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Maize Response to Nitrogen: Timing, Leaf Variables and Grain Yield

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Abstract

The main factors determining plant growth and productivity are decisive to be understood since they contribute to maximize plant nitrogen use efficiency. Thus, more reviews related to the correlation between the real content of chlorophyll and real carotenoids with the values obtained by chlorophyll (SPAD) in the early development stages of the maize are important to be obtained. The relation between the maize crop responses to the nitrogen fertilization at different development stages is of fundamental importance as well. The primary objective of this study was to investigate the responses of maize to the nitrogen application, urea fertilizer (^{15}N), in side-dress at different development stages. The secondary objective was verifying the correlation between chlorophylls and carotenoids with SPAD index and these with total biomass (BM), harvest index (HI), grain yield (GY) and grain N content in response to the nitrogen side-dress at different development stages. The nitrogen fertilization was carried out in plots, with the application of 30 kg ha^{-1} of N at planting and 140 kg ha^{-1} N as side-dress at vegetative stages V4, V6, V8, V10, and V12, without incorporation into the soil, and control treatment consisted of non-nitrogen side-dress application was also utilized. The 2011/2012 season presented higher precipitation than 2012/2013. Maize crop responded similarly for GY to the nitrogen application in side-dress in both seasons, however, the nitrogen application in the early stages caused higher values for leaf variables, leaf pigments, and SPAD. Higher amount of nitrogen in all parts of the plants was observed in the 2011/2012 season than in 2012/2013, influenced by the adequate weather conditions at the nitrogen application moment. Grain N content from ^{15}N fertilizer and N uptake and efficiency were greater for early N applications. SPAD values correlated positively with most pigment variables at V16 in both seasons, thus proving that SPAD was an efficient instrument of indirect evaluation of chlorophylls and carotenoids in maize leaves at early stages. Chlorophyll b at V16 was positively correlated ($P < 0.05$) with grain N content, GY, and BM, and total chlorophyll at V16 was positively correlated with GY and grain N content. However the chlorophylls a and total, evaluated at V14, were negatively correlated with GY. So, measurement of real chlorophyll and carotenoid pigment contents should be done after V14 stage when studies aim to evaluate crop nutritional conditions and prescribe future grain production practices.

Keywords: chlorophylls, carotenoids, SPAD, early growth stages

1. Introduction

Appropriate mineral nutrition is among the factors which have influence on crop productivity improvement. Nitrogen (N) is an essential nutrient required by plants, especially maize, which is one of the crops that mostly respond to fertilization with high increases in productivity. The N non-availability in soil causes several problems to crops such as reduced leaf area, decrease of photosynthetic rate, developmental delays, and reduced yield. On the other hand, excessive N application to the soil results in high production costs and can cause environmental problems such as contamination of the ground water and contribution to the increase of global warming due to the N volatilization.

Maize N requirements vary considerably in different plant development stages (Arnon, 1975). Although it is known that this crop requires about 20 kg ha^{-1} of N for each ton of produced grain (Fancelli, 2000; Sousa & Lobato, 2004), the best time for N application to this crop is still controversy. Some authors state the best time for N application is at seeding or close to this event, but others report it is ideal to apply at later stages, thus avoiding losses by leaching and volatilization, and increasing the efficiency of absorption and use of the nitrogen fertilizer by the plant (Cantarella, 1993; Pauletti & Costa, 2000; Ceretta et al., 2002; Basso & Ceretta, 2000).

Great advances have been made to improve the nitrogen use efficiency (NUE), defined as the ratio of dry matter production per unit of applied N. The use of indirect measurements to determine the nutritional status of plants has been the object of research for many crops. Research studies have shown the concentration of chlorophyll or the greening of leaves is positively correlated with leaf N concentrations, because 70% of N contained in the leaves is in the chloroplasts, participating in the synthesis and the structure of chlorophyll molecules (Marenco & Lopes, 2005). Therefore, the content of chlorophyll in the late vegetative stage has been related to the N nutritional status of various crops (Argenta, Silva, & Sangoi, 2001).

