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Genotype X Environment Interaction Affecting the Physicochemical Quality of *Coffea canephora* Clones Cultivated in Western Amazonia

Interação Genótipo X Ambiente que Afeta a Qualidade Físico-Química de Clones de Coffea canephora Cultivados na Amazônia Ocidental

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Abstract

Coffea canephora is a coffee crop species of economic and social importance in Western Amazonia as a source of income for smallholder farms, which benefits from the proximity to forest ecosystems and their natural pollinators. This study evaluated the physicochemical composition of green beans from the predominant *C. canephora* clones cultivated in the region. Moisture, aqueous extract, total ash, pH, total titratable acidity, crude protein content, lipid content, total phenolic compounds, and total soluble sugars were evaluated in two contrasting environments. The genotype × environment interaction significantly influenced all evaluated traits, with total phenolic compounds showing the largest variation (3.50–5.61 g gallic acid equivalents 100 g^{-1}), reaching up to 50% higher concentrations in the lower-altitude environment (Porto Velho, RO; 88 m) than in São Felipe d'Oeste, RO (276 m). Correlations between aqueous extract and total soluble sugars, and between total phenolic compounds and reducing sugars, were significant in both environments, indicating the absence of environmental effects on these associations. Clone LB80 exhibited the greatest stability in physicochemical composition across environments, whereas clones AR106, LB15, and N16 showed the least stability. The evaluated clones showed considerable genetic diversity, particularly clone AS1, which exhibited the highest crude protein and total soluble sugar contents. The results indicate the importance of selecting genotypes adapted to specific soil and climate conditions to optimize bean quality and enhance the market competitiveness of *C. canephora* produced in Western Amazonia.

Keywords: Amazonian Robusta. Green Bean Composition. Phenolic Compounds. Stability.

Resumo

Coffea canephora é uma espécie de cultivo de café de importância econômica e social na Amazônia Ocidental, servindo como fonte de renda para agricultores familiares e se beneficiando da proximidade com ecossistemas florestais e seus polinizadores naturais. Este estudo avaliou a composição físico-química de grãos verdes dos clones predominantes de *C. canephora* cultivados na região. Umidade, extrato aquoso, cinzas totais, pH, acidez total titulável, teor de proteína bruta, teor de lipídios, compostos fenólicos totais e açúcares solúveis totais foram avaliados em dois ambientes contrastantes. A interação genótipo × ambiente influenciou significativamente todas as características avaliadas, sendo que os compostos fenólicos totais apresentaram a maior variação (3,50–5,61 g equivalentes de ácido gálico 100 g−1), alcançando concentrações até 50% maiores no ambiente de menor altitude (Porto Velho, RO; 88 m) do que em São Felipe d'Oeste, RO (276 m). As correlações entre extrato aquoso e açúcares solúveis totais, e entre compostos fenólicos totais e açúcares redutores, foram significativas em ambos os ambientes, indicando ausência de efeitos ambientais nessas associações. O clone LB80 apresentou a maior estabilidade na composição físico-química entre os ambientes, enquanto os clones AR106, LB15 e N16 demonstraram menor estabilidade. Os clones avaliados apresentaram considerável diversidade genética, especialmente o clone AS1, que exibiu os maiores teores de proteína bruta e açúcares solúveis totais. Os resultados indicam a importância de selecionar genótipos adaptados a condições específicas de solo e clima para otimizar a qualidade dos grãos e aumentar a competitividade de mercado do *C. canephora* produzido na Amazônia Ocidental.

Palavras-chave: Robusta Amazônico. Composição de Grãos Verdes. Compostos Fenólicos. Estabilidade.

1 Introduction

Processed coffee exports in Brazil reached 50.4 million 60-kg bags in 2024, of which approximately 18.5% were *Coffea canephora*. Genetic characteristics of coffee plants, combined with specialty coffee production, can significantly increase export volumes (Francisco et al., 2024). In the state of Rondônia, average yield reached 52.6 bags ha^{-1} in 2024 due to plantation renewal, adoption of crop management technologies, and cultivation of selected clones (Espindula *et al.*, 2022; Lopes Junior *et al.*, 2023).

The predominant clones in Western Amazonia are intervarietal hybrid clones of Conilon and Robusta varieties, selected by local farmers or developed through controlled pollination by the Brazilian Agricultural Research Corporation (Embrapa Rondônia) (Teixeira *et al.*, 2020). These clones are known as Amazonian Robusta, characterized by their high yield, superior beverage quality, and expression of the complementary characteristics of both Conilon and Robusta varieties (Reinicke; Espindula; Rocha, 2023).

Amazonian Robusta coffees exhibit a pronounced sensory profile that results in unique flavors and aromas (Morais *et al*., 2021; Viencz *et al*., 2023). The quality of these coffees is associated with intrinsic fruit characteristics and chemical composition, which, upon roasting, contribute specific combinations of flavor, aroma, acidity, sweetness, and body to the beverage (Figueiredo *et al*., 2015).

Beverage quality varies according to growing location due to genotype × environment interaction (G×E), which directly influences bean chemical composition. Environmental diversity combined with extensive genetic variation results in the production of coffees with diverse chemical and sensory profiles, as observed in Brazil. These attributes, combined with terroir factors, affect marketing positioning, as consumers associate specific climates and environments with quality attributes (Getahun *et al*., 2024).

Understanding bean chemical composition in relation to genotype and environment enables growers to make more informed decisions in selecting clones for cultivation. The clones evaluated in this study are widely cultivated in Rondônia, and information on their physicochemical attributes supports the selection of plant material.

Previous studies have reported significant G×E effects for the bioactive compounds in intervarietal hybrid coffees grown in Rondônia, particularly caffeine, chlorogenic acid, and trigonelline concentrations (Rocha *et al*., 2023), as well as for beverage quality (Reinicke; Espindula; Rocha, 2023). However, G×E effects on standard physicochemical traits of green beans remain undocumented. Therefore, this study evaluated the physicochemical composition of green beans from different *C. canephora* clones cultivated in two contrasting environments in Western Amazonia and quantified the effects of genotype, environmental, and their interaction on the expression of these traits.

