ADÜ ZİRAAT DERG, 2017;14(2):69-73 — doi: 10.25308/aduziraat.323026 Araştırma / Research

The Usage of Native Arbuscular Mycorrhizal Fungi (AMF) in Drought Areas and Low–Input Crop Production Systems

Bülent BUDAK[*1], **Mohammad Ali KHALVATİ**[2], **Şükrü Sezgi ÖZKAN**[3]

[1]*Ege Üniversitesi Ödemiş Meslek Yüksekokulu, İZMİR.*

[2]*Boğaziçi Üniversitesi, Çevre Bilimleri Enstitüsü, İSTANBUL.*

[3]*Ege Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü, İZMİR*

Abstract: With increasing interest in the development of sustainable agriculture in semi–aird regions, low input land use systems are enhanced to be considered as an option on low production site. Amount of soil losses, erosion is an environmental disaster in marginal lands throughout the world. Greatly efficient rhizosphere microorganisms like VAM (vesicular arbuscular mycorrhizal) are of highly importance for sustainable agriculture. They could supply unavailable soil nutrients and create formation of micro aggregates. Also they chemically enmesh and stabilize micro aggregates and smaller macro aggregates into macro aggregate structures. The majority of agricultural activities in Turkey are exposure to nutrient deficiency and soil erosion. Progressing of this problem is in relationship with poor cover of low–input sites with vegetation at drought conditions. Our goal was to apply VAM for low–input vegetation in terms of improving soil nutrients supply and protect soil structure stability. Trap cultures provide a non–molecular approach to baiting cryptic species of VAM present in plant communities. Diversity of arbuscular mycorrhizal fungi in selected habitats using trap culture methodology. In trap culture, we will study the rate of root colonization by VAM as highest and lowest inocula or capability of selected VAM species to symbiosis with other soil bacterial species to nutrients supply and soil aggregation in low–input sites. Based on this situation, it is purpose of this study to combination of classical and molecular methods in order of elucidate VAM species with important soil nutrition and structure stability.

Keywords: *biofertiliser, low–input agricultural system, mycorrhiza, soil*

Arbusküler Mikorizal Mantarların Kurak Bölgelerde ve Düşük Girdili Bitkisel Üretim Sistemlerinde Kullanımı

Özet: *Yarı kurak alanların kullanımı ve sürdürülebilir tarım uygulamalarının geliştirilmesine son dönemlerde yaygın olarak çalışılmaktadır. Bu bağlamda; düşük girdili zirai alanların geliştirilmesi kısıtlı alanlarda zirai üretim yapılabilmesi için uygun bir seçenek haline gelmektedir. Kaybedilen toprak alanlarının, erozyonun değişik karakteristik özelliklere sahip topraklar dahil olmak üzere dünyanın her yerinde rastlanabilen bir çevre felaketlerinden biri olduğu aşikardır. Sürdürülebilir tarım uygulamaları için; veziküler arbusküler mikorizal mantarları (VAM) gibi oldukça verimli olan rizosfer mikroorganizmaları önem arz etmektedir. Bu mikroorganizmalar bitkilerin bünyelerine alamayacak oldukları topraktaki besin maddelerini bitkilerin kullanabileceği mikroagregatların oluşmasını sağlamaktadır. Bunun yanı sıra kimyasal olarak makroagregatların parçalanmasını sağlayarak, yeni oluşan yapıda stabilize edebilmektedir. Türkiye'de otlaklar ve zirai alanların çoğu besin maddeleri bakımından yetersiz ve/veya erozyona maruz kalmaktadır. Düşük girdili alanların yetersiz bitki örtüsü ve kuraklık koşulları arasındaki ilişki ile bu sorunlar zamanla artmaktadır. Amacımız, bu kapsamda veziküler arbusküler mikorizal mantarların düşük girdili sistemlerde uygulanması ve toprak besin maddelerinin zirai amaçla daha etkili kullanılması, ayrıca kuraklık koşulları altında toprak yapısını korumak ve araziyi stabil hale getirerek sürdürülebilir tarım yapılmasına olanak sağlamaktır. Seçilen habitatta arbusküler mikorizal mantarların çeşitliliği kültürlerin ayrılması metodu ile belirlenmiştir. Bu yöntemde, düşük ve yüksek dozdaki aşılamada mikorizal mantarların bitki köklerinde oluşturdukları koloni yüzdeleri kıyaslanarak mikorizal mantarların simbiyoz kapasiteleri, diğer toprak mikroorganizmaları ile etkileşimleri değerlendirilmiştir. Bu kapsamda, bu çalışmanın amacı klasik zirai yöntemler ile moleküler metodların entegrasyonunun, toprak besinlerinin etkili kullanılması ve toprak yapısının korunmasında veziküler arbusküler mikorizal mantarların etkisi ve önemini ortaya koymaktır.*

