

Full Length Research Paper

Efficiency and response of conilon coffee genotypes to nitrogen supply

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Received 7 July, 2016, Accepted 18 August, 2016

The objective of the study was to differentiate genotypes with higher efficiency and responsiveness to nitrogen supply, to understand how the nitrogen supply can impact the dry matter allocation and the accumulation of this nutrient in the different plant compartments of genotypes of conilon coffee, cultivated under contrasting conditions of nitrogen availability in the soil. The plants were cultivated during 150 days in pots containing 10 kg of soil, in greenhouse. The experiment was set up in a 13x2 factorial scheme, following a completely randomized design (CRD) with three replications. The factors were: 13 genotypes and two levels of nitrogen fertilization (0 and 100% of the N recommended level). The N supply increased between 70 and 210% of the total dry matter and between 360 and 680% of the concentration of N content in leaves of the genotypes of conilon coffee. It was possible to observe that the expression of the genotypes was modulated by the availability of N in the soil, since they presented different behaviors in the studied environments (with 0 or 100% of N supply in the soil). The genotypes CV-03, CV-07 and CV-08 were classified as non-efficient and non-responsive, while the genotypes CV-01, CV-04 and CV-09 of conilon coffee were classified as efficient and responsive.

Key words: Alpha parameter, *Coffea canephora* (Pierre ex A. Froehner), mineral nutrition.

INTRODUCTION

Among the species of the genus *Coffea*, the species *Coffea arabica*, *Coffea canephora* and *Coffea liberica* have been widely cultivated for beverage production (Ramalho et al., 2013). During the last decade, the

cultivation of *C. canephora* has been greatly contributing to the increase in the worldwide production of coffee, which has been increasing the need for scientific and technologic advances for the management of genotypes

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of this diverse species.

There is wide intra and interspecific variability among genotypes of *C. canephora*, mainly in characteristics as growth rate, drought tolerance, ripening cycle and crop yield (Ferrão et al., 2008; Fonseca et al., 2004; Marraccini et al., 2012; Martins et al., 2015a; Rodrigues et al., 2012); conferring to conilon coffee, a great possibility of exploration, by means of identifying genetic material adapted to diverse conditions of soil and weather (Martins et al., 2015b). Therefore, the need to understand aspects of the nutritional demand and efficiency of genotypes to use and convert the taken nutrients is undeniable (Martins et al., 2013a; Partelli et al., 2014); however, the breeding programs have not been considering the nutritional efficiency as a selection criterion to identify superior genotypes of conilon coffee worldwide.

Differences in the nutritional efficiency among genotypes of conilon coffee have been reported in several studies (Martins et al., 2013a, 2015a, 2015b). Scientific results indicate that N and P supply in the soil can cause alterations in the biomass allocation patterns, making it possible to discriminate genotypes by their tolerance to the deficit of these nutrients in the soil (Colodetti et al., 2014; Martins et al., 2013b, 2015b). This diverse behavior suggests the possibility of selection of genotypes of *C. canephora* to contrasting soil conditions, especially for areas with low natural fertility, which would be passive of exploration using genotypes of rapid growth and low nutritional demand.

Nitrogen is the second mostly required nutrient by *Coffea* species, being the main component of enzymes, amino acids, proteins, nucleotides, hormones and chlorophyll molecules, among other compounds of the plant metabolism (Carelli et al., 2006). Additionally, nitrogen nutrition is decisive in the protection against the photoinhibition of photosynthesis when the coffee plants are cultivated under high irradiances, promoting and reinforcing the protection mechanisms (Carelli et al., 2006; Ramalho et al., 2000). It is estimated that approximately 75 kg of N are exported per hectare, during one cycle, only by the flowers of *Coffea* spp. (Laviola et al., 2008; Malavolta et al., 2002), and the amount may vary according to the ripening cycle (Partelli et al., 2014), making the study of nutritional efficiency and responsiveness an economic, social and environmental need for all coffee producing regions (Fageria, 1998). The objective of the study was: (i) to understand how the nitrogen supply can impact the dry matter allocation and the accumulation of this nutrient in the different plant compartments of genotypes of conilon coffee; (ii) to evaluate the behavior of genotypes of conilon coffee cultivated under contrasting conditions of nitrogen availability in the soil; and (iii) to differentiate genotypes with higher efficiency and responsiveness to nitrogen supply.