The traditional methods used to determine the amount of chlorophyll in the leaf require sampling and destruction of plant tissue, and the chlorophyll extraction and quantification processes are time-demanding. The portable chlorophyll meter SPAD (Soil Plant Analysis Development) is a nondestructive device that allows measuring instantaneously the chlorophyll amount of leaves, and it is an alternative to estimate the relative content of leaves pigments (Dwyer, Tollenaar, & Houwing, 1991; Argenta, Silva, & Sangoi, 2001). The N concentration in plant leaves has strong and positive relationship with the SPAD values, being more evident in the later growth stages (Argenta, Silva, & Sangoi, 2001). The leaf chlorophyll content shows also high correlation with SPAD results (Dwyer, Tollenaar, & Houwing, 1991; Ciampitti et al., 2012).

The knowledge on the effective influence of the factors that determine the performance of the plant can contribute decisively to minimize the stress caused by nitrogen deficiency. Thus, it is important to obtain reviews related to the correlation between the real content of chlorophyll and real carotenoids with the values obtained by chlorophyll (SPAD) in the early development stages of maize. The maize crop response to the nitrogen fertilization at different development stages is as well as important to be evaluated. Through these assessments, it is possible to have a greater knowledge about the plant relationship with the environment in which it is grown and may lead to increases in the grain yield.

The primary objective of this study was to investigate the maize response to side-dress N application by urea fertilizer (^{15}N) at different development stages. The secondary objective was verifying the correlation between the chlorophylls and carotenoids with SPAD values and these with, total biomass, harvest index (HI), grain yield (GY) and grain N content in response to the nitrogen side-dress in different development stages.

2. Materials and Methods

2.1 Experimental Site and Treatments

The experiments was carried out at Tanquinho Farm, in Piracicaba, SP, Brazil (22°34′13.9″ S, 47°36′14.3″ W) under field conditions during the seasons of 2011/2012 and 2012/2013. The first experiment was installed in December 2011 and completed in March 2012, and the second experiment was carried out from December 2012 to March 2013. The soil was classified as "Latossolo Vermelho Distrófico" (EMBRAPA, 2006), or as Rhodic Hapludox (Soil Survey Staff, 2010). According to Köppen´s classification, the climate of the region is Cwa type, with annual average temperature 23.9 °C and annual precipitation 1,257 mm.

Chemical and physical characterization of the soil was performed for the 0-20 cm layer (Table 1) previously the implementation of the experiments. For both seasons, weed plants were controlled with glyphosate before planting. Mechanical seeding was performed with approximately 3.3 seeds per meter (already accounted for 10% surplus due to losses) in order to obtain a final stand of 60,000 plants per hectare. The hybrid used in this study was the 30F35HR (PIONEER, 2014). The experimental design consisted of random blocks having four replicates managed under conventional tillage, with maize as the preceding crop. Plots had 10 rows of maize with 10 m length, spaced 0.5 m apart, totaling an area of 50 m^2. In each of the plots, mini plots (0.5 m wide and 1.5 m long) were delimited for ^{15}N-urea application at the same rate and time of the commercial urea application to the rest of the plot.

Table 1. Soil analysis in both maize growing seasons, 2011/2012 and 2012/2013, (inorganic nitrogen [NO_3^--N/NH_4^+-N], soil pH, potassium content [K], and phosphorus Bray-P 1 [P]) in from 0-20 and 20-40 of the soil profile

Profile	pH	P	K	Ca	Mg	H+Al	Al	T	V	OM[1]	Silt	Clay
-------cm-------	($CaCl_2$)	---mg dm^{-3}---	---------------mmol$_c$ dm^{-3}---------------						--%--	----------g kg^{-1}----------		
2011/12												
0-20	4.9	27	1.9	30	13	42	1	87	52	29	151	529
20-40	4.5	32	0.6	19	8	58	5	86	32	22	102	548
2012/13												
0-20	4.8	29	1.3	16	9	47	2	73	36	30	-	-
20-40	4.6	21	0.6	10	7	52	3	70	25	24	-	-

Note. [1] = Organic matter.

Planting fertilization was performed with the application of full rates of phosphorus (P) and potassium (K). Nitrogen (N) fertilization was carried out in plots with urea as the source, corresponding to 30 kg N ha^{-1} at planting and 140 kg N ha^{-1} as side-dress, without incorporation. The N, P and K rates were applied aiming at high grain yield (10-12 t ha^{-1}) and following the recommendations for areas with high response to N application (Cantarella, Raij, & Camargo, 1997). The treatments consisted of urea fertilizer application five times as side-dressing, corresponding to the vegetative stages V4, V6, V8, V10 and V12 as described by Ritchie, Hanway, and Benson (2003). A control treatment consisted of non-nitrogen side-dress fertilization.

