SEÇÃO III - BIOLOGIA DO SOLO

NITRATE REDUCTASE AND GLUTAMINE SYNTHETASE ACTIVITY IN COFFEE LEAVES DURING FRUIT DEVELOPMENT[1]

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SUMMARY

Nitrate reductase is the first enzyme in the pathway of nitrate reduction by plants, followed by glutamine synthetase, which incorporates ammonia to glutamine. The purpose of this study was to evaluate the nitrate reductase and glutamine synthetase activity, total soluble protein content, N and Ni content in coffee leaves during fruit development under field conditions to establish new informations to help assess the N nutritional status and fertilizer management. The experimental design was in randomized complete blocks, arranged in a 3 x 6 factorial design, with five replications. The treatments consisted of 3 N rates (0 - control, 150 and 300 kg ha^{-1}) and six evaluation periods (January, February, March, April, May, and June) in six-year-old coffee (*Coffea arabica* L.) plants of Catuaí Vermelho IAC 44 cv. The nitrate reductase and glutamine synthetase activities, leaf soluble protein, and N concentrations increased linearly with the N rates. During fruit development, the enzyme activity, leaf soluble protein and N content decreased, due to the leaf senescence process caused by nutrient mobilization to other organs, e.g, to the berries. Leaf Ni increased during fruit development. Beans and raisin-fruits of plants well-supplied with N had higher Ni contents. Enzyme activities, total leaf N and leaf soluble protein, evaluated during

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the green fruit stage in March, were significantly correlated with coffee yield. These variables can therefore be useful for an early assessment of the coffee N nutritional status as well as coffee yield and N fertilization management.

Index terms: *Coffea arabica*, nitrogen assimilating enzymes, nitrogen fertilizer.

RESUMO: *ATIVIDADE DA REDUTASE DO NITRATO E GLUTAMINA SINTETASE EM FOLHAS DE CAFEEIRO DURANTE O DESENVOLVIMENTO DOS FRUTOS*

A redutase do nitrato (RN) é a primeira enzima na via de redução de nitrato pelas plantas, seguida da glutamina sintetase (GS), a qual incorpora amônia à glutamina. O objetivo deste trabalho foi avaliar a atividade dessas enzimas, o teor de proteína solúvel total e a concentração de N e de Ni em folhas e grãos de cafeeiro durante o desenvolvimento dos frutos, em condições de campo, a fim de estabelecer novas informações para auxiliar a avaliação do estado nutricional do N e manejo da adubação. O experimento foi instalado em blocos ao acaso, em esquema fatorial 3 x 6, com três doses de N (0, 150 e 300 kg ha^{-1}) e seis épocas de avaliação (janeiro, fevereiro, março, abril, maio e junho), com cinco repetições. Foi utilizada a cultivar Catuaí Vermelho IAC 44 com seis anos de idade, no espaçamento de 1,75 m entre linhas e 0,75 m entre plantas. A atividade da RN e da GS e os teores de proteína solúvel e de N total aumentaram com as doses de N. Durante o crescimento dos frutos do cafeeiro, a atividade das enzimas e os teores foliares de proteína e de N diminuíram devido ao processo de senescência das folhas e à mobilização do N para a maturação dos grãos. A concentração de Ni nas folhas e nos grãos aumentou durante a fase de crescimento dos frutos. Plantas com estado nutricional adequado em N apresentaram maiores teores de Ni nos grãos e nos frutos em estádio tipo passa. As atividades das enzimas e os teores foliares de N e de proteína solúvel total, durante o estádio de fruto verde, têm relação direta com a produção, constituindo-se em medidas auxiliares na avaliação do estado nutricional em N, na predição da produção do cafeeiro e no manejo da adubação nitrogenada.

Termos de indexação: Coffea arabica*, enzimas assimiladoras de nitrogênio, adubação nitrogenada.*

INTRODUCTION

Nitrogen assimilation is a vital process for plant growth, directly responsible for crop biomass production and grain yields. The pathway of nitrate assimilation in higher plants involves two sequential biochemical stages. The first consists on the conversion of nitrate to ammonia, measured by the enzyme nitrate reductase (NR), then nitrate is reduced to nitrite, and in turn, nitrite is reduced to ammonia. In the second step, ammonia is incorporated to glutamine and glutamate amino acids, both used to redistribute organic-N from source to sinks (Lam et al., 1996).

Reductive N assimilation is a highly energy-consuming process for plants and for this reason, it occurs mainly in the leaves, where ATP (energy source molecules) and reducing substances, such as NADPH (electron source for the N reduction process), are synthesized.

