







Division - Soil Use and Management | Commission - Soil Fertility and Plant Nutrition

# Nitrogen doses and nutritional diagnosis of virus-free garlic

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**ABSTRACT:** The recommendations of nitrogen (N) fertilization in garlic are still based on different varieties of the current types that are infected with phytopathogenic virus. There are several methods for recommendation of nitrogen (N) fertilization in garlic, but there are not enough methods for N diagnosis in garlic obtained by meristem culture. The objective of this work was to evaluate methods for diagnosing the nutritional status of virus-free garlic subjected to N doses through the use of a specific  $\text{NO}_3^-$  meter in soil solution and foliar sap, portable chlorophyll meter, N content in the leaf, and its relationship with yield and quality of the bulbs. The experiments were conducted with the use of virus-free seed bulbs from the meristem culture from three sites in the 2015 growing season and two locations in the 2016 growing season in South Brazil. The treatments consisted of the application of five nitrogen doses (0, 100, 200, 300, and 400  $\text{kg ha}^{-1}$ ) distributed in three applications during the crop cycle: 1/3 in planting, 1/3 between 30 and 40 days after planting, and 1/3 after visual bulb differentiation. The highest commercial yield was associated with doses between 269 and 307  $\text{kg ha}^{-1}$  of N and the content of 26  $\text{g kg}^{-1}$  of N, in the diagnostic leaf. The relative chlorophyll content was the only diagnostic technology that showed a significant correlation with commercial yield in all experimental conditions. The evaluation of the N status in the virus-free garlic crop by a portable chlorophyll meter can be a quick strategy for recommending N fertilization and ensuring high yields.

**Keywords:** *Allium sativum*, relative chlorophyll content, soil solution, nitrate in foliar sap, nitrate in soil solution.

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## INTRODUCTION

The recommendations of nitrogen (N) for the cultivation of garlic in the South of Brazil are based on the use of cultivars infected with phytopathogenic virus. The presence of virus in plants causes disturbances in the functions of cells, affecting the synthesis of proteins, inhibiting photosynthesis and transport of assimilates, limiting the action of growth regulators, and reducing the production (Kim et al., 2013). Currently, garlic cultivars developed in southern Brazil have undergone virus cleaning by meristem cultivation, which presents significant increases in vegetative vigor, productivity, and bulb quality (Santos et al., 2017). In spite of the significant contribution of the viral cleansing technique, recommendations for N fertilization are not established for these new cultivars under different climatic and soil conditions, especially for the largest producing region in South Brazil.

Nitrogen is the nutrient that contributes most to the increase in the bulb productivity of garlic (Santos et al., 2017). Nitrogen application in inappropriate doses and phases of the garlic development may compromise the number of leaves and bulbs, which will reflect in the quality and productivity. However, Macêdo et al. (2009) emphasize that garlic is extremely sensitive to the minor excess of N, which can induce secondary bulb growth. This physiological disturbance is a process of swelling in the region between the false stem and the true stem, due to the accumulation of reserves at the base of the leaf sheaths, resulting in the formation of a false-bulb (Kim et al., 2013). In addition to excess N, low temperatures and high precipitation also stimulate super-sprouting (Wu et al., 2016).

Given the importance and sensitivity of garlic to N, the application of N should also take into account appropriate diagnostic technologies. Leaves are often employed for assessing garlic nutritional status because, in these organs, main metabolic activities occur and, therefore, they better reflect the variations in nutrient availability. The foliar analysis allows the evaluation and adjustment of the agricultural fertilization practices and is both low cost and efficient in determining the nutritional status of the plant (Arrobas et al., 2018). However, the delay in receiving the analysis may hinder the application of the method for N fertilization in the growth cycle of garlic. From this, alternatives such as the use of real-time diagnostic methods using the plant or soil as indicators have emerged, allowing a rapid evaluation (Westerveld et al., 2003). These tools can be suitable for the diagnosis of the nutritional status of the plants, allowing adjustments in the fertilization application during the crop in which the monitoring was carried out (Zheng et al., 2015; Padilla et al., 2017). The evaluations of relative chlorophyll content in the leaf, nitrate in the foliar sap, and in the soil solution are rapid tests and can be carried out in the field, allowing real-time interpretation of the nutritional status of the plants (Reis et al., 2009).

The shortage of consistent information on N fertilization of the virus-free garlic crop constitutes a major obstacle to the exploitation of this crop more effectively. Additionally, several tools and methodologies for the diagnosis of plant nutritional status are currently available, but few are verified and validated for virus-free garlic. In view of this, the objective of this work was to evaluate the use of technologies of nutritional diagnosis of N from virus-free garlic plants subjected to N doses through the use of a portable chlorophyll meter, specific  $\text{NO}_3^-$  meter in soil solution and foliar sap, electrical conductivity of the soil solution, foliar N content, and its relationship with yield and quality of bulbs.

