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LETTUCE RESPONSE TO NITROGEN FERTILIZERS AND ROOT MYCORRHIZATION

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Abstract

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Two lettuce varieties Ysi 43301/5638 (green lettuce) and Matador 5635 (red lettuce) plants were grown as a spring crop under glasshouse conditions in order to evaluate the effect of different synthetic nitrogen fertilizers applied solely and in combination with mycorrhizal fungi on the yield and quality. Mineral nitrogen was applied as NH_4NO_3, $CO(NH_2)_2$, $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ and an inoculum of arbuscular mycorrhizal fungi, *Glomus intraradices* EEZ 01 was used. The effect of mycorrhizal inoculation on plant biomass more clearly distinguished in variety Ysi 43301/5638, where biomass accumulation significantly increased in the treatments with $CO(NH_2)_2$, $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ in combination with *Gl. intraradices*. The most favorable effect on the plant biomass of the other variety Matador 5635 was observed in plants inoculated with *Gl. intraradices* and fertilized with $(NH_4)_2SO_4$. Deterioration of lettuce quality (reduction of protein content, soluble sugars and increased nitrate content) was observed in Ysi 43301/5638 in the treatments with urea in combination with mycorrhizal fungi. The strong reduction in the values of pigments also in both varieties was estimated as a result of fertilization with urea in non-mycorrhizal and mycorrhizal treatments. The interactive action of nitrogen assimilatory enzymes - nitrate reductase and glutamine synthetase are not in accordance with biomass accumulation.

Key words: Lactuca sativa L., arbuscular mycorrhizal fungi, nitrogen fertilizers, nitrogen assimilatory enzymes
Abbreviations: arbuscular mycorrhizal fungi (AMF); nitrate reductase (NR); glutamine synthetase (GS)

Introduction

Lettuce (*Lactuca sativa* L.) is the most popular crop among the salad vegetables, which requires nitrogen (N) for growth and development. Nitrogen fertilizers such as; ammonium sulfate, ammonium nitrate or calcium nitrate positively affect fresh and dry plant weights, plant diameter and the number of total marketable leaves, whereas the yield and other yield components remained unaffected by N sources (Bozkurt et al., 2009; Gülser et al., 2010).

Application of different nitrogen fertilizer sources makes an impact on yield and quality of lettuce and especially on the content of nitrates (Simeonova et al., 2015). In addition, the use of synthetic fertilizers modifies the balance of the soil chemical properties. During the last years, the role of microorganisms in the conservation of soil fertility increased. Vessey (2003) pointed out that the soil microorganisms were successfully applied as biofertilizers, useful to surrogate chemical fertilization. The arbuscular mycorrhizal fungi (AMF) have an important role in plant nutrient uptake, not only for the availability of P but also of other nutrients such as N, K, Ca and Mg (Clark and Zeto, 2000). The favorable effect of AMF inoculation on the lettuce yield has been reported (Koide et al., 2000). The response of lettuce

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to the production system, organic and phosphate fertilizers and root mycorrhization was evaluated by Brito et al. (2016).

This study evaluates the effect of different synthetic nitrogen fertilizers applied solely and in combination with arbuscular mycorrhizal fungi on the yield and quality of two lettuce varieties.

Material and Methods

Lettuce plants (*Lactuca sativa* L.) Ysi 43301/5638 (green lettuce) and Matador 5635 (red lettuce) were grown under glasshouse conditions during the period 06.11.2015 - 08.03.2016. Lettuce plants were grown in 2 kg plastic pots (2 plants per pot) on the alluvial meadow soil with humus content – 2.65%. The soil possessed slightly alkaline reaction pH(H2O) = 7.5, pH(KCl)-6.9, low to the middle content of mineral nitrogen 30.0 mg/1000g, middle phosphorus content P_2O_5 – 9.9 mg/100g and well supplied with potassium K_2O - 20.2 mg/100g. Mineral fertilization with $N_{300}P_{300}K_{377}$ was applied. Mineral nitrogen was applied as NH_4NO_3, $CO(NH_2)_2$, $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ (2/3 before the planting and 1/3 as a dressing). Phosphorus and potassium were incorporated into the soil before the planting.

The inoculum of AMF, *Glomus intraradices* EEZ 01 was added to the soil (at 2 cm depth under soil surface) before sowing in quantity 0.5 g per pot. The mycorrhizal strain was provided from the collection at the experimental field station of Zaidin, Granada, Spain.

Extraction and determination of nitrates in leaves samples were assayed by zinc reduction method according to Mir (2007). Sugar content was determined refractometrically, and plastid pigments chlorophyll “a”, chlorophyll “b” and carotenoids according to the Lichtenthaler (1987).