2 Material and Methods

The clones evaluated are intervarietal hybrids of *Coffea canephora* exhibiting characteristics of both Conilon and Robusta varietal groups. These genotypes were originally selected and publicly released by coffee growers in the state of Rondônia, Brazil (Table 1).

Table 1 - *Coffea canephora* genotypes selected and released for public use by coffee growers in the state of Rondônia, Brazil, and evaluated for genotype X environment interaction effects

Genotype	Coffee grower
LB15	Laerte Braun
LB80	Laerte Braun
R22	Ronaldo Vitoriano
WP6	Vanderley Peter
GB7	Gilberto Boone
L1	Alcides Rosa
AR106	Aldnei Raasch
AS1	Ademar Schmidt
AS5	Ademar Schmidt
AS7	Ademar Schmidt
R152	Ronaldo Guedes
N13	Nivaldo Ferreira
N16	Nivaldo Ferreira
N8(G8)	Nivaldo Ferreira

Source: research data.

The effects of genotypes, environments, and their interaction were evaluated in a completely randomized design with five replicates, in two distinct environments. Samples were collected at a commercial plantation in São Felipe d'Oeste (276 m altitude) and at the Embrapa experimental field in Porto Velho (88 m altitude), Rondônia, during the 2020–2021 growing season. These plantations were managed according to recommended practices for coffee cultivation (Marcolan; Espindula, 2015). Soil chemical properties and climatic data for the experimental sites were reported by Reinicke, Espindula and Rocha *et al*. (2023).

Fruits were harvested at the cherry stage when 707–80% of the fruits on each plant were fully ripe, considering the specific maturation cycle of each clone. Harvested fruits were sun-dried on raised screens until moisture content reached 12%. Samples were subsequently hulled in a drum huller to obtain green coffee beans.

Green coffee beans were ground in a knife mill equipped with a 20-mesh sieve. Physicochemical analyses were performed in triplicate and included moisture, aqueous extract, total ash, pH, total titratable acidity, crude protein content, lipid content, total phenolic compounds, total soluble sugars, and reducing sugars, following official methods (IAL, 2008; AOAC, 2000). Non-reducing sugar content was calculated as the difference between total soluble sugars and reducing sugars. The ratio (aqueous extract / total titratable acidity) was also calculated.

Individual and joint analysis of variance were conducted to quantify the effects of environments, genotypes, and genotype × environment interaction (G×E). The significance of the genotype effect was tested separately using the following model (Cruz, 2013):

$$Y_{ijk} = m + G_i + e_{ijk}$$

where Y_{ijk} is the observation of the i^{th} genotype in the j^{th} environment at the k^{th} replication, m is the overall mean, G_i is the effect of the i^{th} genotype, and e_{ijk} is the experimental error.

Homogeneity of residual variances was confirmed, and genetic variability relative to environmental variation was assessed through joint analysis of variance, in which the effects of genotype, environment, and G×E were modeled explicitly (Cruz, 2013):

$$Y_{ijk} = m + G_i + E_j + GY_{ij} + e_{ijk}$$

where Y_{ijk} is the observation of the i^{th} genotype in the j^{th} environment at the k^{th} replication, m is the overall mean, G_i is the effect of the i^{th} genotype, E_j is the effect of the j^{th} environment, GY_{ij} is the interaction effect between the i^{th} genotype and the j^{th} environment, and e_{ijk} is the experimental error. The genotype effect was considered fixed, and the environment effect was random.

Correlations of variables were interpreted using Pearson correlation coefficients (Cruz *et al*., 2021). Genetic diversity among genotypes was assessed by principal component analysis (PCA), considering the distribution of the first two principal components, using standardized data to meet statistical assumptions. All statistical analyses were performed using GENES software, version 1990.2023.93 (Cruz, 2013).

3 Results and Discussion

Cumulative rainfall from August 2020 to July 2021 was 1,880.5 mm at São Felipe d'Oeste and 2,193.6 mm at Porto Velho. The climate of both sites is classified as 'Am' (tropical monsoon), according to the Köppen–Geiger system, with September as the hottest month and May as the coldest (Alvares *et al*., 2013). Monthly rainfall was higher in Porto Velho in August, September, November, and December 2020 and in May 2021, whereas São Felipe d'Oeste recorded higher rainfall in October 2020 and from January to April and June 2021. In July 2021, monthly rainfall was similar at both sites.

The soil in Porto Velho exhibited lower pH and lower concentrations of phosphorus, calcium, and aluminum, but higher concentrations of potassium and magnesium than São Felipe d'Oeste. Altitude, temperature, and soil fertility are known to affect plant performance (Mori *et al*., 2018).

Joint analysis of variance (Table 2) of the 14 predominant coffee clones grown in Western Amazonia revealed significant genotype effects for aqueous extract and crude protein content. Environmental effects were not significant for total ash, crude protein content, and lipid content. The genotype × environment interaction (G×E) was significant for all traits, indicating that the relative performance of clones varied across environments. Significant G×E indicates that the environment modifies trait expression differently across genotypes (Rocha *et al.*, 2015). Consequently, clone evaluation and selection should consider specific soil and climatic conditions to identify genotypes showing broad or specific adaptation.

