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ORIGINAL ARTICLE

Nitrogen effect on gas exchange characteristics, dry matter production and nitrate accumulation of *Amaranthus cruentus* L.

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Abstract

Among the mineral nutrients, nitrogen stands out as an essential constituent of amino acids, nucleic acids and proteins, as well as being part of the photosynthetic apparatus. The present study aimed to evaluate the responses of *Amaranthus cruentus* L. cv. BRS Alegria to nitrogen availability in terms of biomass production, photosynthetic characteristics, content of photosynthetic pigments, total nitrogen, ammonium and nitrate in the leaves. Plants of *A. cruentus* were cultivated in plastic pots filled with vermiculite and kept in a greenhouse under natural photo-periodic conditions. The plants were watered with 70% of full strength nitrogen-free Long Ashton solution, containing 1.97, 4.94 or 9.88 kg N ha^{-1} as ammonium nitrate, three times a week. Increased nitrogen availability resulted in higher shoot dry matter, specific leaf mass, total leaf nitrogen, chlorophyll and carotenoids as well as in higher photosynthetic rates. There was a positive correlation between photosynthesis and leaf nitrogen. Higher nitrogen availability resulted in greater stomatal conductance and transpiration, but the CO_2 concentration in the sub-stomatal cavity was not significantly altered. In addition, an increase in ammonium and nitrate content of leaves was observed. The nitrate content reached 176, 193 and 288 mg kg^{-1} of dry matter in low, medium and high nitrogen supply, respectively. The data show that *A. cruentus* plants are sensitive to changes in nitrogen availability during the vegetative phase. The limitation of photosynthesis under conditions of low nitrogen supply cannot be fully explained by the reduction in stomatal conductance; non-stomatic limitations are also involved. In addition, the maximum nitrate content observed in leaves was far below the risk level for human health.

Keywords Amaranth · Growth · Nitrate toxicity · Photosynthesis

1 Introduction

The genus *Amaranthus*, an ancient crop originated from Central and South America, belongs to the Amaranthaceae family and includes about 400 species (Rastogi and Shukla 2013). *Amaranthus* is a plant of great economic importance because both leaves and seeds can be used as food. The nutritional quality of leaves and seeds is related to the large amounts of proteins, vitamins and minerals (Rastogi and Shukla 2013). Amaranth seeds are protein rich and have a better balance in the amino acid composition than cereals such as wheat, maize, and sorghum (Rastogi and Shukla 2013) besides not possessing gluten making it important for celiac people (Alemayehu et al. 2015). In many countries, the consumption of amaranth has been stimulated for the purpose of introducing new sources of nutrients and bioactive compounds (Achigan-Dako et al. 2014). Several species of *Amaranthus* possess antioxidant capacity, free radical scavenging effects and antimicrobial activity (Cherian and Sheela 2017; Yong et al. 2017). In the review by Venskutonis and Kraujalis (2013), it is evident that both leaves and seeds are rich in protein, carbohydrates, lipids and various other constituents, which may have various effects on the quality of plant and human health. In addition, leafy parts of the plants contained many times higher amounts of flavonoids than the seeds. Jung et al. (2006) reported that the antioxidant power was higher in the green parts of the adult plant, whereas seeds and sprouts had a 20-fold lower antioxidant power.

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Nutrient availability is the primary factor limiting nutrient uptake and crop yield (Mueller et al. 2012). Inorganic and organic fertilizers are widely used in agriculture to increase crop productivity in order to meet the growing demand for food by the expanding population. Among the mineral nutrients, nitrogen stands out as an essential constituent of amino acids, nucleic acids and proteins, as well as being part of the photosynthetic apparatus. Nitrogen deficiency affects many aspects of plant development such as the ratio between root and shoot, photosynthetic capacity and dry matter production (Ferreira et al. 2015). The reduction in photosynthesis under nitrogen deficiency is related to reduction in chlorophyll and photosynthetic enzymes activities (Seepaul et al. 2016; Makino and Ueno 2018). Increased nitrogen availability leads to a higher nitrogen concentration in the leaf resulting in a strong positive correlation between photosynthetic capacity and leaf nitrogen in both C_3 and C_4 plants (Evans 1989; Zhao et al. 2005; Ma et al. 2017; Makino and Ueno 2018). The amount of nitrogen application is directly related to photosynthesis, with higher nitrogen availability resulting in higher photosynthetic capacity and such response to nitrogen translated in higher dry matter accumulation (Zhao et al. 2005; Lamptey et al. 2017). The indiscriminate use of high doses of nitrogen in agriculture can result in nitrogen losses via emission of nitrous oxide (Millar et al. 2018) which is a potent greenhouse gas and which also plays a crucial role in human health. In addition, oxalate which is an anti-nutrient that occurs in many cultivated plants including *Amaranthus* that has adverse effects on human health can be affected by supplementation of nitrogen, but this response depends on the form of nitrogen. Leaf accumulation of oxalate in spinach is positively associated with root uptake of nitrate, but not with ammonium thus suggesting that a combination of nitrate and ammonium in the nutrient solution reduces the oxalate accumulation (Liu et al. 2015). Moreover, high doses of nitrogen fertilization can also result in the accumulation of nitrate in the plants, thus affecting nutritional quality and safety of the edible parts. Plants are the main source of nitrate, but there is a wide variation in nitrate content depending on the type of plant and crop conditions (Guadagnin et al. 2005). Large accumulations of nitrate in plants were reported in the literature due to the increase in nitrogen availability (Mazahar et al. 2015; Sareer et al. 2016). The ingestion of high nitrate has adverse effects on human health such as methaemoglobinemia and gastrointestinal diseases (Renseigné et al. 2007).

