Genetics and Plant Physiology – 2016, Volume 6(3–4), pp. 176–185
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Research Article

EFFECT OF SYNERGETIC INTERACTIONS OF ARBUSCULA MICORRHIZA FUNGI AND RHIZOBIA ON SOYBEAN YIELD UNDER SALINE CONDITION

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Received: *29 July 2016* Accepted: *03 November 2016*

Summary: Soil salinity is one of the major constraints on crop productivity, which causes a significant decrease of agricultural production in Uzbekistan. Interactions among microorganisms such as AM fungi and Rhizobium bacteria commonly co-occur in the rhizosphere of legumes, but their combined effects on growth and yield of soybean in salt stressed conditions are still not well understood in field applications. A field experiment was conducted in middle salinated soil to assess the effectiveness of Arbuscular Micorrhiza (AM) fungi and Rhizobium on soybean growth and yield during 2014-2015. The results revealed that in the absence of inoculation, plant growth was significantly lower in the tested soybean plants. Inoculation with AM fungi and Rhizobia resulted in many nodules formed, also increased soybean shoot and root biomass and improved yield formation. Salinity affected plant growth and development particularly in non-inoculated plants, but inoculated plants were healthier in salt stress conditions. Both AM fungi and Rhizobia alone increased soybean growth and yield production to similar levels as compared to the control and exerted additive effects under co-occurrence. AM fungi increased growth of reproductive plant parts and seed production, while Rhizobia increased the biomass of vegetative aboveground plant tissues.

Citation: Khaitov B., 2016. Effect of synergetic interactions of abruscula micorrhiza fungi and rhizobia on soybean yield under saline condition. *Genetics and Plant Physiology*, 6(3–4): 176–185.

INTRODUCTION

Soybean (*Glycine max*) is an important legume crop in human diet grown mainly for its edible bean. Also, with its function in the process of biological N fixation thus improving soil fertility and structure, it is considered as an excellent intercropping crop in agriculture. Soybean production in Uzbekistan is very low, because there are so many factors in soil limiting soybean growth, such as salinity, drought, soil fertility and appropriate rhizobial strains (Egamberdieva, 2012).

Environmental stresses such as soil salinity, drought and decertification are considered the reasons for the great loss in agricultural crop productivity as well as soybean production in arid regions (Aggarwal et al. 2012). In nature, only legume crops could fix N available in the air biologically, but only after they form nodules with rhizobium.

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Different macro and micronutrients are required for the perfect growth and yield of plants, but plants are not always capable to utilize available nutrients from the soil, especially under stress conditions such as salinity. Nutrient uptake by the plants is improved by the associations with AM fungi and Rhizobium bacteria available in the soil which commonly act additively or synergistically (Artursson et al. 2006, Sabannavar and Lakshman 2011). AM fungi commonly colonize the roots of agricultural crop plants including legumes. This fungi increase phosphorous uptake by plants thereby improving yield formation process. Rhizobial strains available in the soil are known with their ability to form symbioses with legumes and improve nodulation and nitrogen fixation process. Therefore, there has been growing interest in the possible use of dual inoculation with AM fungi and Rhizobium on growth and yield of legumes in salinity soil conditions of Uzbekistan.

To date, several researchers have conducted similar experiments with different plant growth promoting bacteria and obtained positive results by inoculation of crops with dual organisms. Mirzakhani et al. (2009) indicated that seed yield and yield components of safflower have been affected significantly by the inoculation with Azotobacter and Micorrhiza, because these biofertilizers can fix nitrogen and phosphorus in soil and enhance the uptake of elements by plants. Grant et al. (2005) pointed out that phosphorus uptake in many crops is improved by associations with AM fungi. In his study, Salem (2003) also revealed that the nutrients phosphorus, nitrogen, zink, copper, and sulphur were absorbed and translocated to the host by micorrhizal fungi.

Dual inoculation with both *Azotobacter Chrocooccum* and *Pseudomonas Putida* caused increasing root system activity, enhanced plant access ability to nutrients and in addition, had the highest protein content of yield (Nasiri et al., 2013). Cropping system and long-term input of P through fertilizers and manures can influence the amount and availability of P in the system and the development of mycorrhizal associations.

The aim of this research was to determine the effect of dual inoculation with AM fungi and Rhizobium on soybean growth and yield in middle salinated soil of Uzbekistan.