In order to prepare crude extracts for determination of leaves nitrate reductase (NR-NADH: EC 1.6.6.2) and glutamine synthetase (GS: EC 6.3.1.2) activity, the extraction medium containing 50 mM Tris-HCl (pH 8.0), 1 mM Na_2MoO_4, 10 mM $MgSO_4.7H_2O$, 1 mM EDTA, 10 mM L-cysteine, 1% PVP-40, 1g Dowex (Frechilla et al. 2002). The extract was filtered through one layer of cheesecloth, centrifuged at 10 000 g for 20 min (4°C), and the supernatant was used for the following assays. NR activity was measured according to Hageman and Reed (1969). GS activity was determined by a biosynthetic assay based on g-glutamil hydroxamate synthesis (O·Neal and Joy, 1973). Protein content was determined according to Bradford (1976) with BSA as a standard.

Data are expressed as means ± standard error where n = 3. Comparison of means was performed by the Fisher LSD test ($P \leq 0.05$) after performing multifactor ANOVA analysis. A statistical software package (StatGraphics Plus, version 5.1. for Windows, USA) was used.

Results and Discussion

Different nitrogen sources resulted in different levels of lettuce biomass in both varieties (Table 1). The effect of mycorrhizal inoculation on plant biomass more clearly distinguished in variety Ysi 43301/5638. In this variety biomass accumulation significantly increased in the treatments with $CO(NH_2)_2$, $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ in combination with *Gl. intraradices*. The most favorable effect on the plant biomass of the other variety Matador 5635 was observed in plants inoculated with *Gl. intraradices* and fertilized with $(NH_4)_2SO_4$.

Kohler et al. (2006) reported that inorganic fertilizer and inoculation with *Gl. intraradices* had significantly increased lettuce shoot and root biomass and foliar nutrient contents of P and Fe. Oehl et al. (2003) suggested that AMF symbiosis is influenced by various management practices, such as the degree and type of fertilization, the host plant species or cultivar, the mycorrhizal species, the type of host plant root system and the crop rotation or soil tillage.

Inoculation with AMF influenced in a different way the parameters, characterized lettuce quality in both varieties in dependence on nitrogen fertilizer source (Table 1). In variety Ysi 43301/5638 fertilization with $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ in combination with AMF increased to significant extent total soluble sugar and protein content in the leaves and at the same time lowered content of nitrates. On the other hand fertilization with $CO(NH_2)_2$ in combination with *Gl. intraradices* had harmful effects on the quality parameters soluble sugars and protein which decreased compared with non-mycorrhizal plants and nitrate content in the leaves rise (Table 1).

In variety Matador 5635 the highest biomass accumulation was observed in the inoculated with AMF plants fertilized with $(NH_4)_2SO_4$ (Table 1). Total soluble sugars and protein content increased significantly as a result of mycorrhizal inoculation and the all applied nitrogen fertilizers but nitrate content in the leaves increased as a result of $CO(NH_2)_2$ application.

Ammonium was a better N-source for non-mycorrhizal plants and nitrate for mycorrhizal plants (Azcón et al., 2003). According to Guertal (2009) reported also that urea application caused greater nitrate content in lettuce plant than the urea-formaldehyde that prevented excessive accumulation of NO_3^- in the vegetables, including lettuce plants and leaching of N from the soil. According to Gonella et al. (2000), inappropriate fertilizer source led to quality deterioration, reduction of carbohydrates and vitamins and elevated nitrate content. Despite the increased nitrate content in the treat-

Table 1
Lettuce fresh biomass and some quality parameters

Treatments	Fresh biomass g plant^{-1}	Total soluble sugars (%)	Protein content mg gFW^{-1}	Nitrates µg NO_3^- g FW^{-1}
Variety Ysi 43301/5638				
1. Control	15.57a	5.2a	3.22b	20.71a
2. NH_4NO_3	36.81c	6.9bc	1.94a	223.87f
3. $CO(NH_2)_2$	29.15b	7.8d	5.93e	65.92b
4. $Ca(NO_3)_2$	39.70cd	6.6bc	3.34bc	283.44j
5. $(NH_4)_2SO_4$	48.22 e	6.9bc	2.01a	84.29c
6. Control + AM fungi	17.5a	6.4b	4.33d	32.01a
7. NH_4NO_3+ AM fungi	40.95d	8.8e	4.68d	115.51d
8.$CO(NH_2)_2$+ AM fungi	38.65cd	7.2cd	3.67c	148.10e
9. $Ca(NO_3)_2$+ AM fungi	52.81f	10.0f	8.23f	265.41g
10. $(NH_4)_2SO_4$+ AM fungi	63.37g	11.3g	3.24b	23.46a
LSD ($P \leq 0.05$)	3.47	0.68	0.38	13.45
Variety Matador 5635				
1. Control	12.34a	4.9a	0.57a	54.92a
2. NH_4NO_3	29.65e	6.4c	1.66b	114.50d
3. $CO(NH_2)_2$	24.31d	5.6b	2.24c	54.61a
4. $Ca(NO_3)_2$	20.96c	5.8bc	0.29a	120.91d
5. $(NH_4)_2SO_4$	18.63c	5.8bc	0.49a	78.89c
6. Control + AM fungi	15.83b	7.8de	4.91de	81.10c
7. NH_4NO_3+ AM fungi	37.92f	9.0f	6.02f	69.59b
8. $CO(NH_2)_2$+ AM fungi	28,64e	9.2f	11.23g	122.13d
9. $Ca(NO_3)_2$+ AM fungi	42.10g	7.3d	5.30e	123.35d
10. $(NH_4)_2SO_4$+ AM fungi	48.53j	8.0e	4.56d	60.12a
LSD ($P \leq 0.05$)	2.56	0.61	0.42	8.99

