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Chlorophyll relative index for diagnosing nitrogen status in corn hybrids

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The aim of the current study was to determine the chlorophyll relative index (CRI) in different leaves and phenological stages of the crop in order to diagnose the status of nitrogen (N) in corn (*Zea mays* L.) hybrids as a function of N rates applied in bands correlating them with N content in the leaves and crop productivity. The field experiment consisted of two corn hybrids (P30R50 and AG8025) and 6 N rates applied in bands (0, 75, 150, 225, 300 and 375 kg N ha^{-1}), under a factorial 2 × 6 experimental design, arranged in a randomized block with 4 replications. The dose of 295 kg N ha^{-1} allowed estimating crop yields corresponding to 13.033 kg ha^{-1}. Hybrids and N rates influenced concomitantly CRI in several leaves and phenological stages. The chlorophyllometer is shown to be quite sensitive to nutritional status in corn hybrids as a response to N rates applied in bands since the early stages of the crop growing season for early diagnosis. At the end of the vegetative phase, as well as the reproductive phenological stage the chlorophyllometer performed well as an indicator of efficiency of nitrogen fertilizer application.

Key words: *Zea mays* L., chlorophyllometer, productivity, nitrogen fertilization.

INTRODUCTION

A high productive potential of the corn crop (*Zea mays* L.) is highly dependent on essential nutrients in the soil solution such as nitrogen (N), which is required by the plants in high quantities and provides a significant rise in crop yields. The amount of N absorbed taken up by the roots of the maize with the goal of reaching the maximum yield corresponds to 0.9% of the N present in dry phytomass of the sprouts (DPS) in compliance with Amado and Mielniczuk (2000), as well as to 1.17% of it according to Subedi and Ma (2005), showing therefore a high demand of the crop by such a macronutrient.

The complexity level and importance of N for the maize crop is consistent, mainly if we consider all the additional information needed to increase its efficiency. One of the alternatives to increase the efficiency of N is by synchronizing its application with the need of the plants. According to Rambo et al. (2007) and Holland and Schepers (2010), it is feasible to manage the amount of

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N to be applied in bands in compliance with the desired synchronism, enhancing its use efficiency by means of applications compatible to the needs of the plants. Such needs can be identified by the physiological parameter known as chlorophyll relative index (CRI) to be measured on the leaves of the crop by the chlorophyllometer throughout different phenological stages.

Measurements of chlorophyll rates (CR) on maize leaves, as well as other species, are relevant in studies carried out to examine the response of plants to nitrogen fertilization management when the aim is to increase crop photosynthetic efficiency (Amarante et al., 2010). In the same fashion, CRI correlates positively with chlorophyll rates and extractable N from the plants, as well as with crop yield. However, such a technique allows us to obtain reference values by means of a non-destructive procedure throughout different crop phenological stages. This will make possible the N monitoring and management whenever it is necessary in such a way as to obtain a positive correlation between the CRI and CR, regardless of the crop growing season and the maize hybrid in consideration.

The usage of indirect measurements aiming at estimating the CR by means of portable devices, which make use of optical principles and are based on the absorbance and/or reflectance of the light, turned out to be more straightforward and rapid, being able to be performed directly in the field without destroying the leaf tissues (Argenta et al., 2001b). CRI might also show a more practical applicability in agronomical trials owing to a high accuracy and low cost throughout its evaluation (Rambo et al., 2004).

Research manuscripts published by Argenta et al. (2002) and Hurtado et al. (2009) demonstrated a positive correlation between the CRI and maize yield, as well as between the N leaf level and productivity for diagnosing of the level of such element in the plants. Rambo et al. (2008) verified that the most precise characteristics for predicting the optimal doses of N to be applied to maize crops in bands are DPS and N accumulated in the plant, followed by the CRI in the leaf, which as a function of its practicability and ease of determination, shows a more pronounced potential for use. Therefore, in a sense, it is possible to increase the use efficiency of N in bands, either by means of a rise in yield or rationalization of nitrogen fertilization in bands, therefore decreasing the contamination of the soil and water, mainly due to nitrate lixiviation.

Owing to the climate and soil variability along with management practices existing in different production environments it is crucial to conduct regionalized research aiming at correlation studies between CRI readings and productive potential of maize hybrids, which will be expressed as a function of the climatic aptness for each region. Therefore, the aim of the current research paper was to determine the CRI and productive potential of maize hybrids in different leaves and throughout of different phenological stages with the purpose of diagnosing the nitrogen status in maize hybrids as a function of the amount of N applied to the plants in bands, correlating the content of N in the leaves with crop yield under field conditions in the State of Paraná, Brazil.

MATERIALS AND METHODS

The field experiment was conducted using a no tillage system at the region of Entre Rios, in the municipality of Guarapuava, PR, Brazil [latitude 25º32'S, longitude 51º28'W and altitude of 1,126 m] throughout the period between October 1st and March 20th of 2010. The climate of the site in the study according to the Köppen's classification is of the humid subtropical type without a dry season and with frequent severe frosts (Peel et al., 2007). Mean annual rainfall in the region is of 1.942 mm. Monthly mean air temperature is of 16.8°C with maximum and minimum values corresponding to 23.1 and 12.4°C, respectively (Simepar, 2011).

Throughout of the crop growing season the overall amount of rainfall was above the 35 year historical average along with variations of 5.1 mm for the month of November and of 114.7 mm for the month of January. Crop rotations over the last year at the experimental area were for the winter/summer year season wheat/soybean (2006), oat/maize (2007), barley/soybean (2008), and in 2009 oat preceded maize crop. The type of soil prevailing in the experiment area is latossol with a depth of roughly 2 m, good physical conditions, and with a high potential for agricultural use. The chemical characteristics of the soil might be seen in the Table 1.

