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Nitrogen Fixation and Yield of Lucerne (*Medicago sativa* L.), as Affected by Co-inoculation with *Sinorhizobium meliloti* and Arbuscular Mycorrhiza under Dry Organic Farming Conditions

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Abstract: This study evaluated the effects of co-inoculation with *Sinorhizobium meliloti* and arbuscular mycorrhizal fungi on the growth, yield and biological nitrogen fixation (BNF) of lucerne (*Medicago sativa* L.) under organic farming and dry weather conditions. The trial was laid out as a factorial experiment in the fields of the University of Natural Resources and Applied Life Sciences, Vienna-Austria at Raasdorf in 2007. The experimental factors of *S. meliloti* and arbuscular mycorrhiza (AM) including *Glomus etunicatum, G. intraradices and G. claroideum* and irrigation levels were tested. Co-inoculation of lucerne with *S. meliloti* and AM increased shoot dry weight as well as BNF at the first and second harvest but for BNF it was not significant. Irrigation resulted in the enhanced growth of some growth parameters. Microbial activities in this organically managed field were high enough for establishing an effective symbiosis with lucerne without any necessity for inoculation. Hence, it can be suggested that the tripartite symbiosis of *S. meliloti*, AM and lucerne can improve the performance of lucerne in organic farming and under dry conditions.

Key words: *Arbuscular mycorrhiza* · Biological nitrogen fixation · Lucerne (*Medicago sativa* L.) · Organic farming · *Sinorhizobium meliloti*

INTRODUCTION

Organic farming has to be self-sufficient in nitrogen (N), because the use of mineral N fertilizers is excluded. The cornerstone for soil fertility in organic farming is the use of legumes to fix atmospheric N_2. Lucerne (*Medicago sativa* L.) is an important forage legume in organic farming systems, mainly under dry conditions. The annual biological nitrogen fixation (BNF) rates, which is the result of symbiosis between legumes (*Fabaceae*) and nodulating bacteria (rhizobia), by lucerne ranges broadly from 85 to 360 kg ha^{-1} [1]. The resulting N benefit to succeeding crops is very variable, depending on the performance of the lucerne crop. Environmental factors and management practices affect the fixation process and the amount of N_2 fixed [2,3].

Fluctuations in weather patterns influence legume growth and N_2 fixation through creating environmental stresses (e.g. drought and high temperatures). Such stresses directly limit N_2 fixation by affecting nodule formation or functioning, or indirectly through the host plant performance [4,5].

Since, forage production in the organically managed field is highly related to the performance of the beneficial microbes, then soil microbial activities are considered very important parameters in functioning of these kinds of agroecosystems.

The diazotroph bacteria such as symbiotic bacteria (*Rhizobium sp.*) are beneficial microorganisms in the root zone of the leguminous plants being reported as very essential for plant establishment and growth, especially under nutrient unbalanced conditions [6,7].

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Arbuscular mycorrhizal fungi are also mutualistic micro symbionts of about 90% of higher plants. Their well documented effects are the improvement of plant-water relations and hence increased water absorption [8,9,10] as well as the enhancement of nutrients uptake [11]. It has also been shown that arbuscular mycorrhiza (AM) (Plant-fungi association) colonisation and activities are enhanced by rhizobium, resulting in enhanced plant performance [12].

AM and *Rhizobium sp.* form an intimate association with leguminous plants, often termed the "tripartite symbiosis". Plants benefit from this association in many ways including enhanced plant growth, yield and nutrient content especially N and P [13,14]. In addition, research reports suggest that plant benefits derived from the tripartite symbiosis are superior to that of un-inoculated control plants or that of plants inoculated with either AM or rhizobium alone and furthermore, differences between co-inoculations results are mediated by specific interendophyte interactions between rhizobium strains and the different AM species, which became evident under N-deficient conditions [13,14].

Rhizosphere / mycorrhizosphere system can therefore help plants to survive under stress conditions such as nutrient deficiency or degraded habitats [15]. Seed or soil inoculation is a common practice for enhancing the growth and development of some agricultural plants and can be very advantageous in organic farming. However, the success of this practice depends strongly on the effectiveness of the indigenous microbes and on the interactions between the participants in the rhizosphere [16]. Therefore, the study and understanding of the interactions between the beneficial microorganisms (especially the diazotrophs and AM fungi) is essential [17].

Plants collaborate with many microorganisms in the rhizosphere to form mutualistic associations. Biofertilizers have been recognized as important inputs in integrated plant nutrition systems. Using different kind of biofertilizers such as diazotrophes and AM for various crops are well reported and these biofertilizers can minimize the use of inorganic nitrogen.