In species of *Amaranthus* large differences have been observed in responses to nitrogen supply in terms of biomass production and nutritional quality (Pospisil et al. 2006; Ayodele et al. 2010). Due to the scarcity of research regarding the physiological responses of this plant to changes in nitrogen supply, the present study aimed to evaluate the responses of *A. cruentus* L. to nitrogen availability in terms of biomass production, photosynthetic characteristics, content of leaf photosynthetic pigments, total nitrogen, ammonium and nitrate.

2 Material and methods

Plant material and growth conditions – BRS Alegria is a new cultivar of *A. cruentus* developed by the Center for Agricultural Research of Cerrados which was originated from the *A. cruentus* strain AM 5189 from the USA (Spehar et al. 2003). Seeds of *A. cruentus* cv. BRS Alegria were sown on October 10, 2016, in 4 dm^3 plastic pots filled with vermiculite and kept in a greenhouse under natural photoperiodic conditions and minimum and maximum average temperature of 21 and 33 °C, respectively. The length of photoperiod at the time of the experiment varied between 12.8 and 13.5 h. Maximum photosynthetic active radiation (PAR) intensity at noontime under a clear sky day was about 2,100 μmol m^{-2} s^{-1}. In the greenhouse, PAR intensity was nearly 70% of that measured outside under clear sky conditions due to both polycarbonate transmission characteristics and greenhouse structural elements. The plants were watered with 70% of full strength nitrogen-free Long Ashton solution (Hewitt 1966), containing different doses of nitrogen as ammonium nitrate. The nitrogen doses used were: 1.97, 4.94 and 9.88 kg N ha^{-1}, which correspond to 20, 50 and 100% of full strength Long Ashton nutrient solution, respectively. The plants were supplied with 300 cm^3 of nutrient solution per pot three times a week, and with water from the supply network for the population of the city on the other days.

Gas exchange measurements and specific leaf mass – A portable infrared gas analyzer (LCpro, ADC, Hoddesdon, UK) was used for measurements of photosynthesis (A), stomatal conductance (g_s), transpiration (E) and intercellular CO_2 concentration (C_i) on the youngest fully expanded leaf after 33 days of growth. Measurements were taken between 8 and 10 am inside the greenhouse under ambient temperature, partial pressure of carbon dioxide and water vapor pressure of air. PAR of 1000 µmol m^{-2} s^{-1} was supplied by a light unit, mounted on top of the leaf chamber. The leaf was kept under this PAR until a steady-state rate was achieved.

The specific leaf mass (SLM) was determined in 4 leaf disks of known area per plant after being oven-dried at 60 °C for 48 h. The SLM was determined according to the equation:

$$\mathrm{SLM} = \frac{\mathrm{DM}}{A} \tag{1}$$

where A is the area and DM is the dry matter of disks.

Chemical analysis – Photosynthetic pigments (Chl *a*, Chl *b* and carotenoids) were extracted in 80% aqueous acetone according to Lichtenthaler (1987) and the concentration expressed on a leaf area basis (g m^{-2}). The determination of nitrate, ammonium and total nitrogen was made in the leaves located in the middle region of the plant. The leaves were oven-dried at 60 °C until constant weight was obtained. The leaves were then finely ground with a mill and sent for analyses at Soil Laboratory of School of Agricultural Sciences, Botucatu, São Paulo/Brazil. The foliar nitrogen, nitrate and ammonium analysis were performed by the semi-micro-Kjeldahl method, after sulphurous digestion of dried and finely ground leaves according to (Malavolta et al. 1997).

Dry mass determination – Thirty-three days after sowing, the plants were collected and divided into stem and leaves before been oven-dried at 60 °C for 48 h.

Statistical analysis – The data were submitted to analysis of variance and Tukey's honestly multiple comparison at 5% significance level by using the Software SPSS/PC 9.0.