Table 2 - Joint analysis of variance for physicochemical traits of predominant *Coffea canephora* genotypes evaluated in two contrasting environments (São Felipe d'Oeste and Porto Velho, RO, Brazil)

Source of Variation	**DF**	**M**	**AE**	**ASH**	**pH**	**TTA**	**CPC**
Genotype	13	0.38 ^{ns}	2.82 *	1.50 ^{ns}	1.54 ^{ns}	0.90 ^{ns}	4.59 **
Environment	1	39.34 **	14.95 **	1.15 ^{ns}	109.34 **	32.67 **	0.08 ^{ns}
G × E	13	124.42 **	52.95 **	94.91 **	8.53 **	28.29 **	7.78 **
Residue	56						
Total	83						
Overall mean		10.06	27.11	4.19	5.68	154.02	15.48
CVe		1.39	0.92	1.28	0.43	2.20	2.55
Source of Variation	**DF**	**EE**	**TPC**	**TSS**	**RS**	**NRS**	**Ratio**
Genotype	13	1.58 ^{ns}	2.02 ^{ns}	0.96 ^{ns}	0.68 ^{ns}	0.99 ^{ns}	1.60 ^{ns}
Environment	1	2.12 ^{ns}	13.33 **	43.45 **	29.85 **	35.13 **	13.71 **
G × E	13	16.45 **	125.49 **	36.41 **	41.47 **	32.94 **	24.03 **
Residue	56						
Total	83						
Overall mean		4.87	4.87	5.63	1.19	4.44	0.17
CVe		4.96	1.19	5.62	4.77	7.02	2.41

* Significant at 5% probability. ** Significant at 1% probability. ns, not significant. DF = degrees of freedom; M = moisture; AE = aqueous extract (%); ASH = total ash (%); TTA = total titratable acidity (mL NaOH 0.1 mol L^{-1} 100 g^{-1}); CPC = crude protein content (%); EE = ether extract (lipids) (%); TPC = total phenolic compounds (g gallic acid equivalents 100 g^{-1}); TSS = total soluble sugars (%); RS = reducing sugars (%); NRS = non-reducing sugars (%); Ratio = aqueous extract / total titratable acidity (% mL^{-1} NaOH). CVe = experimental coefficient of variation.
Source: research data.

The G×E indicates how environmental variation affects the relative performance of genotypes. A significant G×E effect can be classified as simple or complex; a simple interaction occurs when genotypes maintain consistent ranking across environments, whereas a complex interaction results in substantial changes in ranking (Cruz *et al.*, 2021) and clone performance should be evaluated separately for each environment.

Although all the evaluated physicochemical traits showed significant G×E effects, repeated-measures analysis (Table 3) revealed that aqueous extract (r = 0.49) and crude protein (r = 0.68) exhibited small changes in genotype ranking between environments. A separate repeatability study across two environments in Western Amazonia involving 68 publicly released genotypes reported repeatability estimates for aqueous extract and crude protein content of r = 0.57 and r = 0.62 (Lopes Junior *et al.*, 2024). This consistency indicates that the limited rank changes observed for these traits between environments also occur across harvest years.

The G×E for the remaining traits was predominantly complex, with both genotype and environment factors strongly affecting trait expression and causing larger changes in genotype ranking between environments (Table 3).

Table 3 - Repeatability analysis for physicochemical traits of predominant *Coffea canephora* genotypes evaluated in two contrasting environments (São Felipe d'Oeste and Porto Velho, RO, Brazil)

Variables	São Felipe d'Oeste × Porto Velho
Moisture	-0.49 ^{ns}
Aqueous extract (%)	0.49 ^{ns}
Total ash (%)	0.23 ^{ns}
pH	0.22 ^{ns}
Total titratable acidity (mL NaOH 0.1 mol L^{-1} 100 g^{-1})	-0.06 ^{ns}
Crude protein content (%)	0.68 **
Ether extract (lipids) (%)	0.26 ^{ns}
Total phenolic compounds (g gallic acid equivalents 100 g^{-1})	0.37 ^{ns}
Total soluble sugars (%)	-0.04 ^{ns}
Reducing sugars (%)	-0.19 ^{ns}
Non-reducing sugars (%)	-0.06 ^{ns}
Ratio (aqueous extract / total titratable acidity) (% mL^{-1} NaOH)	0.28 ^{ns}

** = significant at 1% probability; ^{ns} = not significant
Source: research data.

Genotype × year interaction for physicochemical traits is often significant, as reported for Conilon coffee in the Federal District, Brazil (Brige *et al.*, 2024), and for Amazonian Robusta in Rondônia, Brazil (Lopes Junior *et al.*, 2024). These results indicate that both harvest year and growing environment affect the chemical composition of green beans. G×E has also been shown to affect beverage quality scores of Amazonian Robusta (Reinicke; Espindula; Rocha, 2023).

Joint experimental coefficients of variation were low for all traits, ranging from 0.43 for pH to 7.02 for non-reducing sugars (Table 2), which reflects high experimental precision. Individual analysis of variance and genetic parameters are presented separately for each environment because of the complex G×E pattern (Table 4).

Table 4 - Individual analysis of variance for physicochemical traits of predominant *Coffea canephora* genotypes evaluated in two contrasting environments

