Australian Journal of Crop Science AJCS
AJCS 8(12):1648-1657 (2014) ISSN:1835-2707

Differential tolerance between genotypes of conilon coffee (*Coffea canephora*) to low availability of nitrogen in the soil

Tafarel Victor Colodetti[1*], Wagner Nunes Rodrigues[1], Lima Deleon Martins[1], Marcelo Antonio Tomaz[2]

[1]Programa de Pós-Graduação em Produção Vegetal do Centro de Ciências Agrárias da Universidade Federal do Espírito Santo (CCA-UFES), Alto Universitário, s/nº, Cx Postal 16, Bairro Guararema, CEP: 29500-000, Alegre, ES, Brasil
[2]Departamento de Produção Vegetal do Centro de Ciências Agrárias da Universidade Federal do Espírito Santo (CCA-UFES), Brasil

***Corresponding author: tafarelcolodetti@hotmail.com**

Abstract

Aspects related to mineral nutrition are gaining space and importance in breeding programs and in studies related to the behavior of coffee genotypes. Therefore, the objective of this study was to evaluate the differential tolerance between genotypes of conilon coffee subjected to low availability of nitrogen in the soil, as well as the gains obtained with the addition of this nutrient in the soil through fertilization. Ten genotypes of *Coffea canephora* were cultivated in greenhouse, without fertilization with N and with fertilization at level of 0.625 g kg^{-1} of soil, tested in a completely randomized design. Based on the results of growth analysis and expression of variability between genotypes, the most appropriate level was selected to discriminate the genotypes for tolerance to low availability of nitrogen in the soil. Following this premise, the genotypes 67, 23 and 02 were classified as intolerant and the genotypes 32, 73, 83, 77, 76, 24 and 153 as tolerant to low availability of nitrogen in the soil. It was also noticed an increased plant growth with nitrogen fertilization and, as consequence, greater biomass accumulation, as well as better development of leaves, in quantity, area and content of photosynthetic pigments. The pattern of biomass allocation between the plant compartments were also altered, favoring leaves and stems.

Keywords: *Coffea canephora*, nitrogen deficiency, mineral nutrition, fertilization.
Abbreviations: CLA_Chlorophyll *a*; CLB_Chlorophyll *b*; CLT_Chlorophyll total; CV_Coefficients of variation; CVg_Coefficient of genetic variation; DML_Dry matter of leaves; DMR_Dry matter of roots; DMS_Dry matter of stems; DMT_Dry matter of total; H^2_Coefficient of genotypic determination; LAR_Leaf area ratio; LMR_Leaf mass ratio; N_Nitrogen; NLE_Number of leaves; PHE_Plant height; RGR_Relative growth rate; RMR_Root mass ratio; SLA_Specific leaf area; SMR_Stem mass ratio; TLA_Total leaf area.

Introduction

Coffee is one of the most valuable commodities traded in the world. Brazil is the world largest coffee producer, cultivating both arabica (*Coffea arabica*) and conilon (*Coffea canephora*) coffees. Considering the national production, the Espirito Santo State stands as the largest producer of conilon coffee, accounting for approximately 75% of the Brazilian production for this species (Conab, 2014). The Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper) has been developing the main breeding program of conilon coffee in Brazil (Fonseca et al., 2008) and being responsible for the development of nine improved cultivars recommended for the State of Espírito Santo so far: eight clonal cultivars and one propagated by seed. The breeding programs are allowing the replacement of old and unproductive cultivars with new improved ones. In that sense, Brazil is considered a world leader in development of improved cultivars of coffee (Borém and Miranda, 2005). The main objective of breeding programs of *C. canephora* has been improving various characteristics of interest, aimed primarily at high crop yield, production stability, resistance to pests and beverage quality (Ferrão et al., 2007; Carvalho, 2008). In addition, issues related to mineral nutrition are gaining space and importance in studies related to the behavior of coffee genotypes. Gerloff and Galbeman (1983) reported that, on condition of nutritional stress, certain genotypes present different responses to nutritional efficiency, demonstrating different capabilities in acquiring nutrients from the environment and use them to produce biomass. Therefore, genotypic differences may result in changes of nutritional efficiency due to morphological and physiological factors associated to the specific nutritional demand of each genotype. Knowledge about these demands and mechanisms are valuable to increase the efficiency of selection of genotypes in breeding programs (Marschner, 1995). Taking into account that chemical fertilization contributes approximately with 30% of total production costs, optimizing the use of fertilizers and exploitation of the potential response of improved genotypes of coffee become very important study targets, generating information to increase the profitability of coffee crops through the rational nutrition management of the cultivars. Among the nutrients applied in greater quantities through fertilization, nitrogen stands out as being one of the essential nutrients that are most required for the full development of the coffee and its

deficiency causes restriction to growth and production of plants (Vaast et al., 1998; Malavolta, 1986).
Conilon coffee is a species with great productive potential and improved cultivars can achieve very high crop yields, this capability comes associated with higher nutritional requirement. Since nitrogen is the most accumulated nutrient in the tissues of coffee plants, the supply of N should be sufficient to meet the demands of fruit and vegetative organs, in order to guarantee the expression of its productive potential (Bragança et al., 2008; Clement et al., 2013; Quintela et al., 2011). Considering the exposed scenario, this experiment was developed aiming to study the differential tolerance between genotypes of conilon coffee subjected to low availability of nitrogen in the soil, as well as the gains obtained with the addition of this nutrient in the soil through fertilization.

Results

Genetic parameters

The individual analysis of variance showed the existence of different behavior between genotypes for most traits. Despite the gains promoted by the addition of nitrogen, no variability among the genotypes for relative growth rate and stem mass ratio were observed. Additionally, there were no significant differences between the means of the genotypes for specific leaf area, in the environment without the addition of nitrogen in the soil; and for content of chlorophyll *a* in the environment supplied with nitrogen. Table 1 presents the estimated parameters, based on the individual analysis, for the environment without the addition of nitrogen in the soil. The coefficients of variation (CV) were relatively low, with 13.08 % being the highest value observed for the variables with significant F. The estimates of the coefficient of genetic variation (CV_g) demonstrate good expression of heterogeneity among the genotypes regarding most of the studied features. For NLE, TLA, DML, DMS, DMR, DMT, LAR, LMR, RMR, CLA, CLB and CLT, the estimated CV_g was superior to CV, resulting in variation indexes (CV_g/CV) greater than 1.00, indicating that genetic factors predominated in the studied variation for these characteristics. Also, based on Table 1, it is observed that estimates of coefficient of genotypic determination (H^2) were high for NLE, TLA, DML, DMR, DMT, CLA, CLB and CLT, demonstrating a reduced environmental influence over those variables. Characteristics greatly influenced by the environment usually have low estimates for H^2, usually less than 30%; in contrast, characteristics that are less influenced by environmental effects have higher values (Ramalho et al., 2004). Therefore, it is safe to assume that the different behavior between genotypes was due, in greater proportion, of the genetic differences between them. For the environment with nitrogen fertilization, the estimated parameters are presented in Table 2. Similarly, the coefficients of variation were relatively low, with the highest value being 9.06% for variables with significant F. The coefficient of genetic variation exceeded the coefficient of variation for PHE, NLE, TLA, DML, DMS, DMR, DMT, LAR, LMR, RMR, SLA and CLB. The estimates of coefficient of genotypic determination were high for PHE, NLE, TLA, DML DMS, DMR, DMT, LAR, RMR and SLA. The chlorophyll content presented little variation in the environment well supplied with nitrogen, indicating that the genotypes, although having different efficiencies to absorb and use nitrogen in poor environments, can product similar levels of photosynthetic pigments when well nourished. Similarly, the promotion of growth allowed the identification of different responses regarding characteristics of canopy size and biomass accumulation, some of which were not expressed when the environment was unfavorable.