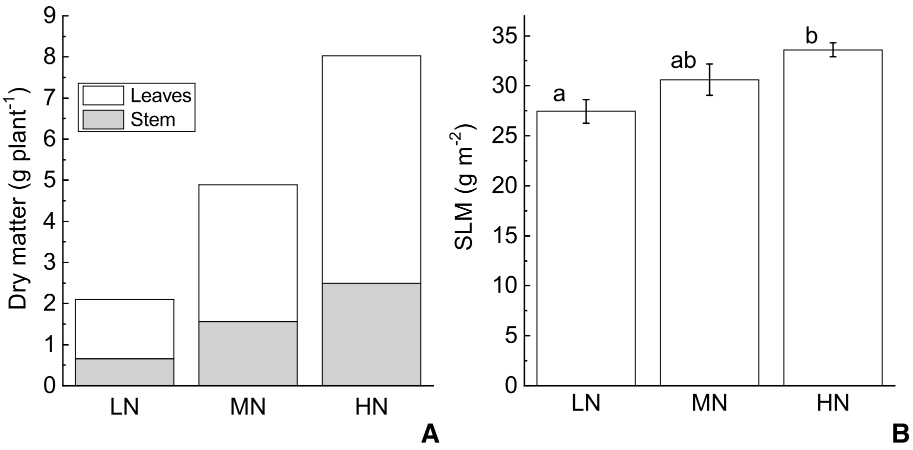
3 Results

Higher nitrogen fertilization had a greater effect on dry matter production in *Amaranthus* plants compared to low and medium fertilization. The above ground biomass responded positively to increasing nitrogen availability, resulting in an increase in shoot dry matter from 2.09 to 8.02 g $plant^{-1}$ from low to high nitrogen supply, respectively (Fig. 1A). Similar effects were found for stem and leaves dry matter with the proportion invested between the stem and the leaves remained practically constant among the different treatments (Fig. 1A). Leaf mass per area (SLM), an indication of leaf thickness, showed the higher values among the plants that received higher amount of nitrogen during the experiment compared to those receiving the lowest nitrogen supply (Fig. 1B). However, SLM of plants grown under medium nitrogen supply did not differ significantly from low and high nitrogen supply.

The content of Chl *a* and Chl *b* was increased as the nitrogen was raised from low to high nitrogen (data not shown). Therefore, the ratio of Chl *a* to Chl *b* did not change significantly. Total chlorophyll concentrations were significantly increased as the nitrogen availability raised from low to high (Fig. 2A). The total chlorophyll of plants grown under high nitrogen was 61% higher than that under low nitrogen while for the medium nitrogen-grown plants was 17% higher. In contrast, the total carotenoids concentration was significantly increased from low to medium nitrogen availability, but it did not differ from medium to high nitrogen supply (Fig. 2B). Significant differences in leaf nitrogen content were observed among nitrogen treatments (Fig. 2C). At higher nitrogen supply, leaves accumulated more nitrogen per unit of mass than medium and low nitrogen-grown plants, the accumulation linearly correlated with the nitrogen availability. It is important to note that although there was an increase in leaf mass production with increase in nitrogen availability, there was no dilution of the total leaf nitrogen content.

A positive linear correlation was found between photosynthesis (*A*) and the concentration of nitrogen supplied to *Amaranthus* plants (Fig. 3A). The application of high nitrogen resulted in a linearly increase in the rate of photosynthesis (*A*) of 41% compared to low nitrogen supply. In addition, leaf stomatal conductance (g_s) was also positively correlated with nitrogen availability (Fig. 3C). In general, changes in g_s are reflected in changes in the measured rate of transpiration (*E*). However, a similar relationship was not observed between *E* and nitrogen supply (Fig. 3B). There was no significant difference in *E* between low and medium nitrogen availability, whereas *E* under high nitrogen differs from the previous ones. It is interesting to note that in plants of *Amaranthus* deficient in nitrogen the low photosynthetic rate was accompanied by a lower g_s when compared with high

Fig. 1 Partitioning of dry matter (g) in the above ground (**A**) and specific leaf mass (**B**) of amaranth plants after 33 days of treatment. Plants were grown with low (LN), medium (MN) or high (HN) nitrogen. Values are means ± SE of 15 and 6 plants, respectively



