Single-head broccoli response to nitrogen application

Resposta de brócolis cabeça única à aplicação de nitrogênio

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Abstract

Information regarding nitrogenous fertilization in broccoli for Santa Catarina is still scarce. Therefore, this study aimed at analyzing the nitrogen effect upon the levels of chlorophyll (*a*, *b* and total), carotenoids and nitrogen (N) forms in leaves, and upon the yield of inflorescence, stem and leaves of single-head broccoli. The experiment was conducted in September, 2011, in Florianópolis (SC), with seedlings of single-head broccoli (BRO 68 – Syngenta), in completely randomized blocks, with five treatments (0, 75, 150, 200 and 250 kg ha^{-1} of N, in the form of urea) and three replications. In the blooming, the fourth leaf from the ground was harvested from each broccoli for the following analyses in triplicate: chlorophyll levels (*a, b* and total), carotenoids and N forms (NH_4^+, NO_3^- and mineral N). At the end of the cycle, the yield of inflorescence, body (leaves + stem) and total were estimate. The levels of N lead to increased levels of chlorophyll *a, b* and total, as well as the N forms in leaves and the yield of inflorescence, leaves, and stem of single-head broccoli. The increased N doses promoted increment of carotenoid levels on leaves until 170 kg ha^{-1} of N, having a decrease for superior doses. The N forms in the plant had been incremented with increasing of N doses supply, reflecting in increased yield of broccoli. The N application in the soil stimulated the synthesis of chlorophylls and carotenoids on leaves, promoting better nutritional quality.

Additional keywords: *Brassica oleracea* L. var. *italica* Plenck; carotenoids; chlorophyll; nitrogenous fertilization; yield.

Resumo

As informações sobre adubação nitrogenada em brócolis para Santa Catarina (SC) ainda são escassas. Por isso, objetivou-se avaliar o efeito do nitrogênio (N) sobre os teores de clorofila (a, b e total), carotenoides e formas de N nas folhas, e sobre a produtividade de inflorescência, talo e folhas de brócolis cabeça única. O experimento foi implantado em setembro de 2011 em Florianópolis (SC), com mudas de brócolis cabeça única (BRO 68 - Syngenta), em delineamento de blocos completos casualizados, com cinco tratamentos (0, 75, 150, 200 e 250 kg ha^{-1} de N, na forma de ureia) e três repetições.No pleno florescimento das plantas, foi coletada a quarta folha a partir do solo para as seguintes avaliações, em triplicata: teores de clorofila (a, b e total), carotenoides e formas de N (NO_3^-, NH_4^+ e N mineral). No final do ciclo, foi estimada a produtividade de inflorescência, corpo (folhas + talo) e total. As doses de N incrementaram os teores de clorofila *a*, *b* e total, as formas de N na folha e a produtividade de inflorescência, talo e folhas de brócolis cabeça única. O aumento das doses de N proporcionou o incremento do teor de carotenoides na folha até 170 kg ha^{-1} de N, decrescendo nas doses superiores. O aumento das doses de N incrementou as formas desse nutriente na planta, refletindo em aumento da produtividade de brócolis. A aplicação de nitrogênio no solo estimulou a síntese de clorofilas e carotenoides nas folhas, promovendo a melhora na sua qualidade nutricional.

Palavras-chave adicionais: adubação nitrogenada; *Brassica oleracea* L. var. *italica* Plenck; carotenoides; clorofila; produtividade.

Introduction

The need and the dose of N for broccoli culture (*Brassica oleracea* L. var. *italica* Plenck) in the states of Santa Catarina (SC) and Rio Grande do Sul (RS), Brazil, are established based on soil organic matter content (CQFS-RS/SC, 2004). However, this criterion provides partial information about the N amount that is potentially mineralized in the soil, and therefore does not provide the values of the different forms of N available to the plants, such as nitrate (NO_3^-) and ammonium (NH_4^+), in a short period of time, as it occurs with broccoli cultivation. Alternatively to organic matter content, it is appropriate to conduct field experiments on fertilization calibration in regional character, in order to define the appropriate doses to obtain a satisfactory production and the desired composition.

Broccoli response to nitrogen fertilization is quite variable. CAMPAGNOL et al. (2009), for example, found no effect on crop yield due nutrient application. On the other hand, many studies establish that N addition in the soil promotes increased broccoli production, although the appropriate dose for obtaining the maximum yield is not unanimous among authors, varying widely from 150 to 625 kg ha^{-1} of N (BOWEN et al. 1999; BÉLEC et al., 2001; BAKKER et al., 2009; SEABRA JUNIOR et al., 2013). Added to this, information about broccoli nitrogen fertilization in SC state is scarce, which reinforces the need to conduct experiments for proper N rate calibration for the culture.

Broccoli roots can absorb N from the soil in NH_4^+ form, which can be assimilated by roots and then translocated to other plant parts, especially in the form of free amino acids. However, the highest N amounts absorbed by roots are in NO_3^- form, which is found in the soil in higher quantities. Before being assimilated, NO_3^- must be reduced to ammonium and, therefore, undergoes a chemical process that requires energy expenditure (BREDEMEIER & MUNDSTOCK, 2000; SHAN et al., 2012; LI et al., 2013.). After reduction, N is assimilated to be used in the synthesis of carbonaceous and nitrogen compounds essential to plant growth, such as amino acids, carbohydrates, proteins and other compounds used in photosynthesis (LIMA et al., 2011; TUNCAY et al., 2011).