MATERIALS AND METHODS

Experimental design

The experiment was conducted in greenhouse, installed in the experimental area of the Centro de Ciências Agrárias of the Universidade Federal do Espírito Santo (CCA-UFES), located at municipality of Alegre, in Espírito Santo State, with a latitude of 20°45' S, a longitude of 41°33' W and altitude of 277.41 m.

The experiment was set up in a 13x2 factorial scheme, following a completely randomized design (CRD) with three replications. The factors were: 13 clones of the clonal cultivar "Vitória Incaper 8142" (CV-01, CV-02, CV-03, CV-04, CV-05, CV-06, CV-07, CV-08, CV-09, CV-10, CV-11, CV-12, and CV-13) and two levels of nitrogen fertilization (0 and 100% of the N recommended level, according to Lani et al. (2007)). The experimental unities were constituted by one seedling per pot.

Soil preparation

Soil was collected at the experimental research area of the CCA-UFES, from a site with wavy topography and covered by pasture vegetation (*Brachiaria* species), from 10 to 40 cm depth, eliminating the first 10 cm of the soil profile to evade the effect of organic matter, more present in this superficial layer. A sample was collected and subjected to chemical and physical analyses (Table 1), and the soil was classified as dystrophic red-yellow clay loam (Embrapa, 1997). The soil was dried in shadow, homogenized using a 2.0 mm mesh sieve, separated in samples of 10 dm³, standardized by weight and packed in sealed plastic pots (14 L). The soil fertility was corrected using liming (2.19 g per pot, 100% of real power of full neutralization) to increase the level of Ca⁺⁺ and elevate the base saturation, following the recommendation of Prezotti et al. (2007).

Multiplication of clones and cultivation

After the soil preparation, conilon coffee plantings provided by Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper), produced at Fazenda Experimental de Marilândia (Marilândia-ES) by vegetative propagation (cutting), were selected at their 120 days of development, presenting two pairs of leaves and good phytosanitary and nutritional aspects. The plantings, from each specific genotype, were accommodated in the pots, identified and cultivated with the different levels of nitrogen fertilization.

Fertilization with the other nutrients, beside nitrogen, was performed following the actual recommendation for the crop in the Espírito Santo State (Prezotti et al., 2007). The irrigation of the pots was performed daily, keeping the soil humidity near the adequate level for the initial growth of coffee plants. The phytosanitary management and spontaneous plants removal was conducted manually, whenever required.

Nitrogen fertilization

The levels of nitrogen fertilization used in the study were chosen based on recommendations proposed by Lani et al. (2007), using the levels of 0 and 100% aiming to effectively differentiating the responses to the nutritional efficiency for nitrogen. For this end, 0.00 or 1.56 g of N were applied to the pots, following the treatments, in the form of urea salt (NH₂CONH₂), diluted in distilled water and applied to the soil. This solution was applied 10 cm away from the plant crown and was parceled into five applications: the

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

Characteristic	Value
Sand (g kg ⁻¹) ¹	386.73
Silt (g kg ⁻¹) ¹	36.61
Clay (g kg ⁻¹) ¹	576.66
Soil density (kg dm ⁻³) ²	1.15
pH ³	5.96
P (mg dm ⁻³) ⁴	6.1
K (mg dm ⁻³) ⁵	79
Ca (cmol _c dm ⁻³) ⁶	0.22
Mg (cmol _c dm ⁻³) ⁶	0.46
Al (cmol _c dm ⁻³) ⁷	0
H+Al (cmol _c dm ⁻³) ⁸	1.98
Sum of bases (cmol _c dm ⁻³)	0.87
Potential CEC (cmol _c dm ⁻³)	2.85
Effective CEC (cmol _c dm ⁻³)	0.87
Base saturation (%)	30.59
Aluminium saturation (%)	0
Organic matter (g kg ⁻¹) ⁹	13.7

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).

first one on the first day and the others periodically at 30, 60, 90 and 120 days after planting.

