



# Methylxanthine and polyphenol distribution in guarana cultivars

Distribuição de metilxantinas e polifenóis em cultivares de guaraná

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Guarana is a plant native to the Amazon, and its seeds are used as soft drinks and other energy drink ingredients. The phytochemical aspects of guarana have been extensively explored for its use in herbal and cosmetic compositions due to the presence of metabolites, such as methylxanthines and polyphenols. This work aims to fill a gap in the information about the presence and accumulation of methylxanthines and polyphenols in leaves and fruits of guarana cultivars. Thus, information will be generated to contribute for future studies on guarana genetic improvement and plant physiology. Methylxanthines (caffeine [Cf], theobromine [Tb], and theophylline [Tf]) and phenolic compounds (catechin [Ct] and epicatechin [EC]) were quantified by HPLC in the leaves and fruits (pericarp and seed) of eighteen guarana cultivars. Tf was not detected in any tissue. Caffeine is present almost exclusively in seeds ( $4.21 \pm 0.69$  g.100g dry weight<sup>-1</sup>), while theobromine is predominant in leaves. Although catechin and epicatechin are present in both tissues, their contents are higher in seeds, with mean values of 1.36 and 0.76 g.100g dry weight<sup>-1</sup>, respectively. In guarana cultivars under study, BRS-Satere and BRS-608 stood out due to the high caffeine and phenolic contents in seeds. On the other hand, in BRS-Luzeia cultivar, the highest catechin and epicatechin contents were observed in leaves. The results provide new perspectives for the commercial exploitation of other guarana tissues, which could favor the industry, since the main source of these compounds are guarana seeds.

Keywords: caffeine, theobromine, catechins.

O guaraná é uma planta nativa da Amazônia, e suas sementes são utilizadas em refrigerantes e outros ingredientes de bebidas energéticas. Aspectos fitoquímicos do guaraná têm sido amplamente explorados para seu uso em composições fitoterápicas e cosméticas devido à presença de metabólitos, como metilxantinas e polifenóis. Este trabalho visa preencher uma lacuna nas informações sobre a presença e acúmulo de metilxantinas e polifenóis em folhas e frutos de cultivares de guaraná. Dessa forma, serão geradas informações para contribuir com novos trabalhos sobre melhoramento genético e fisiologia vegetal do guaraná. Metilxantinas (caféina [Cf], teobromina [Tb] e teofilina [Tf]) e compostos fenólicos (catequina [Ct] e epicatequina [EC]) foram quantificados por HPLC de folhas e frutos (pericarpo e semente) de dezoito cultivares. Tf não foi detectado em nenhum tecido. A caféina está presente quase que exclusivamente nas sementes ( $4,21 \pm 0,69$  g.100g peso seco<sup>-1</sup>), enquanto a teobromina é predominante nas folhas. Embora a catequina e a epicatequina estejam presentes em ambos os tecidos, elas são maiores nas sementes, com médias gerais de 1,36 e 0,76 g.100g de peso seco<sup>-1</sup>, respectivamente. Nas cultivares estudadas, BRS-Satere e BRS-608 se destacaram pelo alto teor de caféina e compostos fenólicos nas sementes. Por outro lado, no BRS-Luzeia a maior detecção de catequinas e epicatequinas está nas folhas. Os resultados trazem novas perspectivas para a exploração comercial de outros tecidos do guaraná podendo favorecer a indústria que tem como principal fonte de exploração apenas a semente do guaraná. Palavras-chave: caféina, teobromina, catequinas.

## 1. INTRODUCTION

Guarana (*Paullinia cupana* Kunth var. *sorbilis* (Mart.) Ducke) is a plant native to the Amazon region, with distribution covering Brazil, Ecuador, Peru, and Venezuela [1]. The traditional consumption of the drink prepared from guarana seeds, mainly by communities in the Brazilian Amazon, is due to the stimulating and medicinal properties of guarana [2, 3].

Several pharmacological studies have investigated the medicinal potential of guarana, focusing on the mechanism of action, especially of alkaloids and tannins [4, 5]. The medicinal and stimulant properties of guarana are due to the high concentrations of methylxanthines. Theobromine (3,7-dimethylxanthine) is found at higher concentrations in green tissues, while seeds contain caffeine (1,3,7-trimethylxanthine), with approximately 3.2% to 7.3% of dry weight [6, 7].

