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Top-dressing nitrogen management decision in potato using the "UFV-80" color chart and SPAD readings

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The chlorophyll meter (SPAD-502) and "UFV-80" color chart can be used to diagnose the nitrogen (N) nutritional status of potato plants to determine the need for fertilizer-N topdressing. The present investigation was focused on how chlorophyll meter and "UFV-80" color chart help in determining optimal N management through real time N application for improving yield and profitable rate in potato crop. The experiments, in two planting seasons (dry and wet), were carried out a split-plot design in a randomized complete blocks with four repetitions. In the whole plot, five N fertilization rates (0, 50, 100, 200 and 300 kg ha^{-1} of N) were applied before planting. In the half of plot area, N fertilization rates (42, 152, 152, 152 and 152 kg ha^{-1} of N at dry season and 133, 93, 69, 58 and 205 kg ha^{-1} of N in wet season) were or did not applied in top-dressing, depending on the UFV-80 color chart reading and SPAD chlorophyll meter reading in the terminal leaflet of the fourth leaf, at 21 day after the plant emergency (DAE). At 77 and 70 DAE for the dry and wet seasons, respectively, the plants were harvested to determine the commercial tuber yield. The SPAD chlorophyll meter reading and "UFV-80" color chart can be used to determine N nutritional status in potato crops in both planting seasons. Both planting seasons, the commercial production was not influenced by the N fertilization rates in pre-planting and top-dressing.

Key words: *Solanum tuberosum* L. fertilization, diagnosis, profitable rate.

INTRODUCTION

Nitrogen (N) is one of the most important nutrient since it has a positive effect on chlorophyll concentration, photosynthetic rate, plant height and dry matter accumulation and higher potato tuber yields (Sinfield et al., 2010; Tremblay et al., 2011), and increase the chance of nitrate contamination on surface and ground water (Muñoz-Huerta et al., 2013). Due to the low availability of N in the superficial layer of the soil and high demand by N of potato plants makes the N one of the most limiting nutrients for plant growth and increase tuber yield.

Potato plants have a high demand on soil nutrients to obtain maximum tuber yield. Proper N management is one of the most important factors required to obtain high

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tuber yields. However, soil N supply is often limited. Then, optimum N fertilization rate applied in top-dressing to obtain high tuber yields is necessary. Until now, many researchers have worked with the SPAD chlorophyll meter, and proposed that SPAD readings could estimate leaf N concentration and potato yield prognosis. In this study, we hypothesized that SPAD chlorophyll meter reading and "UFV-80 color chart" in potato leaves can be used to determine N fertilization rates applied in top-dressing.

Various farmers increase the amount of N fertilizers in order to achieve better crop yield without any criteria, fact that can cause unnecessary expenditure on the part of farmers or deficiency this nutrient in potato plants. Then, according of this hypothesis, it is necessary to have methods for the evaluation of N nutritional status, preferably in real time (Fontes and Araujo, 2007; Busato et al., 2010).

Several diagnostic methods can be used to determine N nutritional status in crops, such as destructive and non-destructive techniques (Fontes, 2001; Muñoz-Huerta et al., 2013). N concentration and critical N dilution curve have been used as destructive techniques. Non-destructive techniques based on leaf reflectance have been proposed as robust alternative and simple methods for pigment quantification in leaves (Silva et al., 2009; Busato et al., 2010; Coelho et al., 2012). The most commonly used equipment for the evaluation of N nutritional status in real time are: the analysis of the N-NO_3 content in the sap of the petiole using microelectrode (Brink et al., 2002) and the indicator strip selected for nitrate (Parks et al., 2012). Additionally, SPAD chlorophyll meter readings have been shown to correlate well to laboratory-extracted chlorophyll (León et al., 2007), and to N status in culture of lettuce (Fontes et al., 1997), wheat (Tremblay et al., 2010), potato (Gil et al., 2002; Silva et al., 2009, Busato et al, 2010), tomato (Fontes and Araujo, 2006) and rice (Cabangon et al., 2011). Other equipment have been used as a tool to evaluate N nutritional status are Dualex® (Coelho et al., 2012) and the digital camera (Li et al., 2010; Wang et al., 2014). Furthermore, the chlorophyll meter conducts fast readings without destroying leaves; however, it has relatively elevated costs for the small farmer (Fontes and Araujo, 2007).

