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SEASONAL VARIATION AND THRESHOLD VALUES FOR CHLOROPHYLL METER READINGS ON LEAVES OF POTATO CULTIVARS

Camilo Busato[a]; Paulo Cezar Rezende Fontes[b]; Heder Braun[b]; Paulo Roberto Cecon[c]
[a] Instituto de Defesa Agropecuária e Florestal do Espírito Santo, IDAF, Colatina, Brazil [b] Departamento de Fitotecnia, Universidade Federal de Viçosa, Viçosa, Brazil [c] Departamento de Informática, Universidade Federal de Viçosa, Viçosa, Brasil

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SEASONAL VARIATION AND THRESHOLD VALUES FOR CHLOROPHYLL METER READINGS ON LEAVES OF POTATO CULTIVARS

Camilo Busato,[1] Paulo Cezar Rezende Fontes,[2] Heder Braun,[2] and Paulo Roberto Cecon[3]

[1]*Instituto de Defesa Agropecuária e Florestal do Espírito Santo, IDAF, Colatina, Brazil*
[2]*Departamento de Fitotecnia, Universidade Federal de Viçosa, Viçosa, Brazil*
[3]*Departamento de Informática, Universidade Federal de Viçosa, Viçosa, Brasil*

□ *Chlorophyll meter readings below a threshold value would indicate the necessity to supply nitrogen (N) to the crop. This study aimed to determine threshold values for chlorophyll meter (SPAD) and nitrogen (N) rate effects on SPAD leaf measurements of potato cultivars through the crop cycle. Five N rates (0, 50, 100, 200, and 300 kg ha^{-1}) were evaluated in a randomized block design, with four repetitions. SPAD index was measured on the oldest (OL) and on the fourth leaf from the apex (FL) at 7, 14, 21, 28, 35, and 49 days after the emergence of 90% of the plants in the plots. SPAD values decreased with plant age and were influenced by N rates and leaf position. At 21 DAE, the SPAD threshold values on FL were 43.0, 44.6, 46.5, and 50.0 for 'Agata', 'Monalisa', 'Atlantic', and 'Asterix', respectively. The corresponding values were 41.9, 43.5, 49.9, and 49.9 on OL. Plant age and leaf position should be standardized for the assessment of SPAD threshold values to diagnose nitrogen status of potato cultivars.*

Keywords: *Solanum tuberosum*, real-time sensing, SPAD, critical values, green intensity

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important crop in Southern Brazil and it usually is fertilized with large amounts of nitrogen (N) to achieve high tuber yields. When N application is not synchronized with crop demand, N losses from the soil-plant system are large, leading to low N fertilizer use efficiency. Even moderate N deficiency will substantially reduce yield and profit, but excessive N can pollute both surface and ground water. Optimizing N management for each cultivar is critically important to maximize

Received 21 January 2009; accepted 23 June 2009.
Address correspondence to Paulo C. R. Fontes, Departamento de Fitotecnia, Universidade Federal de Viçosa, UFV, 36570-000 Viçosa, Minas Gerais, Brazil. E-mail: pacerefo@ufv.br

tuber yield and quality (Joern and Vitosh, 1995) as potato genotypes vary markedly in response to N fertility (Lewis and Love, 1994; Arsenault et al., 2001).

In N-deficient soil, potato plants grow slowly, are stunted, and show a lighter green or chlorotic leaf color (Trehan et al., 2001). Chlorosis is a condition in which leaves produce insufficient amounts of chlorophyll. This phenomenon is related with the N participation in the structure of the chlorophyll molecule. In the potato plant, N deficiency symptoms are initially characterized by a chlorosis of older leaves as N is a mobile nutrient and the plant will transfer N from the older to younger, developing leaves. Both N and chlorophyll deficiencies will lead plant to present low efficiency in using light as energy source for essential functions such as carbohydrate production and nutrient absorption.

The plant N status may be used for estimating supplemental N requirements. The N status of potato plant can be estimated by the analysis of N in the dry matter (Mills and Jones, 1996). The conventional dried tissue analysis is a useful procedure but may have some limitations as the required labor and specialized laboratory equipment (Waskon et al., 1996). Due to the dynamic nature of potato growth, decisions need to be made promptly. Besides that, the current precision agriculture requires real time assessment of plant N status.