The N assimilation process is directly or indirectly regulated by the plant nitrate reduction capacity, which is controlled by the plant metabolic level by sensor molecules and signal transducer pathways (Campbell, 1999). Due to the regulatory role of the available reduced N for the plant metabolism, particularly in soils where nitrate is the main available N source, it has been suggested that NR activity is related to plant productivity and/or to a significant response to N fertilization (Malavolta et al., 2004; Reis et al., 2007).

The N taken up by plants is incorporated into C chains to produce amino acids, which are the structural components of proteins stored in plant tissues. By the time of fruit or grain filling and periods of old tissue senescence, such protein reserves are metabolized and broken into small amino acid molecules and N is quickly redistributed to new tissues resulting in high mobility within the plant (Marschner, 1995).

Nitrogen assimilation is affected by several micronutrients such as Fe, Mo and Ni. Nickel is a constituent of the urease enzyme molecule (originated in the ornithine cycle), which is responsible for urea degradation, resulting in ammonia and CO_2 (Gerendás et al., 1999). Ni-deficient plants

accumulate nitrate as a consequence of the low malate dehydrogenase activity, because this enzyme activity supplies energy for the nitrate reduction process (Brown, 2006). Therefore, there are some evidences for a relationship between nitrate assimilation and Ni content in the plant.

The objective of this work was to evaluate the NR and GS activities, the N and Ni concentrations in leaf and coffee fruit during fruit development under field conditions, for a deeper understanding of the coffee plant N nutritional status and the N fertilization management.

MATERIAL AND METHODS

The experiment was carried out in the growing season of 2006/2007 on an experimental farm of the Universidade de São Paulo (USP/ESALQ), in Piracicaba, State of São Paulo, Brazil (22 ° 42 ' S; 47 ° 38 ' W; 580 m asl). Coffee plants (*Coffea arabica)* of the cultivar Catuaí Vermelho IAC 44 from a six-year-old coffee orchard were used, spaced 1.75 m between rows and 0.75 m between plants (plant density of 7.633 plants ha^{-1}). According to Köppen the region has a Cwa climate, or mountainous tropical, with annual average temperatures of 21.1 ºC, rainfall of 1,257 mm and relative air humidity of 740 g kg^{-1}. There is a rainy period from October to March and a dry period from June to September. Climate data were monitored daily by the computerized agrometeorological station of USP/ESALQ at the experimental site.

The soil in the experimental area is classified as an eutroferric Red Nitosol moderate A horizon with clayey texture. The soil chemical attributes were determined in samples from the 0–20 cm layer, with the following results: pH ($CaCl_2$) = 5.3; OM = 31 g dm^{-3}; P (resin) = 8 mg dm^{-3}; K = 4.3 $mmol_c$ dm^{-3}; Ca = 29 $mmol_c$ dm^{-3}; Mg = 20 $mmol_c$ dm^{-3}; H + Al = 30 $mmol_c$ dm^{-3}; SB = 53.1 $mmol_c$ dm^{-3}; CTC = 83.1 $mmol_c$ dm^{-3}; and base saturation = 64 %.

The experiment was arranged in randomized complete blocks, in a 3 x 6 factorial design, with five replications. The treatments consisted of three N rates (0 = control; 150 and 300 kg ha^{-1}) and six evaluation periods (January, February, March, April, May and June). The highest N rate (T3 – 300 kg ha^{-1}) was estimated based on the yield expectancy (Raij et al., 1997) and an intermediary rate was considered (T2 – 150 kg ha^{-1}).

The data were submitted to analysis of variance using the SAS program of statistics, *System for Windows* 6.11 (SAS, 1996). According to the F test significance level for the treatments (N-rates and evaluation periods), the data were submitted to linear and quadratic regression analysis using the GLM (general linear model) and the Tukey test (0.05) for mean comparisons. The correlations among dependent variables (coffee yield, enzyme activities and leaf protein and N concentrations) were obtained by the CORR procedure (Pearson correlation coefficient) of SAS - *System for Windows* 6.11 (SAS, 1996). Therefore, the polynomial regression model with the highest correlation coefficient (r) and significance for the studied relationship was considered.

Nitrate reductase activity in leaf extracts

The *in vivo* NR activity was determined according to the procedure described by Radin (1974). Leaf samples were collected between 9:00 h and 10:00 h am, to avoid the interference of light intensity with the enzyme activity at different N rates.