Anahtar Kelimeler: *biyogübre, düşük girdili zirai sistem, mikoriza, toprak*

INTRODUCTION

General Aspects

Almost a century has passed since the extensive analysis of the distribution of vesicular arbuscular mycorrhizal (VAM) symbiosis through the plant kingdom. Identification of the probability that VAM populations may be selected is very significant. It runs counter to the prevailing opinion, maybe basically based upon the low levels of speciation seen in the Glomerales. While it may be appropriate to use morphological organisms for the investigation of molecular or physiological processes, recently in ecosystem researches should be remote from the use of ecologically irrelevant genotypes obtained from cultural collections, toward selection of these isolated from the ecosystem being investigated.

VAM and Soil Aggregration

Most AMF fungi produce simple branched hyphae or hyphal networks in soil and extend the root systems of plants (Khalvati and Dincer, 2013). The stability of macroaggregates of several soils was related to the length of these hyphae in soil (Wehner *et al.*, 2014; Kiers *et al.*, 2011; Walder *et al.*, 2015; Zhang *et al.*, 2015). This hyphae produce extracellular polysaccharides to which microaggregates are attached and bound into stable macroaggregates by the network of hyphae (Wu *et al.*, 2014).

VAM and Soil Nutrients Allocation

VAM hyphae form characteristic structures including branched absorbing structures (BAS, formerly named arbuscule-like structures, ALS; Costa *et al.*, 2013) spore–associates BAS (Gopal *et al.*, 2012) and spores. The extra radical mycelia network increases the nutrient uptake root surface of the host plant and allows a more efficient extrication of phosphorus, nitrogen and certain micronutrients (Smith *et al.*, 2011; Smith and Smith, 2011). Recent field studies suggest that nitrogen (N) isotope signatures ($\delta^{15}N$) may reveal plant–mycorrhizal N dynamics. Some studies have suggested that mycorrhizal

***Sorumlu Yazar:** *bulent.budak@ege.edu.tr*
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transfer processes simultaneously created opposite isotopic patterns in mycorrhizal fine roots and foliage (Hobbie and Hogberg, 2012). $\delta^{15}N$ appears to be a particularly useful marker of plant–mycorrhizal N partitioning and could be used to examine species–specific responses to shifts in N supply (Pena and Polle, 2014). Concentration of glomalin is tightly correlated with aggregate stability across many soils (Rillig, 2004; Singh *et al.*, 2013).

VAM and Host Compatibility

In the past decades these various changes in rhizosphere conditions and their consequences for plant growth were intensively studied with continuously improved techniques and well documented in the relevant literature. Nowadays it will be a great challenge to apply this accumulated knowledge to manipulate rhizosphere conditions by innovative techniques for a better plant growth and plant health. Therefore there will be an increasing demand for an adapted rhizosphere management under different site conditions including different climate, soil and management conditions in integrated and sustainable systems with production of high quality food and resource–saving inputs and a minimum of environmental risks (Guo *et al.*, 2010). Many greenhouse studies have demonstrated benefit to plants hosts from (VAM) associations; it has been difficult to unequivocally demonstrate benefit to plants in the field (Fitter, 1991; Gianinazzi *et al.*, 2002; Hamel and Strullu, 2006; Faye *et al.*, 2013).

The goals of this study as following:

a) The aims of the scientific studies are;

- Search and identification of unknown VAM species in plants that highly aggregate soil in the Mediterranean climate.
- Screening tests of indigenous VAM isolates for their impact on improving field crops production in local low nutrient sites in Turkey in contribution with plant growth promoting rhizobacteria (PGPR).
- Screening and validation of erosion ameliorating effects of AMF under local field conditions.
- Affectivity of indigenous AMF appropriate for the given edaphoclimatic conditions of the specific field sites.
- Screening of German indigenous AMF and comparison with Turkey indigenous VAM species beneficial for sustainable management of agriculture.
- Improvement of soil structure stabilities.
- Application of VAM to allocation of nutrients on high–input agriculture soil
- Testing the efficacy of indigenous VAM under different soil climate conditions.

Table 1. Conceptual frame of field, greenhouse and laboratory studies

Work Package	Task	Outcome
Soil analysis	Soil sampling from target sites	Analysis of soil pH, nutrient content
Host plant assay	Plant morphological and physiological analysis	
Mycorrhizal assay	Soil and plant mycorrhizal analysis	
Plant microbe interaction assay	Experimental application on mycorrhizal affects	

Figure 1. The locations of low–input and high–input agricultural sites in this study

b) Scientific goals: To improve soil–structure stabilities and improvement of soil nutrients supply in the Mediterranean area for protection against soil erosion and improve crop production in low-input agricultural activities of Turkey.

