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Foliar application of molybdenum enhanced quality and yield of crispleaf lettuce (*Lactuca sativa* L., cv. Grand Rapids)

Aplicação foliar de molibdênio melhora a produtividade e a qualidade nutricional da alface crespa (*Lactuca sativa* L., cv. Grand Rapids)

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Abstract

Foliar molybdenum (Mo) application may enhance the nitrogen (N) acquisition by the plants and increase the yield and quality of vegetables. A study was conducted to investigate the effect of foliar Mo spray on N nutrition and yield of crispleaf lettuce (*Lactuca sativa* L., cv. Grand Rapids) grown in the spring-summer period. The experiment was carried out at Ourinhos, São Paulo State, Brazil, from October 2013 to January 2014. Treatments consisted of five Mo rates [0 (control), 25, 50, 75, and 100 g ha^{-1} of Mo] divided into two foliar sprays, at 14 and 21 days after planting. Foliar application of Mo rates reduced the nitrate (NO_3^-) concentration, and increased the concentrations of ammonium (NH_4^+), total N and Mo in the lettuce leaves. These data indicate that the foliar Mo application improved N assimilation of lettuce plants, resulting in less accumulation of NO_3^- in the leaves and therefore, improving the quality of the vegetable. Number of leaves per plant was not affected by the foliar Mo application. The 55 to 62 g ha^{-1} of Mo application resulted in increased of the leaves fresh weight (33%), leaves dry weight (28%) and commercial yield (34%) of crisphead lettuce, respectively. Results suggest that Mo deficiency can compromise the N metabolism of plants, and result in lower commercial yield of crispleaf lettuce.

Key words: Molybdoenzymes, nitrate assimilation, plant nutrition, nitrate accumulation, vegetable quality.

Resumo

A aplicação foliar de molibdênio (Mo) pode melhorar a absorção de N das plantas e aumentar a produtividade e a qualidade da hortaliça comercializada. Este estudo foi realizado para avaliar o efeito da aplicação foliar de molibdênio na absorção de nitrogênio e na produtividade da alface crespa (*Lactuca sativa* L., cv. Grand Rapids) cultivado no período de primavera-verão. O experimento foi realizado em Ourinhos (SP), durante os meses de outubro de 2013 a janeiro de 2014. Os tratamentos constituíram da aplicação de cinco doses de Mo [0 (controle), 25, 50, 75 e 100 g ha^{-1}] divididas em duas aplicações foliares aos 14 e 21 dias após o transplantio das mudas. A colheita foi realizada 36 dias após o transplantio das mudas. A aplicação foliar de Mo reduziu a concentração de nitrato (NO_3^-) e aumentou a concentração de amônio (NH_4^+), nitrogênio total (N) e de Mo nas folhas de alface. Estes dados indicam que a aplicação foliar de Mo melhorou a assimilação de N das plantas de alface, resultando na redução do acúmulo de NO_3^- nas folhas e, consequentemente, melhorando a qualidade nutricional da hortaliça. O número de folhas por plantas não foi afetado pela aplicação foliar de Mo. A aplicação de 55 a 62 g ha^{-1} de Mo resultou no aumento da matéria fresca das folhas (33%), material seca das folhas (28%) e na produtividade comercial (34%) da alface crespa, respectivamente, em comparação ao tratamento controle. Os resultados sugerem que a deficiência de Mo pode comprometer o metabolismo de N das plantas, e resultar em menor produtividade comercial da alface crespa.

Palavras-chave: Molibdoenzimas, assimilação de nitrato, nutrição de planta, acúmulo de nitrato, qualidade das hortaliças.