Samples of fresh leaf tissue were collected and stored in plastic bags and transported to the laboratory on ice, and rinsed with deionized water. Afterwards, 100 mg of fresh tissue cut in discs was transferred to assay tubes containing 3 mL of phosphate buffer solution pH 7.4 (50 mmol L^{-1} + 200 mmol L^{-1} KNO_3). These samples were vacuum-infiltrated during 2 min to enhance the solution penetration into tissues. Thereafter, the assay tubes were incubated in a 33 °C water bath for 30 min wrapped in aluminum foil against the light.

The reaction was stopped by adding 1 mL of 1 % sulfanilamide in 2 mol L^{-1} HCl solution and after that, 1 mL of 0.05 % naphtylenediamine solution. The nitrite (NO_2^-) produced was measured in a spectrophotometer at 540 nm, using a nitrite standard calibration curve. The enzyme activity was directly related to the amount of NO_2^- and the results were expressed in µmol NO_2^- g^{-1} h^{-1} FM.

Glutamine synthetase activity in leaf extracts

A 1.0 g leaf tissue sample was collected from each experimental unit and treated with a small portion of liquid N. Before tissue maceration, a 2.0 mL aliquot of the extracting solution (Tris–HCl 200 mmol, pH 7.5) was added. The material was homogenized and centrifuged at 5.000 rpm for 5 min at 4 °C. The supernatant was transferred to an *Eppendorf* flask, which was placed in a recipient containing ground ice, and then, the enzyme activity was determined.

The GS activity was measured using the method proposed by Elliott (1953), which is based on the enzyme biosynthetic activity in the formation of λ-glutamyl hydroxamate. This reaction was carried out in assay tubes under continuous shaking in a water bath at 30 °C, with the addition of: 0.25 mL of extraction buffer solution; 0.1 mL of 50 mmol L^{-1} ATP, pH 7.0; 0.25 mL of 500 mmol L^{-1} sodium glutamate; 0.05 mL of 1 mol L^{-1} $MgSO_4$; 0.05 mL of 100 mmol L^{-1} cysteine; 0.15 mL of 100 mmol L^{-1} hydroxylamine, pH 7.0; and 0.15 mL of the supernatant.

After the incubation period, the reaction was stopped by the addition of 1 mL of ferric chloride – $FeCl_3$ (10 %) : TCA (24 %) : HCl (6 mol L^{-1}), 1:1:1 – forming a brown-yellowish precipitate. Thereafter, the mixture was centrifuged at 5,000 rpm and the supernatant color was analyzed in a spectrophotometer at 540 nm to determine the formation of λ-glutamyl hydroxamate (λ-GH), using a standard calibration curve. The GS activity was expressed in μmol L^{-1} of λ-GH h^{-1} mg^{-1} of protein.

Determination of total soluble protein concentration

The total soluble protein concentration was determined according to the Bradford (1976) procedure, using tissue extracts prepared with Tris-buffer (Tris – 200 mmol L^{-1} HCl, pH 7.5). To a 100 μL aliquot extract sample 5 mL Bradford reagent was added and the color was read with a spectrophotometer (595 nm). The total soluble protein concentrations in the samples were calculated using a bovine serum albumin (BSA) standard calibration curve. The results were expressed in mg g^{-1} fresh tissue.

Determination of total-N and Ni

Leaves and fruits were dried in a forced-air oven at 65 °C during 48 h and ground. Thereafter, the samples were digested and chemically analyzed. Total-N was determined by the Kjeldahl method, which consists in the sulfuric digestion of the plant material to decompose the organic-N into ammonium, which is then converted to ammonia, distilled, and the amount of ammonia (and consequently the amount of N in the sample) is determined by back-titration. Nitroperchloric digestion was performed for the Ni analysis and Ni was determined by atomic absorption spectrophotometry, as described by Malavolta et al. (1997).

RESULTS AND DISCUSSION

NR and GS activities, leaf N and total soluble protein concentrations increased with N-rates, reaching the highest values in January. The average leaf N concentrations for the two N rates (150 and 300 kg ha^{-1}) were 28 and 32 g kg^{-1}, respectively, which are considered adequate for coffee plants (Malavolta et al., 1997). These leaf N concentrations are related to the following mean values for NR, GS and total soluble protein for the two N-rates, respectively: 1.03 and 1.29 μmol NO_2^- g^{-1} FM h^{-1}; 82.50 and 113.13 μmol y GH h^{-1} mg^{-1}; 3.65 and 4.35 mg g^{-1} FM (Table 1).