São Felipe d´Oeste, RO							
Source of variation	**DF**	**M**	**AE**	**ASH**	**pH**	**TTA**	**CPC**
Genotype	13	37.34 **	128.87 **	285.34 **	30.66 **	8.58 **	29.31 **
Residue	28						
Total	41						
H^2		97.32	99.22	99.64	96.73	88.35	96.58
CVg		5.90	5.86	9.74	0.94	3.66	7.77
CVe		1.69	0.89	1.00	0.29	2.30	2.53
CVg/CVe		3.48	6.53	9.73	3.14	1.59	3.07
Source of variation	**DF**	**EE**	**TPC**	**TSS**	**RS**	**NRS**	**Ratio**
Genotype	13	9.59 **	93.31 **	6.25 **	24.87 **	6.02 **	13.71 **
Residue	28						
Total	41						
H^2		89.58	98.92	84.01	95.97	83.39	92.71
CVg		9.09	6.67	7.51	16.21	8.86	5.26
CVe		5.37	1.20	5.67	5.74	6.84	2.55
CVg/CVe		1.69	5.54	1.32	2.82	1.29	2.059
Porto Velho, RO							
Source of variation	**DF**	**M**	**AE**	**ASH**	**pH**	**TTA**	**CPC**
Genotype	13	168.37 **	74.33 **	43.60 **	4.94 **	58.08 **	14.41 **
Residue	28						
Total	41						
H^2		99.4	98.65	97.7	79.77	98.27	93.06
CVg		7.96	4.75	5.76	0.61	8.91	5.43
CVe		1.06	0.96	1.53	0.53	2.04	2.57
CVg/CVe		7.46	4.94	3.76	1.14	4.36	2.11
Source of variation	**DF**	**EE**	**TPC**	**TSS**	**RS**	**NRS**	**Ratio**
Genotype	13	35.41 **	311.12 **	47.88 **	44.30 **	42.27 **	49.46 **
Residue	28						
Total	41						
H^2		97.17	99.67	97.91	97.34	97.63	97.97
CVg		15.46	12.11	21.3	15.55	25.18	9.18
CVe		4.56	1.19	5.38	4.09	6.77	2.28
CVg/CVe		3.38	10.16	3.95	3.79	3.7	4.01
Means for each environment							
		M	**AE**	**ASH**	**pH**	**TTA**	**CPC**
São Felipe d'Oeste		9.02 b	27.88 a	4.25 a	5.60 b	165.28 a	15.51 a
Porto Velho		11.11 a	26.34 b	4.13 a	5.76 a	142.76 b	15.44 a
		EE	**TPC**	**TSS**	**RS**	**NRS**	**Ratio**
São Felipe d'Oeste		4.72 b	5.13 a	4.26 b	0.97 b	3.28 b	0.16 b
Porto Velho		5.03 a	4.61 b	7.01 a	1.41 a	5.60 a	0.18 a

* Significant at 5% probability. ** Significant at 1% probability. ns, not significant. DF = degrees of freedom; H^2 = broad-sense heritability, CVg = genetic coefficient of variation, CVe = experimental coefficient of variation. M = moisture; AE = aqueous extract (%); ASH = total ash (%); TTA = total titratable acidity (mL NaOH 0.1 mol L^{-1} 100 g^{-1}); CPC = crude protein content (%); EE = ether extract (lipids) (%); TPC = total phenolic compounds (g gallic acid equivalents 100 g^{-1}); TSS = total soluble sugars (%); RS = reducing sugars (%); NRS = non-reducing sugars (%); Ratio = aqueous extract / total titratable acidity (% mL^{-1} NaOH). Means followed by the same letter within a column do not differ by Scott-Knott test at 5% probability level.

Source: research data.

These individual analyses revealed significant genotype effects for all traits in both environments, whereas in the joint analysis only aqueous extract and crude protein showed significant genotype effects (Table 2).

Broad-sense heritability estimates ranged from 83.39% to 99.64% at São Felipe d'Oeste, with the highest values for total ash and the lowest for non-reducing sugars, and from 79.77% to 99.67% at Porto Velho, with the highest values for total phenolic compounds and the lowest for pH (Table 4). Individual experimental coefficients of variation (CVe) were below 10% for all traits in both environments (Table 4), which is consistent with the high experimental precision observed in the joint analysis (Table 2). In both environments, pH showed the lowest variation and non-reducing sugars the highest.

The ratio of genetic to experimental coefficient of variation (CVg/CVe) exceeded 1.0 for all traits, which reflects potential for genetic gain through selection (Ferrão *et al.*, 2008). The highest CVg/CVe values were observed for total ash (9.73), followed by total phenolic compounds (5.54) in São Felipe d'Oeste, and for total phenolic compounds (10.16) in Porto Velho (Table 4). High CVg/CVe ratios for total phenolic compounds in green beans were also reported across multiple harvests at a single site in Western Amazonia (Lopes Junior *et al.*, 2024).

Moisture content, pH, ether extract (lipids), total soluble sugars, reducing sugars, non-reducing sugars, and ratio (aqueous extract / total titratable acidity) were higher at Porto Velho than at São Felipe d'Oeste. Consequently, aqueous extract, total titratable acidity, and total phenolic compounds were higher in São Felipe d'Oeste, whereas total ash and crude protein content did not differ significantly between environments (Table 4).

The Scott–Knott clustering test ($p < 0.05$) formed multiple groups for all physicochemical traits. At São Felipe d'Oeste, total ash showed the greatest differentiation (10 groups), followed by total phenolic compounds (8 groups) and aqueous extract (7 groups) (Table 5). At Porto Velho, total phenolic compounds formed 9 groups, followed by moisture, aqueous extract, total titratable acidity, reducing sugars, and ratio, each forming 7 groups.

Moisture content ranged from 7.89% to 12.43% across both environments, with the highest values observed for clones AR106, AS1, and N13 in Porto Velho and the lowest for clone N13 in São Felipe d'Oeste (Table 5). Aqueous extract, an indicator of compounds soluble in boiling water, ranged from 23.84% to 30.54%, with the highest values observed for clones N13 and R22 at São Felipe d'Oeste and the lowest for clone GB7 at Porto Velho.