Response of genotypes

Through the analysis of variance, it is observed the existence of interaction between the effect of genotypes and availability of nitrogen. The study of the means of genotypes in each environment is presented in Table 3. In the environment without nitrogen fertilization, plants of the genotypes 73, 83 and 24 showed homogeneous behavior in relation to height, being taller than plants of other genotypes. With the addition of nitrogen, the configuration of the homogenous groups was changed and the genotypes 67, 23, 73, 77, 76 and 02 showed highest means for height, while genotypes 32 and 24 formed the group of lower means for plant height. No difference between genotypes in relation to relative growth rate was observed, regardless of the environment, as the genotypes showed homogeneity of growth pattern. Regarding the number of leaves, a large number of groups of homogeneous means were observed between the genotypes under the condition of absence of nitrogen fertilization. This expression of variability favors the selection, facilitating the identification of genotypes with better leafiness in that environment. In the environment without N supply, the genotype 77 showed higher production of leaves, contrary, genotype 02 formed the group with the lowest mean alone. With nitrogen fertilization, the genotypes 67, 73, 83, 77, 76 and 02 stood out for having the higher means. Without providing N through fertilization, the genotypes 32, 73, 83, 77, 24 and 153 formed the group with the highest means for the total leaf area. For the environment supplied with this nutrient, the genotype 67 developed larger leaf area than all the others, while the genotype 32 did not show any response in leaf area to addition of N. Considering the accumulation of dry matter in leaves, the genotypes 73, 83 and 153 formed the group with the highest means in the environment without nitrogen fertilization, while the genotypes 67, 23, 73 and 83 stand out with the addition of N. Higher dry matter in the stems was observed in the genotypes 73, 83, 24 and 153 in the environment without N supply. With the nitrogen fertilization, the genotypes 67 and 83 showed higher means for this variable. For dry matter accumulated in the roots, the genotypes 24 and 153 stood out when compared to the others in the condition of absence of nitrogen fertilization. With the addition of N, the genotype 73 alone presented the higher mean. Considering the total production of biomass, the genotypes 83, 24 and 153 showed higher dry matter accumulation in the environment without nitrogen, while the genotypes 67, 23, 73 and 83 presented the highest means in the environment with nitrogen supply. Higher leaf area ratios were observed in the genotypes 32 and 77, in the environment without addition of nitrogen; and in the genotypes 67, 32 and 02, in the environment that received nitrogen fertilization. In the environment without nitrogen supply, the genotypes 32, 73 and 77 showed higher proportion of biomass allocated in the leaves. With the increasing availability of this nutrient in the soil, this ratio has been changed and genotypes 23, 32, 83 and 02 stood out with higher means of LMR. The amount of biomass directed towards the roots was also altered by the nitrogen fertilization. While in the environment without fertilization the genotypes 67, 23, 76, 24, 153 and 02 formed the group with higher RMR, in the environment with nitrogen fertilization the genotypes with higher root mass ratios were

Table 1. F statistics, coefficient of genetic variation (CV_g), coefficient of variation (CV), index variation (CV_g/CV) and coefficient of genotypic determination (H^2) of 16 variables of genotypes of conilon coffee grown without addition of nitrogen in soil, at 150 days of cultivation.

Parameter	PHE[1]	RGR[2]	NLE[3]	TLA[4]
F	3.67**	2.16ns	43.97**	8.24**
CV_g	9.36	15.29	26.76	14.87
CV	9.91	24.59	7.07	9.56
CV_g/CV	0.94	0.62	3.78	1.55
H^2	72.79	53.71	97.72	87.86
Mean	29.40	0.02	14.60	623.81
Parameter	DML[5]	DMS[6]	DMR[7]	DMT[8]
F	13.17**	4.80**	14.76**	17.20**
CV_g	15.30	14.72	19.61	16.20
CV	7.59	13.08	9.15	6.97
CV_g/CV	2.01	1.12	2.14	2.32
H^2	92.41	79.17	93.22	94.18
Mean	5.59	3.73	10.76	20.10
Parameter	LAR[9]	LMR[10]	SMR[11]	RMR[12]
F	6.07**	5.94**	1.97ns	4.02**
CV_g	11.33	8.90	6.08	4.80
CV	8.71	6.93	10.65	4.78
CV_g/CV	1.30	1.28	0.57	1.00
H^2	83.53	83.18	49.47	75.17
Mean	31.28	0.28	0.18	0.53
Parameter	SLA[13]	CLA[14]	CLB[15]	CLT[16]
F	0.66ns	8.67**	22.56**	13.99**
CV_g	-	13.12	27.05	15.45
CV	10.16	8.20	10.08	7.42
CV_g/CV	-	1.59	2.68	2.08
H^2	-	88.46	95.56	92.85
Mean	111.77	27.59	6.06	33.65

**Significant at 1% of probability; nsNon significant at 5% of probability; [1]Plant height (cm); [2]Relative growth rate (cm cm^{-1} dia^{-1}); [3]Number of leaves; [4]Total leaf area (cm^2); [5]Dry matter of leaves (g); [6]Dry matter of stems (g); [7]Dry matter of roots (g); [8]Total dry matter (g); [9]Leaf area ratio (cm^2 g^{-1}); [10]Leaf mass ratio; [11]Stem mass ratio; [12]Root mass ratio; [13]Specific leaf area (cm^2 g^{-1}); [14]Content of chlorophyll a; [15]Content of chlorophyll b; [16]Total content of chlorophyll.

