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Chlorophyll meter reading and total nitrogen content applied as topdressing in parts of the crambe plant

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The present study was aimed to assess the relationship between the reading of the chlorophyll meter and the total nitrogen (N) content in the leaf in different parts of the crambe plant, depending on the doses of nitrogen applied to the canopy. Randomized block design in a split-plot experimental design was used. The plots were composed by five levels of topdressing with nitrogen and the subplots composed of three parts of the plant. The analyzed variables were the values of the simple handheld devices to measure chlorophyll index (SPAD) and N content in the leaf. The nitrogen levels were found to influence the total chlorophyll and nitrogen contents, but there was no correlation between chlorophyll and N contents in the crambe. The middle third of the plant during early flowering was found to be the most suitable location for the analysis of foliar nitrogen in the crambe.

Key words: Nitrogen (N), chlorophyll meter, topdressing.

INTRODUCTION

The crambe (*Crambe abyssinica* Hochst) is an oleaginous plant belonging to the family of Cruciferae, which is characterized by a short cycle, and can be grown in Brazil during the second winter crop (Pitol et al., 2010). However, there is little technical information on its cultivation, for example, criteria for liming and fertilization (Silva et al., 2013). It is known that the availability or supply of nutrients to plants is critical, particularly nitrogen, which is usually the element required in greatest quantity (Taiz and Zeiger, 2006). Nitrogen deficient plants have leaves with color between pale-green and yellowish. Nitrogen is necessary for chlorophyll synthesis and being a part of this molecule is involved in photosynthesis (Epstein and Bloom, 2006).

In the absence of nitrogen, the plant degrades the molecule of chlorophyll, re-translocating this element to regions of active growth where it performs its functions (Malavolta et al., 1997). Thus, if nitrogen deficiency persists, the photosynthetic activity of the plant is impaired, which impacts essential functions such as the

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Table 1. Result of the chemical analysis of the soil used in the experiment at Umuarama-Pr in 2012.

pH	P	C	Ca+Mg	K	Al	CTC	SB	V	Fe	Cu	Mn	Zn	B	S
($CaCl_2$)	(mg dm^{-3})	(g dm^{-3})	(cmol$_c$ dm^{-3})					(%)	(mg dm^{-3})					
4.40	5.10	4.48	2.38	0.05	0.10	6.71	2.43	36.18	48.5	6.1	127.7	13.82	0.4	12.3

absorption of other nutrients and the production of carbohydrates.

The methods traditionally used for determining the chlorophyll content in leaves are destructive and not practical (Godoy et al., 2003). With the invention of the direct-reading portable meter that estimates the chlorophyll content (Minolta, 1989), by measuring the intensity of the green color of the leaf (Simple handheld devices to measure chlorophyll index - SPAD), it is possible to obtain field values instantaneously and non-destructively (Godoy et al., 2003).

Souza et al. (2011) using indirect measure of chlorophyll in citrus plants observed a significant correlation between the indirect measure of chlorophyll and nitrogen (N) content in the leaf. They concluded that the indirect measure of chlorophyll can be used as a fast and non-destructive tool in monitoring and evaluation of nitrogen availability.

Rigon et al. (2012) assessed the relationship between photosynthetic pigments extracted in the laboratory and readings obtained by the portable chlorophyll meter in sesame leaves. They concluded that the referred portable meter can be used to estimate the concentration of photosynthetic pigments with high accuracy, providing resources and time saving.

In their analysis of the relationship between nitrogen nutritional indexes and the productivity of corn grains, in two levels of management, under nitrogen fertilization, Rambo et al. (2011) noticed that the measurement of chlorophyll was related to productivity. Barbosa Filho et al. (2008) and Maia et al. (2012) reported that indirect readings of chlorophyll may indicate the period of greatest demand for nitrogen and whether or not this nutrient should be applied as topdressing.

