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Growth response of maize plants (*Zea mays* L.) to wheat and lentil pre-cropping and to indigenous mycorrhizae in field soil

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Abstract

The presence of indigenous mycorrhizal fungi may have significant effects on the growth and on the root morphology of plants, under arid and semi arid soil conditions. Lentil and wheat are the traditional crops grown in Southeastern Turkey. In this study soil samples from the Harran plain were collected from the 0-15 cm surface layer under wheat or lentil crop residues and used in a pot experiment carried out under greenhouse conditions with four levels of P fertilization: 0, 20, 40 and 80 mg kg^{-1} soil as $Ca(H_2PO_4)_2$. Half of the soil batches were submitted to a heating treatment (80°C, 2 h). The maize variety PX-9540 was grown in the pots for 57 days. At harvest, plant dry weight, root length, P and Zn concentrations in plant tissues were measured and the extent of root colonization by arbuscular mycorrhizal fungi (AMF) was determined. Results showed that maize plants grown in soils where lentil had been previously cultivated grew better than those grown after wheat cultivation. In both cases, P concentration in plant tissues increased with increased P fertilization. There were no significant differences in root AMF colonization between soils with different crop sequences, nor with soils submitted to high temperature. Previous crops had a significant influence on the growth of plants that could be related to differences in the indigenous mycorrhiza inoculum potential and efficacy that can promote P uptake and benefit plant growth.

Additional key words: crop and soil management, crop rotation, P fertilization, temperature.

Resumen

Respuesta de plantas de maíz (*Zea mays* L.) a cultivos previos de trigo y lenteja y a la presencia de micorrizas nativas en el suelo

La presencia de micorrizas nativas puede tener efectos significativos en el crecimiento y la morfología del sistema radicular de las plantas especialmente en zonas áridas y semi áridas. Los cultivos tradicionales en el sureste de Turquía son las lentejas y el trigo. Se obtuvieron muestras de la capa superior de suelo de la llanura de Harran (0-15 cm de profundidad), después del cultivo de lentejas o de trigo, y se utilizaron en un experimento en macetas y bajo condiciones de invernadero, con cuatro niveles de fertilización fosforada (0, 20, 40 y 80 mg kg^{-1} suelo). La mitad de cada muestra compuesta de los suelos se sometió a un tratamiento de calor (80°C, 2 h) y se cultivó la variedad de maíz PX-9540 en los contenedores durante 57 días. Se determinaron los siguientes parámetros: materia seca, colonización de raíz por hongos formadores de micorrizas arbusculares (HMA), longitud de raíz, y concentraciones de P y Zn en las plantas. Crecieron mejor las plantas de maíz cultivadas en suelos con un cultivo previo de lentejas que de trigo. En ambos casos, la concentración de P en las plantas se incrementó con cantidades crecientes de fertilización fosforada. No hubo diferencias significativas en la colonización por HMA de las raíces entre los dos suelos utilizados procedentes de cultivos diferentes y tampoco con el suelo sometido al tratamiento térmico. Se concluyó que los cultivos anteriores pueden tener un efecto en la cosecha siguiente, efecto que puede estar relacionado con diferencias en la eficacia y efectividad de las micorrizas nativas, que pueden contribuir significativamente a mejorar la absorción de P y el crecimiento de las plantas.

Palabras clave adicionales: fertilización con P, manejo de de cultivos y suelos, rotación de cultivos, temperatura.

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Abbreviations used: AMF (arbuscular mycorrhizal fungi), CEC (cation exchange capacity), SAP (Southeastern Anatolian Project).

Introduction

The Southeastern Anatolian Project (SAP, Turkish acronym GAP) is the largest irrigation and development project of Turkey covering about 2 million ha cultivated land. The Harran plain, also known as the 'Fertile Crescent', is located in the upper part of the Mesopotamian plain (Ortas *et al*., 1999) and has the main climatic characteristics of the eastern Mediterranean climate with a strong continental influence. The annual rainfall is about 400 mm with evapotranspiration reaching up to 2,000 mm. Rainfall is irregular and mostly falls in the winter. The SAP is a multi-sector development project that receives good-quality water from Atatürk Dam which is one of the world's largest dams (Çullu *et al*., 2009).