However, in times of low demand by the plant, the absorbed NO_3^- may not be readily reduced to NH_4^+ and, therefore, not be initially assimilated. In this case, NO_3^- is stored in reserve organelles, such as vacuoles, to be metabolized in times of need, such as the flowering period (BOWEN et al., 1999; HAWKESFORD et al., 2012). On the other hand, when the N availability in soil is high - soils with a high organic content and high N mineralization rate, or even under conditions of high doses addition of the nutrient in the soil, for example - N absorbed and accumulated in cellular compartments may exceed the plant metabolic capacity, even in higher demand for nutrient periods, and may not be used in biochemical and physiological reactions (LI et al., 2013). Besides that, when N is applied to the soil in doses above plant necessity, N mineral forms in the soil, mostly NO_3^-, can be lost by leaching. In soils with superficial sandy texture and shallow water table, like many soils where broccoli and other vegetables in the SC state are grown, these losses may be potentiated (SANGOI et al., 2003; KURTZ et al., 2012.).

As the plant nutritional status is reflected in broccoli production and composition, N must be applied to the soil at doses that increase its available forms in the soil, improving its content in tissues (as NO_3^- and NH_4^+) and also increasing photosynthetic pigments - among them, chlorophyll *a* and *b* and carotenoids. Increase in photosynthetic pigments content stimulates photosynthetic rate and the plant biomass production (ENGELS et al., 2012). Furthermore, higher chlorophyll content can promote a larger antioxidant, antimutagenic and chemopreventive activity in humans (LANFER-MARQUEZ, 2003), inducing cancer prevention (SIMONICH et al., 2007; MCQUISTAN et al., 2012) and contributing for healing process (TANAKA et al., 1997). Carotenoids are vitamin A precursors and also powerful antioxidants, working in the prevention of degenerative diseases (RODRIGUEZ-AMAYA, 2001; AMAN et al., 2005).

Broccoli is rich in photosynthetic pigments such as chlorophyll and carotenoids (GUZMAN et al., 2012), especially in the leaves (PADULA et al., 2006). However, works on the production of these pigments in broccoli leaves in function of N doses are still scarce, so it is urgent to carry out studies that contemplate how N may boost its production in the plant.

In this context, the aim of this study was to evaluate the N effect on chlorophyll (*a*, *b* and total), carotenoids and N forms content in leaves, and yield of the inflorescence, stem and leaves on single-head broccoli.

Material and methods

The experiment was carried out in the municipality of Florianópolis (SC), Brazil, coordinates 27°41'02"S and 48°32'34"W, 22 m above sea level. The soil of the experimental area was classified as Quartzipsamments (SOIL SURVEY STAFF, 2010). Prior to the experiment implementation, chemical characteristics of the 0-20 cm layer were of 85.0 g kg^{-1}clay; 43.5 g kg^{-1}organic matter; pHin water of 4.8 (1:1); 10.5 mg kg^{-1} of

available P, and 33.0 mg $kg^{-1}$of exchangeable K (both extracted by Mehlich 1); 1.2 $cmol_c$ kg^{-1} of exchangeable Ca and 0.4 $cmol_c$ kg^{-1} of exchangeable Mg (both extracted by KCl 1 mol L^{-1}).

Data from the weekly average maximum, average and minimum temperatures and accumulated precipitation in the experiment conduction period are shown in Figure 1.

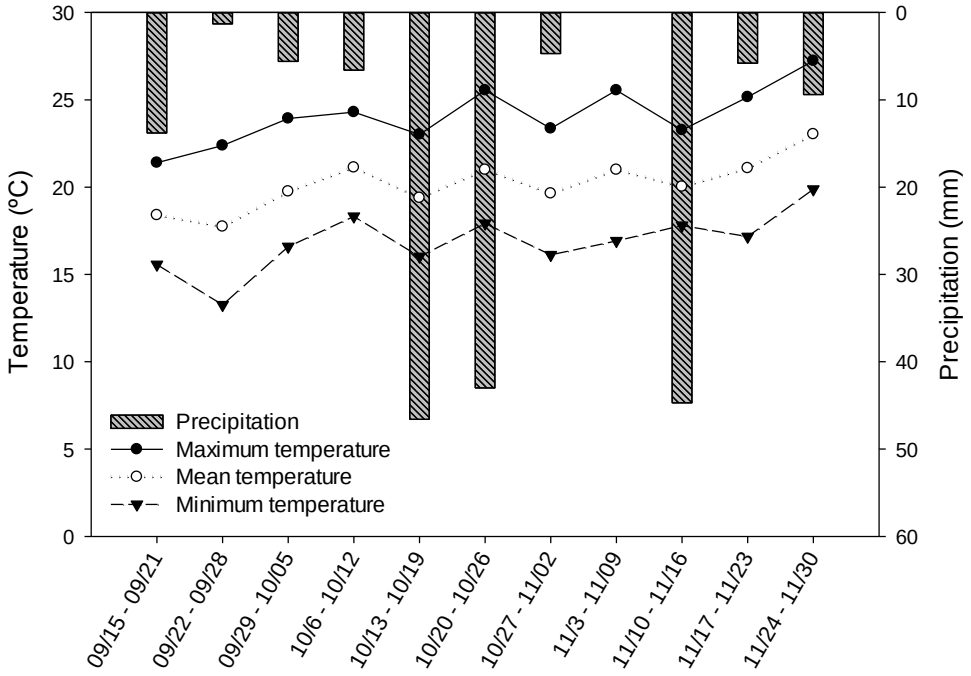

Figure 1 - Weekly averages of maximum, mean and minimum air temperatures, and rain precipitation accumulated during the experiment conduction in 2011 – Florianópolis (SC).