Silva et al. (2012) assessed the correlation of the nitrogen content in the aerial part of the crambe and the reading performed by the chlorophyll meter regarding the nitrogen topdressing. The authors did not find a significant correlation between the parameters. The authors did not find a significant correlation between the measured parameters. However, Nascimento and Marenco (2010) observed that SPAD values may vary in leaves with high levels of chlorophyll, probably due to the uneven distribution of chloroplasts in bright sunlight.

Some criteria were established for visual analysis, such as vegetative stage and site of plant collection (Malavolta et al., 1997). For example, in their attempt to determine the relationship between chlorophyll content in the upper leaves and severity of panicle blast on rice, Silva-Lobo et al. (2012), performed readings of the chlorophyll content in flag leaf. In the determination of the optimal dose of N for the production of mini tubers of potato seed, Souza et al. (2013) measured the SPAD index with the portable chlorophyll meter on the fourth leaf and found that the dose varies with the age of the plant. Maia et al. (2012) assessed the nitrogen content in the aerial part and the SPAD index with the portable chlorophyll meter in the middle third of bean plants. Such information is not available for the crambe though.

The present study was aimed to assess the relationship between the readings provided by the chlorophyll meter and the total N content of the leaves collected from different parts of the crambe plant, as well as to identify the ideal location for collecting these data, according to the levels of nitrogen applied in topdressing.

MATERIALS AND METHODS

The experiment was conducted at the farm of Universidade Estadual de Maringá, Regional Campus of Umuarama-PR, in a greenhouse measuring 6.6 × 27.8 m, right foot in galvanized steel 3.7 m high and arched roof, built with galvanized steel pipes, covered with 150 m thick transparent polyethylene film and overlapping with 50% shade cloth. The sides are surrounded with plastic film up to 2.0 m, and above this height a curtain hung in a lateral opening. The geographic coordinates are: 53º 18' 48 west longitude, 23º 47' 55 south latitude and 430 m altitude. The local climate is humid subtropical (cfa).

The soil used in the vases was collected in the farm and is typical oxisol (Embrapa, 2013), and the characteristics are shown in Table 1. The correction was performed considering soil analysis, and was based on the indications of Pitol et al. (2010) and a study of Silva et al. (2013).

The sowing of the crambe, cultivar *FMS-brilhante*, was performed on February 22, 2012, in polyethylene pots with capacity of 9 dm^3 of soil. Pruning was performed 15 days after the emergence of the plants, with two plants per pot until flowering. Hand weeding for weed control was also performed in this period.

The experimental design was a randomized block design with four replicates in a split plot design. The plots were composed by five levels of topdressing with nitrogen (0, 50, 100, 150 and 200 mg L^{-1}), manually performed 21 days after sowing, with urea as a source, and the subplots composed of three parts of the plant (lower third, middle third and upper third).

Reading of the values of the SPAD index (chlorophyll) was done: the index was determined by the portable meter Chlorophyll Meter, model SPAD-502 (Minolta, 1989). The values obtained in the chlorophyll meter reading are proportional to the chlorophyll content in the leaf because the wavelength bands used in the equipment were based on the two absorbance peaks of chlorophyll "*in vitro*"(Godoy et al., 2003). Its use requires, among other requirements, that foliar N is the only variable that influences chlorophyll content, because, according to Malavolta et al. (1997),