The SPAD chlorophyll meter readings can be an indication of the need for N application in top-dressing, since the critical level below critical is known in which the plant would deprive of this element, and other factor should also be considered to affect the SPAD chlorophyll meter readings, such as: edafoclimatic conditions, attack of plagues, diseases and weeds, growth status and plantation system (Arregui et al., 2000). Due to the high price of equipments for plant N status estimation limits its use by individual income-poor farmers (Yang et al., 2003). Another tool faster and non-destructive for plant N status estimation is a leaf color chart, in analogy to the one originally proposed for Asian rice farmers (Yang et al., 2003; Nachimuthu et al., 2007). The high price of SPAD limits its use by individual income-poor farmers. The color chart is easy, simple to use and has low cost; it is an alternative tool to the chlorophyll meter to establish N nutritional status at any given moment.

In a research conducted by Fontes and Silva (2006) culminated in the development of a color chart for potato crops, not seen before anywhere. This color chart was proposed to growth Monalisa and was called "UFV-80", a tribute to the 80^{th} anniversary of the Federal University of Viçosa.

Initial studies with Monalisa crop have shown that the color green number 4, determined in the terminal leaflet of the fourth leaf completely expanded from the apex at the moment of clumping, it is the "critical color" or when it is not necessary to apply the N in top-dressing (Fontes and Silva, 2006). This recommendation was not tested in the field. However, information on chlorophyll meter and leaf color chart to diagnose N nutritional status of potato plants and determine the need of N in topdressing are scarce. Therefore, the present investigation was focused on how chlorophyll meter and "UFV-80" color chart help in determining optimal N management through real time N application for improving yield and profitable rate in potato crop.

MATERIALS AND METHODS

Two experiments were conducted in the field. One experiment was performed in dry season (105 mm of rainfall), from June 26^{th} to September 26^{th} of 2008, cold weather, with supplementary irrigation; and another experiment was performed during hot and rainy season (1,075 mm of rainfall), from November 4^{th} of 2008 to February 4^{th} of 2009. The experiments were performed in the field of adjacent areas of the Federal University of Viçosa (UFV), in Viçosa, Minas Gerais State, Brazil, located at 693 m altitude, latitude 20°45′ S and longitude 42°51′ E. Fields were located on a Cambic Red-Yellow Argisol clay (Typic Hapludult) (Embrapa, 2006) which characteristics are 1.50 and 1.40 mg kg^{-1} of N-NO_3, 4.52 and 4.21 dag kg^{-1} of organic matter, 170 and 175.4 mg dm^{-3} of available P, 165 and 163 of available K mg dm^{-3}, 4.57 and 5.70 $cmol_c$ dm^{-3} of exchangeable Ca, 0.77 and 0.81 $cmol_c$ dm^{-3} of exchangeable Mg, 0.00 and 0.00 $cmol_c$ dm^{-3} of exchangeable Al, 280 g kg^{-1} and 280 g kg^{-1} of sand, 610 g kg^{-1} and 610 g kg^{-1} of clay and 110 g kg^{-1} and 110 g kg^{-1} of silt, at a layer of 0-20 cm depth, in dry and wet seasons, respectively.

The experiments were carried out in a split-plot design, in a randomized block design with four replications. In both planting seasons, five N fertilization rates (0, 50, 100, 200, and 300 kg ha^{-1} of N) were allocated in the plots, and the five N fertilization rates were applied in top-dressing in dry season (42, 152, 152, 152, and 152 kg ha^{-1} of N) and in wet season (133, 93, 69, 58, and 205 kg ha^{-1} of N). The potato plants were fertilized with ammonium sulfate (20% N). Experiments performed in both growing season, half of the plot was fertilized with N and the other half did not receive the N fertilizer. This was based on the study of true/false diagnosis describe by Beverly and Hallmark (1992).

