Journal of Plant Process and Function, Vol. 10, No. 43, Year 2021

Research Article

Reaction of *Glomus mosseae* and *Glomus intraradicea* to different plantation cultures

Asma Ghaemi [1], Farzaneh [1*] Najafi and Hossein Askari [2]

[1] Department of plant Sciences, Faculty of Biological Sciences, Karazmi University,Tehran, Iran
[2] Department of plant sciences and Biotechnology, Faculty of life sciences and Biotechnology, Shaid Beheshti University, Tehran, Iran.
(Received: 04/12/2020 Accepted: 25/05/2021)

Abstract

A study with complete randomized design block and three replications was carried out in greenhouse condition to examine the effects of inoculation of mycorrhiza fungi (*Glomus intraradicea*) and (*Glomus mosseae*) on growth indices, chlorophyll content, carotenoid, and protein of parsley (*Petroselinum crispum* L.), dill (*Anethum graveolens* L.), and celery (*Apium graveolens* L). The study was conducted in different experimental plantation environments (soil, sand and hydroponic). The highest colonization was seen in celery (100%) in sand culture and the lowest colonization was observed in parsley (33%) in hydroponic culture. There was no significant differences between the two fungi in terms of colonization and its effect on plant growth. Based on the effects of symbiosis of sand culture plants, some of the plants were selected randomly and transferred to hydroponic culture. The first week in the hydroponic culture was featured with a decreased colonization, which had no negative effect on the plant's growth. The traits under consideration were significant at probability level of 5%. The results showed that the effect of colonization on plants' growth was a function of growth culture and the host plant.

Keywords: Celery, Colonization rate, Dill, Hydroponic culture, Parsley

Introduction

Mycorrhiza fungi appeared on the earth 450 million years ago along with the first plants. The term mycorrhiza is from Greek and it is comprised of two words "Mukes" for fungus and "Rhiza" for root (Das, 2015). Fungi constitute 50% of the microbial biomass of soil and 5-36% of soil biomass (Paterson *et al*., 2016). Arbuscular Mycorrhiza fungi (AMF) play a role in increasing host plants ability to absorb relatively immovable nutrients such as phosphorous and other elements that are needed in small amounts (Guissou *et al*., 2016). The AMF generates arbuscules structure by stimulating strigolactones hormones secreted by the host plant (Etesami and Alikhani, 2016). The host plant prefers AMF growth when nutrient is scarce by secreting nutrient from the roots to the soil (Ellouze *et al*., 2012). Arbuscules structure are the sites of nutrients exchange, which are formed on the cortex of plant when there is a shortage of nutrient for plant growth and compensate the shortage to a great extent (Nouri *et al*., 2014). The symbiosis between fungi and plants paves the way to establish a molecular dialogue that benefits the host through activating antioxidant, phenyl propanoid, or carotenoid metabolic pathways (Baslam *et al*., 2011). Some authors believe that the main role of mycorrhiza fungus is to facilitate access to elements such as phosphorous (Weisanny, 2016). The AMF stimulates secretion of growth adjusting hormone and increases photosynthesis rate (Zhu *et al.*, 2014). Studies have shown that inoculation with mycorrhiza fungus increases the volume and biomass of the root, which is related to an increase in growth hormone level (Safapour *et al*., 2012). Mycorrhiza plants can increase drought resistance of the host through improving hydraulic conductance of the roots (Tian *et al*., 2013). An increase in water absorbance in low humidity environment through expansion of fungus strains means osmosis balance and preservation of turgor pressure, an increase in photosynthesis activity, accumulation of carbohydrates and proline, and an increase in nutrient absorption (Deepika and Kothamasi, 2015). Mycorrhiza plants have better performance in absorbing minerals and sustaining biotic and abiotic stresses (Ortas, 2012). Wheat plants inoculated with *G. intraradicea* and 75% NPK fertilizer yielded the same as the samples without *G. intraradicea* and 100% NPK fertilizer. The results indicate that arbuscular mycorrhiza fungi facilitated absorption of minerals and water by the plant and was also more economic in terms of using fertilizers (Sharma *et al*., 2011). Mycorrhiza fungi has phosphorus conductors and the conductors have different types. The increase in absorption and growth in the host plants is different depending on the fungus strains and the phosphor conductors (Elbon and Whalen, 2014). Hydroponic is a plant growth system featured with an

*Corresponding Author, Email: najafi_f@khu.ac.ir

[DOR: 20.1001.1.23222727.1400.10.43.6.0]

accurate control of cultivation. There are several hydroponic systems available such as Arabidopsis. A hydroponic environment is also highly flexible given the control on plant materials. Therefore, it can support plant growth at different stages from seed to mature plant (Conn *et al*., 2013). Different plantation conditions including hydroponic condition are used to create symbiosis relationship in greenhouse. Hydroponic condition is the best choice for controlling mineral combinations and uniform distribution of solved materials to the roots (Alatorre *et al.*, 2014). Experiment have shown that colonization of the root is affected by mycorrhiza fungus (Hamzei and Salimi, 2014). Differences between mycorrhiza colonized plants in hydroponic environment might be due to different percentage of roots colonization and high phosphor concentration in root zone (Maboko *et al*., 2013). The highest colonization level of host plant was in hydroponic environment with the lowest phosphor concentration in nutrient solution (Kowalska *et al*., 2015). Hydroponic greenhouse products in the developed countries constitute a major part of greenhouse industries production. Industrialized greenhouse production is expanding fast and can supply food demands of growing populations. The present study examined the effect of *G. intraradicea* and *G. mossea* symbiosis on growth of parsley, dill, and celery in soil, sand, and hydroponic cultures.

