ATINER CONFERENCE PAPER SERIES No: AGR2013-0774

Athens Institute for Education and Research
ATINER

ATINER's Conference Paper Series
AGR2013-0774

Influence of Mycorrhiza Inoculation on Plant Growths of Triticale (*x Triticosecale Wittmack*) and Clover (*Medicago sativa*) in Sterile and non-Sterile Soils

Onder Ozal
Suleyman Demirel University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition Isparta
Turkey

Ali Ozden
Suleyman Demirel University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition Isparta
Turkey

Ali Coskan
Suleyman Demirel University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition Isparta
Turkey

ATINER CONFERENCE PAPER SERIES No: AGR2013-0774

Athens Institute for Education and Research
8 Valaoritou Street, Kolonaki, 10671 Athens, Greece
Tel: + 30 210 3634210 Fax: + 30 210 3634209
Email: info@atiner.gr URL: www.atiner.gr
URL Conference Papers Series: www.atiner.gr/papers.htm

ISSN **2241-2891**
18/12/2013

An Introduction to ATINER's Conference Paper Series

ATINER started to publish this conference papers series in 2012. It includes only the papers submitted for publication after they were presented at one of the conferences organized by our Institute every year. The papers published in the series have not been refereed and are published as they were submitted by the author. The series serves two purposes. First, we want to disseminate the information as fast as possible. Second, by doing so, the authors can receive comments useful to revise their papers before they are considered for publication in one of ATINER's books, following our standard procedures of a blind review.

Dr. Gregory T. Papanikos
President
Athens Institute for Education and Research

This paper should be cited as follows:

Ozal, O., Ozden, A. and Coskan, A. (2013) **"Influence of Mycorrhiza Inoculation on Plant Growths of Triticale (*x Triticosecale Wittmack*) and Clover (*Medicago sativa*) in Sterile and non-Sterile Soils"** Athens: ATINER'S Conference Paper Series, No: **AGR2013-0774**.

Influence of Mycorrhiza Inoculation on Plant Growths of Triticale (*x Triticosecale Wittmack*) and Clover (*Medicago sativa*) in Sterile and non-Sterile Soils[1]

Onder Ozal
Suleyman Demirel University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition Isparta
Turkey

Ali Ozden
Suleyman Demirel University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition Isparta
Turkey

Ali Coskan
Suleyman Demirel University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition Isparta
Turkey

Abstract

A pot experiment was carried out to determine the effects of mycorrhiza inoculation on mycorrhizal infection, spore abundance as well as micronutrient contents of clover (*Medicago sativa*) and triticale (*x Triticosecale Wittmack*) plants. Clover and triticale was sown on sterile or non-sterile soils either with or without mycorrhiza spore applications.

Results revealed that root and shoot weights of clover and triticale were increased by mycorrhizal inoculation in both sterile and non-sterile conditions. The positive effect of mycorrhizal inoculation on biomass yield was considerable higher in sterile condition. Not surprisingly, mycorrhizal inoculation increased the mycorrhizal infection rate. However, the highest infection rate was observed in non-sterilized and non mycorrhiza applied variant as 86% at clover whereas the highest value in triticale was observed at -sterile +mycorrhiza application as 79%. Results related the nutrient contents of the both plants, represented the effectiveness of mycorrhiza. Therefore inoculation by mycorrhiza enhanced the plant nutrient uptake capability.

Key words: Mycorrhiza, clover, triticale, nutrient uptake

Corresponding Author:

[1]This research was supported by The Scientific and Technological Research Council of Turkey

Introduction

The most proportion of soils in Turkey contain considerable amount of lime; therefore, they have higher pH than the lime-poor soils. Typically, the availability of both phosphors and some micronutrients significantly lower in the soils that have higher pH (Mengel and Kirkby, 1982). Indeed, plant available phosphorus, zinc and iron amount of Turkey reported insufficient by Eyupoglu (1996). According to basic fertilization principles, plant nutrient requirement have to be provided either by fertilization or practices that increase availability of soil insoluble nutrient deposits. On the other hand P and Zn availability is effected not only by soil factors, but also plant nutrient uptake capabilities. Marschner (1991) reported that, plant roots may increase P and Zn availability by modifying chemical and biological properties of rhizosphere. These effects realized by secretion of organic acids and chelates. Recent studies concluded that in addition to root strategies, Arbuscular Mycorrhiza (AM) symbiosis has special role on P and Zn uptake (Li et al., 1991; Kothari et al., 1991). Thus, soil biological activity leads the nutrient uptake in the soils that has enough nutrient; however, in less available forms. Werner (1987) reported that, if there was no limitation factors that prevent successful mycorrhizal infection, P can be taken from the site where plant roots are not able to reach, via mycorrhizal hypha. Moreover George at al. (1992) proved that mycorrhizal hypha expedite transportation of Zn and Cu as well as water to plant roots. Besides, more than 90% of plants are establishing symbiotic relation with mycorrhiza, even this relation is vital for P and Zn uptake of some plants (Li et al., 1991). Although a number of researchers agreed that the function of mycorrhiza is closely related soil phosphorus content, Simith et al. (1992) claimed that the relation between mycorrhiza and plant is also related by genotypes of both parties. As well known, plant-microorganism interaction established in either as parasitism, commensalism or mutualism, where neutralism is quite minor. As an overall conclusion plant mutualistic symbioses have to be taking into consideration in fertilization to successful plant nutrition (Fritsche, 1985). In scope of above mentioned results, it can be said that plants which could not establish good interaction with microorganism, have a difficulties to uptake P as well as micronutrients such as Fe, Cu, Zn and Mn. Therefore, mycorrhizal inoculation would be great potential to overcome these nutrient uptake disorders. Effects of mycorrhizal inoculation were tested in a number of researches using many plant species. In this research, the effects of mycorrhizal inoculation to clover and triticale plants on plant nutrient uptake and biomass yield were investigated.

