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Mycorrhiza Abundance and Biological Activity of Soil Under Iron-Fertilized Apple Cultivar (Red Chief) Grafted on Different Rootstocks Grown on a Calcareous Soil

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The aim of this study was to examine mycorrhizal spore abundance, its infection rate and biological activity of soil depending on different rootstock, Fe sources or Fe doses. A field experiment was carried out to determine the effects of rootstocks on mychorizal abundance and root infection as well as CO_2 production and dehydrogenase activity of soil. Doses of 25, 50 and 75g $tree^{-1}$ Fe-EDDHA or $FeSO_4$ were applied to apple trees (Red Chief cv.) grafted on dwarf (M9 and M26) and semi-dwarf (MM106) rootstocks. At flowering stages soil and root samples were collected and analyzed for their dehidrogenase activity, CO_2 production, microbial biomass-C or mycorrhizal abundance and infection rate. Results revealed that neither rootstock nor Fe applications effect on mycorrhiza number in rhizosphere soil. The higher value was observed in the soil M26 planted and 75 g da^{-1} Fe-EDDHA applied plot (15 spores per g of soil). Therefore, infection rate showed significant variations related to rootstocks and Fe applications. The most adapted rootstocks was MM106 which 37.1 % of the roots infected by mycorrhiza. Fe-EDDHA was more effective than $FeSO_4$ whereas both of them increased infection rate compared to control. There was no statistical difference between rootstocks in CO_2 production; however, Fe-EDDHA is stimulated CO_2 formation. The highest CO_2 formation (11.55 mg CO_2 100 g $soil^{-1}$) observed in 50 g of Fe-EDDHA applied MM106 plot whereas the lowest was in M26 plot where Fe application not realized. Dehydrogenase activity was not affected by Fe sources; however, increased Fe application increased dehydrogenase activity. Rootstock of MM106 is statistically more effective on dehydrogenase and highest dehydrogenase value was observed in 50 g of Fe-EDDHA applied MM106 plot as 328 µg TPF 10 g $soil^{-1}$. The highest biomass-C value was observed in 50 g of Fe-EDDHA applied MM106 plot, whereas the lowest was observed in 50 g of $FeSO_4$ applied M9 plot. In general, Fe-EDDHA application was promote biomass-C more than $FeSO_4$. Comparing to rootstocks, the highest effective rootstock was MM106 and followed by M26 and M29, respectively.

Key Words: Mycorrhiza, Apple, Red chief, Calcareous soil.

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INTRODUCTION

Fruit production is one of the major sectors of Turkish agriculture. Apple cultivation on dwarf rootstock has become widespread and cultivars such as Fuji, Braeburn, Gala, Elstar and Jonagold are preferred by Turkish growers[1].

Arbuscular mycorrhizal fungi (AMF) are obligately symbiotic soil fungi which colonize the roots of the majority of plants. It is well recognized that arbuscular mycorrhizal (AM) fungi can form beneficial associations with the roots of around 80 % of terrestrial plants[2]. One of the most dramatic effects of mycorrhizal infection on the host plant is an increase in the phosphorus[3] and zinc[4-7] uptake mainly due to the capacity of the mycorrhizal fungi to absorb their ions from the soil and transfer it to the host roots[8,9]. The mycorrhizal fungus-plant association provides carbohydrates and some organic materials for the fungus and improves plant access to water and mineral intake[10,11].

Most fruit trees and horticultural plants are naturally associated with arbuscular mycorrhizal fungi (AMF). Positive effects on survival and growth were seen after arbuscular mycorrhizal inoculation both in micropropagated horticultural plants and fruit species, as in apple[12,13]. Fortuna *et al.*[14] inoculated apples grafted on micropropagated MM 106 rootstocks and Mr.S. 2/5 plums with *G. mossae* and *G. intraradices* and observed increased apical shoot growth along with phosphorus uptake. Plenchette *et al.*[15] and Gianinazzi *et al.*[16] reported increase of shoot growth in apples inoculated with mycorrhiza. Studies showed that conservative practices of agriculture promote higher number of spores, inoculum potential and mycorrhizal colonization when compared to conventional agriculture[17,18].

Arbuscular mycorrhizal (AM) associations play important roles nutrient cycling through their microbial activity and their involvement in plant nutrient acquisition[19]. AM colonization can cause physiochemical or microbiological changes to the rhizosphere thus affecting root hyphae uptake of some nutrients[20,21]. Nitrogen and phosphorus levels are considered among the most important factors affecting AM association efficiency. Not only the level but also the balance between nitrogen and phosphorus fertilizer regimes are crucial in establishing an efficient symbiosis.