The biological function of catechins also involves plant defense, as well as environmental plant adaptation [8]. Catechins are generally considered to provide protection for plants from damage by UV rays in sunlight, and their production is seriously affected by the photosynthesis capacity of tea plants [9]. Catechins are a group of tannins that include (–)-epigallocatechin gallate (EGCG), (–)-epicatechin gallate (ECG), (–)-epigallocatechin (EGC), (–)-epicatechin (EC), (–)-gallocatechin (GC), and (+)-catechin (CT), which are considered to be synthesized through phenylpropanoid and flavonoid biosynthetic pathways [10]. Recent studies have highlighted variations in the total and individual content of catechins in guarana seeds, which is comparable to tea leaves [6-11]. Yonekura et al. (2016) [12] showed that these catechins are bioavailable to humans and also attributed them with antioxidant and detoxifying properties.

The guarana genetic improvement program aims, mainly, to develop cultivars that are more productive and resistant to anthracnose [13]. To date, 18 cultivars have been released by Embrapa Western Amazon; however, the content of methylxanthines and phenolic compounds in the fruits of all these cultivars is not yet known [14]. This highlights the chemical diversity of guarana genotypes and the importance of knowing this composition for new breeding programs aimed at seed exploration, as well as the characterization of other tissues for alternative exploration, physiology studies, and breeding. Thus, the objective of the current work was to characterize the composition of methylxanthines and phenolic compounds (catechin and epicatechin) in fruits (seeds and pericarp) and leaves of 18 guarana cultivars.

## 2. MATERIAL AND METHODS

### 2.1 Plant Material

The plant material used in the study comes from the technological showcase of Embrapa Western Amazon, located on the AM 010 highway, km 29, Manaus-AM (02°52' S; 59°59' W). The soil at the site is classified as Yellow Latosol, deep, with high levels of exchangeable aluminum, a clayey to very clayey texture, acidic, with a pH ranging from 3.5 to 4.7, and low levels of calcium, potassium and phosphorus, as well as high aluminum saturation [6]. Ripe fruits and leaves were collected. The experimental design adopted was entirely randomized, with 18 treatments (genotypes) in three replications. After collection, the plant material was stored in properly identified paper bags and transported to the Laboratory of Weed Sciences (LCPD), at the Federal University of Amazonas UFAM, where the fruits were separated into pericarp and seed.

### 2.2 Quantification of Methylxanthines and Polyphenols

The seeds, pericarps, and leaves were packed separately to be dried in a forced circulation oven at 50°C until constant mass and finely ground in a knife mill. Extraction and quantification of caffeine, theobromine, theophylline, catechin, and epicatechin were performed through the methodology used by (Nina et al., 2021) [6]. Approximately 100mg of grouped samples was homogenized in 5 mL of MeOH–H<sub>2</sub>O (80:20 v/v) in screw-capped glass tubes, in a thermal bath

at 50°C for 2 h, with occasional shaking in a vortex tube shaker. After cooling at room temperature, the extracts were centrifuged at 14,000g for 20 min. The supernatant (300 µL) was diluted in Milli-Q ultrapure water in a ratio of 1:1 (v:v) of leaves to pericarp, and a ratio of 5:1 (v:v) to seeds.

Methylxanthines and polyphenols were quantified by high performance liquid chromatography (HPLC Shimadzu System) using a photodiode array detector operating at 272 (methylxanthines) and 280 nm (phenolic). The analyses were carried out in the Interdisciplinary Support Center of the Federal University of Amazonas (CAM-UFAM) in the Biomolecule Purification Laboratory. The separation was performed in an Allcrom® C18 (25 cm × 4.6 mm, 5 µm) reverse-phase column, with the mobile phase (A) using water with 1% acetic acid and (B) methanol under 1.2 ml min<sup>-1</sup>; the gradient used was 0 min for 20% B, 13min for 45% B, 23 min for 100% B, and 30 min for 20% B. For quantification, curves were constructed with known quantities of solutions with commercial standards for CF, TB, TP, CT, and EC (Sigma-Aldrich).

### 2.3 Data analyses

Graphical boxplot analysis was performed to compare theobromine (TB), catechin (CT), caffeine (CF), and epicatechin (EP) values obtained in different regions of the guarana plants. Boxes show the 25% and 75% quartiles, smaller bars show the 10% and 90% quartiles, the larger bar shows the median, and circles show the outliers.