On September 15, 2011, seedlings belonging to the Chinese varietal group, single-head type (hybrid BRO 68 - Syngenta), produced in trays containing four to five full leaves were transplanted in the soil, in flowerbeds.

The experimental design was done incompletely randomized blocks with five treatments (0, 75, 150, 200 and 250 kg ha^{-1} of N) and three replications. The experimental plot consisted of five rows 3.5 m long with 1.0 m between rows. Each row consisted of seven plants spaced 0.5 m apart, establishing a plant density of 20,000 plants ha^{-1}. The useful area in the plot was made by the three inner plot rows, excluding one plant from each rows end.

Nitrogen fertilization was performed using urea, divided into three times in equal amounts, with the first dose applied in seedlings transplanting (September 15, 2011) and the other two in the coverage (October 9 and November 11, 2011). Furthermore, in transplantation, phosphate fertilization (350 kg ha^{-1} de P_2O_5), in triple superphosphate form, and potassium (340 kg ha^{-1} of K_2O), in potassium chloride form, following the recommendation proposed by CQFS-RS/SC (2004), took place.

In the plant blooming, which occurred in mid-November, the fourth leaf from the soil was harvested, as it was fully developed, as described in the collection method performed by CAMPAGNOL et al. (2009). After harvesting, leaves were rinsed with distilled water and separated into two parts. The first part was wrapped in plastic bags and immediately stored at -20 ºC until analysis. The second part was dried in an oven with forced air at 65 °C and subsequently grinded and set aside. At the end of the cycle, which took place in November, the plants inflorescence and body (leaves + stems) were harvested. After weighing, using an electronic precision scale, inflorescence and body plant yield (Mg ha^{-1}) were estimated; total yield was calculated from the sum of the two previous weighing.

Part of the stored leaves green mass was submitted to chlorophyll *a*, *b* and total content evaluation, following the methodology proposed by LICHTENTHALER (1987). For that, circa 500 mg of plant tissue was macerated in liquid N, and after obtaining a homogeneous material, 7 mL of 80% acetone solution was added. The filtration of the added solution to the macerate was performed on a previously moistened filter paper, with 2 mL of 80% acetone and with the

help of a funnel, and subsequently transferred to a volumetric flask. The remaining residue on the filter paper was washed with 4 mL of 80% acetone and the volume was completed to 25 mL. Absorbance was read in a spectrophotometer at two different wavelengths: 663 nm for chlorophyll *a* and 647 nm for chlorophyll *b*. Readings were performed in triplicate for each sample, and the data used for analyzes were made from the triplicates average.

Chlorophyll *a*, *b* and total contents were calculated by Equations (1), (2) and (3), respectively:

$$\text{Chlorophyll } a\ (\mu g\ g^{-1}\ MF) = [12{,}25 \times A_{663}) - (2{,}79 \times A_{647})] \times V \quad (1)$$

$$\text{Chlorophyll } b\ (\mu g\ g^{-1}\ MF) = [(21{,}50 \times A_{647}) - (5{,}10 \times A_{663})] \times V \quad (2)$$

$$\text{Total chlorophyll } (\mu g\ g^{-1}\ MF) = [(7{,}15 \times A_{663}) + (18{,}71 \times A_{647})] \times V \quad (3)$$

where: A is absorbance and V is the sample volume (mL).

The other part of the stored leaves green mass was used for the assessment of carotenoid content in accordance with the procedure proposed by AMAN et al. (2005). For that, 375 mg of plant tissue was macerated in liquid N, to which 15 mL of Hexane:Acetone (1:1 - v/v), containing 100 mg $L^{-1}$of BHT (butylate dhydroxytoluene) was added. The samples remained at rest in the dark for 30 minutes, and after this period, were filtered with the help of a vacuum centrifuge (35 °C) to remove the solvent. The concentrated residue was dissolved in 3 mL hexane, washed three times with distilled water and the supernatant was separated by centrifugation (1 minute at 4000 rpm). To perform the reading, the supernatant was diluted at a ratio of 2 mL hexane to 1 mL solution, and the absorbances were read in a spectrophotometer at a wavelength of 450 nm. Readings were taken in triplicate for each sample, and the data used for analyzes were obtained from the triplicates average.

Total carotenoid content quantification in the organic solvent extracts was determined by Equation (4) (RODRIGUEZ-AMAYA, 2001):

$$\text{Carotenoids } (\mu g\ kg^{-1}\ MF) = \frac{A \times V \times 10^6}{A^{1\%}_{1\ cm} \times 100 \times m} \quad (4)$$

where Carotenoids are equivalent to the total carotenoid content; A is the absorbance obtained in the sample volume V (mL); $A^{1\%}_{1\ cm}$is the absorption coefficient of the carotenoid in the solvent used [lutein absorption coefficient in hexane ($A^{1\%}_{1\ cm}$= 2.589), obtained in CRAFT (1992) was used]; m is the sample mass.

In the dry matter (DM) of stored leaves, the mineral N content was analyzed using the methodology described by TEDESCO et al. (1995). In a distillation tube containing 15 mL of KCl 1 mol L^{-1}, 1 g of dry matter was added. After five minutes of agitation, samples were distilled with 0.2 g of MgO for NH_4^+ determination, and then distilled with 0.2 g of Devarda alloy to obtain NO_3^- in the sample. The product of each distillation was collected in an erlenmeyer flask containing 5 mL boric acid, reaching a final volume of 40 mL. The collected solution was titrated with H_2SO_4 (0.0025 mol L^{-1}), and NH_4^+ and NO_3^- contents in the MS was calculated according to TEDESCO et al. (1995). The sum of NH_4^+ and NO_3^- content resulted in the mineral N content.