Nutritional evaluation and grouping by responsiveness

After 150 days of cultivation, the plants were cut, separating the stems, leaves and roots. These parts were removed from the pots, washed, weighted and dried in shadow, further separately packed in paper bags and taken to laboratory stove, with forced air circulation, at a temperature of 65°C, until a constant weight was obtained to determine the dry matter.

To determine the shoot dry matter (SDM) and the root dry matter (RDM), the plant material was weighted on analytical scale (precision: 0.001 g), obtaining results on grams per plant. As SDM is the sum of the dry matter of leaves, stem and branches, total dry matter (TDM) was obtained by the sum of the SDM to the RDM.

The dry matter of each vegetal component was milled in a Wiley electric mill, equipped with stainless steel sieve having a mesh size of 0.42 mm, until the material became a homogeneous power, and packed in paper bags for chemical analysis of nutrient content (Silva, 1999). The determination of N content in each vegetal component was done according to the semimicro Kjeldahl method (Malavolta et al., 1997). The α parameter was calculated as reported by Fox (1978), in order to classify the clones according to nutritional efficiency and responsiveness to nitrogen fertilization. The α parameter represents the ratio between the difference of SDM accumulated in each level of nitrogen fertilization (SDM_{100%} - SDM_{0%}) and the difference of the amount of applied nitrogen (1.56

g to 0.00 g per pot) adapted by Martins et al. (2013a).

The results of α parameter for each clone were set in the ordinates axis and the means for SDM were set in the abscissas axis, arranging four quadrants that allowed the classification of the clones in 4 groups: ER, efficient and responsive; ENR, efficient and non-responsive; NER, non-efficient and responsive; NENR, non-efficient and non-responsive.

Statistical analyses

The data was subjected to analysis of variance ($p \leq 0.05$), using SISVAR statistic software (Ferreira, 2011) and when the sources of variation were significant, the Tukey test ($p \leq 0.05$) was used to compare the results of nitrogen levels and Scott-Knott test ($p \leq 0.05$) was used to group clones of homogeneous behavior.

RESULTS AND DISCUSSION

The analysis of variance presented significant effect of the interaction between genotypes of conilon coffee and the different scenarios of N supply in the soil (Table 2). This fact indicates that the dry matter accumulation (Table 3) and the N content (Table 4) of coffee plants were mutually influenced by the genetic constitution and nitrogen supply. Similar results have been evidenced by the same genotypes cultivated in environments with different levels of phosphorus supply (Martins et al., 2013a, 2013b).

Supply of de N influence the dry matter accumulation and leaf nitrogen

The N supply in the soils implicated increase of dry matter (RDM, SDM and TDM) and N content (CR, CSP and CT) of the genotypes (Tables 3 and 4), for example, higher N supply caused a gain of 60% over the total dry matter (Table 3) and 76% of the leaf content of the nutrient (Table 4) for the genotype CV-09. These results suggest that genotypes of conilon coffee may be highly exigent in N, which has been related in past decades for the genus *Coffea* (Carelli et al., 2006; Catani and Moraes, 1958) and specifically for genotypes of conilon coffee (Colodetti et al., 2015).

Overall, the adequate N supply caused increase of RDM, SDM and TDM (Table 3), as well as of CR, CSP and CT (Table 4) for all genotypes. This fact may be explained by a higher production of structural carbohydrates and sugars, and by the stabilization of the nitrate assimilation rate which inhibits limitations of root growth over the shoots, designating more energy for the metabolic processes (Carelli et al., 2006; DaMatta et al., 1999).

