

Full Length Research Paper

Genotypes of conilon coffee can be simultaneously clustered for efficiencies of absorption and utilization of N, P and K

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The objective of this research was to group *C. canephora* cv. Conilon coffee genotypes of different ripening cycles for both efficient absorption and utilization of N, P and K in contrasting environments. The experiment was arranged in a factorial scheme 13x4, with four replicates, the factors being: 13 genotypes of Conilon coffee and four types of fertilization (NPK: 0%-100%-100%, 100%-0%-100%, 100%-100%-0% and 100%-100%-100% of the level recommended). The results indicated that conilon coffee genotypes have efficiencies to absorb and use N, P, K modulated by the availability of these nutrients in the soil, furthermore it was possible to assume that conilon coffee genotypes of early ripening cycle have high joint efficiencies of absorption and utilization in environment with adequate NPK supply. To optimize the nutritional management, the genotypes 67 and 76 would be recommended for plantations with low technological potential to better exploit their efficiencies of absorption and use of N, P and K; and the genotypes 02, 48 and 67 would be recommended for crops with high technological potential where, besides the nutritional efficiency, their responsiveness could be explored. For breeding programs, it is recommended the exploitation of conilon genotypes 02 and 67, for presenting simultaneously high absorption and utilization efficiency of NPK.

Key words: *Coffea canephora*, mineral nutrition, fertilization, crop breeding.

INTRODUCTION

In recent years, the coffee world market witnessed significant change with a substantial increase of about

30% of world production (ICO, 2016), driven mostly by *Coffea canephora* Pierre ex A. Froehner cultivars (Robusta type of coffee). This supply growth happened due to the association between greater productivity and lower production costs (Martins et al., 2013a), coupled with an increasing use of coffee in the industry in the manufacture of soluble coffees and blends with *Coffea arabica* L. (Arabica type of coffee) beans, participating up to 50% in the blends proportion.

The *C. canephora* species has a reproduction by allogamy, presenting wide genetic and phenotypic variability (Fonseca et al., 2004; Ferrão et al., 2008). In addition, Conilon, the most used genotypes from this species in Brazil, have different fruit ripening cycles (Bragança et al., 2001). By definition, the maturation period is the time between flowering and fruit ripening, and this period of time can be used to classify the ripening primarily in early, intermediate and late cycle. This characteristic may vary depending on the genotype and environment (Pezzopane et al., 2003). Furthermore, Conilon coffee genotypes of different ripening cycles also show differences in growth and plant vigor, which in turn interferes with the photosynthetic activity and transport of assimilates (Morais et al., 2012), resulting in differential absorption and accumulation of nutrients and biomass, thus possible requiring a different mineral fertilization management (Martins et al., 2013a; Martins et al., 2013b; Prezotti and Bragança 2013; Partelli et al., 2014).

Beside the relationship between genotype and ripening cycle, the supply of nutrients; particularly nitrogen (N), phosphorus (P) and potassium (K); is extremely important. Studies report that nitrogen is the macronutrient that is accumulated in greater quantities in Conilon plants, making the requirement for this nutrient to be very high (Bragança et al., 2008; Bragança et al., 2010; Clemente et al., 2013). There is evidence that Conilon trees cultivated without satisfactory phosphorus supply present uneven and limited development of roots and aerial part (DaMatta et al., 2007; Martins et al., 2013b). Additionally, potassium is the third major macronutrient in order of accumulation by Conilon coffee (Bragança et al., 2008), with importance to control of turgidity and maintenance of fruit (Nogueira et al., 2001). Therefore, its nutritional deficiency is extremely detrimental to plant growth and crop yield.

In this scenario, the study of nutritional efficiency becomes of great importance for research involving genotypes with potential to adapt to conditions of nutritional limitation (Tomaz et al., 2011; Martins et al., 2013c), regarding the plant ability to absorb, translocate and use particular mineral nutrients (Fageria, 1998). However, there are few methods to simultaneously identify genotypes efficient in absorbing and using the

mineral nutrient supplied in the soil (Fageria, 1998; Martins et al., 2013a). Thus, we aim at to group Conilon coffee genotypes of different ripening cycles for both efficient absorption and utilization of N, P and K in contrasting environments.

MATERIALS AND METHODS

Local conditions

The experiment was conducted in greenhouse, located in the experimental area of the Centro de Ciências Agrárias of the Universidade Federal do Espírito Santo (CCA-UFES), in Alegre, ES, Brazil, with coordinates of 20°45' S latitude and 41°33' W longitude, and an average altitude of 277.41 meters over sea level, from September 2012 to March 2013.