Materilals and methods

The experiment was done as factorial with completely randomized design with three replications in chamber room condition with high (25°C) and low (18°C) temperatures and 16/8 day/night photoperiod. The experiment site was the Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Tehran, Shahid Beheshti University (during 2015 and 2016). The treatment included two fungal contamination levels with *G.mosseae* and *G. intraradicea*. Along with a control treatment without inoculation, there were three cultures including sand culture, soil culture, and hydroponic culture. The seeds of celery, parsley, and dill were purchased from Pakan Bazr Isfahan Company. The soil was prepared from the depth zero to 30cm and used after NPK examination (Table 1). To disinfect, the soil and sand were placed in an autoclave (100°C, 1hr, 1atm). Mycorrhizal treatment consisted of 20% of the pot volume AMF-inoculum. AMF-inoculum used in the experiments consisted of *G.mosseae* and *G. intraradicea* were that purchased from Turan Biotic Company, Semnan, Iran. Non-mycorrhizal treatments received mycorrhiza free inoculum. Afterwards, the seeds were surface disinfected in 1% sodium hypochlorite solution for 10 mins. The seeds were planted at 0.5cm depth of sand and soil in 2 L pots. Irrigation was daily until germination based on field capacity (daily, until the pot weight reached field capacity limit). Fourteen days after germination, the roots were marked to examine establishment of symbiosis following Vierheiliga *et al*. (1998). After affirmation of symbiosis, a few plants were randomly selected from soil and sand and transferred to hydroponic culture. Then, the growth was continued in the three cultures for 15 days. The plants in sand and hydroponic cultures were fed by Hoagland solution. To prepare the solution, tap water with EC=18MS/cm was used. With high phosphorus concentration, AMF fails to establish a symbiosis with the host plant. Given this, the concentration of phosphor was lowered by 50% in Hoagland solution. The hydroponic system consisted of nine 2 L rectangular pots and each pot was covered with perforated styrofoam (the holes were 1 cm in diameter). The plants were placed in the holes and the air was supplied using an electromagnetic pump (32 Watt, 62 L/min). The air pump would supply air nonstop. One month after germination of the seeds, colonization percentage of the plants in the three cultures (sand, soil and hydroponic) was calculated using Trouvelot *et al*. (1986) (Table 2). After determining growth characteristics including dry weight of leaf, root, and stem, the plant samples were placed in an oven for 48 hrs. at 70ºC. Afterwards, the samples were weighed on a digital scale (0.001 gr accuracy).

Total protein: 0.1 gr leaf and root samples were homogenized in 2 mL of 0.1 mol.L^{-1} phosphate buffer (pH 6.8). The homogenate was centrifuged at 15000 × g for 20 mins. at 4°C, and the supernatant was used for total protein assessment following the technique described by Bradford (1976).

Photosynthetic pigments: Chlorophyll a, chlorophyll b, total chlorophylls and carotenoid were determined by using the method described by Lichenthaler (1987). 0.2 gr fresh leaves were homogenized in 10 ml of 80% acetone. The absorbance of acetone extract of the leaves was recorded at 470, 647 and 663 nm using a spectrophotometer. Pigment content was calculated as follows:

Chlorophyll *a*: 12.25 A663 - 2.79 A647

Chlorophyll *b*: 21.5 A647 - 5.1 A663

Carotenoids: (1000 A470 - 1.82 Chlorophyll *a* - 85.02 Chlorophyll *b*) /198

Total phenol content: Total phenol content was measured using Folin-Ciocalteu reagent. To do so, 0.5 gr wet tissue was mixed with 3 ml methanol (85%). Then, 300 µl of the obtained extract was mixed with 1500 µl of the reagent (1:10). After 5 min, 1200 µl of sodium carbonate (7%) was added to the solution and 90 min later, absorption level was measured using spectrophotometer at 760 nm. Compared with the standard curve of Gallic acid, total phenol content was expressed based on Gallic acid (mg) in the extract (g) (Singleton and Rossi, 1965).

Total anthocyanin content: To measure anthocyanin, 1 gr of the wet shoot tissue was powdered thoroughly using a mortar and pestle containing 5 ml of acidic methanol (methanol 0.5% and HCl 1% - 99/1 ratio). The obtained extract was poured into a test tube

[DOR: 20.1001.1.23222727.1400.10.43.6.0]

Table 1. Soil physical and chemical properties in 0-30 cm depth

Soil texture	dS m^{-1}	pH	Total nitrogen (%)	Available P (mg kg^{-1})	K^+	Na^+	Organic matter (%)
Silliness and loam	4.3	7.5	0.08	12.5	160	157	0.45

Table 2. Some of the growth parameters and colonization (%) in *Petroselinum crispum* L.(parsley), *Anethum graveolens* L. (dill) and *Apium graveolens* L. (celery) cultivated in different media. Geram in per plant. (D.W: Dry Weight)