Different letters in the values indicate significant differences assessed by Fisher LSD test ($P \leq 0.05$) after performing ANOVA analysis

ments with NO_3^--N and urea in combination with mycorrhiza, concentration of nitrates are lower than acceptable limit concentration 4500 mg kg^{-1} fresh weight grown in the period 01.10-31.03 in the glasshouse (Instruction 31/24.07.2004).

Plastid pigment content (chlorophyll “a”,”b” and carotenoids were affected significantly by the mycorrhizal fungi inoculation in both varieties (Table 2), but much higher values were observed in variety Matador 5635. The strong reduction in the values of pigments also in both varieties was estimated as a result of fertilization with urea in non-mycorrhizal and mycorrhizal treatments. In the leaves of variety Ysi 4330/5638, the highest values of chlorophyll and carotenoids occurred in the plants fertilized with NH_4NO_3 and AMF followed by the values in the non-fertilized treatments in combination with AMF.

In the leaves of another variety - Matador 5635 plastid pigments reached maximal values in the treatments with AMF without fertilization (Table 2). Fertilization with $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ in combination with AMF also have a favorable effect on the plastid pigments levels in the leaves of this variety. Zuccarini (2007) have already observed the enhancement of chlorophyll content and nutrient uptake as a result of mycorrhizal colonization of lettuce plants.

Because the different nitrogen fertilizer forms were used, it is interesting to study the activity of the main nitrogen assimilatory enzymes – nitrate reductase and glutamine synthetase. With the addition of nitrates to the plant tissues ammonia is generated by the concerted reactions of nitrate reductase and nitrite reductase. It is established that the major pathway for ammonia incorporation into non-toxic organic compounds occurs through glutamine synthetase - glutamate synthase (GS-GOGAT) cycle (Ireland and Lea, 1999). The GS-GOGAT cycle converts ammonia by the combined activity of glutamine synthetase and glutamate synthase to produce two molecules of glutamate. Nitrate reductase activity (Figure 1) is much higher in cv. Matador 5635 in comparison with another Ysi 4330/5638 variety. Application of mycorrhizal fungi to lettuce plants of Ysi 4330/5638 variety enhanced NR activity only in the non-fertilized plants as well as in the plants fertilized with $(NH_4)_2SO_4$. Mycorrhization of lettuce of var. Matador 5635 resulted in stimulation of nitrate reductase also in non-fertilized plants and those fertilized

Table 2
Plastid pigments content in lettuce leaves

Treatments	Chlorophyll "a" mg gDW^{-1}	Chlorophyll "b" mg gDW^{-1}	Chlorophyll "a+b" mg gDW^{-1}	Carotenoids mg gDW^{-1}
Variety Ysi 43301/5638				
1. Control	0.32d	0.32f	0.64j	0.51
2. NH_4NO_3	0.65e	0.42g	1.07k	0.21
3. $CO(NH_2)_2$	0.24cd	0.12bc	0.36d	0.11
4. $Ca(NO_3)_2$	0.09ab	0.10b	0.19b	0.12
5. $(NH_4)_2SO_4$	0.13bc	0.11bc	0.23b	0.26
6. Control + AM fungi	0.33d	0.19de	0.52f	0.25
7. NH_4NO_3+ AM fungi	0.35cd	0.21e	0.57g	0.29
8.$CO(NH_2)_2$+ AM fungi	0.07a	0.05a	0.12a	0.12
9. $Ca(NO_3)_2$+ AM fungi	0.17abc	0.13c	0.30c	0.12
10. $(NH_4)_2SO_4$+ AM fungi	0.25cd	0.18d	0.41e	0.14
LSD ($P \leq 0.05$)	0.12	0.02	0.04	0.02
Variety Matador 5635				
1. Control	1.31e	0.89c	2.19d	0.55c
2. NH_4NO_3	1.27e	0.85c	2.12d	0.39a
3. $CO(NH_2)_2$	0.15a	0.15a	0.30a	0.52c
4. $Ca(NO_3)_2$	0.95c	0.80bc	1.75c	0.46b
5. $(NH_4)_2SO_4$	1.12d	0.95bc	2.07d	0.89f
6. Control + AM fungi	2.56g	1.43d	3.99f	0.77e
7. NH_4NO_3+ AM fungi	0.93c	0.69bc	1.61c	0.39a
8. $CO(NH_2)_2$+ AM fungi	0.69b	0.56b	1.25b	0.51bc
9. $Ca(NO_3)_2$+ AM fungi	1.73f	1.17d	2.90e	0.90f
10. $(NH_4)_2SO_4$+ AM fungi	1.63f	1.18d	2.81e	0.71d
LSD ($P \leq 0.05$)	0.12	0.27	0.2	0.06