Inherent differences in cation-anion uptake ratios and, thus rhizosphere pH, are important for the capacity to mobilize phosphorus, zinc and iron in calcareous soils[22].

Biological activities in soils drive many of the key ecosystem processes that govern the global system, especially in the cycling of elements such as carbon, nitrogen and phosphorus[23].

Mycorrhizal fungi, upon root colonization, develop an external mycelium which is a bridge connecting the root with the surrounding soil microhabitats. Therefore, the mycorrhizal symbiosis, by linking the biotic and geochemical portions of the ecosystem, can contribute to nutrient capture and supply. Particularly, the arbuscular mycorrhizal (AM) symbiosis plays a direct role in nutrient cycling rates and patterns in agroecosystems and natural environments.

Environmental factors (such as pH, temperature) affect and alter soil microbial assemblage structure and function. Arbuscular mycorrhizal fungi (AMF) are a major component of rhizosphere microflora in natural ecosystems and play significant roles in the mineralization and cycling of plant nutrients. AMF increase the efficiency of nutrient and water absorption and host plant response to stressful environments. AMF also contribute to the maintenance of good soil structure[24]. In this work, it was aimed to determine the effects of rootstocks on mychorizal abundance and root infection as well as CO_2 production and dehydrogenase activity of soil on a calcareous soil.

EXPERIMENTAL

The study was carried out at Research and Implementation Farm, Suleyman Demirel Universitiy, Isparta, Turkey during 2006 growing seasons. Five years old Red chief cultivar grafted on dwarf (M9, M26) or semi-dwarf (MM106) rootstocks. The orchard was basal fertilized and irrigated as needed to prevent any nutritional disorders and water stress. Totally, 10 kg da^{-1} N, 6 kg da^{-1} P, 14 kg da^{-1} K and 3.5 kg da^{-1} Mg from mono ammonium phosphate, ammonium nitrate, phosphoric acid, potassium nitrate, magnesium sulfate were applied with drip irrigation. For iron fertilization, 0 (Fe0), 25 (Fe25), 50 (Fe50) and 75 (Fe75) g of $FeSO_4$ ($FeSO_4 \cdot 7H_2O$, 19 % Fe) or Fe-EDDHA (6 % Fe) were applied to the each tree root zone and mixed to the soil.

The experimental soil was clay loam having pH 7.8 (1:2.5 soil to water ratio), 17 % $CaCO_3$ (Calcimetric method)[25], 1.5 % organic matter (Walkley & Black method)[26], 30 kg ha^{-1} 0.5 M $NaHCO_3$ extractable P (Olsen's method)[27], 600 kg ha^{-1} 1 N NH_4OAc exchangeable K and Mg (atomic absorption spectrophotometer methods)[28]. The available Fe, Cu, Zn and Mn as determined in DTPA extract[28] on atomic absorption spectrophotometer were 3.1, 1.0, 0.37 and 3.0 mg kg^{-1}, respectively.

Long term mean temperature of Isparta is 12 °C and annual precipitation is 551.8 kg m^{-2}'dir. Most proportion (72.7 %) of precipitation is realized in winter and spring.

At flowering stage, root and soil samples were collected. Soil samples were sieved from 4 mm sieve and analyzed for their dehydrogenase activity (DHA), CO_2 fluxes and microbial biomass carbon. The root samples were washed carefully; they were preserved in a mixture of ethanol, glacial acid and formalin with proportion of 92:2:6 (v/v). The root clearing and staining was done according to the method described by Koske and Gemma[29]. The percentage of AM infection was calculated as a number of 10 mm long root segments out of 100 identified as infected under a stereo microscope at a magnification of 20X[30].

Mychorrizal propagules were isolated from the rhizosphere soil samples by using the wet sieving technique. Spore densities were expressed in terms of the number of spores per g of dry soil. Statistical analyses were performed using Mstatc program. Differences between the means were separated by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Mychorriza spore number and infection rate: Mycorhizal spore abundance of soil was not statistically differing for rootstocks, Fe-sources or Fe doses (Table-1, $p > 0.05$), however, the higher value was observed in the soil M26 planted and 75 g da^{-1} Fe-EDDHA applied. Therefore, infection rate showed significant ($p < 0.05$) variations related to rootstocks and Fe applications. The most adapted rootstocks was MM106 which 37.1 % of the roots infected by mycorrhiza (Table-2). Iron-EDDHA was more effective than $FeSO_4$ whereas both of them increased infection rate compared to control. Although increasing level of iron application was not effective on mychorizal spore abundance, it is significantly effective on infection rate. Thus, iron fertilization would help to sustain mychorrhiza-apple relationship.