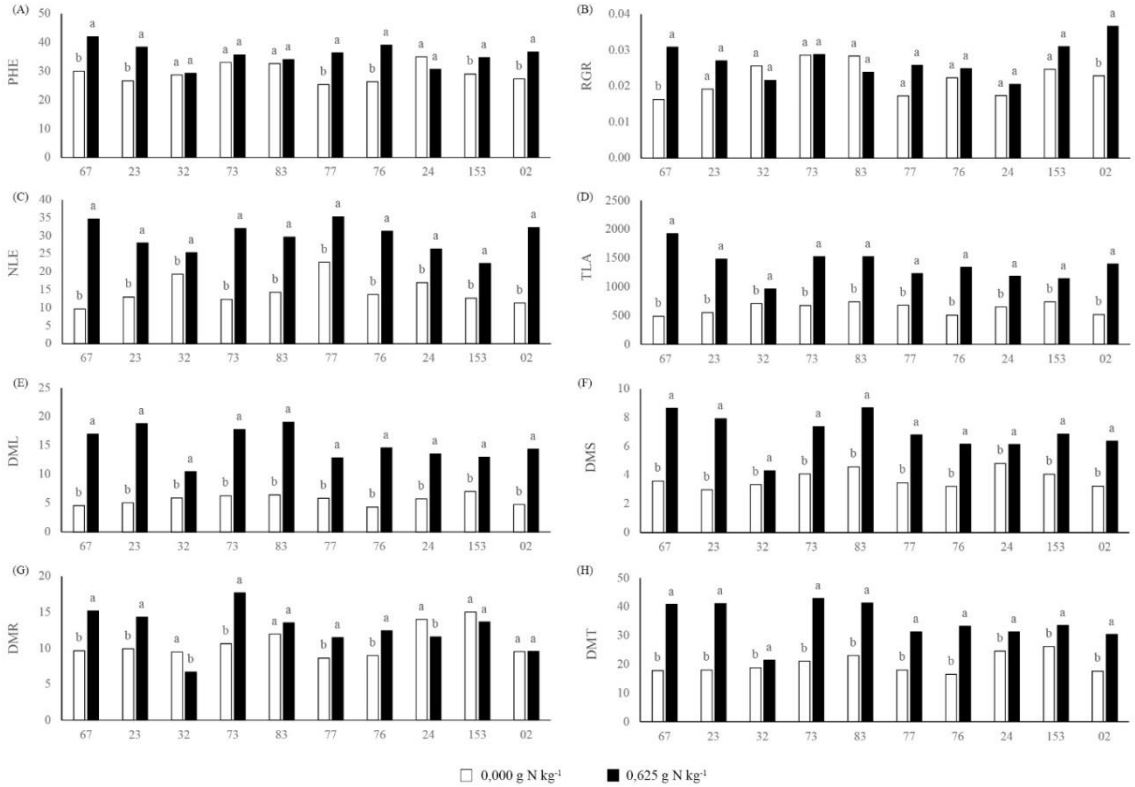


Fig 1. Means of plant height (A), relative growth rate (B), number of leaves (C), total leaf area (D), dry matter of leaves (E), dry matter of stems (F), dry matter of roots (G) and total dry matter (H) of genotypes of conilon coffee, at 150 days of cultivation.

32, 73, 83 and 77. Regarding the biomass to the stem, no change in the pattern (approximately 20% of the dry matter of the plant) among genotypes in any of the environments was observed. It was observed that there was no difference on specific leaf area between genotypes in the environment without nitrogen supply. However, in the environment that received application of that nutrient, different responses were observed, the genotype 67 had higher mean, while genotypes 23 and 83 had lower means of SLA than the others did. The environment without nitrogen fertilization favored the differentiation between genotypes regarding production of chlorophyll *a*, highlighting the genotypes 32, 77, 76, 153 and 02, which showed higher CLA even in the absence of fertilization. Contrary, in the environment with nitrogen addition, there was no differentiation between genotypes. Regarding the production of chlorophyll *b*, different behaviors between genotypes were observed for both environments. However, this differentiation was more noticeable in the environment without the addition of N, which generated a higher number of different groups, configuring a situation that is more favorable for the study of those genotypes. The same behavior described above was observed for the total chlorophyll content of the plants. Under conditions of no nitrogen supply, the genotype 76 showed higher mean of CLT, while the genotypes 67, 23, 73, 83 and 24 were responsible for lower production of chlorophyll. However, this last group of genotypes showed greatest response to nitrogen fertilization, considering this variable, forming the group of genotypes with higher CLT in the fertilized environment.

Gains with the addition of nitrogen

Figure 1 shows the growth gains observed in the genotypes in response to the availability of nitrogen in the soil. Considerable gains for plant height, with the addition of nitrogen, were observed for genotypes 67, 23, 77, 76, 153 and 02 (Figure 1A). Among these genotypes, 67 and 02 also showed gains for relative growth rate, between of 94% and 61%, respectively (Figure 1B). The leafiness of all genotypes was more abundant with the nitrogen fertilization, with gains for number of leaves (Figure 1C), leaf area (Figure 1D) and dry matter accumulated in the leaves (Figure 1D). This fact demonstrates the importance of nitrogen in the development leaves of the coffee plants, this fertilization with this nutrient promotes the rapid growth of new leaves, making them bright green; the growth of leaf area; the increase of photosynthetic rates and, therefore, the higher production of assimilates (Guimarães & Mendes, 1997). The positive effect over the development of the aerial part in response to nitrogen fertilization was not only noticed the foliage. The growth of the stems was also favored, resulting in higher means for dry matter of stem in the environment supplied with this nutrient (Figure 1F). The root development of the genotypes 67, 23, 73, 77 and 76 was also favored by the addition of N, resulting in stronger and heavier root systems (Figure 1G). When considering the total dry matter of the plant, all genotypes showed significant gains with the nitrogen fertilization, with average gains ranging from 15% to 130% (Figure 1H). In addition to the gains in growth, nitrogen fertilization also stimulated the production of photosynthetic pigments and changed the pattern of biomass allocation between the different plant parts (Figure 2). With the exception of the genotypes 73 and 77, leaf area ratio was higher with the addition of nitrogen in the soil (Figure 2A). The biomass allocation of the plants was modified by the fertilization. There was an increase in the proportion of dry matter that was accumulated in the leaves of all genotypes (Figure 2B). Overall, the pattern of allocation of dry matter in the stem was not greatly modulated by the fertilization with nitrogen, only showing significant change for the genotype 153 (Figure 2C). Furthermore, the nitrogen addition caused a reduction in the proportion of biomass allocated in the roots (Figure 2D). This behavior indicates that the development of the aerial part was favored over the root system with the nitrogen fertilization. In conditions of low nitrogen availability, there development of roots was prioritized, which may be related to a survival strategy of the plant, aiming to increase the organ responsible for nutrient acquisition. For most genotypes, the specific leaf area was lower with N fertilization (Figure 2E). Without the nutritional limitation caused by the lack of nitrogen in the soil, the leaves developed more firm structure, with greater thickness and higher accumulation of dry matter per unit of area. In contrast, cultivation without nitrogen reduced the development of the leaves, which, in general, were thinner and presented a less intense green color. The chlorophyll production was greatly favored by the nitrogen fertilization in all genotypes. Gains in the order of 47% for chlorophyll *a* (Figure 2F), 454% for chlorophyll *b* (Figure 2G) and 118% for total chlorophyll content (Figure 2H) were observed. Epstein (1975) reported that nitrogen is present in the formation of amino acids, nucleotides and coenzymes. In the absence of this nutrient, symptoms such as yellowing or chlorosis of leaves occurs due to its involvement in the chlorophyll synthesis, resulting in restriction of the photosynthesis process.

