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Forte Guarani: First conilon cultivar selected for high grain caffeine content

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Abstract: Forte Guarani is a *Coffea canephora* cultivar derived from clones discovered and bred by farmers. It was evaluated in the northern state of Espírito Santo, Brazil, for caffeine content, soluble solids, chlorogenic acids, yield, plant vigor, and pest and disease resistance. This cultivar has a high grain caffeine content as well as good coffee yields.

Keywords: *Coffea canephora*, breeding, coffee, health.

Introduction

Among the 130 species of the genus *Coffea* (Davis and Rakotonasolo, 2021), *C. arabica* L. and *C. canephora* Pierre ex A. Froehner are commercially the most important. Brazil is world-leading in terms of coffee production and exportation (ICO, 2024), representing 36% of world coffee production (average for harvests 2018 to 2022) (Salvador et al., 2024). Espírito Santo State ranks first nationally in *Coffea canephora* production, with 10.2 million bags of green coffee in 2023 (Conab, 2024). This specie has particularly high levels of soluble solids and bioactive compounds, produces beverages with neutral characteristics and lower acidity than *C. arabica*, and can even be used for flavor enhancement (Farah and Lima, 2019).

The *C. canephora* specie also stands out for a high production potential, pest and disease resistance and for developing well in areas with hot and humid climate. Over time, the scenario of Conilon coffee has been changing in that the quality has been optimized and the market space is becoming increasingly relevant, especially after the Brazilian Association of Specialty Coffees (BSCA) accepted the membership of Conilon and Robusta coffee producers in the institution.

Coffee is a widely consumed beverage in Brazil and one of the most appreciated and consumed in the world, due to the organoleptic properties and stimulating effect of caffeine (Oliveira et al., 2014). Tea, chocolate, soft drinks and energy drinks also contain caffeine, but the main source is coffee (Altimari et al., 2001). Among the main effects of caffeine on the human body are reactions of the central nervous system, in that the metabolism is accelerated and catecholamine release increased (Altermann et al., 2008), which can improve concentration, reduce fatigue and increase performance in attention-demanding activities (Paluska, 2003).

Regular caffeine consumption has positive effects on health, e.g., a lower incidence of type 2 diabetes mellitus, kidney stones, liver fibrosis, liver cancer, Alzheimer's disease, Parkinson's disease and cardiovascular risks (Zhang et al., 2011; Cao et al., 2012; Ribeiro et al., 2014; Gunter et al., 2017; Carlström and Larsson, 2018; Cornelis, 2020). In view of these benefits, coffee is a truly functional beverage, and moderate coffee consumption may be considered beneficial for human health (Lima et al., 2010).

Thus, the wide genetic variability of the species *Coffea canephora* (Nowak et al., 2011; Vázquez et al., 2019; Zaidan et al., 2023) allows the identification of plants with distinct characteristics (Giles et al., 2019; Martins et al., 2019; Dubberstein et al., 2020; Silva et al., 2023; Rodrigues et al., 2023; Santos et al., 2024; Silva et al., 2024) and the selection of genotypes for several cultivation purposes.

Breeding process

Over the years that preceded this experiment, a work team addressed the identification of promising Conilon/Robusta trees in the field. At the same time, a coffee clone, discovered by a farmer at the end of the 1990s, was also identified and monitored. This genotype was observed in a seed field, in the rural area of the district of Vila Valério, due to its outstanding production. It was noted/identified at the end of the 1990s, by Hermes Joaquim Partelli and his family. It was later propagated vegetatively and cultivated for years on the family property, by several farmers of the region, at other locations in the north of the state and even in east of Minas Gerais.

The data for this study were collected in two coffee plantations, under similar management and climate conditions. On one of the plantations, cultivation began in April 2014 with 42 genotypes (plantation 1) propagated by cuttings, in the district of Nova Venécia-ES, at approximately 200 m asl. (18° 39' 43" S; 40° 25' 52" W). The other area (plantation 2) consisted of one clone (Forte Guarani) surrounded by rows of different genotypes, all cutting-propagated. Planting was also carried out in 2014, in the district of Vila Valério-ES, at approximately 150 m asl (18° 57' 02" S; 40° 18' 32" W).

The region has an Aw climate, i.e., tropical with dry winters and rainy summers (Alvares et al., 2013). In general, the average minimum temperature is above 16°C (July and/or August) and an average maximum temperature of up to 32°C in January and/or February. Occasionally, temperatures can drop below 13°C or exceed 36°C.

Plantation 1 was arranged in a randomized block design, with three blocks, where each experimental plot consisted of seven plants. Fertilization was applied as indicated by soil analysis. The spacing for planting was 3 x 1m, with an area of 3 m^2 per plant. Excess branches were pruned, to maintain a standard of 12,000 to 15,000 stems per hectare. The experimental area was irrigated every year.