The collected data were submitted to analysis of variance and the means compared by the Scott-Knott test at 5% probability. The following genetic parameters were estimated: coefficient of genetic variation, heritability in the broad sense, selective accuracy, and the ratio between the coefficient of genetic variation and the coefficient of environmental variation, according to Cruz et al. (2014) [15]. The phenotypic correlations between the characters under evaluation were also estimated. All these analyses were performed using the Genes program [16].

The techniques of multivariate analysis of principal components and relative importance of characters, based on the method proposed by Singh (1981) [17], were applied to the quantitative data, and the graphic dispersion of the similarity between the cultivars was performed by the Main Coordinates Method from the program *RStudio* Team (2020) [18].

## 3. RESULTS

Eighteen guarana species were characterized in terms of methylxanthine, catechin, and epicatechin values in leaves and fruits (seed and pericarp). Considering the average of all cultivars, as previously described, guarana seeds mainly accumulate caffeine and the other tissues accumulate theobromine (Figure 1). When detected, theobromine in seeds and theophylline in all tissues, were at very low values and in very few repetitions, so these results are not presented. Regarding phenolic compounds, catechin values are higher in all tissues analyzed in relation to epicatechin, with greater accumulation in seeds (Figure 1).

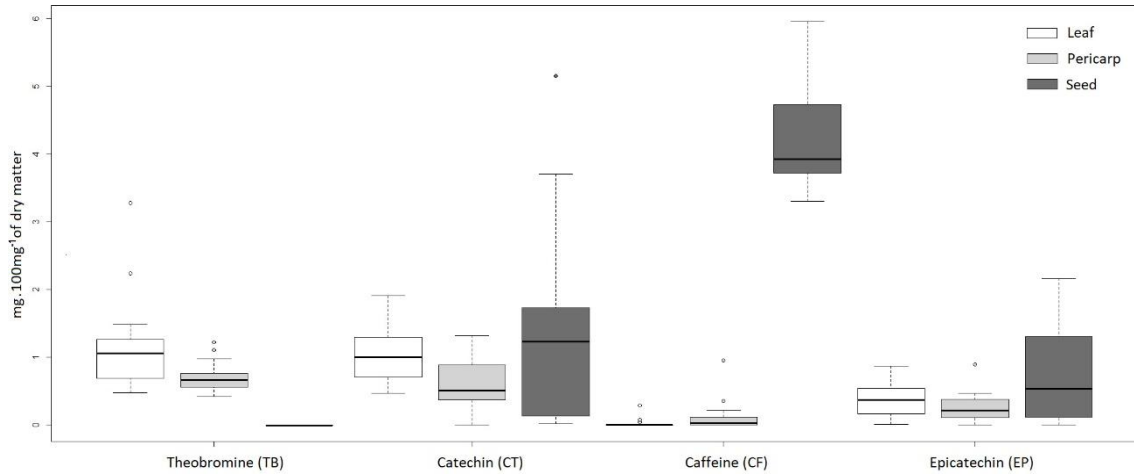


Figure 1. Levels of theobromine (TB), catechin (CT), caffeine (CF), and epicatechin (EP) obtained from different plant tissues in eighteen guarana genotypes.

Analysis of variance for tissue-specific metabolites detected significant differences at 1% probability between cultivars, except for the characteristic caffeine in leaves (CF-L) (Table 1), which highlights the genetic variability between the genotypes studied and makes the use of multivariate analysis techniques possible in the evaluation of genetic material divergence.

In the evaluated traits, with the exception of epicatechin in pericarp (EP-P), the values of heritability in the broad sense ( $H^2$ ) were considered high, which highlights that most of the variations found in the levels of metabolites are of genetic origin (Table 1). The catechin and epicatechin polyphenols in seeds (CT-S and EP-S) and theobromine in leaves and pericarps (TB-L and TB-P) demonstrated the highest heritability and selective accuracy values. In addition, the ratio between CVg/CVe was being greater than unity, a situation considered ideal for selection.

Epicatechin was the most metabolite among the three tissues, with mean values between 0.26 and 0.37 (Table 1). However, the lowest coefficient of experimental variation (CVe%) was for caffeine in seeds, evidencing the stability of this characteristic among guarana cultivars. The coefficient of genetic variation (CVg%), which expresses the amount of existing genetic variation as a percentage of the general average, also presented a low value (14.5%), characterizing that there may be greater expression of genetic variation in future field evaluations.