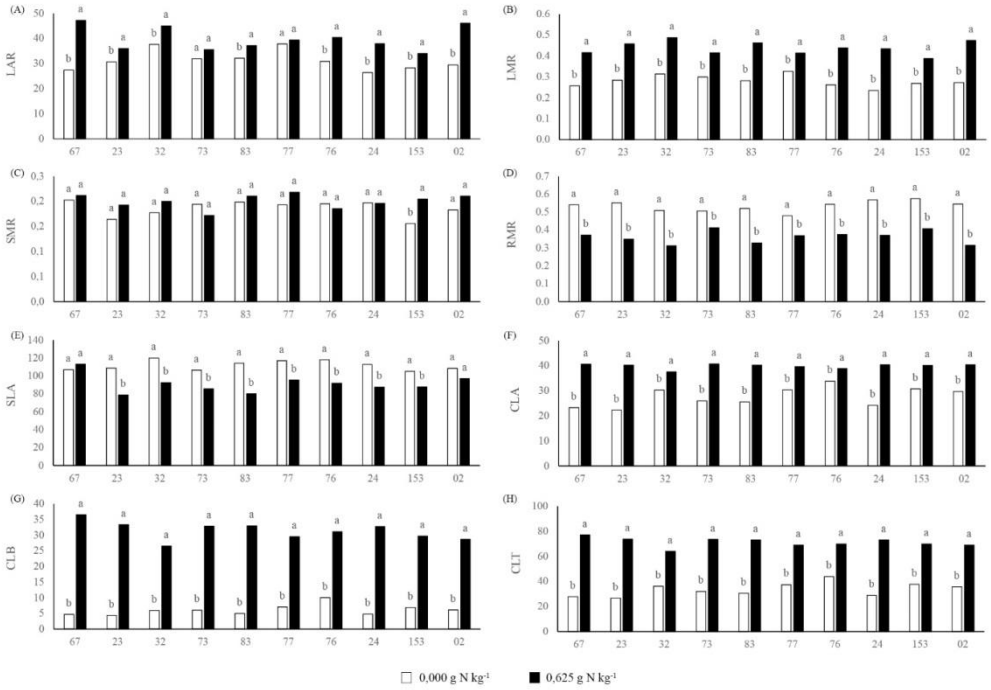
Discrimination analyses

The differences observed in the behavior of those genotypes grown under low nitrogen availability in the soil demonstrate the variability of response existing between them and allow the identification of those genotypes that showed higher development in these conditions, as well as genotypes that do not develop satisfactorily in low availability of this nutrient.
Analyzing the comparisons between means in the environment with nutritional stress, we note that the genotypes 24 and 153 were able to accumulate more dry matter on condition of low supply of N, and simultaneously, those genotypes showed superior performance to other variables. In contrast, genotypes 67, 23 and 02 were greatly compromised by nitrogen deficiency, presenting unsatisfactory growth. The other genotypes showed intermediate behavior, resulting in the preliminary classification presented on Table 4. As tolerance is a complex trait and is result of a number of factors that lead the plant to present biological superiority, it was decided to consider more characteristics related to the plant development to determinate the patterns of tolerance and intolerance in this group of genotypes. In addition to the large accumulation f biomass, the genotype 24 showed good vegetative growth, forming vigorous and tall plants. The genotype 153 showed excellent leafiness and leaves presenting more intense green color. In contrast, genotypes 67, 23 and 02 showed underperformed developments and reduced vigor; being used as standards in the study of intolerance (Table 4). After selecting the variables that allow better differentiation between genotypes and are related with the biological superiority of genotypes, functions were estimated, based on the results of the aforementioned genotypes, to discriminate them regarding the tolerance to low availability of N in the soil.

Table 2. F statistics, coefficient of genetic variation (CV_g), coefficient of variation (CV), index variation (CV_g/CV) and coefficient of genotypic determination (H^2) of 16 variables of genotypes of conilon coffee grown with addition of 0.625 g of nitrogen per kilogram of soil, at 150 days of cultivation.

Parameter	PHE[1]	RGR[2]	NLE[3]	TLA[4]
F	6.27**	1.79ns	7.28**	23.44**
CV_g	9.71	12.02	13.12	19.01
CV	7.32	23.34	9.06	6.95
CV_g/CV	1.32	0.51	1.44	2.73
H^2	84.06	44.32	86.27	95.73
Mean	35.66	0.03	29.73	1.370.83
Parameter	DML[5]	DMS[6]	DMR[7]	DMT[8]
F	20.60**	14.16**	27.21**	43.01**
CV_g	18.49	18.37	23.79	19.24
CV	7.23	8.77	8.04	5.14
CV_g/CV	2.55	2.09	2.95	3.74
H^2	95.14	92.93	96.32	97.67
Mean	15.16	2.09	12.60	34.68
Parameter	LAR[9]	LMR[10]	SMR[11]	RMR[12]
F	8.59**	4.51**	2.35ns	7.90**
CV_g	11.06	6.21	5.59	9.15
CV	6.95	5.74	8.32	6.03
CV_g/CV	1.59	1.08	0.67	1.51
H^2	88.36	77.85	57.55	87.35
Mean	39.88	0.43	0.20	0.36
Parameter	SLA[13]	CLA[14]	CLB[15]	CLT[16]
F	24.82**	0.62ns	4.11**	3.65**
CV_g	10.62	-	8.01	4.32
CV	3.77	5.34	7.86	4.60
CV_g/CV	2.81	-	1.01	0.94
H^2	95.97	-	75.69	72.61
Mean	90.94	39.83	31.35	71.19

**Significant at 1% of probability; nsNon significant at 5% of probability; [1]Plant height (cm); [2]Relative growth rate (cm cm^{-1} dia^{-1}); [3]Number of leaves; [4]Total leaf area (cm^2); [5]Dry matter of leaves (g); [6]Dry matter of stems (g); [7]Dry matter of roots (g); [8]Total dry matter (g); [9]Leaf area ratio (cm^2 g^{-1}); [10]Leaf mass ratio; [11]Stem mass ratio; [12]Root mass ratio; [13]Specific leaf area (cm^2 g^{-1}); [14]Content of chlorophyll a; [15]Content of chlorophyll b; [16]Total content of chlorophyll.



Fie 2. Means of leaf area ratio (A), leaf mass ratio (B), stem mass ratio (C), root mass ratio (D), specific leaf area (E), content of chlorophyll *a* (F), content of chlorophyll *b* (G) and total content of chlorophyll (H) of genotypes of conilon coffee, at 150 days of cultivation.