2.2 Sampling and Analysis

The grain yield (GY), the harvest index (HI) and the plant biomass (BM) were determined at the end of the crop cycle. GY was measured by weighing the grain harvested with moisture correction to 13%. BM was measured based on the wet weight of the residuals, followed by the correction of the previously determined moisture.

Plant material from the shoot was separated into stem, leaf + tassel + ear husk, cob and grain. All material was dried at 60 °C with forced air to a constant weight. Subsequently, the dried material was ground in a Wiley grinder, homogenized and subsampled. In all subsamples, the nitrogen content (g kg^{-1}) was determined by Kjeldahl digestion - distillation and the sulfur content by the nitric perchloric digestion methodology followed by turbidity determination.

Plants collected from the mini plots in the 2012/2013 season were oven dried in laboratory and finally ground for later determination of total-N content and ^{15}N abundance by mass spectrometer (Barrie & Prosser, 1996). Grain nitrogen content derived from the fertilizer (GNCF) as well as the nitrogen fertilizer use and efficiency (NFUE, Equation 3) were calculated according to Gava et al. (2006).

Indirect chlorophyll content index was obtained by using the SPAD-502 chlorophyll meter (Minolta, Japan) (Minolta, 1989; Pestana et al., 2001; Markwell, Osterman, & Mitchell, 1995) on the two uppermost fully developed plant leaves before entering senescence and being photosynthetically active. Six samples per leaf were performed in 4 plants per treatment, carried out at the V14 to V16 stages.

The plant height (PH) was measured with a tape (in meters) placed from soil surface to the highest insertion of the last uppermost leaves on 4 plants per treatment at the flowering period (VT).

The content of pigments in the leaves was evaluated in the laboratory. The same leaves of SPAD evaluation were afterwards used for the analysis of chlorophylls a, b, and total, and carotenoids. Following the methodology adapted from Moran and Porath (1980), two leaves per plant were evaluated from a total of 4 plants per treatment at the V14 and V16 stages.

2.3 Statistical Analysis

Data were tested for normality, as well as the homogeneity of variances, and then subjected to analysis of variance at 5% of significance. Having significant effects of treatments by the F test, comparisons were performed using the t test, also at the level of 5% significance. The Pearson correlation test was also performed among the variables in order to verify the existence of positive correlations.

3. Result and Discussion

3.1 Growing Season and Phenology

The weather conditions during both growing seasons can be seen in Figure 1. The 2011/2012 season presented higher precipitation than 2012/2013, however, in the last one there was regular precipitation during the stages of N application. Figure 1 shows also the occurrence date of plant stages for both growing seasons.

