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The role of *Azospirillum lipoferum* bacteria in sustainable production of sunflower (*Helianthus annuus* L.)

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Abstract

This research was carried out to study the effect of *Azospirillum lipoferum* on declining the consumption of nitrogen fertilizer on two sunflower cultivars Armawireski and Alestar at the Research Station of the Faculty of Agriculture in Baku State University. The nitrogen fertilizer was applied in four levels (0, 70, 140 and 210 kg/ha) with or without inoculation of seeds by *Azospirillum* bacteria. The treated seeds were inoculated with peat-based inoculants of *Azospirillum lipoferum*, with 2x10 ^{7} cells per g of carrier. A factorial experiment, conducted with three replications on the base of randomized complete block design, revealed that nitrogen fertilizer inoculation with *Azospirillum* could lead to increase in grain yield and yield components. Using nitrogen fertilizer and bacteria together caused declining of the use of nitrogen and increasing of the grain yield. Increasing the nitrogen fertilizer amount to 210 kg/ha and inoculation led to declining of yield to less than that of 140 kg/ha treatment. The results revealed that in the case of inoculation with *Azospirillum lipoferum*, the highest grain yield (5.14 t/ha) was produced and without inoculation the lowest grain yield (3.68 t/ha) was obtained. The results showed that the inoculation with *Azospirillum* increases the yield, percentage of nitrogen in grains, number of grains in a row, flag leaf area, head length and number of grains in head. It can be concluded that by using 140 kg of nitrogen fertilizer per hectare and inoculation of maize seeds by *Azospirillum* could decline the application of nitrogen fertilizer, increases the grain yield up to 30 percent and prevents the pollution of environment by extended sustainable agriculture.

Key words: *Azospirillum*, sunflower, nitrogen fertilizer, grain yield.

Introduction

Low irrigation is one of the methods for agricultural production when available water is deficit [1]. Sunflower consumes the greatest amount of water during growth period average, a well growing sunflower crop will use 6 mm of water per day [7]. Nitrogen fixation was the first mechanism proposed to explain improved plant growth following inoculation with *Azospirillum*. *Azospirillum* species occur in soil and are enriched in root surface of many different plants such as wheat, rice, maize, sugar cane [3, 17]. At present, in some countries, this bacterium is used as biological fertilizer to produce agricultural products including cereals and vegetables [9, 16]. *Azospirillum* causes physiological and morphological changes of host plant roots, and the major changes observed in inoculated roots were increasing of cellular division at root and rising number of root hairs [2, 19]. Apparently inoculation allows plants to have a more balanced nutrition and the absorption of nitrogen and other mineral nutrients (such as phosphate, potassium, zinc and manganese) are improved [5, 12, 20]. Spring wheat inoculated with *Azospirillum lipoferum* by using of 40 kg of nitrogen fertilizer per hectare showed positive effect and increased grain yield up to 30 percent [6, 10]. Inoculation with *Azospirillum* increases plant growth, flag leaf area and quantity of chlorophyll in the leaf and led to higher dry weight than non-inoculated plants [13, 14]. The biological fertilizers attribute not only to the materials from dung and plant remainders, but also including all products of microorganisms, e.g. soil bacteria *Azotobacter* and *Azospirillum*. These bacteria help to preserve the health of the plant by controlling the pathogenic agent indirectly [11]. With respect to above explanation, determining the effects of growth stimulating bacteria on the crop yield in the soils of every region are completely necessary for extension of the usage of biological fertilizers and sustainable agriculture. Therefore, the purpose of the present study was to evaluate *Azospirillum* effect on growth, development and decreasing of consumed fertilizer as well as increasing of sunflower yield.