Introduction

Crispleaf lettuce (*Lactuca sativa* L.) is the leaf vegetable most planted in Brazil. This type of lettuce has loosely bunched leaves and is used mainly for salads (Hamerschmidt, Leonardecz, Gheller, Bortolossi, Franco, Harger & Carvalho, 2013). Lettuce is a crop that has high response to N fertilization, resulting in higher yields, in more uniform products and higher commercial value. However, sustainable management of lettuce yield, as of many leafy vegetable crops, requires that N supplies are optimized to maximize crop growth and development, whilst minimizing economic inefficiencies and environmental contamination caused by soil nitrate (NO_3^-) leaching to groundwater (Dahan, Babad, Lazarovitch, Russak & Kurtzman, 2014). Furthermore, the excessive N fertilizer use may result in the accumulation of NO_3^- in the lettuce leaves and reduce the quality of the vegetable (Liu, Sung, Chen & Lai, 2014; Qiu, Wang, Huang, Chen & Yang, 2014). Nitrate by itself is relatively non-toxic; however, it may be endogenously transformed to nitrite, which can react with amines and amides to produce N-nitroso compounds (Santamaria, 2006). These compounds have been related to an increased risk of diseases, such as the methemoglobinemia (Santamaria, 2006). In addition, nitrite may react with certain amine containing substances found in food to form nitrosamines, which are known to be potent cancer causing chemicals. For this reason, nitrate in vegetables has received increasing attention. In recent decades, much research has been conducted to minimize the accumulation of nitrate in vegetables (Liu *et al.*, 2014; Resende, Alvarenga, Yuri & Souza, 2010).

Among the management practices used to reduce the NO_3^- accumulation in the leafy vegetables, the Mo application has been reported as an excellent alternative (Resende *et al.*, 2010). This because the Mo plays a crucial role in nitrogen metabolism of plants because of its involvement in the nitrogen fixation processes, nitrate reduction, and transport of N compounds in plants (Li *et al.*, 2013). Enzymes that require Mo for activity include nitrate reductase, nitrogenase, and sulfite oxidase. In general, the plants require Mo levels of 0.1 to 3.0 mg kg^{-1} in their tissues for optimum growth.

Molybdenum can be applied to crops by various methods in the form of different fertilizer sources. The recommended rate for the foliar spray of Mo in lettuce is 0.5 g L^{-1} sodium molybdate, applied to seedlings one week before transplanting (Hamerschmidt *et al.*, 2013).

Foliar Mo application is often more effective than soil applications, particularly for acid soils due to the adsorption process of this micronutrient in soil, and are most effective if applied at early stages of plant development (Hamerschmidt *et al.*, 2013). Indeed, the effectiveness of foliar spray depends on the nutrient uptake rate by the leaves and its translocation into the plant (Fernández & Brown, 2013). In general, the leaves rapidly absorb Mo applied by leaf spray.

The effect of Mo application on increasing plant yield is often related to an increased ability of the plant to utilize N. Resende *et al.* (2010) found that the highest yields of crisphead lettuce occurred when N fertilization was associated with Mo leaf supply. The nitrogenase and nitrate reductase activities are affected by the Mo status of plants, and their activities are often suppressed in plants suffering from Mo deficiency (Toledo, Garcia, Pereira, Boaro & Lima, 2010). Calonego, Ramos, Barbosa, Leite & Grassi (2010), found that the absence of Mo leaf supply promoted the accumulation of NO_3^- in the plants as result of the increased N availability in the soil, indicating the low efficiency of N assimilation of plants in the absence of this micronutrient. Thus, it is expected that the application of Mo improves the N assimilation of plants, reducing the nitrate concentration in leaves and, consequently, improving the quality and commercial yield of leafy vegetables. However, these effects of crisphead lettuce are inconclusive and incipient (Resende *et al.*, 2010).

The aim of this study was to investigate the effect of Mo foliar spray on N nutrition and yield of crispleaf lettuce (*Lactuca sativa* L., cv. Grand Rapids) grown in the spring-summer period in Ourinhos, São Paulo State, Brazil.