Different letters in the values indicate significant differences assessed by Fisher LSD test ($P \leq 0.05$) after performing ANOVA analysis

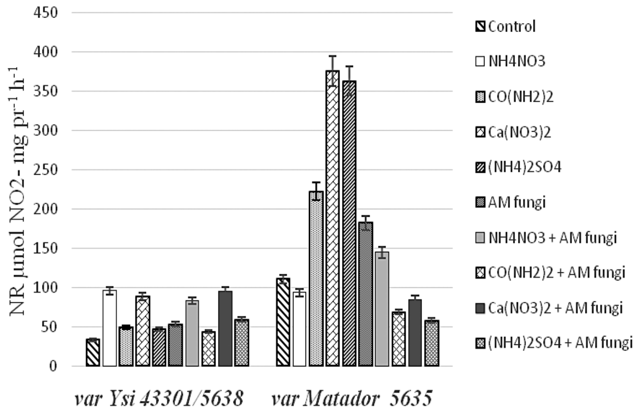

Fig. 1. Nitrate reductase (NR) activity in two lettuce varieties grown at different nitrogen sources. Different letters in the values indicate significant differences assessed by Fisher LSD test ($P \leq 0.05$) after performing ANOVA analysis

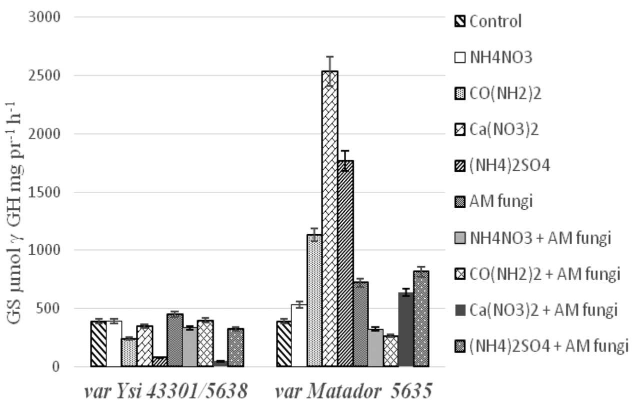

Fig. 2. Glutamine synthetase (GS) activity in two lettuce varieties grown at different nitrogen sources. Different letters in the values indicate significant differences assessed by Fisher LSD test ($P \leq 0.05$) after performing ANOVA analysis

with NH_4NO_3. Enhanced levels of NR activity in the leaves of fertilized plants coincided with lowered levels of nitrates. Almost the same trend of changes was observed regarding GS activity (Figure 2), much higher levels were estimated in the leaves of var. Matador 5635. Inoculation wit AM fungi positively affected enzyme activity in Ysi 4330/5638 variety in the same treatments as NR activity has been affected (non-fertilized plants and those fertilized with $(NH_4)_2SO_4$). In the leaves of Matador 5635 mycorrhization had no effect on the GS activity with the exception of inoculated non-fertilized plants. Both lower levels of nitrogen assimilatory enzyme activities NR and GS did not coincide with higher biomass accumulation in variety Ysi 4330/5638.

Conclusion

Mycorrhizal inoculation resulted in plant biomass accumulation in a different way in both lettuce varieties. In green lettuce variety Ysi 43301/5638, biomass accumulation significantly increased in the treatments fertilized with $CO(NH_2)_2$, $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ in combination with *Glomus intraradices,* In the another variety Matador 5635 (red lettuce) plant biomass increased in the treatments where *Glomus intraradices* was applied in combination with $(NH_4)_2SO_4$, followed by $Ca(NO_3)_2$ and NH_4NO_3. Reduced soluble sugars and protein and elevated content of nitrates were estimated in Ysi 43301/5638 in the treatments with urea in combination with mycorrhizal fungi. Fertilization with urea significantly lowered plastid pigments in both varieties independently of mycorrhization. The lower levels of nitrogen assimilatory enzyme activities NR and GS did not coincide with biomass accumulation especially in variety Ysi 4330/5638.

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