The climate of eastern Austria is stamped by dry summers. The resulting low water supply can considerably impair the BNF rate of legumes, AM activities and their constructive effects on soil fertility. In a preceding study [18], lucerne proved to be the most efficient legume under dry conditions. This study assessed the effects and interactions of inoculations with AM and rhizobia on the growth and performance of lucerne under dry organic farming conditions.

Our objective were (i) to test the single or combined effects of inoculations with AM and *Sinorhizobium meliloti* on lucerne drought tolerance, BNF and yield under dry conditions; and (ii) to assess the effect of water availability on the efficacy of the inoculation treatments. We assumed that, mainly under drought, an inoculation with AM will improve plant water status and nutrition, thus enhancing the response to *S. meliloti* inoculation and the related enhanced BNF and hence plant growth and yield.

MATERIALS AND METHODS

Site Description and Weather Conditions: The trial was conducted on the organically managed fields of the research station 'Gross-Enzersdorf' of the University of Natural Resources and Applied Life Sciences, Vienna, in Rassdorf (longitude: 16°35 ' 32" E , latitude: 48°13 ' 53" N , height: 151 m) in April 2007. Climate of this area is characterized by hot, dry summers with little dew and cold winters with little snow. The mean annual temperature is 9.8°C, the average precipitation is 554 mm. The soil was a Calcaric Phaeozems [19] from loess with a silty loam texture, organic carbon contents of 2.2 % in the A horizon and a pH $CaCl_2$ value of 7.6 in the topsoil. The soils are described in great details by Freyer *et al.,* [20]. The weather data were assessed by the gauging station of the Institute for Agronomy and Plant Breeding, University of Natural Resources and Applied Life Sciences, Vienna (BOKU). The mean temperature in April 2007 (sowing trial) was 12.28°C, which was more than monthly average (9.7°C) and mean precipitation at this time was 0.80 mm, which was a very small amount of precipitation, compared with the monthly average (38.8 mm). Because of this dry condition and the very low precipitation, sprinkler irrigation for all treatments was used, immediately after sowing. During the May-September 2007 period, the temperature was more or less in the long term monthly average. The precipitation was above the long term monthly average most of the times. The amount of precipitation from the beginning of experiment until the first harvest (12 April until 9 July) was 150.8 mm and from the first harvest until the second harvest (9 July-20 September) was 331 mm. It shows that there was a large amount of precipitation (481.8 mm) during the experiment period.

Table 1: Treatment characters

Treatments	Rhizobia	Mycorrhiza	Irrigation
$R_1M_0I_0$	+	-	-
$R_0M_1I_0$	-	+	-
$R_1M_1I_0$	+	+	-
$R_0M_0I_0$	-	-	-
$R_1M_0I_1$	+	-	+
$R_0M_1I_1$	-	+	+
$R_1M_1I_1$	+	+	+
$R_0M_0I_1$	-	-	+

-: control + : treated

Experimental Procedure: Treatment variants differ with respect to the inoculation and irrigation of lucerne (Table 1). The seeding density was 25 kg ha^{-1} in all cases, lucerne cultivar was Sitel (origin and maintainer: Netherlands / Barenburg Holland BV, Atationsstraat 40, 6678 AC, Osterhout; thousand seed weight: 2.3 g).

To estimate BNF by the ^{15}N dilution method, we considered one reference crop plot that was cropped with a grass-mixture for each lucerne plot. The mixture consisted of 25 % of each of the grass species included (perennial Ryegrass *Lolium perenne*, false oats *Arrhenatherum elatius*, cockfoot *Dactylis glomerata* and red fescue *Festuca rubra*) with a seeding density of 25 kg ha^{-1}.

Inoculation with Rhizobia and Mycorrhiza: The rhizobium inoculum originated from the commercial inoculum collection of Becker Underwood Company with the trade name Histick. The carrier material was sterilized peat and contained minimum 2×10^9 viable cells of selected *Sinorhizobium meliloti* per gram. Lucerne seeds of each plot (30.56 g / plot with considering germination rate) were inoculated with 6.5 g of *S. meliloti* inoculum. Multispecific cultures of the AM species included *Glomus etunicatum, G. intraradices and G. claroideum,* produced by INOQ Agri company. The other characteristics of the inoculums are as follows: vermiculite as carrier material; grain size of 1 – 2 mm; specific weight of 570 - 610 (g l^{-1}); pH 5.7; 150±9 of propagules per gram of inoculant, estimated by the Most Probable Number (MPN) method. The inoculums were previously weighed for each AM plot (850g $plot^{-1}$), spread congenial at the soil surface of each plot and then mixed with soil to the 5 cm depth before sowing. After spreading and mixing AM inoculum with the soil, lucerne seeds were sowed in the plots.