Culture Condition	Fungi	plants	leaf D.W	Shoot D.W	Root D.W	Shoot/Root D.W	Colonization Percent
			(g)				
Soil	non-AMF	parsley	0.05±0.00^{d}	0.08±0.00^{d}	0.01±0.00^{de}	5.75±0.1^{c}	0.00±0.0^{d}
		dill	0.09±0.00^{cd}	0.11±0.00^{e}	0.01±0.00^{d}	5.75±0.79^{cde}	0.00±0.00^{f}
		celery	0.13±0.02^{cde}	0.2±0.02^{d}	0.08±0.00^{e}	2.46±0.23^{bc}	0.00±0.00^{d}
	G. mossea	parsley	0.05±0.02^{d}	0.09±0.02^{d}	0.01±0.00^{d}	3.75±0.64^{c}	60.32±.3.18^{abc}
		dill	0.04±0.00^{e}	0.06±0.00^{de}	0.01±0.00^{d}	3.75±0.62^{ef}	65.83±4.65^{b}
		celery	0.16±0.02^{c}	0.25±0.02^{d}	0.09±0.00^{de}	2.88±0.13^{b}	45.14±1.61^{c}
	G. intaradicea	parsley	0.04±0.00^{d}	0.07±0.00^{d}	0.01±0.00^{de}	17.50±1.05^{c}	61.11±12.54a
		dill	0.09±0.00^{cd}	0.18±0.01^{c}	0.01±0.00^{d}	17.50±1.59^{a}	49.90±3.53^{c}
		celery	0.1±0.00^{cde}	0.15±0.00^{d}	0.08±0.00^{e}	1.80±0.05^{de}	34.44±2.94^{c}
Sand	non-AMF	parsley	0.03±0.00^{d}	0.06±0.00^{d}	0.01±0.00^{e}	7.50±0.07^{c}	0.00±0.00^{d}
		dill	0.06±0.00^{de}	0.14±0.00^{d}	0.01±0.00^{d}	7.50±0.50^{cd}	0.00±0.00^{f}
		celery	0.08±0.00^{e}	0.16±0.00^{d}	0.08±0.00^{e}	1.91±0.09^{cde}	0.00±0.00^{d}
	G. mossea	parsley	0.08±0.00^{cd}	0.12±0.000^{d}	0.04±0.00^{c}	6.00±0.21^{c}	91.67±8.34^{abc}
		dill	0.13±0.02^{bc}	0.25±0.02^{c}	0.04±0.00^{b}	6.00±0.50^{de}	72.94±1.43^{a}
		celery	0.08±0.00^{de}	0.15±0.01^{d}	0.11±0.02^{d}	1.37±0.15^{e}	100.00±0.00^{a}
	G. intaradicea	parsley	0.08±0.01^{cd}	0.13±0.01^{cd}	0.04±0.00^{c}	10.00±0.09^{c}	80.00±11.56^{ab}
		dill	0.20±0.00^{a}	0.39±0.00^{b}	0.04±0.00^{b}	10.00±0.53^{bc}	72.72±2.92^{ab}
		celery	0.10±0.00^{cde}	0.19±0.01^{d}	0.15±0.02^{c}	1.34±0.23^{e}	100.00±0.00^{a}
Hydroponic	non-AMF	parsley	0.13±0.03^{c}	0.2±0.04^{c}	0.08±0.00^{b}	1.14±0.41^{c}	0.00±0.00^{d}
		dill	0.06±0.00^{de}	0.07±0.00^{de}	0.08±0.00^{a}	1.14±0.07^{f}	0.00±0.00^{f}
		celery	0.18±0.03^{cd}	1.23±0.11^{c}	0.28±0.01^{b}	4.47±0.47^{a}	0.00±0.00^{d}
	G. mossea	parsley	0.5±0.06^{a}	0.58±0.06^{a}	0.03±0.00^{a}	5.33±0.72^{a}	33.09±21.01^{cd}
		dill	0.17±0.03^{ab}	0.23±0.03^{a}	0.03±0.00^{c}	5.33±0.61^{cde}	19.70±1.62^{e}
		celery	0.55±0.06^{a}	1.57±0.04^{a}	0.58±0.00^{a}	2.71±0.07^{b}	60.38±8.34^{b}
	G. intaradicea	parsley	0.31±0.02^{b}	0.04±0.02^{b}	0.01±0.00^{a}	16.0±0.14^{b}	49.35±21.38^{bc}
		dill	0.09±0.00^{cd}	0.14±0.00^{c}	0.01±0.00^{d}	16.00±2.52^{ab}	41.95±1.82^{d}
		celery	0.35±0.03^{b}	1.38±0.04^{b}	0.58±0.00^{a}	2.37±0.07^{bcd}	61.90±11.92^{b}

Values are the mean ± SE. values with similar letters are no significantly different.

with screw cap and then sealed and covered with aluminum foil. The extract was kept for 24 hrs in the dark at 25ºC. Afterwards, the extract was centrifuged (10min, 4000g). The absorption of the top solution was read at 550 nm. Anthocyanin content was also determined using the following formula using extinction coefficient (= 33 $mol^{-1}cm^{-1}$) based on mmol in 1 gr of wet weight (Krizek DT *et al*., 1998).

