Tripartite symbiosis of Lentil (*Lense culinaris* L.), Mycorrhiza and *Azospirillum brasilense* under Rainfed Condition

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Abstract

A field experiment was conducted aiming to determine the possibility of improving the lentil performance when co-inoculated with Vesicular Arbuscular Mycorrhiza (VAM) fungi and Azospirillum under natural rain-fed conditions, in Iran. Results showed the substantial impact of VAM fungi on grain protein, root colonization and shoot dry weight. Highest value for shoot dry weight recorded in plants which inoculated with G. intraradices and highest values for root colonization and grain protein content was observed in plants which inoculated with G. mosseae. Also, Azospirillum had a significant effect on shoot dry weight and root colonization. A significant differences on grain protein content observed when combination of both microorganisms have been used.

Introduction

Seed inoculation with *Azospirillum brasilense* and soil inoculation with the vesicular arbuscular mycorrhizal fungi have been long recognized as biofertilizer technologies. The classical mutualism relationship of arbuscular mycorrhizal fungi and a majority of crop roots plays a direct role in nutrient cycling rates and patterns in agroecosystems contributing markedly to nutrient capture and supply, water retention, alleviating the environmental abiotic stresses and improving the pathogen resistance resulting in improvements of crop health and yield in sustainable agroecosystems (Azcon et al., 1997; Annaduraia et al., 2002; Daei et al.,2009; Safapour et al., 2011; Ardakani et al., 2009, 2011; Zakikhani et al.,2012). Lentil (*Lens culinaris* Medik.), an old world grain legume food crop that was domesticated in the Near East and is broadly cultivated in semi-arid Mediterranean, south Asia, Indian sub-continent, south America and Iran. Water deficiency is a major limiting factor of crop yield in arid and semi-arid areas (Moghaddam et al., 2012). Iran has an arid or semiarid climates mostly characterized by low rainfall and high potential evapotranspiration. The average annual precipitation over the country was estimated to be around 250 mm, occurring mostly from October to March (Nazemosadat et al., 2006). So, shifting toward the efficient use of water and soil resources and implementing of the principles of sustainable agriculture are highlighted recently. Present paper examines the effects of application of biofertilizers in order to enhance the quantity and quality of yield of the lentil with the aid of mycorrhizal mutualistic and Azospirillum associative relationships under rain-fed conditions.

Material and methods

This field study was conducted in, Khalkhal, Iran, (37º 37′ N latitude, 48º 32′ E longitude and altitudes of 1796 m) on 2011-2012 growing season. The yearly average precipitation (30-years long term period) is 370 mm but the precipitation on this particular growing season has recorded as 230 mm by Khalkhaal synoptic meteorology institute. The treatments were arranged as factorial experiment based on randomized complete block design with 4 replicates. The experimental treatments consisted as follow:
--Mycorrhizal inoculation in 3 levels (M_0 = without inoculation, M_1 = *Glomus intraradices* and M_2 = *G. mosseae*) Mycorrhizaes with the population of 10^5 *spor.g*$^{-1}$
-- *Azospirillum* inoculation in 2 levels (A_0 = without inoculation and A_1 = inoculation with *A. brasilense*) with the population of 10^8 *cfu.g*$^{-1}$
-- Lentil cultivar in 2 levels (L_1 = large grain Mashadi and L_2 = small grain Naaz). Seeds were provided by Agriculture department of the province. Each plot was consisted of 6 rows, 25 cm apart. Distance between plots and replications were arranged to be 1 and 2 meters, respectively. No chemical fertilizers were applied during the course of experiments and weeds were eliminated with mechanical methods. For measuring percentage of root colonization root samples were taken and washed with FAA (Formalin Acetic Acid

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Alcohol) solution for further procedures. Philips and Hayman (1970) method was used for staining the roots. The gridline intersect method was applied introduced by Giovannetti and Mosse (1980) and results expressed in percentage.

Results

Mean comparison of treatments imparted significant differences between different levels of Mycorrhizal inoculations and application of *G. intraradices* resulted in the foremost value (115.78 g m^{-2}) while the non-inoculated treatments showed the least value (108.61 g.m^{-2}) for shoot dry weight. Mean comparison of treatments revealed that inoculation with Azospirillum substantially (2.8% more) affected the shoot dry weight (114.97 g m^{-2}) in comparison with control (111.73 g m^{-2}). Mean comparison of treatments imparted significant differences between different levels of Mycorrhizal inoculations as in application of *G. mosseae* resulted in the foremost percentage of root colonization (34.56%) and the inoculated treatments with *G. intraradices* showed (33.1%) of myco-colonization whilst the measured colonization in non-inoculated treatments recorded as (22.24%). Inoculation with Azospirillum increased the percentage of root colonization (30.30%) statistically significant over non-inoculated treatments. The maximum measured value (24.31%) for percentage of grain protein content was recorded in treatments with *G. mosseae* inoculations while the minimum value was (20.03%). Applications of Mycorrhiza and Azospirillum did not resulted in improvement of grain yield albeit the drastic decline in annual precipitation (230 mm) vis-à-vis the long term average (370 mm) might be the reasoned to be main cause. It can be assumed that application of biofertilizer under rain-fed and severe drought will not result in improvement of biological yield, but rather it aids the survival of stressed plant.

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