## MATERIALS AND METHODS

Five experiments were conducted in commercial crops of virus-free garlic in the municipalities of Fraiburgo and Frei Rogério, located in mid-eastern Santa Catarina, in the growing seasons of 2015 and 2016. According to the climatic classification of

Köppen, the climate of the region is “Cfb”, temperate and constantly humid with a mild summer. The soils where the experiments were conducted are classified as *Nitossolo Bruno Distrophic* according to the Brazilian Soil Classification System (Santos et al., 2013), clay texture, moderately plain to slightly undulated landscape, which corresponding to a Typic Hapludox (Soil Survey Staff, 1999). The physical and chemical properties of the soils of the five experiments are presented in table 1.

The recommended dose of 200 kg ha<sup>-1</sup> was adopted (CQFS-RS/SC, 2004), and the other treatments were: D0 = zero; D1 = half of the recommended dose; D2 = the recommended dose; D3 = 1.5 time the recommended dose; D4 = 2 times the recommended dose, which corresponded, respectively, to the doses of 0, 100, 200, 300, and 400 kg ha<sup>-1</sup>. The N source was ammonium nitrate (32 % N), distributed in three applications during the crop cycle: 1/3 in planting, 1/3 applied by side-dressing between 30 and 40 days after planting, and 1/3 applied by side-dressing after visual bulb differentiation, according to CQFS-RS/SC (2004). The experimental design used in every experiment was completely randomized blocks with four replications.

The experiments were installed inside commercial fields, with plots arranged in plant beds and constituted by three double lines of planting of 5 m in length. The planting density was 45 bulb-seed m<sup>-2</sup>, and the useful area consisted of the six rows, excluding one meter from the ends of each row. The cultural practices followed the current bases of production of the commercial fields of the region. Virus-free garlic bulb-seed obtained by meristem culture from the cultivar Chonan was used in experiments 1, 2, 4, and 5 and from cultivar Ito in Experiment 3.

Nitrate content (NO<sub>3</sub><sup>-</sup>) in soil solution and foliar sap, the total N content, and the relative chlorophyll content in the diagnostic leaf and the electrical conductivity of the soil solution were used as technologies for the diagnosis of N status of garlic plants. The soil solution was collected in the differentiation phase of the plants [about 125 days after differentiation (DAP)], immediately before the application of the second N dose was applied by side-dressing. To obtain the samples, two porous capsules extractors were installed in the central area of each plot, to the depth of 0.20 m, measured from half the height of the porous capsule. After 24 h, the extracted solutions were collected, stored in expanded polystyrene boxes and kept on ice, and the levels of NO<sub>3</sub><sup>-</sup> determined by selective ion meters, model LaquaTwin B-743 (Horiba Ltd., Kyoto, Japan) and electrical conductivity, by means of a Benchtop Portable model AK51 (Akso, Porto Alegre, Brazil). All samples were collected from the soil within the soil field capacity measured by a tensiometer.

**Table 1.** Physical and chemical properties of soils used in the experiments with nitrogen doses in the 2015 and 2016 growing seasons

Growing seasons	Experiments	Coordinates	Clay	OM	pH(H <sub>2</sub> O)	P	K	Ca <sup>2+</sup>	Mg <sup>2+</sup>	CEC <sub>pH 7.0</sub>
			g kg <sup>-1</sup>	%		mg dm <sup>-3</sup>	cmol <sub>c</sub> dm <sup>-3</sup>			
2015	1	-26.981696 S -50.825729 W	55	3.9	6.2	10.5	248.0	9.3	1.7	13.18
2015	2	-27.048185 S -50.782615 W	62	4.3	5.7	7.4	116.6	6.9	2.0	13.56
2015	3	-27.160526 S -50.798687 W	63	3.9	5.8	9.9	138.2	6.1	2.6	13.94
2016	4	-26.981812 S -50.825656 W	55	4.9	6.1	3.1	216.0	9.2	3.6	18.50
2016	5	-27.005582 S -50.847597 W	58	3.7	5.8	21.8	348.0	8.5	2.0	13.48

Clay: pipette method; OM: organic matter determined according to the Walkley-Black method; pH(H<sub>2</sub>O) at a soil:solution ratio of 1:1; P and K were extracted with Mehlich-1; Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Al<sup>3+</sup> were extracted by KCl 1 mol L<sup>-1</sup>; CEC: cation exchange capacity at pH 7.0.

Samples of the foliar sap and the measurement of relative chlorophyll content were performed at the same time as the soil solution collection. Ten diagnostic leaves (4th youngest fully expanded leaf) were randomly collected in each plot, as indicated by CQFS-RS/SC (2004). To obtain the foliar sap, fragments of 1.5 cm from the basal part of each leaf were pressed with a manual equipment (garlic squeezer), until the collection of an aliquot higher than 0.1 mL that was placed in an 'Eppendorf' tube and stored in polystyrene boxes and kept on ice. The contents of  $\text{NO}_3^-$  were quantified by a selective ion meter, as already described.

The determinations of the leaf relative chlorophyll contents were performed in the central part of the ten leaves collected for the quantification of  $\text{NO}_3^-$ . The reading per sheet was given by the average of two readings, one in the lower half and the other in the upper half of the leaf, using chlorophyll meter model ClorofiLOG CFL1030 (Falker, Porto Alegre, Brazil).