Statistical analysis: All analyses were done using MSTATC and comparison of mean scores was carried out using least significant difference (LSD) at 5% level.

Results and discussion

Growth: The results of ANOVA indicated that dry weight of aerial organs of mycorrhizal plants in hydroponic environment was significantly different from the plants in soil and sand environments. The highest dry weight of the mycorrhizal plants root was observed in the samples in hydroponic environment followed by the samples in sand and soil environments. In hydroponic the type of fungi used in the study did not cause a significant change in dry weight of aerial organs and roots (Table 2). Growth and expansion of mycorrhiza fungus during the exposure and the reciprocal effects on the host plant were functions of the condition of the association that was dictated by the culture environment. Dry weights of root and aerial organs of the mycorrhizal plants in hydroponic environment were higher than those of the control plants (Dugassa *et al.,* 1995). Dry weight of mycorrhizal tomato (*Solanum lycopersicum*) associated with *G. mosseae* was higher than the samples associated with *G. intaradices* and dry weights of the both was higher than that of the non-mycorrhizal samples (Utkhed, 2006). Due to aeration in hydroponic environment, development of the roots was better than the samples planted in soil (David *et al*., 1997). Among the reasons for the higher height and larger biomass observed in the hydroponic environment comparing with the samples in sand, richer mineral elements and steady access to mineral materials are notable (Hawkins *et al.,* 1999). Several studies have reported the positive effect of

[DOR: 20.1001.1.23222727.1400.10.43.6.0]

Table 3. Some of the physiological parameters in *Petroselinum crispum* L.(parsley), *Anethum graveolens* L. (dill) and *Apium graveolens* L. (celery) cultivated in different media mg.g^{-1} FW per plant.

Culture Condition	Fungi	plants	Ch.l *a*	Chl. *b*	Total chlorophyll	Anthocyanins	Phenol	Protein
			(mg.g^{-1})					
Soil	Non-AMF	parsley	0.08±0.00^{ab}	0.04±0.00^{ab}	0.14±0.00^{b}	2.86±0.352^{bc}	47.52±2.37^{d}	107.90±2.33^{c}
		dill	0.30±0.00^{d}	0.18±0.05^{b}	0.14±0.01^{e}	1.74±0.21^{c}	79.29±1.09^{a}	128.08±1.7^{bc}
		celery	0.48±0.02^{zb}	0.31±0.01^{ab}	0.87±0.03^{a}	3.45±0.28^{abc}	40.59±2.8^{d}	44.40±5.84^{a}
	G. mossea	parsley	0.08±0.00^{a}	0.03±0.00^{bc}	0.11±0.00^{b}	3.62±0.25^{a}	36.06±1.41^{e}	164.22±11.8^{b}
		dill	0.28±0.01^{d}	0.12±0.00^{b}	0.11±0.02^{d}	1.78±0.06^{c}	44.91±7.50^{cd}	151.49±3.97^{a}
		celery	0.60±0.03^{a}	0.25±0.01^{bc}	0.85±0.03^{a}	2.61±0.09^{a}	34.15±1.30^{e}	47.56±4.69^{c}
	G. intaradicea	parsley	0.07±0.00^{bc}	0.03±0.00^{c}	0.1±0.00^{b}	3.08±0.06^{abc}	49.68±0.88^{b}	128.6±1.95^{c}
		dill	0.51±0.04^{b}	0.19±0.04^{b}	0.10±0.04^{bc}	1.66±0.06^{c}	78.49±5.08^{a}	149.13±8.90^{a}
		celery	0.73±0.02^{bc}	0.28±0.01^{c}	1.01±0.03^{a}	3.14±0.18^{abc}	47.27±2.03^{cd}	55.30±5.23^{b}
Sand	Non-AMF	parsley	0.06±0.00^{c}	0.04±0.00^{a}	0.11±0.01^{b}	3.30±0.39^{ab}	51.82±3.33^{bcd}	100.87±3.63^{c}
		dill	0.27±0.02^{d}	0.15±0.01^{b}	0.11±0.01^{e}	2.03±0.33^{bc}	50.80±4.17^{bc}	73.07±8.83^{b}
		celery	0.71±0.02^{c}	0.31±0.01^{a}	1.05±0.05^{a}	3.10±0.16^{ab}	43.27±1.77^{bcd}	110.35±4.33^{f}
	G. mossea	parsley	0.06±0.00^{c}	0.03±0.00^{abc}	0.10±0.01^{b}	2.97±0.19^{abc}	64.08±4.98^{a}	187.84±2.3^{ab}
		dill	0.43±0.02^{bc}	0.16±0.02^{b}	0.10±0.00^{e}	1.69±0.08^{c}	56.04±2.13^{b}	141.0±5.63^{ab}
		celery	0.72±0.03^{c}	0.33±0.00^{abc}	0.91±0.09^{a}	2.80±0.31^{abc}	45.14±2.46^{a}	102.45±4.09^{g}
	G. intaradicea	parsley	0.07±0.00^{bc}	0.02±0.00^{c}	0.39±0.00^{a}	3.08±0.44^{abc}	59.56±1.06^{ab}	177.7±10.9^{ab}
		dill	0.33±0.07^{cd}	0.11±0.01^{b}	0.39±0.05^{d}	1.83±0.12^{c}	56.59±1.81^{b}	113.51±4.59^{c}
		celery	0.68±0.03^{bc}	0.31±0.00^{c}	1.05±0.06^{a}	2.90±0.40^{abc}	57.14±6.05^{ab}	113.67±9.04^{c}
Hydroponic	Non-AMF	parsley	0.07±0.00^{c}	0.02±0.00^{c}	0.09±0.00^{b}	2.25±0.31^{c}	51.82±3.33^{bcd}	194.55±6.94^{a}
		dill	0.44±0.04^{bc}	0.17±0.02^{b}	0.09±0.02^{cd}	2.49±0.25^{ab}	39.06±4.70^{d}	128.1±10.5^{bc}
		celery	0.82±0.03^{c}	0.51±0.02^{c}	1.36±0.19^{a}	2.57±0.26^{c}	36.03±0.57^{bcd}	117.38±7.10^{e}
	G. mossea	parsley	0.08±0.00^{a}	0.03±0.00^{abc}	0.1±0.01^{b}	2.61±0.10^{bc}	52.31±1.88^{bcd}	192.1±14.1^{ab}
		dill	0.74±0.03^{a}	0.30±0.01^{a}	0.10±0.03^{b}	2.83±0.23^{a}	58.58±0.99^{b}	117.03±1.30^{c}
		celery	0.80±0.04^{a}	0.47±0.02^{abc}	1.09±0.09^{a}	2.76±0.26^{bc}	43.18±6.77^{bcd}	111.94±5.93^{d}
	G. intaradicea	parsley	0.08±0.00^{ab}	0.03±0.00^{c}	0.39±0.00^{a}	2.77±0.24^{ab}	57.08±2.24^{abc}	183.5±18.3^{ab}
		dill	0.79±0.05^{a}	0.39±0.03^{a}	0.39±0.09^{a}	2.60±0.12^{a}	40.78±0.00^{b}	112.62±7.12^{c}
		celery	0.87±0.03^{ab}	0.43±0.00^{c}	2.03±0.45^{b}	2.73±0.36^{abc}	37.97±4.96^{abc}	107.18±1.10^{f}