The SPAD chlorophyll meter readings and "UFV-80 color chart" were carried out in the morning, between 8:00 and 11:00 a.m at 21 days after the completely emergency (DAE). On the same day, the N fertilization rates applied in top-dressing were performed. Twentytwo days after plant emergence (DAE), the plants were hilled by hoeing soil up around the plant stems. The N fertilization rates applied in top-dressing were calculated based on SPAD chlorophyll meter reading according to results obtained by Silva et al. (2009). The SPAD chlorophyll meter readings obtained in this experiments, color of terminal leaflet of the fourth leaf completely expanded from the apex, measured with the "UFV-80" color chart, and critical level of SPAD chlorophyll meter readings are shown in Table 1.

The decision to fertilize (yes or no) with N was based on the "UFV-80 color chart" (Fontes and Silva, 2006). According to authors, images of terminal leaflet of the fourth leaf completely expanded from the apex were captured using a digital still color camera (Nikon 135 TL) with high resolution. Soon after, the images were digitalized in scanner, model HP 1315, with resolution of 300 dpi. All images from the experiments were saved in JPEG and stored in CorelDRAW 12 software. With this program, images were attributed degree of colors from 1 to 5 to terminal leaflet of the fourth leaf completely expanded from the apex from each subplot, using the color scale, in which 1= yellowed-green, 2= light green, 3= opaque green, 4= dark green and 5= very dark green.

Immediately before N application in top-dressing, SPAD index was determined using chlorophyll meter SPAD-502 to calculate N fertilization rates in top-dressing at 21 DAE (Table 1). The SPAD chlorophyll meter readings were measured in the terminal leaflet of the fourth leaf completely expanded from the apex, in four plants of each subplot. Each leaf five readings were performed, from which the average was calculated.

The ammonium sulphate (20% of N) was the source of N applied in the experiments. The application of N fertilization rates in pre-planting was made in the planting grooves. The N fertilization rates applied in top-dressing was distributed next to the plants, throughout the rows, at 21 DAE, making the clumping afterwards.

Plots (5 m × 3 m) were consisted of four rows with 20 plants each, spacing of 0.75 m between rows and 0.25 m between plants in the row. The two outer rows and the outermost two plants at each end of the inner rows were used as borders, totalizing 32 useful plants per plot. Half of the subplot was fertilized with N and the other half did not receive N fertilizer.

The soil was prepared with a mouldboard plow and two passages of leveling blade. In both planting seasons, the quantity of fertilizers applied in the planting grooves were: 2000 kg ha^{-1} of simple super phosphate (18% of P_2O_5), 500 kg ha^{-1} of potassium chloride (60% of K_2O), 200 kg ha^{-1} of magnesium sulphate (9% of Mg), 10 kg ha^{-1} of borax (11% of B), 10 kg ha^{-1} of zinc sulphate (22% of Zn), 10 kg ha^{-1} copper sulphate (24% of Cu) and 250 g ha^{-1} sodium molybdate (39% of Mo). During the experimental period, pulverization was made for plague control and diseases, as needed.

The irrigation, when needed, was made using an aspersion system. The irrigation blade was established using the evaporation-transpiration estimate and the culture coefficient (Kc). The evaporation-transpiration estimate was calculated by the Penman-Monteith method. The soil was kept with moisture close to field capacity.

The precision in diagnosis was verified following the procedure cited by Fontes (2001) and adapted by Beverly and Hallmark (1992). Basically, the method was based on the comparison between the decision of fertilizing or not, and the plant's response to the application of N. In the proposition, the criterion of false (F) or true (V) incidence of diagnosis was used.

One week after complete drying of the areal part, which occurred at the 77th and 70th day after the emergency, for dry and wet seasons respectively, the plants in the useful area of each subplot were harvested to determine the production of commercial tubers.

Economic analysis of the production was also made; prices of tubers and ammonium sulphate were also checked in several establishments in the city of Viçosa, Minas Gerais State, in both planting seasons. The prices of potato paid to the farmer varied from R$ 0.80 and 1.00 kg^{-1}, and the N fertilizer had the purchase values from R$ 6.60 and 3.0 kg^{-1}, in dry and wet seasons, respectively. The production costs were based on Fontes (2005) that considered the value of R$ 10.000,00 ha^{-1} in both planting seasons.

The data was submitted to variance analysis (ANOVA) and regression. The regression models were chosen based on the biological occurrence of the response, the significance of the regression coefficients, using the "t"-test up to 10% of probability, and on the equation's coefficient of the determination value. Tukey's multiple range test (P<0.05) was used to separate the means when the ANOVA *F-test* indicated a significant effect on the treatment. The statistical software used to perform the statistical analysis was the System for Statistical and Genetic Analysis (SAEG version 9.1).