The results were submitted to analysis of variance, and when the effects were significant by F test ($p<0.01$), polynomial regression equations were set at $p<0.05$. Moreover, linear correlation tests between all variables were performed.

Results and discussions

The results submitted to analysis of variance were significant, indicating that there were effects of N doses on all variables (Table 1).

Table 1 – Mean, minimum and maximum values, F values and coefficients of variation of the variables associated with levels of pigments, forms of nitrogen and yield. 2011 - Florianópolis (SC).

Parameter	Mean	Minimum[(1)]	Maximum[(1)]	F value	CV (%)
Carotenoids (µg g^{-1})	244.92	222.94	262.19	13.74**	3.09
Chlorophyll a (µg g^{-1})	162.58	118.09	252.56	55.76**	8.33
Chlorophyll b (µg g^{-1})	72.18	52.88	97.95	12.84**	11.89
Totalchlorophyll (µg g^{-1})	234.76	170.97	350.52	72.23**	6.57
NH_4^+ (mg kg^{-1})	135.19	79.57	183.63	75.11**	6.25
NO_3^- (mg kg^{-1})	33.13	3.73	80.73	61.15**	20.53
Total mineral N (mg kg^{-1})	168.33	83.30	248.50	101.38**	7.12
Yield of inflorescence (Mg ha^{-1})	3.57	0.00	7.01	7.86**	45.37
Yield of body (Mg ha^{-1})	10.38	2.28	14.63	38.76**	14.28
Total yield (Mg ha^{-1})	13.94	2.28	24.40	130.03**	8.45

(1) Three repetitions mean. (**)Significative by F Test ($p<0.01$).

The values of chlorophyll *a*, *b* and total in broccoli leaves increased with N doses applied to the soil (Figure 2A), adjusting to the quadratic function. The broccoli leaves grown in soil and subjected to higher N doses (250 kg ha^{-1}) had chlorophyll *a*, *b* and total levels of, respectively, 2.14, 1.85 and 2.05 times higher in relation to content than those observed in the broccoli leaves without nutrient application. Increasing chlorophyll values in the leaves with increased N dose applied in the soil can be attributed to an increased N content in the plants interior (Table 2), as diagnosed by foliar analysis of NH_4^+, $NO_3^-$and mineral N content (Figure 3). The N in the tissue is used in the synthesis of many structural compounds, such as lipids, amino acids and proteins used in photosynthesis (TUNCAY et al., 2011). Furthermore, it is the major component of chlorophyll. The presence of N in the leaves favors the assimilation of CO_2 in the photosynthetic process, promoting an increase in the liquid photosynthetic rate and, consequently, increasing chlorophyll content in leaves (LI et al., 2013).

