranking across measurements. Clones with higher outturn, including LB30, BRS1216, LB12, N7, LB10, LB20, BRS3220, and AS5, exhibited an average outturn of 25.51%, reflecting a gain of 12.17%. Conversely, clones with lower outturn, such as BG180, GJ30, GJ20, AS7, AS10, P42, N1, and P60, had an average of 19.15%, indicating a reduction of 14.02% compared to the general mean. Analyzing the distribution of the outturn values, 19.4%, 62.7%, and 17.9% of genotypes were classified as high, medium, and low outturn, respectively, providing valuable insights for optimal cultivation strategies.

Key words: Conilon, Robusta, outturn index, plant selection, breeding.

Diversidade genética das características de processamento do café em *Coffea canephora*

RESUMO: Com o avanço da cafeicultura, múltiplas características devem ser consideradas na seleção de plantas, que devem apresentar um conjunto de atributos favoráveis. O rendimento, definido como a relação de massas entre os frutos maduros e os grãos processados, é um importante componente da produtividade. Este estudo tem como objetivo caracterizar o rendimento ao longo de duas colheitas de 57 clones comercializados em domínio público e 10 cultivares registradas. A análise considera efeitos de genótipos, medições e estimativas de ganhos com a seleção. De acordo com o ciclo de maturação de cada clone, amostras de café cereja foram colhidas considerando um delineamento fatorial inteiramente ao acaso para a caracterização dos efeitos de genótipos, anos e interação genótipo × anos (GA). A secagem, o descascamento e o rendimento foram avaliados individualmente. Apesar dos efeitos significativos da interação GA, os genótipos apresentaram pequenas mudanças no ordenamento de uma medição para a outra. Clones com maior rendimento, entre eles, LB30, BRS1216, LB12, N7, LB10, LB20, BRS3220 e AS5 apresentaram rendimento médio de 25,51% associado a estimativas de ganho de 12,17%. Por outro lado, clones de menor rendimento, como BG180, GJ30, GJ20, AS7, AS10, P42, N1 e P60 tiveram uma média de 19,15% associada a uma redução de 14,02%. A partir da distribuição dos valores, 19,4, 62,7 e 17,9% dos genótipos apresentaram rendimento classificados como alto, médio e baixo, subsidiando o melhor cultivo dos genótipos avaliados.

Palavras-chave: Conilon, Robusta, rendimento, ganho de seleção, melhoramento.

INTRODUCTION

For many years, coffee plants of the *Coffea canephora* species have borne the unfortunate stigma of being associated with lower-quality produce, resulting in depressed market prices (VIENCZ et al., 2023). Consequently, producers have often opted for cost-minimization strategies, historically contributing to the cultivation of coffee characterized by inferior quality and indistinct characteristics (OLIVEIRA & ARAÚJO, 2015).

However with increased adaptability to tropical climates, the cultivation of this coffee species has undergone a transformative modernization process. This transformation has been facilitated by the adoption of advanced management practices and the cultivation of highperformance genetic materials (PARTELLI et al., 2021; FERRÃO et al., 2021). The evolution of coffee farming in the Amazon region finds a noteworthy example in Rondônia, marked by a substantial reduction in cultivated area and a

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remarkable surge in productivity. Over the period from 2001 to 2023, there was a staggering 78% reduction in cultivated area (from 318,000 to 70,000 hectares) and an approximately fourfold increase in productivity (from 9.0 to 50.3 bags of processed coffee per hectare) (ESPINDULA et al., 2022; CONAB, 2023). This enhanced cultivation efficiency is intricately linked to the improved management of coffee plantations and the judicious selection of genetically superior materials.