RESULTS AND DISCUSSION

There was a significant correlation between the SPAD chlorophyll meter readings performed in terminal leaflet of the fourth leaf completely expanded from the apex with "UFV-80 color chart" for both growing seasons. These results are presented in Figure 1. In this figure, it is shown a good and high correlation coefficient for both planting seasons (r=0.96 and r=0.97, for the dry and wet seasons, respectively) regardless of the N fertilization rates. Then, "UFV-80 color chart" could substitute for SPAD readings in estimating leaf N status in leaf potato plants. These results are in accordance with Cabangon et al. (2011), that reported r=0.84, in two years of crops, in rice crops, as well as the results presented by Yang et al. (2003). These authors reported that for diagnosing leaf N status in real-time N management, leaf color chart and SPAD readings can be used directly for determining the timing of N topdressing without adjusting for specific leaf weight (dry matter/leaf area), and if leaf color chart or SPAD readings is used to estimate N (per unit dry weight), specific leaf weight has to be considered.

Although in both planting season the critical color of the leaves obtained value 4, the values of SPAD chlorophyll meter readings were 45.3 and 38.9 in both dry and wet seasons, respectively (Table 1). In wet season, SPAD chlorophyll meter readings were 14.5% lower when compared to dry seasons. This probably occurred due to the higher volume of precipitated rain in the wet season, which may have led to losses of N by leaching. Cabangon et al. (2011) reported that for rice crop the critical values of 3 and 3.5 for leaf color chart corresponded SPAD chlorophyll meter readings of 35 and 38, respectively. Furthermore, the foliar color chart as well as the SPAD chlorophyll meter readings can diagnose N nutritional status of the potato in a practical and non-destructive manner.

The SPAD chlorophyll meter readings of 47.3 is above the SPAD chlorophyll meter readings of 44.9 (N fertilization rates of 144.3 kg ha^{-1} of N in pre-planting) found by Gil et al. (2002) during the winter in Viçosa, Minas Gerais State. Difference in N fertilization rates and the number of SPAD chlorophyll meter readings in the terminal leaflet of the fourth leaf completely expanded

