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Production of basic potato seed minitubers in substrate and different nitrogen rates

Production of basic potato seed minitubers in substrate and different nitrogen rates[1]

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ABSTRACT

The optimal dose of nitrogen (N) in potato crop depends on the production system. The objective of this study was to determine the optimal dose of N for the production of basic potato seed minitubers and evaluate the effect of N rates on physiological and nitrogen indices in the youngest fully developed leaf (fourth leaf) and in the oldest leaf of the plants at 60 days after planting. The experiment was conducted in a greenhouse at the Departamento de Fitotecnia da Universidade Federal de Viçosa. The treatments consisted of five N rates (0, 45, 90, 180 and 360 mg dm^{-3}), with 10% of each dose applied at planting and the remainder through irrigation water, daily, for 30 days. The nitrogen rates positively influenced the physiological indices (length, width, leaf area, number of leaves, fresh mass and dry mass) and nitrogen (level and content of N and N-NO^3 in the dry mass and SPAD) both in the fourth leaf and in the oldest leaf. Likewise, the N rates positively influenced the number and mass of harvested tubers. The largest number (5.44 tubers/plant) and the maximum mass of tubers (243.5 g/plant) were obtained with 360.0 and 332.9 mg N dm^{-3}, respectively. Therefore, the mass and number of tubers were not optimized by the same N rate. The critical SPAD index was 38.8 in the fourth leaf, which was more sensitive to the effect of N rates than the oldest leaf.

Key words: *Solanum tuberosum* L., propagation, diagnosis, chlorophyll meter, nutrition, vase.

RESUMO

Produção de minitubérculos de batata-semente básica em substrato em razão de doses de nitrogênio

A dose ótima de nitrogênio (N) na cultura da batata depende do sistema de produção. O objetivo deste trabalho foi determinar a dose ótima de N para a produção de minitubérculos de batata-semente básica e avaliar o efeito de doses de N sobre índices nitrogenados e fisiológicos determinados na folha mais jovem completamente desenvolvida (quarta folha) e na folha mais velha da planta, aos 60 dias após o plantio. O experimento foi realizado em ambiente protegido, no Departamento de Fitotecnia da Universidade Federal de Viçosa. Os tratamentos foram constituídos por cinco doses de N (0, 45, 90, 180 e 360 mg dm^{-3}), sendo 10% de cada dose aplicada em pré-plantio e o restante via água de irrigação, diariamente, por 30 dias. As doses de N influenciaram positivamente os índices fisiológicos (comprimento, largura, área foliar, número de folíolos, massas de matérias fresca e seca) e nitrogenados (teor e conteúdo de N e N-NO$_3$ na matéria seca e índice SPAD) tanto na quarta folha quanto na folha velha. Da mesma forma, doses de N influenciaram positiva-

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mente o número e a massa de tubérculos colhidos. O maior número (5,44 tubérculos/planta) e a máxima massa de tubérculos (243,5 g/planta) foram obtidos com 360,0 e 332,9 mg dm^{-3} de N, respectivamente. Portanto, número e massa de tubérculos não foram otimizados pela mesma dose de N. O índice crítico SPAD na quarta folha foi 38,8, sendo essa mais sensível ao efeito de doses de N do que a folha mais velha.

Palavras-chave: *Solanum tuberosum* L., propagação, diagnóstico, clorofilômetro, nutrição, vaso.

INTRODUCTION

The basic potato seed used in Brazil is either imported from other countries, especially the Netherlands, or produced inside the country by tissue culture. The production by tissue culture demands a specialized laboratory, which is not accessible to all producers.

In the production cost of potato crops, the seeds are the most costly production factor consisting of 30% of the final cost. The high cost and low availability of quality seeds in the country lead producers to use inadequate material for propagation without the desired productivity.

Potatoes are mainly propagated by vegetative methods from seed tubers, which ensure the obtaining of material identical to the original. However, when infected, seed tubers favor the spread of diseases, particularly viruses, leading to early crop degeneration and directly influencing tuber quality and production.