Irrigation: The first irrigation was applied to all the plots by a sprinkler on 12 April, 2007. After 7 weeks, irrigation for the respective treatments was started. During the experimental period, five irrigations with 12 mm / plot were applied.

Experimental Design and Statistical Methods: The experiment was laid out as a factorial experiment in a completely randomized block design (CRBD) with 4 replicates (8 x 4 =32 plots). The area of each plot was 3×3 meters (9 m^2). Within each lucerne plot, part of the area was designed for yield measurements without any destruction till end of the project. Soil sampling and other plant sampling for studying AM colonization was done from other parts of the plots considering 50 cm distance from each side of the plot as a margin.

Following a 4-way ANOVA [21] with the factors "AM inoculation", "*S. meliloti* inoculation", "irrigation" and "block", means of the interaction combinations were compared by Duncan's Multiple Range Test [22].

Plant Sampling: The crops were harvested two times during the experiment (H1: 09.07.2007 and H2: 20.09.2007), according to the development stage of the lucerne crop at beginning of flowering. The shoot and the stubble dry matter yield were determined by harvesting two separate areas per plot with a 1 m × 0.5 m area. The second harvest represented the shoots material regrown from the stubbles left after the first harvest. An aliquot was dried at 105°C to constant weight. Shoots were harvested 5 cm above the soil surface. Stubbles were harvested from soil surface at the second harvest. Part of the dried plant material was ground to a fine powder and analysed for isotopic ratio by an isotope ratio mass spectrometer (IRMS- ThermoQuest Finnigan DELTA plus). Root samples to study root parameters were taken at the first and second harvest, using a root auger (10 cm diameter, 30 cm deep) in two replicates per plot, one in the row and one between the rows. Root dry weight was calculated as weighed means from the in and between rows of dried roots. The roots were subsequently separated from the soil by a hydro- pneumatic elutriation system (Gillison´s Variety Fabrication Inc., USA) through a sieve with a 760 µm mesh.

AM Colonization (%): Usually, vesicular arbuscular mycorrhizal roots are observed in the upper 0-30 cm of the soil profile. In order to evaluate AM colonization in lucerne roots, lateral roots were separated and stained with an ink-vinegar staining according to Vierheilig *et al.* [23]. For quantification of root colonization by AM fungi, the gridline intersection method was used [24].

Estimation of BNF and Total Nitrogen Fixation by Lucerne Using 15_n Dilution Method: In this experiment BNF was estimated by the ^{15}N dilution method. The earliest application of $^{15}N_2$ in N_2 fixation studies was done by Burris and Miller [25]. This method can be used to provide direct evidence for N_2 fixation because the ^{15}N concentration in N_2 fixing and non-fixing plants differs according to the $^{15}N/ ^{14}N$ ratio of their N source.

In a preliminary work at the experimental site, it was found that the N-value of the plant-available soil N pool is below 5 %. The BNF and total nitrogen fixation were therefore estimated using the ^{15}N dilution method [26]. In order to more accurate evaluation of BNF, for each plot of lucerne treatment, a plot of mixed grass considered in this trial as the reference crop. The soil was labelled with ^{15}N by applying 0.1 kg ^{15}N ha^{-1}as 1 kg potassium nitrate ha^{-1} with 10 % ^{15}N at the beginning of the vegetation period in April 2007 for all Lucerne and grass plots at the same level.

Both the legume and the reference crop (grass-mixture) were grown on the ^{15}N-labelled soil. The percentage of legume N content derived from the air (N_{dfa}) was calculated using the isotopic differences between the two crops according to McAuliffe *et al.* [27].

$$N_{dfa} = [1-(\text{atom \% } ^{15}N \text{ excess}_{\text{legume}} / \text{atom \% } ^{15}N \text{ excess}_{\text{reference crop}})] * 100\%$$

$$\text{atom\% } ^{15}N \text{ excess} = \text{atom\% } ^{15}N_{\text{legume or reference crop}} - 0.3663$$

The Amount of N from BNF Calculated as Follows:

N_{fix} total [kg ha^{-1}] = N_{dfa} x N content x DM yield [kg ha^{-1}]
N_{fix} total = Amount of N from BNF, sum of N_{fix} at harvest 1 and 2 (= seasonal N fixation value)
N_{dfa} = N derived from the atmosphere in % of the legume plant

N content = N content in % of legume shoots at harvest 1 + 2 and of legume stubbles and roots at harvest 2 DM yield = Dry matter yield in kg ha^{-1} of legume shoots at harvest 1 + 2 and legume stubbles and roots at harvest 2.