Treatments resulting from the combination of two simple hybrids classified as a precocious cycle for maize - Pioneer 30R50 and Agroceres 8025 (P30r50 and AG8025) - and 6 doses of N to be applied in cover (0, 75, 150, 225, 300 and 375 kg of N ha^{-1} in the form of urea), comprising a factorial design 2×6 designed in randomized blocks with four replications. Plots consisted of 8 lines 5 m long spaced 0.75 m occupying a total area of 30 m^2. Sowing was performed manually on October 1st of 2009 shortly after the incorporation of the 8-30-20 fertilizer formula plus 0.4% of Zn in order to reach a plant population of 69.722 plants ha^{-1}. Cultural practices were implemented according to its occurrence and recommendation for the crop in the field.

Nitrogen doses were applied manually in the total area in just one application on September 9th of 2009 with the plants at V_5 stage. Under the V_3 stage out of the two central lines 2 plants $plot^{-1}$were selected and identified for determination of CRI by means of the chlorophyllometer-chlorophy LOG.

Fifteen readings of CRI were made in compliance with the following sequence: 1st reading: Leaf 3 at stage V_3 (L_3V_3); 2nd reading: L_5V_5; 3rd reading: L_3V_5; 4th reading: L_5V_7; 5th reading: L_7V_7; 6th reading: L_7V_9; 7th reading: L_9V_9; 8th reading: L_5V_9; 9th reading: L_9 grain filling stage (L_9GFS); 10th reading: corn tassel emission L_{11}CTE; 11th reading: $L_{11}R_1$; 12th reading: $L_{13}R_2$; 13th reading: $L_{15}R_2$; 14th reading: $L_{13}R_3$; 5th reading: $L_{15}R_3$. For all analyzed leaves readings were taken in two different stages, except for the leaf 5, which were made in V_5, V_7 and V_9.

For each evaluation of CRI one single leaf $plant^{-1}$ was used from four localized points in the central part of the leaf, between the edge and central nerve, obtaining the average from 8 readings per plot (2 plants). For the first assessment of CRI leaves were identified for the second reading, leaf 3 was the one taken into consideration for the identification of the other leaves.

Ten leaves per plot at the R_1 stage were used for the determination of leaf N content, always taking into account the below and opposite leaf oriented to the primary ear according to Malavolta et al. (1997). Leaves were stored in plastic bags, dried in

Table 1. Chemical characteristics of the soil in the experimental area.

Attributes	Unities	Depth (cm)		
		0-10	10-20	20-40
pH in $CaCl_2$		5.4	4.7	4.7
H + Al	$cmol_c\ dm^{-3}$	5.35	8.36	9.01
Al changeable	$cmol_c\ dm^{-3}$	0.0	0.3	0.4
Ca changeable	$cmol_c\ dm^{-3}$	6.9	4.0	3.1
Mg changeable	$cmol_c\ dm^{-3}$	2.4	1.5	1.3
K changeable	$cmol_c\ dm^{-3}$	0.57	0.38	0.25
P	$mg\ dm^{-3}$	22.9	6,0	2.4
C-organic	$g\ dm^{-3}$	32.0	21.0	19.0
CCC at pH 7,0	$cmol_c\ dm^{-3}$	15.22	14.24	13.66
CCC efetiva	$cmol_c\ dm^{-3}$	9.87	6.18	5.05
Sat. for bases (V)	%	64.8	41.3	34.0
Sat. for Al (m)	%	0.0	4.9	7.9
Sat. for Ca	%	45.3	28.1	22.7
Sat. for Mg	%	15.8	10.5	9.5
Sat. for K	%	3.7	2.7	1.8
Relation Ca/Mg		2.9	2.7	2.4
Relation Ca + Mg/K		16.3	14.5	17.6

H + Al: Buffer solution SMP; Al, Ca and Mg changeable: KCl 1 mol L^{-1}; P and K: Mehlich-1; C-organic: Walkley-Black. Source: Laboratory of Soil Fertility - State University of Ponta Grossa - UEPG.

a greenhouse with air circulation at 65°C up until the constant DPS was reached, then ground in a knife mill and analyzed by the semi-micro Kjeldhal method. Final productivity was assessed by means of the manual harvest of the ears from a useful area of 13.5 m^2, mechanical threshing, determination of DPS and extrapolation of the values for kg ha^{-1}, correcting it to a water content of 13%.

Experimental data obtained from each variable was subjected to analysis of variance through the SAS statistical program (SAS, 2008). Whenever the interaction between the hybrids and N doses was significant, and also when the effect of N doses was observed, a study of regression was carried out by means of illustrations provided by graphs made by an Excel program. The degree of correlation and agreement between the variables measured herein was expressed by the coefficient of the Pearson correlation.

RESULTS AND DISCUSSION

Interactions between the maize hybrids and doses of N to be applied in bands for the variable CRI occurred throughout the phenological stages: $L_{05}V_7$, $L_{07}V_7$, $L_{07}V_9$, $L_{09}V_9$, $L_{11}VT$, $L_{11}R_1$, $L_{13}R_2$, $L_{15}R_2$ e $L_{15}R_3$. Subedi and Ma (2005) obtained interactions only for the CRIs whenever N was applied in bands at different phenological stages of maize hybrids at V_8 four weeks after flowering.