MATERIAL AND METHODS

Experimental site location and soil properties

The experiment was conducted in irrigated agricultural farmer's field located in Syrdarya Region (42°00′N, 64°00′E,) in north-eastern Uzbekistan. The region climate is semi-arid, annual rainfall is about 200±40 mm. The air temperatures in winter and summer periods are 0°C and 36–38°C, respectively. A typical characteristic of the summer climate is drought.

The electro conductivity (EC) values of soil were around 560 ± 61 mS per metre, pH 8.0. Soil has suffered continuous cotton monoculture production for many decades in this area, and its fertility was low (Table 1). In general, the high concentrations of Ca^{2+}, K^{+}, and Na^{+} are associated with $CO_3^{2-}$and Cl^{-} ions, reflecting the dominance of carbonates

Table 1. Soil chemical analysis.

Soil horizons [cm]	Total content [%]				Mobile forms [mg/kg]		
	Humus	N	P	K	N-NO_3	P_2O_5	K_2O
0-30	0.925	0.082	0.153	2.30	4.8	30.0	180.5
30-50	0.715	0.066	0.139	1.80	3.2	15.0	141.0

and chlorides in saline soil. On average, soil contained 46±9 g kg^{-1} sand, 668±12 g kg^{-1} silt, and 213±13 g kg^{-1} clay. The main chemical soil properties were: organic matter 0.77%; Ct 2.43%; Nt 0.11%; CO_3^{2-}C 1.43%, Ca^{2+} 44.7 g/kg; Mg^{2+} 18.1 g/kg; K^+ 6.3 g/kg; P 1.1 g/kg; Cl^- 0.1 g/kg; Na^+ 0.8 g/kg; The high concentrations of Ca^{2+}, K^+, and Na^+ are associated with $CO_3^{2-}$and Cl- reflecting the dominance of carbonate and chloride in the saline soil.

Germination of seeds

Soybean seeds of "Orzu" variety were provided by Seed Production Laboratory of Plant Science Department of Tashkent State Agrarian University (TSAU) and planted manually in the middle of April.

Four treatments were involved: no inoculation (control), inoculation with AM fungi, inoculation with Rhizobium, and dual inoculation with AM fungi + Rhizobium.

The seeds of soybean were first sorted to eliminate broken, small seeds. Then they were surface-sterilized with a solution of 75 mL chloride + 25 mL water for 2-3 min, rinsed five times with sterile distilled water and then put in 0.5-L pots filled with perlite, previously autoclaved for 20 min at 121°C for pregermination (25 seeds/pot). All pots and saucers were disinfected with 75% ethanol before use. After 9-10 days seedlings with primary true leaves were transplanted in the field after inoculation with appropriate Rhizobium and AM fungi. The inoculated and control plants were set-up in a randomized complete block design with three replicates. Two months after sowing the number of nodules per soybean plant was estimated. At the end of the growing season plants were harvested to determine soybean seed yield as well as seed content.

Inoculation process

Rhizobial bacteria of *Mesorhizobium ciceri* strain R6 were previously isolated from the rhizosphere of soybean plants grown in salinated soil of Uzbekistan. The strain was obtained from the Biotechnology Department of Tashkent State Agrarian University, Tashkent, Uzbekistan.

In order to inoculate Rhizobium, the bacterial strain was cultured in YEM medium. The growth rate of bacteria isolates was determined spectrophotometrically after 24, 48, 72 h. The suspension used for the inoculation was adjusted to a final concentration of approximately 10^7 CFU mL^{-1}.

We used single isolates of *Glomus mosseae* to inoculate the plants with AMF, which were obtained from the Biotechnology Department of Tashkent State Agrarian University, Tashkent, Uzbekistan.

For treatments AMF+/RB+ and AMF+/RB– approximately 5 g of *G. mosseae* inoculum were added to each pot containing three plants. After the experiment, the roots of all plants were checked for mycorrhizal colonization, which allowed assigning a specific fungal colonization level to each pot used in the experiment. Plants were removed from the field and the substrate and the roots were rinsed off with cold tap water. To assess the root length colonized (RLC) by AMF, the roots were cleared by boiling for 10 min in 10% KOH and stained by boiling for 5 min in 5% black ink (Sheaffer, Ft. Madison, Iowa), household vinegar solution (equal to 5% acetic acid) (Vierheilig et al., 1998). The RLC was estimated according to Newman (1966) and the modified gridline intersect method (Giovannetti and Mosse, 1980).