Material and Methods

The study was carried out in climate chamber as a pot experiment using clover and triticale as a test plant. Soils were placed to the pots either as natural or just after sterilization at 121 °C, 1/2 h. The soil used in the experiment was

not have salinization problem with clayey loam texture, having pH 7.5 (1:2.5 soil to water ratio), 28% $CaCO_3$ (Caglar, 1949), 1.3% organic matter (Jackson, 1962). The experiment was set up in a completely randomized experimental design with 3 replications. The cocktail mycorrhiza culture consisted of *G. mossea, G. etinicatum and G. clarium* was used as an inoculant. Mycorrhizal inoculation (+M) was realized by placing culture at 4 cm deep, as 2 cm thick sub layer. In non mycorrhiza inoculated pot (-M), same amount of soil directly placed to the pots. At the end of the experiment plants were dried at 65°C until they reached constant weights. Afterwards biomass weight was determined using analytical balance and the plant materials were grinded to prepare analyses. Samples were analyzed to determine Zn, Cu and Mn contents according to DTPA extraction (Lindsay & Norvell, 1978) method on atomic absorption spectrophotometer. Phosphorus analyzes were done spectrophotometric method using vanadomolybdophosphoric acid (Olsen et al., 1954).

Mychorrizal propagules were isolated from the rhizosphere soil samples by using the wet sieving technique (Gerdemann & Nicolson 1963). Spore densities were expressed in terms of the number of spores per 10 g of dry soil. The root clearing and staining was done according to the method described by Koske & Gemma, (1989). The percentage of AM infection was calculated as a number of 10 mm long root segments out of 10 identified as infected under a stereo microscope at a magnification of 20X (Giovannetti & Mosse, 1980).

Results

Biomass yield

Following the harvest, taken plant root and shoot samples were dried and weighted separately. Results obtained were presented in Table 1.

Root weight of clover plant was not effected by mycorrhizal inoculation in non-sterile soils. Sterilization diminished the root weights considerably; however, mycorrhizal inoculation supported plant to sustain root weight as much as those obtained in non-sterilized variant. Even in the highest root weight value was observed in the pot that sterilized and mycorrhiza inoculated. According to shoot weight values, mycorrhizal inoculation was increased plant shoot weight in both sterile and non-sterile soils. Root weight of triticale represents the similar values of clover; therefore, the lowest shoot weight value in triticale was observed in the pot that sterilized but not inoculated. Although mycorrhizal inoculation increased the root weight of triticale in sterile soils; these favorable effect was not observed in shoot weight. Root weight of triticale was negatively effected by mycorrhizal inoculation in sterilized and mycorrhiza applied soils whereas in non-sterile condition mycorrhizal inoculation increased the root weight. This negative effect was not observed in shoot weight, mycorrhizal inoculation increased shoot weight.

Table 1. *Root and shoot biomass weight (g plant^{-1})*

		Clover				Triticale			
		Root		Shoot		Root		Shoot	
Sterile	+ M	7,99	±1,68	9,88	±0,56	2,62	±0,55	10,13	±0,74
	- M	3,43	±1,35	3,75	±0,51	2,87	±0,83	9,45	±0,58
non sterile	+ M	7,83	±0,97	7,55	±0,29	2,37	±0,27	7,83	±0,35
	- M	7,39	±2,53	6,36	±0,61	1,64	±0,21	7,52	±0,36

Mycorrhizal infection and spore abundance

Determined mycorrhizal infection rate and mycorrhizal spore number values are presented in Table 2 and Table 3, respectively.