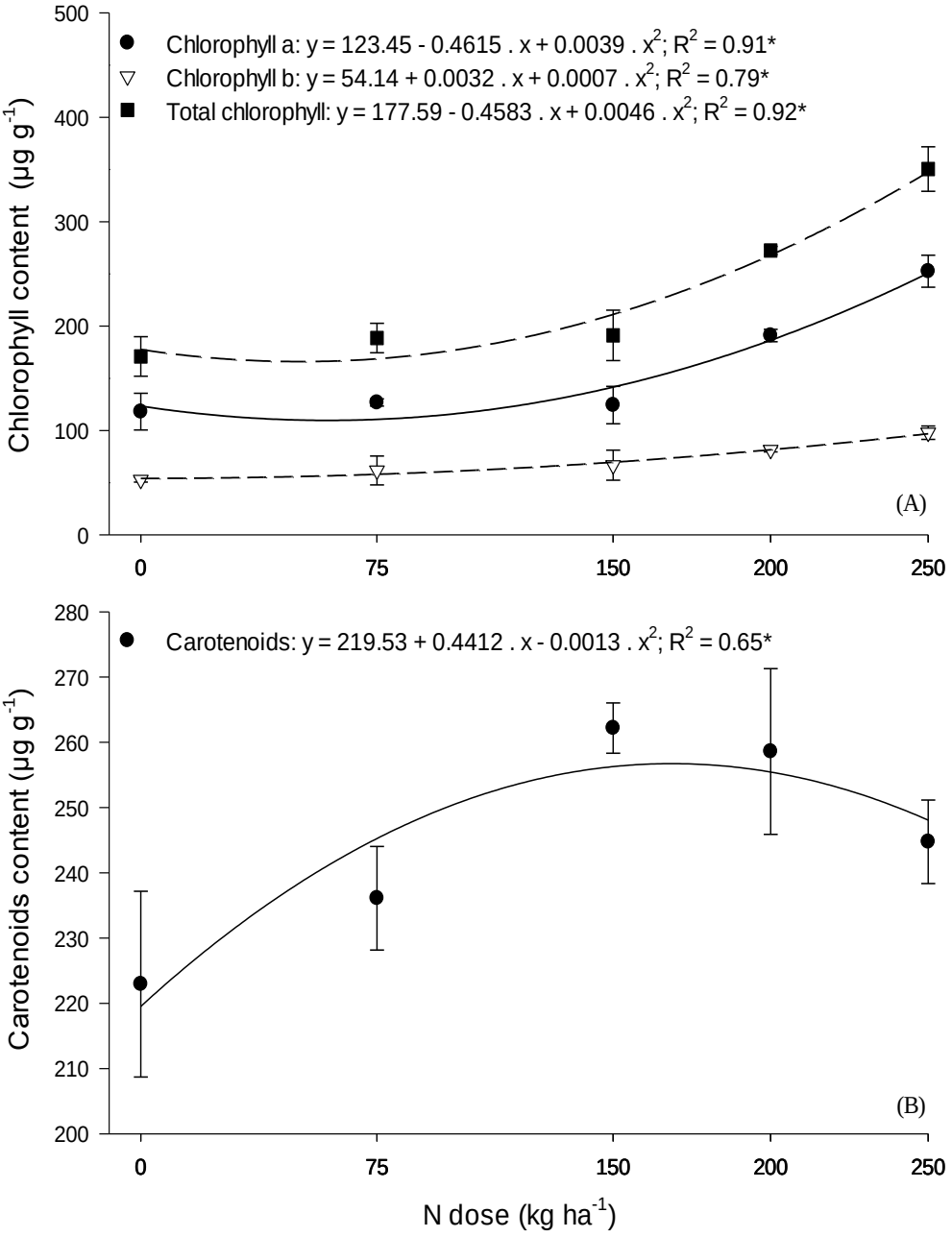

Figure 2 - Contents of chlorophyll *a*, *b* and total (A) and content of total carotenoids (B) in leaves of single-head broccoli submitted to N application. 2011 - Florianópolis (SC).

The carotenoid content in broccoli leaves also set to the quadratic function, increasing with the increased N doses in the soil and having the maximum level observed at a 170 kg ha^{-1} of N (Figure 2B) dose. The carotenoid content increase occurred in leaves because these pigments are nitrogen compounds, and when increasing the N forms ($NH_4^+$and NO_3^-) in tissues (Figure 3), there is also an increase in photosynthetic pigments (WIESLER, 2012; LI et al., 2013). At the same time, high N doses applied to the soil increase biomass yield (stems + leaves) (Figure 4), promoting the shading of some middle and bottom parts of the plant leaves and, therefore, may reduce carotenoids concentration. This effect was observed in the leaves located in the middle part of the plant - collected and used in the analysis - submitted to treatments with200 and 250 kg ha^{-1} of N application. This may be due to the decrease of the photosynthetic rate of shaded leaves, which led to carotenoids production inhibition (BASLAM et al., 2013). Moreover, with the increase of plants biomass, the dilution of carotenoids in the tissue may have occurred (WIESLER, 2012). On the other hand, chlorophyll had the inverse responses, i.e., even plants that received smaller amounts of light showed high chlorophyll *a*, *b*and total contents, which may be an adaptive response of plants to increase light absorption in the shading position, so as to maintain the photosynthesis effective (LIMA et al., 2011).

In single-head broccoli, the consumed part is the inflorescence. Leaves and stem are usually discarded. However, photosynthetic pigments production (chlorophyll and carotenoids) in the leaves, increased by N dose increase, indicates that this part should be exploited as food, mainly because chlorophyll and carotenoids are beneficial to human health (LANFER-MARQUEZ, 2003; SIMONICH et al., 2007; MCQUISTAN et al., 2012). In addition, broccoli leaves are a source of polyphenols, which are substances known for its antioxidant activity, as well as stems and leaves are rich in fiber and lipids (STORCK et al., 2013). All of this is a good argument to reaffirm that the body (stem + leaves) of broccoli plants should be more harnessed for human consumption, as is already done with cabbage leaves

NH_4^+, NO_3^- and mineral N content in leaves were set in the quadratic function, increasing according to the increase of the N dose applied to the soil (Figure 3). This may have happened because urea addition increased the content of mineral N forms in the soil, such as NH_4^+ and, especially NO_3^-, which may have been absorbed by plant roots, especially in periods of intense dry matter accumulation, such as flowering (BOWEN et al., 1999). Urea applied to the soil is rapidly hydrolyzed by the urease enzyme action, promoting NH_4^+ release (WITTE, 2011). However, this N form is unstable in the soil and is transformed into NO_3^-, which is the form of mineral N observed with highest concentration in the soil and, consequently, the most absorbed by plants (LI et al., 2013). On the other hand, in soils saturated with water, the predominant form of N is NH_4^+, with it becoming a major nutrients source for the plants (LI et al., 2013). Therefore, it is valid to point out that the soil where the broccoli was cultivated has shallow water table, especially during rainy periods and, moreover, that the last urea application in the soil happened on November 11, when a period with high precipitation began (Figure 1). These associated factors may have favored NH_4^+ absorption by plants, with consequent accumulation of this N form in the leaves (Figure 3), which were collected on November 15 (end of the rainy season).

The application of increasing N doses in the soil increased inflorescence and body yield, which was reflected in the total yield (Figure 4). In fact, the plants absorbed and accumulated greater amounts of N forms on its tissues when the N dose in the soil was increased (Figure 3) which, in turn, resulted in the increase of chlorophyll content in leaves (Figure 2A). As a result, the plants photosynthetic rate may increase, as well as the production of structural compounds, such as amino acids, carbohydrates and lipids (LIMA et al., 2011; TUNCAY et al., 2011).

Photosynthesis increase stimulates leaf area increase, photosynthate synthesis promotion (especially before flowering) and floral tissues initiation, reflecting in the production of the reproductive organs, such as inflorescence (ENGELS et al., 2012). On the other hand, low mineralization of soil organic matter and, consequently, the insufficient amount of mineral N in the soil to meet the needs of broccoli explains the absence of inflorescence formation in treatment plants that did not receive nitrogen fertilization (Figure 4). WOJCIECHOWSKA et al. (2005) and BAKKER et al. (2009) corroborated that broccoli plants require high N doses to express its maximum production potential.

