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INVESTIGATING THE EFFECT OF ARBUSCULAR MYCORRHIZA *GLOMUS* Sp. AS A BIOFERTILIZER ON LETTUCE PRODUCTION

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ABSTRACT

Arbuscular mycorrhiza used recently as biopesticide has shown beneficial effects on plant growth. An experiment was conducted in West Bekaa in 2016 in order to investigate the effect of a commercial biostimulant (MYCOSAT) containing 5 *Glomus* species on the production of two lettuce varieties: Romaine and Iceberg. Plant growth and nutritional quality were compared between mycorrhizal plants (mycorrhizal Romaine: MR and mycorrhizal Iceberg: MI) and non-mycorrhizal plants (non-mycorrhizal Romaine: NMR and non-mycorrhizal Iceberg: NMI). Measurements were done on root and leaf parameters and results showed a significant positive effect of mycorrhizal inoculation on plant growth. Best results were obtained for root parameters as well as leaf area and leaf weight in mycorrhizal plants of both varieties compared to non-mycorrhizal plants. An improvement was found in root length, root diameter, number of secondary roots and root weight by 81%, 81%, 61% and 60% for MR plants and of 80%, 88%, 84% and 94% in MR and MI in comparison to NMR and NMI. Leaf number was only enhanced in MI plants. The improved crop performance was associated with an ameliorated nutritional status with higher percentages of N, P, and K in leaves and was correlated to a stronger root development in mycorrhizal plants due to the action of arbuscular mycorrhiza. Finally, the application of the biostimulant MYCOSAT could provide a biological tool for improvement of growth and quality of lettuce grown in clay soils of West-Bekaa.

Keywords: *Bekaa, Lactuca sativa, Arbuscular mycorrhiza, Root growth, Plant Growth*

INTRODUCTION

Sustainable agricultural production is based on enhancing natural biological processes above and underground. Symbiotic associations are underground biological processes considered as important approach for a profitable sustainable agriculture (Smith *et al.*, 1968). Arbuscular mycorrhiza is one biological strategy (Cekic *et al.*, 2012) that could be suitable in Mediterranean regions including semi-arid conditions where soil is poor in organic matter and soil particles inhibit root development and spread (Coons *et al.*, 1990). Arbuscular mycorrhizal colonization is reported to promote plant growth (Evelin *et al.*, 2011); In fact, the association of AMF with plant roots creates an intimate link between plant roots (Johnson et *al.*, 1997) and improves plant adsorption of water and nutrients, consequently plants are better fed, watered and are more tolerant to biotic and abiotic stress factors. Therefore, this strategy induces a reduction in the use of chemical fertilizers and pesticides resulting in higher crop sustainability (Candido *et al.*, 2013).

AMF (Arbuscular Mycorrhizal Fungi) is associated with the vast majority of higher plants. It is the most common endomycorrhiza (Brundrett *et al.*, 1996) and it stimulated the hormones regulating plant growth and accelerates the rate of photosynthesis (Al-Karaki, 2006). Glomus is one very common genus of the AM (Torrey, 1992). The potential of mycorrhization differs among species (Ma *et al.*, 2001). For instance, the beneficial effect of AMF on plant growth has been proven in the traditional cultivation of lettuce (Kowalska *et al.*, 2015). This shallow-rooted crop is sensitive to any soil deficit and has the ability for a beneficial symbiotic mycorrhizal fungi association (Ma *et al.*, 2001). Lettuce (*Lactuca sativa* L.) is widely grown under greenhouse and open-field conditions in the Mediterranean region, particularly in Lebanon where it is a valuable crop, and its high quality yield is an essential prerequisite for its economic success. In 2014, harvested area reached 1417 ha with a total production of 37709 tonnes (FAOSTAT, 2017). Bekaa plain is one the main areas of lettuce cultivation in Lebanon that is characterized by heavy clay soils. Lettuce production in this region employs a heavy chemical fertilization to promote plant growth. Therefore, the study investigated the effect of applying a commercial biostimulant of symbiotic endomycorrhizal fungi (MYCOSAT TAB, VALLE D'AOSTA, CCS) with 5 *Glomus* sp. (*Glomus coronatum, Glomus Caledonium, Glomus intraradices, Glomus mosseae, Glomus viscosum*) on the performance of two common lettuce varieties: Romaine and Iceberg as an environmentally safe method that could alternate heavy chemical fertilization in Bekaa.

MATERIALS AND METHODS

The experiment was carried out in open field, under summer conditions at an open-field situated in West Bekaa where soil was a silt-clay soil, rich in calcium, poor in organic matter and containing acceptable values of nitrogen, potassium and phosphorus. Seedlings were transplanted at the stage of 2-3 leaves on 20 May 2013 into previously prepared seedbeds.