Relative Field AM Dependency: The relative field mycorrhizal dependency (RFMD) was defined as the degree to which a plant responds to mycorrhizal inoculation [28] and it was calculated according to the formula:

RFMD (%) = [(shoot dry weight of mycorrhizal plant - shoot dry weight of non-mycorrhizal plant) / shoot dry weight of mycorrhizal plant] x 100.

RESULTS AND DISCUSSION

At the first harvest, effect of double interaction of rhizobium×irrigation on shoot dry weight varied from 4158 to 5629 $kgha^{-1}$ and R0I0 resulted in the lowest amount. At the first harvest, rhizobium inoculation increased shoot dry weight only in non-irrigated treatments (Fig. 2), indicating a compensating effect of rhizobium inoculation on water limitation but in the second harvest R0I1 and R1I1 with 3853 and 3876 kg ha^{-1} shoot dry weight resulted in the highest amount. Results show that effect of irrigation was stronger than rhizobium to increase shoot dry weight. Also, for the double interaction of AM×irrigation, treatments M0I1 and M1I1 resulted in the highest amounts at first harvest and M1I1 with 4088 $kgha^{-1}$ produced the highest amount in second harvest.

Shoot dry weight was higher at the first than at the second harvest because during this time higher temperature cause earlier flowering of lucerne with shorter growth duration. On the opposite, root dry weight increased from first to second harvest (Fig. 2). At the Rassdorf site, Pietsch *et al.* [29,30] found twice as much below ground biomass than above ground biomass yield. A possible explanation for that obviously is the reduced water availability to plants during the vegetation period. Plants can transfer assimilates from the shoot to the roots at water deficit conditions and the root system will be extended [31] meaning that plants allocate more carbon to their roots during stress [32-36]. The stubble yield was nearly equal in all treatments (evaluated only in the second harvest). This result seems reasonable, since the stubbles were harvested at a height of approximately 5 cm in all treatments.

AM colonization varied from 33 % in the non-inoculated treatment at harvest 1 to 66 % in the AM treated plots at harvest 2 (Fig. 3). AM inoculation significantly increased AM colonization at P= 0.05 and 0.01 at the first and second harvest, respectively (Tables 2 and 3).

At harvest 1, the effect of AM inoculation on mycorrhizal colonization was more pronounced on irrigated than on non-irrigated plots (Fig. 3). This can be very favourable for higher water and nutrient uptake

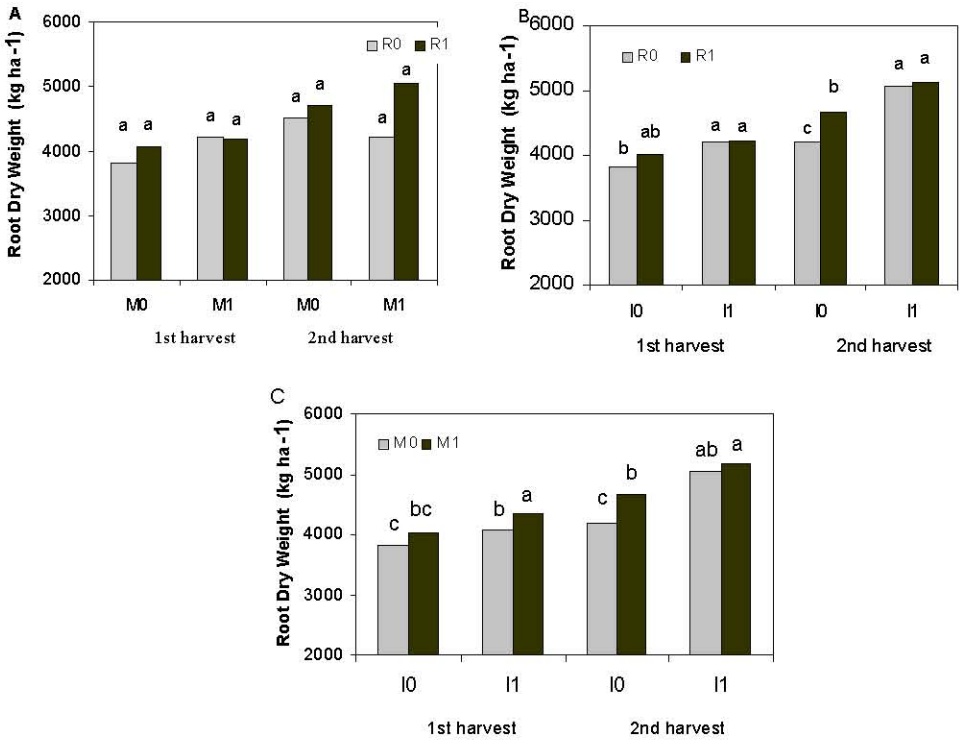

Fig. 1: Mean comparisons of double interactions for root dry weight at first and second harvest by Duncan's multiple range test. Means with the same letter are not significantly different