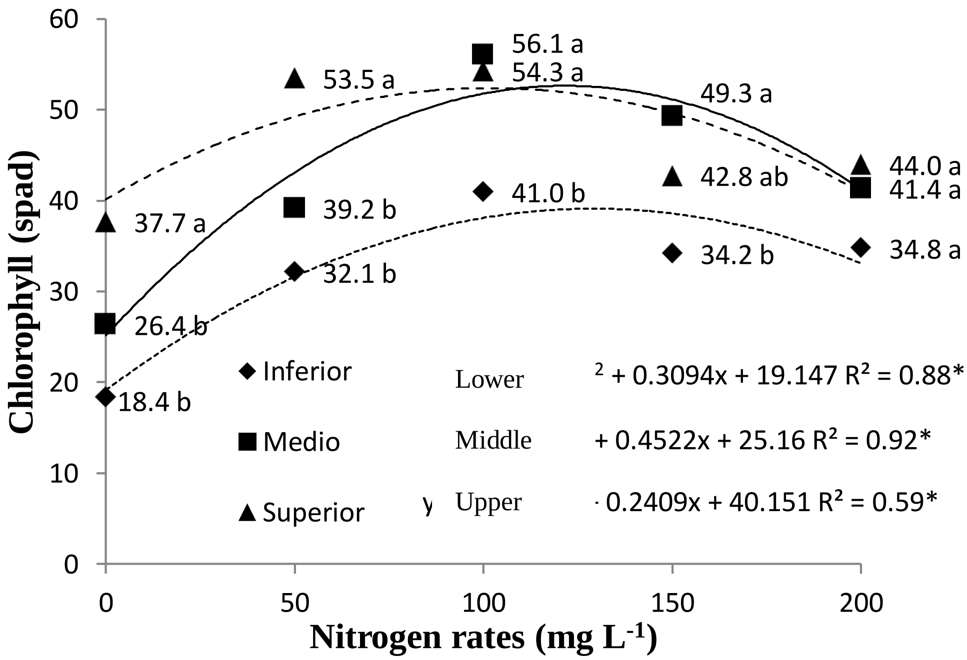

Figure 1. Chlorophyll content (SPAD) in three parts of the crambe plant (lower, middle and upper third) under application of nitrogen doses as topdressing at Umuarama (PR) in 2012. * = significant at 5% probability. Means followed by the same letter, within each dose do not differ by Tukey test at 5% probability. C.V. (Plot) = 21.3%; C.V. (Subplot) = 13.3%.

almost all deficiencies and in some cases the excesses cause chlorosis.

The readings were made in the morning, in the shade, to avoid direct incidence of sunlight on the chlorophyll meter. Two plants per pot were used, with three points per plant, and necrotic areas or areas attacked by pests and diseases, leaf edges and midrib were avoided. The phenological stage of the plant during sampling was early flowering.

The total nitrogen content in the leaf was assessed: immediately after the reading of the values of the SPAD index, the plants were cut in three parts, packed in paper bags and placed in an oven at a temperature of 60°C, with forced air circulation for 48 h. Then, the material was ground using a Willey mill, for analysis of N content, according to the method described by Malavolta et al. (1997).

The data were subjected to analysis of variance. Tukey test at 5% was performed for secondary treatment of the portion of the plant, while analysis of linear and quadratic regressions was performed for the N levels in topdressing (main treatments), and regressions with higher coefficient of determination (r^2) selected among those found to be significant by F test. The results obtained with the values of the SPAD index and the total N content in the leaf were also subjected to the study of simple linear correlation (r).

RESULTS AND DISCUSSION

As seen in Figure 1, increase in the nitrogen level led to an increase in the relative content of chlorophyll in the parts of the plant until a maximum point was reached, which occurred, for the lower part at the dose of 128.01 mg L^{-1}, middle part at 119 mg L^{-1} and upper part of the plant at 100.37 mg L^{-1}. From these points there was no increase in the chlorophyll values for any part of the plant. These results confirm the relationship between nitrogen and chlorophyll, where the greatest concentration of N promoted the increase in chlorophyll concentration to the point of maximum efficiency technique (PMET), since the portable meter SPAD-502 cannot identify the luxury consumption of N. The explanation for this is that the device measures the intensity of the green color, and nitrogen not incorporated into chlorophyll molecules will not reflect the change in the intensity of this color (Godoy et al., 2003; Rigon et al., 2012).

This fact corroborates those of Souza et al. (2011) who reported that nitrogen participates in the synthesis and structure of molecules of chlorophyll, so that increasing the N supply to plants, to a limited extent, increases the amount of chlorophyll and the intensity of green color in the leaves.

With nitrogen doses of 0 to 150 mg L^{-1}, the upper third of the plant showed the greatest chlorophyll content according to the SPAD index (Figure 1). This indicates that metabolic activity is greater in younger regions of the plant (Malavolta et al., 1997; Epstein and Bloom, 2006; Taiz and Zeiger, 2006). At the nitrogen dose of 200 mg L^{-1} there was no difference in the chlorophyll content for all the parts of the plant, probably because the dose is high, culminating in equal coloring of the leaves of crambe.