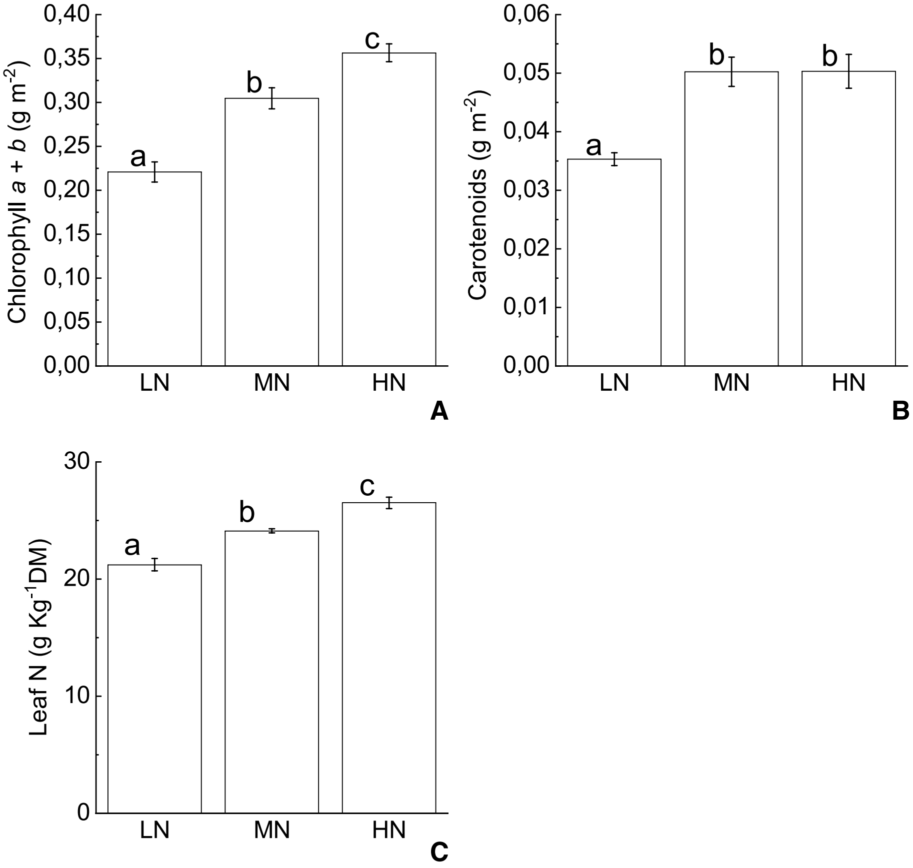


Fig. 2 Content of total chlorophyll (**A**), total carotenoids (**B**) an total leaf nitrogen (**C**) of amaranth plants after 33 days of treatment. Plants were grown with low (LN), medium (MN) or high (HN) nitrogen. Values are means ± SE of 6 plants for chlorophyll and carotenoids and 4 for leaf nitrogen content

nitrogen availability (Fig. 3A, C), but the intercellular CO_2 concentration (C_i) did not differ significantly between treatments (Fig. 3D). Increasing nitrogen availability resulted in a significant increase in leaf nitrogen content (Fig. 2C). This increase in leaf nitrogen content was reflected in a linear correlation with A (Fig. 4).

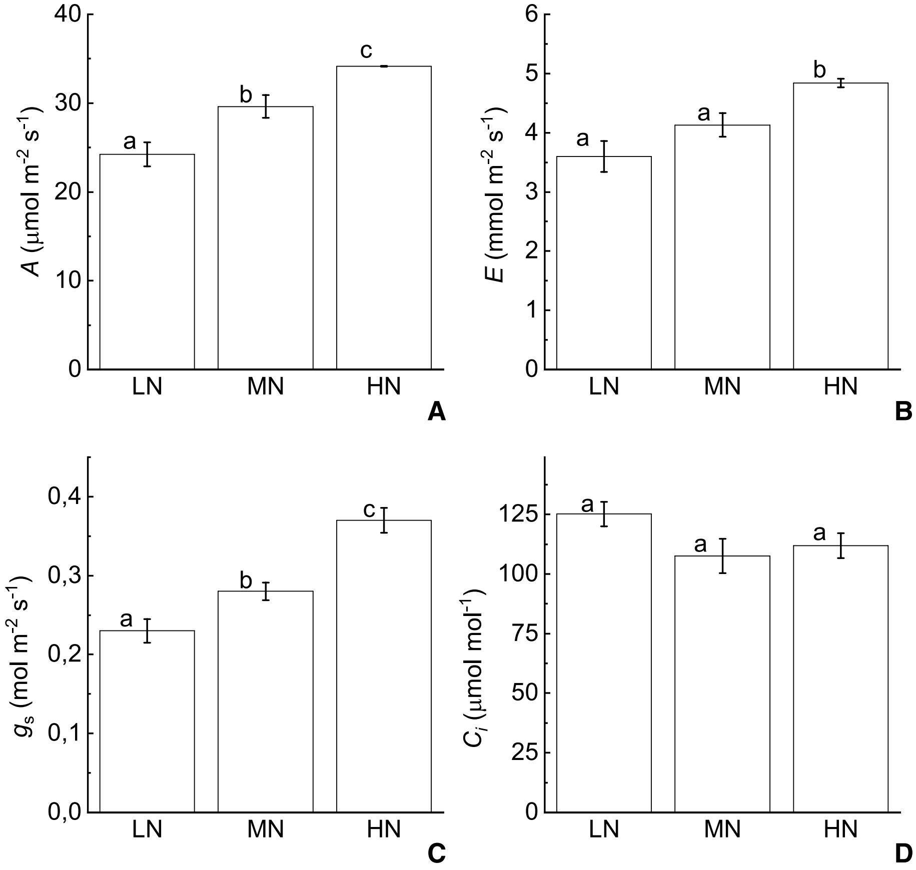
A linear relationship between A and g_s represents the contribution of g_s on photosynthetic CO_2 assimilation. A positive linear relationship between A and g_s was also observed in leaves of *Amaranthus* plants grown under low and medium nitrogen supply (Fig. 5A). However, there was no relationship between the two variables in plant grown under high nitrogen supply. The relationship between A and C_i had an inverse behavior than A and g_s for low and medium nitrogen-grown plants while there was no relationship between the two variables in plant grown under high nitrogen supply (Fig. 5B). As the photosynthetic rates declined in leaves under low and medium nitrogen supply, the CO_2 concentration in the sub-stomatal cavity of the leaf increased.