Table 1. Mean squares and genetic parameters of theobromine (TB), catechin (CT), caffeine (CF), and epicatechin (EP) metabolites, obtained from different plant regions (L - leaf, P - pericarp, and S - seed) in eighteen genotypes of guarana.

Variation Source	GL	Metabolites (mg. 100 mg of dry weight <sup>-1</sup> )										
		Mean squares										
		TB-L	TB-P	CT-L	CT-P	CT-S	CF-L	CF-P	CF-S	EP-L	EP-P	EP-S
Genotype	17	1.36**	0.14**	0.44**	0.42**	6.24**	0.01 <sup>ns</sup>	0.16**	1.41**	0.12**	0.13 <sup>ns</sup>	1.52**
Residue	36	0.17	0.02	0.13	0.14	0.27	0.02	0.05	0.29	0.04	0.10	0.12
CVe (%) <sup>1</sup>		34.77	19.23	34.21	62.35	38.14	530.8	198.07	12.76	55.63	123.76	45.51
Mean		1.18	0.71	1.05	0.60	1.36	0.03	0.11	4.21	0.36	0.26	0.76
Minimum		0.46	0.26	0.00	0.00	0.00	0.00	0.00	2.90	0.00	0.00	0.00
Maximum		4.15	1.34	2.34	1.80	5.48	0.86	1.45	6.28	1.09	2.31	2.31
Genetic parameters												
H <sup>2</sup> (%) <sup>2</sup>		87.66	86.75	70.87	67.30	95.66	-	68.24	79.48	76.83	25.07	92.18
Acc <sup>3</sup>		0.94	0.93	0.84	0.82	0.98	-	0.83	0.89	0.88	0.50	0.96
CVg (%) <sup>4</sup>		53.52	28.42	30.81	51.65	103.37	-	167.63	14.50	58.48	41.33	90.20
CVg/CVe <sup>5</sup>		1.54	1.48	0.90	0.83	2.71	-	0.85	1.14	1.05	0.33	1.98

The average theobromine contents among the studied cultivars ranged from 0.48% to 3.28% for leaves and 0.42 to 1.22% in pericarp, with the cultivar BRS-CG610 showing the highest accumulation of these metabolites in both tissues (Table 2). Regarding the main alkaloid of commercial interest in guarana, caffeine was detected in leaves of only three cultivars, of which two, BRS-Saterê and BRS-CG608, showed the highest caffeine content in pericarp and seeds. The caffeine content in pericarp ranged from 0.01% to 0.95% among the 13 cultivars in which this compound was detected. In seeds, the lowest value found was 3.31% in BRS-CG648, and the highest was 5.95% in BRS-Saterê (Table 2).

Regarding polyphenols, levels of catechin and epicatechin were detected in the tissues studied in all cultivars (Table 2). It is not possible to identify a predominant accumulation of these compounds in the plant, which indicates a metabolic alteration, as occurs for methylxanthines. On the contrary, there is a trend towards equivalent values of these compounds, such as the cultivar BRS-Luzeia, which showed the highest accumulation of catechin and epicatechin in leaves, and the cultivar BRS-CG608 in seeds, 5.16 and 2.17%, respectively (Table 2). It is noteworthy that the cultivar BRS-CG608 also has one of the highest levels of caffeine in seeds. However, BRS-CG189, also with the highest levels of CF in seeds, does not have high amounts of polyphenols in this tissue.

Table 2. Mean<sup>1</sup> of theobromine (TB), catechin (CT), caffeine (CF), and epicatechin (EP) metabolites, obtained in different plant regions (L - leaf, P - pericarp, and S - seed) in eighteen guarana genotypes.