Analyses

The fresh weight of shoots, roots and pods were measured immediately after removing the plants from the field and cleaning the roots, respectively. During cleaning the roots, we verified the presence of nodules in inoculated plants with Rhizobium bacteria and also checked the controls keeping in mind the possibility for inoculation with indigenous rhizobial strains. Subsequently, shoots, roots, and pods were dried for 14 days, and their dry weights were measured. Matriz-I laser electron equipment was used to analyse protein, sugar and oil content in the seeds at the Crop Science Department Lab of the University of Natural Resources and Life Sciences, Tulln, Austria.

Statistical procedures

Data were tested for statistical significance using the analysis of ANOVA. Comparisons were done using Student's *t*-test. Mean comparisons were conducted using a least significant difference (LSD) test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Inoculation with Rhizobium is known to improve growth, development and yield formation of legumes. AM fungi increase absorption surface area of the root, improve phosphorus uptake by plants and thereby facilitate fruit organs formation (Khaitov, 2015). Arbuscular mycorrhizal fungi play a significant beneficial role in 95% of the vascular plants on earth (Schausberger et al, 2012).

Our results showed that salinity affected plant growth by increasing stress rate at the salt concentrations used particularly in non-inoculated plants (controls). Inoculated plants were healthier at medium salt concentrations; however, dual inoculation with AM and Rhizobium led to enhanced growth and pod formation (Fig. 1). Our results showed that Rhizobium bacterial strains and AM fungi increased shoot and root dry weight by 17.4% and 8.7%, respectively, and the combined effect of both microorganisms resulted in a 32% increase over control. Rhizobium inoculated plants had greater shoot and root mass compared to those inoculated with AM fungi. These two microorganisms helped each other to develop microbial communities under salt stress conditions and promoted colonization of bacterial strains in plant roots (Khaitov et al, 2015). This process can facilitate plant growth

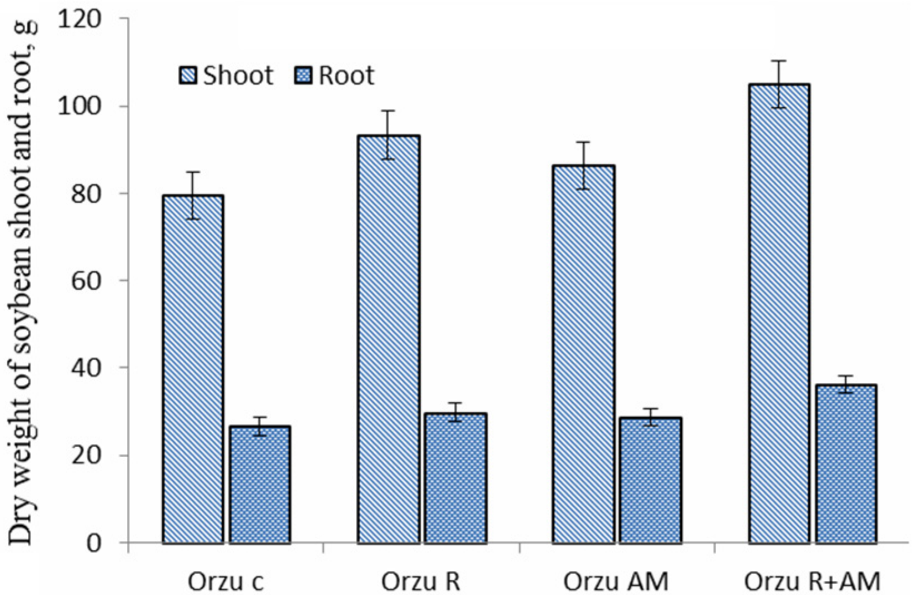

Figure 1. Dry weight of soybean shoot and root inoculated by AM fungi in the presence or absence of Rhizobium bacteria (R). Error bars represent the SE (n = 3).

and development under salt stress conditions. There are many reports indicating that the beneficial effects of mycorrhiza can alleviate the pathogenic effects significantly, and also there is a competitive interaction between the pathogenic and symbiotic fungi (Norouzi et al, 2011).

The increase of nodule number highly correlated with shoot and root weights, which is an indication of the connection between the nodules and plant growth (Table 2). The soybean variety "Orzu" used in this experiment showed good performance in nodulation and micorrhization under soil salinity conditions. AMF produced extensive extraradical hyphae in the plant root, which are a habitat for other microbes in the soil (Gahan and Schmalenberger, 2015). Thus, mutual cooperation exists between AMF and their associated microbes especially in harsh soil-climatic conditions. Many researchers reported

Table 2. Nodule number, weight and micorrhization level in soybean.