A significant influence of the hybrids was observed over the $CRIL_{03}V_4$, $CRIL_{05}V_9$, $CRIL_{11}R_1$, $CRIL_{13}R_2$, $CRIL_{13}R_3$ and CRIL15R_2. However, this occurred with a higher frequency for the determinations performed throughout the reproductive phase. The only assessment of the CRI performed shortly before the application of N in bands was $CRIL_{03}V_4$. Nevertheless, the maize hybrid P30R50 showed a $CRIL_{03}V_4$ higher in 2.24 in relation to the AG8025 (Table 2). Average values obtained for the hybrids P30R50 and AG8025 was higher and similar to a CRI of 45.4% as cited by Argenta et al. (2003) at the stage V_3 to V_4, indicating that the content of N in the plant is quite adequate. The reading $CRIL_{03}V_4$ did not correlate with the N content in the leaf, as well as with yield (Table 3), corroborating with Argenta et al. (2002) and Hurtado et al. (2010).

The $CRIL_{03}V_5$ was remarkably affected by the doses of N applied in bands to the leaves, observing, therefore, an increasing linear effect (Figure 1a), occurring 4 days after its application (DAA). According to Godoy et al. (2007), a CRI of 46.6 between V_4 and V_5 triggered a more pronounced accumulation of DPS in maize. Once the mean value obtained for $CRIL_{03}V_5$ was of 46.87, such a value may be considered as perfect for the phenological stage in question.

The $CRIL_{03}V_5$ responded to the doses of N; however, during the assessment of the $CRIL_{05}V_5$ such a response did not show any statistical significance. This might be due to the fact that F_{05} is a very young phenological stage, suggesting then that the N added to the tissues will be utilized first to increase the number of cells of new leaves in conjunction with the cellular differentiation process. This final process will enhance the production of chloroplasts - the site of synthesis of chlorophyll - being confirmed during the evaluation of $CRIL_{05}V_7$ and $CRIL_{05}V_9$. Since the F_{03} was to be in a more advanced growth stage along with the fact that its final size was not

Table 2. Leaf chrophyll relative index at the leaf #3 at the phenological stage V_4 ($CRIL_{03}V_4$), $CRIL_{05}V_9$, $CRIL_{13}R_3$ and yield as a function of maize hybrid.

Hybrids	$CRIL_{03}V_4$	$CRIL_{05}V_9$	$CRIL_{13}R_3$	Yield (kg ha^{-1})
P30R50	48.07^a	63.56^a	64.58^b	10.929^b
AG8025	45.83^b	61.23^b	68.95^a	12.439^a

*Averages followed by the same letters do not differ among themselves by the F test at 5% confidence level.

Table 3. Correlation coefficients of the chlorophyll relative indices (CRI) at different leaves and phenological stages with leaf N content and yield.

Coefficients	Leaf N content (g kg^{-1})	Yield (kg ha^{-1})
$CRIL_{03}V_4$	0.24^{ns}	-0.10^{ns}
$CRIL_{03}V_5$	0.42**	0.36*
$CRIL_{05}V_5$	0.21^{ns}	-0.01^{ns}
$CRIL_{05}V_7$	0.40**	0.47**
$CRIL_{05}V_9$	0.72**	0.57**
$CRIL_{07}V_7$	0.39**	0.33*
$CRIL_{07}V_9$	0.58**	0.58**
$CRIL_{09}V_9$	0.41**	0.27^{ns}
$CRIL_{09}$GFS	0.73**	0.67**
$CRIL_{11}$CTE	0.63**	0.60**
$CRIL_{11}R_1$	0.67**	0.82**
$CRIL_{13}R_2$	0.65**	0.80**
$CRIL_{13}R_3$	0.69**	0.86**
$CRIL_{15}R_2$	0.71**	0.85**
$CRIL_{15}R_3$	0.71**	0.77**
Leaf N content (g kg^{-1})	-	0.69**

^{ns}, Not significant; *,** Significant at 0.05 and 0.01 probability level, respectively.

reached at all, its cells were at a more advanced differentiation stage, possessing more chloroplasts per cell and as a result of that having the synthesis of chlorophyll intensified by the supply of N. First of all N is used to assure cellular division plus cellular differentiation processes in the new leaves of the plants. Throughout the differentiation process cells get their perimeter enlarged, as well as the number organelles such as chloroplasts and photosynthesizing parenchyma. As a result of that, the content of chlorophyll rises concomitantly with cellular differentiation from tissue to organ. In this particular case, the organ in consideration is the leaf, being therefore incorporated into the membranes of the thylakoids as reaction centers called components of photo systems II and I (Taiz and Zeiger, 2013).

Mean values of $CRIL_{03}V_5$ (46.87) and $CRIL_{05}V_5$ (55.69) were to be very close to that one obtained by Hurtado et al. (2010) at V_4 to V_5 phenological stages, causing the maize crop to reach yields of 11.300 kg ha^{-1} found by such scientists, as well as by the current research (roughly corresponding to 11.684 kg ha^{-1}). However, either $CRIL_{05}V_5$ or mean yields were similar to the outcomes obtained by these authors. This might be explained by the fact that the amount of 32 kg ha^{-1} of N applied to the crop during the sowing date was sufficient to meet the initial physiological needs of the plants up to the stage of V_5, as well as owing to the fact that soil type in study possessed a content of organic matter capable of making available a higher amount of N to the soil solution. $CRIL_{03}V_5$ correlated positively with leaf N content and yield, showing coefficients equivalent to 0.42** and 0.36*, respectively, as opposed to the $CRIL_{05}V_5$ (Table 3). Similarly, Hurtado et al. (2010) observed a positive correlation between $CRIV_4$-V_5 with yield.