An alternative method for potato propagation is the use of detached sprouts from seed potato tubers. The sprout technique was pioneerly proposed and used in Brazil by Souza-Dias (2001, 2004, 2006a; 2006b). This technique allow reduction in the importation and the production of new basic minitubers, enhancing up to 200% the productivity rate for each unit of imported potato seed (Souza-Dias, 2006a). Therefore, sprout planting can be an important advance in the production process of basic seed potatoes.

The cultivation of basic seed potatoes in substrate is widespread among producers (Grigoriadou & Leventakis, 1999; Lommen, 1999) because it results in higher nutrient utilization and improved product quality. The production of seed potatoes in substrate involves the use of different propagation material, including the sprout. Greenhouse sprout planting in substrate is practical and of low cost. Each sprout cutting originates minitubers that can be directly planted in the field (Dias, 2002).

Some authors found that nitrogen (N) influences both the mass and the number of tubers per plant (Errebhi et al., 1998; Meyer & Marcum, 1998). Nitrogen also positively affects the size of the aerial part of the plant, which will influence the intercepted radiation and dry matter accumulation. Nitrogen also has a negative effect by providing dry matter accumulation in other parts of the plant, other than the tuber (Alonso, 1996). The excess of N can delay tuber production extending the cycle and reducing productivity (Gil *et al.* 2002; Busato, 2007, Silva, 2007). The growth and development of stems, leaves and leaf area influence plant production of tubers (Oliveira, 2000).

The use of N criteria or indices is important in monitoring the plant nitrogen status and properly manage the potato crop fertilization program. The chemical analysis of dry leaf matter to obtain N and nitrate levels is common in this monitoring, which are expensive and time demanding procedures that need to be performed by qualified technicians. However, they can be replaced by rapid tests that allow sensing the nutritional status of the plant in real time (Fontes, 2011).

The SPAD index, measured by the chlorophyll meter can indirectly indicate the nitrogen status of the plant (Guimarães *et al.*, 1999; Sources, 2001). It is a rapid test accomplished in the field and allows sensing the nutritional status of plants in real time. Therefore, it can become a viable alternative for the production system of seed potatoes in vases containing substrate. Another hypothesis is that the morphological (number of leaves, plant height and length of the fourth leaf) or physiological plant characteristics may be used as an index of plant N status (Fontes, 2011).

Although there are a number of studies establishing indices to assess the nitrogen status of potato plants (Guimarães *et al.*, 2008; Junior Sampaio et al., 2008, Silva *et al.*, 2009, 2011; Braun *et al.*, 2010; Busato *et al.*, 2010; Coelho et al., 2010; Fontes *et al.*, 2010; Moreira *et al.*, 2011a) and the nitrogen management in minitubers production in vases and other systems containing substrate (Junior Sampaio, 2005 ; Moreira, 2008), there is still a lack of research to determine the critical value of these indices in the production system of basic seed potatoes multiplication by sprouts in substrate.

This study aimed to determine the optimal dose of nitrogen (N) in the production of basic seed potatoes minitubers and to evaluate the effect of nitrogen (N) rates on nitrogen and physiological indices both in the youngest fully developed leaf (fourth leaf) and in the oldest leaf at 60 days after planting.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Horticultural Department of the Federal University of Viçosa, in Viçosa, Minas Gerais. The chapel type greenhouse had the following dimensions: width of 9 m, length of 40 m, side posts height of 3 m, ridge height 3.8 m, with the top, front and side closed with a crystal clear polyethylene film, 0.1 mm thick. The treatments consisted of 5 N rates (0, 45, 90, 180 and 360 mg dm^3), with 10% of each dose applied at planting and the remainder through daily irrigation water, for 30 days.

Each dose was divided into 30 portions and diluted in 200 mL of water, applied to each vase daily, starting 20 days after planting (DAP). Ammonium nitrate was the nitrogen source. The experiment was a randomized block design with six replications. Each plot consisted of two vases, with one sprout planted, cultivar Asterix, detached from a basic potato seed. After the detachment, the sprout was disinfested following the methodology described by Bryan *et al.* (1981).

The substrate was uniformly fertilized with macro and micronutrients and quantity was expressed in mg dm^{-3}: 3,400 of Simple Superphosphate, 3,600 Magnesium Sulphate, 1,320 Potassium Chloride, 2.5 Boric Acid, 2.5 Zinc Sulphate, 2.5 copper sulphate, 2.5 ferrous sulphate, 2.5 manganese sulphate and 0.25 of ammonium molybdate, irrigated when needed.