The discriminant functions for tolerant genotypes, called $D_t(x)$, and intolerant genotypes, called $D_i(x)$, were:

$D_t(x)$ = 13.10**ALT** + 2.65**NFL** + 1.601.84**MSF** + 1.549.51**MSC** + 1.560.39**MSR** + 3.967.53**CLA** + 3.947.51**CLB** - 1.564.05**MST** - 0.43**AFT** - 3.958.68**CLT** - 222.26

$D_i(x)$ = 12.78**ALT** + 2.53**NFL** + 1.600.49**MSF** + 1.547.41**MSC** + 1.557.89**MSR** + 3.955.77**CLA** + 3.934.34**CLB** - 1.561.62**MST** - 0.45**AFT** - 3.946.71**CLT** - 203.19

The classification of the genotypes for tolerance to low availability of nitrogen in the soil, based on the scores of the discriminant functions, is presented in Table 5. Observe that the genotypes that were used as the standard of tolerance and intolerance maintained their rating correctly. This shows the statistical consistency of the generated functions and validates the inferences and proposed classification for the genotypes of undefined behavior. Following this criteria, the genotypes 32, 73, 83, 77 and 76 were all included in the tolerant class, presenting growth parameters, in general, closer to the tolerant genotypes.The results reinforce the importance of a proper management of the nitrogen fertilization during the early development of the coffee plants, especially for some genotypes that cannot tolerate conditions of low availability of the same, being more drastically impaired by mineral deficiency than others. It is worth mentioning that even genotypes classified as tolerant in this study also presented limited development when subjected to conditions of nutritional stress. Even if this limitation is less intense during early stages, compared to intolerant genotypes, with the advance of development of the stress and the increase of metabolic demands of the plant with time, the lack of nitrogen will result in losses for the crop. The cultivation of clonal cultivars of conilon coffee requires the use of a selected group of improved clones, hence the existence of intolerant genotypes within this group makes the selection of appropriate management systems for fertilization even more important. The delay in the fertilization or the deficient nitrogen fertilization may affect the initial development of these genotypes, impairing the formation of a productive clonal crop.

Discussion

Coffea canephora is a species that presents high genetic variability for many agricultural traits and morphological and physiological characteristics (Cecon et al., 2008; Ferrão et al., 2008; Ferrão et al., 2009; Rodrigues et al., 2012; Marraccini et al., 2012; Rodrigues et al., 2013). There are scientific reports showing that genotypes of conilon coffee can also have differential growth and crop yield when cultivated with different levels of fertilization, presenting different nutritional efficiencies and responses to fertilization (Mattiello et al., 2008; Martins et al., 2013a; Martins et al., 2013b). Studies made by Partelli et al. (2014) demonstrate the existence of different behaviors among genotypes of conilon coffee from different groups of ripening cycle. These authors concluded that genotypes of early cycle might present higher rate of accumulation of nutrients and biomass than genotypes of intermediate or late cycle. As nitrogen is essential to the photosynthetic process and the formation of new plant organs, participating in the composition of amino acids, proteins, enzymes, RNA, DNA, ATP and other molecules (Marschner, 1995; Taiz and Zeiger, 2013), this nutrient has a direct effect over vegetative growth (Malavolta et al., 1997). This effect was observed in this study, the growth of the plants were altered by the availability of nitrogen in the soil, with increase of the vegetative growth in the environment with higher availability of the nutrient. The smaller growth observed in the environment without nitrogen fertilization may also be explained by the lack of nitrogen blocking the synthesis of cytokinin hormone, responsible for growth (Mengel and Kirkby, 2001; Clement et al., 2013).

The major portion of the total N found in the leaves is part of enzymes that are associated with chloroplasts (Stoking and Ongun, 1962). Corresponding to this fact, the environment with higher supply of N favored the development of leaves with stronger coloration and higher content of chlorophyll.

Several factors may influence the acquisition of nutrients by plants, such as the morphology of roots, its architecture and distribution in the soil profile. These features can undergo genetic changes as a mechanism of adaptation to environments with different availability of nutrients (Chun et al., 2005; Hammer et al., 2009; Martins et al., 2013a). The availability of N to the level of 0.625 g kg^{-1}, through fertilization, altered the development of the roots of many genotypes, in early stages of development, as observed for the means of dry matter that was allocated to the root system. For some genotypes, the fertilization with nitrogen favored the growth of the plant as a whole, causing the root system to also present gains. However, it is also possible that environments with a limited supply of nutrients promote an adaptation of the system root, stimulating the allocation of biomass to the roots in order to increase its ability to find and absorb nutrients for the plant survival (Taiz and Zeiger, 2013). Many other studies reported significant and important interactions occurring between the effect of genotypes and levels of nitrogen supply (Bänziger et al., 1997; Bertin and Gallais 2000). Those studies also support that the process of selection considering the nutrition for nitrogen should be conducted in conditions of low supply of this nutrient, as the heterogeneity of the genotypes tend to increase (Bänziger et al., 1997; Brun and Dudley 1989). This scenario increases the chance of success to classify genotypes regards their tolerance, exploring the phenotypic variability that is expressed in this conditions. The use of morphological and physiological characteristics in the early selection of genotypes to increase the nutritional efficiency of new cultivars have been largely recommended by the scientific community (Sattelmacher et al., 1994; Bänziger and Lafitte 1997; Feil et al., 1993; Oliveira et al., 1999). The utilization of those characteristics to select genotypes with higher tolerance or efficiency, and, therefore, a differential adaptation to different systems of cultivation, can help to improve the understanding of the mechanisms that are involved in the determination of this complex agronomic trait.

Material and Methods

Experimental design

The experiment was conducted in greenhouse, located at the experimental site of the Centro de Ciências Agrárias da Universidade Federal do Espírito Santo (CCA/UFES), in the municipality of Alegre, southern Espírito Santo State (latitude 20°45'S, longitude 41°33'W, 277.41 meters of altitude). The experiment followed a 10x2 factorial scheme, with a completely randomized design and four replications. The factors studied were 10 genotypes of conilon coffee and two conditions of availability of soil nitrogen. The conditions

Table 3. Means of 16 variables of genotypes of conilon coffee, grown without or with addition of 0.625 g of nitrogen per kilogram of soil, at 150 days of cultivation.