In January, the berries were in the fruit expansion/filling stage and the NR activity in leaves was high, as observed also by Carelli et al. (1989), when studying NR activity in coffee plants. In February, although the leaf N concentrations were adequate, the enzyme activity values were lower than in January, probably due to the lower rainfall and a slight decrease in the average air temperature (Figure 1). These results agree with those observed by Reis et al. (2007), when studying coffee plants under field conditions.

NR activity was positively influenced by N rates, in all evaluation periods, and the enzyme activity data fit a linear regression model (Table 1). On the other hand, decreasing enzyme activity values were observed in the months from January to June (Figure 2), a period in which the average air temperature and rainfall tend to decrease.

Environmental variations might alter the plant nitrate assimilation capability, once the NR enzyme activity is affected by the substrate NO_3^- concentration as well as by light intensity and temperature (Taleisnik et al., 1980; Da Matta et al., 1999).

Taleisnik et al. (1980) observed seasonal variation of the leaf NR activity and a significant correlation with the period of higher rainfall in Costa Rica. The authors also reported that the enzyme activity values were highest when the soil moisture was close to field capacity, while lowest NR activity values were reported during the low-rainfall seasons, corresponding to the fruit ripening period.

Similar results were observed in the present study. The highest NR activity values were observed in January (0.83; 1.37 and 1.78 μmol NO_2^- g^{-1} FM h^{-1}, at rates of 0–150 and 300 kg ha^{-1} N, respectively), which was the period of fruit expansion/filling (Pezzopane et al., 2003). Furthermore, NR activity was positively correlated with coffee yield, indicating that this enzyme activity might be a useful tool (biochemical test) to evaluate the plant N nutritional status and to predict coffee productivity (Table 2, Figure 7a).

In the period between fruit expansion/filling and ripening, NR activity decreased in all treatments, independent of the N rate (Figure 2), whereas the leaf Ni concentration increased (Figure 3). It is known that Ni activates the oxide-reductase of NAD-malate to produce NADH, which is required for NO_3^- reduction (Brown et al., 1990). This fact could explain the increasing NR activity from March onwards (beginning of fruit ripening), since a significant leaf Ni increase was also observed in the same period (Figure 3), independent of the N rate. It has been reported that Ni has a specific role in the N metabolism during seed germination, and for this reason, Ni is redistributed during senescence to the reproductive organs, mainly to the fruits (Welch, 1999). This evidence suggests that N metabolism in coffee plants, particularly the NR activity in fruits, may be affected by Ni concentrations in the plant.

In the N-deficient plants (control) the average leaf N concentration of 15 g kg^{-1} was below the range of 29 to 32 g kg^{-1} considered adequate for coffee

Table 1. Leaf N concentration, nitrate reductase (NR) and glutamine synthetase (GS) activities, and total soluble protein concentration (TSP), considering N-rates, R^2 values and linear models for each variable response

Month	N-rate	N	NR	GS	TSP
	kg ha^{-1}	g kg^{-1}	µmol NO_2^- g^{-1} FM h^{-1}	µmol $_y$ GH h^{-1} mg^{-1} of protein	mg g^{-1} FM
Jan	0	15.66	0.83	82.48	0.62
	150	31.40	1.37	95.31	5.66
	300	33.30	1.78	136.72	6.52
	R^2	0.83	0.99	0.91	0.86
	Equation	$\hat{y}$ =17.967+0.0588 x	$\hat{y}$ =0.852+0.0032 x	$\hat{y}$ =77.713+0.1808 x	$\hat{y}$ =1.3157+0.0197 x
Feb	0	14.70	0.76	75.87	0.53
	150	30.03	1.28	93.14	4.92
	300	32.71	1.48	131.82	5.92
	R^2	0.86	0.94	0.95	0.88
	Equation	$\hat{y}$ =16.81+0.06 x	$\hat{y}$ =0.8095+0.0024 x	$\hat{y}$ =72.372-0.1865 x	$\hat{y}$ =1.0921+0.018 x
Mar	0	15.80	0.62	70.92	0.42
	150	25.48	1.07	89.65	4.11
	300	30.59	1.37	120.67	5.55
	R^2	0.97	0.98	0.98	0.94
	Equation	$\hat{y}$ =16.562+0.0493 x	$\hat{y}$ =0.6443+0.002 x	$\hat{y}$ =68.871+0.1658 x	$\hat{y}$ =0.7944+0.0171 x
Apr	0	14.86	0.52	64.31	0.33
	150	25.01	0.96	82.40	2.95
	300	31.27	1.18	113.99	3.22
	R^2	0.98	0.96	0.97	0.81
	Equation	$\hat{y}$ =15.508+0.0547 x	$\hat{y}$ =0.5567+0.0022 x	$\hat{y}$ =62.062+0.1656 x	$\hat{y}$ =0.7269+0.0096 x
May	0	18.83	0.42	54.01	0.30
	150	28.00	0.85	69.54	2.04
	300	31.20	0.96	90.04	2.33
	R^2	0.93	0.89	0.99	0.84
	Equation	$\hat{y}$ =19.825+0.0412 x	$\hat{y}$ =0.4695+0.0018 x	$\hat{y}$ =53.181+0.1201 x	$\hat{y}$ =0.5423+0.0068 x
Jun	0	14.68	0.28	52.25	0.27
	150	24.90	0.68	64.81	2.22
	300	27.09	0.77	85.56	2.57
	R^2	0.88	0.88	0.97	0.86
	Equation	$\hat{y}$ =16.018+0.0414 x	$\hat{y}$ =0.329+0.0017 x	$\hat{y}$ =50.889+0.111 x	$\hat{y}$ = 0.5333+0.0077 x