The evaluation began at 26 days after planting and then every seven days until final harvest. The following determinations were made: leaf green color intensity, length, width and number of leaflets of the fourth leaf (FL) and old-leaf (OL), stem length and number of leaves of the potato plant. Leaf green colour intensity was determined in the terminal leaflet of the FL and OL, between 8 and 11 a.m. with the SPAD-502 portable chlorophyll meter (Soil-Plant Analysis Development 502).

Ten days after the final application of N, at 60 days after planting (DAP), one plant was collected from each treatment. The SPAD index was determined in the terminal leaflet of the FL and OL. Following, the plant was harvested and separated into fourth leaf, old leaf, leaves, stems and tubers. The number of leaflets was counted and length, width and area of FL and OL were determined. Leaf area of FL and OL was obtained using the LICOR 3100 meter.

The masses of FL and OL were weighed (fresh matter), placed in paper bags and dried to a constant weight in a forced air oven set at 70 ℃, and dry matter was determined.

After drying, the material was ground in a Wiley mill with a 20-mesh sieve to determine nitrogen concentration after sulphuric digestion, using the Nessler's reagent (Jackson, 1958). In another subsample, $N-NO_3$ was extracted with demineralized water in a water bath at 45 °C for 1 h, determining the concentration of $N-NO_3$ by colorimetry, using a spectrophotometer at 410 nm (Cataldo *et al.*, 1975). At 60 days after planting, the agronomic characteristics of the plant were also determined: leaf area, stem length, number of leaves and tubers, fresh matter mass and dry matter mass of leaves, stems and tubers.

The final harvest occurred at 89 days after planting. The plants were harvested, separated into fourth leaf, old leaf, leaves, stems, roots and tubers and weighed to determine fresh matter mass, then, after drying the dry matter mass was determined. The tubers were counted and classified according to the largest diameter: type V (16 to 23 mm); type VI (13 to 16mm); type VII (10 to 13 mm); and type VIII (<10 mm), according to IMA (2003).

Data were submitted to analysis of variance and regression, using the software SAEG (SAEG, 1993) to fit the model that best describes the relationship between dependent and independent variables.

RESULTS AND DISCUSSION

Throughout the cycle and during the nine assessment periods, the number of leaflets in OL was the only non-destructive index that was not affected by N and, therefore, cannot be considered a suitable index to evaluate the variability of N supply to plants. Indices that were most early (47 DAP) influenced by N rates were: SPAD index, fourth leaf width, stem length and number of leaves (Tables 1, 2 and 3).

The increase in the SPAD index suggests an increase in the green color intensity of the plant. According to Fontes (2001), this index indirectly measures SPAD chlorophyll content, indicating the N status of the plant. Chlorophyll is the pigment involved in photosynthesis, and positive correlations between the photosynthetic rate and N content of the plant have been reported by several authors (Stol & Keulen, 1991; Makino *et al.*, 1994). Vos & Bom (1993) also found a positive correlation between chlorophyll concentration in the plant and N addition in the potato crop, indicating that the chlorophyll content in the plant is related to the nitrogen nutritional status (Minotti *et al.*, 1994).

The N dose that resulted in the highest gain of tuber fresh mass (332.9 mg dm^{-3}) promoted an increase in the SPAD index values in FL and OL of up to 47 DAP, reaching values of 46.9 (FL) and 47.2 (OL). From this period on, the

values decreased to 18.5 (FL) and 5.0 (OL) (Table 1). It was observed that after the application of N, which occurred at 50 DAP, the green color of the leaf was reduced due to either initial senescence or the absence of N to stimulate leaf development. The plant also reached its peak nitrogen accumulation. Similar results were found by several authors (Minotti *et al.*, 1994; Rodrigues, 2004; Junior Sampaio, 2005; Moreira *et al.*, 2011a).