Correlation analyzes (Table 2) showed that NH_4^+ leaves content were not correlated with chlorophyll content. However, NO_3^- and mineral N contents were positively correlated with chlorophyll *a*, *b* and total contents. In addition, NO_3^- content were positively correlated with yield of inflorescence, and NH_4^+ and mineral N contents correlated positively with inflorescence, body and total yield. There was also a positive correlation between chlorophyll *a*, *b* and total contents with yield of inflorescence, and of chlorophyll *b* with total yield. These results support the claim that foliar N increase stimulates photosynthetic rate increase, promoting plant biomass yield. In addi-

tion, high correlations between mineral N and yield of inflorescence, body and total broccoli indicate that the leaf nutrient content is a good criterion for predicting the need for nitrogen fertilization for broccoli culture.

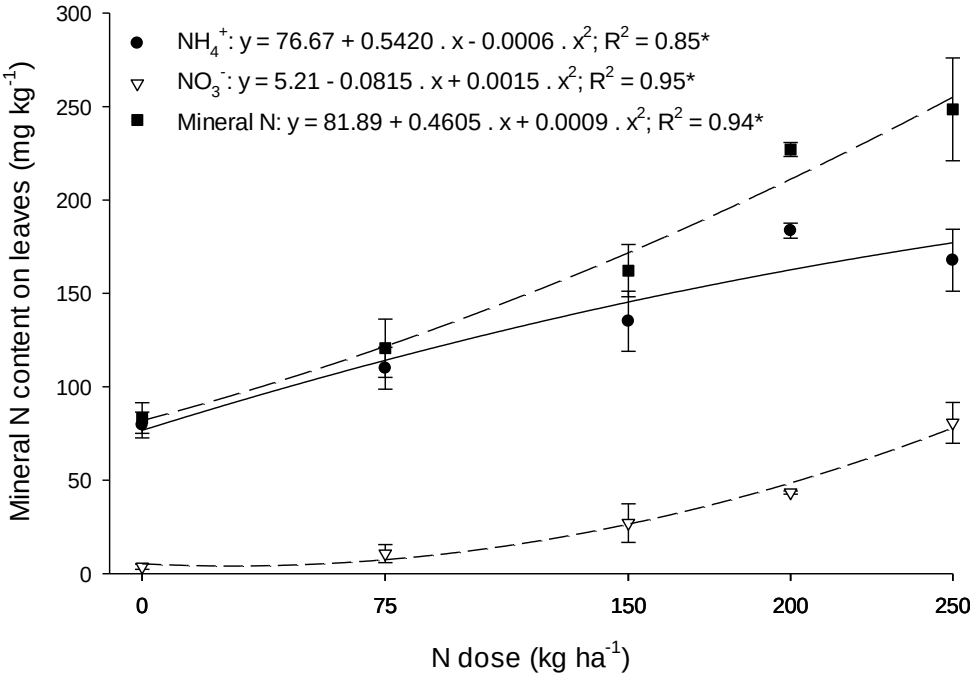

Figure 3 - Contents of NH_4^+, NO_3^- and mineral N in leaves of single-head broccoli submitted to N application. 2011 - Florianópolis (SC).

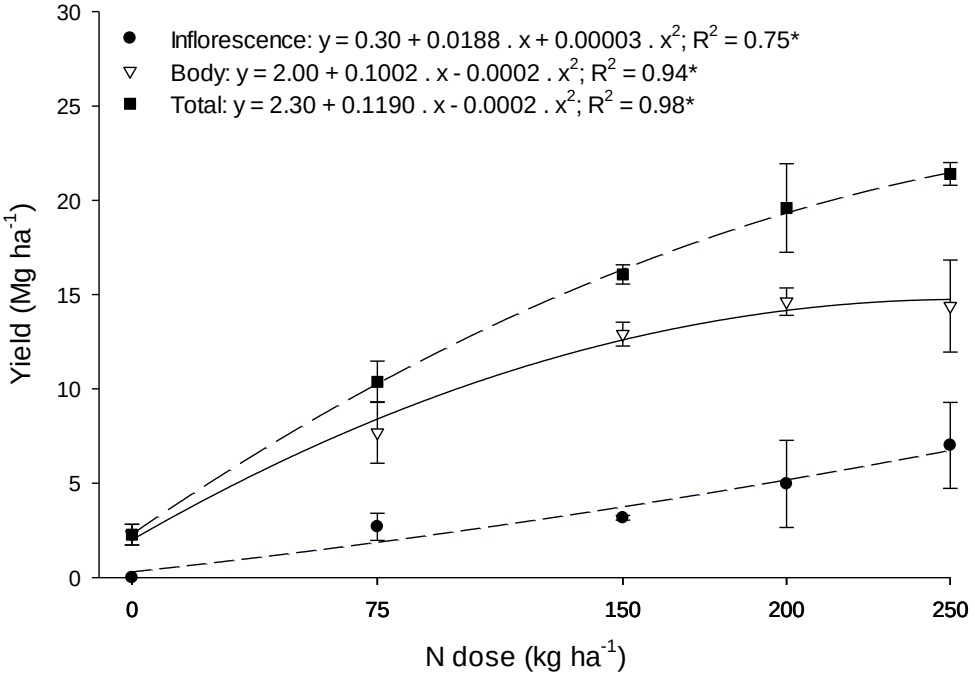

Figure 4 -Yield of inflorescence, body (leaves + stem) and total of single-head broccoli submitted to N application. 2011 – Florianópolis (SC).