Table 2. *Mycorrhizal infection rate (%)*

		Yonca		Tritikale	
Sterile	+ mycorrhiza	76	±21,0	76	±17,0
	- mycorrhiza	20	±15,0	30	±22,0
non sterile	+ mycorrhiza	67	±14,0	79	±15,0
	- mycorrhiza	86	±13,0	74	±13,0

Table 3. *Mycorrhiza spore number (spore per 10 g soil)*

		Yonca		Tritikale	
Sterile	+ mycorrhiza	89	±37	73	±37
	- mycorrhiza	30	±15	58	±26
non sterile	+ mycorrhiza	92	±30	63	±46
	- mycorrhiza	68	±32	51	±18

According to mycorrhizal infection rate values that presented in Table 2, it is clearly seen that mycorrhizal inoculation increased the infection rate. The lowest values were determined in non-inoculated and sterilized soils. Although zero infection rate was expected in sterilized variants; still rather lower infection rate as 20% in clover and 30% in triticale were observed. These infections probably took place either contamination or less effective sterilization process in spot that steam would not penetrate sufficiently. Mycorrhizal inoculation following sterilization process yielded up to 4 time higher infection rate. In non-sterile soils, infection rate was negatively influenced by mycorrhizal inoculation. The reason of these effects is most likely due to the antagonistic relation between indigenous and inoculated mycorrhiza species. Based on the results obtained from clover, there was significant adaptation between plant and indigenous mycorrhiza spores; therefore, clover was infected adequately by indigenous spores, even without introduction the well-known mycorrhiza species. Infection rate in sterile soil was higher than non-sterile one in case of mycorrhizal inoculation. In triticale, mycorrhizal inoculation was not effective on infection rate.

The variation on spore number was quite high; therefore, it is hard to conclude the effectiveness of sterilization or mycorrhizal inoculation. Nevertheless mycorrhizal inoculation increased, whereas sterilization decreased the spore abundance of soil in both clover and triticale. Due to the destructive effects of high temperature, spores are denatured in sterilized variants. On the other hand mycorrhizal inoculation enhanced entire spore number of soil at non-sterile variants. The highest spore number in clover was observed in the pot that mycorrhiza inoculated and non-sterilized. However the highest number in triticale was determined in sterilized and mycorrhiza inoculated pots.

Nutrient uptake

At the harvest stage, plants were oven dried at 65° C and grinded to determine their phosphorus, copper, zinc and manganese contents. Results obtained were presented in Table 4, 5, 6 and 7, respectively.

Table 4. *Phosphorus contents (%)*

		Clover				Triticale			
		Root		Shoot		Root		Shoot	
Sterile	+ M	0,18	±0,01	0,27	±0,01	0,25	±0,11	0,37	±0,05
	- M	0,10	±0,03	0,21	±0,02	0,20	±0,08	0,25	±0,02
non sterile	+ M	0,19	±0,01	0,30	±0,01	0,27	±0,13	0,35	±0,07
	- M	0,14	±0,02	0,21	±0,07	0,35	±0,10	0,33	±0,09

Table 5. *Cupper contents (mg kg^{-1})*

		Clover				Triticale			
		Root		Shoot		Root		Shoot	
Sterile	+ M	11	±2,1	11	±2,1	31	±3,4	4	±0,7
	- M	11	±3,0	7	±0,1	31	±2,9	4	±0,4
non sterile	+ M	6	±0,4	6	±0,6	28	±1,4	2	±0,1
	- M	8	±2,8	6	±0,3	37	±5,0	2	±0,5

Table 6. *Zinc contents (mg kg^{-1})*

		Clover				Triticale			
		Root		Shoot		Root		Shoot	
Sterile	+ M	28	±2,7	45	±1,6	85	±10,9	41	±11,4
	- M	42	±14,7	25	±4,8	97	±11,5	24	±6,7
non sterile	+ M	33	±13,6	34	±7,6	83	±6,6	39	±14,6
	- M	39	±17,8	23	±1,0	103	±9,5	34	±3,8

Table 7. *Manganese contents (mg kg^{-1})*

		Clover				Triticale			
		Root		Shoot		Root		Shoot	
Sterile	+ M	114	±22,9	157	±21,7	206	±16,3	178	±14,2
	- M	184	±69,5	146	±13,6	320	±2,1	278	±40,1
non sterile	+ M	38	±11,9	71	±0,9	150	±46,7	60	±5,5
	- M	87	±19,9	68	±7,3	101	±3,5	49	±4,6