The soil was collected at a depth of 10 to 40 cm, with the first 10 cm of the soil being discarded to reduce the effect of the organic matter present on the surface layer. A soil sample was sent to laboratory for chemistry and physic characterization, performing according to the methodology described by Embrapa (2006) (Table 1). The soil was characterized as a clayey red-yellow latosol.

After the characterization, the soil was dried under shade and homogenized in a 2.0 mm mesh sieve. Subsequently, the soil was separated into samples of 10 dm³, standardized by weighing on a precision balance and placed in sealed plastic pots (with a capacity for 12 dm³).

Along the assays, the pots were irrigated daily, maintaining the soil moisture at 60% of the total pore volume, obtained by particle density and soil density determination using the cylinder method, according to Embrapa (1997).

Plant material

The genotypes have high compatibility gametophytic, high adaptability, high visual evaluation index (VEI > 6.5), different ripening cycles (early, intermediate and late), tolerance to drought, moderate resistant to rust of coffee, high yield potential (average 70.4 bags of 60 kg ha⁻¹), large grain and production stability (Fonseca et al., 2004) (Table 2).

The conilon coffee seedlings were provided by the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural – INCAPER, produced at Fazenda Experimental de Marilândia-ES (with coordinates of 19°24' S latitude and 40°32' W); seedlings were planted in pots with 120 days of development, with two pairs of leaves and good phytosanitary and nutritional aspects

Experimental design for the study of nutrition with NPK

The experiment was arranged in a factorial scheme 13x4, with four replications, the factors being: 13 genotypes of conilon coffee (02, 23, 32, 48, and 67 of early ripening cycle; 22, 31, 73, 77, and 83 of intermediate ripening cycle; 24, 76, and 153 of late ripening cycle) and four types of fertilization (NPK: 0%-100%-100%, 100%-0%-100%, 100%-100%-0% and 100%-100%-100% of the level recommended by Lani et al., 2007), following a completely randomized design. The experimental plot has consisted of one

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Table 1. Physical and chemical characteristic of the soil used as substrate.

Characteristics	Value
Coarse sand (g kg ⁻¹) ¹	395.30
Fine sand (g kg ⁻¹) ¹	157.70
Silt (g kg ⁻¹) ¹	43.60
Clay (g kg ⁻¹) ¹	403.40
Soil density (kg dm ⁻³) ²	1.20
pH ³	5.40
P (mg dm ⁻³) ⁴	2.00
K (mg dm ⁻³) ⁵	93.0
Ca (cmol _c dm ⁻³) ⁶	1.70
Mg (cmol _c dm ⁻³) ⁶	1.10
Al (cmol _c dm ⁻³) ⁷	0.00
H+Al (cmol _c dm ⁻³) ⁸	2.10
Sum of bases (cmol _c dm ⁻³)	3.37
Potential CEC (cmol _c dm ⁻³)	5.45
Effective CEC (cmol _c dm ⁻³)	3.37
Base saturation (%)	61.80

¹Pipette method (slow mixing); ²Graduated cylinder method; ³pH in water (relation 1:2.5); ⁴Extracted by Mehlich-1 and determined by colorimetry; ⁵Extracted by Mehlich-1 and determined by flame photometry; ⁶Extracted with 1 mol L⁻¹ potassium chloride and determined by titration; ⁷Extracted by oxidation, humid route, with potassium dichromate in sulfuric medium, and determined by titration (Embrapa, 1997).

Table 2. Characterization of ripening cycles, visual evaluation index (VEI) and mean productivity (bags of 60 kg ha⁻¹) of the genotypes of *Coffea canephora*.

Genotypes	Ripening cycle	VEI ¹	Mean productivity ²
67	Early	6.5	66.0
73	Intermediate	8.3	78.0
83	Intermediate	10.0	83.7
77	Intermediate	6.6	86.1
76	Late	7.1	84.1
48	Early	7.3	72.4
22	Intermediate	6.9	62.3
23	Early	7.3	64.2
24	Late	7.7	69.7
31	Intermediate	7.3	62.3
32	Early	7.8	62.5
02	Early	7.0	62.0
153	Late	7.0	62.0

¹VEI, Visual evaluation index: Scale from 1 to 9 (1 = poor and 9 = very good) (Fonseca et al., 2004). ²Annual mean productivity (bags of 60 kg ha⁻¹) of genotypes in eight successive harvests in the different studied environments in the state of Espírito Santo (Fonseca et al., 2004).

plant per pot with three replications.

The study of nutrition with nitrogen (Sub experiment 1 - 0% and 100% of the N) fertilization was performed with NH₂CONH₂ p.a., diluted in distilled water and applied over the soil surface, distant 10 cm of the plant collar, following levels consistent with the treatments of 0 and 100% of the N (respectively 0.00 and 2.15 g dm⁻³ of N). The fertilization was divided into four applications, performed at 30,

60, 90, and 120 days after planting. In this study, the fertilization with phosphorus and potassium was provided to all parcels in a single application before planting, using KH₂PO₄ P.A. diluted in water and applied in the entire volume of soil, according to the recommendation (Lani et al., 2007).