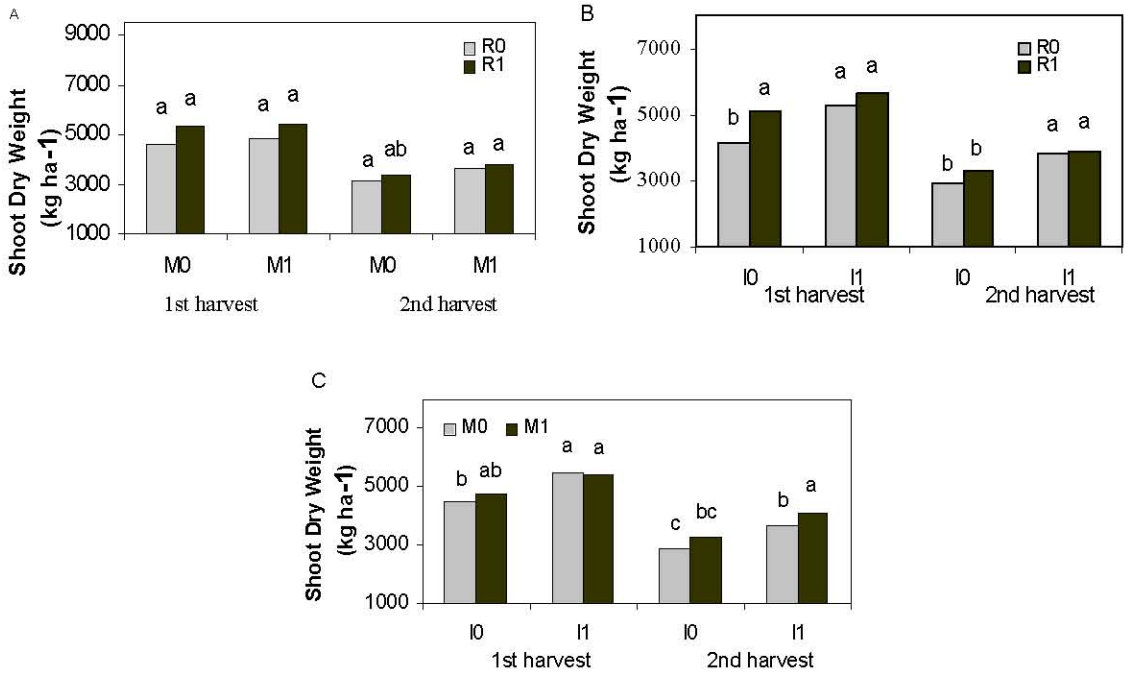

Fig. 2: Mean comparisons of double interactions for shoot dry weight at first and second harvest by Duncan's multiple range test. Means with the same letter are not significantly different

Table 2: Analysis of variance for shoot dry weight (SDW), mycorrhizal colonization (MC), root dry weight (RDW) at first (09.07.2007) and second (20.09.2007) harvest , total fixed nitrogen (Nfix total) and nitrogen derived from atmosphere (Ndfa)

	Mean Square (MS)							
S.O.V	df	SDW_1	SDW_2	MC_1	MC_2	RDW_1	RDW_2	Nfix total	Ndfa
Replication	3	609210.92	403408.01	11.76	9.623	337.00	595.00	1729.00	46.77
Rhizobium (R)	1	3463159.30 *	299828.32 ns	21.61 ns	58.401 ns	994.58 ns	4974.28 ns	7871.86 ns	17.11 ns
Mycorrhiza (M)	1	99750.34 ns	1313212.69 *	64.64 *	934.30 **	4645.99 *	7510.17 *	4587.00 ns	0.63 ns
Irrigation (I)	1	5287849.56 **	4822453.32 **	0.47 ns	108.89 ns	6683.41 **	36904.33 **	13153.74 ns	0.024 ns
R × M	1	22729.25 ns	25453.32 ns	0.11 ns	46.344 ns	1507.28 ns	130.85 ns	1202.55 ns	90.99 ns
R × I	1	753703.44 ns	231625.19 ns	3.03 ns	535.22 **	658.66 ns	3575.41 ns	4796.67 ns	16.71 ns
M × I	1	279759.48 ns	14556.44 ns	21.42 ns	76.973 ns	116.28 ns	1867.65 ns	2535.78 ns	211.85 ns
R × M × I	1	49148.96 ns	657661.13 *	0.70 ns	598.840 *	829.87 ns	95.32 ns	215.11 ns	97.02 ns
Error	21	493882.05	176815.89	8.98	58.653	455.00	1335.00	3807.00	59.366
CV	-	13.92	12.09	8.58	12.75	5.24	7.67	23.79	16.09