Gen.	PHE[1]		RGR[2]		NLE[3]		TLA[4]	
	0.000	0.625	0.000	0.625	0.000	0.625	0.000	0.625
67	30.00 b	42.00 a	0.016 a	0.031 a	9.67 e	34.67 a	486.97 b	1.922.47 a
23	26.67 b	38.33 a	0.019 a	0.027 a	13.00 d	28.00 b	552.30 b	1.478.03 b
32	28.67 b	29.33 c	0.026 a	0.022 a	19.33 b	25.33 b	706.40 a	964.17 d
73	33.00 a	35.67 a	0.029 a	0.029 a	12.33 d	32.00 a	671.43 a	1.525.47 b
83	32.67 a	34.00 b	0.028 a	0.024 a	14.33 d	29.67 a	736.93 a	1.526.07 b
77	25.33 b	36.33 a	0.017 a	0.026 a	22.67 a	35.33 a	678.27 a	1.229.80 c
76	26.33 b	39.00 a	0.022 a	0.025 a	13.67 d	31.33 a	505.13 b	1.341.03 c
24	35.00 a	30.67 c	0.017 a	0.021 a	17.00 c	26.33 b	647.53 a	1.184.27 c
153	29.00 b	34.67 b	0.025 a	0.031 a	12.67 d	22.33 b	737.90 a	1.139.60 c
02	27.33 b	36.67 a	0.023 a	0.037 a	11.33 e	32.33 a	515.27 b	1.397.40 b
	DML[5]		DMS[6]		DMR[7]		DMT[8]	
	0.000	0.625	0.000	0.625	0.000	0.625	0.000	0.625
67	4.55 c	16.99 a	3.60 b	8.64 a	9.62 c	15.16 b	17.77 c	40.79 a
23	5.09 c	18.82 a	2.97 b	7.92 b	9.93 c	14.33 b	17.99 c	41.07 a
32	5.88 b	10.45 c	3.33 b	4.30 d	9.48 c	6.67 e	18.69 c	21.42 c
73	6.29 a	17.82 a	4.08 a	7.37 b	10.60 c	17.68 a	20.97 b	42.86 a
83	6.46 a	19.06 a	4.57 a	8.68 a	11.96 b	13.51 b	22.99 a	41.25 a
77	5.85 b	12.89 b	3.46 b	6.80 c	8.59 c	11.49 c	17.90 c	31.19 b
76	4.31 c	14.58 b	3.22 b	6.15 c	8.96 c	12.44 b	16.49 c	33.18 b
24	5.76 b	13.58 b	4.81 a	6.12 c	13.99 a	11.57 c	24.57 a	31.26 b
153	7.03 a	12.99 b	4.05 a	6.85 c	15.01 a	13.64 b	26.09 a	33.48 b
02	4.75 c	14.42 b	3.23 b	6.37 c	9.54 c	9.56 d	17.52 c	30.34 b
	LAR[9]		LMR[10]		SMR[11]		RMR[12]	
	0.000	0.625	0.000	0.625	0.000	0.625	0.000	0.625
67	27.44 b	47.14 a	0.26 b	0.42 b	0.20 a	0.21 a	0.54 a	0.37 b
23	30.72 b	35.96 b	0.28 b	0.46 a	0.16 a	0.19 a	0.55 a	0.35 c
32	37.69 a	45.08 a	0.31 a	0.49 a	0.18 a	0.20 a	0.51 b	0.31 c
73	31.98 b	35.55 b	0.30 a	0.42 b	0.19 a	0.17 a	0.51 b	0.41 a
83	32.11 b	37.14 b	0.28 b	0.46 a	0.20 a	0.21 a	0.52 b	0.33 c
77	37.85 a	39.47 b	0.33 a	0.41 b	0.19 a	0.22 a	0.48 b	0.37 b
76	30.85 b	40.40 b	0.26 b	0.44 b	0.20 a	0.19 a	0.54 a	0.38 b
24	26.50 b	37.94 b	0.23 b	0.43 b	0.20 a	0.20 a	0.57 a	0.37 b
153	28.32 b	34.05 b	0.27 b	0.39 b	0.16 a	0.20 a	0.57 a	0.41 a
02	29.46 b	46.09 a	0.27 b	0.47 a	0.18 a	0.21 a	0.54 a	0.31 c
	SLA[13]		CLA[14]		CLB[15]		CLT[16]	
	0.000	0.625	0.000	0.625	0.000	0.625	0.000	0.625
67	107.03 a	113.19 a	23.23 b	40.57 a	4.63 d	36.48 a	27.87 c	77.05 a
23	108.69 a	78.66 d	22.25 b	40.23 a	4.32 d	33.37 a	26.57 c	73.60 a
32	119.94 a	92.30 b	30.27 a	37.57 a	5.90 c	26.47 b	36.17 b	64.03 b
73	106.44 a	85.57 c	25.95 b	40.68 a	6.02 c	32.87 a	31.97 c	73.55 a
83	114.25 a	80.12 d	25.53 b	40.15 a	4.98 d	32.95 a	30.52 c	73.10 a
77	117.00 a	95.45 b	30.30 a	39.57 a	7.07 b	29.47 b	37.37 b	69.03 b
76	118.11 a	91.99 b	33.75 a	38.78 a	10.00 a	31.03 b	43.75 a	69.82 b
24	112.69 a	87.35 c	24.15 b	40.35 a	4.72 d	32.70 a	28.87 c	73.05 a
153	105.23 a	87.71 c	30.78 a	40.07 a	6.90 b	29.65 b	37.68 b	69.72 b
02	108.38 a	97.12 b	29.70 a	40.37 a	6.07 c	28.60 b	35.77 b	68.97 b

Means followed by the same letter do not differ by the Scott-Knott test, at 5% of probability; [1]Plant height (cm); [2]Relative growth rate (cm cm^{-1} dia^{-1}); [3]Number of leaves; [4]Total leaf area (cm^2); [5]Dry matter of leaves (g); [6]Dry matter of stems (g); [7]Dry matter of roots (g); [8]Total dry matter (g); [9]Leaf area ratio (cm^2 g^{-1}); [10]Leaf mass ratio; [11]Stem mass ratio; [12]Root mass ratio; [13]Specific leaf area (cm^2 g^{-1}); [14]Content of chlorophyll a; [15]Content of chlorophyll b; [16]Total content of chlorophyll.

of availability of nitrogen were absence of fertilization and addition of nitrogen to the level of 0.625 g kg^{-1}, which, according to preliminary trials, are fertilization levels below and above optimal that allowed better expression of variability for accumulation of dry matter by the plants.

Selection of genotypes

The 10 genotypes of *Coffea canephora* were selected from different groups of ripening cycle: genotypes 67, 23 and 32 of early cycle; 73, 83 and 77 from intermediate cycle; 76, 24 and 153 of late cycle; and, additionally, the genotype 02 (early cycle), which present high yield potential and is widely cultivated in the Espírito Santo State. These genotypes are originated from the breeding program developed by Incaper, featuring desirable agronomic traits and adaptation to the cultivation systems used in Brazil.

The genotypes were multiplied asexually through cuttings and the plantlets were formed in nurseries registered and certified by the Ministério da Agricultura, Pecuária e Abastecimento.

Fertilization and conduction

The soil used in the experiment was collected at a depth of 40 cm, discarding the first 10 cm of in order to reduce the effect of organic matter present in the superficial layer. A sample of this soil was sent to laboratory for chemical and physical

Table 4. Preliminary classification of the genotypes of conilon coffee regarding the development under low availability of N in the soil.

Genotype	Cycle	Classification
67	Launched by Incaper in 2004, Early ripening cycle	Intolerant
23	Launched by Incaper in 1999, Early ripening cycle	Intolerant
32	Launched by Incaper in 2004, Early ripening cycle	Intermediate tolerance
73	Launched by Incaper in 2004, Intermediate ripening cycle	Intermediate tolerance
83	Launched by Incaper in 2004, Intermediate ripening cycle	Intermediate tolerance
77	Launched by Incaper in 2004, Intermediate ripening cycle	Intermediate tolerance
76	Launched by Incaper in 2004, Late ripening cycle	Intermediate tolerance
24	Launched by Incaper in 1999, Late ripening cycle	Tolerant
153	Launched by Incaper in 1993, Late ripening cycle	Tolerant
02	Launched by Incaper in 1993, Early ripening cycle	Intolerant

Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural, Incaper, Brazil.