The study of nutrition with phosphorus (Sub experiment 2 - 0% and 100% of the P₂O₅), consisted of 0.00 and 1.75 g dm⁻³ of P₂O₅,

Table 3. Mean squares, coefficients of variation (CV) and overall means for efficiency of absorption of nitrogen, phosphorus and potassium (EAN, EAP and EAK respectively) and for efficiency of utilization of nitrogen, phosphorus and potassium (EUN, EUP and EUK respectively) for genotypes of conilon coffee grown in environments with discriminating levels of N, P and K in the soil.

Variation source	EAN	EUN	EAP	EUP	EAK	EUK
Genotypes (G)	282.87*	1.71*	39.11*	334.62*	876.33*	263.18*
Fertilization (A)	20591.94*	12.44*	51.49*	2740.45*	14748.25*	8137.74*
Interaction G*A	181.62*	1.02*	39.31*	61.82*	254.17*	160.03*
Residue	9.07	0.02	0.87	2.02	15.37	4.46
CV (%)	6.77	5.13	6.71	41.04	5.87	5.89
Overall mean	44.51	3.08	13.89	3.46	66.74	39.86

*Significant at 5% probability by F test.

were applied using KH₂PO₄ p.a., diluted in distilled water and homogenized completely the volume of soil in the pot. In this study, fertilization with nitrogen and potassium was performed in four cover applications, the first at 30 days after planting, and the others, at an interval of 30 days. In all fertilizations, the nutrients were supplied via p.a. salts (KNO₃, KH₂PO₄ and NH₂CONH₂), seeking to establish the nutritional balance of the soil, according to the recommendation (Lani et al., 2007).

The study of nutrition with potassium (Sub experiment 3 - 0% and 100% of the K), consisted of 0.00 and 1.5 g dm⁻³ of potassium, were applied using KCl p.a., diluted in distilled water and homogenized completely the volume of soil in the pot. In this study, the fertilization with phosphorus was provided to all parcels in a single application before planting, using CaHPO₄ p.a., (recommendation by Lani et al., 2007), and fertilization with nitrogen was performed in four cover applications, the first at 30 days after planting, and the others, at an interval of 30 days, using NH₂CONH₂ p.a., (recommendation by Lani et al., 2007); both diluted in water and applied in the entire volume of soil.

Evaluation of the study and calculate indices

After 150 days of cultivation, the plant materials (leaves, stems and roots) were collected and separated in paper bags, which were then dried in laboratory oven, with forced air circulation at 60.0°C (STF SP-102/2000 CIR), until constant weight. After drying, the plant materials were weighed on analytical balance (SHIMADZU AUW-220D; precision: 0.00001 g) and triturated (CIENLAB EC-430, 8 blades, 1725 rpm, 20 mesh) to obtain a homogeneous powder.

To quantify the nitrogen content, 0.5 g (+/-0.001g) of the prepared material, in triplicate samples, were transferred to Taylor tubes (25 mm x 200 mm) and submitted to the stages of sulfuric digestion (H₂SO₄), distillation (NaOH 40%) and titration (NaOH 0.02 mol L⁻¹) of nitrogen in "Kjeldahl" distillers (Marconi MA-036), according the Kjeldahl method (Ma and Zuazaga, 1942).

To quantify phosphorus and potassium content, 0.5 g (±0.001 g) of the prepared material, in triplicate samples, were transferred to Taylor tubes (25 mm x 200 mm) and submitted to the stages of nitroperchloric digestion (HNO₃ and HClO₄) in a digestion block (Tecnal, TE-007D) at 180 to 190°C for 3 h; afterwards, 3 mL of ascorbic acid (C₆H₈O₆, 0.87M) were added and the determination was done by spectrophotometry at 725 nm (Femto, 700 Plus) (Defelipo and Ribeiro, 1996).

The following indices were calculated: Absorption efficiency = (total nutrient content in the plant)/root dry matter) (Swiader et al., 1994) and use efficiency = (total dry matter)²/(total nutrient content in the plant) (Siddiqi and Glass, 1981).

For each nutrient (N, P and K), the genotypes were classified in 4 groups according to the efficiencies of absorption (horizontal axis) and utilization (vertical axis). The grouping was made into two

different scenarios of nutrient supply (for N, P and K separately), creating a set of contrasting environments. Therefore, there was cluster analyses in scenarios with low supply of N, P and K (0% of the recommended by Lani et al. (2007) and cluster analyses in scenarios with standard supply of N, P and K (100% recommended by Lani et al., 2007). The grouping, in each scenario, was performed with the intersection of the axes, defined with the overall means for each variable.