Nitrogen doses applied in bands resulted in a linear increase of $CRIL_{05}V_7$ (Figure 1b). Mean $CRIL_{05}V_7$ was of 63.7, regardless of the hybrid and N dose in consideration, revealing a superiority of 22.26% in relation to the CRI of 52.1 obtained by Argenta et al. (2003) for maize at V_6 to V_7, which corresponded to a rise of 6.7% in productivity. With a rise in availability of N an increment of $CRIL_{05}V_7$ occurred in conjunction with a rise in yield, a fact that might be firmed up by the positive

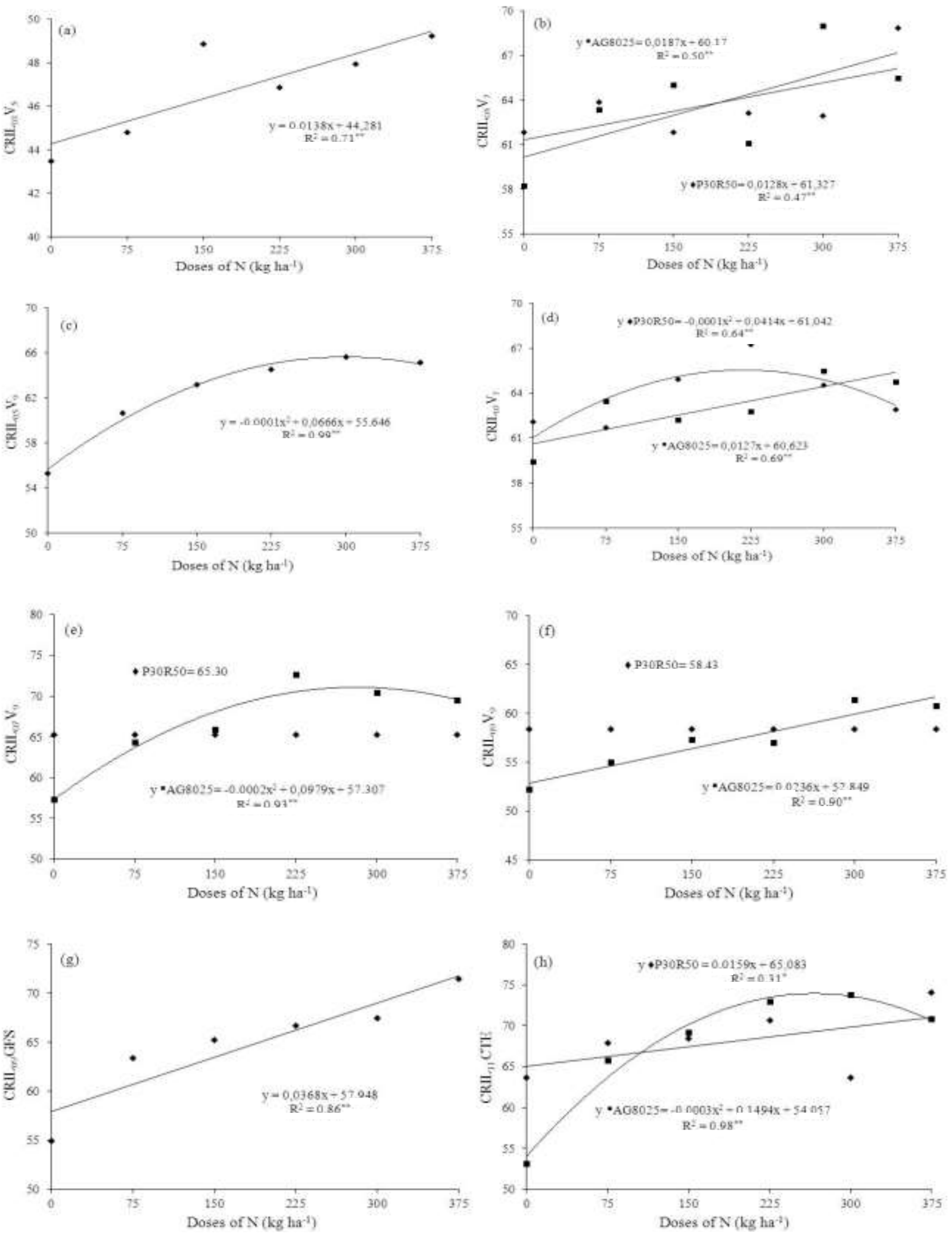

Figure 1. Leaf chrophyll relative index at the leaf #3 at the phenological stage V_5 ($CRIL_{03}V_5$) (a), $CRIL_{05}V_7$ (b), $CRIL_{05}V_9$ (c), $CRIL_{07}V_7$ (d), $CRIL_{07}V_9$ (e), $CRIL_{09}V_9$ (f), at leaf # 09 at grain filling stage ($CRIL_{09}GFS$) (g) and at leaf # 11 at corn tassel emission ($CRIL_{11}CTE$) (h) as a function of maize hybrids and N doses applied in bands. $^{*}p< 0.05$ and ** $p < 0.01$.

correlation established between $CRIL_{05}V_7$ readings and leaf N content, as well as productivity (Table 3).