Material and methods

Study area

The experiment was carried out in Ourinhos, São Paulo State, Brazil (24°55’20” S, 49°54’24” W, altitude of 480 m), from October 2013 to January 2014, where the environmental conditions were: minimum and maximum mean temperature of 18 °C and 31 °C, respectively; and air relative humidity of 72% (± 8%) and light fluence of 1480 μmol m^{-2} s^{-1} (±345 μmol m^{-2} s^{-1}) photosynthetic photon flux density (PPFD).

Soil samples

The soil was a Rhodic Hapludox with 520 g kg^{-1} clay, 200 g kg^{-1} silt, and 280 g kg^{-1} sand. Before starting the experiment, soil samples were taken from the surface depth (0–0.20 m) of seedbeds, air-dried, sieved through a 2.0 mm mesh, and subjected to chemical analysis. Soil analysis showed pH in $CaCl_2$ 0.01 mol L^{-1} of 5.1, 22 g dm^{-3} of organic matter (OM), 12 mg dm^{-3} P-resin, 7 mg dm^{-3} of S-SO_4, 32 $mmol_c$ dm^{-3} of Ca, 17 $mmol_c$ dm^{-3} of Mg, 2.8 $mmol_c$ dm^{-3} of K, 53 $mmol_c$ dm^{-3} of H+Al, 50% of base saturation, 9.5 mg dm^{-3} of Cu-DTPA-TEA, 2.6 mg dm^{-3} of Zn-DTPA, 41 mg dm^{-3} of Fe-DTPA, 128 mg dm^{-3} Mn-DTPA, 0.45 mg dm^{-3} of B (hot water), and 0.21 mg dm^{-3} of Mo (1.0 mol L^{-1} ammonium acetate).

Foliar application

Foliar application rates were defined based on the responses of crisphead lettuce foliar Mo spray reported by Resende *et al.* (2010). As Mo source was used ammonium molybdate $[(NH_4)_6Mo_7O_{24}]$. Applications were performed with a CO_2 pressurized sprayer with 150 kPa working pressure capacity, equipped with flat fan nozzle, adjusted to apply 350 L ha^{-1} broth. The plants were sprayed at dusk due to a lower likelihood of drift by wind speed reduction and higher relative humidity.

Plant seedlings

Lettuce seedlings (*Lactuca sativa* L., cv. Grand Rapids) produced in 288-cell expanded polystyrene trays filled with commercial substrate (Plantmax®) were used. At 26 days after sowing, when they presented four leaves, the seedlings were transplanted on seedbeds of 0.20 m in height. Each experimental plot consisted of seedbeds with four rows 1.2 m long, spaced 0.25 m apart with plants being 0.30 m apart. Four central plants formed the area to be studied. Irrigation was performed daily by the sprinkler system. The basic fertilization was carried out by applying 1,000 kg ha^{-1} 05-20-10 (N-P_2O_5-K_2O) formulation at transplanting, and 40 kg ha^{-1} N topdressing as urea at 21 and 28 days after transplanting.

Lettuce was harvested 36 days after transplanting the seedlings. The plants were cut close to soil surface, weighed to obtain leaves fresh weight and commercial yield. The collected leaves were then dried in a forced-air oven for four days at 55 °C, weighed and ground in a Willey type mill. Concentrations of nitrate (NO_3^-) and ammonium (NH_4^+) were determined by vapor distillation, and total N by sulfuric acid digestion and vapor distillation, as previously described by Tedesco, Gianello, Bissani, Bohnen & Volkweiss (1995). Molybdenum (Mo) was determined using the graphite furnace as described by Carmo, Araújo, Bernardi & Saldanha (2000).

Statistic analysis

The experimental design was randomized blocks with four replicates. Treatments consisted of five Mo rates [0 (control), 25, 50, 75, and 100 g ha^{-1} of Mo] divided into two foliar sprays, at 14 and 21 days after planting.

Data were submitted to analysis of variance (ANOVA), F test, and polynomial regression, and significant equations ($p \leq 0.05$) with the higher coefficient of determination, which were adjusted. All analyses were performed using SigmaPlot 11.0 software for Windows (Systat Software®, Inc., San Jose, CA, USA).