The ten leaves used to extract the foliar sap were washed and dried in a forced-air circulation oven at  $65 \pm 5$  °C to constant mass; they were then ground and subjected to chemical analysis of N in the Chemical Test laboratory of the Experimental Station of Caçador (Epagri) according to the methodology of Tedesco et al. (1995).

The bulbs were harvest in a linear meter within the plot. After harvesting, the plants were left to cure for about 40 days, and the size was determined according to commercial yield size classes of Ordinance No. 242, of September 17, 1992, of the Mapa (Luengo, 1999) and bulbs with secondary growth and damaged, are considered non-commercial. The total production was the sum of the commercial and non-commercial garlic production. Percent relative commercial yields were calculated as the ratio between the maximum commercial yield of a given N dose treatment and the maximum yield corresponding to a given N dose treatment multiplied by 100.

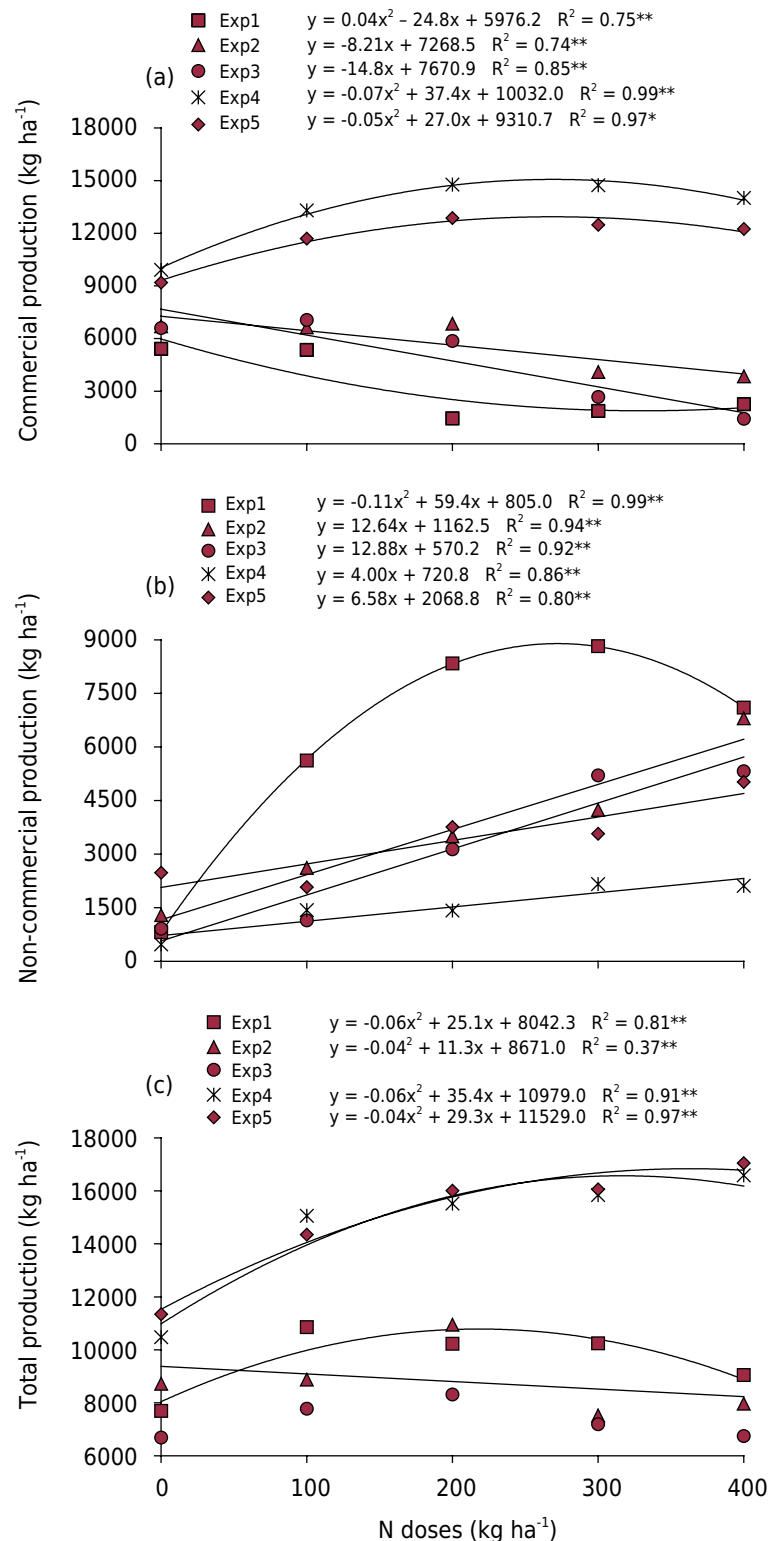
The critical level of  $\text{NO}_3^-$  in the foliar sap and the soil solution, electrical conductivity of the soil solution, relative chlorophyll content and N content in leaf tissue was estimated by the regression equation obtained associating the maximum relative commercial yield with N doses. The variables were also correlated with each other by Pearson's correlation.

The data were subjected to analysis of variance by F-test at least 5 % probability of error. Quantitative factors were subjected to polynomial regression analysis, selecting the model with the highest significance in the F-test. The statistical software R, version 3.0.3 was used (R Development Core Team, 2014).