A number of field experiments in the region with different crops including maize (*Zea mays* L.), wheat (*Triticum aestivum* L.) and cotton (Gossypium spp.), have repeatedly shown that increasing fertilizer application not always results in a yield increase (Ortas, 2003). Early written evidence shows that the Harran plain has been cultivated since 10,000-4,000 B.C. Ancient agricultural practices illustrate that under non-irrigation regime, plant production depends on soil and crop management systems such as plant rotation and land fallow. Due to water deficiency and low precipitation, the present day main crops in the area are monoculture field crops such as wheat, barley (*Hordeum vulgare* L.) and lentil (*Lens culunaris* L.) (Ortas *et al*., 1999). Crop rotation, as a soil management system would most probably improve crop yields in the area enhancing the benefits from soil beneficial microbiota such as mycorrhiza.

Crop species, soil type, and management technologies can play a significant role in soil microbial diversity (Ortas, 2006). Li *et al.* (2007) found that the dominant species of arbuscular mycorrhizal fungi (AMF) were different in three habitats in China. The results of Su and Guo (2007) suggested that the AMF diversity is greatly affected by long-term over-grazing and that fencing of degraded areas partly restores plant cover and AMF diversity in grassland ecosystems.

In general, mycorrhizae will increase the uptake of plant nutrients that move from soil to plant by diffusion and thus play a critical role in nutrient cycling and ecosystem functioning (Kernaghan, 2005). They improve plant growth and survival through a mutualistic relationship that facilitate the access to water and nutrients. The benefits of AMF for the host have most often been ascribed to enhanced uptake of immobile nutrients from the soil, notably P, Zn, and Cu (Ortas *et al*., 2002; Ortas and Akpinar, 2006; Ortas and Warma, 2007). Mycorrhizal diversity can positively contribute to nutrient and water use efficiency (Brussaard *et al*., 2007). In the Harran plain, P and Zn deficiency has been identified as the major nutrition problem (Ortas, 2003).

The research results of Reynolds *et al.* (2006) suggested that the degree of AMF benefit to a plant host depends not only on AMF species, but also on the plant species and on the soil phosphorus availability. There is a general perception that P uptake by plants occurs as a direct consequence of uptake from the soil by root cells. However, in more than 90% of land plants, symbiotic associations are formed with mycorrhizal fungi (Smith and Read, 1997). In these plants the fungal hyphae play an important role in the acquisition of P and other nutrients for plant growth (Schachtman *et al.,* 1998).

Uhlmann *et al*. (2006) analyzed soil and root samples from three arid sites in southern Namibia, they recovered twelve AMF species whose presence was linked to the geographic origin of the samples and not to different management systems. Douds and Millner (1999) found that the study of AMF diversity in agricultural soils presents many challenges, including the difficulty in identifying field collected spores, the detection of non-sporulating members of the community, and the lack of correlation between functional and morphological features of the spores used to define the different species. Dhillion and Anderson (1993) showed that in sand prairies the abundance of spores was always significantly lower in burned than in unburned sites early in the growing season. Ortas *et al.* (2000) reported that soil and crop management can help to get maximum benefit from indigenous mycorrhiza for sustainable crop production.

The aim of this research was to examine the effect of two different cropping systems on the secondary planted crop. In this study, an experiment using containerized plants was conducted in order to determine the effect of indigenous mycorrhizae on maize grown in heated and non-heated soils that were collected from field sites previously cultivated with wheat or lentil crops.

Material and methods

Wheat and lentil are the main crops cultivated under drought conditions in the Harran Plain, which is the largest fertile region of Şanlıurfa, Southern Turkey.