Figure 2 shows that the application of nitrogen doses as topdressing changed the N content in the leaves in the entire aerial part of the crambe, given the increased

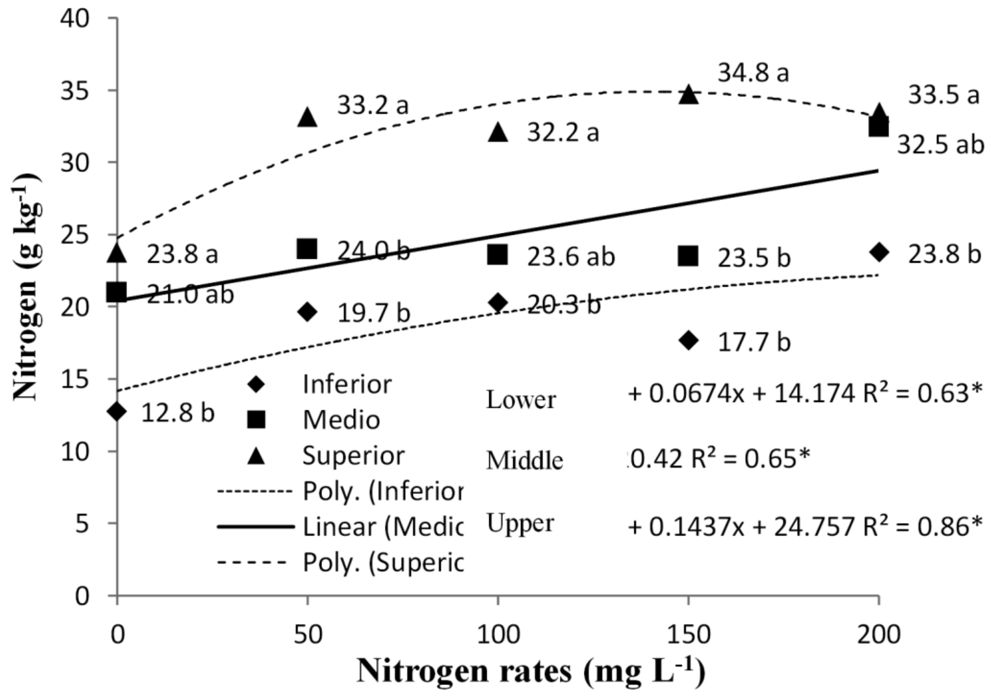

Figure 2. Nitrogen content (g kg^{-1}) in three aerial parts of the crambe plant (lower, middle and upper third) under application of nitrogen doses as topdressing at Umuarama (PR) in 2012. * = significant at 5% probability. Means followed by the same letter, within each dose, do not differ by Tukey test at 5% probability. C.V. (Plot) = 20.1%; C.V. (Subplot) = 21.2%.

availability of the element in the soil, leading to greater absorption. However, the points of maximum efficiency technique of nitrogen varied widely between the parts collected for analysis.

In the lower third, the PMET was 337 mg L^{-1}, that is, higher than doses applied. This occurred because the lower third was already in vegetative decline, because the bodies were older, and the nitrogen had already been trans-located to regions that were in greater need of the nutrient, which corroborates the statements of Malavolta et al. (1997), and Esptein and Bloom (2006), who reported the translocation of mobile elements from old to new bodies.

In the upper third, PMET was 143.7 mg L^{-1}, demonstrating that there is luxury consumption of nitrogen above this value (Godoy et al., 2003; Rigon et al., 2012).

In the middle third, the nitrogen doses were adjusted in linear regression, that is, in this part of the plant there was no luxury consumption. Therefore, the higher the dose of nitrogen, the higher the content of this element in plant tissue. This fact was observed in other cultures such as beans (Maia et al., 2012), corn (Rambo et al., 2011), citrus (Souza et al 2011), millet (Lima et al., 2007) and rice (Silva-Lobo et al., 2012).