Table 2 - Linear correlations between chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Total Chl), carotenoids, NH_4^+, NO_3^-, mineral N (Min. N), yield of inflorescence (Yield infl.), yield of body and total yield of single-head broccoli submitted to N application. 2011 - Florianópolis (SC).

	Chl *a*	Chl *b*	Total Chl	Carote-noids	NH_4^+	NO_3^-	Min.N	Yield infl.	Yield of body	Total yield
Chl *a*	1.00									
Chl *b*	0.97**	1.00								
Total Chl	0.99**	0.98**	1.00							
Carotenoids	0.21ns	0.41ns	0.21ns	1.00						
NH_4^+	0.78ns	0.88ns	0.81ns	0.72ns	1.00					
NO_3^-	0.97**	0.99**	0.98**	0.38ns	0.82ns	1.00				
Min. N	0.90*	0.97**	0.92*	0.60ns	0.98**	0.94*	1.00			
Yield infl.	0.91*	0.98**	0.93*	0.58ns	0.91*	0.95*	0.97**	1.00		
Yield of body	0.68ns	0.83ns	0.72ns	0.88ns	0.95*	0.79ns	0.93*	0.90*	1.00	
Total yield	0.77ns	0.90*	0.81ns	0.80ns	0.96*	0.86ns	0.96**	0.95*	0.99**	1.00

ns not significative; * significative at 5% error probability; ** significative at 1% error probability

Conclusions

The nitrogen forms in the plant have increased with the dose of nutrient applied, reflecting in increased single-head broccoli yield. The application of nitrogen stimulates the synthesis of photosynthetic pigments (chlorophylls and carotenoids) in single-head broccoli leaves, promoting improvement in their nutritional quality.

References

AMAN, R.; CARLE, R.; CONRAD, J.; BEIFUSS, U.; SCHIEBER, A. Isolation of carotenoids from plant materials and dietary supplements by high-speed counter current chromatography. **Journal of Chromatography A**, Amsterdam, v.1074, n.1-2, p.99-105, 2005.

BAKKER, C. J.; SWANTON, C. J.; MCKEOWN, A. W. Broccoli growth in response to increasing rates of pre-plant nitrogen. I. Yield and quality. **Canadian Journal of Plant Science**, Ottawa, v.89, n.3, p.527-537, 2009.

BASLAM, M.; MORALES, F.; GARMENDIA, I.; GOICOECHEA, N. Nutritional quality of outer and inner leaves of green and red pigmented lettuces (*Lactuca sativa* L.) consumed as salads. **Scientia Horticulturae**, Amsterdam, v.151, p.103-111, 2013.

BÉLEC, C.; VILLENEUVE, S.; COULOMBE, J.; TREMBLAY, N. Influence of nitrogen fertilization on yield, hollow stem incidence and sap nitrate concentration in broccoli. **Canadian Journal of Plant Science**, Ottawa, v.81, n.4, p.765-772, 2001.

BOWEN, P. A.; ZEBARTH, B. J.; TOIVONEN, P. M. A. Dynamics of nitrogen and dry-matter partitioning and accumulation in broccoli (*Brassica oleracea* var. *italica*) in relation to extractable soil inorganic nitrogen. **Canadian Journal of Plant Science**, Ottawa, v.79, n.2, p.277-286, 1999.

BREDEMEIER, C.; MUNDSTOCK, C. M. Regulação da absorção e assimilação do nitrogênio nas plantas. **Ciência Rural**, Santa Maria, v.30, n.2, p.365-372, 2000.

CAMPAGNOL, R.; NICOLAI, M.; MELLO, S. C.; ABRAHÃO, C.; BARBOSA, J. C. Boro e nitrogênio na incidência de hastes ocas e no rendimento de brócolis. **Ciência e Agrotecnologia**, Lavras, v.33, n.6, p.1477-1485, 2009.

CQFS-RS/SC – Comissão de Química e Fertilidade do Solo. **Manual de adubação e de calagem para os estados do RS e SC**. 10.ed. Porto Alegre: Sociedade Brasileira de Ciência do Solo - Núcleo Regional Sul, 2004.394p.

CRAFT, N. E. Relative solubility, stabily, and absorptivity of lutein and β-carotene in organic solventes. **Journal of Agricultural and Food Chemistry**, Easton, v.40, n.3, p.431-434, 1992.

ENGELS, C.; KIRKBY, E.; WHITE, P. Mineral nutrition, yield and source-sink relationships. In: MARSCHNER, P. **Marschner's mineral nutrition of higher plants.** 3rd ed. London: Academic Press, 2012. p.85-133.

GUZMAN, I.; YOUSEF, G.G.; BROWN, A.F. Simultaneous extraction and quantitation of carotenoids, chlorophylls, and tocopherols in *Brassica* vegetables. **Journal of Agricultural and Food Chemistry**, Easton, v.60, n.29, p.7238-7244, 2012.

HAWKESFORD, M.; HORST, W.; KICHEY, T.; LAMBERS, H.; SCHJOERRING, J.; MOLLER, I.S.; WHITE, P. Functions of macronutrients. In: MARSCHNER, P. **Marschner's mineral nutrition of higher plants.** 3rd ed. London: Academic Press, 2012. p.135-189.