Genotypes	Metabolites (mg.100mg of dry mass <sup>-1</sup> )										
	TB-L	TB-P	CT-L	CT-P	CT-S	CF-L	CF-P	CF-S	EP-L	EP-P	EP-S
BRS-Amazonas	1.08 c	0.67 b	0.61 b	0.28 b	1.23 c	0.00 a	0.00 b	3.71 c	0.36 a	0.10 a	1.45 b
BRS-Andirá	1.10 c	0.66 b	1.46 a	1.33 a	0.57 d	0.00 a	0.07 b	3.64 c	0.61 a	0.31 a	0.24 d
BRS-Cereçaporanga	0.65 d	0.70 b	0.54 b	0.47 b	1.74 c	0.00 a	0.00 b	3.74 c	0.06 b	0.15 a	1.31 b
BRS-CG189	2.24 b	0.48 c	0.48 b	0.00 b	0.13 d	0.00 a	0.12 b	5.15 b	0.12 b	0.15 a	0.12 d
BRS-CG372	1.26 c	0.82 b	0.96 b	1.13 a	0.14 d	0.00 a	0.02 b	4.74 b	0.40 a	0.41 a	0.01 d
BRS-CG505	1.01 c	0.77 b	0.60 b	1.04 a	2.12 c	0.00 a	0.02 b	4.27 c	0.22 b	0.38 a	0.87 c
BRS-CG608	0.49 d	0.56 c	1.03 b	0.05 b	5.16 a	0.08 a	0.95 a	5.10 b	0.46 a	0.01 a	2.17 a
BRS-CG610	3.28 a	1.23 a	1.30 a	0.60 b	1.24 c	0.04 a	0.01 b	3.96 c	0.56 a	0.23 a	0.27 d
BRS-CG611	1.21 c	0.57 c	0.71 b	0.99 a	1.27 c	0.00 a	0.00 b	3.89 c	0.16 b	0.43 a	0.75 c
BRS-CG612	0.60 d	0.74 b	0.82 b	0.39 b	0.09 d	0.00 a	0.04 b	3.88 c	0.01 b	0.47 a	0.20 d
BRS-CG648	1.04 c	0.55 c	1.35 a	0.46 b	1.64 c	0.00 a	0.22 b	3.31 c	0.39 a	0.89 a	0.86 c
BRS-CG850	1.46 c	0.67 b	0.96 b	0.49 b	3.30 b	0.00 a	0.02 b	4.75 b	0.24 b	0.06 a	1.85 a
BRS-CG882	1.24 c	0.60 c	1.24 a	0.89 a	0.19 d	0.00 a	0.06 b	4.06 c	0.50 a	0.11 a	0.12 d
BRS-Luzeia	1.04 c	0.98 a	1.92 a	0.89 a	3.71 b	0.00 a	0.00 b	4.45 c	0.87 a	0.26 a	1.84 a
BRS-Marabitaná	0.48 d	0.74 b	1.50 a	0.74 a	0.02 d	0.00 a	0.12 b	3.84 c	0.29 b	0.25 a	0.12 d
BRS-Maués	0.87 d	0.42 c	0.99 b	0.17 b	0.47 d	0.00 a	0.00 b	3.73 c	0.56 a	0.06 a	0.33 d
BRS-Mundurucânia	0.69 d	0.47 c	1.12 a	0.54 b	0.12 d	0.00 a	0.05 b	3.62 c	0.04 b	0.21 a	0.03 d
BRS-Saterê	1.49 c	1.11 a	1.24 a	0.37 b	1.44 c	0.29 a	0.35 b	5.96 a	0.72 a	0.13 a	1.10 b
Overall average	1.18	0.71	1.05	0.60	1.36	0.03	0.11	4.21	0.37	0.26	0.76
CVe (%) <sup>2</sup>	34.77	19.23	34.21	62.35	38.14	530.85	198.07	12.76	55.63	123.76	45.51

In guarana, the correlation of metabolites between the cultivars studied does not provide conclusive information regarding a pattern of accumulation of methylxanthines and polyphenols, since each metabolite presented different correlation values between tissues (Figure 2). The highest correlations found were positive for catechin and epicatechin levels in leaves (0.70) and in seeds (0.92) (Figure 2). A strong negative correlation was observed in

caffeine from the pericarp and seeds. On the other hand, caffeine from leaves and seeds presented a high correlation (0.70), because caffeine content was detected in only three of the 18 cultivars, of which BRS-CG608 and BRS-Saterê showed the highest levels of caffeine in the seed (Figure 2).

Data on methylxanthines and polyphenols from all tissues per cultivar were normalized and subjected to principal component analysis in order to enable simpler and more direct visualization of the genetic diversity between the evaluated genotypes, through two Cartesian axes (Figure 3), in addition to verifying which metabolite could best explain the variation between cultivars.

Seed catechin presents a relative contribution to the model of 57.52%, followed by theobromine in pericarp (13.39%) and in leaf (10.01%). The cultivar BRS-608, which is isolated in the lower right corner of the PCA, has the highest content of theobromine in pericarp and leaf and of catechin in seed (Figure 3), with a content of 5.16%, while the general average is 1.36% (Table 2). The cultivars BRS-Satere and BRS-Luzeia, which have high amounts of caffeine in the leaf and catechin in the seed, respectively, are also positioned further away from the other cultivars (Figure 3).