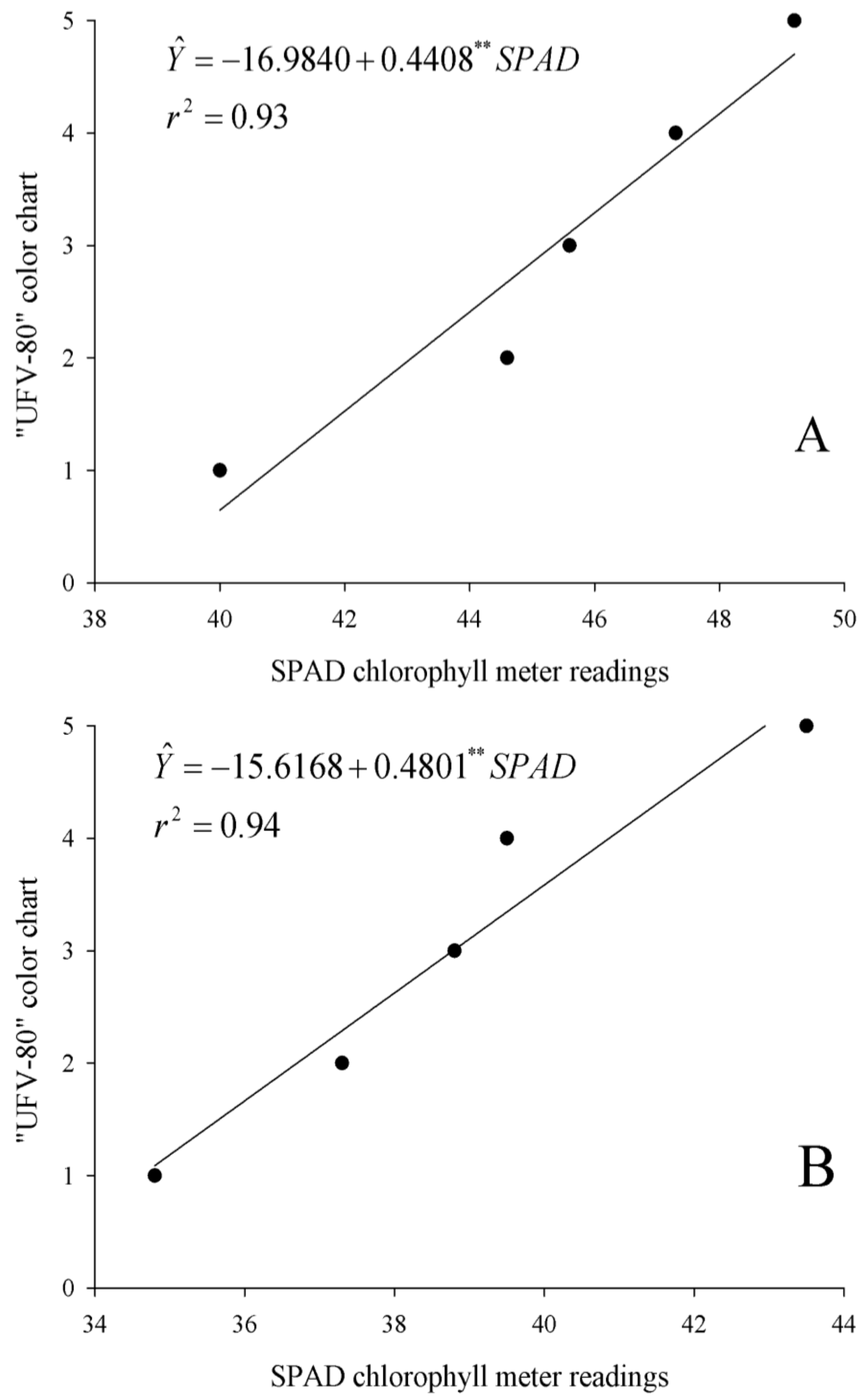

Figure 1. Relation between the "UFV-80" color chart and SPAD chlorophyll meter readings in the terminal leaflet of the fourth leaf completely expanded from the apex of the 'Monalisa' potato, at 21 DAE, in the dry (A) and wet (B) seasons. **: Statistically significant by the 't' test (P<0.01).

from the apex could have caused a variation in the data of the present work in comparison to the results from Gil (2001). However, Arregui et al. (2000) suggests the reading to be made in each leaflet of the potato without reaching the main nerve of the leaf, since it can cause variation in SPAD chlorophyll meter readings. In this study, SPAD chlorophyll meter readings was performed in leaflet of terminal leaflet of the fourth leaf completely expanded from the apex, in which each leaflet was measured four times, totaling 16 readings with SPAD

Table 1. Nitrogen (N) fertilization rates applied in pre-planting, SPAD chlorophyll meter readings obtained, color of terminal leaflet of the fourth leaf completely expanded from the apex measured with the "UFV-80" color chart (LCC), critical level (CL) of the SPAD chlorophyll meter readings, and N fertilization rates applied in top-dressing in the "Monalisa" cultivar at 21 days after the emergency, during dry and wet seasons.

N rates in pre-planting (kg ha^{-1})	SPAD Index at 21 DAE	LCC	CL SPAD at 21 DAE	Difference between the index and CL SPAD	N rates in top-dressing (kg ha^{-1})
			Dry Season		
0	40.0	1	42.1 *	2.1 [#]	42 [&]
50	44.6	2	42.1	- 2.5	152
100	45.6	3	42.1	- 3.5	152
200	47.3	4[•]	42.1	- 5.2	152
300	49.2	5	42.1	- 7.1	152
			Wet Season		
0	34.8	1	43.1	8.3	133
50	37.3	2	43.1	5.8	93
100	38.8	3	43.1	4.3	69
200	39.5	4	43.1	3.6	58
300	43.5	5	43.1	- 0.4	205

[•] Critical color of the terminal leaflet of the fourth leaf completely expanded from the apex, according to (Fontes and Silva, 2006). * Critical level SPAD obtained in Silva et al. (2009). [#] Negative value indicates no need for N application, however, applied for the true/false study (Beverly and Hallmark, 1992). [&] The quantity of N applied in top-dressing followed the recommendations of Fontes (1999) (152 kg ha^{-1} of N), except when the dose 0 kg ha^{-1} of N was applied, according to Silva et al. (2009) 1 unit of SPAD is equal to 20 and 16 kg ha^{-1} of N in the dry and wet seasons, respectively.