Statistical analysis

The data were subjected to analysis of variance (p≤0.05) and the analyses were performed using the statistical software SISVAR (Ferreira, 2011).

RESULTS

Effect of genotypes and fertilizations over the nutritional efficiency

The analysis of variance showed significant interaction for the parameters of nutritional efficiencies for N, P and K between genotypes of conilon coffee and scenarios of nutrient supply in the soil (Table 3). This fact shows that the rates of absorption and utilization of N, P and K are influenced by the genetic material as well as by the supply in the soil, which may allow separation of genotypes between distinct groups for efficiencies in each condition of nutrient supply.

Nutritional efficiency for N, P and K of genotypes of conilon coffee

Figure 1 shows the distribution of the genotypes of conilon coffee as function of the efficiencies of absorption (AE) and utilization (UE) of nitrogen in environment with low supply of this nutrient (control level - 0% N), allowing their separation into four groups: Group 1, consisting of genotypes with higher efficiencies for both absorption and utilization of N (67 and 76); Group 2, with lower efficiency of absorption and higher efficiency of utilization of this nutrient (23, 73, and 153); Group 3, with higher efficiency of absorption and reduced efficiency of utilization of this

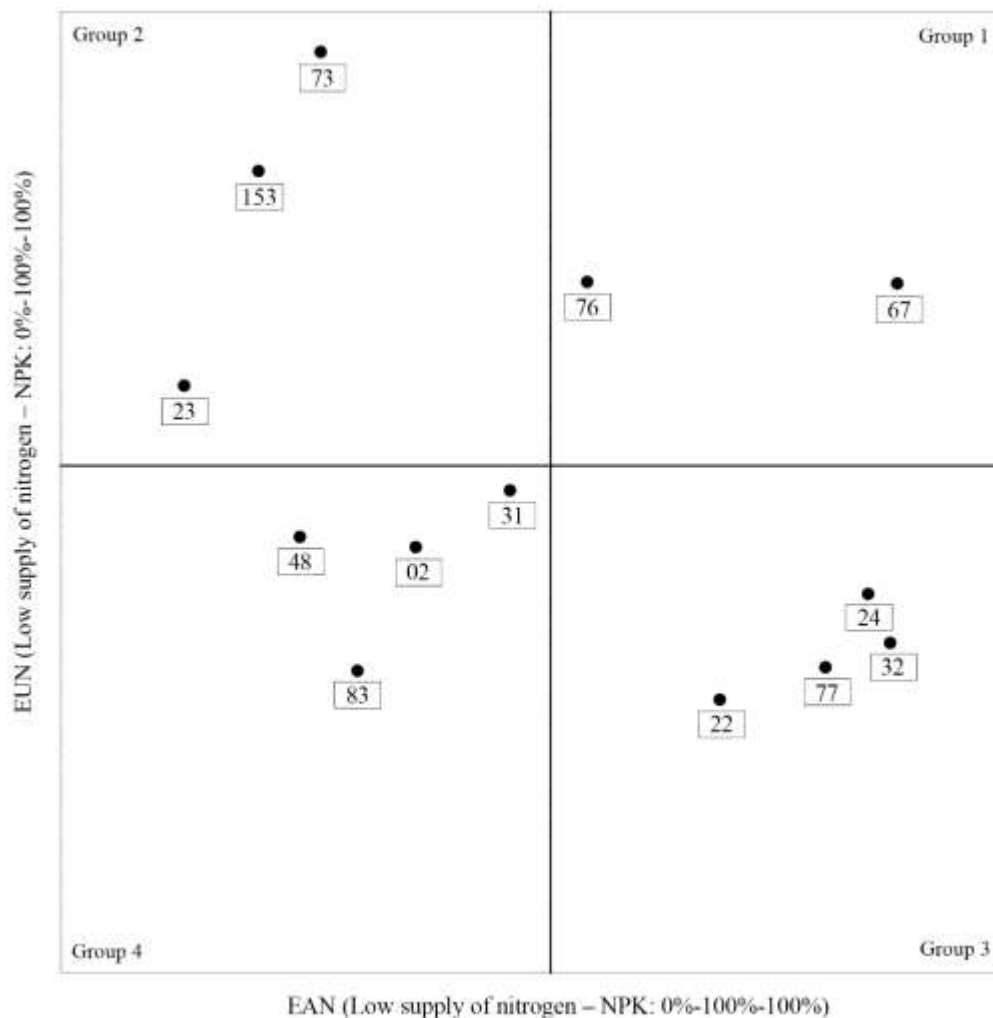


Figure 1. Distribution of genotypes of conilon coffee as function of the efficiencies of absorption (EAN) and utilization (EUN) of nitrogen in environment with low supply of this nutrient (control level - 0% N).

nutrient in the production of dry matter (22, 24, 32, and 77); and Group 4, with low efficiencies for both parameters (02, 48, 31 and 48).