TABLE-1
MYCORRHIZA SPORE NUMBERS DETERMINED FROM DIFFERENT APPLE ROOTSTOCK, IRON-SOURCES AND DOSES APPLIED PLOT

Iron sources	Dose (g)	Root stocks			Average
		M9	MM106	M26	
$FeSO_4$	0	7.8 abc	9.5 abc	5.2 bc	7.5 a
	25	7.3 abc	7.3 abc	10.7 abc	8.4 a
	50	9.2 abc	11.0 abc	5.3 bc	8.5 a
	75	11.3 abc	10.8 abc	7.5 abc	9.9 a
	Average	8.9 A	9.7 A	7.1 A	8.6 A
Fe-EDDHA	0	7.8 abc	9.5 abc	5.2 bc	7.5 a
	25	13.0 abc	6.3 abc	7.5 abc	8.9 a
	50	4.2 c	10.0 abc	14.5 ab	9.6 a
	75	9.3 abc	9.0 abc	15.0 a	11.1 a
	Average	8.6 A	8.7 A	10.5 A	9.3 A
Average		8.7 A	9.2 A	8.8 A	

TABLE-2
MYCORRHIZAL COLONIZATION RATE DETERMINED FROM DIFFERENT APPLE ROOTSTOCK, IRON-SOURCES AND DOSES APPLIED PLOT

Iron sources	Dose (g)	Root stocks			Average
		M9	MM106	M26	
$FeSO_4$	0	13.3 f	13.3 f	13.3 f	13.3 b
	25	18.3 ef	25.0 def	23.3 def	22.2 b
	50	40.0 bcd	40.0 bcd	40.0 bcd	40.0 a
	75	30.0 def	60.0 a	25.0 def	38.3 a
	Average	25.4 A	34.6 A	25.4 A	28.5 B
Fe-EDDHA	0	13.3 f	13.3 f	13.3 f	13.3 b
	25	40.0 bcd	40.0 bcd	35.0 cde	38.3 a
	50	55.0 ab	50.0 abc	25.0 def	43.3 a
	75	35.0 cde	55.0 ab	20.0 ef	36.7 a
	Average	35.8 A	39.6 A	23.3 A	32.9 A
Average		30.6 AB	37.1 A	24.4 B	

CO_2 Flux and dehydrogenase activity: Values of CO_2 production and dehydrogenase activity affecting by different rootstock, different iron doses and sources are presented in Tables 3 and 4, respectively. According to mean values, there was no statistical difference between rootstocks in CO_2 production; however, Fe-EDDHA is stimulated CO_2 formation. The highest CO_2 (11.55 mg CO_2/100 ds) formation observed in 75 g of Fe-EDDHA applied MM106 plot whereas the lowest (3.24 mg CO_2/100 ds) was in M26 plots where Fe application not realized.

TABLE-3
CO_2 FORMATION OF SOIL DETERMINED FROM DIFFERENT APPLE ROOTSTOCK, IRON-SOURCES AND DOSES APPLIED PLOT

Iron sources	Dose (g)	Root stocks			Average
		M9	MM106	M26	
$FeSO_4$	0	6.73 b-f	7.77 a-e	3.24 f	5.91 b
	25	6.78 b-f	6.61 b-f	9.45 ab	7.61 ab
	50	4.51 def	7.54 b-e	10.22 ab	7.42 ab
	75	7.62 b-e	5.50 c-f	6.70 b-f	6.61 b
	Average	6.41 D	6.85 BCD	7.40 ABC	6.89 B
Fe-EDDHA	0	6.73 b-f	7.77 a-e	3.24 f	5.91 b
	25	7.55 b-e	3.82 ef	9.81 ab	7.06 ab
	50	10.00 ab	3.84 ef	8.90 abc	7.58 ab
	75	7.11 b-f	11.55 a	8.11 a-d	8.92 a
	Average	7.85 A	6.75 CD	7.51 AB	7.37 A
Average		7.13 A	6.80 A	7.46 A	