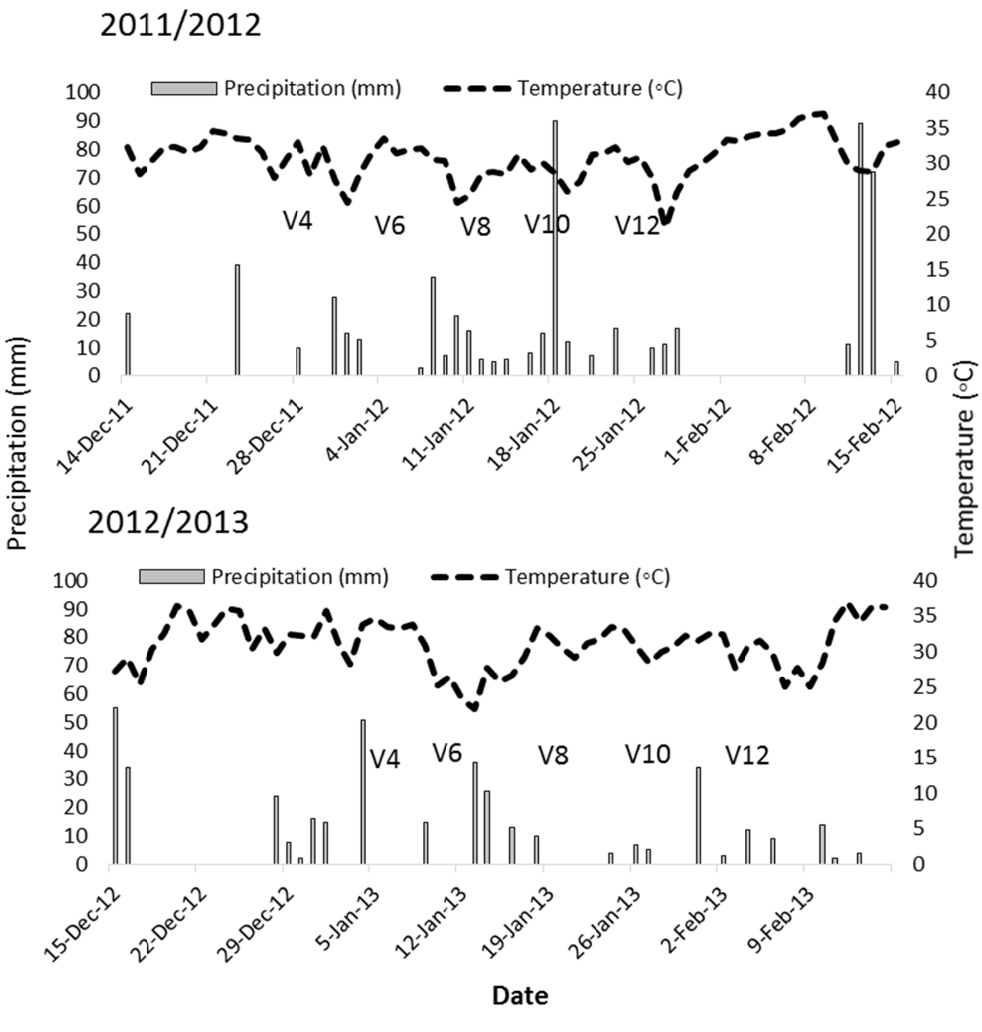

Figure 1. Days of development stages (moment of nitrogen application in side-dress) and weather conditions (maximum air temperature and mean precipitation) for the 2011/2012 and 2012/2013 maize growing seasons at *Tanquinho* Farm, Piracicaba, Sao Paulo, Brazil

3.2 Nitrogen and Sulfur Uptake

During the 2011/2012 season (Table 2), the treatments were significant for most variables, but not for cob N content. For leaf N content there was only difference between the control and all N applications, in all development stages. But for stem N content only the N application in V4 and V6 were different in relation to the control (V4 = V6 > control). Grain N content and total plant N uptake were greater for V6 and V10 in relation to the control. However, the N application at V8 resulted in similar values in relation to the control for both variables. Nonetheless, the total plant sulfur uptake was similar for all treatments with nitrogen application. The N amount in all parts of the plants was higher in the 2011/2012 season than in 2012/2013 (Table 3). The average total N plant uptake value 50 kg ha^{-1} of N was evidently greater in the first season (2011/2012).

Maize responded differently between seasons due to water availability. After the initial stages that presented less variable means of the measured plant parameters, a well-distributed precipitation spell was observed (very close to the nitrogen application in side-dress), while in the other stages there was a lack or small amount of precipitation that could have influenced the N uptake and partitioning. For urea application in dry soil conditions, Black, Sherlock, and Smith (1987) observed 70% of the applied nitrogen remained in the soil in the hydrolyzed form.

Fertilizer N content (GNCF) in grain and N fertilizer use and efficiency (NFUE) calculated from ^{15}N data were greater for the early applications (V4 = V6 > V8, V10, and V12) $p < 0.0001$.These variables presented lower values for the later N application, the V12 application being the lowest one for GNCF, lower than the N application at V4 stage. This could have happened because plants had a longer period to perform the N uptake from the soil, nevertheless the weather conditions had a greater influence on this.