Soil samples were collected from adjacent fields located on the Harran soil series which is a dominant soil type in the plain. In June, after harvest, the soil samples were collected from five different sites in lentil and wheat fields and from the 0-15 cm surface layer to establish a pot experiment. Immediately after collection the soil samples were analyzed and the soil physical and chemical properties determined. The soil had pH 7.5 (soil: H_2O 1:2.5), 26% calcareous (moderate to high), with a 58% cation exchange capacity (CEC) and 1.3% organic matter content (low), 0.25 mg kg^{-1} DTPA extractable Zn, and 42.8 kg ha^{-1} Olsen-P [0.5 mol L^{-1} sodium bicarbonate ($NaHCO_3$)-extractable]. The particle-size distribution (19% sand, 35% silt and 46% clay) classified the soil as heavy clay (Çullu *et al.*, 2009). Also 34 AMF spores g^{-1} soil were found in the soil sampled.

The soil moisture regime of the study site is xeric and the temperature regime is mesic (Soil Survey Staff, 1999). The Harran soils are classified as Vertisols according to Soil Taxonomy (Soil Survey Staff, 1999) and World Resource Base (ISSS/ISRIC/FAO, 1998) classification systems.

The wet sieving method described by Gerdemann and Nicolson (1963) was used to quantify the number of mycorrhizal spores. Half of the soils batches were submitted to a heating treatment (80°C for 2 h) and left for 2 weeks before sowing, in order to reduce the mycorrhizal inoculums of the native endophytes. The pot experiment carried out at the Çukurova University greenhouse followed a completely randomized factorial design with 3 replications. Soils were fertilized with four levels of P: 0, 20, 40 and 80 mg kg^{-1} soil as Ca $(H_2PO_4)_2$. Each container was filled with 2 kg of soil and received 100 mg kg^{-1} N (50 mg kg^{-1} $(NH_4)_2SO_4$ and 50 mg kg^{-1} KNO_3) at sowing and 100 mg kg^{-1} N twenty days after sowing. Also all the containers received 5 mg Zn as ($ZnSO_4$) and 40 mg kg^{-1} K (K_2SO_4). Five seeds of PX-9540 maize variety were sown per pot and after germination, two plants per pot were left and they were irrigated daily with deionized water. Plants were harvested 57 days after sowing. At harvest, plants were cut at 0.5 cm from the pot surface and plant dry matter, root length (Tennat, 1975), and P (Murphy and Riley, 1962) and Zn concentrations were measured and the percentage of mycorrhizal root colonization (Koske and Gemma, 1989) was determined.

The effects of the treatments on plant parameters were tested using ANOVA and data were analyzed by Statistical Analysis System (SAS Inst, 1987).

Results and discussion

Results showed that the development of maize plants in the soil where lentil had previously been cultivated was much better than in the soil where wheat had been harvested. The growth of plants in the heated soil was lower than in the non-heated soil. In fact, after the wheat harvest, there was a strong reduction in the maize dry matter production. In heated, lentil grown soil, the dry matter production of maize was 3.23 g pot^{-1}, while in

Table 1. Effect of P fertilization on maize growth in Harran soil submitted or not to heating treatment (80°C, 2 h), after wheat or lentil pre-cropping

$Ca(H_2PO_4)_2$ (mg kg^{-1} soil)	Shoot dry weight (g pot^{-1})		Root dry weight (g pot^{-1})		Shoot/root	
	Non- heated soil	Heated soil	Non-heated soil	Heated soil	Non-heated soil	Heated soil
After wheat						
0	3.62 ± 0.05	3.82 ± 0.07	1.70 ± 0.14	1.63 ± 0.03	2.13	2.34
20	3.96 ± 0.26	3.20 ± 0.05	1.73 ± 0.09	1.67 ± 0.09	2.29	1.92
40	3.86 ± 0.24	3.99 ± 0.09	1.93 ± 0.07	1.57 ± 0.06	2.00	2.54
80	3.55 ± 0.31	4.03 ± 0.06	1.85 ± 0.01	1.73 ± 0.10	1.92	2.33
After lentil						
0	4.58 ± 0.09	3.23 ± 0.04	1.65 ± 0.08	1.70 ± 0.12	2.78	1.90
20	5.36 ± 0.17	4.16 ± 0.03	1.78 ± 0.06	1.97 ± 0.14	3.01	2.11
40	4.57 ± 0.13	3.64 ± 0.19	2.30 ± 0.06	2.09 ± 0.19	1.99	1.74
80	4.20 ± 0.26	3.72 ± 0.26	2.37 ± 0.28	2.25 ± 0.10	1.77	1.65

Data are means of three samples ± standard deviation.