Clones cultivated in the Western Amazon region are distinguished by their hybrid nature, arising from the cross-pollination of plants from the Conilon and Robusta botanical varieties (OLIVEIRA et al., 2018; ROCHA et al., 2015). These botanical varieties exhibit complementary agronomic traits. The Conilon botanical variety, originating from regions near sea level in the African continent is characterized by its smaller stature, heightened susceptibility to pests and diseases, and greater resilience to water deficits (MONTAGNON et al., 2012; BERTHAUD & CHARRIER, 1998). Similarly sourced from equatorial forest regions of Africa, the Robusta botanical variety presents complementary characteristics, including a larger stature, lower resistance to water deficits, and increased resilience to pests and diseases (MONTAGNON et al., 2003). In Amazon region, Conilon botanical variety seeds were introduced by local coffee farmers, while Robusta botanical variety seeds from the Campinas Agronomic Institute (IAC) were disseminated by Embrapa (ESPINDULA et al., 2022).

As coffee farming has modernized, additional facets of production have come under scrutiny (PARTELLI et al., 2022; ALKIMIM et al., 2021). A new cultivar must possess a variety of advantageous characteristics to be considered worthwhile (SPINELLI et al., 2018). In this context the outturn index, which represents the relationship between the masses of harvested ripe fruits and processed beans, may emerge as a key determinant of productivity (FIALHO et al., 2022). This fruitto-bean ratio may significantly influence crop productivity, as genotypes with similar productive potential may exhibit substantial variations in fruit husk content and moisture content (LOURENÇO et al., 2022; ROCHA et al., 2015).

Two distinct stages are part of the processing of fruits: drying and hulling. In the first stage, during drying, the fruit loses most of its water allowing the identification of plants that retain lower water content in their fruits. In the subsequent

hulling stage, the dried cherry fruit is pulped, and the study of weight reduction at this stage allows inference about the relationship between husk and beans content. Through these two stages, the mass ratio between harvested coffee and processed beans can be estimated.

In the literature, few studies have dedicated themselves to studying this characteristic. Intrinsic outturn considers only the mass reduction that occurs during hulling, disregarding the drying of the fruits. GASPARI-PEZZOPANE et al. (2004) and VACARELLI et al. (2003) evaluated the intrinsic outturn of 79 and 138 genotypes, respectively, observing variations of 48% to 62% in the weight retained after pulping. When evaluating diversity between different species, GASPARI-PEZZOPANE et al. (2004) observed a higher outturn in *C. canephora* coffee plants compared to *Coffea arabica*.

Considering the hulling and drying stages, PARTELLI et al. (2021) observed a range from 21.7% to 31.2% in the evaluation of 43 genotypes in the Nova Venécia-ES environment, categorizing them into four distinct clusters. Also, in the context of the drying and pulping stages, LOURENÇO et al. (2022) scrutinized the outturn index of Amazonian Robusta cultivars. These researchers recorded average values of mass after drying and hulling of 46.12% and 53.01%, respectively, with an average outturn index of 24.4%. This study considered estimates of genetic parameters and experimental precision.

In light of this situation, the objective of this study is to comprehensively characterize the outturn index evaluated over time for registered cultivars and clones marketed in the public domain. This investigation takes into account the nuanced effects of genotypes, measurements, and the genetic progress achieved through the cultivation of selected genotypes.

MATERIALS AND METHODS

In January 2019, a clonal competition trial was set up in the experimental field of the Empresa Brasileira de Pesquisa Agropecuária (Embrapa) at 8o 48'05.5'' S and 63o 51´02.7'' W at 88 m above sea level. The predominant climate in the region is tropical rainy with a dry winter, type "Am" (Köppen), with a mean temperature of 26.0 °C and mean annual rainfall of 2095 mm. September is the hottest month of the year (27.1 °C) and May is the coldest month (24.9 \degree C) (ALVARES et al. 2013). Crop treatments were C) (ALVARES et al., 2013). Crop treatments were carried out according to ESPINDULA et al. (2022).

During the period of flowering, bean formation, and maturation of the fruit, climate data were recorded in Ambient Weather WS2902 smart weather stations. The climate data and soil chemical properties are showed elsewhere (LOURENÇO et al., 2022).