Table 2. 2011/2012 Season: Nitrogen (N) partitioning into leaf, stem, cob, grain components and total plant; and total plant sulfur (S) uptake at physiological maturity, in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages

Treat	Leaf N	Stem N	Cob N	Grain N	Total N	Total S
	---kg ha^{-1}---					
Control	40.9 b	15.2 b	8.4	85.7 bc	150.2 c	8.9 c
V4	50.0 a	24.0 a	10.5	115.7 ab	200.3 ab	12.9 ab
V6	55.5 a	25.9 a	11.6	125.6 a	218.6 a	13.2 ab
V8	55.2 a	23.8 ab	8.7	78.9 c	166.0 bc	11.3 b
V10	53.5 a	21.2 ab	10.9	131.7 a	217.4 a	13.5 a
V12	49.9 a	18.0 ab	11.7	110.5 abc	190.1 abc	12.3 ab
ANOVA						
Treat.	*	*	ns	*	*	**
CV%	11.4	27.3	29.4	21.8	15.4	12.9

Note. ns = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.0001$.

Table 3. 2012/1013 Season: Nitrogen partitioning into leaf, stem, cob, grain components and total plant; Sulfur total plant uptake; grain nitrogen content from the fertilizer (GNCF), and nitrogen fertilizer use and efficiency (NFUE) at physiological maturity, in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages

Treat.	Leaves N	Stem N	Cob N	Grain N	Total N	Total S	GNCF	NFUE
	---kg ha^{-1}---							----%----
Control	35.3	10.6 c	6.6	129.6	182.1	13.6 c	-	-
V4	45.5	15.1 ab	6.8	127.6	195.0	14.7 bc	46.1 a	48.9 a
V6	43.4	16.3 a	7.6	130.7	198.1	16.4 ab	49.7 a	51.8 a
V8	39.0	15.2 ab	8.3	121.9	184.4	15.5 abc	31.9 b	31.2 b
V10	39.3	15.8 ab	9.0	135.6	199.7	17.7 a	27.0 bc	26.7 bc
V12	41.8	13.4 bc	8.8	145.7	209.8	17.4 a	17.7 c	17.8 c
ANOVA								
Treat.	ns	**	ns	ns	ns	*	***	***
CV%	13.7	12.8	15.5	7.7	7.8	11.3	20.56	19.9

Note. ns = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.0001$.

3.3 Grain Yield, Biomass and Harvest Index

In 2011/2012, total plant biomass was not influenced by treatments (Table 4), only cob weight had significant differences ($p < 0.05$). On the other hand, in 2012/2013 (Table 5) there was only difference for HI, the V12 application being better than most applications, but not better than V8. França et al. (1994) reported that the splitting of N does not affect the efficiency of nitrogen fertilizer or use of N from the soil, and the results were similar when they applied up to 106 kg of N per ha in a single rate at the stage where the plant had 6 leaves or, when fertilizer is subdivided twice, half at the 6-leaf stage and the other half at the 10 leaf stage. These authors also observed most of the N in the plant was accumulated until flowering, reaching values of up to 93%. They concluded that the nitrogen side-dressing should be made after seeding until early flowering, a period during which the rate of absorption is virtually linear. The N application efficiency prior to maize planting was studied by many authors (Pauletti & Costa, 2000; Ceretta et al., 2002). All of them found little difference among time N applications, but Ceretta et al. (2002) warned that the early application can compromise yield in years of high rainfall, in the early stage of crop development. However, Jokela and Randall (1989) concluded that there was less response of maize to N when it was applied at theV2 stage than at the V8 stage. Maize starts to take up N rapidly at the middle vegetative growth period (V10) and the maximum rate of N uptake occurred near to silking (Hanway, 1963; Settimi & Maranville, 1998). Hence, application of N at V8-V10 stage should be one of the best ways of supplying N to convene this high demand.

Table 4. 2011/2012 Season: Plant biomass of leaf, stem, cob, grain, and total plant components; grain yield (GY); and grain harvest index (HI) at physiological maturity, in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages

Treat.	Leaf	Stem	Cob	Total	GY	HI
	--kg ha				--	------%------
Control	4.6	3.1	1.2 c	16.7	6.7	43.1
V4	5.3	4.1	1.5 ab	19.2	8.2	42.9
V6	5.3	4.2	1.6 ab	18.7	7.6	40.5
V8	4.8	3.6	1.3 bc	15.4	5.6	36.2
V10	5.1	3.8	1.6 a	20.0	9.4	47
V12	5.2	3.7	1.5 ab	18.9	8.4	44.1
ANOVA						
Treat.	ns	ns	*	ns	ns	ns
CV%	9.5	17.7	12.6	16	15.5	11.6

Note. ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.0001.