Alternatively, the potato N status may be assessed by quick procedures as the petiole nitrate sap (Errebhi et al., 1998) and leaf greenness. The analysis of leaf green color intensity by a portable chlorophyll meter that can make instant and non-destructive reading in plant leaves appears as an alternative. One the most common portable chlorophyll meter is the SPAD-502 (Minolta Co., Tokyo, Japan). The SPAD meter is a simple, portable tool that measures the greenness or relative chlorophyll content of leaves. Meter readings are given in values that indicate relative chlorophyll contents. Several authors have shown chlorophyll content in leaves, measured with a chlorophyll meter, was correlated with both leaf N contents and yield of several species (Scheepers et al., 1992; Peng et al., 1996; Guimarães et al., 1999; Schröder et al., 2000; Azia and Stewart, 2001; Sexton and Carrol, 2002), including potato (Vos and Bom, 1993; Minotti et al., 1994; Rodrigues et al., 2000; Gil et al., 2002; Gianquinto et al., 2003; Rodrigues, 2004; Silva, 2007). Recently, a system to improve crop N efficiency in potato based on the use of leaf chlorophyll meter as a plant N status indicator was proposed (Olivier et al., 2006).

The chlorophyll meter can be used as a tool to monitor plant N status *in situ* in the field and to assist in decision-making on N fertilization as the right time of N topdressing in several plant species (Balasubramanian et al., 1999; Fontes and Araújo, 2006). One approach to apply fertilizer N is when SPAD value is less than the set critical value below which N supply is necessary to avoid yield losses. SPAD threshold value of 45 was set for potato plants by using data obtained in Italy (Giaquinto et al., 2003), under unique conditions of weather, soil type and genotype which all influence potato growth.

Significant differences among potato cultivars in N-use efficiency has been detected (Kleinkopf et al., 1981; Errebhi et al., 1998; Zvomuya et al., 2002; Zebarth et al., 2004) and several factors can interfere with plant growth and leaf chlorophyll content such as plant age and genotype (Mauromicale et al., 2006) making it difficult to establish a critical level of wide validity (Bullock and Anderson, 1998). Additionally, variation in the leaf N contents occurs among different leaf position on the plant. The objectives of this study were to determine the threshold values for chlorophyll meter (SPAD) and the effect of N rates on SPAD values measured at different leaf age of potato cultivars through part of the crop cycle.

MATERIALS AND METHODS

Four field experiments were simultaneously conducted at Federal University of Viçosa, Viçosa, Brazil (20″45′ S; 42″51′ W; 693-m altitude), in a Cambic Red-Yellow Argisol during the fall/winter season.

Potato cultivars 'Atlantic', 'Agata', 'Monalisa', and 'Asterix' were assigned to separate experiments with five treatments (0, 50, 100, 200 and 300 kg ha^{-1} of N) arranged in a randomized block design, with four repetitions. Plots (3×1.75 m) consisted of 28 plants, 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.

Prior to the experiment, the soil was limed and cultivated with two successive plantings of corn without N fertilization to lower soil N availability as it is common in soil planted with potato. The soil presented 47% clay, 2.74% organic matter, 4.6 mg kg^{-1} N-nitrate (NO_3) and 9.15 cmol dm^{-3} cation exchange capacity (CEC). Following the corn harvest, soil was prepared by deep moldboard plowing and two harrowing operations. Three days before planting, macro and micronutrients and N rates, as ammonium sulfate, were applied in-furrow.

Pre-sprouted seed tubers were hand-planted on 13 April and the crop was managed following standard production practices, including irrigation. The moisture content of the soil was maintained close to field capacity and the ridging was carried out twenty-two days after plant emergence (DAE).

Soon after complete plant emergence, a green index was measured in the fourth fully expanded leaf from the apex (FL) and in the oldest leaf (OL). Five readings were recorded for each leaf in four randomly selected plants of each plot. The green index was determined by the SPAD-502 portable chlorophyll meter (Minolta Co, Tokyo, Japan) The SPAD readings were carried out in the morning, between 8:00 and 11:00 a.m at 7, 14, 21, 28, 35 and 49 days after complete plant emergence (DAE) at the same plants.