Table 1. Adjusted equations for SPAD index (S) measured in the fourth leaf (FL) and old-leaf (OL) of potato plants at 26, 33, 40, 47, 54, 68, 75, 82 and 89 days after planting, as a function of nitrogen (N), and coefficients of determination

Characteristics	Adjusted equations	R^2/r^2
Fourth leaf		
SFL26	$\hat{Y} = 34.4$	—
SFL33	$\hat{Y} = 35.0$	—
SFL40	$\hat{Y} = 35.4$	—
SFL47	$\hat{Y} = 30.1318 + 0.05988705**N - 0.00009963**N^2$	0.95
SFL54	$\hat{Y} = 28.3825 + 0.02072222**N$	0.85
SFL68	$\hat{Y} = 20.7975 + 0.03838889**N$	0.92
SFL75	$\hat{Y} = 13.5233 + 0.05281**N$	0.95
SFL82	$\hat{Y} = 18.2$	—
SFL89	$\hat{Y} = 4.3829 + 0.042398148**N$	0.74
Old leaf		
SOL26	$\hat{Y} = 28.1$	—
SOL33	$\hat{Y} = 39.3$	—
SOL40	$\hat{Y} = 38.5$	—
SOL47	$\hat{Y} = 34.4121 + 0.04800524**N - 0.00008703*N^2$	0.80
SOL54	$\hat{Y} = 30.4517 + 0.02077778**N$	0.69
SOL68	$\hat{Y} = 16.0208 + 0.04416667**N$	0.84
SOL75	$\hat{Y} = 11.0688 + 0.04119444**N$	0.83
SOL82	$\hat{Y} = 8.4$	—
SOL89	$\hat{Y} = 5.0$	—

* and ** - significant at 1 and 5% probability by "t" test, respectively.

Table 2. Adjusted equations for width (W) measured in the fourth leaf (FL) and number of leaflets (NL) of the fourth leaf of potato plants at 26, 33, 40, 47, 54, 68, 75, 82 and 89 days after planting, as a function of nitrogen (N), and coefficients of determination

Characteristics	Adjusted equations	R^2
Fourth leaf width		
FLW26 (cm)	$\hat{Y} = 3.4$	—
FLW33 (cm)	$\hat{Y} = 7.0$	—
FLW40 (cm)	$\hat{Y} = 9.4$	—
FLW47 (cm)	$\hat{Y} = 8.929 + 0.030532028**N – 0.000064608*N^2$	0.61
FLW54 (cm)	$\hat{Y} = 11.4$	—
FLW68 (cm)	$\hat{Y} = 9.8577 + 0.036812058**N – 0.000081549**N^2$	0.64
FLW75 (cm)	$\hat{Y} = 11.8$	—
FLW82 (cm)	$\hat{Y} = 12.1$	—
FLW89 (cm)	$\hat{Y} = 8.1$	—
Fourth leaf leaflet number		
FLNLF26	$\hat{Y} = 2.0$	—
FLNLF33	$\hat{Y} = 6.0$	—
FLNLF40	$\hat{Y} = 7.0$	—
FLNLF47	$\hat{Y} = 8.0$	—
FLNLF54	$\hat{Y} = 8.0$	—
FLNLF68	$\hat{Y} = 6,3359 + 0,021181877*N – 0,000038599***N^2$	0,60
FLNLF75	$\hat{Y} = 5,3872 + 0,026253102**N – 0,000051262*N^2$	0,60
FLNLF82	$\hat{Y} = 7,0$	—
FLNLF89	$\hat{Y} = 5,0$	—

, * and * - significant at 1, 5 and 10% probability by "t" test, respectively.

Fourth leaf length (FLL) and old leaf length (OLL) was influenced by N rates at 75 DAP for the FL and at 82 DAP for OL, represented by the following equations: $FLL = 14.064 + 0.0850235^{**}N - 0.0001823^{**}N^2; R^2 = 0.78$ and $OLL = 9.080 + 0.089010^{**}N - 0.000232^{**}N^2; R^2 = 0.73$. Nitrogen levels affected the width of the FL at 47 and 68 DAP (Table 2) and OL only at 82 DAP, represented by the equation: $LFV = 3.931 + 0.067055^{**}N - 0.000180^{**}N^2; R^2 = 0.71$.