Table 5. 2012/2013 Season: Plant biomass of leaf, stem, cob, grain, and total plant components; grain yield (GY); and grain harvest index (HI) at physiological maturity, in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages

Treat.	Leaves	Stem	Cob	Total	GY	HI
	--kg ha^{-1}---					------%------
Control	4.6	3.2	1.5	19.9	11.1	53.1 b
V4	4.7	3.6	1.6	20.9	11.1	52.5 bc
V6	4.8	3.8	1.6	21.2	10.9	51.5 c
V8	4.4	3.2	1.6	20.0	10.1	53.9 ab
V10	4.6	3.4	1.6	20.9	10.6	53.4 b
V12	4.6	3.1	1.5	20.8	11.1	55.1 a
ANOVA						
Treat.	ns	ns	ns	ns	ns	**
CV%	8.2	10.7	7.2	6.9	5.8	2

Note. ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.0001.

3.4 Leaf Pigment Contents, SPAD, and Plant Height

Early N application triggered the highest value for most variables in both seasons. In 2011/2012 (Table 6), lower values than in 2012/2013 (Table 7) were observed for all variables. The differences were more expressive for the V14 stage than for V16 for most of the variables. However, SPAD had significant differences for these two stages in both seasons, being greater from V4 to V10 than at the V14 stage and having difference only between N fertilized treatments with the control for V16 in 2011/2012. However in 2012/2013 differences for SPAD were larger than in the first season. At the V14 sample stage, V6 was greater than V8, V10, V12, and control. Similar results for V16 were found, however, V6 presented higher values for SPAD only in relation to V4, V12, and the control. PH in 2011/2012 was only different from the control, and in 2012/2013 only V4 presented differences in relation to the control (V4 > Control). Chlorophyll a, b and total were also lower in the non-fertilized treatment than in treatments with N.

Table 6. 2011/2012 Season: Leaf pigment contents (mg g^{-1} fresh leaf mass): chlorophyll a (CA), chlorophyll b (CB), chlorophyll total (CT), carotenoids (Carot), SPAD, evaluated at V14 and V16 stages; and plant height (PH) at VT in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages

Treat.	CA V14	CA V16	CB V14	CB V16	CT V14	CT V16	Carot V14	Carot V16	SPAD V14	SPAD V16	PH
	--mg g^{-1}--										---m---
Control	0.770 c	0.77	0.25 c	0.24	1.02 c	1.01	0.16 c	0.17 b	41.1 c	38.0 b	2.59 b
V4	1.130 a	0.88	0.46 a	0.32	1.59 a	1.20	0.23 a	0.21 ab	55.2 a	51.5 a	2.90 a
V6	1.050 ab	0.9	0.37 ab	0.30	1.42 ab	1.19	0.22 ab	0.19 ab	51.0 a	51.2 a	2.83 a
V8	1.04 ab	0.92	0.39 ab	0.31	1.43 ab	1.23	0.22 ab	0.22 a	52.1 a	50.1 a	2.82 a
V10	0.947 abc	0.9	0.31 bc	0.28	1.26 bc	1.19	0.20 abc	0.21 ab	50.2 ab	50.0 a	2.87 a
V12	0.880 bc	0.83	0.29 bc	0.26	1.17 bc	1.09	0.19 bc	0.20 ab	45.0 bc	48.5 a	2.81 a
ANOVA											
Treat.	*	ns	**	ns	*	ns	*	*	**	**	*
CV%	13.9	17.9	20.2	19.6	15.1	18.1	13.2	15.6	7.7	6.7	4.1

Note. ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.0001.

Table7. 2011/2012 Season: Leaf pigment contents: chlorophyll a (CA), chlorophyll b (CB), chlorophyll total (CT), carotenoids (Carot), SPAD, evaluated at the V14 and V16 stages; and plant height (PH) at VT in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages

Treat.	CA V14	CA V16	CB V14	CB V16	CT V14	CT V16	Carot V14	Carot V16	SPAD V14	SPAD V16	PH
	--mg g^{-1}--										----m----
Control	1.26 c	1.18	0.40 b	0.35	1.66 b	1.53	0.29	0.21	50.6 e	45.9 c	2.26 b
V4	1.44 ab	0.98	0.61 a	0.38	2.04 a	1.30	0.32	0.12	58.3 ab	51.8 bc	2.42 a
V6	1.43 ab	1.19	0.52 ab	0.39	1.95 a	1.58	0.32	0.23	58.8 a	61.5 a	2.37 ab
V8	1.53 a	0.98	0.51 ab	0.28	2.04 a	1.26	0.31	0.12	56.3 bc	55.5 ab	2.37 ab
V10	1.43 ab	1.11	0.52 ab	0.31	1.94 a	1.42	0.35	0.14	56.0 c	54.8 ab	2.32 ab
V12	1.36 bc	1.22	0.55 ab	0.36	1.91 a	1.58	0.31	0.23	53.0 d	53.8 b	2.36 ab
ANOVA											
Treat.	**	ns	*	ns	*	ns	ns	ns	***	**	ns
CV%	5	27.8	23.4	30.4	8.5	26.3	15.4	63.7	2.64	9.22	3.7

Note. ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.0001.

3.5 Person Correlations

SPAD at V16 had significant positive correlations with all pigments at both stages evaluated in 2011/2012 (Table 8), this variable correlated also positively and significantly (both stages, V14 and V16) with total chlorophyll evaluated in laboratory conditions. The strongest SPAD V16 correlation related to pigments was with chlorophyll a in the V14 stage (0.61, $p < 0.05$). In the same way SPAD at V14 also presented a strong correlation with other pigments, however, this variable only had correlation with the variables evaluated at the same stage. The strongest correlation for SPAD evaluated at V14 with pigments was with total chlorophyll (0.80, $p < 0.0001$). Nevertheless, in 2012/2013 (Table 9), there were significant correlations for SPAD with pigments only between SPAD/V14 with chlorophyll a and total (0.40, $p < 0.05$, for both).

Table 8. 2011/2012 Season: Pearson correlation analysis for leaf pigment contents: chlorophyll a (CA), chlorophyll b (CB), chlorophyll total (CT), carotenoids (Carot), SPAD, evaluated at V14 and V16 stages; total plant biomass (BM), grain harvest index (HI), grain yield (GY), and grain nitrogen content (GNC) in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages

	GNC	GY	HI	BM	SPAD16	SPAD14	Carot16	Carot14	CT16	CT14	CB16	CB14	CA16	CA14
CA14	0.06	0.2	-0.30	0.44	0.61**	0.76***	0.14	0.95***	0.20	0.99***	0.32	0.92***	0.14	1.00
CA16	0.13	0.17	-0.20	0.11	0.42*	0.23	0.08	0.07	0.99***	0.12	0.93***	0.08	1.00	
CB14	0.02	0.12	-0.22	0.05	0.52**	0.71***	0.90***	0.90***	0.12	0.97***	0.27	1.00		
CB16	0.1	0.14	-0.19	0.07	0.50*	0.40	0.24	0.12	0.97***	0.31	1.00			
CT14	0.05	0.15	-0.25	0.05	0.59**	0.80***	0.12	0.95***	0.20	1.00				
CT16	0.12	0.12	0.15	0.10	0.44*	0.23	0.84***	0.13	1.00					
Carot14	0.01	0.20	-0.30	0.04	0.60**	0.70***	0.13	1.00						
Carot16	0.01	0.05	-0.55	0.14	0.50**	0.4	1.00							
SPAD14	0.33	0.34	-0.05	0.34	0.75***	1.00								
SPAD16	0.50*	0.30	-0	0.45*	1.00									
BM	0.92***	0.70***	0.68	1.00										
HI	0.80***	0.50*	1.00											
GY	0.60**	1.00												
GNC	1.00													

Note. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.0001$.