Treatments	Nodule number	Nodule weight [g/plant]	Micorrhization [%]
Control	11±1.4	0.15±0.1	9.6±0.2
R6	43±5.3*	0.49±0.3	10.2±0.7
AMF	31±3.5	0.43±0.4	23.2±1.1*
R6+AMF	59±7.4*	0.89±0.4	31.6±1.4*

*Significantly different from untreated control plants at $P<0.05$.

that the inocula of the AM fungi species applied in soil on a weight basis increased nutrient uptake by agricultural crops (Wu et al., 2005; Wu et al., 2005; Schausberger et al., 2012). The improvement of plant nutrition might be due to the stimulation of root growth or the elongation of root hairs by specific microorganisms.

Both AMF and Rhizobia applied alone increased pod weight by 27.6% and 10.7%, respectively as compared to the control and under co-occurrence the effects were much higher (29.8%) (Fig. 2). Mycorrhiza inoculated plants had higher pod mass compared to Rhizobium inoculated ones and this confirms that AMF primarily enhances the uptake of P and K (Hoffmann et al, 2011). This result also showed that AM fungi enhanced the growth of reproductive tissue, indicating the strong dependency of seed formation on P and K availability.

Similar results were observed by Miri and colleagues (2013) for wheat yield that was significantly increased after all three inoculations with Azotobacter; the increase was by 17.4%, micorrhiza increased the yield up to 21%, whereas after dual inoculation it was by 23.3%. The highest protein and P yields were obtained upon micorrhiza fungus inoculation treatment in normal irrigation conditions.

Soybean primarily is grown for the excellent seed quality, rich in protein (40%) and oil (20%). The increase of protein, oil and sugar contents in soybean seeds are important properties to assess soybean quality.

The dual inoculation with AM fungi and Rhizobium increased significantly the overall soybean yield, oil, protein and sugar content of soybean seeds compared to single inoculation with

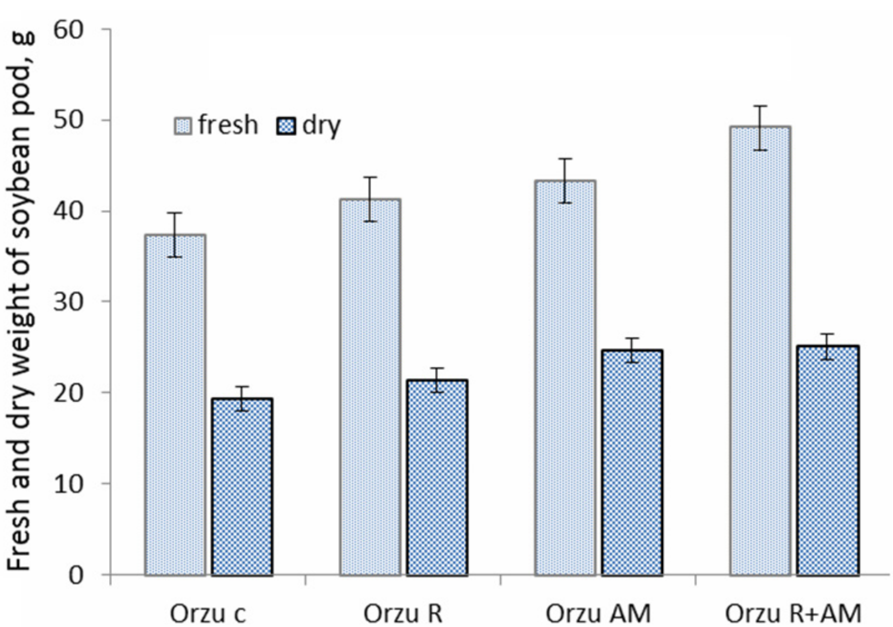

Figure 2. Fresh and dry weight of soybean pod inoculated by arbuscular mycorrhizal fungi (AM) in the presence or absence of Rhizobium bacteria (R). Error bars represent the SE ($n = 3$).

each of the organisms or the control. The increase in soybean yield was by 10.9%, 18.1% and 25.9% due to Rhizobium, AMF or dual inoculation, respectively. The content of oil, protein and sugar of soybean seeds was increased by 12.8%, 4.0% and 12.9%, respectively after dual inoculation of soybean plants. In AMF inoculated plants, the increase in seed oil, protein and sugar content was by 11.0% and 1.9%, respectively while Rhizobium inoculation led to 9.7%, 3.6%, and 8% increase over the control, respectively (Table 3).