Values are the mean ± SE. values with similar letters are no significantly different

arbuscular mycorrhiza on stimulation of growth and increase of dry mass of plants (Pandey and Garg, 2017). It has been proven that mycorrhizal plants had higher whole plant dry weight, height, and leaf area comparing with non-mycorrhizal plants. Consistent results were also reported by Ortas *et al*. (2012).

Photosynthesis pigments: The results of ANOVA indicated that the effect of environment on chlorophyll content of the three plants was significant. The effects of arbuscular mycorrhizal fungi on chlorophyll level, total chlorophyll, and carotenoid were higher comparing with non-mycorrhizal plants except for carotenoid of inoculated parsley in soil environment. Increase in chlorophyll contents might be due to an increase of photosynthesis in mycorrhizal plants. In comparison with *G. mossaeae* and *G. intraradices* fungus had the highest effect on the increase of photosynthesis pigments of plants in all the three environments. The increase was highest in hydroponic environment and lowest in soil environment (Tables 3). *G. intraradices* and *G. mosseae* fungi improved chlorophyll contents of the host plants (Habibzadeh *et al.,* 2015). Chlorophylls *a*, *b*, total chlorophyll and carotenoids were higher in mycorrhizal plants comparing with non-mycorrhizal plants (Mathur, 1995). *G. mosseae* increases absorption of soil magnesium, chlorophylls contents of the host plant, which leads to an increase of biomass (Xiao *et al*., 2014).

Anthocyanin: *G. mosseae* had the highest anthocyanins effect on mycorrhizae plants in hydroponic and soil environments. In addition, *G. intraradices* had the highest anthocyanin effect on mycorrhizae plants in sand environment. The results listed in (Table 3) are ANOVA results for dill (*anethum graveolens*), which is the only subject for which the effect of environment on anthocyanin is significant (Table 3). Anthocyanin is one of the widest groups of chromatic flavonoids and there are doubts about their role as the essential signaling compounds in symbiotic relationship with arbuscular mycorrhizae fungus (Becard *et al*., 1995). On the other hand, it has been proven that specific flavonoids in mycorrhizae plants improve germination of mycorrhizae fungi (Harborne, 1980). Antioxidant compounds like phenol and anthocyanin increase under stress (Kim *et al*., 2005).

Phenol content: The results of measuring phenol content on total aerial limb content of mycorrhizae and non-symbiotic plants showed that the total phenol in the symbiotic samples with mycorrhizae was higher than that of the control non-mycorrhizae samples. The

[DOR: 20.1001.1.23222727.1400.10.43.6.0]