Seedlings of Romaine and Iceberg varieties were subjected or not to mycorrhizal inoculation. The experimental design was a randomized complete block design and investigated treatments were four: MR: Mycorrhizal Roman, NMR: Non-Mycorrhizal Roman, MI: Mycorrhizal Iceberg, and NMI: Non-Mycorrhizal Iceberg with 200 plants per treatment. The commercial biostimulant was added at the same day of transplantation. It was dissolved with water to prepare a solution of a concentration 5g/l, thus of 5ml of the solution were provided to each plant. Data was recorded twice: 25 DAT (days after transplanting): date 1 and 50 DAT: date 2 on a sample of 15 plants from each treatment. Diameter and length of the principal roots were measured using a sliding caliper. Then, plants were cut at the junction level to measure roots and leaves weight separately. Leaf area was calculated by multiplying the length and width of leaves. Finally, the macronutrients composition was analyzed on the mill of oven-dried leaves; nitrogen content was determined using Kjeldhal digestion procedure, phosphorus content was measured using Vanadate-Molybdate-Yellow method and potassium content was evaluated through flame photometer. The effects of the factors (variety and mycorrhizal application v.s. mycorrhizal absence) and their interactions on the averages of the measurements were analyzed using Factorial ANOVA. Correlations between aboveground and underground parameters were also studied.

RESULTS AND DISCUSSIONS

Results of the factorial analysis (Table 1) showed that the non-interactive effect of the experimental factors was significant regarding all parameters. In addition, for the first (line 4 to line 6) and second (line7) orders, interactive effects of the different investigated factors were mostly significant with some exceptions:
For the first order interactive effects: the combination effect of time and variety on number of leaves, the combination effect of time and mycorrhizal application on the number of leaves and the combination effect of variety and mycorrhizal application on root diameter and root length. For the second order interactive effects: the combination effect of time, variety and mycorrhizal application on root length and leaf area.

Table 1. ANOVA null hypothesis rejection probability (P_{value}≤0.05) for the effects of the experimental factors and their interactions on the different measurements averages.

Effect	N.S.R	R.D	R.L	R.W	L.N	L.W	L.A
Date (d)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Variety (v)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Mycorrhiza (m)	0.0000	0.0000	0.0000	0.0000	0.0312	0.0000	0.0000
d*v	0.0000	0.0000	0.0000	0.0000	0.0955	0.0000	0.0000
d*m	0.0000	0.0000	0.0000	0.0000	0.4542	0.0000	0.0000
v*m	0.0000	0.1046	0.1503	0.0000	0.0102	0.0000	0.0029
d*v*m	0.0000	0.0000	0.0720	0.0000	0.0218	0.0000	0.7660

N.S.R.=Number of Secondary Roots, R.D=Root Diameter (cm), R.L=Root Length (cm), R.W= Root Weight (g), L.N=Leaf Number, L.W=Leaf Weight (g), L.A=Leaf Area(cm^2).

Results (Figure 1) reflected a positive effect of mycorrhizal inoculation on the majority of parameters and this effect was mainly evident at the second date of sampling. It improved the diameter of the root neck and its effect differed among both varieties according the date of sampling. At date 1, root neck diameter did not differ significantly between mycorrhizal and non-mycorrhizal plants of each variety and between treated plants of both varieties, however at date 2 it differed significantly for both cases with superiority for mycorrhizal plants of both varieties (MR: 31.52 cm v.s NMR: 25.46 cm and MI: 24.81 cm v.s NMI: 21.85 cm). The same tendency was observed for the variation of the average values of the following parameters: root length, number of secondary roots and weight of leaves where superiority was maintained for mycorrhized plants of both varieties at date 2. Also, root weight was obviously not affected by mycorrhizal application at date 1 since there was no significant difference between average values related to MR and MI compared to NMR and NMI. However, at date 2, differences in root weight were observed only for the Romaine variety where MR recorded a significantly higher average compared to NMR (35.23 g and 21.03 g respectively). In addition, at date 2, arbuscular mycorrhiza enhanced the average number of leaves of Iceberg lettuce (MI: 35.0 v.s NMI: 31.6) while it did not create any significant difference of leaf number of Romaine lettuce during the whole vegetative cycle. Finally, at date 1 average leaf area was almost similar in Romaine and Iceberg plants with or without mycorrhizal application while at date 2 it was significantly higher in treated plants of both varieties (MR: 125.03 cm^2 v.s NMR: 75.94 cm^2 and MI:164.55 cm^2 v.s NMI: 106.34 cm^2).