## RESULTS

There was effect of N doses in all five experiments for garlic production (commercial, non-commercial, and total), except in experiment 3, in which there was no effect in the total production. The production of commercial garlic showed a distinct behavior in the two growing seasons. In the 2015 growing season, a quadratic decrease was observed in the commercial production in Experiment 1 and a linear decrease in experiments 2 and 3 with the increment of N levels (Figure 1a). On the other hand, in the 2016 growing season, this variable increased in quadratic form in experiments 4 and 5. The maximum commercial production was obtained with the doses 307 and 269  $\text{kg ha}^{-1}$  of N, in experiments 4 and 5, respectively, with a production of 15,776 and 12,938  $\text{kg ha}^{-1}$ .

Regardless of the growing season, the production of non-commercial bulbs was augmented with the increase of N doses (Figure 1b), mainly in experiment 1, with almost all harvested garlic considered of low commercial value. For experiments 1, 2, 4, and 5, there was a quadratic increase in the total production of bulbs with the increase of N



**Figure 1.** Commercial (a), non-commercial (b), and total production (c) of garlic virus-free due to the application of increasing N doses in five experiments. \*, \*\*, ns: significant at 5 and 1 % of probability, respectively.

level (Figure 1c), and only in experiment 3 there was no effect of N application. Total production was expressively superior in 2016 growing season, reaching 16,570 and 16,834 kg ha<sup>-1</sup> of garlic with the application of 316 and 362 kg ha<sup>-1</sup> of N, respectively, in experiments 4 and 5.

The N doses influenced the results of a greater number of nutritional diagnosis technologies in the 2016 season in relation to the 2015 growing season. In the 2015 growing season, the effect was observed only for the  $\text{NO}_3^-$  of the foliar sap in Experiment 3 (Figure 2c) and for foliar N content (Figure 2e) and leaf relative chlorophyll content (Figure 3d) in experiments 1, 2, and 3. Conversely, in the 2016 growing season, there was an effect of N levels for all of the nutritional diagnosis technologies, except for foliar N content (Figure 3e) in experiment 4.

In the 2016 growing season, there was a quadratic increment in the levels of  $\text{NO}_3^-$  of the soil solution in experiments 4 and 5 (Figure 3a) according to the doses of N. Considering the commercial production of experiments 4 and 5 and the N dose for maximum commercial production (Figure 1a), the content of  $\text{NO}_3^-$  in the soil solution corresponding to these experiments were 286.7 and 133.5  $\text{mg L}^{-1}$ , respectively. The increase in the  $\text{NO}_3^-$  levels in the soil solution may explain the increase of the electrical conductivity observed in these two experiments as a function of the N doses, which reached 2.80 and 1.66  $\text{mS cm}^{-1}$  (Figure 3b), for doses of 307 and 269  $\text{kg ha}^{-1}$  of N that was the maximum commercial production.