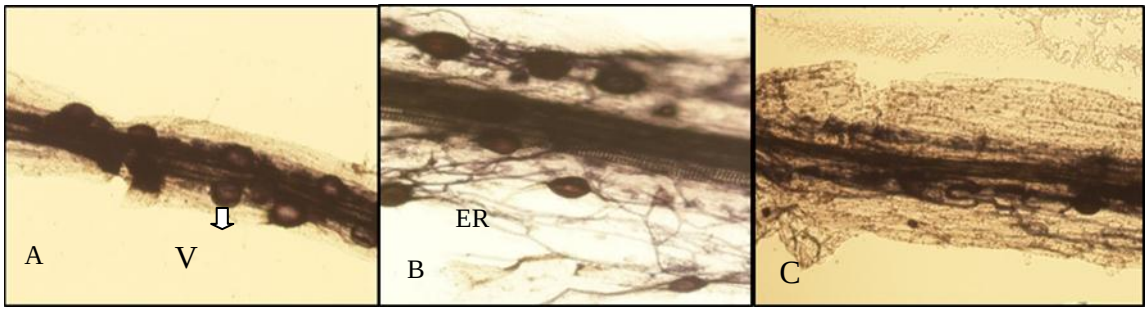

Figure 1. Functional mycorrhizal structures in roots of the plants host *Petroselinum crispum* L. (parsley), *Anethum graveolens* L. (dill) and *Apium graveolens* L. (celery). Fungal colonization was both inter- and intracellular and only involved the cortical cells of the roots. A, the plant host *Petroselinum crispum* L. (parsley) detail of a vesicle (V) and arbuscule in soil culture. B, root of *Anethum graveolens* L. (dill) showing extraradical hyphae (ER), arbuscules (*G. mosseae*) in sand culture. *G. mosseae* in *Apium graveolens* L. (celery), roots grown in hydroponic culture.

highest phenol content was observed in mycorrhizae dill sample in soil environment followed by mycorrhizae parsley and celery in sand environment. On an average, the effect of *G. intraradices* on phenol content of the samples in the three environments was higher compared to that of *G. mosseae*. The highest level of phenol in mycorrhizae parsley was observed in samples planted in sand environment followed by the samples in hydroponic and soil environments. The results of ANOVA showed that the effect of fungous and its reciprocal effect on environment were significant on dill and celery and insignificant on parsley (Table 3). The results indicated that phenols are signs of plants development and interaction between plant and microbe (Lynn *et al*., 1990). An increase in adsorption of phosphor in the mycorrhizae plants leads to an increase in the percentage of phenol compared to non-mycorrhizae plants (Sharma *et al*., 2006). Arbuscular mycorrhizae fungi affect the early reactions of the host plant and physiological mechanisms of aggregation of secondary metabolites (carotenoid and phenols) (Schliemann *et al*., 2008).

Protein : Protein content of total mycorrhizae plants was higher than that of the non-mycorrhizae plants. In general, the effect of *G. mosseae* on the protein content of the plants in the three environments was higher than that of *G. intraadices*. The ANOVA showed that the effects of environment, fungus, and their reciprocal effects were significant on dill and parsley. In addition, the effect of environment on protein content in parsley was significant. Mycorrhizae fungi symbiosis helped the host plant to keep a higher concentration of protein content in its shoot (Kizhaeral and Charest, 2005). Mycorrhizae trifolium showed 17% increase in protein content (Arienes *et al*., 1993). Arbuscular mycorrhizae fungi improved nitrogen adsorption and since the element is needed for protein production, an increase in protein content is expectable (Sanchez-Blanco *et al*., 2004) (Table 3).

Colonization percentage: The highest colonization percentage was found in sand environment followed by soil and hydroponic. Colonization percentage of the plants in the three environments was significantly different (Table 2). Figures 1, illustrates different types of arbuscular mycorrhizae fungi with different capacity in colonization. Clearly, their effect on plant growth is not the same (Ortas *et al*., 2012). The decrease in colonization level in the hydroponic environment, comparing with sand, is consistent with fungal colonization percentage (*G. intraradices*) in other studies (Li *et al*., 2004). Over time, the percentage of penetration of *G.intraradices* increased in flax (*Linum usitatissimum*) sand, and hydroponic environments (Dugassa *et al*., 1995). There was a correlation between colonization of plant's root and capillary roots, so that colonization rate increased over time in hydroponic environment along with roots development (Michelsen, 1993). The results showed that the rate of colonization decreased over time in hydroponic environment.

Conclusion

The results showed that symbiosis of parsley, celery, and dill with mycorrhizae in soil, sand, and hydroponic cultures increased photosynthesis pigment, as well as protein, phenol, and anthocyanin contents compared with non-mycorrhizae plants. Hoagland solution was used in hydroponic, sand, and soil environments; however, colonization rate of the plants in hydroponic environment was less than sand and soil environments. On the other hand, the highest and lowest growth rates were observed in hydroponic and soil environments. The plants in different environments had different growth and colonization rates. Therefore, the growth environment can solely affect the growth and development of mycorrhizae fungus, supply of water and mineral to the host plant, and yield product. Colonization rate can be a function of the type of plant, so that different plants in the same environment had different rate of colonization..

References

Alatorre, F., Vazques, C., Laclette, E., Villalobos, L., Torres, C., Aburto, A., Bravo, A., Morales, S., Alanis, D., Lopez,

[DOR: 20.1001.1.23222727.1400.10.43.6.0]

A., Ocana, B. and Estrella, L. (2014) An important, low- cost, hydroponic system for growing Arabidopsisn and other plant species under aspecting condisions. BMS Plant Biology 14: 69.

Arienes, J., Palma, J. M. and Varion, A. (1993) Camparison protein pattern in nonmycorrhizal and VA mycorrhizal root of red clover. New Phytologist 123: 763-768.