MATERIALS and METHODS

Targets Sites

The work schedule is described in Table 1. The study in this project was located in:

- Bogazici University, Institute of Environmental Sciences, Istanbul.
- Agricultural activities sites (low and high–production sites): Bornova and Odemis/Izmir location of field research stations.
- Low–input pasture lands (low and high–production sites): Bergama/Izmir and Soke/Aydin.
- Agriculture area (low and high–production sites): Bornova-Odemis/Izmir.
- The experimental sites: Bornova-Izmir and Odemis/Izmir.

Including 18 fields of low–input and high–input agricultural sites in Turkey (Figure 1):

- Bornova/Izmir and Odemis/Izmir, 6 fields in the low and high–input sites.
- Bergama/Izmir, 6 fields in the low and high–input sites.
- Soke–Aydin, 6 fields high soil erosion sites.

The host plants in fields were selected with commercial grasses and as like as Barley as commercial spices using in Turkey.

Milestone A: Soil–sampling and elemental analysis measurements (field and greenhouse between 2014 and 2016):

Soil sample points were taken at intervals of 5 cm from each selected field. A sampling depth was determined as 15 cm. AMF propagate densities are located in the first 15 cm of the soil. Six cores (2.5 cm diameter x 15 cm deep) were collected from each sample point. First three cores were used for bioassay and were placed directly into plastic growth tubes. The remaining three cores were used for analysis of spore population, content of soil P, K and pH. The soil sample for a second bioassay was collected in May 2015. For this purpose, a total of 15 cores (five cores from each of the three sample points and 6 cm diameter x 10 cm deep) was collected from each selected field.

Milestone B: Host plant–sample and preparation of indigenous AMF–host plant & description and identification of AMF species (between 2014 and 2016):

- Staining roots with chlorazol black E (CBE) or trypan blue.
- Sample storage and slide preparation.
- Development of molecular markers: Using VAM and spores isolated from trap cultures material from single–spore cultures belonging to species of selected VAM.

Milestone C: Spore population (greenhouse between 2014 and 2015):

Structure and diversity of VAM communities was surveyed either directly on spores isolated from the field soil or on spores isolated from trap cultures, planted with different host plants. Spores was extracted, counted, and identified from composite soil samples collected from each of these sample points along 18 field sites. Spores were extracted from 25 ml aliquots of soil by wet- sieving followed by sucrose centrifugation based on the previous methods (McGraw and Hendrix, 1984). An aliquot of each soil sample was air dried, and bulk density was determined. Total spore counts will expresses as spores per gram dry soil. The relative abundance (%) of species at each site was calculated by the Shannon-Weaver index. The time of sampling: October 2014 and May–October 2015. The sampling includes plant fresh roots. To identify of AMF species in root samples, the samples was sent to Bank Europe Glomus (BEG).

- To identify of VAM species in root samples: The samples was sent to Bank Europe Glomus (BEG).
- Assessment in a greenhouse bioassay: The soil was placed in 2–3 kg pots with several replicates and roots was planted both in sterilized and unsterilized soil. In additional pots with untreated soil was inoculated with isolated fungal. A condition for this methodology is the simultaneous evaluation of several AMF populations to obtain a relative estimation for each indigenous population.

Milestone D: Selection of AMF for field application (greenhouse between 2015 and 2016): Selected AMF species used for management of sustainable agriculture should have especial features such as: Sufficiently symbiosis rate with crops on high–input agricultural sites, environmental adaptability with the barley, compatibility with the grasses, positive effects on growth of barley and total yields under harsh conditions.

Method: Growing of VAM with a living host plant (clover and plantago) in soil pot culture usually propagates Glomeralean (VAM) fungi. These pot cultures, which consist of soil, spores, root pieces and hyphal fragments, can be used as inoculums for experiments or to introduction of fungi into plots.

Milestone E: Testing of AMF–Plants (Field and greenhouse between 2015 and 2016):

In this study, application of VAM are planned in the experimental sites at Odemis in Turkey was prepared to testing of AMF plants to improvement reproduction, and soil aggregation (soil–structure stabilization).