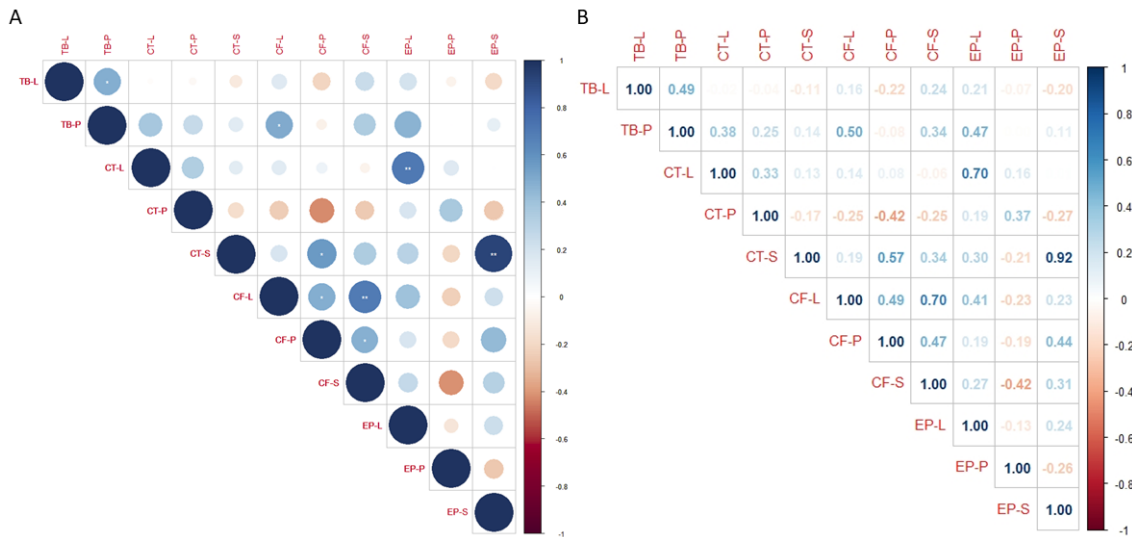


Figure 2. Estimates of the phenotypic correlation coefficients for theobromine (TB), catechin (CT), caffeine (CF), and epicatechin (EP) metabolites, obtained in different plant regions (L-leaf, P-pericarp, and S-seed) in eighteen guarana genotypes. A: Significance relations \*\*: \*  $p \leq 0.01$ ,  $p \leq 0.05$ , respectively, using the *t* test. B: correlation coefficient value.