TABLE-4
DEHYDROGENASE ACTIVITY OF SOIL DETERMINED FROM DIFFERENT APPLE ROOTSTOCK, IRON-SOURCES AND DOSES APPLIED PLOT

Iron sources	Dose (g)	Root stocks			Average
		M9	MM106	M26	
$FeSO_4$	0	152 bc	194 bc	152 bc	166 ab
	25	108 cd	199 bc	160 bc	156 ab
	50	172 bc	203 bc	151 bc	176 ab
	75	110 cd	151 bc	158 bc	140 b
	Average	135 C	187 AB	156 BC	159 A
Fe-EDDHA	0	152 bc	194 bc	152 bc	166 ab
	25	203 bc	150 bc	187 bc	180 ab
	50	36 d	328 a	200 bc	188 ab
	75	239 ab	264 ab	119 cd	207 a
	Average	158 BC	234 A	165 BC	185 A
Average		147 B	210 A	160 B	

Dehydrogenase activity was not affected by iron sources. However, increased iron doses application increased dehydrogenase activity particularly in Fe-EDTA applications. Acording to general avareges Rootstock of MM106 is statistically more effective on dehydrogenase, whereas no differences were obtain between M9 and M26.

Microbial biomass carbon: Microbial biomass carbon of soil was statistically affecting by different rootstocks, iron-sources or iron doses (Table-5, $p > 0.05$). The highest biomass-carbon value was observed in 50 g of Fe-EDDHA applied MM106 plot, whereas the lowest was observed in 50 g of $FeSO_4$ applied M9 plot. In general, Fe-EDDHA application was promote biomass-carbon more than $FeSO_4$. Comparing to rootstocks, the highest effective rootstock was MM106 and followed by M26 and M29 respectively.

TABLE-5
MICROBIAL BIOMASS C DETERMINED FROM DIFFERENT APPLE ROOTSTOCK, IRON-SOURCES AND DOSES APPLIED PLOT

Iron sources	Dose (g)	Root stocks			Average
		M9	MM106	M26	
$FeSO_4$	0	2.93 d-g	8.12 a-f	4.39 b-g	5.15 b
	25	2.49 efg	6.48 a-g	12.70 a	7.22 ab
	50	1.21 g	1.90 fg	10.06 abc	4.39 b
	75	2.89 d-g	8.56 a-f	9.29 a-e	6.91 ab
	Average	2.38 D	6.27 BC	9.11 A	5.92 B
Fe-EDDHA	0	2.93 d-g	8.12 a-f	4.39 b-g	5.15 b
	25	9.11 a-e	9.57 a-d	3.25 c-g	7.31 ab
	50	11.08 ab	12.90 a	6.26 a-g	10.08 a
	75	6.95 a-g	7.13 a-g	6.82 a-g	6.97 ab
	Average	7.52 AB	9.43 A	5.18 C	7.38 A
Average		4.95 C	7.85 A	7.14 B	

Microbial life in soil is influenced highly from root exudes. Thus, MM106 seems to be exuding more organic compounds than others. Although nutrient availability is not subjected in this research, increasing microbial population is not only increased soil's microbial biomass-carbon but also nutrient bioavailability. Due to the mycorrhiza needs organic compounds that plant supplies, it is expected to AMF spore number in soil is also affected from different rootstocks. Somehow there was not relation between biomass-carbon and AMF spore number. Root exudes is not only the factor for AMF spore number in rhizosphere soil. Mycorrhiza should need to be having successful infection to produce spore. Mycorrhizal infection of MM106 is also statistically higher than others. Concerning the infection rate and biomass-carbon, MM106 would be more beneficial rootstock for healthy relation with mycorrhiza, however, according to spore number determined in rhizosphere, there was no evidence that indicate success relationship.

Conclusion

Results revealed that different rootstocks are one of the main factors for successful relationship between plant and mycorrhiza. Thus, determining the most effective rootstock variety would help mycorrhiza-plant community and so plant

nutrition. Soil biomass-carbon also indicates that different plant variety has different effect on soil microbial life which has special role many nutrient turnovers relating plant nutrition. Finally, Fe-EDDHA shuld preferred to $FeSO_4$ for maintain mycorrhiza-plant community. According to infection rate, dehydrogenase activity and microbial biomass-carbon, the most effective rootstock was MM106 and it is advised to farmers for good association between soil microbial life and plant.

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