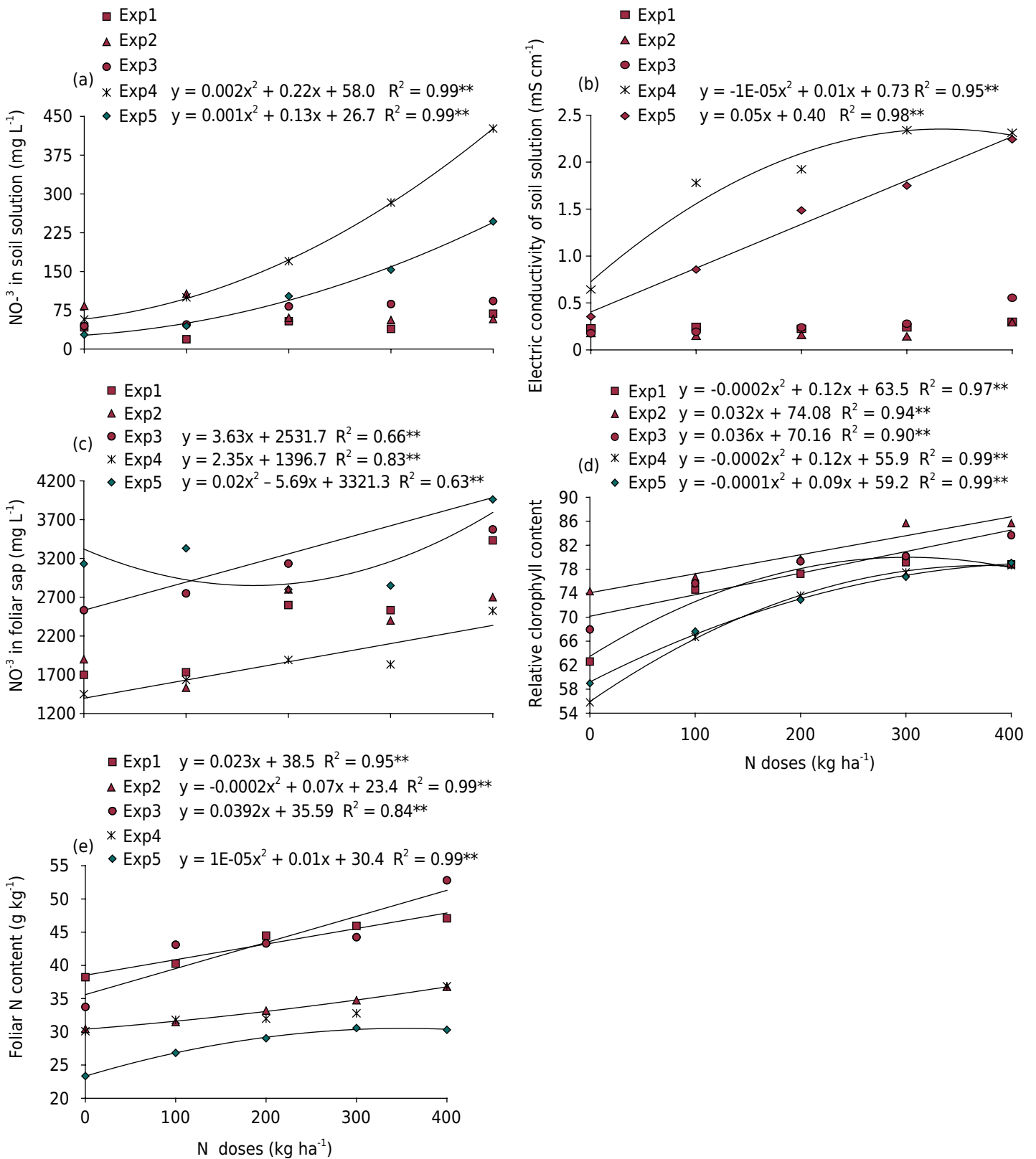
Regarding the technologies of plants nutritional diagnosis, the contents of  $\text{NO}_3^-$  of the foliar sap increased linearly in experiments 3 and 4 and quadratic mode in experiment 5 with the increment of N (Figure 2c). Considering the commercial production of experiments 4 and 5 and the N dose for maximum commercial production, the levels of  $\text{NO}_3^-$  in the foliar sap corresponding to these two experiments were 2,118.2 and 3,037.6  $\text{mg L}^{-1}$ , respectively. The leaf relative chlorophyll content increased linearly in experiments 2 and 3 and in a quadratic model in experiments 1, 4, and 5 (Figure 2d). Considering the commercial production of experiments 4 and 5 and the N dose for maximum commercial production, the relative chlorophyll content in the corresponding garlic leaves were 74 and 76, respectively. Foliar N content increased linearly in experiments 1 and 3 and quadratic form in experiments 2 and 5 (Figure 2e). With foliar N content corresponding to 29  $\text{g kg}^{-1}$ , the maximum commercial production of experiment 5 was obtained.

The contents of  $\text{NO}_3^-$  in the soil solution and foliar sap, the foliar N and relative chlorophyll content, and electrical conductivity of the soil solution correlated positively with a production of non-commercial garlic bulbs in all five experiments (Table 2). The exception was  $\text{NO}_3^-$  in the soil solution in experiment 2 and the  $\text{NO}_3^-$  in the foliar sap in experiment 5 that did not correlate with the non-commercial bulbs production.

On the other hand, commercial production showed totally distinct correlations in the two growing seasons. In 2015, the correlation was negative for all diagnostic technologies in experiments 1 and 3. However, in experiment 2, the  $\text{NO}_3^-$  in the soil solution and foliar sap and electrical conductivity did not correlate with commercial production. Otherwise, in the 2016 growing season, the relative chlorophyll content and electrical conductivity were positively correlated with the commercial production in the experiments 4 and 5, as well as the  $\text{NO}_3^-$  in the soil and foliar sap were also correlated with the commercial production in experiment 4. Regarding total production, it was found that there was no correlation with any of the diagnostic technologies in the 2015 growing season experiments. For the 2016 growing season, total production showed a positive correlation with all the results of N variables and electrical conductivity in soil solution, except for foliar N content in experiment 4 and  $\text{NO}_3^-$  in the foliar sap in experiment 5.

It was found, in general, that the results provided by technologies of nutritional diagnosis presented positive correlations among themselves, however, with a greater number of positive correlations in experiments 4 and 5. Among these technologies, the relative chlorophyll content was highlighted, which only did not correlate with the electrical conductivity in experiment 2 and the  $\text{NO}_3^-$  in the foliar sap in experiment 5.





**Figure 2.** Nitrate levels (NO<sub>3</sub><sup>-</sup>) (a), electrical conductivity of the soil solution (b), nitrate levels in foliar sap (c), relative chlorophyll content (d), and foliar N content (e) as a function of the application of increasing doses of N in five experiments of virus-free garlic. \*, \*\*: significant at 5 and 1 % probability.