The P30R50 hybrid showed a $CRIL_{05}V_9$ 3.8% higher in relation to the one yoked to the AG8025 hybrid, corresponding to a CRI difference of 2.33 (Table 2). Genetic variability between maize hybrids and discrepancies in chlorophyll synthesis was also observed by Sunderman et al. (1997) and Subedi and Ma (2005). In so far as N in bands increased up to an estimated dose of 325 kg of N ha^{-1} $CRIL_{05}V_9$ increased as well, resulting in a fraction of 66.73 of $CRIL_{05}V_9$ (Figure 1c). $CRIL_{05}V_9$ was associated to a higher coefficient of correlation concerning leaf N content (0.72^{**}) rather than with yield (0.57**) (Table 3).

Nitrogen doses unfolding for $CRIL_{07}V_7$ allowed to identify the differential responsiveness of hybrids to the N doses applied in bands, since the P30R50 hybrid responded well up to an estimated dose of 205 kg of N ha^{-1}, which would be corresponding to a CRI of 65.33, and the AG8025 hybrid responded linearly to the increasing N doses applied (Figure 1d). The estimated value for the hybrid P30R50 was superior to the mean value of 59.7, a fact that was evidenced also by Hurtado et al. (2010) between V_7 and V_8.The correlation of $CRIL_{07}V_7$ with leaf N content and yield was of 0.39^{**} and 0.33^{**}, respectively, being therefore quite below the one for $CRIL_{05}V_9$ (Table 3).

Faced with the unfolding of N doses applied in bands within maize hybrids for the variable $CRIL_{07}V_9$, P30R50 did not respond to the N doses, leading to a mean CRI of 65.3, whereas for the AG8025 hybrid there was a consistent rise in the $CRIL_{07}V_9$ up to an estimated dose of 245 kg of N ha^{-1} when CRI was expected to be of 69.29 (Figure 1e). The coefficient of correlation between $CRIL_{07}V_9$ and leaf N content and yield was the same (0.58^{**}) (Table 3).

The differentiated responsiveness of $CRIL_{07}$ for the hybrids at all N doses observed between V_7 and V_9stages might be ascribed to the fact that AG8025 hybrid was more productive than P30R50 (Table 2), once for such a genotype $CRIL_{07}$ between V_7 and V_9 was roughly the same. Such a similarity was established throughout the time and for the AG8025 hybrid an increment of 3.9 was achieved in such a fashion as to enhance the positive correlation between the CRI and leaf N content and yield at the stage V_9 in comparison to V_7 (Table 3). The difference between the CRI values estimated at F_{07} between the Stages V_7 and V_9 for the AG8025 hybrid demonstrated that there was an increase in the chlorophyll synthesis due to N incorporation into the tissues.

$CRIL_{09}V_9$ readings obtained for the P30R50 hybrid were not remarkably influenced by the N doses, showing an average of 58.43, whereas for the AG8025 hybrid $CRIL_{09}V_9$ expressed an increasing linear response to the N doses (Figure 1f). $CRIL_{09}V_9$ was strongly correlated to leaf N content in 0.41^{**} (Table 3). The lack of correlation between $CRIL_{09}V_9$ and yield might be attributed to the fact that CRI readings for the P03R50 hybrid were not influenced by the N doses, in spite of the linear responsiveness of the AG8025 hybrid. Nevertheless, low amplitude of $CRIL_{09}V_9$ among the maize hybrids was observed herein. Such a fluctuation translates that while for the P30R50 hybrid the mean value was of 58.43, the estimated $CRIL_{09}V_9$ in 61.7 by means of the fitted equation with a dose of 375 kg of N ha^{-1} would have been obtained for the AG8025 hybrid.

The mean value of $CRIL_{09}V_9$ was of 57.85 irrespective of the genotype and N dose to be applied, being higher in 9.15% to that one observed by Godoy et al. (2007) between V_8 to V_9, as well as lower in 8.03% of that obtained by Hurtado et al. (2010) between V_9 and V_{10}. The comparisons revealed that the indicator leaf of the phenological stage, no matter how young it is, usually does not respond to N applied in bands as to the CRI likely due to its utilization firstly for cellular division and differentiation. The $CRIL_{09}GFS$ regardless of the hybrid responded linearly to the increment of N applied in bands (Figure 1g). $CRIL_{09}GFS$ readings were positively correlated to both leaf N content and yield, showing coefficients of 0.73^{**} and 0.67^{**}, respectively (Table 3).

The AG8025 hybrid responded in an increasing manner up to the estimated dose of 250 kg of N ha^{-1} for the $CRIL_{11}CTE$, corresponding to a CRI of 72.66. The $CRIL_{11}CTE$ obtained for the P30R50 hybrid increased linearly with the increment of N applied in bands (Figure 1h). Since N takes part in the chlorophyll molecule with an overall of four atoms, it is well known that a rise in the requirements of N to the plants up to a certain point provides an increase in the tonality of the green color of the leaves, which will increase the chlorophyll content or CRI, as well as leaf N content and yield. Such a fact might be confirmed by means of the correlation analysis, once the coefficients between $CRIL_{11}CTE$ and leaf N content and yield were of 0.63^{**} and 0.60^{**}, respectively (Table 3).

Argenta et al. (2003) obtained a CRI of 58 in the leaves below and opposite to the stalks at the filling physiological stage of maize crop. Therefore, the mean $CRIL_{11}CTE$ of 67.84 was 16.96% higher. Chlorophyll synthesis is triggered by the light, being iron the co-factor of some enzymes, depending upon N, Mg and carbonic skeletons from the photosynthesis itself, in conjunction with the extrinsic factors (availability of N, Mg, Fe, water, solar radiation, photoperiod, management practices, etc.) and intrinsic factors (genetic variability). Water availability was an average of 8 mm day^{-1} throughout the month of December during the CTE stage. According to Matzenauer et al. (1998) such a water supply to the maize plants coincides with the crop needs and contributes to the plants in order to synthesize and maintain a high content of chlorophyll, expressed by the CRI.