Table 5 - Scott–Knott clustering ($p < 0.05$) for physicochemical traits of predominant *Coffea canephora* genotypes evaluated in two contrasting environments

São Felipe d´Oeste												
	M	**AE**	**ASH**	**pH**	**TTA**	**CPC**	**EE**	**TPC**	**TSS**	**RS**	**NRS**	**Ratio**
AR106	8.82d	27.73e	4.32e	5.57c	168.82b	15.90b	5.49a	4.86f	4.28a	0.88c	3.40a	0.16b
AS1	8.85d	29.50b	3.86h	5.61b	162.30c	17.15a	4.43c	5.21c	4.00b	1.04b	2.95b	0.18a
AS5	8.89d	28.26d	3.77i	5.58c	159.73c	16.99a	4.75b	4.65h	4.41a	0.94c	3.46a	0.17a
AS7	9.55b	27.37f	4.59d	5.52d	164.17c	15.45c	3.87d	5.00e	4.35a	0.91c	3.43a	0.16b
GB7	9.97a	25.44g	4.83b	5.59c	156.77c	15.82b	4.81b	4.68h	3.79b	0.79d	3.00b	0.16b
L1	9.19a	25.60g	4.30e	5.61b	157.48c	14.89d	4.65b	5.46b	3.99b	0.84d	3.15b	0.16b
LB15	9.88a	27.77e	5.02a	5.51d	158.61c	13.82e	4.94b	4.93f	4.70a	0.95c	3.75a	0.17a
LB80	9.20c	28.37d	4.04g	5.58c	173.80a	17.18a	5.08a	5.45b	4.54a	1.01b	3.52a	0.16b
N13	7.89f	30.54a	4.15f	5.74a	170.92b	16.94a	3.97d	5.56a	4.08b	1.37a	2.71b	0.17a
N16	8.65e	28.92c	3.98g	5.59c	177.23a	13.43e	4.83b	5.56a	4.45a	1.10b	3.35a	0.16b
N8(G8)	8.94d	25.40g	4.32e	5.62b	166.42b	14.48d	5.22a	4.90f	3.79b	0.76d	3.03b	0.15c
R152	9.12c	27.05f	4.74c	5.61b	170.66b	14.75d	4.67b	4.80g	3.81b	0.81d	3.00b	0.15c
R22	8.63e	30.23a	3.96g	5.60c	167.09b	15.47c	5.02a	5.61a	4.86a	1.06b	3.79a	0.18a
WP6	8.65e	28.19d	3.65j	5.62b	159.96c	14.90d	4.31c	5.12d	4.53a	1.09b	3.43a	0.17a
Porto Velho												
AR106	12.43a	27.50b	4.10d	5.81a	136.97e	15.44c	6.29a	4.50e	7.30b	1.28e	6.02b	0.20b
AS1	12.37a	29.22a	4.03d	5.76b	154.42c	16.65a	3.90e	5.33a	9.18a	1.78a	7.40a	0.18d
AS5	11.24c	26.88c	4.37b	5.71b	145.25d	15.33c	5.39b	4.10g	7.39b	1.31e	6.08b	0.18d
AS7	10.67d	26.02d	4.35b	5.73b	168.98a	15.84b	4.64c	4.74d	8.93a	1.66b	7.27a	0.15g
GB7	9.91f	23.84g	3.91e	5.69b	143.20d	16.65a	6.23a	3.95h	5.30d	1.20f	4.09d	0.16f
L1	9.51g	26.94c	4.10d	5.79a	139.76e	14.84c	5.13b	4.97c	7.72b	1.36d	6.36b	0.19c
LB15	11.37c	26.61c	4.32b	5.74b	135.49e	14.59d	3.86e	4.21f	8.84a	1.44d	7.39a	0.19c
LB80	11.28c	25.43e	3.81e	5.75b	128.50f	16.97a	5.49b	5.25a	4.34e	1.40d	2.94e	0.19c
N13	12.26a	26.66c	4.41b	5.75b	143.60d	15.91b	5.60b	4.76d	7.44b	1.36d	6.07b	0.18d
N16	11.62b	26.78c	4.18c	5.75b	151.71c	15.06c	4.78c	5.16b	6.85b	1.51c	5.34c	0.17e
N8(G8)	10.51e	24.95f	4.54a	5.83a	161.75b	15.16c	4.29d	4.55e	7.87b	1.60c	6.27b	0.15g
R152	10.79d	26.25d	4.04d	5.78a	123.75g	15.15c	4.39d	5.24a	5.04d	1.68b	3.36e	0.21a
R22	10.38e	26.18d	3.85e	5.80a	134.64e	14.65d	5.73b	4.23f	5.67c	1.07g	4.60d	0.19c
WP6	11.12c	25.45e	3.79e	5.79a	130.60f	13.97d	4.71c	3.50i	6.28c	1.04g	5.23c	0.19c

M = moisture; AE = aqueous extract (%); ASH = total ash (%); TTA = total titratable acidity (mL NaOH 0.1 mol L^{-1} 100 g^{-1}); CPC = crude protein content (%); EE = ether extract (lipids) (%); TPC = total phenolic compounds (g gallic acid equivalents 100 g^{-1}); TSS = total soluble sugars (%); RS = reducing sugars (%); NRS = non-reducing sugars (%); Ratio = aqueous extract / total titratable acidity (% mL^{-1} NaOH). Means followed by the same letter within a column do not differ by Scott-Knott test at 5% probability level.

Source: research data.

Total ash (mineral content) ranged from 3.65% to 5.02%, with clone WP6 showing the lowest and clone LB15 the highest value, both at São Felipe d'Oeste. Bean pH ranged from 5.51 to 5.83, with the lowest values for clones AS7 and LB15 at São Felipe d'Oeste and the highest for clones N8(G8), AR106, R22, WP6, L1, and R152 at Porto Velho. Total titratable acidity ranged from 123.75 to 177.23 mL NaOH 0.1 mol L^{-1} 100 g^{-1}, with the highest values recorded for clones N16 and LB80 at São Felipe d'Oeste and the lowest for clone R152 at Porto Velho (Table 5).