In Figure 2 it can also be seen that the nitrogen content in the leaf of the upper third of the crambe plant was significantly higher than in the lower third, while intermediate values were observed in the middle third, for all doses. The explanation for this, as already reported, is that N is translocated to regions of the plant in greater need of the nutrient (Malavolta et al., 1997; Epstein and Bloom, 2006; Taiz and Zeiger, 2006).

There was no significant correlation between nitrogen content in the leaf and chlorophyll (SPAD) in the aerial parts of the plant (A= lower third, B = middle and C= upper) as it can be seen in Figure 3. Silva et al. (2012) in a study of N fertilization in crambe plants, also failed to find a correlation between nitrogen content and indirect measure of chlorophyll. Lavres Junior et al. (2005) in an experiment to evaluate macronutrient deficiencies in the nutritional status of castor bean cultivar Iris observed that the deficiencies influenced chlorophyll meter readings, though without correlation.

The middle third of the plant was more constant in the assessments, or else, chlorophyll contents were average and nitrogen content increased depending on the doses applied, which was expected. (Maia et al., 2012, Silva et al., 2013), thus, this region of the plant is representative of its real metabolic activity. There are recommendations on the ideal location for collecting many plant species. For beans, the ideal location is the middle third, in corn the location is the leaf above and opposite the cob (Malavolta et al., 1997). Therefore, in what regards the crambe plant, it can be inferred that the middle third is the ideal location for collecting leaves in the nutritional analysis.

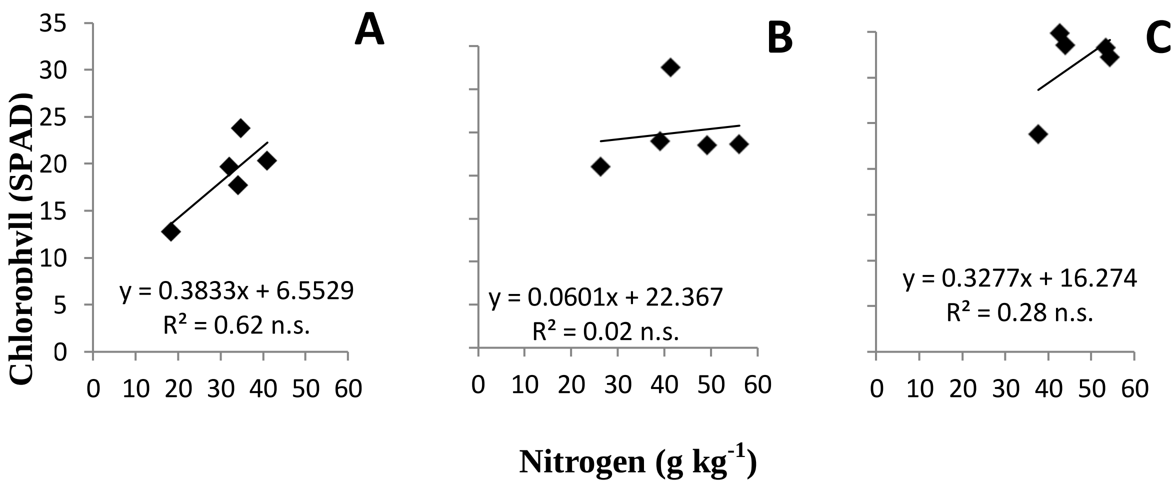

Figure 3. Linear correlation between the nitrogen content (g kg^{-1}) and chlorophyll (SPAD) in three aerial parts of the crambe plant (A= lower third, B = middle and C = upper) under application of nitrogen doses as topdressing. Umuarama (PR) – 2012. n.s. = non significant at 5% probability.

Conclusion

The nitrogen doses influenced the amount of chlorophyll and nitrogen in the leaves. There was no correlation between chlorophyll and N content in the leaves of crambe plants. The middle third during the flowering of crambe was found to be the most suitable location for evaluations of foliar analysis.

Conflict of interests

The authors have not declare any conflict of interest.

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