In contrast, the unsatisfactory performance of the genotypes when cultivated in the environment with low N availability's (Tables 3 and 4) may be explained by the

Table 2. Mean squares, coefficients of variation (CV) and overall means of dry matter (g/plant) of roots (RDM), shoots (SDM) and total (TDM); and N content (mg/plant) of root (CR), shoots (CSP) and total (CT), for genotypes of conilon coffee grown in environments with discriminating levels of N supply.

Variation source or parameter	df ¹	RDM	SDM	TDM	CR	CSP	CT
Genotypes (G)	12	25.90*	180.76*	263.18*	0.003*	0.010*	0.018*
Levels of N (N)	3	424.12*	5617.47*	8137.74*	0.310*	6.329*	9.422*
Interaction G*N	36	24.69*	84.48*	160.03*	0.002*	0.007*	0.013*
Residue	104	0.86	3.25	4.46	0.0001	0.0006	0.001
Overall Means		15.39	29.22	44.62	0.17	0.40	0.57
CV (%)		6.05	6.17	4.73	7.03	6.37	4.89

*Significant at 5% probability by F test. ¹degrees of freedom.

Table 3. Mean values of dry matter (g/plant) of roots (RDM), shoots (SDM) and total (TDM) for genotypes of conilon coffee grown in two levels of N supply (0 and 100% of the recommended values for the crop, respectively N1 and N2).

Genotype	RDM		SDM		TDM	
	N ₁	N ₂	N ₁	N ₂	N ₁	N ₂
CV-01	10.37 ^{bb}	18.59 ^{ba}	16.55 ^{ab}	42.42 ^{aA}	26.92 ^{aB}	61.01 ^{bA}
CV-02	12.79 ^{aB}	22.09 ^{aA}	15.42 ^{aB}	38.15 ^{bA}	28.21 ^{aB}	60.24 ^{bA}
CV-03	9.62 ^{bb}	16.48 ^{ca}	9.65 ^{bb}	34.74 ^{ca}	19.28 ^{bb}	51.23 ^{dA}
CV-04	12.19 ^{aB}	18.07 ^{ba}	12.20 ^{bb}	40.87 ^{aA}	24.39 ^{aB}	58.94 ^{bA}
CV-05	12.83 ^{aB}	15.20 ^{ca}	15.50 ^{ab}	35.28 ^{ca}	28.33 ^{aB}	50.49 ^{dA}
CV-06	11.95 ^{aB}	18.90 ^{ba}	10.66 ^{bb}	39.59 ^{ba}	22.61 ^{bb}	58.49 ^{bA}
CV-07	9.57 ^{bb}	16.09 ^{ca}	11.21 ^{bb}	31.15 ^{dA}	20.78 ^{bb}	47.24 ^{eA}
CV-08	13.56 ^{aB}	16.60 ^{ca}	11.34 ^{bb}	30.34 ^{dA}	24.90 ^{aB}	46.94 ^{eA}
CV-09	8.59 ^{bb}	17.25 ^{ca}	12.84 ^{bb}	37.03 ^{ba}	21.43 ^{bb}	54.28 ^{ca}
CV-10	10.74 ^{bB}	16.42 ^{ca}	12.38 ^{bb}	39.87 ^{ba}	23.12 ^{bb}	56.29 ^{ca}
CV-11	9.30 ^{bb}	16.00 ^{ca}	12.88 ^{bb}	39.00 ^{ba}	22.18 ^{bb}	55.00 ^{ca}
CV-12	9.40 ^{bb}	19.85 ^{ba}	11.13 ^{bb}	43.93 ^{aA}	20.54 ^{bb}	63.79 ^{aA}
CV-13	12.09 ^{aB}	18.45 ^{ba}	13.91 ^{ab}	30.69 ^{dA}	26.00 ^{aB}	49.14 ^{dA}

Means followed by the same letter in each variable, uppercase letters in lines (Tukey) and lowercase letters in columns (Scott-Knott), are not different ($p < 0.05$).

imposition caused by this low supply, which results in decrease of chlorophyll concentration and Calvin-cycle enzymes, which by itself leads to a lower capacity of carbon assimilation and increase the sensibility for photo-inhibition (Carelli et al., 2006; Ramalho et al., 1998, 1999, 2000), compromising the whole carbohydrate accumulation and dry matter production (Amaral et al., 2001).