Each experiment was evaluated separately and data were examined by analyses of variance and regression analysis by SAEG software program (SAEG for Statistical Analysis System, Version 5.0, Fundação Arthur Bernardes, Viçosa, Brazil). 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 determination value. The SPAD values were correlated with the N rates to calculate the SPAD threshold or critical values associated to maximum commercial tuber yield following procedures described by Fontes and Ronchi (2002).

RESULTS AND DISCUSSION

Potato plant emergence started on 11 April and was considered complete at 17, 12, 15, and 15 days after planting for 'Atlantic', 'Agata', 'Monalisa' and

TABLE 1 Relationships between SPAD values (Y) measured on the fourth fully expanded leaf from the apex of Atlantic, Agata, Monalisa, and Asterix potato cultivars and nitrogen (N) rates, at 7, 14, 28, 35, and 49 days after plant emergence (DAE)

DAE	Adjusted model	R^2
	Atlantic	
7	$\hat{Y} = 41.4583 + 0.0711509^{**}N - 0.000138392^{**}N^2$	0.99
14	$\hat{Y} = 39.5029 + 0.0743827^{**}N - 0.000126381^{**}N^2$	0.92
28	$\hat{Y} = 36.0604 + 0.079808^{**}N - 0.000149601^{**}N^2$	0.92
35	$\hat{Y} = 35.035 + 0.0792026^{**}N - 0.000164872^{**}N^2$	0.89
49	$\hat{Y} = 32.8868 + 0.0518335^{**}N - 0.00007749^{*}N^2$	0.88
	Agata	
7	$\hat{Y} = 41.5898 + 0.0184149^{**}N$	0.96
14	$\hat{Y} = 38.9449 + 0.0515469^{**}N - 0.0000910886^{**}N^2$	0.99
28	$\hat{Y} = 36.1097 + 0.0534173^{**}N - 0.0000966309^{**}N^2$	0.99
35	$\hat{Y} = 35.67 + 0.055529^{**}N - 0.000112297^{**}N^2$	0.98
49	$\hat{Y} = 36.4488 + 0.0562771^{**}N - 0.000101983^{**}N^2$	0.99
	Monalisa	
7	$\hat{Y} = 39.0734 + 0.0546986^{**}N - 0.000099444^{**}N^2$	0.90
14	$\hat{Y} = 36.0802 + 0.069618^{**}N - 0.000116596^{**}N^2$	0.96
28	$\hat{Y} = 34.7403 + 0.798357^{**}N - 0.000158209^{**}N^2$	0.99
35	$\hat{Y} = 34.5242 + 0.0730707^{**}N - 0.00013813^{**}N^2$	0.99
49	$\hat{Y} = 30.6208 + 0.0591805^{**}N - 0.00010243^{**}N^2$	0.97
	Asterix	
7	$\hat{Y} = 43.0688 + 0.0581581^{**}N - 0.000124698^{**}N^2$	0.94
14	$\hat{Y} = 43.1901 + 0.061842^{**}N - 0.000121788^{**}N^2$	0.94
28	$\hat{Y} = 43.2329 + 0.0214296^{**}N$	0.92
35	$\hat{Y} = 41.3181 + 0.0486183^{**}N - 0.00010461^{**}N^2$	0.94
49	$\hat{Y} = 39.1164 + 0.0439701^{**}N - 0.0000848258^{*}N^2$	0.99

** and * are significant at 1 and 5% of probability by the t-test, respectively.

'Asterix', respectively. The N rates affected the SPAD readings in the fourth fully expanded leaf from the apex (Table 1) and in the oldest leaf (Table 2) for all cultivars and evaluation times. Increase in SPAD readings in potato leaves with increase in N rate were also reported by other authors (Minotti et al., 1994; Gil et al., 2002).