The leaves of *Amaranthus* presented an accumulation of nitrate of 397.6 mg kg^{-1} of dry mass in response to an increase in nitrogen availability from low to high nitrogen supply (Fig. 6), but there was no significant difference between low and medium nitrogen availability. Similar to nitrate, ammonium also accumulated in response to increasing nitrogen application with no significant difference between low and medium nitrogen (Fig. 6). However, the accumulation in nitrate was 5.3 times higher while the accumulation in ammonium was only 1.6 times compared to low nitrogen fertilization.

4 Discussion

Nitrogen fertilizer has significant effects on the agronomic traits, grain yield and leaf photosynthetic capacity of several plants (Zhao et al. 2005; Fang et al. 2018). Lower nutrients availability, particularly nitrogen, decreases growth more than photosynthesis (Poorter et al. 2009). In *Amaranthus*, lower nitrogen availability reduced the above ground dry matter by 74%, whereas photosynthetic rate was reduced only 29% when compared with high nitrogen. Under nitrogen deficiency, there is a decline in the transport of reduced nitrogen to the aerial part, resulting in a decrease in meristematic activity and initiation of new leaves, favoring root growth (Rufty et al. 1990). On the other hand, plants cultivated under higher nitrogen content showed higher rate of total nitrogen deposition in the leaf growing zone than those without nitrogen suggesting that leaf expansion rate is very sensitive to nitrogen concentrations in zones of cell

Fig. 3 Photosynthesis (*A*, **A**), transpiration (*E*, **B**), stomatal conductance (g_s, **C**) and intercellular CO_2 concentration (C_i, **D**) of amaranth plants after 33 days of treatment. Plants were grown with low (LN), medium (MN) or high (HN) nitrogen. Values are means ± SE of 8 plants



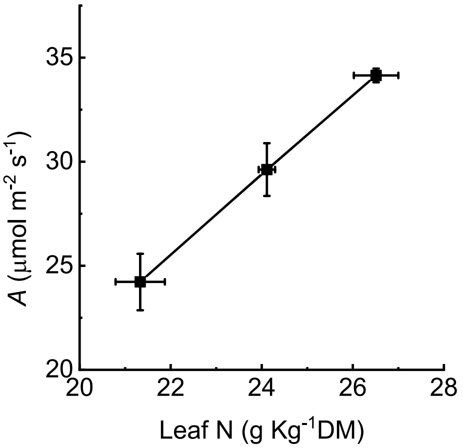


Fig. 4 Relationship between photosynthesis (*A*) and total leaf nitrogen concentration (Leaf N) of amaranth plants after 33 days of treatment. Plants were grown with low (LN), medium (MN) or high (HN) nitrogen. Values are means ± SE of 8 and 4 plants for photosynthesis and for leaf nitrogen, respectively. The regression equation is $y = -16 + 1.91x$; $R^2 = 0.99$

division and expansion (Gastal and Nelson 1994). Although the root to shoot ratio was not measured, it is well known that, in general, when nitrogen availability increases, plants allocate relatively less to their roots, meaning that less effort is required to acquire this resource. In this study, the above ground biomass of *Amaranthus* responded positively to increasing nitrogen availability with the proportion invested between the stem and the leaves remained practically constant between the different treatments. Comparisons between the various published studies correlating increased nitrogen availability with *Amaranthus* productivity are difficult to make because the vast majority of these studies were conducted under field conditions where these are very adverse among the different studies. However, the higher biomass production detected in this study confirms the *Amaranthus* responses to the nitrogen supply observed under field conditions (Brambilla et al. 2008; Abbasi et al. 2012). Although there are some studies related to biomass production in several amaranth species in response to nitrogen supply, less is known about the morphological and physiological responses to variation in nitrogen availability.