Results

Foliar Mo application did not affect the number of leaves per plant of lettuce (Figure 1A). The crispleaf lettuce yield was significantly affected by the foliar Mo application (Figure 1). Leaves fresh weight increased from 85.6 g $plant^{-1}$ in the control treatment to a maximum of 113.8 g $plant^{-1}$ with the 61.2 g ha^{-1} of Mo application, indicating mean increase of 33% (Figure 1B). Leaves dry weight increased from 4.25 g $plant^{-1}$ in the control treatment to a maximum of 5.5 g $plant^{-1}$ with the application of 55.0 g ha^{-1} of Mo, indicating mean increase of 28% (Figure 1C). The commercial lettuce yield increased from 1.04 kg m^{-2} in the control treatment to a maximum of 1.39 kg m^{-2} with the application of 61.3 g ha^{-1} of Mo, indicating mean increase of 34% (Figure 1D).

Foliar Mo application affects the concentrations of nitrate (NO_3^-), ammonium (NH_4^+), total nitrogen (N), and molybdenum in the leaves dry weight of lettuce (Figure 2). Nitrate concentrations decreased progressively with increasing Mo rates in the foliar spray (Figure 2A). Nitrate reduced from 19.8 g kg^{-1} in the control treatment to minimum of 13.9 g kg^{-1} with the 100 g ha^{-1} of Mo application (30% reduction).

Foliar Mo application resulted in the significant increase of NH_4^+ and total N concentrations in the lettuce leaves. Ammonium concentration increased from 14.2 g kg^{-1} in the control to maximum of 17.9 g kg^{-1} with the application of 63.9 g ha^{-1} of Mo, indicating mean increase of 26%.

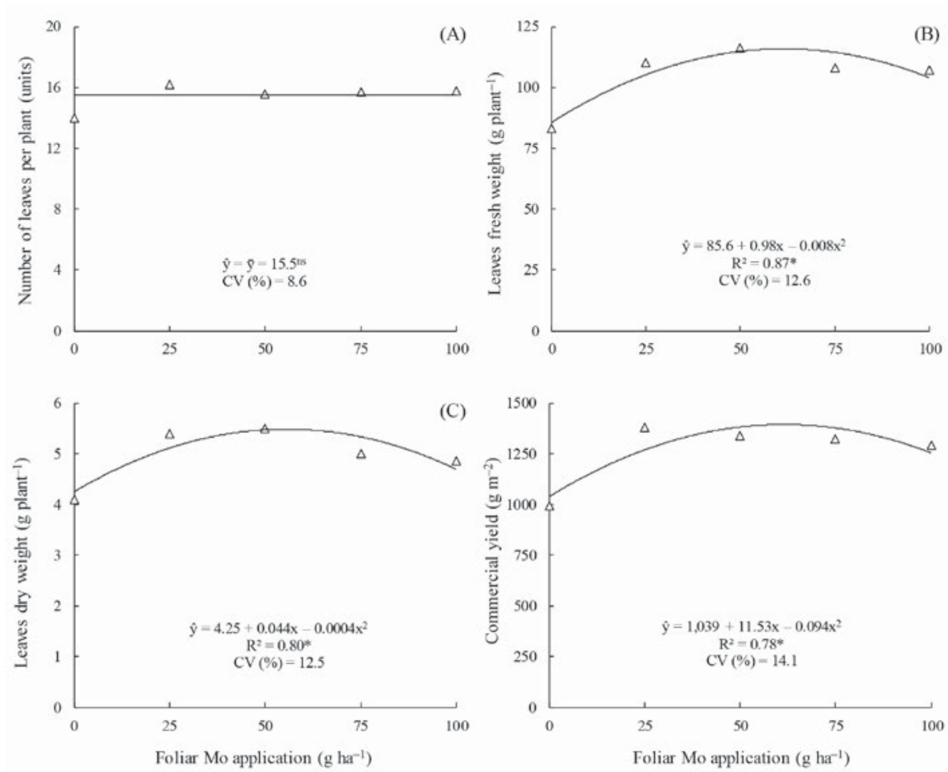

Figure 1. Effect of foliar molybdenum application on number of leaves per plant (A), leaves fresh weight (B), leaves dry weight (C) and commercial yield (D) of crispleaf lettuce (*Lactuca sativa* L., cv. Grand Rapids) grown in the spring-summer period.