The experiment extended over 36 months to consider both genotype and year effects, also known as measurements. To evaluate the impact of genotypes and measurements, as well as the interaction between these factors, a fully randomized experimental design with six replications was employed. In this study, 67 genotypes were assessed (Table 1), including registered cultivars (10) and clones marketed in the public domain, widely cultivated in the Amazon region (57). These cultivars, bearing the 'BRS' prefix, are categorized into three distinct compatibility

Table 1 - Identification of 67 genotypes studied based on their cultivation status and origin: whether they are cultivars or commercially available in the public domain. Mass ratios between ripe cherry fruits and processed grains were assessed in two harvests over 36 months, taking into account the stages of drying and peeling. The study was conducted at the Embrapa Rondônia experimental field in Porto Velho, RO.

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groups and exhibit diverse maturation cycles, including early, intermediate, and late maturation stages (ROCHA et al., 2021; TEIXEIRA et al., 2020).

Considering the maturation cycle of each genotype, harvesting occurred when approximately 80% of the fruits reached the red-cherry stage. Unique samples of washed cherry coffee were collected for each clone and naturally sun-dried on elevated drying beds for 10 to 15 days until achieving a moisture content of around 12%. Moisture content was assessed using a Gehaka (G600) grain moisture meter. Subsequently, 250 g samples of dry cherry coffee underwent processing with a manual coffee pulping machine, followed by separation using a set of sieves. Outturn index estimates were computed based on the reduction in weight observed during drying and pulping, adjusted to 12% moisture. The estimates for the outturn index were derived using the subsequent expression:

$$
Output turn index = \left(\frac{m_{dry \, cherry}}{m_{from \, field}}\right) \cdot \left(\frac{m_{beans}}{m_{beans} + m_{hull}}\right) \cdot F_{moist12\%}
$$
\n
$$
(1)
$$

where Outturn index was estimated by the relation between the mass of the dry cherry coffee (*^mdry cherry*) and the mass of the coffee from the field (*^mfrom field*), together with the relation between the mass of the coffee beans (*^mbeans*) and the mass of the dried fruits (*^mbeans* + *^mhull*), corrected to 12% moisture (*^Fmoist12%*).

The significance of the clone effects and the homogeneity of residual variances was verified before the combined analysis to quantify the GY interaction effect, according to the following model described by CRUZ et al. (2013).

$$
Y_{ijk} = m + G_i + Y_j + GY_{ij} + e_{ijk}
$$
\n⁽²⁾

where *Yijk* refers to the observation of the i*th* genotype in the j*th* measurement, *m* is the experimental average, G_i is the effect of the ith genotype, E_j is the effect of the jth measurement, GY_{ii} is the effect of the interaction between the ith genotype and the jth measurement, and e_{ijk} is the experimental error.

From the estimates of the mean square expected values, repeatability was estimated as follows (CRUZ et al., 2021):

$$
r = \frac{COV(Y_{ij}Y_{ij'})}{\sqrt{\hat{V}(Y_{ij})\hat{V}(Y_{ij'})}} = \frac{\sigma_p^2}{\sigma_p^2 + \sigma_{et}^2}
$$
\n(3)

where: r is the repeatability coefficient; σ_p^2 is the genotypic variance combined with the variance of permanent environmental effects; σ_{et}^2 is the temporary environmental variance associated with experimental error.

Estimates of the experimental coefficient of variation and the genetic coefficient of variation were utilized to assess the precision of the experiments. The performance of the clones was evaluated through scatter plots and correlation estimates between the initial and subsequent measurements. Genotypes were then classified based on their performance using the Scott-Knott test, with a significance level of 5%.

Genotypic values served as the basis for quantifying the selection gain (SG) in the first and second measurements, as well as the average gain over time. Estimates of SG took into account the selection differential and the repeatability coefficient within all measurements. All statistical analyses were performed using the software GENES (CRUZ et al., 2013) and Selegen (RESENDE, 2016).