SLM, an indication of leaf thickness, is a leaf trait that is dependent on environmental conditions, and it was found to increase or decrease in response to high nitrogen (Amanullah et al. 2013; Liu and Li 2016). It has been reported that nitrogen increased mesophyll cell number more than epidermal cell number (MacAdam and Volenec 1989). Additionally, as shown by Makino and Ueno (2018) for other C_4 plant, the higher leaf thickness under high nitrogen supply

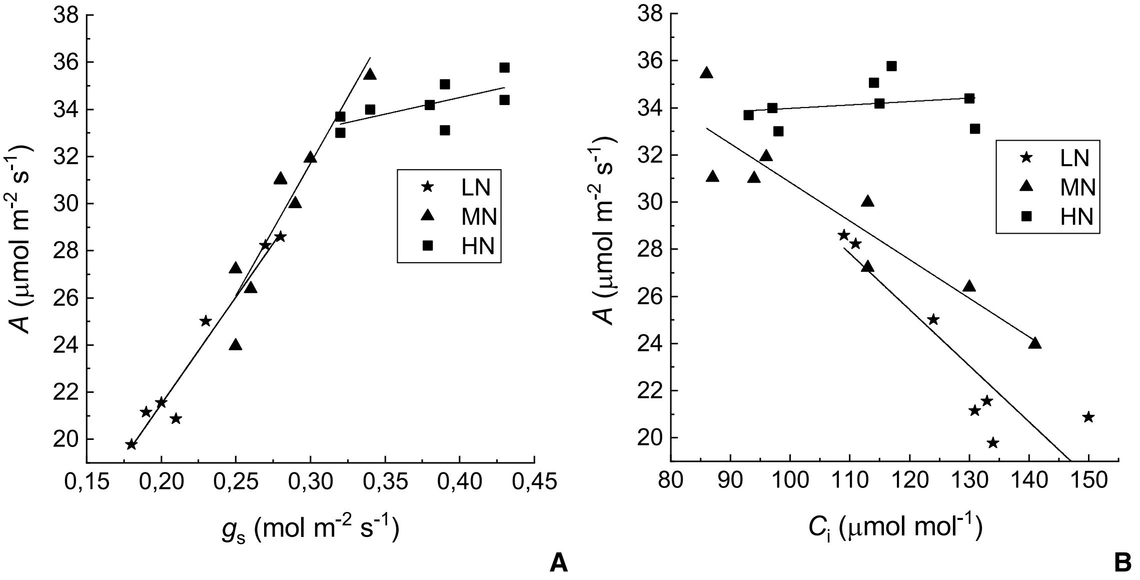


Fig. 5 Relationship between photosynthesis (A) and stomatal conductance (g_s) (**A**) and between photosynthesis (A) and intercellular CO_2 concentration (C_i) (**B**) of amaranth plants after 33 days of treatment. Plants were grown with low (LN), medium (MN) or high (HN) nitrogen. Values are means ± SE of 8 plants for each nitrogen supply. The regression equations are: Fig. 5A, filled star $y = 3.32 + 90.85x$, $R^2 = 0.966$; black up-pointing triangle, $y = -1.93 + 112.12x$, $R^2 = 0.865$; black square, $y = 28.86 + 14.09x$, $R^2 = 0.445$. Figure 5B, filled star $y = 53.99 - 0.24x$, $R^2 = 0.836$; black up-pointing triangle $y = 47.21 - 0.16x$, $R^2 = 0.847$; black square $y = 32.53 + 0.01x$, $R^2 = 0.051$

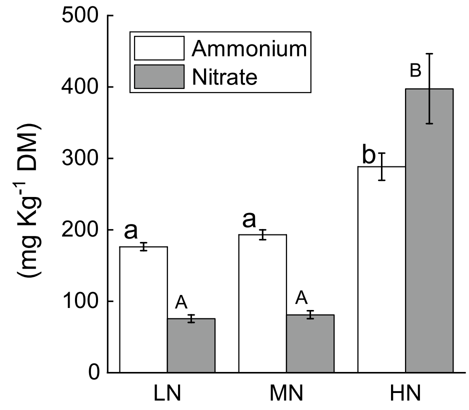


Fig. 6 Content of ammonium and nitrate of leaf of amaranth plants after 33 days of treatment. Plants were grown with low (LN), medium (MN) or high (HN) nitrogen supply. Values are means ± SE of 4 plants, respectively

was also accompanied by higher interveinal distance and increased length, area and number of chloroplasts of both mesophyll and bundle sheath cells but reduced the thickness of both mesophyll and bundle sheath cells. In *Amaranthus*, SLM was increased in plants that received higher amount of nitrogen during the experiment compared to those receiving the lowest nitrogen concentration. In addition, there was an increase in the content of total chlorophylls and carotenoids similar to lettuce (Konstantopoulou et al. 2012) and total nitrogen per unit of dry mass thus corroborating with the results of others authors (Liu et al. 2014; Peng et al. 2016). Although there was an increase in leaf mass production with increase in nitrogen availability, there was no dilution of the total photosynthetic pigments or leaf nitrogen content suggesting that the increase in SLM under high nitrogen supply may reflect the increase in the accumulation in photosynthetic pigments and total nitrogen along with any effect of nitrogen on mesophyll thickness.