ns: not significant. *statistical significance at 5%. CV: coefficient of variation.

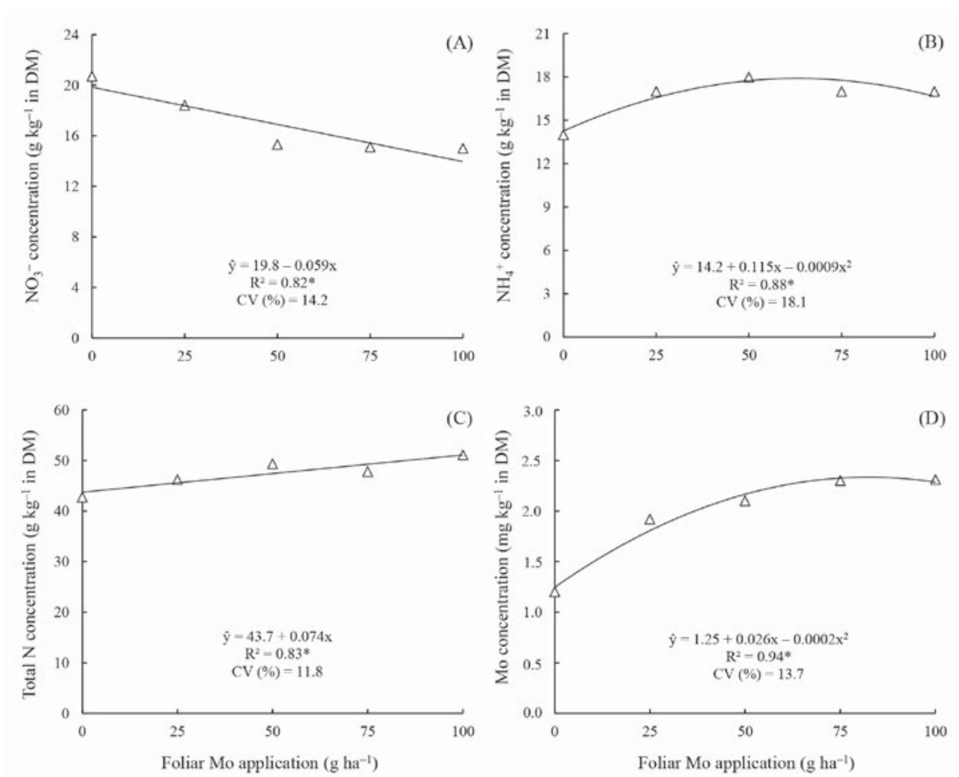

Figure 2. Effect of foliar Mo application on nitrate (A), ammonium (B), total nitrogen (C) and molybdenum (D) concentrations in the leaf tissue of crispleaf lettuce (*Lactuca sativa* L., cv. Grand Rapids) grown in the spring-summer period. *statistical significance at 5%. CV: coefficient of variation.

Total N concentration increased from 43.7 g kg^{-1} in the control treatment to maximum of 51.1 g kg^{-1} with the 100 g ha^{-1} of Mo application, indicating mean increase of 17%.

Molybdenum concentration on the lettuce leaves was significantly affected by foliar Mo rates (Figure 1D). The Mo concentration increased from 1.25 mg kg^{-1} in the control treatment to maximum of 2.10 mg kg^{-1} with the application of 65.0 g ha^{-1} of Mo, indicating mean increase of 68%. These results were expected due to the low availability of Mo in the soil and its application on the leaves.