However, inoculation of soybean seeds with AM fungi significantly increased the content of oil and sugar than Rhizobium inoculation showing that AM fungi enhance the uptake of P and thereby serve for the improvement of seed quality properties. As nitrogen fixing bacteria Rhizobium enhanced the uptake of N by the plants and resulted in higher protein content in soybean seeds when compared to AM fungi inoculation. Behl et al. (2012) observed nitrogen-fixing and phosphate-mobilizing bacteria, as well as mycorrhizal fungi which can influence plant nutrition beneficially and thus could be used as biofertilizers in agriculture. Biomass and yield improvement due to symbioses depends on nutrient absorbing efficiency of the fungal symbiont. Application of AM fungi and plant growth promoting bacteria (PGPB) in legume crop production is one of the best options to overcome stress imposed by salinity in arid regions (Khaitov et al, 2014). Inoculation with Rhizobacteria had little influence on the metal concentrations in plant tissues, but produced much greater above-ground biomass and altered metal bioavailability in the soil (Wu et al., 2006). As a consequence, higher efficiency of phytoextraction was obtained compared to control.

There are many reports showing a positive interaction between AM fungi and Rhizobium in legumes leading to improved nodulation, growth and yield. Patale and Shinde (2012) found that mycorrhizal fungi play a significant role in the reduction of salt stress effects in plants. Ortas (2012) reported that plant species belonging to Solanaceae, Leguminosae and Cucurbitaceae showed

Table 3. Yield of soybean, oil, protein and sugar content of soybean seeds.

Treatments	Yield [dt/ha]	Oil content [%]	Protein content [%]	Sugar content [%]
Orzu control	26.37^{c}	17.20^{c}	37.60^{c}	5.37^{d}
Orzu R	29.20^{b}	18.87^{b}	38.97^{a}	5.80^{c}
Orzu AM	31.13^{ab}	19.10^{ab}	38.33^{b}	6.10^{b}
Orzu R+AM	33.20^{a}	19.40^{a}	39.10^{a}	6.33^{a}
LSD 0.05	2.10	0.31	0.36	0.22
CV(%)	3.5	0.8	0.5	1.9

Data are means of 3 replicates/plot analysed. Means between treatments in the same row followed by the same letter are not significantly different according to the LSD test ($P \leq 0.05$).

effective responses to mycorrhizal inoculation and plants had higher nutrient content than non-inoculated plants.

Muller (2000) found that plant hormones are known to play roles in different developmental processes and may therefore be involved in the regulation of this symbiosis. Little is known about the function of plant hormones during the colonization process, although there is evidence that they are involved in signalling events between AM fungi and host plants.

In this study, the sole application of AM fungi or Rhizobial strain, and their combination promoted growth and yield formation in soybean plants by increasing shoot and root dry matter weight, yield, and seed content in soil salinity conditions. Our results showed that the combined application of AM fungi and Rhizobial strain holds a lot of promises in contrast to their sole application which soybean producers can exploit. It is apparent that the combined application resulted in a synergy in terms of soybean growth, development and yield formation.

It has been established that for field crops, seed inoculation with beneficial mycorrhiza and beneficial bacterial strains makes a great contribution to improvement of plant growth in soil salinity conditions. Our findings confirm the hypothesis that arbuscular mycorrhizal fungi have a higher capacity to alleviate the saline stress effects in plants grown in saline soil, and in combination with Rhizobial strains the efficiency could be increased significantly. These results suggest the feasibility of using a combined inoculation with AM fungi and Rhizobium strains for improving legume crop production under soil salinity conditions.

The findings above demonstrate a more profitable application of beneficial bacterial fertilizers which are environmentally friendly in many salinated fields in Uzbekistan, in general, as possible alternatives or for replacing in part the mineral fertilizers now in use in agricultural production. Further field researches should be conducted to assess the effects of the application of AM fungi and Rhizobial bacterial strain on the yield of other legumes, grain crops, and on soil quality.

ACKNOWLEDGEMENTS

The author thanks to Dr. Kh. Nazarov for his help in the analyses of seed oil, protein and sugar content of soybean seeds and also to Dr. Dolores Ledesma from the World Vegetable Center for her kind contribution to the statistical analyses process.

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