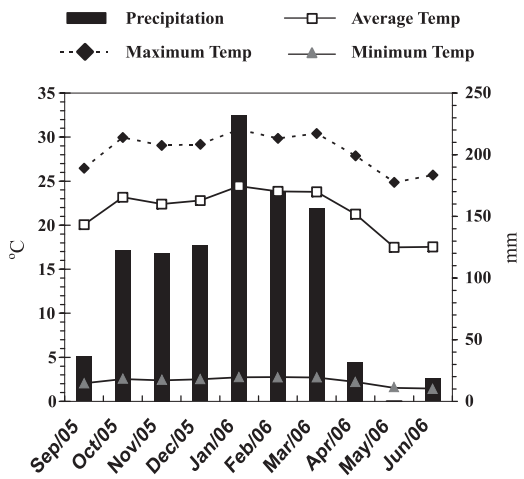

Figure 1. Climatic data of the experimental area of "Areão" Farm, in Piracicaba, State of São Paulo.

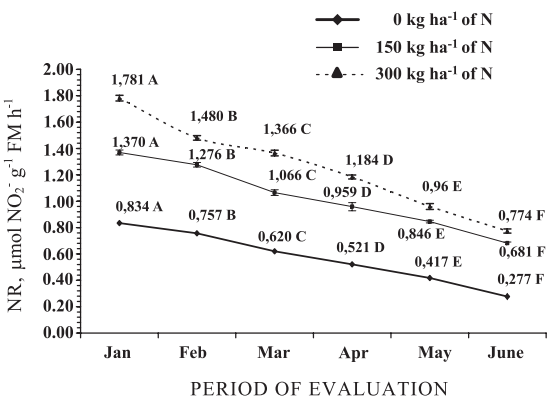

Figure 2. Nitrate reductase activity during fruit development, considering different N-rates (control, 150 and 300 kg ha^{-1}). Means followed by the same letter do not differ by the Tukey test (0.05).

Table 2. Correlation between coffee yield, N-rates, leaf N and Ni concentrations, nitrate reductase (NR) and glutamine synthetase (GS) activities, and leaf total soluble protein concentration (TSP), during the period of evaluation (January to June)