The AG8025 hybrid is the one that would reach a

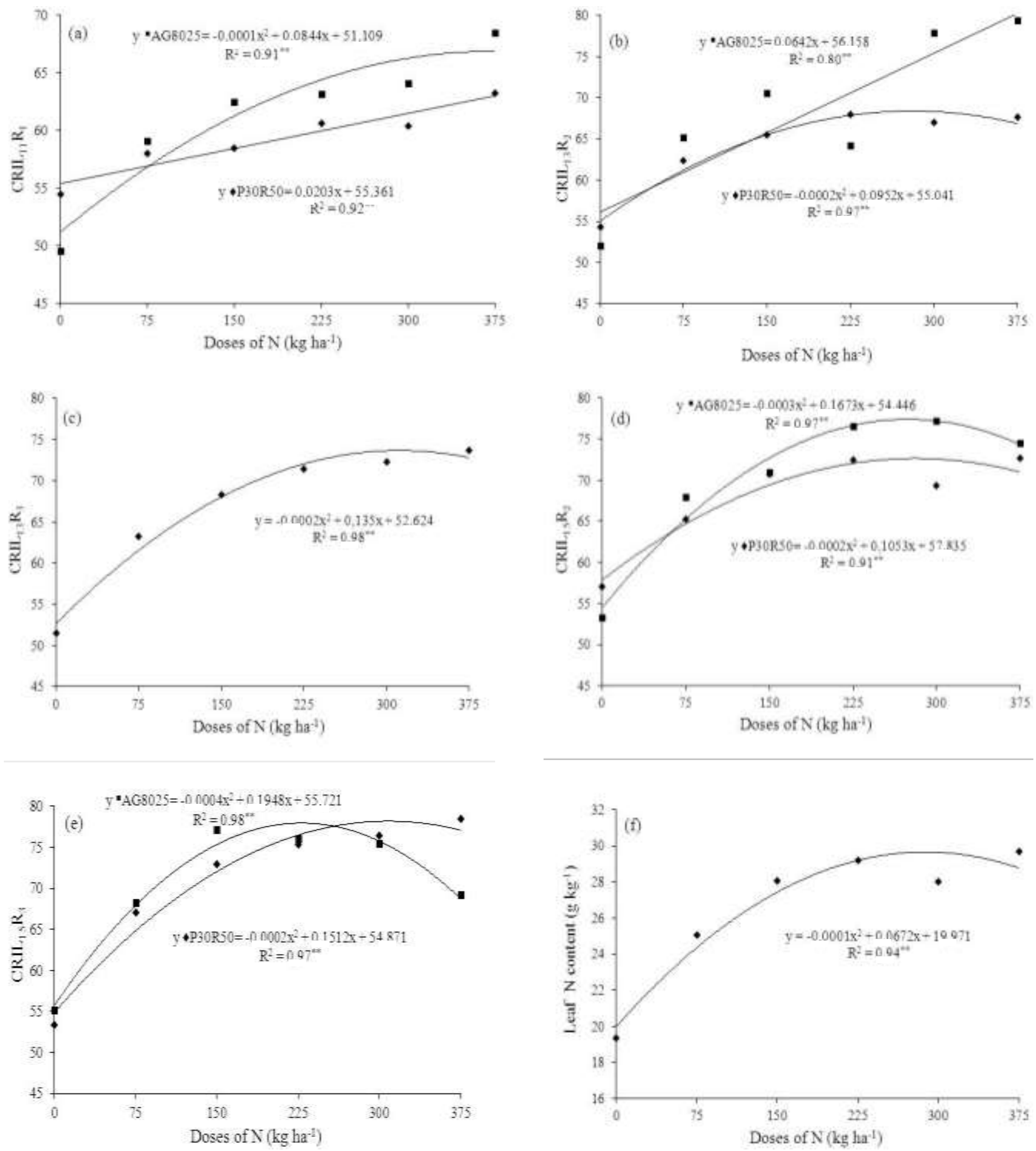

Figure 2. Leaf chlorophyll relative index at the leaf #11 at the phenological stage R_1 ($CRIL_{11}R_1$) (a), $CRIL_{13}R_2$ (b), $CRIL_{13}R_3$ (c), $CRIL_{15}R_2$ (d), $CRIL_{15}R_3$ (e) and leaf N content (g kg^{-1}) at reference leaf determined at phenological stage R_1 (f) as a function of maize hybrids and doses of N applied in bands. ** $p< 0.01$.

technical efficiency maximum dose (TEMD) in 375 kg of N ha^{-1} under an estimated $CRIL_{11}R_1$ of 68.70 (Figures 1h), and for the P30R50 hybrid there was an increment of the $CRIL_{11}R_1$ with N doses applied in bands (Figure 2a).