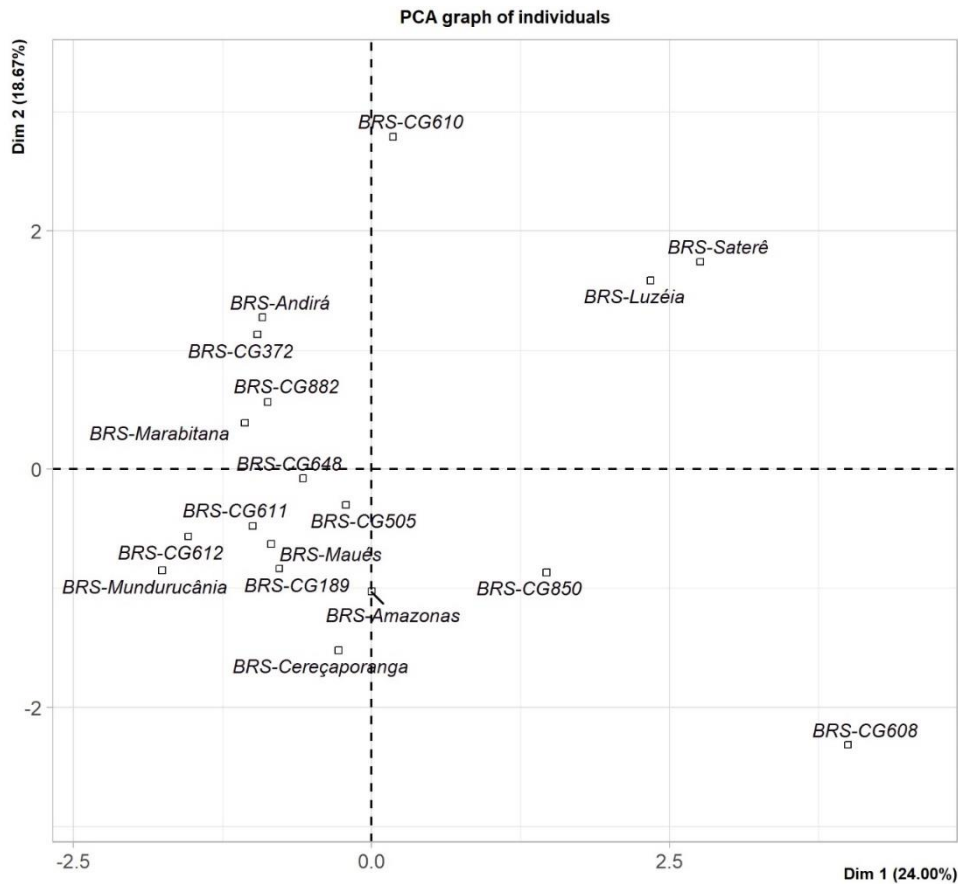


Figure 3. Principal component analysis (PCA) of the compound averages of theobromine, catechin, and epicatechin in tissues by guarana plant cultivar.

#### 4. DISCUSSION

Guarana cultivars accumulate methylxanthines and polyphenols differentially in their tissues; the seed is the tissue with the highest accumulation of caffeine, catechin, and epicatechin, while theobromine is found in higher amounts in leaves (Figure 1, Table 1). The values of methylxanthines are within the range previously described in other studies, from 3.2% to 7.35% of caffeine in mature seeds and very low values of theobromine in leaves, between 1.0% and 2.5% in mature leaves, reaching up to 5% theobromine in young leaves [5-7, 19].

With regard to polyphenols, catechin and epicatechin presented a lower general average in seeds compared to other studies, but with variations ranging from 0.02% to 5.19% between cultivars [5-7, 12, 20]. Due to the number of genotypes, large variation was expected in the levels of metabolites in the analyzed tissues.

In most plant species in which the presence of methylxanthines occurs, caffeine is the predominant compound, but in some species, such as cocoa and cola, as well as in guarana, other methylxanthines are differentially detected in tissues or developmental stages [19, 21, 22]. In *Citrus paradisi* plants, the presence of caffeine, theophylline, theobromine, and paraxanthine was detected in flowers [23]. The accumulation of caffeine in plant tissues can be regulated both kinetically by the speed of the degradation pathway X via biosynthesis and through genetic control by the expression of genes that encode key enzymes in the step of converting theobromine to caffeine [24]. In the case of tea, the natural allelic variation in the *TCS1* gene that encodes caffeine synthase in these plants plays a crucial role in caffeine biosynthesis. In low-caffeine-accumulating tea germplasms [25].

The biosynthetic pathway of caffeine from guarana has not yet been fully elucidated, Schimpl et al. (2014) [7] characterized the enzyme caffeine synthase (PcCS), produced heterologously from the *PcCS* gene, and identified the ability to convert 7-methylxanthine into

theobromine and theobromine into caffeine, which suggests that it is a bifunctional enzyme that acts in the last two steps of the pathway, such as TCS1 of tea. However, Huang et al. (2016) [26] when studying evolutionary aspects of caffeine-accumulating species, suggest that the precursor of theobromine in guarana would be 3-methylxanthine, and not 7-methylxanthine, converging on a pathway shared with cocoa and not with tea and coffee. Other aspects of caffeine metabolism need to be studied in order to understand the dynamics of caffeine/theobromine accumulation in guarana plant tissues.

The detection of compounds of commercial interest in other plant tissues, such as leaves and pericarp, in the case of guarana can shed light on the plant metabolism of the species, as well as serve as a starting point for new improvement projects, and may also be used as early markers. In coffee, there is a correlation between the caffeine content of seedling leaves and adult plants with the caffeine content of the seed [27], which enables selection of plants with potential for the desired characteristic in relation to this compound in the initial phase of development, without having to wait for the plant to reach fruiting. However, no significant correlation was found between seed caffeine and methylxanthines in the leaf of adult plants to support this hypothesis (Figure 2). On the other hand, it is necessary to investigate whether there is a relationship between caffeine in seeds and methylxanthine content in the first pairs of seedling leaves, since this relationship occurs for coffee and methylxanthine metabolism is different through the tissue development stages [7-28].

