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Improvement of growth and yield of maize under water stress by co-inoculating an arbuscular mycorrhizal fungus and a plant growth promoting rhizobacterium together with phosphate fertilizers

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ABSTRACT

There is little information about the effect of arbuscular mycorrhizal fungi (AMF) and phosphate solubilizing bacteria (PSB) on maize productivity in the presence of sparingly soluble forms of phosphorus (P) under water stress in natural conditions. The aim of this study was to investigate the effect of *Funneliformis mosseae* and *Pseudomonas fluorescens*, triple superphosphate (TSP) (as an easily available form of P) and rock phosphate (RP) (as a poorly soluble form of P) on vegetative and reproductive parts of maize, root colonization, content of P and N in the plant tissue, and grain yield of maize plant under conditions of water deficit stress. For this purpose, a field experiment was carried out as split–split plot arrangement based on completely randomized block experimental design with three replications for 100 days. The results demonstrated that water deficit stress inhibited the growth and biomass of vegetative and reproductive parts and grain yield of maize; however, co-inoculation of maize with *F. mosseae* and *P. fluorescens* resulted in a significant increase in the vegetative and reproductive traits, root colonization, the grain yield of maize, content of P and N nutrients in plant tissue under water deficit and normal conditions compared with non-inoculated controls and single inoculation treatments, indicating AMF and *P. fluorescens* could make the plants more tolerant to water stress. Efficiency of TSP in combination with microbial inoculants on all measured traits was higher than that of RP. The results indicated that interactions of inoculants depend upon high or low solubility of the used P source (P availability). In general, the results of this study showed that the plants inoculated with a combination of *P. fluorescens* and *F. mosseae* expressed synergistic effect to increase maize yield under water deficit stress, while keeping safe natural resources such as P stocks.

1. Introduction

Drought is one of the main problems worldwide, which limits the production of crops especially in arid and semi-arid areas (Cimmyt, 2011). Maize (*Zea mays* L.) as a direct staple food for millions of people is considered as a necessary component of global food security (Boomsma and Vyn, 2008). This plant has high irrigation requirements and its morphophysiology at the cellular level and whole-plant level is affected by drought stress (Boomsma and Vyn, 2008). In addition, maize is one of the most important crops in semiarid areas of the world (Cavero et al., 2000). Due to the dominance of calcareous soils and high pH along with dry climate and recent drought conditions, amount of available phosphorus (P) is low in most arid and semi-arid (agricultural) regions of the world. In order to increase the P availability for plants, large amounts of chemical P-fertilizers on a regular basis are required. However, large amounts of P in fertilizers may immediately be converted to insoluble phosphate by reaction with calcium in the soil, which is unavailable to plants (Zaidi et al., 2009). In addition, the immoderate utilization of chemical P-fertilizers has resulted in several environmental problems including surface runoff of P, eutrophication of aquatic ecosystems, reduction in biodiversity, and abnormal changes in the salt concentration and pH of soils (Adesemoye and Kloepper, 2009).

Due to the increase in the price of phosphate fertilizers and their low recovery (10–30%), the use of indigenous reactive ground rock phosphate (RP) as inexpensive alternative source, proposed for sustainability purposes, is increasing in developing countries. Although the indigenous phosphate source is less expensive, the main problem of which (the sparingly soluble form of P) is its low effectiveness, particularly in calcareous soils (Khasawneh and Doll, 1979). Some of these problems can be decreased by using bio-fertilizers (*e.g.,* enhanced fertilizer-use efficiency, improvement of mineral nutrition, and reduced

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application rates of chemical fertilizers) (Adesemoye and Kloepper, 2009; Etesami and Alikhani, 2016), which are ecologically friendly, natural, and beneficial (Singh, 2013).

Arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPRs) are considered as two potentially important components of bio-fertilizers (Adesemoye and Kloepper, 2009). AMF establish beneficial symbiosis with the root system of nearly 80% of terrestrial plants. These fungi are almost ubiquitous in all of the agricultural soils. It has been found that AMF can increase P nutrition of plants by their high affinity P uptake mechanisms and by scavenging the available P through the large surface area of their hypha (Gyaneshwar et al., 2002). AMF have the ability to change water relation of their host plants and influence metabolism, protective adaptation, and morphology of host plants in water deficit stress conditions or drought stress (Al-Karaki, 2006; Kirono et al., 2011; Mickan et al., 2016). In addition, these fungi can enhance drought tolerance of host plants through mechanisms such as improvement of soil properties in rhizosphere, enlargement of root areas, enhancement of water use efficiency and uptake of P and other nutrient elements, quickly activation of defense system and protection against oxidative damages created by drought (Augé, 2001). Some researchers reported improved water relations of host plants colonized with AMF and with PGPRs (Kaushal and Wani, 2016) under moisture stress conditions (Augé et al., 2015). Because of their ability to consistently enhance mycorrhizal development, some of the *P. fluorescens* isolates are known to function as mycorrhization helper bacteria (MHB) (Garbaye, 1994). Due to their multifarious bio-fertilizing activities of increasing soil nutrient status, excretion of plant growth regulators and control of soil-borne pathogens, *Pseudomonas* species have been known as the most important bio-inoculants and the most efficient phosphate solubilizing bacteria (PSB) throughout the world (Vyas and Gulati, 2009). All of the characteristics noted above along with their diversity and tolerance in some environmental stresses such as drought stress (Marulanda et al., 2009) have shown them as a beneficial bio-fertilizer. Use of PSB as bio-inoculants in agriculture may increase the available P in soil, decreases the P-fertilizer application, minimizes environmental pollution, and finally promotes sustainable agriculture (Chen et al., 2006).