Against the accentuated differences observed in the experiments conducted in the two growing seasons, the relative commercial yields were compared to the diagnostic technologies in each growing season. The two diagnostic technologies associated with the soil solution, levels of NO<sub>3</sub><sup>-</sup> and electrical conductivity, showed a relationship with the relative commercial yield only in the 2016 growing season. Relative commercial

**Table 2.** Correlation coefficient matrix (Pearson) among the properties of non-commercial production (NCP), commercial production (CP), total production (TP), nitrate in soil solution (NSS), nitrate in the foliar sap (NFS), electrical conductivity of the soil solution (ECSS), foliar nitrogen content (FNC), relative chlorophyll content (RCC) in five experiments with increasing doses of nitrogen in the crop of garlic in two growing seasons

Properties	CP	TP	NSS	NFS	ECSS	FNC	RCC
Experiment 1							
NCP	-0.89**	-0.03	0.58*	0.83**	0.67**	0.83**	0.77**
CP		-0.33	-0.53*	-0.71**	-0.81**	-0.62**	-0.72**
TP			0.01	0.11	-0.40	0.13	0.11
NSS				0.52*	0.35	0.41	0.64*
NFS					0.40	0.40	0.69**
ECSS						0.60*	0.72**
FNC							0.76**
Experiment 2							
NCP	-0.76**	-0.30	-0.50	0.59*	0.57*	0.78**	0.87**
CP		0.64**	0.35	-0.42	-0.48	-0.74**	-0.79**
TP			-0.01	0.09	-0.24	-0.45	-0.38
NSS				-0.59*	-0.00	-0.36	-0.54*
NFS					0.42	0.58*	0.62*
ECSS						0.46	0.35
FNC							0.66**
Experiment 3							
NCP	-0.89**	-0.03	0.58*	0.83**	0.67**	0.58*	0.77**
CP		0.33	-0.53*	-0.71**	-0.81**	-0.62*	-0.72**
TP			0.01	0.11	-0.40	0.13	0.11
NSS				0.52*	0.35	0.41	0.64*
NFS					0.40	0.40	0.69**
ECSS						0.60*	0.72**
FNC							0.76**
Experiment 4							
NCP	0.63**	0.73**	0.74**	0.50**	0.75**	0.45*	0.86**
CP		0.89**	0.49*	0.53*	0.71**	0.17	0.75**
TP			0.67**	0.66**	0.83**	0.41	0.87**
NSS				0.72**	0.72**	0.52*	0.83**
NFS					0.50*	0.50*	0.63**
ECSS						0.37	0.87**
FNC							0.47*
Experiment 5							
NCP	0.18	0.57**	0.84**	0.27	0.75**	0.69**	0.72**
CP		0.80**	0.42	-0.13	0.61**	0.41	0.61**
TP			0.76**	0.13	0.89**	0.70**	0.86**
NSS				0.37	0.91**	0.65**	0.81**
NFS					0.25	0.04	0.16
ECSS						0.70**	0.92**
FNC							0.86**

\*\* Significant ( $p < 0.01$ ); \* Significant ( $p < 0.05$ ).

yield increased in a quadratic mode with the increase in the levels of  $\text{NO}_3^-$  of the soil solution and electrical conductivity, obtaining 100 % of the relative yield with a content of 206  $\text{mg L}^{-1}$  and 1.98  $\text{mS cm}^{-1}$ , respectively (Table 3).



**Table 3.** Relationship between the values of the critical level of nitrogen in the leaf and the relative yield of virus-free garlic bulbs in two agricultural growing seasons

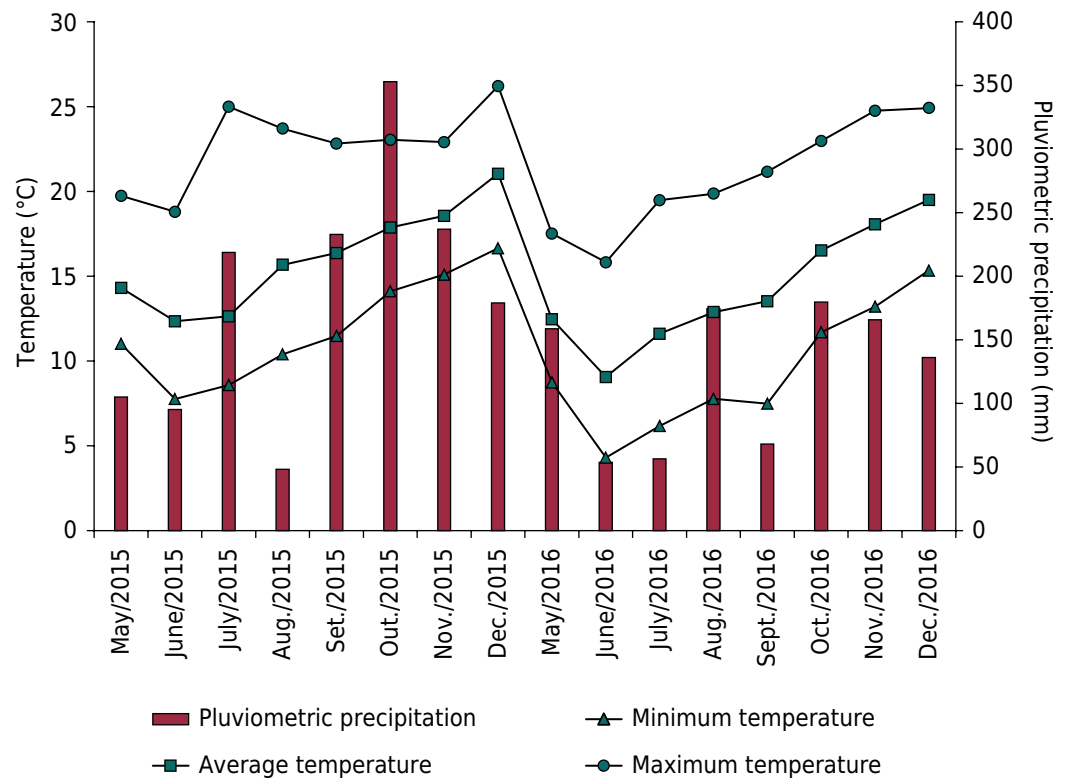
Diagnostic technologies	Seasons	Regression equations	R <sup>2</sup>	Values to obtain the highest relative commercial yield
Electrical conductivity of soil solution	2016	$y = -11.43x^2 + 44.93x + 53.57$	0.78	1.98 mS cm <sup>-1</sup>
NO <sub>3</sub> <sup>-</sup> in soil solution	2016	$y = -0.0004x^2 + 0.23x + 70.48$	0.57	206 mg L <sup>-1</sup>
Relative chlorophyll content	2015	$y = -0.06x^2 + 6.6x - 62.3$	0.63	-
	2016	$y = -0.08x^2 + 12.9x - 389.3$	0.96	76
Foliar N content	2016	$y = -0.29x^2 + 18.86x - 210$	0.82	32.74 g kg <sup>-1</sup>
NO <sub>3</sub> <sup>-</sup> in foliar sap	2015	$y = 3E-06x^2 - 0.043x + 160.2$	0.40	-