RESULTS

The stages of drying and peeling were individually analyzed in this study. Significant effects of genotypes, years, and the genotype x years interaction (GY) were observed for all processing traits (Table 2). The notable effect of the GY interaction indicates the presence of clones that exhibited changes in their performance between measurements. This interaction could lean towards a complex type, where changes in performance result in shifts in the ranking of clones, or a simpler type, where changes lead to minor shifts in clone rankings from one measurement to another.

The estimates of the coefficient of experimental variation (CVe) were 1.37 for drying, 2.06 for peeling, and 2.46 for the outturn (Table 2). These low estimates suggested high precision in evaluating these processing traits. Beyond precision was observed a predominant genotypic variance, with repeatability (r) estimates of 86.4, 92.05, and 92.83 for drying, peeling, and outurn, respectively (Table 2). This high repeatability suggested that drying was more influenced by environmental effects than peeling (Table 2). Estimates of the ratio between genetic and experimental coefficients of variation (CVg/CVe) greater than one indicate favorable conditions for achieving gains through plant selection.

To interpret clone performance over time, mass levels maintained after drying and peeling were assessed at each measurement (Figures 1 and 2).

Table 2 - Summary of the analysis of variance and genetic parameter estimates for drying, peeling, and outturn of mature fruits to processed grains for 10 cultivars and 57 clones cultivated in public domain, assessed in two measurements: 1st (2021-2022) and $2nd$ (2022-2023). Embrapa, Porto Velho - RO, Brazil.

SV	DF	Drying	Peeling	Outturn
Genotypes (G)	66	$7.35***$	$12.58***$	13.94**
Years (Y)	$\mathbf{1}$	329.67**	$6.36***$	$157.50***$
GY	66	$11.97***$	5.34^{**}	$5.17***$
Residual	268			
Total	401			
Mean		42.37	53.03	22.67
Mean 1° year		43.07a	53.15a	22.90a
Mean 2° year		42.01a	52.87a	22.20a
CVe		1.37	2.06	2.46
\mathbb{R}		86.4	92.05	92.83
CVg		4.89	6.64	8.24
CVg/Cve		3.56	3.21	3.34

SV: Source of Variation, DF: Degrees of Freedom, CVe: Experimental Coefficient of Variation, CVg: Genetic Coefficient of Variation, r: repeatability coefficient. Means of measurements in the 1st and 2nd years followed by the same lowercase letter do not differ according to the Scott Knott test at a 5% probability.

Despite significant effects of the GY interaction, we observed a positive and high-magnitude association between the two measurements (Figures 1 and 2). This aligns with the observed high repeatability estimates and a simple type of GY interaction, where changes in clone performance between measurements are less tied to shifts in clone rankings. The means of the first and second measurements showed no significant differences according to the Scott Knott test at a 5% probability level (Table 2).

Mass dispersion values suggested that the majority of the mass was lost during the drying stage, resulting in an average retention of 42.37% of the original mass (Figure 3). Smaller amplitude was observed in this stage (9.5%), compared to larger amplitudes in peeling (19.45%). In turn, the outturn showed an amplitude of 10.74%, quantifying the difference in processed bean production between the clone with the highest and lowest performance (LB30 and BG180). Contrasts among clones are interpreted as follows to derive selection gain estimates, mitigating the impact of environmental and measurement errors on the traits.

Average performance of each genotype was interpreted using the Scott Knott mean grouping test, creating mutually exclusive groups ordered from the best-performing clone. Low estimates of experimental error resulted in grouping genotypes into 12 different average groups for the outturn index (Table 3). LB30 showed the highest outturn and grouped separately from other genotypes. BRS1216 and LB12 grouped in the second-best outturn group, followed by genotypes N7, LB10, LB20, BRS3220, AS5, LB88, GJ25, and SK80 in the third group of higher outurn (Table 3). Groups formed by genotypes WP6, GJ5, AS2, SK244, P60, N1, P42, followed by clones AS10, AS7, GJ20, and GJ30, BG180 correspond to lower-outturn genotypes observed in this study.