Stem length increased during the cycle, reaching 46.7 cm with a dose of 332.9 mg dm^{-3} of N (Table 3). Moreira (2008) found that plant height also showed a linear response to the application of increasing doses of N throughout the crop cycle, but the plants were smaller. This difference in the results can be explained by the use of cultivar Agate.

The average leaf number of 21.5 was lower than that found by Junior Sampaio *et al.* (2008) using cultivar

Table 3. Adjusted equations for stem length (SL) and leaf number (LN) of potato plants at 26, 33, 40, 47, 54, 68, 75, 82 and 89 days after planting, as a function of nitrogen (N), and coefficients of determination

Characteristics	Adjusted equations	R^2/r^2
	Stem length	
SL26 (cm)	$\hat{Y} = 6.3$	—
SL33 (cm)	$\hat{Y} = 11.3$	—
SL40 (cm)	$\hat{Y} = 19.1$	—
SL47 (cm)	$\hat{Y} = 15.4844 + 0.13973082^{**}N - 0.00024337^{**}N^2$	0.87
SL54 (cm)	$\hat{Y} = 15.6169 + 0.16921138^{**}N - 0.00027055^{**}N^2$	0.94
SL68 (cm)	$\hat{Y} = 16.9318 + 0.17078908^{**}N - 0.00025907^{**}N^2$	0.95
SL75 (cm)	$\hat{Y} = 17.0813 + 0.17200551^{**}N - 0.00025666^{**}N^2$	0.95
SL82 (cm)	$\hat{Y} = 17.1123 + 0.17814254^{**}N - 0.00027117^{**}N^2$	0.95
SL89 (cm)	$\hat{Y} = 17.3790 + 0.18122259^{**}N - 0.00027966^{**}N^2$	0.95
	Leaf number	
LN26	$\hat{Y} = 5.8$	—
LN33	$\hat{Y} = 8.4$	—
LN40	$\hat{Y} = 11.2$	—
LN47	$\hat{Y} = 10.8000 + 0.01703704^{**}N$	0.61
LN54	$\hat{Y} = 10.7292 + 0.02916667^{**}N$	0.85
LN68	$\hat{Y} = 10.7500 + 0.03222222^{**}N$	0.90
LN75	$\hat{Y} = 9.8583 + 0.034629630^{**}N$	0.89
LN82	$\hat{Y} = 8.7000 + 0.036296296^{**}N$	0.89
LN89	$\hat{Y} = 6.9500 + 0.032222222^{**}N$	0.80

** - Significant at 1% probability by "t" test.

Table 4. Adjusted equations for area (LA), length (L), width (W), number of leaflets (LN), fourth leaf and old leaf fresh matter mass (FM) and dry matter mass (DM) at 60 days after planting, as a function of nitrogen (N), and coefficients of determination

Characteristics	Adjusted equations	R^2
	Fourth leaf	
LA (cm^2)	$\hat{Y} = 60.3164 + 0.77728404^{**}N - 0.00189585^{*}N^2$	0.86
L (cm)	$\hat{Y} = 14.9650 + 0.06477967^{**}N - 0.00015627^{**}N^2$	0.91
W (cm)	$\hat{Y} = 9.3635 + 0.051988075^{**}N - 0.000159099^{**}N^2$	0.88
LN	$\hat{Y} = 6.0078 + 0.025116481^{**}N - 0.000065545^{*}N^2$	0.64
MF (g)	$\hat{Y} = 2.8978 + 0.030868852^{**}N - 0.000095979^{**}N^2$	0.85
DM (g)	$\hat{Y} = 0.1730 + 0.0026092316^{**}N - 0.000007059^{**}N^2$	0.87
	Old leaf	
LA (cm^2)	$\hat{Y} = 9.1$	—
L (cm)	$\hat{Y} = 8.7$	—
W (cm)	$\hat{Y} = 3.8$	—
LN	$\hat{Y} = 2.0$	—
MF (g)	$\hat{Y} = 0.6$	—
DM (g)	$\hat{Y} = 0.04$	—

* and ** - significant at 1 and 5% probability by "t" test, respectively.

Monalisa, with 25.8 leaves/plant (Table 3). The number of leaves is influenced by the nitrogen availability in the plant. This means that within certain limits the greater the amount of nitrogen supplied to the plant, the greater the stimulus for leaf production (Vos & Putten, 1998).