Materials and Methods

The study was conducted at the Research Station of the Faculty of Agriculture, Baku State University, in 2008. Planted sunflower cultivars were Armawireski and Alestar. These cultivars were grown with a plant density of 66,000 per hectare. The experiment was conducted as factorial, based on randomized complete block design with three replications. The nitrogen fertilizer was applied in four levels, 0, 70, 140 and 210 kg/ha, with or without inoculation of seeds by *Azospirillum* bacteria. The treated seeds were inoculated with peat-based inoculants of *Azospirillum lipoferum*, with 2x10 ^{7} cells per g of carrier. Inoculated seeds were planted directly to soil. The first irrigation was done immediately after seed planting. All treatments were irrigated every 7 days once until harvesting time. Also nitrogen fertilizer was applied as topdressing in two stages, emergence and stamen appearance.

Before harvesting plant height and flag leaf area (flag leaf measured by leaf area meter) and after harvesting seed weight, head length, head diameter, grain number per head and number of seeds in row, percentage of nitrogen in grains and shoots and grain yield were measured. Percentage of nitrogen was evaluated in Institute of Soil and Water, Ministry of Agriculture. The data were tested for normal distribution and subjected to statistical analysis using SAS statistical software. When analysis of variance showed significant difference, Duncan's multiple range test was used to compare the means at $p \leq 0.05$ [18].

Results and Discussion

Analysis of variance for data collected in the experiment is shown in Table 1. The main effect of nitrogen fertilizer was significant for all traits studied. *Azospirillum* inoculation had also significant effect on majority of traits, except the thousand seed weight. However, their interactions were not significant for all traits, except flag leaf area and head length.

Nitrogen fertilizer inoculation with *Azospirillum* could lead to increase in grain yield and yield components (Fig. 1). Nitrogen fertilizer and bacteria applied together caused declining use of nitrogen and increasing grain yield. Mean comparison of nitrogen fertilizer levels and bacterial inoculation (Tables 2-3) showed that inoculated plants produced more grain yield than control. Increase of the nitrogen fertilizer amount to 210 kg/ha and inoculation led to declining of yield to less than that of 140 kg/ha treatment (Fig. 2). Use of the nitrogen fertilizer amount higher than this level enhanced vegetative growth and accumulated nitrogen in vegetative organs so that plants mature later in cold weather and could not fill seeds well and so the grain yield decreases considerably [6]. It is clear that the amount of nitrogen fertilizer level could vary with regard to various regions [15]. Mean comparison of interactions between nitrogen and *Azospirillum* in sunflower (*Helianthus annuus* L.) showed that plants inoculated with *Azospirillum lipoferum* produced higher grain yield and grain number per head.

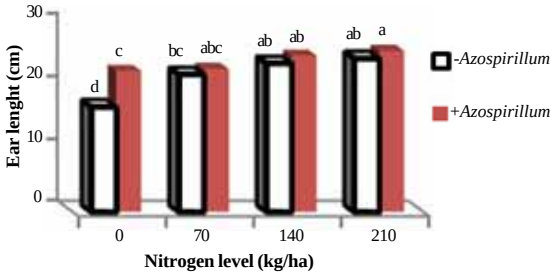

Figure 1. Mean of treatment combinations of nitrogen and *Azospirillum* for head length on sunflower.

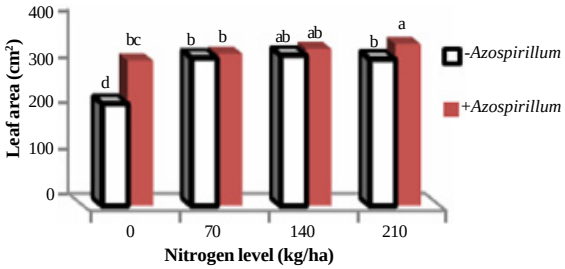

Figure 2. Mean of treatment combinations of nitrogen and *Azospirillum* for leaf area of sunflower.

Enhanced early growth and higher photosynthesis in bacterial treatments are the main factors for yield increase and this was in agreement with results obtained by other researchers [4, 20]. Applying chemical fertilizer together with bacteria could reduce nearly 50 percent of the use of nitrogen fertilizers. Accumulation of mineral nitrogen in soils not only enhances environmental hazards but causes nitrogen losses from soil through ammonification and denitrification. Pollution of underground waters and increase of nitrate concentration in agricultural products are other hazardous effects of excess nitrogen for human and animal health [8, 9]. Environmental pollution reduction, less cost, simple availability

Table 1. Analysis of variance for traits of inoculated and non-inoculated sunflower.