LB30, LB88, LB12, BRS1216, GJ25 presented the highest masses after peeling, while GJ8, LB07, BRS3137, GB7, CA1 presented higher masses after drying. Clones with higher masses after peeling showed greater alignment with clones with higher outturn. Higher genotypic effects emphasize the importance of genotype effects in expressing husk levels. While the drying (1) and peeling (2) stages showed a weak association $(r_{1x} = 0.03^{NS})$, the correlation estimates between these stages and outturn (3) were significant and of high magnitude $(r_{1x3}=0.60^{**}, r_{2x3}=0.78^{**}).$

To estimate gains with selection, progress estimates with the cultivation of eight genotypes with the highest and lowest performance were considered (Table 4). The new average of clones selected for higher outturn (LB30, BRS1216, LB12, N7, LB10,

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LB20, BRS3220, AS5) was 25.51%, associated with a selection gain of 12.17% (Table 4). In turn, the cultivation of clones with lower outturn (BG180, GJ30, GJ20, AS7, AS10, P42, N1, and P60) is associated with an average of 19.15%, representing a reduction of 14.02% compared to the average of all evaluated clones.

The first quartile referring to the top 25% of clones showed an amplitude from 23.72 to 27.92. The second and third quartiles, covering 50% of the evaluated clones, ranged from 21.55 to 23.72. Finally, the lowest 25% of clones in terms of outturn are found in the range of 17.18 to 21.55. Based on the distribution of values, clones were classified according to their performance (Table 5).

DISCUSSION

Cultivated across the global tropical belt, encompassing many developing nations, *C. canephora* coffee farming has witnessed significant transformations in the past decade (FERRÃO et al., 2017). Enhancements in cultivation efficiency, stemming from improved management practices and plant breeding, have

led to the consideration of several traits in plant selection. New cultivars are now expected to exhibit a range of favorable characteristics.

This study addressed an important yet underexplored facet of productivity. Cultivated for bean production, the dry husk of coffee fruits once discarded in the past, is a significant source of nutrients and organic matter that returns to the field in the best production systems. However, during fruit processing, it is desirable for fruits to possess lower husk content, resulting in a higher mass of beans post-drying and husking.

The findings underscore the dual influence of genetics and the environment on moisture, husk, and bean content. While genetic variability plays a crucial role in drying, husking exhibits greater genetic determination, reflecting the unique husk levels associated with each genotype. In examining the outturn expression across different environments, LOURENÇO et al. (2022) observed that fruits from irrigated trials experienced a more pronounced mass reduction after drying due to higher water content.

Estimates of genetic parameters and experimental error contribute to a nuanced

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understanding of the genetic variability. Unlike previous literature, this study considers the evaluation of outturn over time. Measurements taken in the 2021-2022 and 2022-2023 harvests reveal that while some clones showed performance fluctuations from one year to another, this did not significantly alter the ranking of clones between harvests. The consistent ranking reflects predominantly genotypic effects, supporting the case for selecting superior clones.

Despite their intensive cultivation, the clones trade in public domain are unknown in many aspects. A 10% contrast, as observed between higher and lower-performing clones, translates to a 10% reduction in final productivity in a plantation, simply due to the different in the outturn index.

Among the top eight high outturn clones are two cultivars developed by Embrapa, BRS1216 and BRS3220, and six clones traded in public domain: LB30, LB12, LB10, LB20, N7, and AS5. Registered cultivars have undergone evaluations in diverse environments over several years and exhibit known behaviors (TEIXEIRA et al., 2020). For instance, BRS1216 is characterized by high productivity, rigid stems, intermediate maturation cycle, resistance to nematodes and rust, full-bodied flavor, and falls

under compatibility group I. Conversely, BRS3220 features flexible stems, intermediate maturation cycle, nematode susceptibility, rust resistance, fullbodied flavor, and belongs to compatibility group III (ROCHA et al., 2021). LOURENÇO et al. (2022) also noted the superior outturn of these cultivars across different environments.