At 60 days after planting (DAP), one plant was harvested to determine the effect of N levels on plant growth and development. At this time, the N had been fully applied, and the indexes in FL (SPAD, leaf area, length, width, number of leaves, fresh and dry matter, N-NO_3 and N-NH_4 content) were significantly influenced by the N dose. Inversely, the same indices in OL did not respond to the application of increasing doses of N.

The SPAD index in the fourth leaf, at 60 DAP, increased as the N rates increased (Figure 2). The estimated critical value for the SPAD index, at 60 DAP, at calculated dose of 332.9 mg dm^{-3} of N was of 37.3 for FL and 25.7 for OL.

The level and the content of N-NO_3 in the fourth leaf at 60 DAP, increased with increasing dose of N (Table 5). The content of N-NO_3 was not determined for the oldest leaf, for there was not enough material to perform the analysis. No significance was found for the N-NH_4 content in FL and OL, but the N content in the FL positively

responded to N rates (Table 5). This can be partially explained by the response to N rates in the production of leaf dry matter, since to obtain the content, it is necessary to multiply the N concentration by the dry matter production of organ studied. The results for the level and content of N-NO_3 and the N-NH_4 content of the fourth leaf at 60 DAP are in accordance with the results found by Moreira *et al*. (2011b) who observed that the N-NO_3 and N-NH_4 concentration in FL and OL varied with increasing N doses. Similar results were found by Porter & Sisson (1993) and Gil (2001).

Therefore, the use of these indices and the N-NH_4 content in OL at 60 DAP did not reflect the positive effect of N levels verified on tuber production at the final harvest. Busato (2007) and Moreira et al. (2011b) found higher SPAD index values at 60 DAP, which may be explained by the use of a different cultivar, the 'Asterix'. The SPAD indices reported by these authors were 43.02 and 38.2, respectively. In the work by Busato (2007) this value was 168.2 kg ha^{-1} for potato field production.

Due to N mobility in the phloem, when the N concentration in the OL is equal or higher than the concentration in FL, it is assumed that there is satisfactory N in the plant environment. In the present study, the

Table 5. Adjusted equations for the nitrate level (N-NO_3) and content (N-NO_3c) in the dry matter of the fourth leaf, organic nitrogen level (N-NH_4) and organic nitrogen content (N-NH_4c) in the fourth leaf (FL) and old leaf (OL) dry matter, at 60 days after planting, as a function of nitrogen (N), and coefficients of determination

Characteristics	Adjusted equations	R^2/r^2
N-NO_3·FL (g kg^{-1})	$\hat{Y} = -0.053 + 0.005181513**N$	0.76
c N-NO_3·FL (g/plant)	$\hat{Y} = -0.0016 + 0.00015525**N$	0.90
N-NH_4 FL (g kg^{-1})	$\hat{Y} = 38.4$	—
N-NH_4 OL (g kg^{-1})	$\hat{Y} = 17.3$	—
N-NH_4c FL (g/plant)	$\hat{Y} = 0.3740 + 0.0133329418**N - 0.0000278598**N^2$	0.64
N-NH_4c OL (g/plant)	$\hat{Y} = 0.06$	—

** - Significant at 1% probability by "t" test.

Table 6. Adjusted equations for leaf area (LA), stem length (SL), leaf number (LN), number of tubers (NT), fresh matter mass (FM) and dry matter mass (DM) of leaf (L), stem (S), root (R) and tuber (T), at 60 DAP, as a function of nitrogen (N), and coefficients of determination

| Characteristics	Adjusted equations	R^2/r^2
LA (cm^2)	$\hat{Y} = 322.5537 + 6.775538**N$	0.96
SL (cm)	$\hat{Y} = 15.9438 + 0.10654853**N$	0.91
LN (ud/pl)	$\hat{Y} = 9.5790 + 0.035791826**N$	0.87
TN (ud/pl)	$\hat{Y} = 2.5613 + 0.032247493**N - 0.000080901**N^2$	0.76
FML (g)	$\hat{Y} = 11.2995 + 0.42832774**N - 0.00062132***N^2$	0.93
DML (g)	$\hat{Y} = 1.0654 + 0.021866635**N$	0.98
FM S(g)	$\hat{Y} = 3.0718 + 0.061494253**N$	0.94
DMS (g)	$\hat{Y} = 0.1479 + 0.0037375479**N$	0.96
DMR (g)	$\hat{Y} = 0.2392 + 0.001740000**N$	0.92
FMT (g)	$\hat{Y} = 25.5679 + 0.72756851**N - 0.00139023*N^2$	0.87
DMT (g)	$\hat{Y} = 4.0776 + 0.115885126**N - 0.000267349*N^2$	0.81