In Figure 2, the distribution of the genotypes of conilon coffee shows the differentiation of the genotypes in an environment with low supply of P (control level - 0% P). In this condition, only a genotype, the 67, showed higher efficiencies for absorption and utilization of P (Group 1); the genotypes 22, 23, 24, 31, 48 and 76 were allocated in Group 2, characterized for reduced efficiency of absorption of P but higher utilization of this nutrient; the genotypes 02, 32, 77, 83, and 153 presented inverse behavior (Group 3), and the genotype 73 presented low absorption and utilization efficiency of P (group 4).

The distribution of the genotypes regarding the efficiencies for potassium, in environment with low supply, is presented in Figure 3. The results show that the genotypes 67, 76, 77, and 83 with higher efficiencies

for the nutrition with K (Group 1), the genotype 22 and 73 were placed in Group 2, characterized by lower efficiency of absorption and higher efficiency of utilization of potassium; the genotypes 23 and 48 formed the Group 3, with higher efficiency to absorb and lower to utilize K to produce dry matter. Group 4 was formed by the genotypes 02, 24, 31, 32, and 153, with low absorption and utilization efficiencies for potassium (Figure 3).

The distribution of genotypes as function of the efficiencies of absorption and utilization of nitrogen, phosphorus and potassium in environment with adequate nutrient supply (100% of N, P and K supply) formed the following groups (Figure 4): Group 1, with the genotypes 02, 48, and 67 (high of absorption and utilization efficiency of N, P and K); Group 2, with the genotypes 22, 23, 24, and 76 (low absorption efficiency and high utilization efficiency of N, P and K); Group 3, with the genotypes 31, 32, 83, and 153 (high absorption efficiency