Table 2. Effect of P fertilization on mycorrhizal root colonization of maize plants in heated and non-heated Harran soil submitted or not to heating treatment (80°C, 2 h) after wheat or lentil pre-cropping

$Ca(H_2PO_4)_2$ (mg kg^{-1} soil)	Root colonization (%)		Root length (m pot^{-1})	
	Non-heated soil	Heated soil	Non-heated soil	Heated soil
After wheat				
0	65.3 ± 4.7	45.2 ± 4.6	42.23 ± 11.71	56.20 ± 12.27
20	72.1 ± 16.8	30.8 ± 8.2	46.62 ± 2.27	61.88 ± 36.62
40	62.8 ± 3.1	31.6 ± 12.7	55.62 ± 7.42	49.72 ± 12.22
80	61.5 ± 1.4	25.6 ± 2.8	68.84 ± 5.93	71.40 ± 17.17
After lentil				
0	68.4 ± 3.6	20.1 ± 4.2	51.19 ± 22.56	63.32 ± 17.01
20	67.9 ± 12.2	47.9 ± 22.9	54.41 ± 20.48	111.58 ± 26.63
40	39.6 ± 18.2	36.2 ± 3.9	84.21 ± 25.72	120.02 ± 28.00
80	55.8 ± 14.1	50.3 ± 7.8	73.50 ± 11.70	97.97 ± 41.26

Data are means of three samples ± standard deviation.

non-heated soil it was 4.58 g pot^{-1} dry matter. In heated, wheat grown soil the harvested plant dry matter was 3.62 g pot^{-1} whilst in non-heated soil the harvested dry matter was 3.82 g pot^{-1} (Table 1).

Similarly, the maize plants grown in the sites where wheat and lentil had previously been cultivated were significantly different in terms of shoot and root growth. Plants grown after a lentil crop had higher shoot: root ratio than those grown after a wheat crop (Table 1).

Ortaş *et al.* (2000) reported that indigenous mycorrhizal fungi were present in the same Harran soil. Ortas (2003) used the same sterile and non-sterile soil and found that indigenous mycorrhizae colonized maize plants and significantly increased plant growth. Although there was a significant root colonization in heated soils, the mycorrhizal colonization was higher in the non-heated soils. Root colonization data showed that the heating treatment applied was not enough to completely avoid mycorrhization by indigenous mycorrhizal propagules, however plants grown in non-heated soil showed up a higher percentage of root colonization by AMF than plants grown in heated soil (Table 2).

In both crop management systems, plants grown in heated soil had higher root length than in non-heated soil. In the lentil cultivated heated soil, root length of maize was higher than in the wheat cultivated heated soil. The different growth of maize obtained after pre-cropping with two different plant species in the same soil may be related to their different nitrogen dynamics. With heating, competition between soil organisms and plant roots is minimized, thus maize grown in heated soil had higher root length. Plants grown under a previous wheat crop had shorter root length than plants grown after a lentil crop, indicating that rotation effects may also alter root length and root colonization of subsequent crops. Bagayoko *et al*. (2000) showed that roots of cereals grown in rotation systems had higher early AMF infection rates compared to cereals grown as a continuous monocrop. However, Vestberg *et al*. (1999)

Table 3. Significance of F-values (probability) from analysis of variance for different plant parameters

Sources	DF	Shoot weight	Root weight	Root length	Root colonization	Shoot P	Root P	Total P content
Non-heated soil (N)	1	0.001	0.001	0.001	0.057	0.874	0.869	0.001
Heated soil (H)	1	0.001	0.119	0.004	0.041	0.022	0.018	0.001
Phosphorus level (P)	3	0.001	0.19	0.028	0.016	0.001	0.001	0.001
N*H	1	0.001	0.967	0.045	0.066	0.634	0.62	0.001
N*P	3	0.001	0.578	0.115	0.177	0.005	0.086	0.001
H*P	3	0.001	0.614	0.493	0.527	0.921	0.912	0.004
N*H*P	3	0.002	0.44	0.559	0.002	0.777	0.755	0.176