KURTZ, C.; ERNANI, P. R.; COIMBRA, J. L. M.; PETRY, E. Rendimento e conservação de cebola alterados pela dose e parcelamento de nitrogênio em cobertura. **Revista Brasileira de Ciência do Solo**, Campinas, v.36, n.3, p.865-875, 2012.

LANFER-MARQUEZ, U. M. O papel da clorofila na alimentação humana: uma revisão. **Brazilian Journal of Pharmaceutical Sciences**, São Paulo, v.39, n.3, p.227-242, 2003.

LICHTENTHALER, H. K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. **Methods in Enzymology**, New York, v.148, p.350-382, 1987.

LIMA, M. C.; AMARANTE, L.; MARIOT, M. P.; SERPA, R. Crescimento e produção de pigmentos fotossintéticos em *Achillea millefolium* L. cultivada sob diferentes níveis de sombreamento e doses de nitrogênio. **Ciência Rural**, Santa Maria, v.41, n.1, p.45-50, 2011.

LI, S. X.; WANG, Z. H.; STEWART, B. A. Responses of Crop Plants to Ammonium and Nitrate N. In: SPARKS, D. L. **Advances in agronomy.** San Diego, chap.5, p.205-397. 2013.

MCQUISTAN, T. J.; SIMONICH, M. T.; PRATT, M. M.; PEREIRA, C. B.; HENDRICKS, J. D.; DASHWOOD, R. H. Cancer chemoprevention by dietary chlorophylls: A 12,000-animal dose-dose matrix biomarker and tumor study. **Food and Chemical Toxicology**, Oxford, v.50, p.341–352, 2012.

PADULA, M. L.; CARCIOFI, B. A. M.; DANNENHAUER, C. E.; STRINGARI, G. B.; MONTEIRO, A. L. Influência de diferentes tipos de embalagens nas características físico-químicas e composição gasosa de brócolis (*Brassica oleracea* L. var *italica*) orgânicos minimamente processados e armazenados sob refrigeração. **Alimentos e nutrição**, Araraquara, v.17, n.3, p.259-268, 2006.

RODRIGUEZ-AMAYA, D. B. **A guide to carotenoid analysis in foods**.Washington: ILSI Press, 2001. 54p.

SANGOI, L.; ERNANI, P. R.; LECH, V. A.; RAMPAZZO, C. Lixiviação de nitrogênio afetada pela forma de aplicação da uréia e manejo dos restos culturais de aveia em dois solos com texturas contrastantes. **Ciência Rural**, Santa Maria, v.33, n.1, p.65-70, 2003.

SEABRA JUNIOR, S.; LALLA, J. G.; GOTO, R.; MARINGONI, A. C.; VILLAS BOAS, R. L.; ROUWS, J. R. C.; ORIANI, E. E. Suscetibilidade à podridão negra e produtividade de brócolis em função de doses de nitrogênio e potássio. **Horticultura Brasileira**, Brasília, v.31, n.3, p.426-431, 2013.

SHAN, A. Y. K. V.; OLIVEIRA, L. E. M.; BONOME, L. T. S.; MESQUITA, A. C. Assimilação metabólica de nitrogênio em plântulas de seringueira cultivadas com nitrato ou amônio. **Pesquisa Agropecuária Brasileira**, Brasília, v.47, n.6, p.754-762, 2012.

SIMONICH, M. T.; EGNER, P. A.; ROEBUCK, B. D.; ORNER, G. A.; JUBERT, C.; PEREIRA, C.; GROOPMAN, J. D.; KENSLER, T. M.; DASHWOOD, R. H.; WILLIAMS, D. E.; BAILEY, G. S. Natural chlorophyll inhibits aflatoxin B1-induced multi-organ carcinogenesis in the rat. **Carcinogenesis**, London, v.28, n.6, p.1294--1302, 2007.

SOIL SURVEY STAFF. **Keys to soil taxonomy**. 11 ed. Washington: United States Department of Agriculture, 2010. 338p.

STORCK, C. R.; NUNES, G. L.; OLIVEIRA, B. B.; BASSO, C. Folhas, talos, cascas e sementes de vegetais: composição nutricional, aproveitamento na alimentação e análise sensorial de preparações. **Ciência Rural**, Santa Maria, v.43, n.3, p.537-543, 2013.

TANAKA, K.; YAMADA, A.; NODA, K.; SHOYAMA, Y.; KUBO, C.; NOMOTO, K. Oral administration of an unicellular green algae, *Chlorella vulgaris*, prevents stress-induced ulcer. **Planta Medica**, Stuttgart, v.63, n.5, p.465-466, 1997.

TEDESCO, M.J. **Análise de solo, plantas e outros materiais**. Porto Alegre: UFRGS, 1995. 174p.

TUNCAY, Ö.; ESIYOK, D.; YAGMUR, B.; OKUR, B. Yield and quality of garden cress affected by different nitrogen sources and growing period. **African Journal of Agricultural Research**, Nairobi, n.3, v.6, p.608-617, 2011.

WIESLER, F. Nutrition and Quality. In: MARSCHNER, P. **Marschner's mineral nutrition of higher plants.** 3rd ed. London: Academic Press, 2012. 651p.

WITTE, C. Urea metabolism in plants. **Plant Science**, Limerick, v.180, n.3, p.431-438, 2011.

WOJCIECHOWSKA, R.; ROŜEK, S.; RYDZ, A. Broccoli yield and its quality in spring growing cycle as dependent on nitrogen fertilization. **Folia Horticulturae**, Krakow, v.17, n.2, p.141-152, 2005.