The catechin and epicatechin content in the leaves is also not related to the content in seeds (Figure 2), reinforcing the idea of testing these compounds in the early stages of plant development, to understand the metabolism in guarana as well as to support new programs of improvement. In tea, the highest levels of catechins are mainly accumulated in young leaves, and it is possible to associate the levels of catechins with expression patterns of biosynthetic genes during the different stages of leaf development, which may, in the future, direct an approach to the study of the biosynthetic pathway of catechins in guarana, so far unexplored [10].

The high correlation between the amounts of catechin and epicatechin in the specific tissue, leaf and seed (Figure 2), indicates that regulation of the metabolism of these compounds in guarana occurs during steps in the direct substrate pathway. In tea, approximately seven genes of the polyphenol and flavonoid pathway control catechin biosynthesis during plant development and three other transcription factors are also involved in the regulation of the pathway [10].

Depending on the variability of the levels of methylxanthines and polyphenols found in the breeding populations and the intensity of selection practiced, the results suggest the possibility of high genetic gains from the phenotypic selection in the initial stages of breeding of the species. Regarding the initial and intermediate phases of an improvement program, it is preferable to have accuracy values close to or greater than 70% [29]. In this sense, the accuracy values obtained in the present work (mostly >80%) are promising. For breeding programs based on hybridization, it is also important to recommend crosses between genetically divergent genotypes, and it is desirable that both parents present good productivity combined with the presence of complementarity for quality traits.

There is no knowledge about the genetic diversity of these cultivars, dissimilarity analyses between the 18 cultivars studied here are based only on agro-morphological characters, which brings little information for a more in-depth discussion in this sense [14]. It is important to emphasize that the genome of the variety *sorbilis* is polyploid and presents a high level of polymorphism among the genotypes of the Active Germplasm Bank (BGA) [30-31]. Nina et al. (2021) [6], considering the seed metabolites, studied the phytochemical divergence between eight genotypes of guarana plants, of which only two are part of this study, BRS-Maues and BRS-372. These authors were able to group the genotypes, based on the metabolic profile, into three main chemotypes: energetic guarana (with a higher proportion of caffeine compared to polyphenols - CT and EP), antioxidant guarana (with a higher proportion of polyphenols than caffeine), and energetic-antioxidant guarana (with equivalent proportion of caffeine and polyphenols).



Among the 18 cultivars studied here, this classification of chemotypes cannot be applied, since some cultivars would not represent the groups proposed by Nina et al. (2021) [6], such as the cultivar BRS-608 which has above average caffeine content, presenting an energetic characteristic, but due to the high accumulation of catechin and epicatechin it would be classified as a cultivar with a predominantly antioxidant characteristic. On the other hand, PCA expresses the distribution of characteristics among the cultivars, in which the cultivar BRS-608 is in the fourth quadrant, mainly due to the high values of EP-S and CT-S, antioxidant characteristics of the seeds, while for the cultivars of the first quadrant, BRS-Luzeia and BRS-Satere, high values of EP and CT are present in the leaves and above-average values for caffeine in leaves and seeds, thus the other cultivars are grouped in the other quadrants, without much differentiation (Figure 3).

The grouping of genotypes based on the metabolite profile is not just about genetic knowledge, phenolic profile in guarana seeds can indicate the geographical origin and be used to assist in the botanical authentication of these materials and geographical indication signs in the future [11]. Considering the individual agronomic potential of the cultivars, BRS-Satere stands out with high caffeine content in seeds, BRS-Luzeia with catechins, and BRS-608 with both. However, the productivity of the cultivars cannot be ruled out, which will impact the metabolite yield per hectare, as analyzed for 8 genotypes per hectare [6]. The use of other parts of the plant to exploit these metabolites can also be considered. The pericarp, which represents 6 parts of the fresh weight of the guarana fruit for 1 part of the seed, could be used as a by-product of the seed processing process [32]. The catechin and epicatechin content present in guarana leaves opens new perspectives for the use of this plant, in which consumption is based only on products made from the seeds.

## 5. CONCLUSION

This study highlighted the chemical diversity of guarana genotypes. However, the tissue specific accumulation follows a standard, which is related with conserved metabolisms. The knowledge about methylxanthines and phenolic composition in guarana cultivars provides subsidies for new breeding works aimed at seed exploration focusing one or another compound, as well as the characterization of other tissues for alternative exploration. The results showed cultivars as good alternatives mainly for the soft drink and energy drink industry as well as the pharmaceutical industry. Additionally, this work contributes to insights for studies on the physiology and metabolism of guarana plants.

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