	Rate	N	Ni	NR	GS	TSP	Yield
				January			
Rate	1	0.83**	-0.38 ns	0.99**	0.91**	0.86**	0.96**
N		1	-0.65*	0.94**	0.78*	0.99**	0.77*
Ni			1	-0.43 ns	-0.18 ns	-0.63 ns	-0.17 ns
NR				1	0.95**	0.95**	0.94**
GS					1	0.81**	0.99**
TSP						1	0.80**
Yield							1
				February			
Rate	1	0.86**	-0.44 ns	0.94**	0.95**	0.88**	0.97**
N		1	-0.33 ns	0.99**	0.83**	0.99**	0.79*
Ni			1	-0.38 ns	-0.45 ns	-0.36 ns	-0.48 ns
NR				1	0.89**	0.99**	0.87**
GS					1	0.85**	0.99**
TSP						1	0.82**
Yield							1
				March			
Rate	1	0.97**	0.36 ns	0.98**	0.98**	0.97**	0.97**
N		1	0.20 ns	0.98**	0.93**	0.98**	0.89**
Ni			1	0.28*	0.46 ns	0.21 ns	-0.50 ns
NR				1	0.96**	0.99**	0.92**
GS					1	0.92**	0.99**
TSP						1	0.87**
Yield							1
				April			
Rate	1	0.98**	-0.55 *	0.96**	0.97**	0.81**	0.97**
N		1	-0.51*	0.99**	0.95**	0.89**	0.92**
Ni			1	-0.47 ns	-0.65*	-0.17 ns	-0.67*
NR				1	0.94**	0.92**	0.90**
GS					1	0.74*	0.99**
TSP						1	0.66*
Yield							1
				May			
Rate	1	0.93**	-0.63*	0.89**	0.99**	0.84*	0.96**
N		1	-0.58*	0.98**	0.90**	0.93**	0.81**
Ni			1	-0.65*	-0.62*	-0.55 ns	-0.59*
NR				1	0.92**	0.94**	0.83**
GS					1	0.74*	0.98**
TSP						1	0.61 ns
Yield							1
				June			
Rate	1	0.88**	0.74*	0.88**	0.97**	0.86**	0.96**
N		1	0.81**	0.99**	0.94**	0.98**	0.88**
Ni			1	0.84**	0.68*	0.85**	0.60*
NR				1	0.89**	0.99**	0.83**
GS					1	0.86**	0.99**
TSP						1	0.79**
Yield							1

** and *: significant at 0.01 and 0.05 levels; ns: non-significant.

production (Malavolta et al., 1997). The N leaf concentrations on the fruit-bearing branches decreased from the moment fruits started to expand, in January (Figure 3), and then, N was accumulated in the green beans, coffee-berry and dry fruits (Figure 4). These results agree with those reported by Valarini & Bataglia (2005) and evidenced the high N mobility and fast redistribution within the plant.

The N rates did not affect the leaf Ni concentrations, which only increased after fruit ripening in May, when the leaf Ni concentration increased to approximately 11 mg g^{-1} (Figure 3). This value is above the range (0.5 to 5 mg kg^{-1}) reported by Malavolta et al. (2006b). But it is worth mentioning that Ni concentrations of about 35 mg kg^{-1} are considered toxic to coffee plants (Pavan & Binghan, 1982).

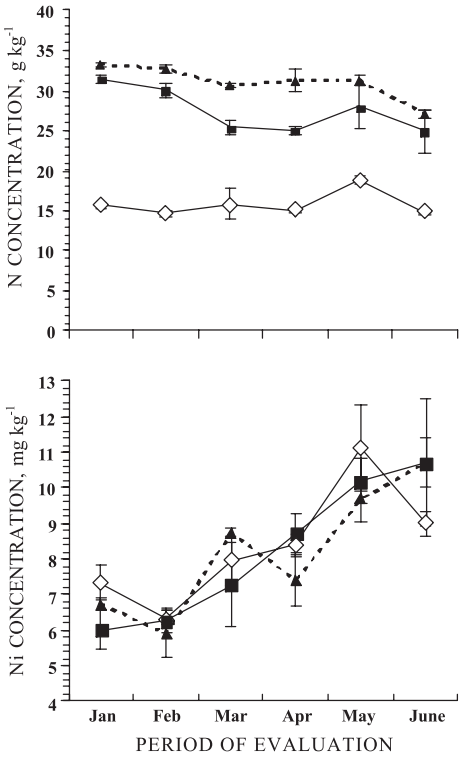

Figure 3. Leaf N and Ni concentrations during the coffee fruit development, considering different N-rates (◇ control, ■ 150 kg ha^{-1} and ▲ 300 kg ha^{-1}). Vertical bars indicate mean standard deviations.

The observed increase in the leaf and bean Ni concentrations in this study can be explained by the element redistribution to the fruits (strong sink). Riesen & Feller (2005) and Page et al. (2006) reported a higher relative mobility of ^{63}Ni than of ^{57}Co, ^{65}Zn, ^{54}Mn and ^{109}Cd in the phloem of wheat and white lupin. Malavolta et al. (2006a) evaluated nutrient partitioning in citrus branches, leaves and flowers and observed a higher Ni proportion in the flowers, from where Ni is possibly transferred to the developing fruits. Kabata-Pendias & Pendias (2001) suggested that Ni transport and storage might be metabolically controlled, and probably accumulated in leaves and seeds, due to the high mobility within the plant. During senescence, about 70 % of leaf Ni is redistributed to the seeds (Cataldo et al., 1978; Malavolta, 2006). This fact would explain the Ni accumulation in fruits from the green to dry stages (Figure 4), because once Ni is transported to reproductive organs through the phloem, it can be accumulated in seeds and beans (Welch, 1999, Riesen & Feller, 2005; Page et al., 2006).