Discussion

Foliar Mo application improved the crispleaf lettuce yield (Figure 1). These results are similar to those reported by Yuri, Resende, Mota, Gonçalves & Souza (2004), who reported significant effect of the foliar Mo application on increase of commercial yield of crisphead lettuce. Resende *et al.* (2010) reported increase of 11% in the dry weight of the commercial part of crisphead lettuce with the 75.3 g ha^{-1} of Mo foliar application. The increase in commercial yield of crispleaf lettuce with Mo rates may be due to the increase in N assimilation and nutrition of plants (Figure 2). The Mo fertilization effect on increasing lettuce yield is associated with increased ability of the plant to utilize N (Resende *et al.*, 2010).

The reduction of NO_3^- concentration in the lettuce leaves with Mo rates (Figure 2A) occurred because this micronutrient plays an indispensable role in the NO_3^- assimilation taken up by plants (Li *et al.*, 2013). Molybdenum is component (cofactor) the nitrate reductase – enzyme that catalyzes the conversion of inorganic N in form of nitrate to nitrite (Mokhele, Zhan, Yang & Zhang, 2012).

Nitrate taken up by the roots is readily mobile in plants and can be accumulated in vacuoles; however, for nitrate to be used in the synthesis of proteins and other organic compounds in plants, it must be reduced to ammonium (NH_4^+). The reduction is catalyzed by enzymes in two steps: the first step takes place in the cytoplasm by nitrate reductase (NR) transforming NO_3^- into nitrite (NO_2^-), and the second occurs in chloroplasts (shoots) or proplastids (roots) by nitrite reductase (NiR) converting NO_2^- to NH_4^+ (Rosales, Iannone, Groppa, & Benavides, 2011). The NO_3^- reduction to NO_2^- is the rate-limiting step for primary nitrate assimilation, and reductive ratios in roots and shoots depend on plant species, carbohydrates in plants, and nitrate reductase activity (NRA) as well as environmental conditions such as NO_3^- concentration, medium soil pH, complementary ions, light, and ambient CO_2 concentration (Li *et al.*, 2013). Toledo *et al.* (2010) showed that the foliar Mo application enhanced the nitrogenase and nitrate reductase activities resulting in increase of N accumulated in the soybean leaves. Calonego *et al.* (2010), reported that the absence of Mo supply promoted the nitrate accumulation in the common bean (*Phaseolus vulgaris* L.) leaves, indicating the low efficiency of N assimilation by the plants in deficiency conditions of Mo.

Considering that the lettuce leaves showed 4.5 to 5% dry weight (Figure 1B and 1C), the NO_3^- concentration in the fresh matter in lettuce leaves ranged 680 to 1020 mg kg^{-1}, with application of 100 g ha^{-1} of Mo and control treatment (Figure 2A). Thus, the maximum values of 1020 mg kg^{-1} of NO_3^- in fresh matter of lettuce obtained in this study are well below of the maximum limits 3500-4500 mg kg^{-1} in fresh weight accepted by the European Union (EU) for this vegetable (Schröder & Bero, 2001). Not compromising, therefore their quality. In this case, to had achieved the Maximum Acceptable Daily Intake index - that is, 3.7 mg $kg^{-1\ body}$ weight, a consumer of 70 kg may consume up to 250 g or more than two heads of lettuce per day.

The increase of NH_4^+ and N concentration in leaf tissue of crispleaf lettuce plants with Mo rates suggests that this micronutrient improved the plant nitrogen assimilation, i.e., the NO_3^- reduction to NH_4^+ and subsequently converted in amino acids, proteins and other organic compounds in plants (Li *et al.*, 2013). All inorganic N taken up by plants is first reduced to NH_4^+, because it is the only reduced N form available to plants for assimilation into N-carrying amino acids (Ruiz, Rivero & Romero, 2007).