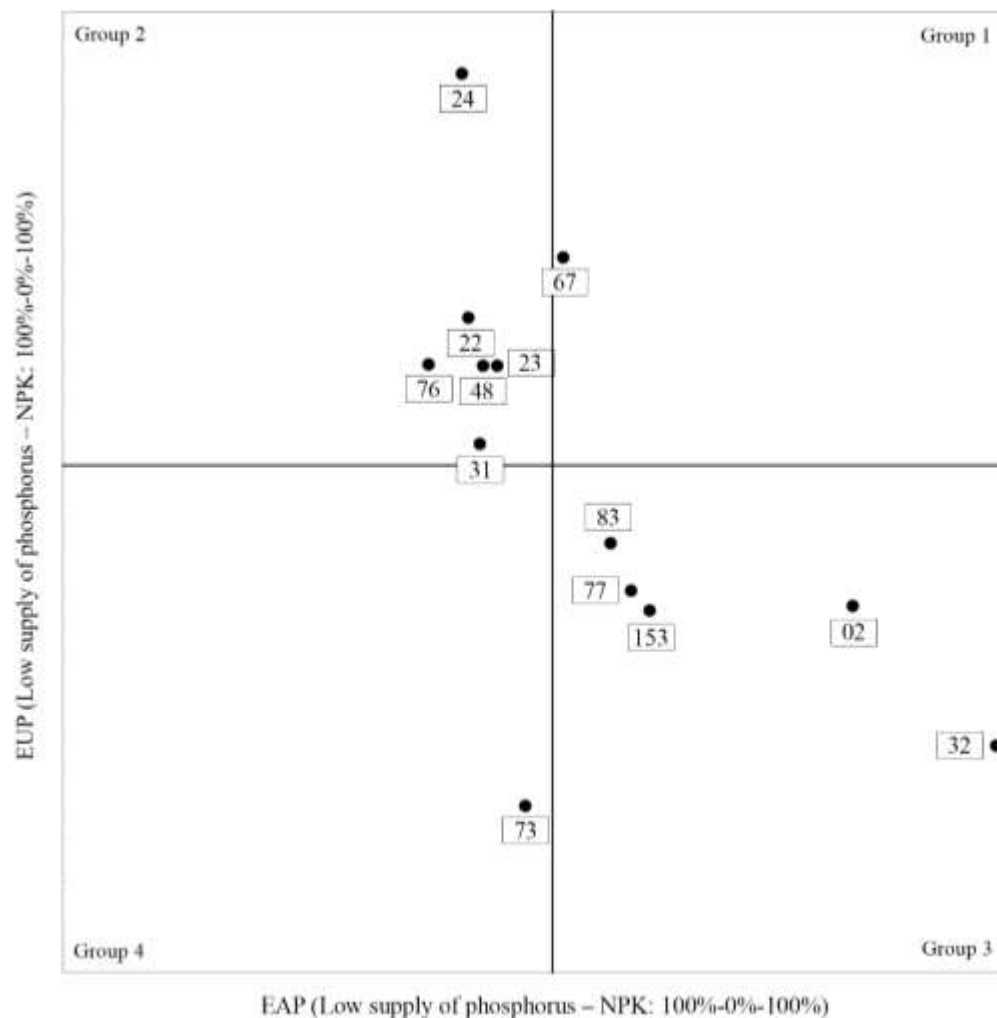


Figure 2. Distribution of genotypes of conilon coffee as function of the efficiencies of absorption (EAP) and utilization (EUP) of phosphorus in environment with low supply of this nutrient (control level - 0% P).

and low utilization efficiency of N, P and K); Group 4, formed with the genotypes 73 and 77 (low of absorption and utilization efficiency of N, P and K).

DISCUSSION

A tendency was observed that conilon coffee genotypes of intermediate and early ripening cycles presented, respectively; low and high joint efficiencies of absorption and utilization of NPK in the environment with adequate supply (Figure 4), and this trend repeated itself in environments with low P supply (Figure 2).

The correspondence between genotypes with low joint efficiencies of absorption and utilization of NPK in an environment with adequate supply, with the characteristic of the cycle of fruit ripening is new, since the behavior of conilon coffee genotypes at low supply of nutrients in soil

has always been linked to the morphology of the root system, architecture and diameter of roots (Amaral et al., 2011; Martins et al., 2013c; Colodetti et al., 2014).

Some results suggest that, in environments with adequate nutrient supply, the behavior of genotypes seems to have a relationship with the characteristic of the ripening cycle, indicating that genotypes of early cycle have more efficiency regarding the accumulation of nutrients and dry matter (Partelli et al., 2014, Martins et al., 2015). Higher efficiency of absorption and utilization of nutrients may be linked to earliness of the cycle, since there is some evidence that precocity of fruit maturation may be governed by a larger demand by the metabolic drains, thus creating more transport of photoassimilates to the fruits, and thus increasing the intensity of the sources, which would trigger an increase in net assimilation of carbon, associated with positive changes in stomatal conductance, mainly supported by greater