In the 2016 growing season, the relative commercial yield was associated with the maximum content of 32.74 g kg<sup>-1</sup> of N (Table 3), a value 11.4 % above the indication of experiment 5 (29 g kg<sup>-1</sup> of N; figure 2e). Such discrepancies may be associated with the adequacy of the mathematical model adjustment, which, despite being all significant, have coefficients of determination (R<sup>2</sup>) of 0.82 and 0.99. The leaf relative chlorophyll content was the only technology that presented a significant relationship with the relative commercial yield in the two growing seasons. The behavior was distinct in the two growing seasons; in the 2015 season, the maximum commercial yield was obtained with the lowest values of relative chlorophyll content; in the 2016 growing season, there was a quadratic increase in commercial yield with the increase of the relative chlorophyll content, obtaining the maximum yield with index 76.

## DISCUSSION

The commercial yield of garlic showed a very distinct behavior between the two growing seasons. In the 2015 growing season (Experiments 1, 2, and 3), a negative response in commercial production and the highest production of non-commercial garlic, as well as a lower total production with higher doses of N, can be explained by unfavorable climatic condition occurring during these experiments. In this crop, there were late negative temperatures occurring on September 13th and 14th (-2.5 °C, in both days), coinciding with the phase of differentiation of garlic, and high rainfall precipitation from half to the end of the production cycle of garlic (months from October to December) where the accumulated monthly rainfall was 397, 291, and 218 mm, and the number of days of the months with rainfall being 20, 23, and 21, respectively (Figure 3). As adverse weather conditions, associated with increased N doses applied in the experiments, decreased commercial production and favored the appearance of the plant disturbance false-branching or super-sprouting, in addition to a high incidence of bacteriosis (Wu et al., 2016). These two conditions deteriorate the quality of garlic, and therefore the product is destined for industrial use (Luengo, 1999), and thus classified as non-commercial garlic. Hence, high N fertilization may reduce the profit of producers as it may increase the production of non-commercial garlic bulbs.

For the experiments conducted in the 2016 growing season, favorable climatic conditions provided high yield of commercial garlic, with a similar prolific output of garlic observed that year in two main producing states of the Brazilian Cerrado, Minas Gerais (15.0 t ha<sup>-1</sup>) and Goiás (13.3 t ha<sup>-1</sup>), respectively, the first and second largest producers in Brazil (Epagri/Cepa, 2016). As the 2016 growing season occurred in climatic conditions considered normal for the production of garlic in the south of Brazil, the results obtained with the application of N can be used to establish N recommendations for commercial crops in the region.



**Figure 3.** Precipitation, maximum temperature, minimum temperature, and average monthly temperature during the period the experiments were conducted.

The experiments of the 2016 growing season (4 and 5) indicate that the doses of 307 and 269 kg ha<sup>-1</sup> of N, provided, respectively, the maximum commercial yields of garlic (Figure 1a). Similar results were observed in a study conducted in the Brazilian Cerrado, with the maximum commercial yield and the average weight of bulbs obtained with doses of 320 and 321 kg ha<sup>-1</sup> of N (Fernandes et al., 2011). Backes et al. (2008), also in the Brazilian Cerrado, observed similar yields with the application of 268 kg ha<sup>-1</sup> of N. The N doses observed in the present study, are close to the current N recommendations for the cultivation of garlic for southern Brazil (CQFS-RS/SC, 2016), which establishes a total dose of 300 kg ha<sup>-1</sup> of N to be applied in the crop cycle in soils with 2.5 to 5.0 % of organic matter. This interval in the MO doses is understood from the five locations where the experiments were conducted (Table 1) and represents a large part of the soils with the cultivation of garlic in Santa Catarina. However, CQFS-RS/SC (2016) recalls that the dose to be applied after the differentiation of the plants (second by side-dressing) may vary according to the vigor of plants, diseases, and predisposition of plants to secondary bulb growth. Thus, the indication of N fertilization according to CQFS-RS/SC 2016) for years with high precipitations is excessive, as demonstrated by the results obtained in the 2015 growing season.