Table 5. Classification of the genotypes of conilon coffee regarding the development under low availability of N in the soil, according to estimates of the discriminant functions $D_t(x)$ and $D_i(x)$ for tolerant and intolerant genotypes, respectively.

Genotype	$D_t(x)$	$D_i(x)$	Classification
67	182.80	185.21	Intolerant
23	182.32	184.35	Intolerant
32	237.72	235.68	Tolerant
73	252.04	249.86	Tolerant
83	187.09	184.87	Tolerant
77	202.53	200.42	Tolerant
76	209.03	206.88	Tolerant
24	240.95	238.77	Tolerant
153	216.75	214.70	Tolerant
02	233.89	235.86	Intolerant

Discriminant functions estimated by Anderson method.

analysis, being characterized as a dystrophic oxisol of clayey texture (Embrapa, 2006). After the characterization, the entire volume of soil was dried in shade and homogenized with a 2.0 mm mesh sieve. It was subsequently separated into samples of 10 dm^3 and accommodated into sealed pots. The fertilization, except for nitrogen, was performed according to the recommendation for nutritional studies in controlled environment (Novais et al., 1991). The nutrients were supplied through solutions prepared with salts (KNO_3, KH_2PO_4, NH_4NO_3, $CaHPO_4$) to establish the nutritional balance of the soil. From the early development of the third pair of leaves, the plantlets were transplanted to the pots and subjected to differential nitrogen fertilizations. The fertilization with nitrogen was performed in coverage, with application of urea (purity: 98%), split in four applications, starting 15 days after replanting and spaced at intervals of 30 days. Irrigation was performed by keeping the soil moisture near 60% of the total pore volume during the experiment. The total pore volume was obtained using the particle density and soil density, determined by the test tube method, according to Embrapa (1997). The other cultural practices were performed manually according to the need.

Traits measured

The plants were conducted for 150 days and their growth were studied with determination of: number of leaves (NLE), obtained by counting; plant height (PHE), measured with a ruler graduated in centimeters and total leaf area (TLA), obtained using a digital integrator (Area meter, Licor 3100, Nebraska, USA, precision: 0.01 cm^2). The contents of chlorophyll *a* (CLA), chlorophyll *b* (CLB) and total (CLT) were determined using a portable chlorophyllometer (ClorofiLOG 1030, Falker). After chose evaluations, the plants were collected, the compartments were separated, placed in paper bags and dried in a drying oven with forced air circulation at temperature of 65 ºC until constant weight in order to determine the dry matter accumulated in leaves (DML), stems (DMS), roots (DMR) and total (DMT). The specific leaf area (SLA) was obtained from the ratio between leaf area and dry weight of the same, measured in leaf discs of known area. The relative growth rate (RGR) was calculated based on the temporal variation of plant height. The leaf area ratio (LAR) was calculated as the ratio between leaf area and total dry matter of the plant. The stem mass ratio (SMR), leaf mass ratio (LMR) and root mass ratio (RMR) were calculated by establishing the ratio between the dry matter of stems, leaves and roots, respectively, and the total biomass of the plants.

Statistical analyses

The data were subjected to analysis of variance, both individual and combined, by the F test at 1 and 5 % probability, to identify the characteristics for which there is differentiation between the genotypes. Based on the individual analysis, using the fixed model: $Y_{ij} = \mu + g_i + \varepsilon_{ij}$, estimative of the coefficient of genetic variation, variation index and genotypic coefficient of determination for each characteristic were calculated. Based on the results of the growth analysis and expression of variability between genotypes, the most appropriate level for discrimination of genotypes for tolerance to low N availability in the soils was selected. The classification of the genotypes regarding the tolerance to low N availability in the soil was done using two criteria. Initially, the different characteristics of the plants were used to compare their growth through the Scott-Knott test ($p \leq 0.05$) to allow the identification genotypes that were able to grow under conditions of low availability of N (higher tolerance) and genotypes that were not able to develop satisfactorily (lower tolerance). Sequentially, a multivariate analysis, employing discriminant functions of Anderson, were performed to classify the genotypes whose tolerance had intermediate behavior in one of two known groups

(tolerant and intolerant). For this analyses, a set of characteristics were selected, based on their relevance to diversity, in order to estimate the discriminant functions, which were used to calculate the scores and rankings, the genotypes. All analyzes considered 5% of probability and were done with the statistical software Genes (Cruz, 2013).

Conclusions

Through the classification of genotypes for tolerance to early development in soils with low availability of nitrogen, the genotypes 67, 23 and 02 are intolerant and the genotypes 32, 73, 83, 77, 76, 24 and 153 are tolerant. Increasing the availability of nitrogen in the soil promotes the plant growth and, as consequence, causes greater biomass accumulation, as well as better development of leaves, in quantity, area and content of photosynthetic pigments. In addition, it alters the pattern of biomass allocation between the plant compartments, favoring leaves and stems.

Acknowledgments

The authors are grateful to Centro de Ciencias Agrárias of the Universidade Federal do Espírito Santo for supporting this research, providing access to the necessary facilities and laboratories.

References

Bänziger M, Beãrn FJ, Lafitte HR (1997) Efficiency of high-nitrogen selection environments for improving maize for low-nitrogen target environments. Crop Sci. 37: 1103-1109.

Bänziger M, Lafitte HR (1997) Efficiency of secondary traits for improving maize for low-nitrogen target environments. Crop Sci. 37: 1110-1117.

Bertin P, Gallais A (2000) Genetic variation for nitrogen use efficiency in a set of recombinant maize inbred lines I. Agro Physi Results. 45: 53-66.

Borém A, Miranda GV (2005) Melhoramento de plantas. 4th Edn. UFV, Viçosa. 525p.

Bragança SM, Martinez HHP, Leite HG, Santos LP, Sediyama CS, Alvarez VVH, Lani JA (2008) Accumulation of macronutrients for the conilon coffee tree. J Plant Nutr. 3(1):103-120.

Brun EL, Dudley JW (1989) Nitrogen response in the USA and Argentina of corn populations with different proportions of flint and dent germplasm. Crop Sci. 29: 565-569.

Carvalho CHS (2008) Cultivares de café. Embrapa Café, Brasília. 334 p.

Cecon PR, Silva FF, Ferreira A, Ferrão RG, Carneiro APS, Detmann E, Faria PN, Morais TSS (2008) Repeated measure analysis in the clonal evaluation in 'Conilon' coffee. Pesq Agropec Bras. 43(9): 1171-1176.

Chun L, Mi GH, Li JS, Chen FJ, Zhang FS (2005) Genetic analysis of maize root characteristics in response to low nitrogen stress. Plant Soil. 276(2): 369-382.

Clemente JA, Martinez HEP, Alves LC, Lara MCR (2013) Effect of N and K doses in nutritive solution on growth, production and coffee bean size. Rev Ceres. 60(2): 279-285.

CONAB – Companhia Nacional de Abastecimento (2014) Acompanhamento da safra brasileira: café. CONAB, Brasília. 21p.

Cruz CD (2013) GENES - a software package for analysis in experimental statistics and quantitative genetics. Acta Sci Agron. 35: 271-276.