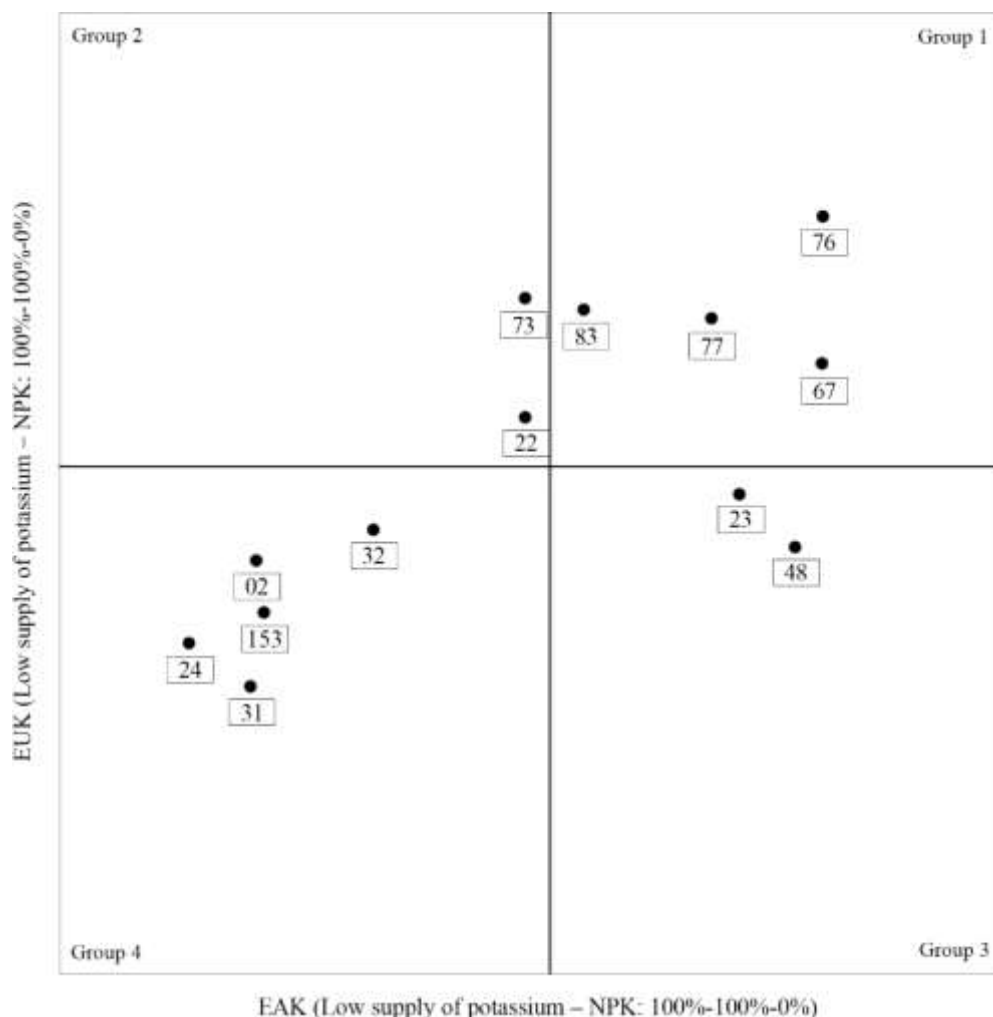


Figure 3. Distribution of genotypes of conilon coffee as function of the efficiencies of absorption (EAK) and utilization (EUK) of potassium in environment with low supply of this nutrient (control level - 0% K).

stomatal aperture (Morais et al., 2012).

As the nutritional efficiency is linked to the accumulation of dry matter, mainly the efficiency of utilization, another factor that gives advantages to early ripening genotypes is the longer period of time that the plant can spend in vegetative recovery compared to their counterparts, resulting in optimization of the process of remobilization of assimilates (DaMatta et al., 2008).

This observation may also explain, at least in part, the responsiveness achieved by genotypes of early cycle, because the increment in the source, with the adequate supply of water and nutrients in the soil, may help stabilizing the sink-source relation of these genotypes, thus meeting the high demand of the fruit, without facing the limitation imposed by the need for metabolic substrates.

Under condition of nutritional limitation, the genotype 02 had low joint efficiencies of absorption and utilization

of N and P - Group 4 (Figures 1 and 2), and also low K utilization efficiency - Group 3 (Figure 3), however, in environment with adequate supply, the same genotype showed high joint efficiencies of absorption and utilization of NPK - Group 1 (Figure 4), and this fact indicates a characteristic of responsiveness and may be related to its ripening cycle of this genotype (Table 2).

In general, it has been noted a tendency for genotypes of early cycle being intolerant and inefficient when cultivated with low supply of nutrients in soil, however, they can be highly responsive to a balanced supply of nutrients (Partelli et al., 2014, Martins et al., 2015). Revisiting other results, it was possible to verify that the genotype 02 (also referred as CV-12) is characterized as non-efficient for N and P (Martins et al., 2013a; Machado et al., 2016) and intolerant to the deficit of N and P soil (Colodetti et al., 2014; Martins et al., 2015), but this genotype is responsive to the soil fertilization with N and