Differential responsiveness of genotypes to N supply

The genotypes presented different behavior regarding the N supply in the soil, resulting in distinct groups of homogeneous means for dry matter (RDM, SDM and TDM) and N content in the plant tissues (CR, CSP and

CT) (Tables 3 and 4).

Overall, the genotypes CV-01, CV-02, CV-04 and CV-13 presented higher accumulation of dry matter (Table 3), while the genotypes CV-01 and CV-05 presented higher N accumulation in the plant tissues (Table 4), when grown with low supply of N. For the environment with adequate N supply, the genotype CV-02 developed high accumulation of dry matter and N content, while CV-04 presented only high N content (Table 4).

Several reports describe a genetic control of the efficiency to use nutrients and this expression occurs in conformity with the level of availability of nutrients in the soil, which implicates the determination of heterogenic groups between cultivars of the same species, or even inside the same cultivar (Carelli et al., 2006; Martins et al., 2013a, 2015a, 2015b; Neto et al., 2016; Rodrigues

Table 4. Mean values of N content (mg/planta) of roots (CR), shoots (CSP) and total (CT) for genotypes of conilon coffee grown in two levels of N supply (0 and 100% of the recommended values for the crop, respectively N1 and N2).

Genotype	CR		CSP		CT	
	N ₁	N ₂	N ₁	N ₂	N ₁	N ₂
CV-01	0.09 ^{bB}	0.22 ^{dA}	0.16 ^{aB}	0.78 ^{aA}	0.25 ^{aB}	1.00 ^{bA}
CV-02	0.11 ^{bB}	0.32 ^{aA}	0.13 ^{aB}	0.76 ^{aA}	0.23 ^{bB}	1.08 ^{aA}
CV-03	0.09 ^{bB}	0.29 ^{bA}	0.09 ^{cB}	0.68 ^{bA}	0.18 ^{bB}	0.97 ^{cA}
CV-04	0.17 ^{aB}	0.26 ^{bA}	0.12 ^{bB}	0.78 ^{aA}	0.29 ^{aB}	1.05 ^{aA}
CV-05	0.13 ^{aB}	0.24 ^{cA}	0.15 ^{aB}	0.70 ^{bA}	0.27 ^{aB}	0.94 ^{cA}
CV-06	0.12 ^{aB}	0.27 ^{bA}	0.10 ^{cB}	0.66 ^{cA}	0.22 ^{bB}	0.93 ^{dA}
CV-07	0.11 ^{bB}	0.19 ^{fA}	0.11 ^{bB}	0.61 ^{cA}	0.22 ^{bB}	0.80 ^{eA}
CV-08	0.13 ^{aB}	0.21 ^{eA}	0.10 ^{cB}	0.57 ^{dA}	0.23 ^{bB}	0.78 ^{eA}
CV-09	0.09 ^{bB}	0.24 ^{cA}	0.11 ^{bB}	0.66 ^{cA}	0.21 ^{bB}	0.90 ^{dA}
CV-10	0.12 ^{aB}	0.23 ^{dA}	0.10 ^{cB}	0.69 ^{bA}	0.22 ^{bB}	0.92 ^{dA}
CV-11	0.11 ^{bB}	0.18 ^{fA}	0.12 ^{bB}	0.71 ^{bA}	0.23 ^{bB}	0.89 ^{dA}
CV-12	0.08 ^{bB}	0.24 ^{cA}	0.10 ^{cB}	0.74 ^{aA}	0.18 ^{bB}	0.98 ^{cA}
CV-13	0.09 ^{bB}	0.19 ^{fA}	0.12 ^{bB}	0.56 ^{dA}	0.22 ^{bB}	0.75 ^{eA}

Means followed by the same letter in each variable, uppercase letters in lines (Tukey) and lowercase letters in columns (Scott-Knott), are not different ($p < 0.05$).

et al., 2015).