Method and intensity of field preparation: At the beginning of the season, the experimental area was prepared with a moldboard plow followed by disking. Composite soil samples were taken to a depth of 30 cm and were analyzed for major soil properties and indigenous AMF fungal spores (see milestone A). Plot dimensions were about 20 m^2 (2m x 10m) with four rows in each plot. Nitrogen was applied on all plots and incorporated below the soil surface at a rate of 75 kg N ha^{-1} as NH_4NO_3. No phosphorous was added to the plots in order to maximize the mycorrhizal benefit.

Experimental design: In the field experiments, there were three replicates. Treatments including, three AM fungal species and two cultivars of selected crop here barley like cultivation procedures such as mounding, deep ripping or trenching may be used to prepare soil for our crop.

VAM fungal treatments include of inoculation or no inoculation with selected VAM. Before planting, mycorrhizal inoculum was uniformly distributed in the furrows opening at a depth of 7–10 cm and a spacing of 0.25 m throughout the whole plot. The VAM fungus fungi inoculum placed in the furrows beneath the seeds were lightly covered on the day of planting. The inoculum consisting of VAM–colonized root pieces, spores and hyphae were mixed with soil. No inoculum application was made to the control plots (Al-Karaki *et al.*, 2004).

Milestone F: Trap culture, compatibility and inoculum potential, screening of VAM with respect to N and P acquisition:

Plots layout to employ of trap culture, compatibility & inoculum potential, screening of AMF plants with respect to N and P acquisition were conducted at the experimental site in Turkey. Testing of efficiency of well–known or isolated indigenous AMF fungus on plant growth promotion under conditions of low nutrient supply; comparing the growth promoting capability of those VAM with different Turkish grass varieties. Testing contribution of well-known or isolated indigenous VAM and their capacity to symbiosis with soil bacterial for transfer nitrogen assimilation to Turkey. Measurements of morphological parameters of crops: number of tillers per plant, shoot and root fresh weight and dry weight, shoot/root ratio were analyzed at the end of application.

RESULTS and DISCUSSION

Plant Growth and Biomass

At the end of this study we measured plant shoot weight as a result of biomass production. Results shows that VAM (mycorrhizal) plants provided higher shoot biomass in well–watered and drought conditions in compared to non–VAM. Plants grown in the drought circumstance revealed almost the same biomass in comparison with non–VAM plant under well-watered conditions (Figure 2). In the similar dry matter study researchers found higher dry matter in the corn plants (Koca and Erekul, 2016).

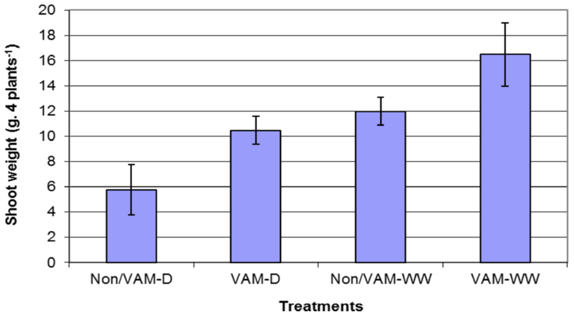

Figure 2. Interactive effect of VAM on shoot fresh weight in non-VAM and VAM plants under well–watered and drought conditions (error bar indicator standard division)

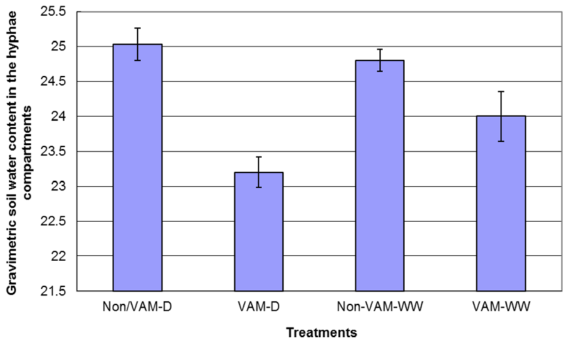

Figure 3. Hyphae compartment gravimetric soil water content of AMF changed during 90 days compared to non–VAM chambers under well-watered and drought conditions (error bar indicates standard deviation)

Soil Water Content

We determined also soil water content during 90 days of experiment. Therefore Figure 3 shows different water contents in soil around the plants with or without VAM inoculation. In this figure lower water content around the VAM plants rhizosphere are highlighted by comprising with non–VAM plants rhizosphere. These data illustrate water uptake up to 20% absorbed by VAM plants higher than non–VAM rhizosphere. These results are agreed with some recent studies where mycorrhizal funguses contribute plant water relations (Auge, 2001; Khalvati *et al.*, 2005; Ruth *et al.*, 2011).