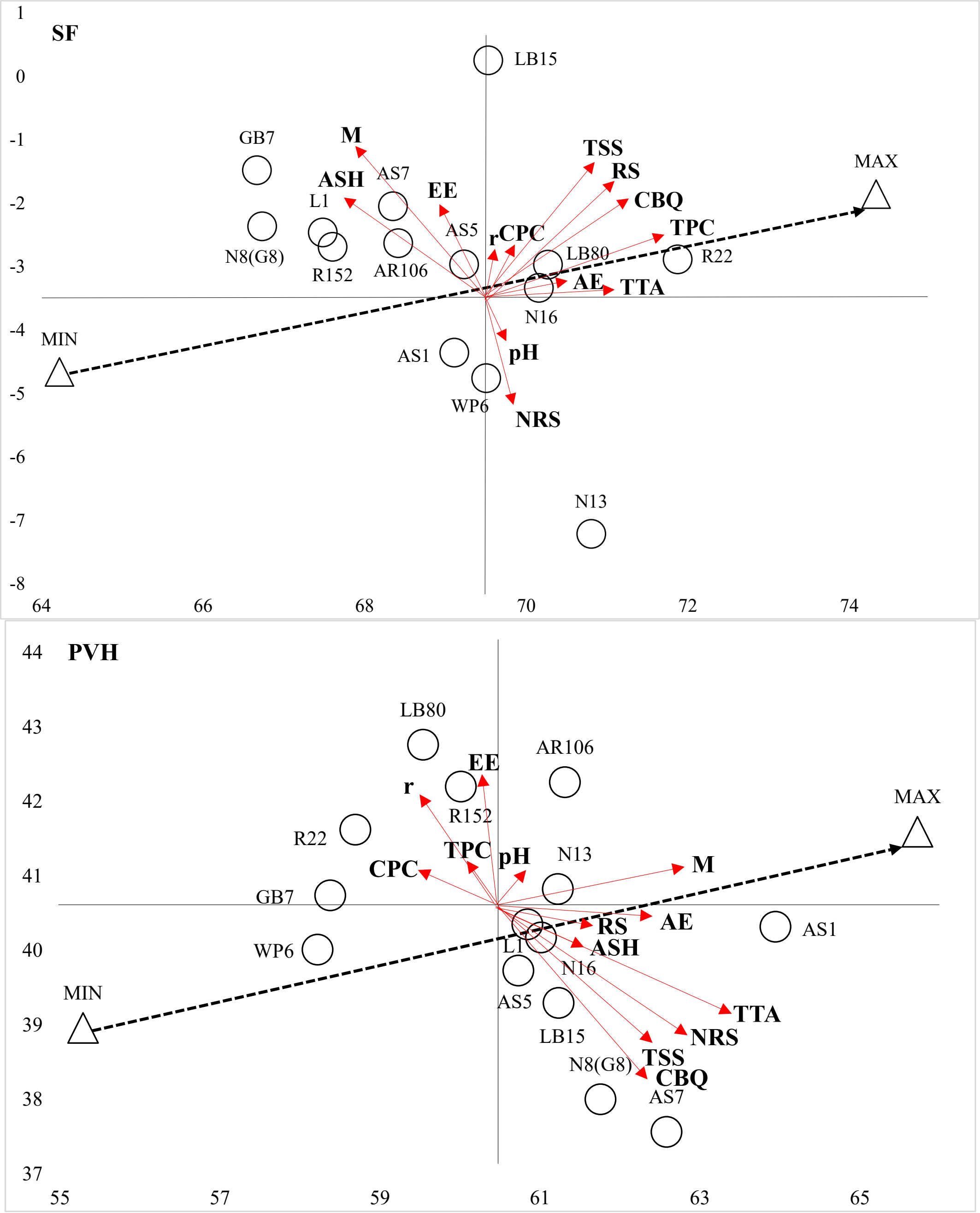
Crude protein content ranged from 13.43 to 17.18%. Approximately 57% of the highest values were observed at São Felipe d'Oeste, with clone LB80 showing the highest values in both environments, followed by clone AS1, which reflects a predominantly simple G×E for this trait (Table 3). Ether extract (lipids) ranged from 3.86% to 6.29%, with the highest content in clones AR106 and GB7 at Porto Velho and the lowest in clones N13 and AS7 at São Felipe d'Oeste and in AS1 and LB15 at Porto Velho (Table 5). High crude protein and lipid content in green beans are associated with the formation of aroma and flavor precursors during roasting (Hall; Trevisan; Hall, 2022).

Total phenolic compounds ranged from 3.50 to 5.61 g gallic acid equivalents 100 g^{-1}. The highest concentrations were observed for clones R22, N16, and N13 in São Felipe d'Oeste and the lowest for clone WP6 in Porto Velho. Total phenolic compounds in coffee beans have been associated, directly and indirectly, with coffee quality. Higher levels are often linked to lower green-bean quality (Barbosa *et al.*, 2019). However, chlorogenic acids—the predominant phenolic compounds—are essential for roasted coffee color and aroma development and are involved in astringency, which is desirable at low levels in high-quality beverages (Kurniawan *et al.*, 2017).

Total soluble sugars ranged from 3.81% to 9.18%, with the highest values observed for clones AS1, AS7, and LB15 at Porto Velho. Reducing sugars ranged from 0.76% to 1.78%, with clone AS1 showing the highest content at Porto Velho. Non-reducing sugars ranged from 2.71% to 7.40%, with the highest values observed for clones AS1, LB15, and AS7 at Porto Velho. Sucrose is the predominant free sugar in green coffee beans, followed by glucose and fructose (Redgwell; Fischer, 2006). Soluble sugars in green beans act as precursors of aroma and flavor during roasting; however, high soluble sugar levels alone do not guarantee superior beverage quality (Iwasa *et al.*, 2015). The ratio (aqueous extract / total titratable acidity) ranged from 0.15 to 0.21, with clone R152 showing the highest value. High ratios are associated with superior green bean quality (Barbosa *et al.*, 2019).