* and **: Significant at 5% and 1% Probability ns: non- Significant

Table 3: Mean comparisons of main effect of Irrigation, Mycorrhiza and Rhizobium for evaluated parameters

	SDW (kg ha^{-1})		MC (%)		RDW (kg ha^{-1})			
Treatments	H1	H2	H1	H2	H1	H2	Ndfa (%)	Nfix total (kg ha^{-1})
Rhizobia								
-	4718.40	3379.22	34.11	58.68	4012.00	4639.61	51.42	247.88
+	5376.34	3572.81	35.75	61.38	4123.50	4888.97	52.89	279.25
Mycorrhiza								
-	4991.53	3273.44	33.51	54.63	3947.26	4611.09	52.30	251.60
+	5103.20	3678.59	36.35	65.43	4188.24	4917.49	52.02	275.54
Irrigation								
-	4660.862	3087.81	35.05	58.20	3923.23	4424.7	52.13	243.30
+	5453.87	3864.22	34.81	61.88	4212.27	5103.89	52.18	283.84

H_1: Harvest 1 (09.07.2007); H_2: Harvest 2 (20.09.2007); -: control; +: treated; SDW: shoot dry weight; MC: mycorrhizal colonization; RDW: root dry weight; Ndfa: nitrogen derived from atmosphere; Nfix total: total nitrogen fixation

under stress conditions. At both harvests, the effects of AM on the non-irrigated plots were similar to the non-inoculated and irrigated plots. This indicates that AM and irrigation similarly affected AM colonisation.

The rhizobium × irrigation interaction was significant at P= 0.01 only at the second harvest (Table 2). Rhizobium inoculation increased AM colonization on non-irrigated plots and had the opposite effect on irrigated plots (Fig. 3). The triple interaction of rhizobium × AM × irrigation also was significant (Table 2). The AM colonization in treatment R1M1I1 was higher than in all other treatments at both harvests. Data show that, at both harvests, the effect of AM with irrigation (R0M1I1) was stronger than the effect of rhizobium with irrigation (R1M0I1) (Fig. 4).

There were only few significant differences between treatments. The main effect of rhizobium at the first harvest, the main effect of AM at the second harvest, the main effect of irrigation at both harvests and the triple interaction of rhizobium × AM × irrigation at the second harvest were significant (Tables 2 and 3). It shows that broadcast of fungal mycelia ameliorate the absorption of nutritional elements by root system and through this process AM create an interaction with internal tissues of root system producing additional water absorption system. In the second harvest the amount of root weight was 3148 to 3747 $kgha^{-1}$ and in both harvests R0M0 resulted in the lowest amount. In first harvest all the treatments were in the same group but in the second