In N-deficient plants, no variations in fruit Ni concentrations were observed during the green-fruit, coffee-berry and dry-fruit stages. Nevertheless, in well N-nourished plants, higher Ni concentrations were found in coffee-berry and dry-fruits compared to the green-fruits. This reinforces the probable Ni role in plant N-metabolism and the high Ni mobility in the phloem of N-satiated plants.

Leaf glutamine synthetase (GS) activity (Figure 5) and total soluble protein concentration (Figure 6) decreased in all treatments during fruit development and may be explained by the simultaneous start of the leaf senescence process in this period. In January, higher GS activity was observed during fruit expansion/ bean filling. The GS values were lowest in April, during the coffee-berry phase, and in July during the dry-fruit phase.

GS enzyme is responsible for the production of glutamine in senescent tissues and the results found here are corroborated by findings reported in previous studies (Kamachi et al., 1991; Souza et al., 1998; Morot-Gaudry et al., 2001), that is, leaf GS activity is lower during fruit development, due to the leaf senescence and the high carbohydrate, sugar and protein demand for the fruit endosperm formation (sink).

Similarly to the NR and GS activity, the observed decrease in the leaf total soluble protein content (Figure 6) can be partly explained by the leaf

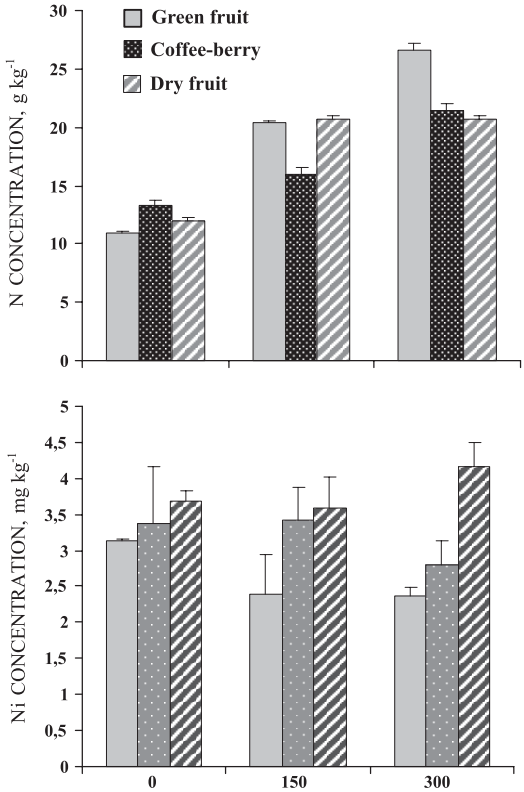

Figure 4. Fruit N and Ni concentrations in different phenological stages, considering different N-rates (control, 150 and 300 kg ha^{-1}). Vertical bars indicate mean standard deviations.

senescence during fruit development, because intense amino acid mobilization also occurs in this period, as a result of protein degradation. These amino acids are reused in other plant organs (amino acid anabolism and ureide catabolism) e.g., in grain endosperm formation (Rochat, 2001).

Correlations were found between coffee yield (60 kg ha^{-1} of coffee bags) and NR activity, GS activity, and leaf total soluble protein content, as well as between coffee yield and leaf N concentration (Table 2 and Figure 7) at all evaluation periods. On the other hand, March was considered the most appropriate period to evaluate enzyme activity for N-nutritional diagnosis and to predict coffee productivity, when green-beans are expanding/filling, because higher values of positive and significant correlation coefficients were found for the cited relationships in this month.

Leaf NR and GS activities, leaf N and leaf total soluble protein concentrations related to higher coffee

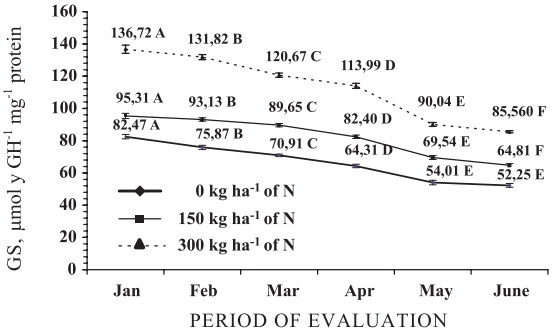

Figure 5. Glutamine synthetase activity during the fruit development stadium, considering different N-rates (control, 150 and 300 kg ha^{-1}). Means followed by the same letter do not differ by the Tukey test (0.05).