EMBRAPA – Empresa Brasileira de Pesquisa Agropecuária (1997) Manual de métodos de análises de solo. 2nd Edn. Ministério da Agricultura e do Abastecimento, Rio de Janeiro.

EMBRAPA – Empresa Brasileira de Pesquisa Agropecuária (2006) Sistema brasileiro de classificação de solos. 2nd Edn. Centro Nacional de Pesquisa de Solos, Rio de Janeiro. 306p.

Epstein E (1975) Nutrição mineral de plantas: princípios e perspectivas. Livros Técnicos e Científicos, Rio de Janeiro. 341p.

Feil B, Thiraporn R, Stamp P (1993) In vitro nitrate reductase activity of laboratory-grown seedlings as an indirect selection criterion for maize. Crop Sci. 33: 1280-1286.

Ferrão MAG, Ferrão RG, Fonseca AFA, Verdin Filho AC, Volpi PS (2007) Origem, dispersão geográfica, taxonomia e diversidade genética de *Coffea canephora*. In: Ferrão RG, Fonseca AFA, Bragança SM, Ferrão MAG, Muner LH (ed) Café Conilon. Incaper, Vitória. p.65-92.

Ferrão MAG, Fonseca AFA, Ferrão RG, Barbosa WM, Souza EMR (2009) Genetic divergence in Conilon coffee revealed by RAPD markers. Crop Breed Appl Biotechnol. 9: 67-74.

Ferrão RG, Cruz CD, Ferreira A, Cecon PR, Ferrão MAG, Fonseca AFA, Carneiro PCS, Silva MF (2008) Genetic parameters in Conilon coffee. Pesq Agropec Bras. 43(1): 61-69.

Fonseca AFA, Ferrão RG, Ferrão MAG, Volpi PS, Verdin Filho AC, Fazuoli LC (2008) Cultivares de café robusta. In: Carvalho CHS (ed) Cultivares de café: origem, características e recomendações. Embrapa Café, Brasília. p. 255-280.

Gerloff GC, Gabelman WH (1983) Genetic basis of inorganic plant nutrition. In: Laüchli A, Bieleski RL (ed) Inorganic plant nutrition: Encyclopedia of Plant Physiology. Springer-Verlag, Berlim, New York, Tokyo. p. 453-486.

Guimarães RJ, Mendes ANG (1997) Nutrição Mineral do cafeeiro. UFLA/FAEPE, Lavras. 70p.

Hammer GL, Dong ZS, Mclean G, Doherty A, Messina C, Schusler J, Zinselmeier C, Paszkiewicz S, Cooper M (2009) Can changes in canopy and/or root system architecture explain historical maize yield trends in the US Corn Belt? Crop Sci. 49(1): 299-312.

Malavolta E (1986) Nutrição, adubação e calagem para o cafeeiro. In: Rena AB, Malavolta E, Rocha M, Yamada T (ed) Cultura do cafeeiro: fatores que afetam a produtividade. Potafos, Piracicaba. p. 136-274.

Malavolta E, Vitti GC, Oliveira AS (1997) Avaliação do estado nutricional das plantas: princípios e aplicações. 2nd Edn. Associação Brasileira para Pesquisa da Potassa e Fosfato, Piracicaba. 201p.

Marraccini P, Vinecky F, Alves GSC, Ramos HJO, Elbelt S, Vieira NG, Carneiro FA, Sujii PS, Alekcevetch JC, Silva VA, DaMatta FM, Ferrão MAG, Leroy T, Pot D, Vieira LGE, Silva FR, Andrade AC (2012) Differentially expressed genes and proteins upon drought acclimation in tolerant and sensitive genotypes of *Coffea canephora*. J Exp Bot. 1: 1-22.

Marschner H (1995) Mineral nutrition of higher plant. 2nd Edn. Academic Press, London. 889p.

Martins LD, Tomaz MA, Amaral JFT, Bragança SM, Reis EF, Rodrigues WN (2013a) Nutritional efficiency in clones of conilon coffee for phosphorus. J Agr Sci. 5: 130-140.

Martins LD, Tomaz MA, Amaral JFT, Christo LF, Rodrigues WN, Colodetti TV, Brinate SVB (2013b) Alterações morfológicas em clones de cafeeiro conilon submetidos a níveis de fósforo. Sci Plena. 9: 1-11.

Mattiello EM, Pereira MG, Zonta E, Mauri J, Matiello JD, Meireles PG, Silva IR (2008) Dry matter production, root growth and calcium, phosphorus and aluminum absorption by *Coffea canephora* and *Coffea arabica* under influence of aluminum activity in solution. Rev Bras Ciênc Solo. 32(1): 425-434.

Mengel K, Kirkby EA (2001) Principles of plant nutrition. 5th Edn. Kluwer Academic Publishers, Dordrecht. 849p.

Novais RF, Neves JCL, Barros NF (1991) Ensaio em ambiente controlado. In: Oliveira AJ, Garrido WE, Araújo JDE, Lourenço S (ed) Métodos de pesquisa em fertilidade do solo. Embrapa-Sae, Brasília. p.189-254.

Oliveira VR, Casali VC, Pereira PRG, Cruz CD, Pires NM (1999) Genotype tolerance of sweet pepper to low phosphorus content in soil. Bragantia. 58(1):125-139.

Partelli FL, Espindula MC, Marré WB, VieiraHD (2014) Dry matter and macronutrient accumulation in fruits of conilon coffee with different ripening cycles. Rev Bras Ciênc Solo. 38: 214-222.

Quintela MP, Silva TJA, Bonfim-Silva EM, Silva EFF, Bebé FV (2011) Parâmetros produtivos e nutricionais do cafeeiro submetido adubação nitrogenada na região de Garanhuns. Rev Caatinga. 24(4): 74-79.

Ramalho MAP, Santos JB, Pinto CABP (2004) Genética na agropecuária. 3rd Edn. UFLA, Lavras. 472p.

Rodrigues WN, Tomaz MA, Ferrão RG, Ferrão MAG, Fonseca AFA, Martins LD (2013) Crop yield bienniality in groups of genotypes of conilon coffee. Afr J Agric Res. 8: 4422-4426.

Rodrigues WN, Tomaz MA, Ferrão RG, Ferrão MAG, Fonseca AFA, Miranda FD (2012) Genetic parameters estimation in groups of conilon coffee clones. Coffee Sci. 7(2): 177-186.

Sattelmacher B, Horst WJ, Becker HC (1994) Factors that contribute to genetic variation for nutrient efficiency of crop plants. Z Pflanzenernähr. 157: 215-224.

Stoking CR, Ongun A (1962) The intracellular distribuition of some metallic elements in leaves. Am J Bot. 49(3): 284-289.

Taiz L, Zeiger E (2013) Fisiologia vegetal. 5th Edn. Artmed, Porto Alegre. 918p.

Vaast P, Zasoski RJ, Bledsoe CS (1998) Effects of solution pH, temperature, nitrate/ammonium rates and inhibitors on ammonium and nitrate uptake by Arabica coffee in short term solution culture. J Plant Nutr. 21(7):1551-1564.