Table 2. Potato marketable yields (g/plant) and economic analysis of "Monalisa" cultivar as influenced by N fertilization rates applied in pre-planting and in top-dressing in dry and wet seasons.

N rates in pre-planting (kg ha^{-1})	Potato marketable yields (g/plant		Expenditure with N (R$ ha^{-1})	Total cost	Gross profit	Net profit
	With	Without		(R$ ha^{-1})		
			Dry season			
0	681.75[a]*	677.50[a]	0.00[#]	8,746.00	28,906.40	20,160.40
50	772.75[a]	792.25[a]	330.00	9,076.00	33,802.66	24,726.66
100	685.00[a]	823.75[a]	660.00	9,406.00	35,146.66	25,740.66
200	732.75[a]	798.75[a]	1.320.00	10,066.00	34,080.00	24,014.00
300	619.75[a]	585.50[a]	1.980.00	10,726.00	24,981.33	14,255.33
			Wet season			
0	554.25[a]	462.00[a]	0.00	8,746.00	24,640.00	15,894.00
50	620.25[a]	551.75[a]	150.00	8,896.00	29,426.66	20,530.66
100	627.75[a]	551.75[a]	300.00	9,046.00	29,426.66	20,380.66
200	660.00[a]	552.75[a]	600.00	10,030.00	29,480.00	19,450.00
300	645.75[a]	563.75[a]	900.00	10,570.00	30,066.66	19,496.66

*Averages in the lines followed by the same letter do not differ statistically between them by the Tukey test (P<0.05). [#] The economic analysis was only made when N fertilizer was used in pre-planting, seen that there was no significant statistical difference when the N was applied in top-dressing.

chlorophyll meter readings in each subplot, however Gil et al. (2002) conducted only 6 readings per subplot.

For both planting seasons (dry and wet), there was no interaction between N fertilization rates applied in pre-planting and in top-dressing over the productivity (Table 2). It was noticed that each N fertilization rates in pre-planting, the commercial production did not differ statistically, in relation to the N fertilization in top-dressing. Therefore, the N applications in pre-planting as well as the N applications in top-dressing did not influence the production of commercial tubers of potatoes.

In Table 2, the expenditure with N and the total cost has increased linearly with the applied N fertilization rates

Table 3. Nitrogen (N) fertilization rates applied in pre-planting to verify the diagnosis of N nutritional status (DENN) of the potato plant at 21 DAE, need for application of N in top-dressing (NANC), response of the commercial production, false or true and their implication, in the dry and wet seasons.

N rates in pre-planting (kg ha^{-1})	DENN	NANC	Production response	False or true	Implications
		Dry season			
0	Deficient	Yes	No *	F + #	FAD &
50	Non deficient	No	No	V -	NND
100	Non deficient	No	No	V -	NND
200	Non deficient	No	No	V -	NND
300	Non deficient	No	No	V -	NND
		Wet season			
0	Deficient	Yes	No	F +	FAD
50	Deficient	Yes	No	F +	FAD
100	Deficient	Yes	No	F +	FAD
200	Deficient	Yes	No	F +	FAD
300	Non Deficient	No	No	V -	NND

*Values of commercial production of the potato in Table 2. # Adapted from Beverly and Hallmark (1992). & FAD=Fertilizer applied unnecessarily and NNF=No need for fertilizer.

in pre-planting, in both planting seasons. Regarding the variables of gross and net profits, in dry season the dose that provided the highest gross and net profits was the dose of 100 kg ha^{-1} of N in pre-planting. However, in wet seasons the best N fertilization rates were 300 and 50 kg ha^{-1} of N for gross and net profits, respectively.

Compared to the average yield in Minas Gerais State in 2007, which was 29.90 ton ha^{-1} (Agrianual, 2009), the values found in the present work were superior, with an average commercial yield of 38.23 and 30.88 ton ha^{-1} in dry and wet seasons, respectively. This is due to the accounting of Agrianual (2009) of yields in every region in Minas Gerais State, using several N fertilization rates in different planting seasons, many crops of potatoes, distinct soils and seeds.