, * and * - significant at 1, 5 and 10% probability by "t" test, respectively

opposite was observed, indicating the occurrence of N mobilization.

From these results it can be inferred that the N doses used at 60 DAP were adequate for plant development and, therefore, this period can be used for nutritional analysis in the estimation of tuber production.

The following characteristics from the fourth leaf at 60 DAP can be used as optimum growth standards: 108.97 cm^2 of area, 19.2 cm of length, 9.0 cm of width, 7.1 leaflets; 2.50 g of fresh matter mass, 0.26 g of dry matter mass, 1.8 g kg^{-1} of N-NO_3 in dry matter; 38.4 g kg^{-1} of N-

NH_4 in dry matter, and 37.3 of SPAD index. In the same period, the plant must present the following characteristics: 2578.1 cm^2 of leaf area, 51.4 cm of stem length, 21.5 leaves, 4.3 tubers/plant, 85.0 g of leaf fresh matter mass; 23.5 g of stem fresh matter mass; 113.70 g of tuber fresh matter mass, 5.2 g kg^{-1} of N-NO_3 in leaf dry matter, 26.1 g kg^{-1} of N-NO_3 in stem dry matter, 0.2 g kg^{-1} of N-NO_3 in root dry matter, 43.4 g kg^{-1} of N-NH_4 in dry leaf, 14.1 g kg^{-1} of N-NH_4 in stem dry matter, 11.4 g kg^{-1} of N-NH_4 in root dry matter and 6.2 g kg^{-1} of N-NH_4 in tuber dry matter.

$$Y = 52,039 + 1,15004595**N - 0,00172727**N^2$$
$$R^2 = 0,94$$
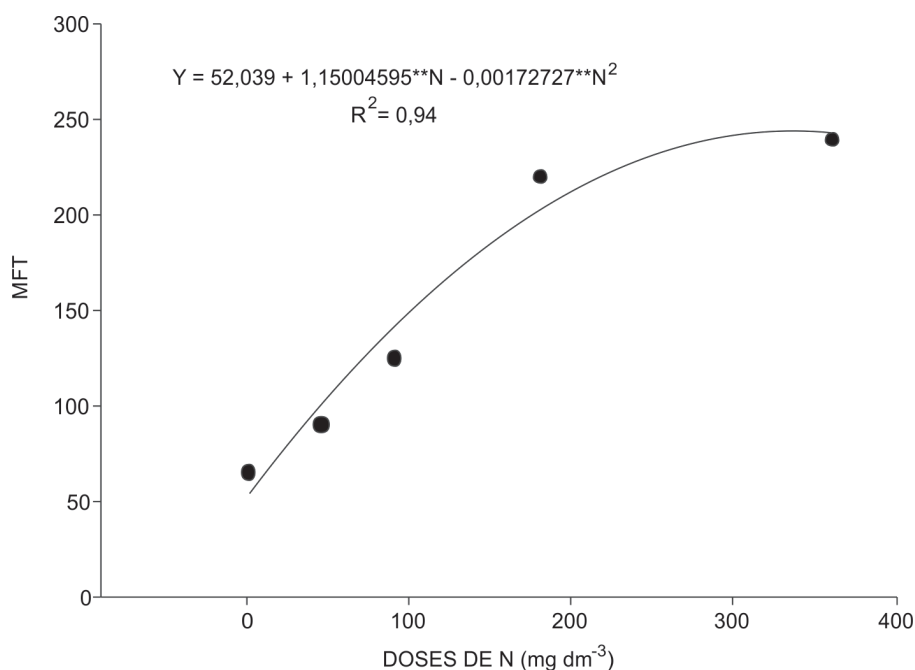
Figure 1. Fresh matter mass of tubers (FMM) at the final harvest (89 days after planting) as a function of nitrogen (N).