The evaluation of control plants with known behavior enhances the interpretation of the studied clones' performance. For the evaluated population, outturn values between 24% and 28% are considered high.

One of the first outturn assessments conducted by GASPARI-PEZZOPANE et al. (2004) studying accessions from the Germplasm Bank at the Agronomic Institute of Campinas, revealed that the Robusta botanical variety had slightly lower outturn than the Conilon botanical variety. PARTELLI et al. (2021), without discriminating between drying and husking stages, noted outturn values higher than those observed in this study, in a breeding population with a higher frequency of Conilon plants.

Among the lower outturn genotypes are clones traded in public domain: BG180, GJ30, GJ20, AS7, AS10, P42, N1, and P60. Cultivating these clones together results in reduced population Table 3 - Performance in terms of drying (Stage 1), peeling (Stage 2), and outturn from ripe fruits to processed grains for 10 cultivars and 57 clones cultivated in public domain in Western Amazon, assessed over two years (2021-2022) (2022-2023) at the Embrapa experimental field located in the municipality of Porto Velho - RO.

performance. The primary distinction in this set lies in their lower bean mass after husking. These clones retained 40.12% of mass after drying compared to the average of 42.37%, and 47.76% of mass after husking compared to the average of 53.04%.

While mass reduction during drying is associated with water loss from husk, mucilage, and beans until reaching an ideal moisture content of 12%, mass reduction during husking is linked to the separation of husk and beans. Mass retained after husking proves more critical in determining the

outturn and is less influenced by the environment, making it pivotal for selecting clones with a favorable set of characteristics.

Despite their intensive cultivation, clones traded in public domain remain unknown in many aspects, and characterizing them is vital for their optimal cultivation and the sustainability of coffee production. The results of this study contributed to a deeper understanding of the genetic variability of this important component influencing productivity and quality.

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Table 4 - Estimates of gain through selection for outturn from ripe fruits to processed grains, involving the selection of eight genotypes with the highest outturn and eight genotypes with the lowest outturn, characterized over two years (2021-2022) (2022-2023) at the Embrapa experimental field in the municipality of Porto Velho - RO.

Mo: overall mean, Ms: mean of selected clones, SD: selection differential, H2: genotypic determination coefficient, SG: selection gain in the original unit, SG%: percentage selection gain. 1° year: first measurement conducted in the agricultural year 2020-2021, 2° year: second measurement conducted in the agricultural year 2021-2022, 1° stage: retained mass after drying, 2° stage: retained mass after peeling.

Table 5 - Ranking of the outturn for 10 cultivars and 57 clones cultivated in public domain in Western Amazon, evaluated in two measurements: 1^{st} (2021-2022) and 2^{nd} (2022-2023), at the Embrapa experimental field located in the municipality of Porto Velho - RO.

CONCLUSION

This study addressesed a crucial yet underexplored component of productivity. The results demonstrate that moisture, husk, and bean content levels are influenced by both plant genetics and the environment. While genetic variability is significant for both drying and husking, husking exhibits greater genetic determination due to the distinctive husk levels of each genotype. Minor shifts in the ranking of clones between measurements, along with high estimates of genetic parameters contribute to a better understanding of this variability. The selected high outturn clones— LB30, BRS1216, LB12, N7, LB10, LB20, BRS3220, AS5—showed an average outturn of 25.51%, representing a gain of 12.17%. In contrast, the clones of lower performance—BG180, GJ30, GJ20, AS7, AS10, P42, N1, P60—averaged 19.15%, indicating a reduction of 14.02%. Based on the distribution of outturn values, genotypes were classified according to their performance, providing valuable insights for the optimal use of cultivated clones.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflicts of interest to disclose.

AUTHORS' CONTRIBUTION

All authors have contributed equally to the research.

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