SV		Mean square										
	df	Grain yield	Seed weight	Nitrogen in seeds	Nitrogen in shoots	Plant height	Leaf area	Number of seeds in head	Number of seeds in row	Row number	Head length	Head diameter
Replication	2	1.201	94.67	0.086	0.0846	45.42	19.43	1113	7.718	0.656	0.686	0.067
Nitrogen	3	35.85**	3054**	0.565**	0.246**	4397**	6167**	53314**	137.4**	1.204*	35.42**	0.443**
Bacteria	1	12.85*	395 ns	0.273+	1.788**	562.2+	8571**	8331*	70.89**	0.510ns	0.004ns	
Nitrogen*Bacteria	3	1.63 ns	408 ns	0.039 ns	0.035 ns	103.6ns	2197**	825.2 ns	2.398ns	0.065ns	8.212**	0.012ns
Error	14	2.128	177.7	0.066	0.036	142.9	247.9	1873	4.802	0.322	1.694	0.03
CV%	-	12.78	5.96	16.93	10.30	5.67	4.95	55.5	4.39	3.65	5.71	3.58

*, **, +, significant at 5, 1 and 10% levels and ns non-significant, respectively.

Table 2. Mean comparison of traits evaluated on sunflower cv. Armawireski.

Treatment	Grain yield (t/ha)	Thousand seed weight (g)	Nitrogen in seeds (%)	Nitrogen in shoots (%)	Flag leaf area (cm^2)	Grain number per head	Number of seeds in row	Head length (cm)
+Azospirillum	5.14 a	72.345 a	1.030 a	1.58 b	336.4 a	798.2 a	15.62 a	23.92 a
-Azospirillum	3.68 b	67.871 b	0.670 a	2.12 a	198.6 b	759.9 b	13.18 b	21.64 b
N 210 (kg/ha)	5.84 a	70.072 a	1.370 a	2.23 a	240.0 a	835.4 a	16.75 a	25.02 a
N 140 (kg/ha)	4.88 a	64.891 b	0.970 a	2.04 a	231.4 a	863.4 a	14.00 a	24.24 a
N 70 (kg/ha)	3.51 b	54.980 c	0.081 b	1.97 a	227.0 a	763.7 b	10.58 b	22.31 b
N 0 (kg/ha)	2.42 c	40.460 d	0.032 c	1.81 b	170.2 b	652.0 c	8.29 c	19.56 c

*Means in each column followed by different letters are significantly different ($P \leq 0.05$).

Table 3. Mean comparison of trait evaluated on sunflower cv. Alestar.

Treatment	Grain yield (t/ha)	Thousand seed weight (g)	Nitrogen in seeds (%)	Nitrogen in shoots (%)	Flag leaf area (cm^2)	Grain number per head	Number of seeds in row	Head length (cm)
+*Azospirillum*	4.14 a	64.345 a	0.97 a	2.58 b	287.4 a	893.2 a	23.49 a	17.62 a
-*Azospirillum*	2.68 b	58.871 b	0.27 a	3.37 a	123.9 b	819.4 b	20.73 b	15.13 b
N 210 (kg/ha)	4.73 a	61.128 a	1.06 a	2.00 a	209.03 a	892.31 a	18.55 a	21.31 a
N 140 (kg/ha)	3.91 a	57.571 b	0.90 a	1.97 a	202.68 a	916.7 a	15.83 a	20.07 a
N 70 (kg/ha)	2.98 b	49.074 c	0.57 b	1.86 a	194.74 a	627.05 b	11.29 b	18.11 b
N 0 (kg/ha)	1.86 c	36.948 d	0.12 c	1.56 b	143.08 b	547.19 c	9.02 c	15.37 c

*Means in each column followed by different letters are significantly different (P≤0.05).

power of reproduction and propagation are the major important factors of growth stimulation by bacteria and biological fertilizers compared to chemical fertilizers.

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