Ammonium is then assimilated into glutamine and glutamate, which serve to translocate organic N from sources to sinks in legumes and non-legumes (Mokhele *et al.*, 2012). The main enzymes involved are glutamate synthase (GS), or glutamine-2-oxoglutarate amino transferase (GOGAT), and glutamate dehydrogenase (GDH) (Mokhele *et al.*, 2012). Cao, Fan, Sun, Xu, Hu & Shen (2008), found that GS has a vital role in NH_4^+ assimilation and the activity of the enzyme is considered critical and possibly rate-limiting step in NH_4^+ assimilation. Ammonium may be toxic to plants, because it can cause proton extrusion, which is associated with NH_4^+ uptake, changes in cytosolic pH and uncoupling of photophosphorylation in plants (Wang *et al.*, 2007). Glutamine and asparagine are the preferential form in which N is assimilated and translocated (Mokhele *et al.*, 2012). This is because these molecules show low C:N ratio, which represents an advantage for NH_4^+ incorporation to non-toxic forms (Frechilla, Lasa, Aleu, Juanarena, Lamsfus & Aparicio, 2002).

These results were expected due to the low availability of Mo in the soil and its application on the leaves. In general, Mo concentration between 0.1 and 3.0 mg kg^{-1} is considered adequate for normal growth of plants. Deficient plants showed leaf concentrations between 0.01 and 0.1 mg kg^{-1} (Tedesco *et al.*, 1995).

Conclusion

This study evaluated the effects of foliar Mo spray on N nutrition and yield of crispleaf lettuce (*Lactuca sativa* L., cv. Grand Rapids). Bearing this in mind, the 55 to 60 g ha^{-1} of Mo foliar application proved to be sufficient to improve N assimilation, yield and quality parameters of crispleaf lettuce grown in the spring-summer period in Ourinhos, São Paulo State, Brazil. Given these concerns, the evaluated rate applied the more Mo concentration in the lettuce leaves, which remained in the range considered adequate for optimum growth and development of plants.

References

Calonego, J.C., Ramos-Junior, E.U., Barbosa, R.D., Leite, G.H.P. & Grassi-Filho, H. (2010). Adubação nitrogenada em cobertura no feijoeiro com suplementação de molibdênio via foliar. *Rev Ciênc Agron,* 41(3), 334-340. *http://dx.doi.org/10.1590/s1806-66902010000300003*

Cao, Y., Fan, X.R., Sun, S.B., Xu, G.H., Hu, J. & Shen, Q.R. (2008). Effect of nitrate on activities and transcript levels of nitrate reductase and glutamine synthetase in rice. *Pedosphere,* 18(5), 664-673. *http://dx.doi.org/*10.1016/s1002-0160(08)60061-2

Carmo, C.A.F.S., Araújo, W.S., Bernardi, A.C.C. & Saldanha, M.F.C. (2000). Métodos de análise de tecidos vegetais utilizados na Embrapa solos. Rio de Janeiro: Embrapa Solos, 41p.

Dahan, O., Babad, A., Lazarovitch, N., Russak, E.E. & Kurtzman, D. (2014). Nitrate leaching from intensive organic farms to groundwater. *Hydro Earth System Sci,* 18(1), 333-341. *http://dx.doi.org/10.5194/hess-18-333-2014*

Fernández, V. & Brown, P.H. (2013). From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. *Front Plant Sci,* 4, 289. *http://dx.doi.org/10.3389/fpls.2013.00289*

Frechilla, S., Lasa, B., Aleu, M., Juanarena, N., Lamsfus, C. & Aparicio-Tejo, P.M. (2002). Short-term ammonium supply stimulates glutamate dehydrogenase activity and alternative pathway respiration in roots of pea plants. *J Plant Physiol,* 159(8), 811-818. *http://dx.doi.org/10.1078/0176-1617-00675*

Hamerschmidt, I., Leonardecz, A., Gheller, J.A., Bortolossi, J.L., Franco, M.J., Harger, N. & Carvalho, N.R.L. (2013). Manual técnico de olericultura. Curitiba: Emater, 266p.