In high nitrogen-grown plants, the total leaf nitrogen content increased 25% while A increased about 41% compared with low nitrogen supply. The positive response of A to nitrogen application and leaf nitrogen content was expected, since this nutrient is positively correlated with A. The increase in A is related to the fact that the majority of leaf nitrogen is located in the chloroplasts (Evans 1989) and the influence that high nitrogen has on chloroplasts development (Bondada and Syvertsen 2003). In addition, in higher nitrogen-grown C_4 plants such as *Amaranthus* there is a greater nitrogen allocation in the Rubisco enzyme (Tazoe et al. 2006) and greater activity of Rubisco and PEPc (Ma et al. 2017). As the concentration of nitrogen supply increased, the photosynthetic pigments content increases in parallel with the increase in A and SLM. The higher A in the leaves exhibiting higher SLM may reflect the presence of a greater amount of photosynthetic components in the leaves.

The effects of nitrogen on g_s in the literature are contradictory. Although nitrogen deficiency has no effect on g_s in some species (Grassi et al. 2002), in others the g_s can be increased (Ciompi et al. 1996) or reduced (Shahrokhnia and Sepaskhah 2017). These contradictory results can be explained by the fact that plant responses to nitrogen supply depend on several parameters such as species and growth conditions. In the present study, the increase in nitrogen availability resulted in higher g_s and higher transpiration rate. An increase in g_s implies a greater availability of CO_2 in the sub-stomatal cavity but also a greater amount of water vapor lost through stomata. Stomatal closure under low nitrogen supply without changes in the mesophyll capacity should reduce the C_i. It is interesting to note that in plants of *Amaranthus* deficient in nitrogen the low photosynthetic rate was accompanied by a lower g_s when compared with high nitrogen availability, but C_i did not differ significantly between treatments. If CO_2 conductance was exclusively limiting photosynthesis, C_i would be expected to decrease under low nitrogen as a result of removal of CO_2 from intercellular spaces and reduction in the resupply through the stomata. The amount of CO_2 in the sub-stomatal cavity depends on the stomatal conductance and on the mesophyll conductance from sub-stomatal cavities into the chloroplasts and by metabolic processes. The content and the activity of photosynthetic enzymes which represent the metabolic processes are increased under supplementation with nitrogen in C_4 plants (Tazoe et al. 2006; Ma et al. 2017). Thus, the numerically lower C_i under high and medium nitrogen compared to low nitrogen suggests that the mesophyll capacity for CO_2 assimilation in those former plants is high thus resulting in a lower C_i even if the g_s is high. These data show that in *Amaranthus* nitrogen deficient plants the stomatal closure is not totally responsible for the reduction in the photosynthetic rate, suggesting a biochemical and/or photochemical dysfunction in addition to any stomatal limitation of photosynthesis. Non-stomatal limitation can be attributed to decreased mesophyll conductance from sub-stomatal cavities into the chloroplasts and by metabolic processes. Although there were no significant differences in C_i between the treatments, the relationship between *A* and C_i showed that under lower and medium nitrogen as the photosynthesis decreased the C_i increased. This suggest that under these conditions the stomatal conductance is not the only factor affecting *A*. The photosynthetic performance requires proteins for all steps of the process including formation of the light-harvesting chlorophyll–protein complexes of the antenna and photosynthetic enzymes. The photosynthetic enzymes limitation to *A* can be through a change in activity or quantity (Tazoe et al. 2006; Ma et al. 2017). A linear relationship between *A* and g_s which represents the contribution of g_s on photosynthetic CO_2 assimilation was also observed in leaves of *Amaranthus* plants in this study. The slope of the relationship between the two variables was steeper in low and medium nitrogen-grown plants with no significant relationship between *A* and g_s under high nitrogen-grown plants, indicating that in high nitrogen-grown plants variation in g_s from 0.32 to 0.42 does not result in alterations in CO_2 fixation. The photosynthesis of C_4 plants is already saturated with a concentration of CO_2 around 100 µmol mol^{-1} in the intercellular spaces. This is due to the high carboxylation efficiency of the PEPcase, combined with the inhibition of photo-respiration promoted by the CO_2 concentrating mechanism. The lack of relationship between *A* and C_i under high nitrogen shows that the mesophyll capacity had already reached the full capacity at low C_i. However, plants under low and medium nitrogen presented an inverse relationship between *A* and C_i showing that as *A* decreased, the C_i increased. This reduced capacity for assimilation of CO_2 as C_i increased is an indication of non-stomatal limitation as mentioned above.