The additive and non-additive effects of the genetic control are linked directly to the accumulation and allocation of dry matter and nutrients in the green photosynthetic tissues (Colodetti et al., 2015; Martins et al., 2013b, 2015b; Neto et al., 2016), which may partially explain the occurrence of differential behavior among genotypes of conilon coffee as function of the N supply in the soil (Tables 3 and 4).

Furthermore, the modulation of biomass production and N content in leaves of genotypes of conilon coffee imposed by the availability of N in the soil may be governed by different traits. Revisiting other studies, it is possible to observe that different performances of genotypes can be attributed to morphology, architecture and diameter of the root system for environments characterized by having low availability of nutrients (Amaral et al., 2011; Colodetti et al., 2015; Martins et al., 2013b). However, in environments with ideal nutritional supply, the differential performance of the genotypes seems to be governed by traits related to the ripening cycle, with evidences that early genotypes may be more efficient in the allocation of biomass and nutrients (Martins et al., 2015b; Partelli et al., 2014), but without evidences of changes in tolerance indexes.

Efficiency and responsiveness of genotypes to N

The genotypes CV-01, CV-04 and CV-09 were classified as efficient and responsive (ER), presenting satisfactory development in soils with low supply of N and also a

significant increase in dry matter when cultivated in soils with adequate N supply (Figure 1). The genotypes CV-02, CV-05 and CV-13 were classified as efficient but non-responsive (ENR), indicating that these genotypes do not respond as much to the increase in the nitrogen supply, but are efficient to grow in conditions of low availability of this nutrient (Figure 1).

The genotypes CV-06, CV-10, CV-11 and CV-12 are non-efficient but responsive, indicating that these genotypes may not tolerate low supply of N, but present considerable gain as function of the increased supply of N in the soil. The genotypes CV-03, CV-07 and CV-08 were classified as non-efficient and non-responsive (Figure 1).

Revisiting results of other nutritional studies, it is possible to observe that the genotype CV-04 was also characterized as efficient and responsive to P (Martins et al., 2013a) and tolerant to low supplies of N and P in the soil (Colodetti et al., 2014; Martins et al., 2015b), demonstrating that this genotype may express desirable characteristics in terms of nutritional efficiency for both nutrients. This is in addition to the other agronomic traits, indicating that this genotype may be explored by breeding programs aiming to improve the nutritional efficiency of future cultivars.

Another intriguing observation is that CV-08 was classified in opposite groups regarding the nutrition with N and P, being characterized as NENR for N (Figure 1) and intolerant to the deficit of N in the soil (Colodetti et al., 2014), but ER for P (Martins et al., 2013a) and tolerant to the deficit of P in the soil (Martins et al., 2015b). This fact is indicative that nutritional efficiency