Plantation 2 was organized in alternating rows of cv. Forte Guarani with more than 50 plants, surrounded by several coffee genotypes cutting-propagated. Fertilization was carried out according to soil analysis. The plants were spaced at 3.5 x 1.5m, i.e., 5.25 m^2 per plant. Excess branches were pruned to a standard level of 12,000 to 15,000 stems per hectare. The area was irrigated every year, except in 2015, due to a severe drought; at this point, the crop was replanted, followed by good regrowth and recovery.

For both plantations, laboratory analyses of coffee bean samples were carried out in triplicate over two years. To assess the stability and adaptability of the genotypes used in this study, the chemical properties were analyzed in coffee bean samples from two harvests (2018 and 2019). A variation in caffeine levels between the two years of less than 5%, indicated stability of the trait bean caffeine content over the years, and consequently, the genetic nature of the trait.

The caffeine content, the focus of this study, was analyzed by the following procedures: coffee beans were ground, sieved and extracted in triplicate, with 40% methanol, under stirring, and clarified using Carrez solutions; caffeine standard was purchased from Sigma-Aldrich (St Louis, MO, United States). The caffeine content of the methanolic extracts was analyzed using an isocratic HPLC (High- Performance Liquid Chromatography; Shimadzu LC-10-AD, Japan) system, at 272nm, in 40% methanol mobile phase, with a quantification limit of 5 µg/100g. Soluble solids were also quantified by Digital Refractometry and expressed in °Brix, and chlorogenic acid levels by High Performance Liquid Chromatography.

Performance

Cultivar Forte Guarani consists of a high-yielding clone with a high grain caffeine content. The genotype was chosen for its vigor and pest and disease resistance, satisfactory productivity and the highest grain caffeine content of all 42 genotypes evaluated on the same occasion. The cultivar Forte Guarani had a 44.1% higher caffeine content than the overall mean of the other evaluated genotypes (Table 1). This highlights the relevance of this genotype for consumers who wish to include caffeine in their diet, with a view to the various benefits of this alkaloid for human health (Lima et al., 2010), apart from improving concentration, reducing fatigue and enhancing the performance in attention-demanding tasks (Paluska, 2003).

Table 1. Caffeine, soluble solids and total chlorogenic acids contents in beans of cultivar Forte Guaraní, in the harvests of 2018 and 2019.

Genotypes	Caffeine		Mean values of two years		
	2018	2019	Caffeine	SS	CGA
Units	(g $100g^{-1}$)	(g $100g^{-1}$)	(g $100g^{-1}$)	(°Brix)	(g $100g^{-1}$)
FORTE GUARANI	2.74a	2.63a	2.68a	4.07a	6.37a
Mean of the other genotypes	1.88b	1.84b	1.86b	3.95a	7.08a
CVe (%)	2.01	1.96	1.98	3.95	8.72

SS: soluble solids; CGA: total chlorogenic acids (sum of CQA + FQA + diCQA). Means followed by the same letter in a column do not differ by the Dunnett test at 5% probability.

Cultivar Forte Guarani has a mean internode distance of 5.30 cm of plagiotropic branches, a mean leaf length of 15.86 cm and mean leaf width of 6.50 cm. During the evaluation period, a good adaptation of the genotype to the cultivation conditions was confirmed by the good growth and yield performance. No severe attack by any of the main pests and diseases was observed and tree growth was vigorous (Figure 1).

Therefore, the new cultivar has desirable characteristics, above all, high caffeine levels (our study focus) and satisfactory yields, which may promote acceptance among coffee growers. The cultivar can be grown under similar climatic conditions as where it was originally cultivated. It is recommended for the State of Espírito Santo, southern Bahia and eastern Minas Gerais, for elevations of less than 500 m asl. It should be planted in rows with up to 66% of cv. Forte Guarani and 33% of a blend of other genotypes to ensure fertilization. It is noteworthy that this is the first study focused on the registration of a cultivar with high caffeine levels in Conilon coffee beans.

Figure 1. Cultivar Forte Guarani in the district of Vila Valério, Espirito Santo State, Brazil.

Clone Maintenance and Distribution

Cultivar Forte Guarani was registered as no. 49301, in 2021, by the National Registry of Cultivars (*Registro Nacional de Cultivares*, RNC) of the Brazilian Ministry of Agriculture, Livestock and Food Supply (*Ministério da Agricultura, Pecuária e Abastecimento*). The Federal University of Espírito Santo (UFES) is in charge of maintaining cv. Forte Guarani.

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