The average commercial yield was practically 20% lower in the wet season, when compared to the dry season (Table 2), probably due to more elevated temperature conditions, which has favored a larger growth of the vegetative part, in detriment to the productive part of the potato, the tuber. According to Ewing (1997), temperature higher to 20°C is a limiting factor to the tuberization, the average temperature in the experiments during dry and wet seasons were 17 and 22°C, respectively. Furthermore, Parr et al. (2014) reported that the effect of different planting dates on infestation and damage of various sweet potato cultivars can be a sustainable way to obtain good yields coupled with low Cylas sp. damage.

According to Table 2, in both planting seasons, it is only recommended to apply N in pre-planting, despite the savings by the farmer when N is only applied in the foundation, inconveniences might occur if only this application is made. The reason is that the application of all N in pre-planting makes corrections impossible according to the current requirements of the crops during the entire plantation period (Olivier et al., 2006), consequently, it is not possible to manage the application of the N in top-dressing.

The answer of the culture yield to a determined nutrient can be affected at the planting season, inefficient application of the fertilizer, absorption and interaction of the nutrients, plant's characteristic, or stress caused by draught (Beverly and Hallmark, 1992).

The highest net profit of the potato (R$ 25.740,66 ha^{-1}) became evident in dry season due to the pre-planting application and in top-dressing of 100 and 0 kg ha^{-1} of N, respectively, which provided the production of 823.75 g $plant^{-1}$. However, it did not differ when applied N rates of 152 kg ha^{-1} of N in top-dressing, obtaining the yield of 685 g $plant^{-1}$ (Table 2). In wet season, the application in pre-planting and in top-dressing was 50 and 0 kg ha^{-1} of N, respectively, providing the yield of 551.75 g $plant^{-1}$, giving the highest net profit (R$ 20.530,66 ha^{-1}), in this planting season. These differences are due to productions, cost of fertilizer and value of the potato paid by the rural producer, in which prices fluctuate every planting season.

In Table 3 in dry season, only N rates of 0 kg ha^{-1} of N in pre-planting caused a deficient N nutritional status diagnosis (DENN), considering that the difference between the index and the SPAD critical level was positive (Table 1), thus the need for the application N fertilizer, however, due to the false/true study, N in top-dressing was applied only in half of the plot (with or without N in top-dressing) (Table 2), nevertheless, no difference was seen between the commercial production in dry season, with or without application of N in top-dressing, implying unnecessary application of fertilizer.

In wet season, only N rates of 300 kg ha^{-1} of N in pre-planting caused a non-deficient N nutritional status diagnosis (DENN) (Table 3), considering that the difference between the index and the SPAD critical level was negative (Table 1), therefore, there would be no need to apply N fertilizer, but, due to the false/true study, remained only one half of the plot (subplot) without fertilizer and the other half the N was applied (Table 2), there was no statistical difference between the commercial production of these subplots.

It was verified that there was no deficient DENN, application of fertilizer (yes), production response (yes) and true (V +), however, according to Beverly and Hallmark (1992) it would imply in a decrease in yield (Table 3). This occurred, probably due to the use of a very high critical level (Table 1), which caused a low probability of response to the nutrient and no frequency of diagnosis V + (Beverly and Hallmark, 1992).

The use of a deficient DENN, that is, very low rate of N in the terminal leaflet of the terminal leaflet of the fourth leaf completely expanded from the apex, cause a high probability of response to the N, therefore, bringing lower frequency of F + and V - diagnosis. However, there will be a higher frequency of F – and V + diagnosis. This can reduce the opportunity of fertilize and consequently elevate the production (Fontes, 2001).

Conclusions

The ‘UFV-80’ color chart for Monalisa potato can be used to diagnose N nutritional status of the potato plant in both planting seasons. The higher net profit was obtained when applied 100 kg ha^{-1} of N in pre-planting plus 152 kg ha^{-1} of N in top-dressing for the dry season and 50 plus 93 kg ha^{-1} in pre-planting and top-dressing, respectively, in wet season. The commercial production was not influenced by the N fertilization rates in pre-planting and in top-dressing in dry and wet seasons.

Conflict of Interest

The authors have not declared any conflict of interest.

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