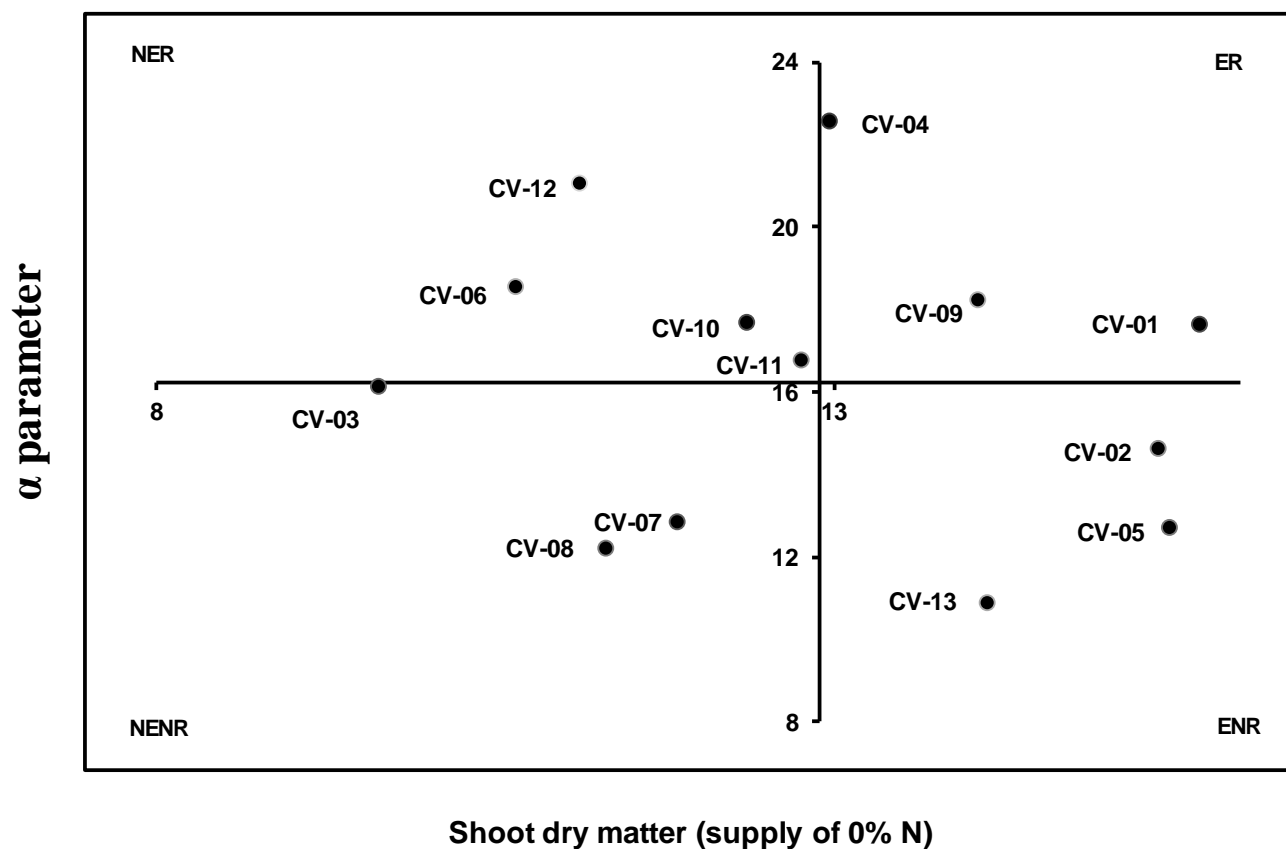


Figure 1. Classification of conilon coffee genotypes according to nutritional efficiency and responsiveness to nitrogen supply. (ER: Efficient and responsive; ENR: efficient and non-responsive; NER: non-efficient and responsive; and NENR: non-efficient and non-responsive).

may present specificities related to the uptake and use of each nutrient in isolate routes and in different magnitudes; making it possible for the same genotype (same morphology and architecture of roots), to present different capacities to acquire and metabolize specific nutrients.

Furthermore, it was possible to notice correlation between the use efficiency of N with the classification of the genotypes regarding their ripening cycle. Save for some exceptions, genotypes of late cycle presented a tendency of being classified as efficient and genotypes of early cycle presented a tendency to be classified as responsive (Martins et al., 2015b).

Conclusion

The nitrogen supply modulates the production of dry matter and nitrogen accumulation for conilon coffee genotypes. The conilon coffee genotypes CV-01, CV-04 and CV-09 are efficient and responsive to nitrogen fertilization.

ACKNOWLEDGEMENT

The authors acknowledged the supported by Ufes, Incaper and CBPD-Café to support the project; CAPES (LSM – doctor scholarship), CNPq (LDM – post-doc scholarship; MAT - scholarship of research productivity), and FAPES (LDM – post-doc scholarship and project financial support; WNR – post-doc scholarship).

Conflicts of interests

The authors declared that they have no conflict of interests.

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