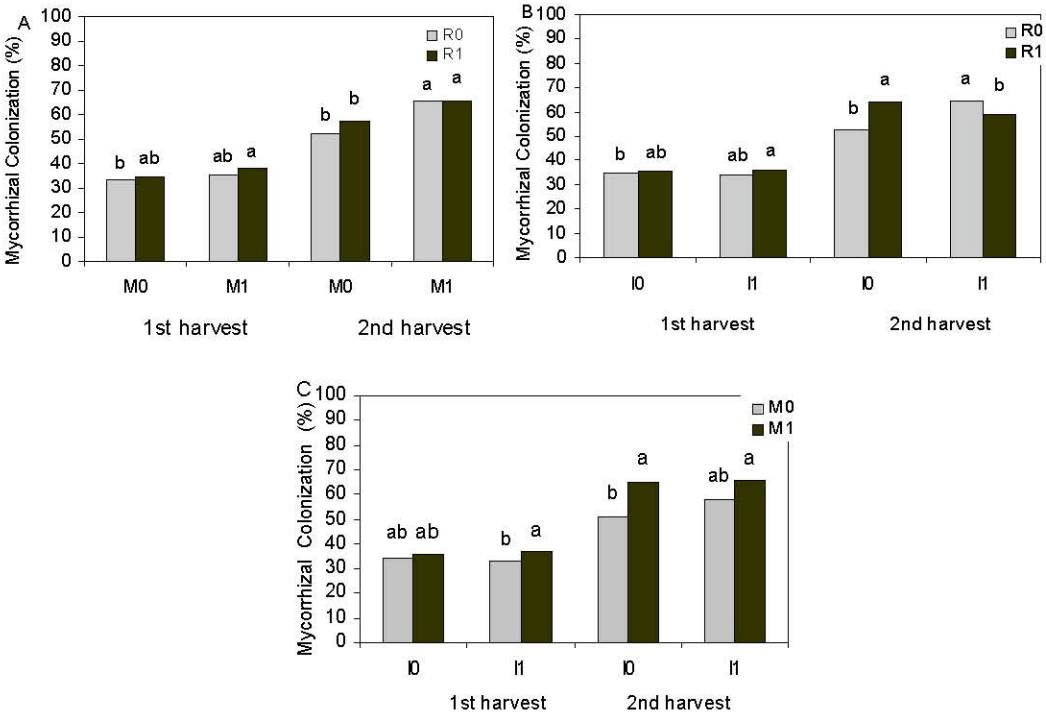

Fig. 3: Mean comparisons of double interactions for mycorrhizal colonization at first and second harvest by Duncan's multiple range test. Means with the same letter are not significantly different

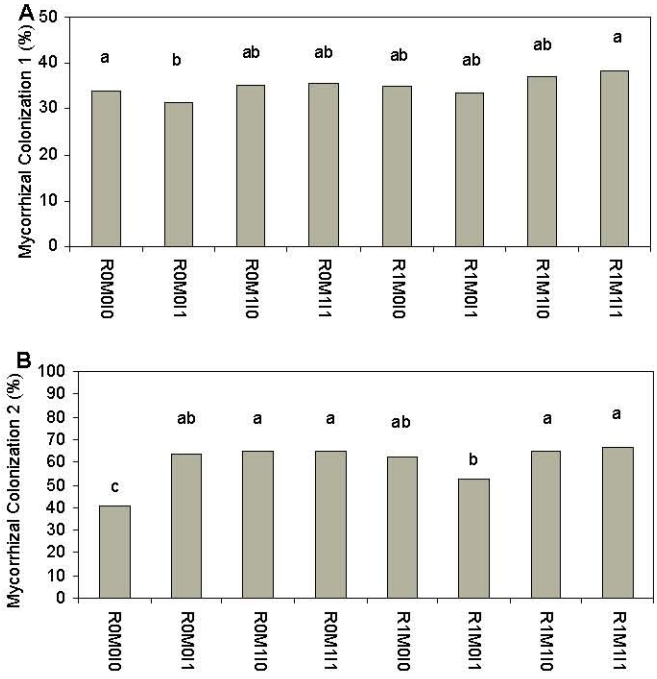

Fig. 4: Mean comparisons for triple interactions of treatments on mycorrhizal colonization at first and second harvest by Duncan's multiple range test. Means with the same letter are not significantly different

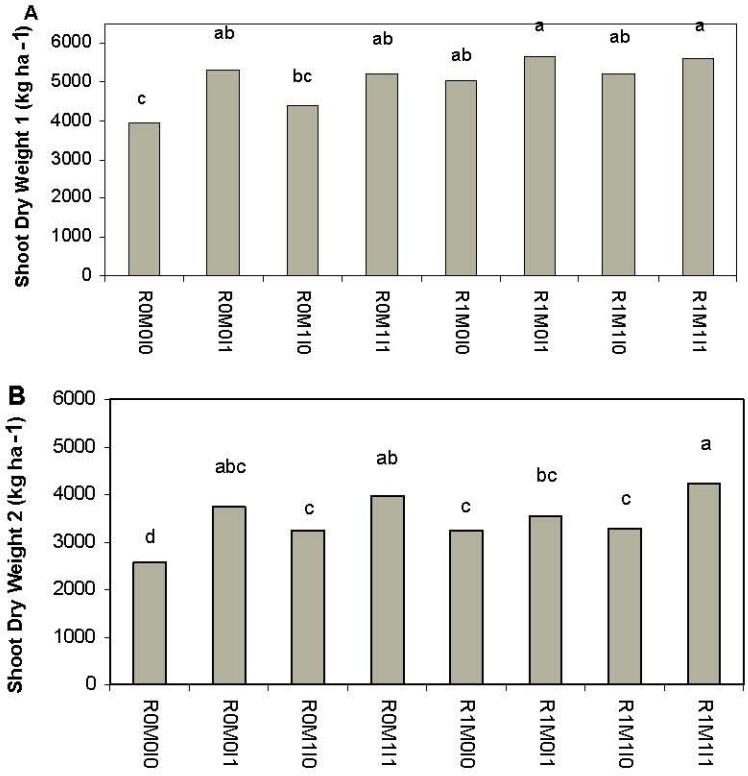

Fig. 5: Mean comparisons for triple interactions of treatments on shoot dry weight at first and second harvest by Duncan's multiple range test. Means with the same letter are not significantly different

harvest treatments R0M1 and R1M1 resulted in higher relative to the other treatments, producing the amount of 3610 and 3747 kg ha^{-1} yield, respectively.