The combined use of PGPRs and AMF has been shown a cumulative and synergistic effect. These beneficial microorganisms have performed in a better way in terms of sustainable plant growth on nutrient-deficient sites (Lee et al., 2015; Mohamed et al., 2014; Xun et al., 2015; Zarei et al., 2006). Most combined applications of PGPRs and AMF are used to improve the nutrient efficiency of fertilizers, enhance phytoremediation (Xun et al., 2015), increase the yields of crops (Mäder et al., 2011), enhance fruit quality (Bona et al., 2016; Ordookhani et al., 2010) and allow reduced application of chemical fertilizers (Adesemoye et al., 2009); however the combination has not been much studied under field conditions (Berta et al., 2014; Bona et al., 2016), where inoculant fungi and bacteria must compete with the indigenous fungal and bacterial population. In addition, there is also little knowledge on the effects of the combined use of AMF and PSB on maize productivity under water stress in field conditions.

Investigating the beneficial effects of microorganisms on water stress affected-plants under field conditions gives us the opportunity to study the real detrimental effects of water stress and the real beneficial effects of inoculated microorganisms for any length of time (Marulanda et al., 2009). Although several bacterial and fungal formulations are currently available as commercial products for agricultural production, their efficiency in open field conditions depends greatly on the viability and survival of the inoculated fungi and bacteria, compatibility of these microorganisms with their host and with each other, and activity of which along the root systems (Bever et al., 2009; Gamalero et al., 2005; Rodriguez and Sanders, 2015).

Keeping in view the above details, the aim of this research was to evaluate the effect of application of AM fungus *F. mosseae* (formerly known as *Glomus mosseae*) and plant growth promoting (PGP) bacterium *P. fluorescens* along with using balanced application of mineral P-fertilizers on the vegetative and reproductive characteristics of maize, nutrient (P and N) contents in the plant tissue, the percentage of root colonization, and finally yield of this plant to achieve the sustainable management of agro-ecological systems through integrated plant nutrition under water deficit stress in field conditions.

2. Materials and methods

2.1. Experimental site and climatic conditions

The study was carried out at the research farm of University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran (latitude: 35°48′N, longitude: 51°10′E, elevation of 1312 m). The growing season was from May to August. No rainfall was received throughout the season. The mean annual rainfall in the area was below 250 mm, and more than 70% falls in February and March. Fresh water is used for irrigation in this area. The mean annual air temperature and the average temperature of summer and winter were about 14.4, 34.6 and 11.2 °C, respectively. In addition, the mean annual potential evapotranspiration and the mean relative humidity (RH) of this region were 2184 mm and 53%, respectively.

2.2. Soil sampling and analysis

Before starting the experiment, soil samples (five soil cores) were collected from 0 to 30 cm depth from each plot and then pooled together to characterize the soil in terms of physical, chemical, and biological characteristics. The soil samples used for determining physical and chemical traits were air-dried at room temperature and sieved through a 2 mm mesh before determination of soil characteristics. Accordingly, the experimental site had field capacity (FC) of 30.0% and permanent wilting point (PWP) of 16.14% with average total available water (TAW) 13.9% in volume percentage. The soil (pH = 8.1) was a predominantly clay loam (36% sand, 34% silt, and 30% clay). The electrical conductivity (EC) was 1.6 dS m^{-1}; organic carbon content was 7.3 g kg^{-1}; calcium carbonate equivalent (CCE) was 98 g kg^{-1}; total N (N_{tot}) content was 0.7 g kg^{-1}; Olsen available P was 8.2 mg kg^{-1}; available potassium (K_{avail}) was 124.0 mg kg^{-1}; and total microbial population density and the number of phosphate solubilizing microorganisms (PSMs) were 4.7×10^5 colony forming units (CFU) $g^{-1}$and 180 CFU g^{-1} soil, respectively, determined using the most probable number method. Soil organic carbon was measured by the Walkley–Black method (Walkely and Black, 1934). The pH and EC of saturated soil paste extract were determined by using a pH meter (EYELA, Japan) and an EC meter 4320 (JENWAY, UK), respectively. The N_{tot} was measured by the Kjeldahl method (Bremner and Mulvaney, 1982) and available P (P_{avail}) was determined according to Olsen P method (Olsen et al., 1954), with soil extracted with 0.5 M $NaHCO_3$ and the P in the extract was colorimetrically measured by using the molybdenum blue method (Murphy and Riley, 1962). In addition, K_{avail} was determined after extracting soil with ammonium acetate (Knudsen et al., 1982).

2.3. Microorganisms and preparation of bacterial and fungal inocula

Bacterium *P. fluorescens* was selected as bacterial treatment in this study. This bacterium was obtained from Soil Biology and Biotechnology laboratory, University of Tehran, Iran. This isolate, isolated from maize rhizosphere, was studied previously by Lotfi et al. (2016). This PGPR strain was selected on the basis of its PGP attributes. The bacterium was positive in terms of solubilizing insoluble organic and inorganic phosphates, production of siderophore, indole-3-acetic acid (IAA), and 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme. The 250-ml flask containing 100 ml nutrient broth (NB) medium was used to grow this bacterial strain. After incubation for 48 h