According to phosphor contents mycorrhizal inoculation increased plant P uptake (Table 4). In non-sterile pots, mycorrhizal inoculations increased both root and shoot P contents of clover; however, in triticale shoot P concentration increased while root P concentration was decreased. This subsidence in phosphor possible realized due to the higher growing rate of inoculated plants (Table 1); therefore, dilution the consumed phosphor. Cupper contents revealed similar results observed in phosphorus, whereas mycorrhizal inoculation increased plant cupper uptake in clover and triticale (Table 5). Sterilization increased copper contents of clover; however, this effect seems to be due to the mobilization of copper deposits under high temperature. In other word, main factor of the copper increment was not subject to mycorrhizal infection. In triticale planted pots, sterilization has similar effects of clover; but, in non-sterile variant mycorrhizal inoculation decreased copper uptake. This finding has a special importance for copper contaminated soils, because of indicating mycorrhizal infection would help the plant to prevent excessive copper uptake. Zinc concentration of clover shoot determined higher in mycorrhiza inoculated plants whereas root zinc concentration was negatively effected by mycorrhizal inoculation. In general, sterilization improved zinc uptake in triticale, nevertheless tihsi effect was not observed in triticale. Sterilization significantly stimulated manganese uptake of both clover ant triticale. As mentioned earlier, most probably higher temperature triggered manganese dissolution; therefore, stimulate manganese uptake. Although mycorrhizal inoculation reduced manganese concentration of clover roots, shoot Mn was positively affected by inoculation.

Conclusions

Results demonstrated that mycorrhizal inoculation enhanced nutrient uptake capacity of both plants studied herein. Plants that infected by mycorrhiza was looking greener and healthier; therefore biomass weight was higher than non-inoculated ones. Generally, sterilization improved plant nutritional status except phosphorus. On the other hand mycorrhiza was not responsible from this favorable situation, most likely depends on the heat applications, nutrient availability was increased. Results revealed that both of the plants are mycorrhizal dependent, thus in case of inoculation, plants may use unavailable nutrient deposits in soil. On the other hand mycorrhiza is

limiting unwanted elements uptake. In this research we observed that sterilization increased soil Mn contents, but mycorrhizal inoculation prevent excessive Mn consumption. Human nutrition in Turkey is mostly based on cereals, thus results obtained from triticale plants has a special importance, because of its opportunity to apply other cereals. Although highly promising results obtained from this relatively narrow experiment, more experiment should carry out to determine the best spore-plant interaction.

References

Caglar, K.O., (1949). Toprak Bilgisi. University of Ankara Press, Nr:10, pp: 230.

Eyupoglu, F., Kurucu N. & Talaz, S., (1996). The general status of some microelements (Fe, Cu, Zn Mn) of the soils in Turkey. Soil and Fertilizer Research Institute Press, Nr: 217, Anlara/Turkey.

Fritsche, W., (1985). Umweltmikrobiologie. Akademic-Verlag. Berlin.

George, E., Ussler, H., Kothari, S.K., Li, X. -L & Marschner, H., (1992). Contribution of mychorrizal hyphae to nutrient and water uptake of plants. In: Read, D.J., Lewis, D.H., Fritter, A.H., Alexender, I.J. (eds.); Mycorrhizas in ecosystems. CAM International Wallingford, Oxon, UK. 419 pp.

Gerdemann, J. W. & Nicolson, T. H., (1963). Spores of mycorrhizal endogamy species extracted from soil by wet sieving and decanting. Trans Brit. Mycol. Soc. 46, 235-244.

Giovannetti, M. & Mosse, B., (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phyt. 84, 489-500.

Jackson, M. L., (1962). Soil chemical analysis, Prentice-Hall, Inc., Englewood Cliffs, N.S.

Koske, R.E. & Gamma, J.N., (1989). A modified procedure for staining roots to detect VAM. Mycol. Res. 92, 486-505.

Kothari, S.K., Marschner, H. & Romheld, V. (1991). Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil. Plant and Soil 131(2), 177-185.

Li, X. L., Marschner, H. & George, E. (1991). Acquisition of phosphorus and copper in VA-mycorrhizal hyphae and root-to-shoot transport in white clover. Plant and Soil 135, 49-57.

Lindsay, W.L. & Norvell, W.A., (1978). Development of a DTPA soil test for zinc, iron, manganese. Soil Sci. Soc. Am. J. 42, 421-428.

Marschner, H. (1991). Root-induced changes in the availability of micronutrients in the rhizosphere. In: Y. Waisel, A. Eshel and K.Kafkafi (eds.). plant Roots. Marcel Dekker, pp. 503-528.

Mengel K., and E.A. Kirkby., (1982). Principles of Plant Nutrition .3 rd Edition.International Potash Institute Bern, Switzerland.

Olsen, C.R., Cole, C.V., Watanabe, E.S. & Dean, L.A., (1954). Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate, USDA Circ. 939, U.S. Government Printing Office, Washington DC.

Smith, S.E., Robson A. & Abbott, L.K., (1992). The involvement of mycorrhizas in assessment of genetically dependent efficiency of nutrient uptake and use. Plant and Soil 146,169-179.

Werner, D., (1987). Pflanzliche und mikrobielle Symbiosen. George ThimeVerlag-Stuttgart New York, 48-102.