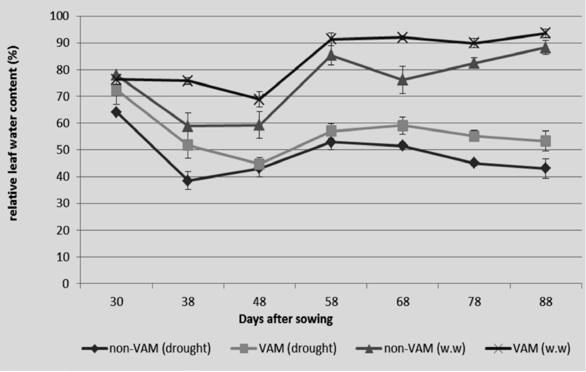

Figure 4. Interactive effect of VAM on relative leaf water content in VAM and non–VAM plant under well–watered and drought conditions (error bar indicates standard deviation)

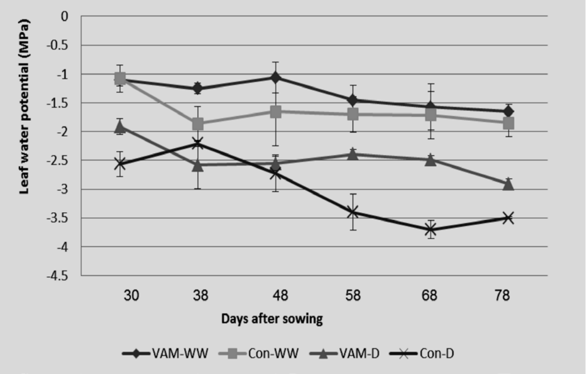

Figure 5. Interactive effect of VAM on leaf water potential in VAM and non–VAM plants under well-watered and drought conditions (error bar indicates standard deviation)

Relative Leaf Water Content

As we discussed plant rhizosphere water content in the previous discussion researcher were curious about the out–coming of plant water content at whole. Figure 4 shows a significant different leaves relative water content in the both VAM and non–VAM plants under well–watered and drought conditions while they were growing. The above results in Figure 4 reveal carefully the different between leaves water content in VAM and non–VAM plants. Furthermore, water content in VAM plants under drought conditions showed 10–15% higher than non–VAM plants. This can useful for photosynthesis and growth metabolisms during the harsh condition such as drought. These findings are in agreed with many recent researches results where mycorrhizal fungi uptake unavailable water for the plant inoculated with VAM (Abdelmoneim *et al.*, 2014; Rahimi *et al.*, 2017). The results of these scientists was showing to decrease in the properties of borage medicinal plant as a result of drought stress. But the application of mycorrhizal fungi could increase leaves water contents in drought stress conditions in the our study and decrease of the negative effects of drought stress.

Leaf Water Potential

VAM and non–VAM plants behave differently in terms of water pressure in leaves under drought conditions. Therefore, VAM plants were able to tolerate high water pressure during the water restriction. Figure 5 reveals significantly low water pressure in the VAM plants due to 1MPa at the highest value in compare to non–VAM plants. This result is in corresponding with high root mycorrhization of barley plants 60 days after sowing. The mycorrhization results shows 59% root inoculation in the VAM plants at the 60-78 days after sowing. VAM symbioses protect host plants against the detrimental effects of drought avoidance (Ruiz-Sanchez *et al.*, 2010; Li *et al.*, 2014). That kind of strategies was found in our VAM–plants under drought conditions.

Root Mycorrhization

Figure 6 is shown the root mycorrhiztion of barley plants associated with mycorrhizal fungus determined at the end of study. The observation approved present of mycorrhizal fungus and hyphal network in the VAM plants rhizosphere.

CONCLUSION

An overview of the results and data enable us to approach in the final discussion by remarking interesting role of VAM

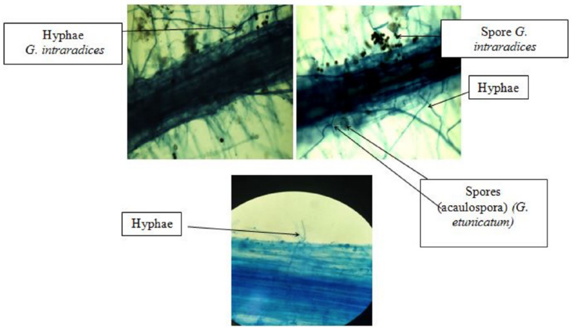

Figure 6. VAM–Plants root mycorrhization including hyphae and spores

in plants tolerance to climate hardness. This could be a short but intensive guideline for local researches and VAM applicants agricultural companies in order to understand beneficial impact of VAM on native crops. Climate changing and increasing concern on weather behaviour in Turkey might encourage indigenous people and agricultural companies.

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