The estimated SPAD values in FL and OL associated with the N rate (data not shown) that gave the maximum commercial tuber yield, or threshold or critical values, are shown (Table 3). At 21 DAE, which is the usual time under Brazilian conditions to decide on carrying out N side-dressing in the potato crop, the relationship between SPAD reading in FL and N rates was quadratic function for all varieties (Figure 1) with the SPAD critical values varying from 43.02 to 49.95 (Table 3). Minotti et al. (1994) reported SPAD critical values ranging from 49 to 56 at 29 and 37 days after planting, depending on the year, cultivar, and locality. For the cultivar 'Monalisa', Gil et al. (2002) found 44.9 SPAD units as the threshold value. In nutrient solution, Rodrigues et al. (2000) found a critical level of 39.6 SPAD units associated with the N rate that gave the maximum potato shoot dry weight. In substrate-filled pots, to

TABLE 2 Relationships between SPAD values (Y) measured on the oldest leaf of Atlantic, Agata, Monalisa, and Asterix potato cultivars and nitrogen (N) rates, at 7, 14, 28, 35, and 49 days after plant emergence (DAE)

DAE	Adjusted model	R^2
	Atlantic	
7	$\hat{Y} = 45.9894 + 0.0248283^{**}N$	0.87
14	$\hat{Y} = 44.8519 + 0.0649409^{**}N - 0{,}000130761^{**}N^2$	0.93
28	$\hat{Y} = 40.5483 + 0.0794808^{**}N - 0{,}000166578^{**}N^2$	0.87
35	$\hat{Y} = 38.8122 + 0.071968^{**}N - 0{,}000144932^{**}N^2$	0.88
49	$\hat{Y} = 33.4755 + 0.0303807^{**}N$	0.91
	Agata	
7	$\hat{Y} = 44.691 + 0.0185981^{**}N$	0.89
14	$\hat{Y} = 41.4719 + 0.058726^{**}N - 0.000112765^{**}N^2$	0.97
28	$\hat{Y} = 36.2523 + 0.0394064^{**}N - 0.0000584274^{**}N^2$	0.99
35	$\hat{Y} = 33.8609 + 0.0693112^{**}N - 0.000147768^{**}N^2$	0.93
49	$\hat{Y} = 29.1032 + 0.0599644^{**}N - 0.000120943^{**}N^2$	0.90
	Monalisa	
7	$\hat{Y} = 44.9802 + 0.0186042^{**}N$	0,90
14	$\hat{Y} = 42.6683 + 0.0218114^{**}N$	0,87
28	$\hat{Y} = 34.3671 + 0.0596783^{**}N - 0.0000898345^{**}N^2$	0,98
35	$\hat{Y} = 34.9611 + 0.0287098^{**}N$	0,95
49	$\hat{Y} = 29.7257 + 0.0519159^{**}N - 0.0000875355^{*}N^2$	0,96
	Asterix	
7	$\hat{Y} = 44.9964 + 0.0398754^{**}N - 0{,}0000707103^{*}N^2$	0.89
14	$\hat{Y} = 44.9001 + 0.0469463^{**}N - 0{,}0000908102^{**}N^2$	0.92
28	$\hat{Y} = 42.0384 + 0.0614909^{**}N - 0{,}000114098^{**}N^2$	0.97
35	$\hat{Y} = 40.3435 + 0.0677644^{**}N - 0{,}000143026^{**}N^2$	0.90
49	$\hat{Y} = 37.5886 + 0.0268822^{**}N$	0.88

** and * are significant at 1 and 5% of probability by the t-test, respectively.

TABLE 3 Estimated SPAD threshold values (SPAD-TV) measured on the fourth and on the oldest leaves of Atlantic, Agata, Monalisa, and Asterix potato cultivars at 7, 14, 21, 28, 35, and 49 days after plant emergence (DAE)

	SPAD-TV in the fourth leaf				SPAD-TV in the oldest leaf			
DAE	Atlantic	Agata	Monalisa	Asterix	Atlantic	Agata	Monalisa	Asterix
07	49.68	44.68	45.92	49.79	50.34	47.81	48.57	50.27
14	48.66	45.03	45.17	50.82	52.22	48.15	46.88	50.77
21	46.48	43.02	44.64	49.95	49.87	41.94	43.48	49.85
28	45.47	42.36	44.25	47.78	49.36	41.22	42.54	49.94
35	43.85	41.83	43.48	46.92	46.97	41.33	40.50	48.28
49	39.59	37.55	38.23	44.62	38.80	35.76	36.48	43.29