Effect of irrigation was stronger than AM on shoot dry weight. In the triple interaction of rhizobium x mycorrhiza x irrigation, R1M0I1 and R1M1I1 produced the highest amount at first harvest. Mean comparison of R0M1I1 (5228 $kgha^{-1}$) and R1M0I1 (5655 $kgha^{-1}$) shows that the interaction effects of rhizobium and irrigation on shoot dry weight was stronger than the interaction effect of AM and irrigation at the first harvest. But at the second harvest, shoot dry weight in R0M1I1 (3961 $kgha^{-1}$) was higher than R1M0I1 (3537$kgha^{-1}$) (Fig. 5). Obviously, plants in symbiosis with a more developed mycorrhizal mycelium during longer time were able to absorb more water and minerals from soil and hence produced more dry matter.

Root dry weight was on average 4000 kg ha^{-1} at harvest 1 and 4500 kg ha^{-1} at harvest 2. It increased from harvest 1 to harvest 2 because of root growth (Fig. 1a, b, c). AM inoculation and irrigation significantly increased root dry weight at both harvests (Tables 2 and 3). *S. meliloti* inoculation slightly increased root dry weight at the first and second harvest (Table 3), but not significantly (Table 2).

AM inoculation increased root dry weight . Efficient water uptake is an important determinant to drought stress. Water uptake depends on root size (length or mass), activity and spatial distribution. Therefore, extensive deep rooting often has been emphasized in relation to drought resistance [37,38]. Root development and distribution in soils are important for root-water and nutrient uptake studies in soil plant systems [39]. In this way, AM inoculation may alleviate drought stress by enhancing water and nutrient uptake similar to the alleviating effects of AM on plant growth under saline conditions [40].

AM inoculation did not affect lucerne root growth upon rhizobium inoculation significantly (Table 2). Still, root dry weight was higher in the *S. meliloti* and AM treatment, relative to the control treatment at harvest 1 (Fig. 1), indicating a slight positive interaction.

Neither rhizobium inoculation nor rhizobium x irrigation interaction affected root weight significantly

(Table 2). But increased root weight in rhizobium treated plots versus control plots on the non-irrigated plots at harvest 2 (Fig. 1) showed a positive rhizobium inoculation effect only under non-irrigated conditions.

The amount of total fixed N evaluated at the end of the experiment showed no significant effect of treatments and their interactions. Still, the main effect of using rhizobium and AM inoculums and irrigation increased the amount by 31, 24 and 41 $kgha^{-1}$, respectively. Although all of treatments in double and triple interactions were at the same group (a), using AM could slightly increase total N fixation. In general, results from many studies have shown that dual inoculation with AM and rhizobia increases plant growth and N_2 fixation to a greater extent than single inoculation [41-43]. As a consequence of the largest number of studies already available, it is commonly accepted that there is a mutualistic tripartite symbiosis between AM, rhizobia and legumes.

Response of plants to inoculation with AM differed markedly with respect to functional compatibility, measured as AM formation, root colonization, external hyphal length, relative mycorrhizal dependency, hyphal P transport and P concentrations in shoots [44,45]. Consideration of RFMD for plants is one of the most important factors determining the magnitude of benefits from improved management of mycorrhiza [46,47]. RFMD is often related to the different morphological properties of plant roots and is regulated by the effectiveness of the AM and P availability in the soil . It is therefore useful to determine whether or not a plant derives or does not benefit from AM symbiosis and to know how to manage it accordingly. RFMD for shoot dry weight in first and second harvest, nitrogen derived from atmosphere (Ndfa) ,Total fixed nitrogen (Nfix Total) and nitrogen yield was 1.07%, 9.30%, 5.67%, 12.17% and 6.88%, respectively. These results show that using mycorrhizal inoculant could increase mentioned parameters scientifically but from economic view using mycorrhiza in this condition was not very reasonable. These results show that AM inoculants significantly increase different parameters related to plant growth and production under dry conditions when in a tripartite symbiosis with *S. meliloti*.

CONCLUSION

Using co-inoculation of mycorrhiza with rhizobia increased shoot dry weight at the first and second harvest, N derived from atmosphere, total fixed nitrogen and nitrogen yield. Irrigation increased some of the growth parameters. Results of AM colonization and total N fixation showed a high level of natural mycorrhization and BNF in the experimental field (33.51%-54.63% and 51-248 kg ha^{-1}, respectively). Microbial activities in this organically managed field were good enough for establishing an effective symbiosis with lucerne under moderately dry conditions without a necessity for inoculation. Also, according to the results of Fig. 5 A&B through providing enough water by irrigation the production would be increased by the participation of native soil microorganisms in Rassdorf organic fields.

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