Liu, C.W., Sung, Y., Chen, B.C. & Lai, H.Y. (2014) Effects of nitrogen fertilizers on the growth and nitrate content of lettuce (*Lactuca sativa* L.). *Int J Environ Res Public Health,* 11(4), 4427-4440. *http://dx.doi.org/*10.3390/ijerph110404427

Mokhele, B., Zhan, X., Yang, G. & Zhang, X. (2012). Review: Nitrogen assimilation in crop plants and its affecting factors. *Can J Plant Sci,* 92(2), 399-405. *http://dx.doi.org/*10.4141/cjps2011-135

Qiu, W., Wang, Z., Huang, C., Chen, B. & Yang, R. (2014). Nitrate accumulation in leafy vegetables and its relationship with water. *J Soil Sci Plant Nutr,* 14(4), 761-168. *http://dx.doi.org/*10.4067/s0718-95162014005000061

Resende, G.M., Alvarenga, M.A.R., Yuri, J.E. & Souza, R.J. (2010). Yield and postharvest quality of winter growing crisphead lettuce as affected by doses of nitrogen and molybdenum. *Hortic Bras,* 28(4), 441-445. *http://dx.doi.org/*10.1590/s0102-05362010000400011

Rosales, E.P.M.F., Iannone, M., Groppa, D.M. & Benavides, P. (2011). Nitric oxide inhibits nitrate reductase activity in wheat leaves. *Plant Physiol Biochem,* 49(2), 124-130. *http://dx.doi.org/*10.1016/j.plaphy.2010.10.009

Ruiz, J.M., Rivero, R. & Romero, M.L. (2007). Comparative effect of Al, Se, and Mo toxicity on NO_3^- assimilation in sunflower (*Helianthus annuus* L.) plants. *J Environ Manag,* 83(2), 207-212. *http://dx.doi.org/*10.1016/j.jenvman.2006.03.001

Santamaria, P. (2006). Nitrate in vegetables, toxicity, content, intake and EC regulation. *J Sci Food Agr,* 86, 10-17. *http://dx.doi.org/*10.1002/jsfa.2351

Schröder, F.G. & Bero, H. (2001). Nitrate uptake of *Lactuca sativa* L. depending on varieties and nutrient solution in hydroponic system PPH. *Acta Hortic,* 548, 551-555. *http://dx.doi.org/*10.17660/actahortic.2001.548.67

Tedesco, M.J., Gianello, C., Bissani, C.A., Bohnen, H. & Volkweiss, S.J. (1995). Análises de solos, plantas e outros materiais. 2nd ed. Porto Alegre: UFRGS, 174 p.

Toledo, M.Z., Garcia, R.A., Pereira, M.R.R., Boaro, C.S.F. & Lima, G.P.P. (2010). Nodulação e atividade da nitrato redutase em função da aplicação de molibdênio em soja. *Biosci J Uberlândia,* 26(6), 858-864. *http://www.seer.ufu.br/index.php/biosciencejournal/article/view/7220/6605.*

Wang, Z.Q., Yuan, Y.Z., Ou, J.Q., Lin, Q.H. & Zhang, C.F. (2007). Glutamine synthetase and glutamate dehydrogenase contribute differentially to proline accumulation in leaves of wheat (*Triticum aestivum*) seedlings exposed to different salinity. *J Plant Physiol,* 164(6), 695-701. *http://dx.doi.org/*10.1016/j.jplph.2006.05.001

Yuri, J.E., Resende, G.M., Mota, J.H., Gonçalves, L.D. & Souza, R.J. (2004). Doses e épocas de aplicação de molibdênio na produção e qualidade de alface. *Hortic Bras,* 22(3), 589-592. *http://dx.doi.org/10.1590/s0102-05362004000300017*