The main sources of nitrogen for most plants species are nitrate and ammonium with nitrate being the preference of most species (Andrews et al. 2013). The nitrate absorbed by the roots is assimilated in the roots or transported to aerial parts, depending on its availability and plant species. The assimilation process results in the transformation of nitrate into nitrite and then into ammonium. Both ammonium absorbed by the roots and produced by the assimilation process are converted into glutamine and then into glutamate for production of many organic compounds. A large naturally variability in nitrate accumulation has been found between plant species and among cultivars of the same species (M'hamdi et al. 2016; Razgallah et al. 2016; Sareer et al. 2016). Large accumulations of nitrate in plants also occur when there is an excess in the absorption due to the great availability of nitrogen in the soil in relation to the transformation capacity into organic compounds. Leafy vegetables as lettuce and parsley and forage plants such as *Brassica oleracea* L. var. Acephala, *Brassica napus* L. and *Amaranthus hypochondriacus* L. accumulate nitrate in response to an increase in nitrogen availability (Petropoulos et al. 2008; Abbasi et al. 2012; Liu et al. 2014; Chakwizira et al. 2015). In this study, the leaves of *A. cruentus* presented an accumulation of nitrate of 397.6 mg kg^{-1} of dry mass in response to an increase in nitrogen availability from low to high nitrogen application showing consistency with the reports found in the literature. Plants usually accumulate ammonium when nourished with ammonium as the only form of nitrogen. Although the plants received equal doses of nitrate and ammonium, there was also accumulation of ammonium in the leaves indicating saturation of the capacity of the enzymes involved in the assimilation of nitrogen into organic compounds. Plants can tolerate high levels of nitrate in their tissues without harmful effects, but high ammonium levels are toxic resulting in growth reduction (Lasa et al. 2001). Despite the accumulation of ammonium in the leaves, no toxic effects were observed in *Amaranthus*.

The consumption of nitrate-rich foods poses a health risk because it can cause methemoglobinemia disease (Renseigné et al. 2007) due to the production of nitrite by the liver which in turn binds with hemoglobin making it unable to bind to oxygen. In addition, the formation of nitrite from ingested nitrate can result in several adverse health effects and implies a genotoxic risk as a consequence of endogenous formation of carcinogenic N-nitroso compounds (Vermeer et al. 1998). More recent scientific evidence suggests that there is no increased risk of stomach cancer due to the ingestion of nitrate because it can undergo metabolic conversion to nitrite and nitric oxide thus performing a useful protective effect to stomach and prevent vascular disease (Gilchrist et al. 2010; Bryan et al. 2012). These new evidences require a reconsideration of food safety in relation to nitrate and nitrite safety (Bryan et al. 2012). To reduce the risk of nitrate intake, the United Nations (FAO) and the World Health Organization (WHO) established the daily limit of nitrate intake of 3.7 mg kg^{-1} body mass (WHO 1995). Considering this limit and the highest amount of nitrate accumulated in *Amaranthus* leaves in this study (397.6 mg Kg^{-1} dry mass), a person with a body mass of 60 kg could ingest up to 558 g of dry mass of *Amaranthus* leaves without presenting health risks. The risk of excess nitrate in the leaves can be reduced by cooking the leaves before using them for edible purposes (Rastogi and Shukla 2013) or by selecting the right time for collecting the leaves since there is a change in the content of nitrate as a function of plant age (Abbasi et al. 2012).

Nitrogen is an indispensable element for the good performance of *A. cruentus* cv. BRS Alegria. High levels were fundamental for higher accumulation of biomass in the aerial parts and higher capacity for CO_2 assimilation. The limitation of photosynthesis under conditions of non-optimal nitrogen availability cannot be fully explained by the reduction in stomatal conductance. The data suggest that non-stomatic limitations such as decreased mesophyll conductance from sub-stomatal cavities into the chloroplasts and by metabolic processes may also involved. Additional studies, especially regarding the possible biochemical changes in the photosynthetic apparatus due to the alteration in the nitrogen supply, are necessary. Considering that the daily intake limit acceptable to the World Health Organization for nitrate by man is 3.7 mg kg^{-1} body weight, it is concluded that the amount of nitrate accumulated in the leaves of plants of *A. cruentus* grown in the higher dose of nitrogen does not pose any risk to human health.

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Author's contribution (1) IC and EMV contributed to experimental work and analyzed the data; (2) IC prepared the manuscript; (3) IC and EMV read, corrected and approved the manuscript in its final form.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict interest.

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