Table 4. Effect of different P fertilization levels on maize P concentration (%) in heated and non-heated soils

$Ca(H_2PO_4)_2$ ($mg\ kg^{-1}$ soil)	Shoot P (%)		Root P (%)	
	Non-heated	Heated	Non-heated	Heated
After wheat				
0	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01
20	0.20 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.01
40	0.23 ± 0.02	0.23 ± 0.01	0.23 ± 0.01	0.22 ± 0.01
80	0.24 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.01
After lentil				
0	0.18 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.17 ± 0.01
20	0.21 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.01
40	0.23 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.21 ± 0.01
80	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.01

Data are means of three samples ± standard deviation.

reported that the mycorrhizal diversity did not differ significantly between cropping systems.

The identity of the previous crop and the soil heating treatment were not statistically significant on mycorrhizal root colonization, but the addition of P significantly modified root colonization ($P = 0.016$, Table 3).

In the soils obtained from different previous crops, P concentration in plant tissues increased with P fertilization (Table 4). Phosphorus uptake by plants was calculated using P content and dry matter production per plant, when considering total P-uptake (Fig. 1), the difference between non-heated and heated soils was significant in soils previously cropped with lentils. In wheat pre-cropped soils, there were no significant differences although plant P uptake in non-heated soil was marginally higher than in the soil submitted to a heating treatment. Increasing P fertilization increased P uptake of plants grown in both wheat and lentil cultivated soils.

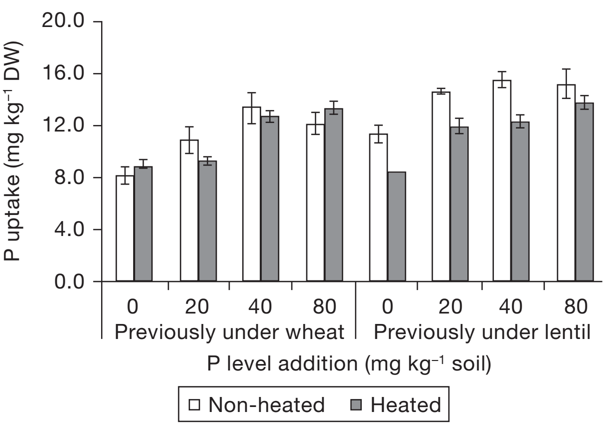

Figure 1. Effect of soil heating treatment (80°C, 2 h) on P uptake by maize plants grown in soils after wheat or lentil pre-cropping. DW: dry weigth.

The lentil grown soil had 60.4 spores g^{-1} soil and the wheat grown soil had 18.9 spores g^{-1} soil, The abundance of indigenous AMF spores in the lentil pre-cropped soil resulted in a higher AMF root colonization of the maize sown as the second crop. Moreover, lentil, as a nitrogen fixing legume plant, might have influenced the soil organic matter and nitrogen contents. Consequently, pre-cropping with lentil is beneficial for the second crop's development. Bagayoko *et al.* (2000) reported that soil N_{min} levels in the topsoil were consistently higher in cereal plots previously sown with legumes (rotation cereals) compared to plots under continuous cereal cultivation.

Our results agree with those of Kitt *et al.* (1988) and Ortas and Harris (1996) who found that soil heating treatments affect the indigenous mycorrhizal fungi and consequently the second crop performance. Previous crops have significant effects on the subsequent crop and this effects can be partly related to the indigenous mycorrhiza population. Ortas (2006); Ortas and Varma (2007) have shown that soil and crop management are important issues to consider to maximize benefits from indigenous mycorrhizae. Our results demonstrate that indigenous mycorrhiza can significantly contribute to plant growth and P uptake. We suggest that legume plant species, such as lentil, should be included in rotation systems, improving crop production with a smaller input of fertilizers, thus reducing phytochemical inputs and increasing the sustainability of the system.

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