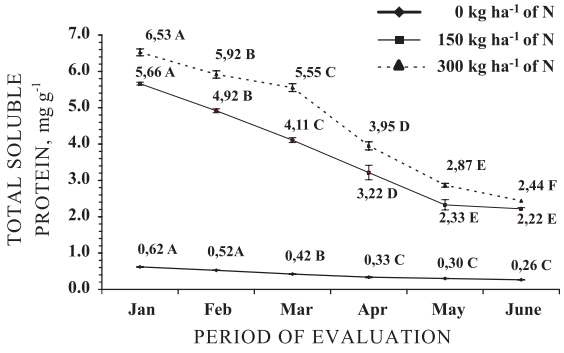

Figure 6. Leaf total soluble protein concentration during the fruit development stadium, considering different N-rates (control, 150 and 300 kg ha^{-1}). Means followed by the same letter do not differ by the Tukey test (0.05).

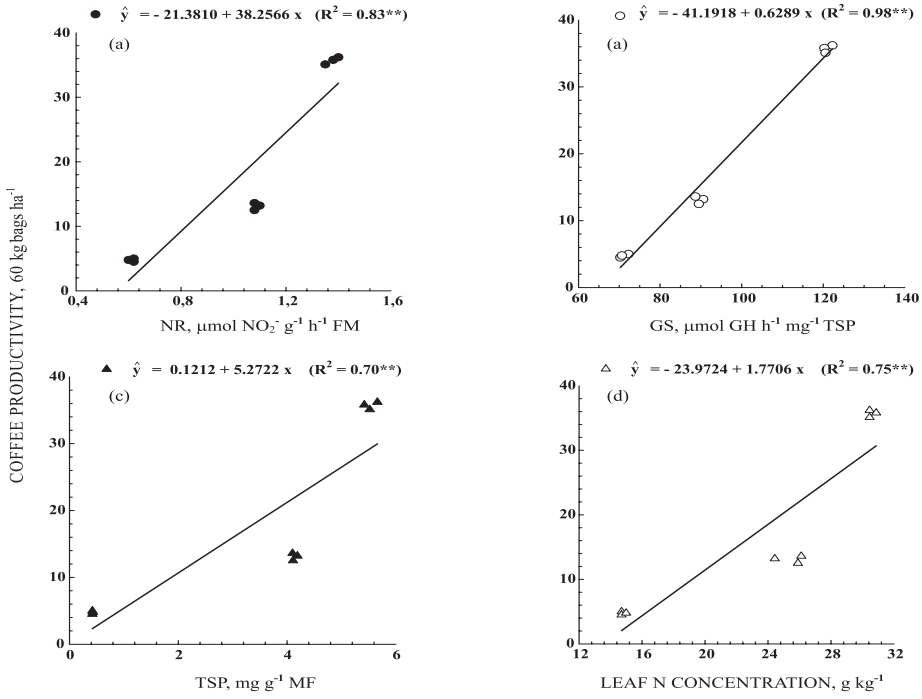

Figure 7. Relationships between: coffee yield and nitrate reductase activity (NR) (a); coffee yield and glutamine synthetase (GS) activity (b); coffee yield and leaf total soluble protein (TSP) (c), coffee yield and leaf N concentration (d), considering the period of March, the month of highest significant relationships.

yields (2160 kg ha^{-1}) were, respectively: 1.38 µmol NO_2^- g^{-1} MF h^{-1}; 121.0 µmol y GH h^{-1} mg^{-1}; 5.5 mg g^{-1} FM; and 31 g kg^{-1} (Figure 7). It was therefore concluded that the NR and GS enzyme activities can represent an additional tool for coffee plant N diagnosis and the early prediction of coffee productivity.

CONCLUSIONS

1. Leaf nitrate reductase and glutamine synthetase activities, leaf N and total soluble protein concentrations tend to decrease as fruit ripening increases and these variables are positively and significantly correlated with coffee yield.

2. Leaf enzyme activities and total soluble protein concentrations, as well as leaf concentrations of 31 mg kg^{-1} N associated to a Ni concentration in green fruit of 2.4 mg kg^{-1} and of 6.0 mg kg^{-1} in leaves, determined during the fruit expansion/ filling phase, are effective for the N nutritional diagnosis of coffee plants and for N fertilization monitoring.

3. Leaf and fruit Ni concentrations increase during the fruit development phase and highest Ni values were found in dry fruits.

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