Baslam, M., Garmendia, I. and Goicoechea, N. (2011) Arbuscular Mycorrhizal Fungi (AMF) improved growth and nutritional quality of greenhouse-grown lettuce. Journal of Agricultural and Food Chemistry 59: 5504-5515.

Becard, G., Taylor, A., Douds, D., Pfeffer, P. E. and Doner, L. W. (1995) Flavonoids are not necessary plant signal compounds in arbuscular mycorrhizal. symbiosis. Molecular Plant-Microbe Interactions 8: 252-8.

Bradford, M. (1976) A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein- dye binding. Analytical Biochemistry 72: 248-254.

Conn, S., Hocking, B., Dayod, M., Xu, B., Athman, A., Henderson, S., Aukett, L., Conn,V., Shearer, M., Fuentes, S., Tyerman, S. and Gilliham, M. (2013) protocol: Optimising hydroponic growth systems for nutritional and physiological analysis of Arabidopsis thaliana and other plants. Plant Methods 9: 4.

Das, D. (2015) A contribution to the vesicular arbuscular mycorrhizal fungal status on twenty selected medicinal plants of Pandam forest in Darjeeling Himalaya, West Bengal, India. Journal of Environmental Science. Toxicology and Food Technology 9: 61-71.

David, M., JohnHulett, G., Cramer, G. R. and Seemann, J. (1997) Maximal biomass of *Arabidopsis thaliana* using a simple, low-maintenance hydroponic method and favorable environmental condeitions. Plant Physiology 115: 317-319.

Deepika, S. and Kothamasi, D. (2015) Soil moisture-a regulator of arbuscular mycorrhizal fungal community assembly and symbiotic phosphorus uptake. Mycorrhiza 25: 67-75.

Dugassa, D. G., Grunewaldt, S. and Schonbeck, F. (1995) Growth of *Glomus intraradices* and its effect on linseed (*Linum usitatissimum* L.) in hydroponic culture. Mycorrhiza 5: 279-282.

Elbon, A. and Whalen, J. K. (2014) Phosphorus supply to vegetable crops -from arbuscular mycorrhizal fungi: A review. Biological Agriculture and Horticulture: An International Journal for Sustainable Production Systems 26: 73-90.

Ellouze, W., Hamel, C., Cruz, A. F., Ishii, T. and Gan, Y. (2012) Phytochemicals and spore germination: At the root of AMF host preference? Applied Soil Ecology 60: 98-104

Etesami, H. and Alikhani, H. A. (2016) Co-inoculation with endophytic and rhizosphere bacteria allows reduced application rates of N-fertilizer for rice plant. Rhizosphere 2: 5-12.

Guissou, T., Babana, A. H., Sanon, K. B. and Ba, A. M. (2016) Effects of arbuscular mycorrhizae on growth and mineral nutrition of greenhouse propagated fruit trees from diverse geographic provenances. Biotechnologie, Agronomie, Societe et Environnement/Biotechnology, Agronomy, Society and Environment (BASE) 20: 417-426.

Habibzadeh,Y., Jalilian, J., Zardashti, M., Pirzad, A. and Eini, E. (2015) Some morpho-physiological characteristics of mung bean mycorrhizal plants under different irrigation regimes in field condition. Journal of Plant Nutrition 38: 1754-1767.

Hamzei, J. and Salimi, F. (2014) Root colonization, yield and yield components of milk thistle (*Silybum marianum*) affected by mycorhizal fungi and phosphorus fertilizer. Journal of Acricultural Science and Sustainable Prosution 24: 85-96.

Harborne, J. B. (1980) Plant Phenolics. Secondary Plant Products. Springer Verlag, Berlin.

Hawkins, H., Cramer, M. and George, E. (1999) Root respiratory quotient and nitrate uptake in hydroponically grown non-mycorrhizal and mycorrhizal wheat. Mycorrhiza 9: 57-60.

Kim, S. Y., Lim, J. H., Park, M. R., Kim, Y. J., Park, T., Seo, Y. W., Choi, K. G. and Yun, S. J. (2005) Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in barley roots under saline stress. Journal of Biochemistry and Molecular Biology 38: 218-224.

Kizhaeral, S. and Charest, S. C. (2005) Arbuscular mycorrhizae and nitrogen assimilation in maize after drought and recovery. Physiology Plantarum 2: 285-296.

Kowalska, I., Konieczny, A., Gastol, M., Sady, W. and Hanus-Fajerska, E. (2015) Effect of mycorrhiza and phosphorus content in nutrient solution on the yield and nutritional status of tomato plants grown on rackwool or coconut coir. Agricultural and Food Science 24: 39-51.

Krizek, D. T., Britz, S. J. and Mirecki, R. M. (1998) Inhibitory effects ambient levels of solar UV-A and UV-B radiation on growth of cv. New Red Fire lettuce. Physiologia Plantarum 103: 1-7.

Li, Y., Chen, Y. L., Li, M., Lin, X. G. and Liu, R. J. (2012) Effects of arbuscular mycorrhizal fungi communities on soil quality and the growth of cucumber seedlings in a greenhouse soil of continuously planting cucumber. Pedosphere 22: 79-87.

Lichenthaler, H. K. (1987) Chlorophylls and cartenoides pigments of photosynthesis. Method in enzymology. INRA, EDP, Sciences 57: 245-250.