In the 2016 growing season, there was a greater effect of N doses on the results provided by nutritional diagnosis technologies used in the differentiation phase of the plants. Of the diagnosis technologies associated with soil, the increasing doses of N influenced the contents of NO<sub>3</sub><sup>-</sup> and the electrical conductivity of the soil solution. Blanco et al. (2008) found an increase in electrical conductivity and NO<sub>3</sub><sup>-</sup> concentration in the soil solution proportional to the increasing doses of N applied to tomato crop in protected cultivation. Thus, the electrical conductivity and the concentration of NO<sub>3</sub><sup>-</sup> in the soil solution present a high potential for nutritional diagnosis of N, especially in years with normal climatic conditions.

All diagnosis technologies associated with plants, the content of  $\text{NO}_3^-$  in the foliar sap, relative chlorophyll content, and foliar N content, were influenced by the doses of N applied in planting and in the first side-dressing. Results obtained by Gaviola and Lipinski (2002), using the garlic cultivar 'Fuego INTA' in Argentina, point out that the levels of  $\text{NO}_3^-$  of the foliar sap were correlated with the application of N fertilizer and a quadratic model was adjusted for the bulb yield. Westerveld et al. (2003) indicate that the application of N results in increased levels of  $\text{NO}_3^-$  in the foliar sap in beet, carrot, and onion. On the other hand, the relative chlorophyll content also presented a significant relationship with N fertilization in garlic cultivated in the Brazilian Cerrado (Fernandes et al., 2010). Wu et al. (2007) observed in three growing seasons the increase in the levels of  $\text{NO}_3^-$  in the sap of the stems and the readings of the relative chlorophyll content of potato with increased N doses, however, the levels of  $\text{NO}_3^-$  in the stem sap responded more quickly to N fertilization compared to the relative chlorophyll content.

In the present study, the technologies of soil and plant diagnosis showed significant correlations with the commercial, non-commercial, and total garlic production according to the growing season. Among these technologies, the relative chlorophyll content was correlated with commercial production in all experiments (Table 2). In garlic (Fernandes et al., 2010) and potato cultivation (Coelho et al., 2010), a linear and positive correlation between chlorophyll content values and commercial production was verified, indicating that there is a good perspective in the use of this technology for nutritional diagnostics. With garlic, positive correlations between relative chlorophyll content and the N content were also verified in Fernandes et al. (2010) and Backes et al. (2008).

For an N technological nutritional diagnosis to present high potential for use, it will need to have a high relationship with commercial production in different scenarios, be it yield, climatic condition, and fertility levels. The diagnostic technologies associated with soil were highly correlated to commercial yield only in the 2016 growing season (Table 3), with normal climatic conditions, demonstrating potential use in years with a favorable climate to the growth and production of garlic. In these conditions, it was established that critical levels can be used for monitoring N. However, as these values were obtained in the differentiation phase of the plants, before the second application of N by side-dressing, the next step will be to establish N doses to be applied in the second application by side-dressing if the levels of  $\text{NO}_3^-$  and electrical conductivity are below critical levels.

Among the nutritional diagnosis technologies associated with plants, the  $\text{NO}_3^-$  in foliar sap presented a significant relationship with the relative commercial yield only in the 2015 growing season (Table 3). Reciprocally, foliar N content had a positive relationship only in the 2016 growing season. In view of these results, these two technologies have potential for diagnosing the nutritional status of garlic according to climatic conditions. The relative chlorophyll content was the only technology that presented a significant relationship with the relative commercial yield in the two growing seasons. In this way, it is the technique with the potential to evaluate the nutritional status in relation to the N content of the plant in real time, efficiently and economically. In the 2016 growing season, the maximum commercial yield was obtained with relative chlorophyll index 76. Similar values were found by Fernandes et al. (2010) evaluating the Caçador cultivar of virus-free garlic in Guarapuava-PR, southern Brazil. The authors obtained a correlation coefficient of 0.73 between the relative chlorophyll content, foliar N content and total yield of garlic plants.

The critical level of the relative chlorophyll content established in the present study (0.76) allows the determination of side-dressing N demanded by the garlic crop. Thus, it is expected to increase the efficiency in the use of N in the production of garlic and with a lower potential for water contamination with this nutrient.

## CONCLUSIONS

The highest commercial production was associated with the doses of 269 e 307 kg ha<sup>-1</sup> of N and the content of 32.74 g kg<sup>-1</sup> of N, in the diagnostic leaf. Among the technologies evaluated, the relative chlorophyll content was the only one that presented a significant correlation with commercial yield in all experimental conditions. The evaluation of the diagnosis of the N status in the culture of the virus-free garlic by a portable chlorophyll meter can be a quick strategy for recommending N fertilization and guaranteeing high yields.

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