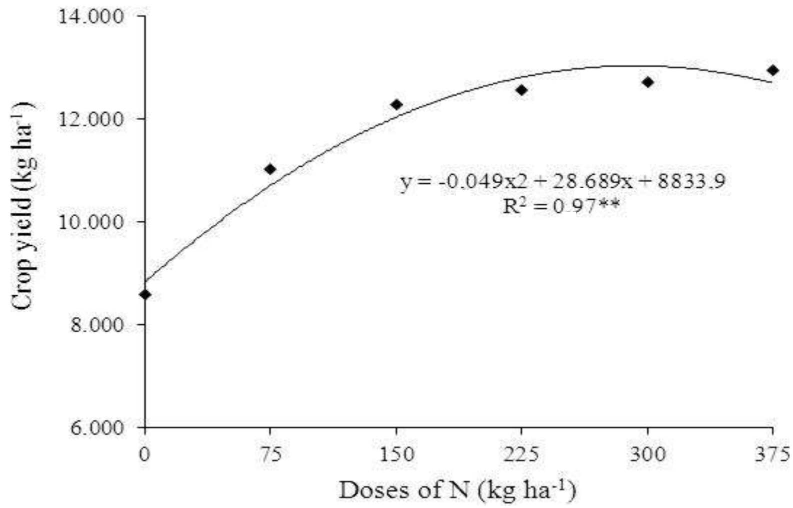

Figure 3. Maize crop yield (kg ha^{-1}) as a function of doses of N applied in bands. ** $p< 0.01$.

The Pearson correlation coefficients between $CRIL_{11}R_1$ and leaf N content and yield were of 0.67^{**} and 0.82**, respectively (Table 3).

The mean value found for $CRIL_{11}R_1$ was of 60.13, an index higher than the mean CRI of 57.9 and lower than the maximum CRI of 68.9, as evidenced by Sunderman et al. (1997) for hybrids of maize at the same phenological stage under controlled water supply conditions. Such environmental conditions led to a mean yield of 14.455 kg ha^{-1}, showing a significant superiority to the maximum estimated yield observed in the current trial as 13.033 kg ha^{-1} (Figure 3).

For the variable $CRIL_{13}R_2$the unfolding of the interaction revealed a squared response for the P30R50 hybrid, having estimated a technical efficiency maximum dose in 235 kg of N ha^{-1} and a CRI in 66.37 (Figure 2b). The Pearson correlation coefficients between $CRIL_{13}R_2$ and leaf N content and yield were of 0.65^{**} and 0.80^{**}, respectively. The $CRIL_{13}R_3$ obtained for the AG8025 hybrid was higher in 4.37 for the P30R50 hybrid, corresponding to a value of 6.77% (Table 2). Regression analysis on the $CRIL_{13}R_3$ allowed to estimate the dose that would be quite conducive to the highest $CRIL_{13}R_3$ (75.4) as the one equivalent in 335 kg of N ha^{-1} (Figure 2c). In the current study, there was a positive correlation of the $CRIL_{13}R_3$ with the leaf N content given by a Pearson correlation coefficient of 0.69^{**}, along with a higher correlation coefficient of 0.86^{**} for yield. Hurtado et al. (2010) obtained in R_3 a CRI of 65.6, having been similar to the mean CRI of 66.76, as well as to the values obtained for each one of the hybrids in study (Table 2), but lower than the estimated value as a function of N doses (Figure 2c).

The highest $CRIL_{13}R_3$ obtained for the AG8025 hybrid evidences a more accentuate chlorophyll synthesis (Table 2). The fact that the plants show a higher content of chlorophyll, mainly of chlorophyll a, results in the highest photosynthetic rates. Consequently, yields will be the highest as shown in Table 3, for the AG8025 hybrid was the most productive. In order to reinforce such evidence in terms of physiological responsiveness, it may be observe in Table 3 that the highest Pearson correlation coefficient obtained for yield was just corresponding to the $CRIL_{13}R_3$, indicating that F_{13} is an important resource of photoassimilates for the grains.

Those doses estimated by means of equations that would be conducive to the maximum $CRIL_{15}R_2$ were of 260 and 275 of N ha^{-1} for the P30R50 and AG8025 hybrids, respectively, corresponding to a CRI of 71.69 and 77.77 (Figure 2d). The effect of N doses applied in bands for each hybrid on the $CRIL_{15}R_3$, shortly after the unfolding of the interaction, allowed to identify the doses of 375 and 245 kg of N ha^{-1} as being those that caused the P30R50 and AG8025 hybrids to present the highest values of $CRIL_{15}R_3$ (83.45 and 79.44, respectively) (Figure 2e).

CRI increased significantly throughout the maize crop growth season, corroborating the outcomes obtained by Argenta et al. (2010). Moreover, the results found in the current research, irrespective of the maize hybrid, leaf or phenological stage of the crop, showed higher CRI values than those obtained by Argenta et al. (2010), Godoy et al. (2007), Hurtado et al. (2009) and Hurtado et al. (2010). Fluctuations in the values of CRI reported by the literature might be ascribed to the equipment used, age of the leaf, position of the leaf, position reading on the leaf, phenological stage, cultural practices, and soil and climate conditions.

The mean leaf N content of 26.56 g kg^{-1} or 2.66% of the DPS determined in the reference leaf R_1 was found to be roughly above the adequate threshold (27.5 to 32.5 g kg^{-1}) in agreement with Malavolta et al. (1997). Ferreira et al. (2009) in a site under no-tillage system for 18 years observed for the AG9020, AG6018 and AG8021 hybrids mean leaf N contents of 23.17, 24.13 and 25.53 g kg^{-1}, respectively. Such indices were also above the range prescribed by Malavolta et al. (1997).