$$Y = 23,015 + 0,08101128**N - 0,0001145**N^2$$
$$R^2 = 0,85$$
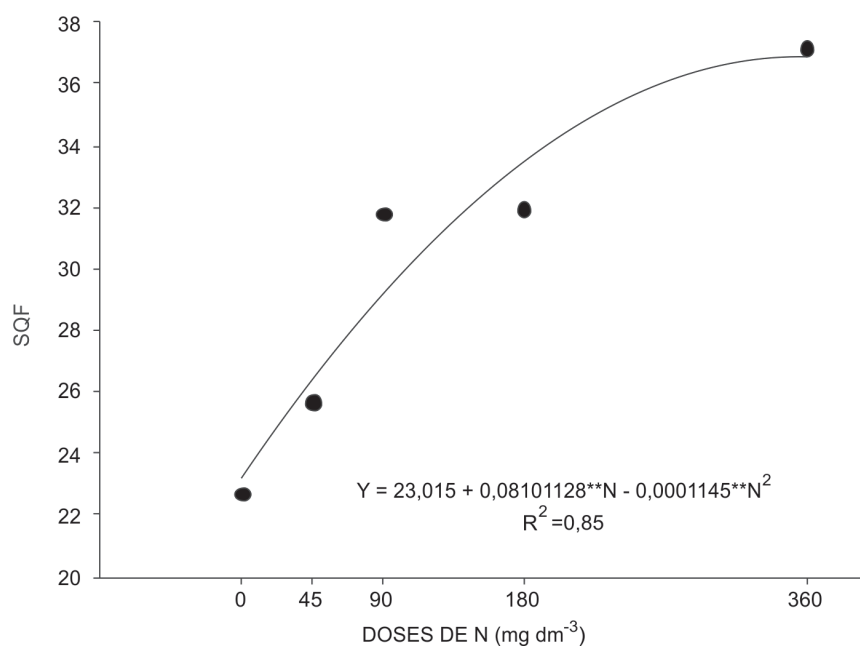
Figure 2. SPAD index in the fourth leaf (SFL), at 60 days after planting, as a function of nitrogen (N).

At the final harvest, at 89 DAP, tuber number and fresh and dry matter masses of leaves, stem, roots and tubers increased with increasing dose of N. Only the fresh matter mass of minitubers showed quadratic response to N application (Figure 1). The number of tubers and the dry matter masses of leaf, stem and tuber reached the highest values   at the dose of 360   mg dm^{-3}. Regarding tuber classification, 84% of the produced tubers were below type VI; and the highest percentage was found for type IV (31%). The results for the number of tubers and tuber dry matter mass are consistent with those observed by Gil (2001) and Moreira *et al.* (2011b) who found that increasing doses of N increased the number of tubers. In field, the N fertilization also increases tuber productivity, as found by Bélanger *et al.* (2000) and Rodrigues (2004).

The optimal N dose is usually established as a function of the production of fresh matter mass of tubers. In this case, the optimal dose of N or the maximum production dose was 332.9 mg dm^{-3}. In the present study, the linear model was adjusted and, therefore, we did not achieve the necessary N dose to obtain the maximum number of tubers. The highest N rate (360.0 mg dm^{-3}) was considered optimal.

CONCLUSIONS

Nitrogen positively influences the number and mass of tubers produced and the physiological indices of both the fourth leaf and old-leaf.

The number of tubers and tuber mass was not optimized by the same dose of N. The highest number of tubers per plant (5.44 pc) was obtained at 360 mg dm^{-3} N and maximum fresh matter mass of tubers per plant (243.5 g) was obtained at 332.9 mg dm^{-3} N.

The green color intensity (determined by the SPAD index) and physiological indices (leaf area, length, width, number of leaflets, fresh and dry matter mass and nitrogen content in dry matter), determined in the fourth leaf and old-leaf were positively influenced by N rates, and the fourth leaf was more sensitive to the effects of N rate than the old-leaf.

The critical SPAD index in the fourth leaf varied according to plant age and the highest value of 38.8 was obtained at 47 days after planting.

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