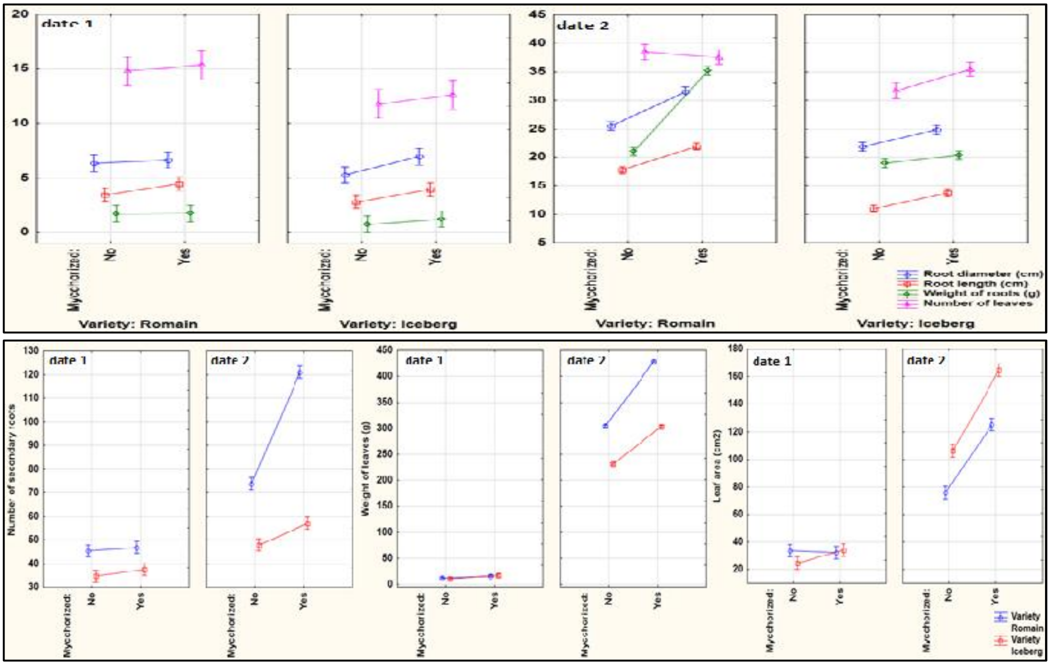

Figure 1. Variation of the average measurements under the effect of variety at date 1 (25 DAT) and date 2 (50 DAT)

All investigated plant parameters were positively correlated (Table 2). This positive correlation was strong between measurements of the root system. For instance, the weight of roots was strongly correlated to the number of secondary roots (R=0.85), length of main root (R=0.86), and diameter of root neck (0.78). Moreover, all measurements of root development were positively correlated with those of leaf development with more or less different strength of correlations. Leaf area and leaf number were less positively correlated to the number of secondary roots than root diameter, root length and root weight.

Table 2. Correlations between plant parameters

	N.S.R	R.D	R.L	R.W	L.N	L.W	L.A
N.S.R	1.000000	0.785703	0.866678	0.858275	0.695628	0.824803	0.519864
R.D		1.000000	0.954215	0.972208	0.956144	0.987667	0.878394
R.L			1.000000	0.956239	0.921144	0.970094	0.757169
R.W				1.000000	0.909891	0.984640	0.851637
L.N					1.000000	0.947818	0.824247
L.W						1.000000	0.863898
L.A							1.000000

N.S.R.=Number of Secondary Roots, R.D=Root Diameter (cm), R.L=Root Length (cm), R.W= Root Weight (g), L.N=Leaf Number, L.W=Leaf Weight (g), L.A=Leaf Area(cm^2).

Extraradical hyphae of *Glomus* sp. that formed the main components of the product MYCOSAT used in various treatments can metabolize both organic and inorganic sources of nitrogen by glutamate synthetase activity (Hawkins *et al.*, 2000). Also, plants cannot readily utilize P in an organic or complex inorganic form due to its low solubility and mobility, thus arbuscular fungi intervene to hydrolyze any available sources of P through the spread with the aid of secreted enzymes like phosphatase (Carlite *et al.*, 2001). Mycorrhizal symbiosis can facilitate the absorption of various other minerals like potassium. Therefore, the percentage of nitrogen, phosphorus and potassium was higher in leaves of MR and MI compared to NMR and NMI leaves (Table 3).

Table 3. Macronutrient content in lettuce leaves of various treatments

	MR	NMR	MI	NMI
N (%)	3.2	2.2	2.5	2.0
K (%)	7.5	5.4	8.0	7.5
P (%)	1.0	0.6	0.9	0.5

Arbuscular mycorrhiza symbiosis improved nutrient uptake by improving soil exploration (Beltrano *et al.*, 2013) by allowing plants to explore larger volumes of soils. It induced amelioration in root length, root diameter, number of secondary roots and root weight of 81%, 81%, 61% and 60% for MR plants and of 80%, 88%, 84% and 94% in MR and MI in comparison to NMR and NMI. It helped roots of mycorrhizal plants to grow deeper and ramify more and consequently to absorb more water and immobile mineral elements like phosphorus and to improve water-use efficiency (Beltrano *et al.,* 2003). Improvement in nutrition of mycorrhizal plants was coupled with an increase in leaf number of the Iceberg variety as well as an increase in leaf area and total leaf biomass in Romaine and Iceberg with an improved nutritional quality which confirmed the findings confirmed of Baslam *et al*. (2011).

CONCLUSIONS

Arbuscular mycorrhiza application can improve crop conditions of Romaine and Iceberg lettuce and contribute to reduction or even to prevent the fertilizers usage to this type of Lebanese horticulture. The application of the biostimulant MYCOSAT could provide a key to a safe improvement of lettuce production and to a biologically-based sustainable farming in Lebanon. However, it could be better to apply it twice during the vegetative cycle in order to promote a higher benefit on root development and consequently on head formation.

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