The leaf N content determined at R_1 was influenced by the N doses applied in bands (Figure 2f) without therefore expressing interactions with maize hybrids. Increments in N supplementation up to the estimated dose as TEMD was of 330 kg of N ha^{-1}, in which the reference leaf would reach a leaf N content of 31.26 g kg^{-1}. Hurtado et al. (2009) obtained a leaf N content of 30.25 g kg^{-1} by the time TEMD for yield was replaced with 242 kg of N ha^{-1}, corresponding therefore to a yield of 9.210 kg ha^{-1}. In the current work the estimated dose for maximum yields was of 295 kg of N ha^{-1}, culminating to a yield of 13.033 kg ha^{-1}. However, when such a dose was replaced with the one obtained by the fitted equation for the leaf N content (Figure 2f) in compliance with the proposition of Hurtado et al. (2009), the leaf N content to be obtained would be of 31.09 g kg^{-1}, reaching an index quite similar to that found by the aforementioned authors, but with a difference of 3.823 kg ha^{-1} in the grain yield.

By assessing four distinct field experiments, Argenta et al. (2010) obtained the following Pearson correlation coefficients between the readings of CRI performed in the reference leaf corresponding to the filling stage and maize yield: 0.69^{*}, 0.80^{*}, 0.87^{*} and 0.93^{*}. Table 3 reveals that readings of $CRIL_{11}CTE$, $CRIL_{11}R_1$, $CRIL_{13}R_2$ and $CRIL_{13}R_3$ were associated to Pearson correlation coefficients of 0.60^{**}, 0.82^{**}, 0.80^{**} and 0.86^{**}, respectively. The correlation coefficient of 0.69^{**} obtained between leaf N content and yield was lower than those coefficients reported by Argenta et al. (2002) in their four field trials (0.73^{*}, 0.76^{*}, 0.83^{*} and 0.91^{*}).

Argenta et al. (2010) obtained better correlations between CRI and leaf N content at more advanced phenological stages of the crop. Nevertheless, it can be noticed that such correlations get stronger with the physiological age of the leaves. This was observed during three evaluations at F_{05} for there was an increase of the correlation at the stage V_5 in relation to the V_7, as well from such stage to V_9 when the leaves 7, 9, 11 and 13 expressed the same behavior. This physiological responsiveness allows obtaining high correlations throughout the initial crop growth phases, such as vegetative phenological stage. Thus the observation on the age of the leaves for the assessment of CRI readings infers the efficiency of evaluation of N status in corn plants by means of CRI, regardless of the phenological stages related to vegetative and reproductive phases (Table 3).

The AG8025 hybrid productivity was higher than that of P30R50 in 1.510 kg ha^{-1}, corresponding to a difference of 13.8% (Table 2). Argenta et al. (2010) obtained a difference of 2.900 kg ha^{-1} between the P32R21 and Premium hybrids with final yields of 12.400 and 9.500 kg ha^{-1}, respectively. Discrepancies in productivity among maize hybrids were also found by Ferreira et al. (2009), whose outcomes report that hybrids such as AG9020 and AG8021 were more productive than AG6018.

The identification of the dose 295 kg of N ha^{-1} that would express maximum yields, equivalent to 13.033 kg ha^{-1} (Figure 3), corroborates the assumptions of Fontoura and Bayer (2009), since both scientists report that for yields above 12.000 kg ha^{-1} it is necessary an application within the range from 130 to 300 kg of N ha^{-1} in bands. The maximum yield estimated in 9.210 kg ha^{-1} obtained by Hurtado et al. (2009), which corresponded to 242 kg of N ha^{-1} in bands, resulted in a saving of 53 kg of N ha^{-1} causing therefore a reduction of 3.823 kg ha^{-1} in the productivity of grains. Different results reported by the literature might be attributed to differences in climate and soil conditions, management and cultural practices among studied sites in conjunction with genetic variability among hybrids. An inferior responsiveness was also found by Silva et al. (2005) with a TEMD estimated in 166 kg of N ha^{-1}, leading to a final yield of 6.709 kg ha^{-1}.

Over three years of evaluation, Holand and Schepers (2010) evidenced that the dose of 200 kg of N ha^{-1} brought about yields of 11.530, 12.110 and 13.660 kg of N ha^{-1} for maize crops. In the work published by Sangoi et al. (2009) the application of two doses of 100 kg of N ha^{-1} in bands at the phenological stages V_4 and V_{10} resulted in a yield of 12.634 kg of N ha^{-1} for the P30F53 simple hybrid. Such outcome was quite similar to that obtained for the AG8025 hybrid (Table 2) and for the maximum estimated yield with the dose of 295 kg of N ha^{-1} in bands (Figure 1) but with only one application at V_5. This appears to be an outstanding observation to surmise because P30F53 simple hybrid reveals an important participation in cultivated areas of the region of Campos Gerais of Paraná in order to assure the sustainability of agriculture in the southern regions of Brazil.

Conclusions

CRIs determined in the same leaf and at different phenological stages revealed that there is a rise in the values of such a physiological parameter, although the hybrids of maize did respond differently to the doses of N applied in bands. The correlation between CRI and leaf N content, as well as yield, increases with the age of the leaves, being useful to indicate the status of N in maize crop fields. A positive correlation between CRIs and leaf N content along with yield in different leaves and at different phenological stages made possible the identification of CRI for diagnosis of N status in maize hybrids with mean values ranging from 46.87 ($L_{03}V_5$) to 70.40 ($L_{15}R_3$). The necessity of N can be identified by means of CRI right on the initial stages of crop development (V_5, V_7 and V_9), causing its utilization to be rather feasible for precocious diagnosis of N status in maize crop. Moreover, when CRI determinations are performed at the end of vegetative stage, as well as throughout their productive phase it might indicate nitrogen fertilization efficiency in bands.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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