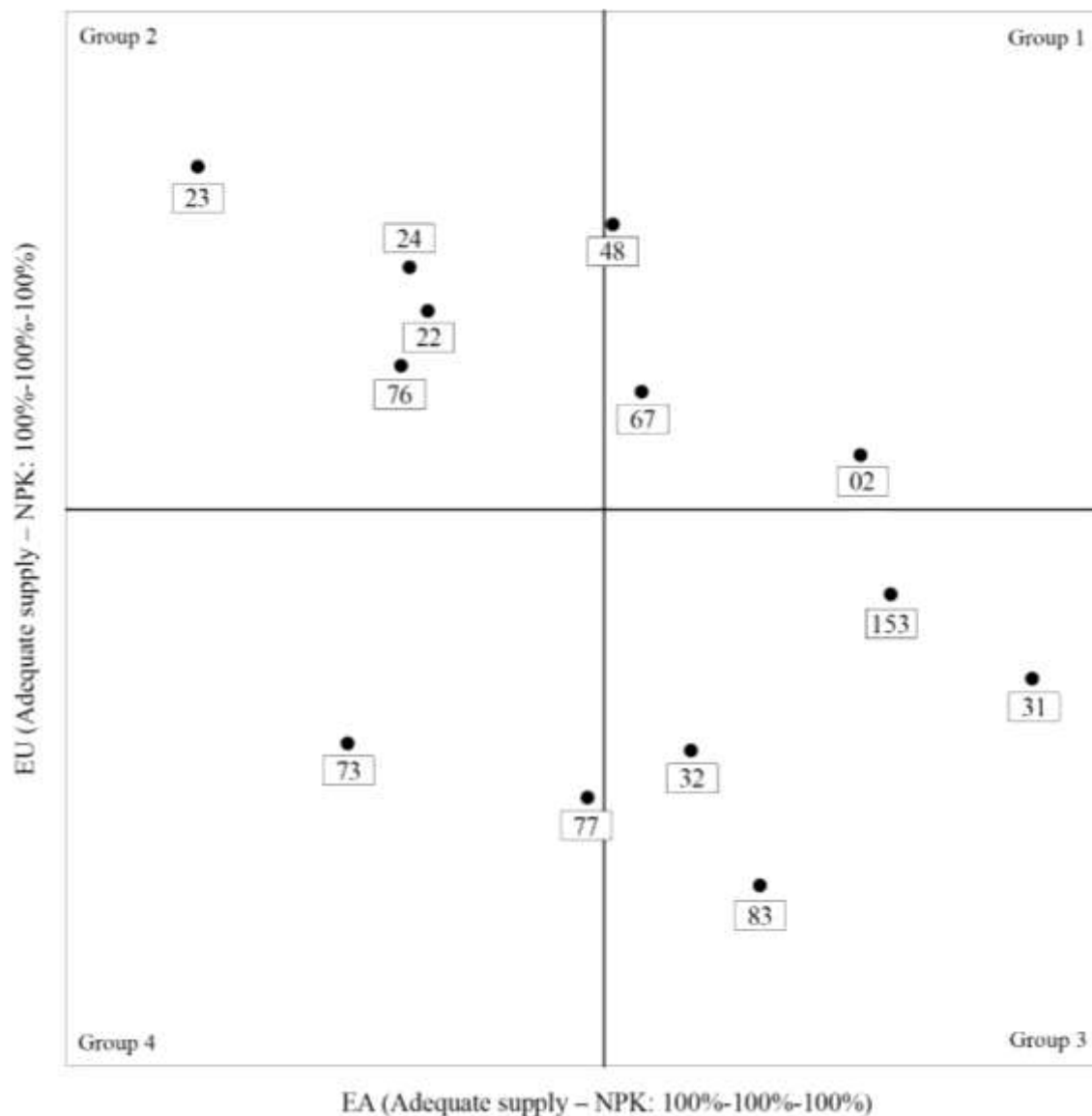


Figure 4. Distribution of genotypes of conilon coffee as function of the combined efficiencies of absorption (AE) and utilization (UE) of nitrogen, phosphorus and potassium in environment with adequate supply of this nutrient (100% of supply N, P and K).

P (Martins et al., 2013a; Machado et al., 2016). Additionally, it has been observed that the genotype 02 has a high yield potential under field conditions (composing three recommended clonal cultivars of conilon coffee), but always linked to an intense demand for nutrients and water.

The behavior of the genotype 67 is also interesting among the displayed cluster, because it has shown high joint efficiency of absorption and utilization for N, P, K, and also NPK, respectively in conditions of low supply of N (Figure 1), P (Figure 2), and K (Figure 3), and also in environment with adequate supply of NPK (Figure 4). This finding was surprising, because it was expected that this genotype had high joint efficiencies of absorption and

utilization of NPK in an environment with adequate supply of nutrients, since it is genotype of early ripening cycle, and for not being tolerant to deficit of N in the soil (Colodetti et al., 2014) and not presenting high growth of root system (Martins et al., 2013b; Martins et al., 2013c), which could possibly characterize a different potential to acquire nutrients. However, the results have indicated, at least for N and P, that the genotype 67 (also referred as CV-01) has characteristics of utilization efficiency of N and P under conditions of low supply (Martins et al., 2013a; Machado et al., 2016).

The genotype 76 was clustered, almost in all nutritional scenarios, in groups of high efficiency of utilization, presenting high efficiencies of absorption and utilization

for N and K in conditions of low supply of these nutrients (Figures 1 and 3). This genotype also presented low absorption efficiency and high utilization efficiency for P in condition of limitation on the supply of this nutrient (Figure 2), with the same behavior for NPK in conditions of adequate nutrient supply (Figure 4). This fact raises the hypothesis that the absorption efficiency may be compromised by the metabolism that governs the delay in the maturation of genotype of late ripening cycle and that may possibly exist an intensified use of absorbed nutrients, mainly due to its classification as tolerant to a deficit of N and P in the soil, and provide the high efficiency of utilization of N and P when cultivated in environments with nutritional restrictions (Martins et al., 2013a; Colodetti et al., 2014; Martins et al., 2015).

Conclusions

In conclusion, to optimize the nutritional management, the genotypes 67 and 76 would be recommended for plantations with low technological potential to better exploit their efficiencies of absorption and use of N, P and K; and the genotypes 02, 48 and 67 would be recommended for crops with high technological potential where, besides the nutritional efficiency, their responsiveness could be explored. For breeding programs, it is recommended the exploitation of conilon genotypes 02 and 67, for presenting simultaneously high absorption and utilization efficiency of NPK.

Conflicts of Interests

The authors have not declared any conflict of interests.

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