The performance evaluation of each clone within each environment revealed that 13 of the 14 clones at São Felipe d'Oeste showed one or more physicochemical traits in the highest Scott-Knott group. Clone N13 ranked highest for aqueous extract, pH, crude protein, total phenolic compounds, reducing sugars, and ratio, whereas clone R22 ranked highest for aqueous extract, ether extract (lipids), total phenolic compounds, total soluble sugars, non-reducing sugars, and ratio. At Porto Velho, all clones except AS5 ranked in the highest group for at least one trait; clone AS1 ranked highest for moisture, aqueous extract, crude protein, total phenolic compounds, total soluble sugars, reducing sugars, and non-reducing sugars.

Figure 1 - Biplot of the first two principal components showing the distribution of predominant *Coffea canephora* genotypes evaluated for physicochemical traits in two contrasting environments.



M = moisture; AE = aqueous extract (%); ASH = total ash (%); TTA = total titratable acidity (mL NaOH 0.1 mol L^{-1} 100 g^{-1}); CPC = crude protein content (%); EE = ether extract (lipids) (%); TPC = total phenolic compounds (g gallic acid equivalents 100 g^{-1}); TSS = total soluble sugars (%); RS = reducing sugars (%); NRS = non-reducing sugars (%); Ratio = aqueous extract / total titratable acidity (% mL^{-1} NaOH); CBQ = coffee beverage quality.
Source: research data.

These patterns are supported by the biplot of the first two principal components (Figure 1), in which clone R22 at São Felipe d’Oeste and clone AS1 at Porto Velho are positioned closest to the vector representing the hypothetical genotype with maximum expression of all measured physicochemical traits.

Physicochemical traits of green beans often show correlations that reflect common metabolic and physiological pathways. Non-reducing sugars and ratio were excluded for correlation analysis because they are derived variables, and beverage quality variables (Reinicke; Espindula; Rocha, 2023) were included for comparison. Eleven of the 57 possible pairwise correlations at São Felipe d'Oeste were significant ($p < 0.05$), and seven were significant at Porto Velho (Figure 2).

Among these correlations, aqueous extract × total soluble sugars and total phenolic compounds × reducing sugars were significant in both environments (Figure 2). These consistent relationships suggest genetic control of the associations between aqueous extract and soluble sugar content and between phenolic compound concentration and reducing sugar content, regardless of the environment. All other significant correlations were environment-specific.

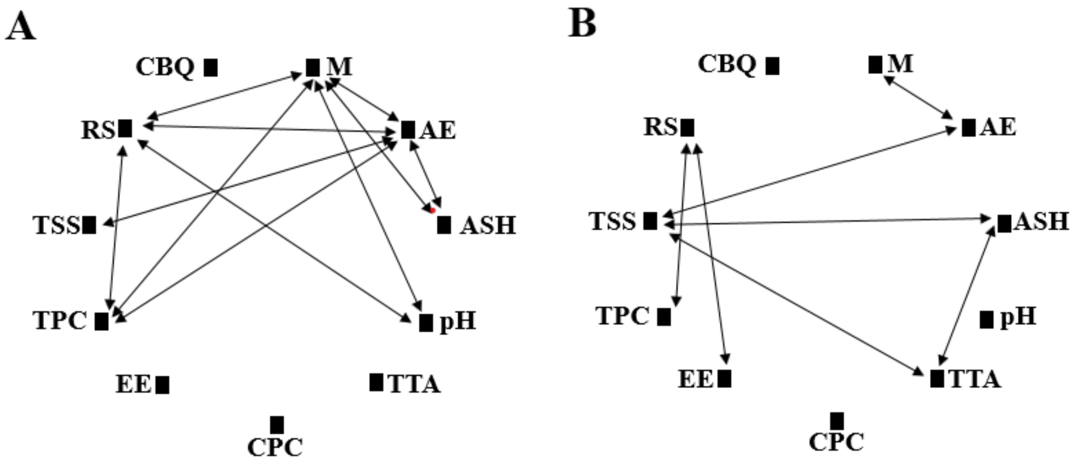
Roasted beverage quality of the same clones, harvest, and environments was reported by Reinicke, Espindula and Rocha (2023). Phenotypic correlations revealed no significant association between beverage quality scores and any of the measured physicochemical traits (Figure 2). This absence of direct correlation was anticipated, because sensory quality in coffee depends on multiple attributes (aroma, flavor, aftertaste, acidity, bitterness/sweetness balance, body, overall impression, uniformity, and cleanliness) that are influenced by complex precursor interactions during roasting (UCDA, 2010).

A single physicochemical variable is rarely sufficient to predict beverage quality (Lemos *et al.*, 2020), reflecting the complex precursor chemistry that determines the flavor and aroma of roasted coffee. Individual precursors in green beans can contribute to the formation of certain sensory compounds while reducing the formation of others; consequently, the relative proportions of these constituents often provide greater insight into sensory differences than absolute concentrations (Hall; Trevisan; Vos, 2022).

Principal component biplot analysis (Figure 1) revealed that, in both environments, clones with higher beverage quality scores were positioned in the same quadrant as vectors for soluble sugars, non-reducing sugars, and total titratable acidity, indicating a positive association among these variables. Thus, superior beverage quality was associated with high content of organic acids and non-reducing sugars in green beans.

Similar correlations between chemical composition and sensory quality of Arabica coffee have been reported by (Borém *et al.* (2016), with PCA showing that samples with higher sensory scores exhibited greater sucrose and organic acid content. Sucrose metabolism in green beans is linked to the accumulation of flavor- and taste-related compounds (Huang *et al.*, 2021) and correlates positively with sensory attributes such as aroma, aftertaste, and overall score.