Lynn, D. J. and Chang, M. (1990) Phenolic signals in cohabitation: Implication for plant development. Annual Review of Plant Physiology and Plant Molecular Biology 41: 497-526.

[DOR: 20.1001.1.23222727.1400.10.43.6.0]

Maboko, M. M., Bertling, I. and Du Plooy, C. P. (2013) Abuscular mycorrhiza has limited effect on yield and quality of tomatoes grown under soilless cultivation. Acta Agriculture Scandinavica, Section B-Soil and Science 63: 261-270.

Mathur, N. and Vyas, A. (1995) Influence of VAM on net photosynthesis and transpiration of *Ziziphus mauritiana*. Plant Physiology 147: 328-330.

Michelsen, A., Lisanework, N. and Friss, I. (1993) Impact of tree plantation in the Etiopian highland on soil fertility, shoot and root growth, nutrient utilization and mycorrhizal colonization forest ecology and management. Forest Ecology and Management 61: 229-324.

Nouri, E., Breuillin-Sessoms, F., Feller, U. and Reinhardt, D. (2014) Phosphorus and nitrogen regulate arbuscular mycorrhizal symbiosis in *Petunia hybrida*. Plos One 9: e90841.

Ortas, I. (2012) The effect of mycorrhizal fungal inoculation on plant yield, nutrient uptake and inoculation effectiveness under long-term field conditions. Field Crops Research 125: 35-48.

Pandey, R. and Garg, N. (2017) High effectiveness of *Rhizophagus irregularis* is linked to superior modulation of antioxidant defence mechanisms in *Cajanus cajan* (L.) Millsp. genotypes grown under salinity stress. Mycorrhiza 1-14.

Paterson, E., Sim, A., Davidson, J. and Daniell, T. J. (2016) Arbuscular mycorrhizal hyphae promote priming of native soil organic matter mineralisation. Plant and Soil 408: 243-254.

Safapour, M., Ardakani, M. R., Khaghani, S., Teymoori, M., Hezaveh, H. and Mafakheri, S. (2012) Phytohormonal and polyamines changes of three red bean (*Phaseolus vulgaris* L.) genotypes as affected by Tripartite symbiosis with mycorrhiza and rhizobium. Archives Des Sciences 65: 235-240.

Sanchez-Blanco, M. J. T., Ferrandez, M. A., Morales, A. M. and Alarcon, J. J. (2004) Variations in water status, gas exchange, and growth in *Rosmarinus officinalis* plants infected with *Glomus deserticola* under drought conditions. Journal of Plant Physiology 161: 675-682.

Schliemann, W., Strack, D. and Ammer, C. (2008) Metabolite profiling of mycorrhizal roots of *Medicago truncatula*. In: Phytochemistry of the 4th International Conference on Plant Metabolomicsn.

Sharma, A. K. (2002) Biofertilizers for sustainable agriculture. Agrobios, India.

Sharma, S. D. and Sharma, N. C. (2011) Occurrence and distribution of AM Fungi in citrus orchards of northwestern Himalayan region of India grown under diverse management systems. Journal of Hill Research 19: 40-43.

Sharma, S. D. and Sharma, N. C. (2006) Occurrence and distribution of AM fungi in citrus orchards of northwestern Himalayan region of India grown under diverse management systems. Journal of Hill Research 19: 40-43.

Singleton, V. and Rossi, J. (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture January 16: 144-158.

Tian, M., Chen, Y. L., Li, M. and Liu, R. J. (2013) Structure and function of arbuscular mycorrhiza: A review. Ying Yong Sheng Tai Xue Bao 24: 2369-2376.

Trouvelot, A., Kough, J. L. and Gianinazzi, V. (1986) Mesure de taux de mycorhization VA d'un système radiculaire. Recherche de methods d'estimation ayant une signification fonctionnelle. In: Physiological and Genetic Aspects of Mycorhizical (eds. Gianinazzi Pearson, V. and Gianinazzi, S.) Pp. 217-221. INRA, Paris.

Utkhed, R. (2006) Increased growth and yield of hydroponically grown greenhouse tomato plants inoculated with arbuscular mycorrhizal fungi and *Fusarium oxysporum* f. sp. radicis-lycopersici. Bio Control 51: 393-400.

Vierheiliga, H., Schweigerb, P. and Brundrettc, M. (1998) An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. Physiologia Plantarum 125: 393-404.

Weisanny, W., Raei, Y., Zehtab-Salmasi, S., Sohrabi, Y. and Ghassemi-Golezani, K. (2016) Arbuscular mycorrhizal fungi induced changes in rhizosphere, essential oil and mineral nutrients uptake in dill/common bean intercropping system. Annals of Applied Biology 1-14.

Xiao, J., Cheng, Y., Hu, Y., Chen,Y., Jun, H. and Yang, B. (2014) Growth and nutrient content of Trifoliate orange seedlings influenced by arbuscular mycorrhizal fungi inoculation in low magnesium soil. New Phytologist 667-676.

Zhu, X. Q., Wang, C. Y., Chen, H. and Tang, M. (2014) Effect of arbuscular mycorrhizal fungi on photosynthesis, carbon content, and calorific value of black locust seedlings. Photosynthetica 52: 247-252.

[DOR: 20.1001.1.23222727.1400.10.43.6.0]