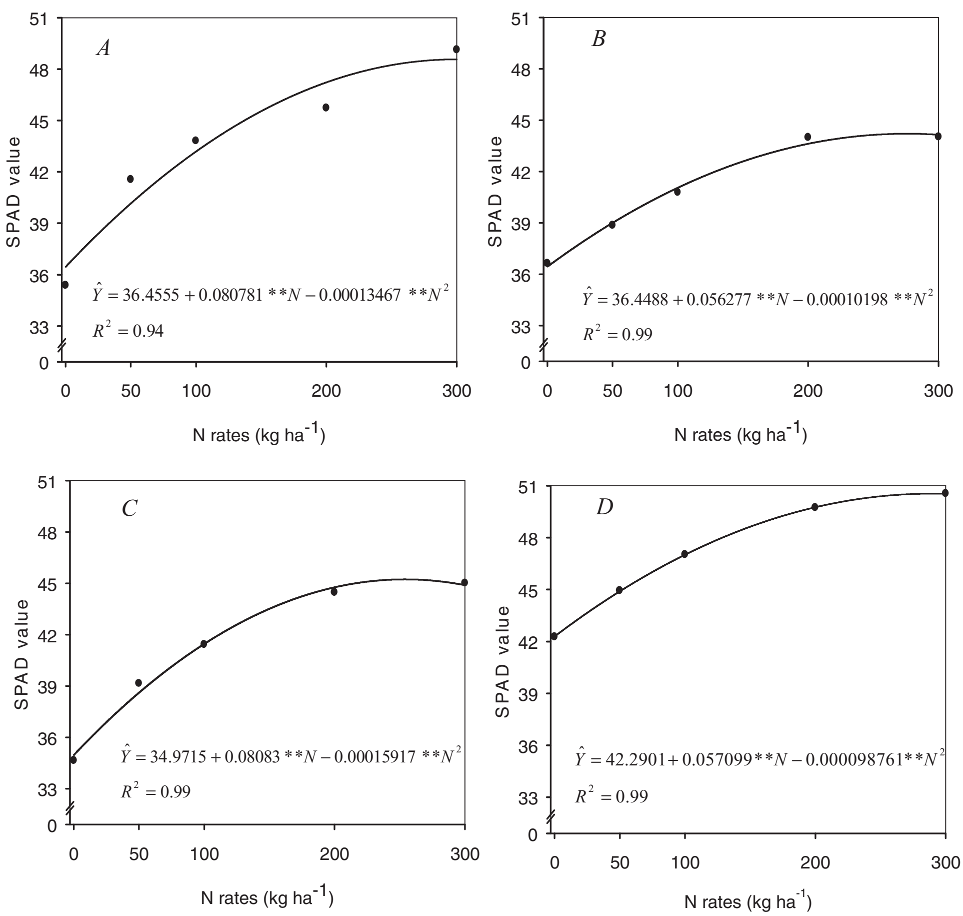

FIGURE 1 Relationships between SPAD values measured on the fourth leaf of A) Atlantic, B) Agata, C) Monalisa and D) Asterix potato cultivars and nitrogen (N) rates, at 21 days after plant emergence. **: significant at 1% of probability by the t-test.

produce 'Monalisa' potato basic seed, SPAD critical values in the fourth leaf at 37 DAE were 41.5, 47.9, and 45.7 for plants propagated by tissue culture plantlets, sprouts and minitubers respectively (Sampaio, 2005).

The SPAD index indirectly measures the chlorophyll content in the plant, which can indicate the crop N status (Fontes, 2001) and provides the possibility of early N diagnosis in potato (Rodrigues, 2004). Leaf chlorophyll content has been shown to vary with several factors such as potato variety (Mauromicale et al., 2006). These authors found positive linear relationships between chlorophyll content and nitrogen supply and potato tuber yield.

The SPAD values decreased with plant age for all cultivars, with more marked reduction in OL than in FL. Several authors had found decrease in SPAD readings with plant age (Minotti et al., 1994; Rodrigues, 2004; Mauromicale et al., 2006). This could have been caused by the N remobilization from the oldest to the youngest leaves, which occurred more markedly in the treatments receiving smaller N rates. Reduction in leaf N content with plant age can be explained by mechanisms involving N absorption, accumulation and distribution within the plant, as well as the plant development (Fontes, 2001).

CONCLUSIONS

The SPAD threshold values in potato leaves vary from 35.76 to 52.22 indicating the need for standardization of cultivar, plant age and leaf position to determine SPAD threshold values to diagnose potato nitrogen status.

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