Figure 2 - Pearson correlation coefficients among physicochemical traits and beverage quality of predominant *Coffea canephora* genotypes evaluated in two contrasting environments (São Felipe d’Oeste and Porto Velho, RO, Brazil). Arrows indicate significant correlations



Correlations	A	B
M × AE	-0.65 *	0.61 *
M × ASH	0.70 **	0.14 ^{ns}
M × pH	-0.77 **	0.01 ^{ns}
M × TPC	-0.53 *	0.22 ^{ns}
M × RS	-0.71 **	0.22 ^{ns}
AE × ASH	-0.52 *	0.13 ^{ns}
AE × TPC	0.56 *	0.45 ^{ns}
AE × TSS	0.56 *	0.55 *
AE × RS	0.87 **	0.38 ^{ns}
ASH × TTA	-0.19 ^{ns}	0.62 *
ASH × TSS	-0.29 ^{ns}	0.65 *
pH × RS	0.57 *	-0.05 ^{ns}
TTA × TSS	0.11 ^{ns}	0.67 **
EE × RS	-0.46 ^{ns}	-0.65 *
TPC × RS	0.65 *	0.74 **

* = Significant at $p < 0.05$; ** = significant at $p < 0.01$; ns = not significant. M = moisture; AE = aqueous extract (%); ASH = total ash (%); TTA = total titratable acidity (mL NaOH 0.1 mol L^{-1} 100 g^{-1}); CPC = crude protein content (%); EE = ether extract (lipids) (%); TPC = total phenolic compounds (g gallic acid equivalents 100 g^{-1}); TSS = total soluble sugars (%); RS = reducing sugars (%); CBQ = coffee beverage quality.

Source: research data.

At São Felipe d’Oeste, clones LB15, N16, R22, and LB80 were positioned in the same biplot quadrant, and all achieved quality scores above 80 points, except LB80. These clones, together with AS5, exhibited the highest total soluble sugar and non-reducing sugar content. At Porto Velho, clones L1, N16, AS5, LB15, N8(G8), AS7, and AS1 were positioned in the same quadrant, and all achieved quality scores above 80 points and higher total soluble sugar and non-reducing sugar content, except N16. Clones scoring above 80 points are classified as Fine Robusta.

Total soluble sugar and non-reducing sugar were highly correlated because non-reducing sugar is dominated by sucrose. Sucrose concentration in green coffee beans is strongly correlated with total soluble sugar (Malta *et al*., 2020). Mean total soluble sugar content at Porto Velho was 39.23% higher than at São Felipe d'Oeste, with clones differing by up to 5.17 g 100 g^{-1} between environments. Non-reducing sugar content was approximately 41% higher at Porto Velho, with differences of up to 4.44 g 100 g^{-1} (Table 5). This result indicates that high sugar content alone is not a sufficient indicator of specialty-grade coffee; the overall balance of chemical constituents is critical for achieving superior beverage quality scores. Higher lipid content has also been associated with improved beverage quality (Alves *et al*., 2018).

Significant genotype × environment interaction for sucrose and organic acid content was also reported in four Brazilian Arabica genotypes grown at different sites, confirming the combined influence of genotype and environment on green-bean chemical composition (Borém *et al*., 2016). *C. arabica* generally contains higher total soluble sugars, non-reducing sugars, and total titratable acidity than *C. canephora*. Studies have reported values above 8% for total soluble sugars and non-reducing sugars and 123 mL NaOH 0.1 mol L^{-1} 100 g^{-1} for total titratable acidity for Arabica (Agnoletti *et al*., 2019), and above 9%, 8%, and 184 mL NaOH 0.1 mol L^{-1} 100 g^{-1}, respectively, in *C. canephora* (Lopes Junior *et al*., 2024). The high concentrations observed in green beans of *C. canephora*, which are consistent with high-quality coffees, were previously considered characteristic only of *C. arabica*.

Clone LB80 exhibited the greatest stability, with crude protein, ether extract (lipids), total soluble sugars, and non-reducing sugars belonging to the same Scott–Knott group in both environments. It was followed by clone GB7 (moisture, total titratable acidity, and ratio), L1 (moisture, crude protein, and ether extract), N13 (pH, reducing sugars, and ratio), N8(G8) (aqueous extract, total titratable acidity, and ratio), R22 (total ash, ether extract, and reducing sugars), AS1 (ether extract and total phenolic compounds), AS5 (ether extract and ratio), AS7 (total titratable acidity and crude protein), R152 (crude protein and non-reducing sugars), and WP6 (ether extract and reducing sugars). In contrast, clones AR106, LB15, and N16 were the least stable, each maintaining the same Scott–Knott group for only one trait across environments (aqueous extract, crude protein, and ether extract, respectively).

Among the measured traits, ether extract (lipids) and crude protein are noteworthy because they participate in thermal degradation reactions during roasting. Ether extract was stable across environments in 50% of the clones. Lipids contribute to the generation of volatile compounds (Wang *et al*., 2020) and, during roasting, migrate to the bean surface where they form an oil layer that helps retain volatile aroma compounds until grinding. Crude protein content was stable in 35% of the clones. Although green-bean protein concentration has received limited attention in relation to beverage quality, some studies suggest that higher levels can either enhance or reduce beverage quality (Barbosa *et al*., 2019; Franca *et al*., 2005). However, crude proteins are important for sensory attributes because their constituent amino acids, together with reducing sugars, undergo Maillard reactions during roasting and contribute to roasted-coffee color, aroma, and flavor (Kitzberger *et al*., 2016).

The Porto Velho environment favored higher sweetness in green beans—an important precursor of aroma and flavor—whereas São Felipe d'Oeste favored greater water solubility (of interest to the instant-coffee industry) and higher total phenolic compound content, which also serve as aroma and flavor precursors.

4 Conclusion

The results reveal substantial genetic variation in the physicochemical composition of green beans among *C. canephora* clones cultivated in Western Amazonia and confirm a strong genotype × environment interaction affecting all measured traits. Clone LB80 exhibited the highest stability across environments and is therefore a promising candidate for breeding programs. In contrast, clones AR106, LB15, and N16 showed the least stability across environments. These findings are highly relevant to *C. canephora* cultivation in Rondônia and demonstrate that green bean physicochemical composition is influenced by both genotypic differences and environmental conditions.

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