Table 9. 2012/2013 Season: Pearson correlation analysis for leaf pigment contents: chlorophyll a (CA), chlorophyll b (CB), chlorophyll total (CT), carotenoids (Carot), SPAD, evaluated at V14 and V16 stages; total plant biomass (BM), grain harvest index (HI), grain yield (GY), and grain nitrogen content (GNC) in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages

	GNC	GY	HI	BM	SPAD16	SPAD14	Carot16	Carot14	CT16	CT14	CB16	CB14	CA16	CA14
CA14	-0.35	-0.61*	-0.35	-0.22	0.23	0.40*	-0.13	0.29	-0.18	0.83***	-0.22	0.41*	-0.14	1.00
CA16	0.40	0.38	0.22	0.24	0.12	-0.10	0.85***	-0.004	0.97***	0.23	0.59***	-0.25	1.00	
CB14	0.06	-0.22	-0.16	0.13	0.05	0.3	-0.12	0.34	-0.12	0.84***	-0.11	1.00		
CB16	0.61*	0.55**	-0.01	0.53*	0.31	0.12	0.075***	0.21	0.75***	-0.23	1.00			
CT14	-0.16	-0.50*	-0.30	-0.04	0.16	0.40*	-0.15	0.34	-0.24	1.00				
CT16	0.48*	0.50*	0.18	0.34	0.20	-0.05	0.90***	0.05	1.00					
Carot14	0.31	-0.14	-0.31	0.36	0.19	0.31	0.03	1.00						
Carot16	0.50**	0.36	0.08	0.28	0.19	-0.05	1.00							
SPAD14	-0.01	0.01	-0.38	0.34	0.47*	1.00								
SPAD16	0.26	0.01	-0.06	0.36	1.00									
BM	0.82***	0.60*	0.00	1.00										
HI	0.26	0.31	1.00											
GY	0.60**	1.00												
GNC	1.00													

Note. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.0001$.

Even with less correlation between SPAD and pigments in the second season, we can consider the SPAD measuring device as efficient to evaluate the actual amount of these pigments. These results are in accordance with other studies. According to Piekielek et al. (1995) and Dwyer, Tollenaar, and Houwing (1991) this indirect evaluation of chlorophyll content in the leaf can be used to predict the nutritional N level in plants, because the correlation with the amount of pigment was positive in relation to N concentration. There is a strong positive relationship between the SPAD and N concentration in the leaves of the plants, although this is more evident in the later growth stages (Argenta, Silva, & Sangoi, 2001), and there is also a high correlation of SPAD with chlorophyll content (Dwyer, Tollenaar, & Houwing, 1991; Ciampitti et al., 2012). A significant positive correlation between SPAD/V16 and grain N content was found in the first season, but there was no significant correlation between SPAD with grain yield, harvest index, and grain N content in the second season (2012/2013). Chlorophyll b at V16 presented significant positive correlation with grain N content (0.61, $p < 0.05$), GY (0.55, $p < 0.01$), and total biomass (0.53, $p < 0.05$). The chlorophyll total at V16 also presented a positive correlation with GY (0.50, $p < 0.05$) and grain N content (0.48, $p < 0.05$), however, the chlorophylls a and total evaluated at V14 presented a negative significant correlation with GY. Thus, the measurement of pigment contents aiming to study nutritional crop conditions and predict grain production should be performed after the V14 stage.

4. Conclusion

This growth and development experiment for maize (*Zea mays* L.) evidenced a similar response to the treatments for grain yield, harvest index and total plant biomass in both seasons with distinct climatic conditions. However, as a result of the different climatic conditions and the N application moment, a higher amount of nitrogen was observed in all parts of the plants in the 2011/2012 season in relation to the 2012/2013.

Fertilizer N in grain and nitrogen fertilizer use and efficiency were greater for the early applications, at stages V4 and V6.

Chlorophyll contents estimated through SPAD measurements and leaf pigments were largely influenced by the development stage. Evaluations made at V14 presented more differences in treatments than at V16, and the N application in the early stages caused higher values for most of the leaf variables (mainly pigments and SPAD).

SPAD correlated positively and significantly with most pigment variables at V16, for both seasons, mainly in the first season in which this variable was correlated with all chlorophylls and carotenoids, showing that the SPAD-502 chlorophyll meter is an efficient instrument for the indirect evaluation of chlorophylls and carotenoids in maize leaves. Also, SPAD in V16 sample stage had a positive correlation with grain nitrogen content and total plant biomass.

Chlorophyll b at V16 presented a significant and positive correlation with grain N content, grain yield, and total biomass. Total Chlorophyll at the same stage also presented a positive correlation with grain yield and grain N content, however, the chlorophylls a and total, evaluated at V14, presented a negative significant correlation with grain yield. Therefore, we recommend that measurements of real pigment contents aiming to study the nutritional maize crop conditions and predict grain production should be made after the V14 stage.

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