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Effects of *Azospirillum* Bacteria and Gibberellin Hormone on Morpho-physiological properties, Yield and Yield Components of Corn (*Zea mays* L.)

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ABSTRACT: To investigate the Effects of *Azospirillum* bacteria and Gibberellin hormone on Morpho-physiological properties, yield and yield components of Corn, a factorial experimental based on RB design was carried out in the research farm of Miyandoab agricultural office during 2014 growing season. *Azospirillum* bacteria in three levels (control 25g *Azospirillum* applying with seed and soil) and gibberellin hormone in three levels (control, 100 and 200 mg/Liter) were evaluated. Variance analysis showed that the effect of *Azospirillum* bio-fertilize on ear length, ear weight, cob weight, seed number per row, 1000 grain weight, biological yield, grain yield and harvest index and the effect of gibberellin hormone on all traits except Ear weight and grain yield were significant. Also, the interaction *Azospirillum* × Gibberellin had significant effects on Chlorophyll a and b, cob weight, seed number per row, biological yield, grain yield and harvest index.Means comparison revealed that *Azospirillum* applied with soil had the highest number ear weight, ear length, seed number per row, 1000 grain weight and grain yield, also treatment of 200 mg/liter Gibberellin hormone had the best value in respect of all understudy traits. Means comparison of interaction effects also showed that *Azospirillum* mixed with seed × 200 mg/liter Gibberellin hormone in compared with the other interactions had the highest value in traits seed number per row, biological yield and grain yield.

Keywords: *Azospirillum*, Gibberellin, Corn, Seed Yield.

INTRODUCTION

Corn is the most powerful cultivation plant and the greatest attraction and storage of free energy in the earth and in terms of energy, it is a good food for livestock and it is full of energy and it is the main food of a great number of people as directly or indirectly via livestock and vegetable products and it has the highest position in comparison with other cereals. Due to this, it is called the lord of cereals (Nourmohammadiand Kashani, 1998).

One of the major limitations in realizing potential yield of crops and achieving high yield is to supply adequate nutrients. In conventional and high input agriculture, this problem has been solved by the use of chemical fertilizers. Environmental problems caused by the excessive use of chemical fertilizers, energy and their consumption and production costs and their adverse effects on biological cycles and sustainability of ecological cropping systems are the causes of approaching bio-fertilizers application. Application of bio-fertilizers in feeding crops has been recently considered as a fundamental solution to develop the integrated management system of plant nutrition and to enhance the quality and quantity of food per area unit through the integration of organic and inorganic methods of feeding crops. Accordingly, agricultural development during the transition from conventional agriculture to sustainable agriculture with adequate input as the integration of chemical and organic fertilizer consumption especially bio-fertilizers has been acceptably proposed as an alternative approach to agricultural production and yield maintenance. By definition, bio-fertilizers are composed of one or several kinds of helpful microorganisms in combination with preservatives or their metabolic products that are used to provide nutrient for crops. Different kinds of bio-fertilizers contain symbiotic bacteria, Mycorrhizal fungi, and plant growth promoting Rhizobacteria (Zahir *et al.*, 2000).

Biological products and especially the use of *Aszosprilium* spp appeared among the new the technologies for optimizing plant implantation, *Aszosprilium* is growth promoting Rhizobacteria (PGPR) capable of colonizing the root and stimulating root growth thus enhancing mineral and water uptake plants (Puente, 2009). (Zahir *et al.*, 2004) reported that the seed yield of corn increased 8.19% due to use combined bacteria Pseudomonas and *Azospirillum*.

Mahfouz and Sharaf-Eldin, (2007) reported that the application of bio-fertilizer: Azotobacter, *Azospirillum* and *Bacillus* increased seed yield and essence content in Fennel plant. In another study on Fleawort medicinal plant cleared that the use of bio-fertilizer *Azospirillum* increased the quality and quantity yield, significantly (Khalil, 2006). (Soleimani Fard *et al.,* 2012) studied the effect of growth stimulating bacteria on phenology, yield and yield components of maize hybrids. The recent years is done many efforts to improve conditions for germination and vigor of seeds and seedlings, one of these efforts is seed priming, that can increase the germination and growth of seeds (Foti *et al.,* 2002). Growth parameters such as speed and uniformity of emergence are very important factors for achieving to high quantity and quality yield especially in annuals. Use of hormones and growth regulators in agricultural practices are the modern and customary methods in order to increase the yield of crops. Plant growth regulators, chemical compounds that are used in small rate, cause that the plant growth and development be better (Muniralzaman, 2000). Nowadays, Phytohormones have been found to play an important role in plants development. Plant growth regulators have plentiful applications in agriculture such as delaying or accelerating maturity, stimulation, flowering, abscission, controlling weeds and so on. Gibberellins induce flowering in long-day plants which require chilling (Harkess & Lyons, 1994). Heading was delayed by addition of Gibberellic acid (GA3) to the root zone in super-dwarf rice (Frantz *et al.,* 2004). Gibberellins are probably one of the growth regulators that have a significant effect on flowering (Takahashi *et al.,* 1991). Seed soaking with 20 ppm Gibberellic acid (GA20) solution for 30 minutes improved seedling vigor (i.e., seedling height and growth),

(Khaleghi *et al.,* 2012) observed an increasing 1000 seed weight, number of grains per ear, biological yield and grain yield in corn due to use Gibberellic hormone. (Subedi and Ma, 2005) reported seed soaked for 30 min with gibberellin solution increased plant height, power and seedling vigor, but did not lead to the attainment of high yield. (Keshavarzi *et al.,* 2014) observed with increasing concentration of gibberellins fresh weight of plant, plant height, stalks and leaves protein, were increased.

The purpose of this study was to evaluate the effects of *Azospirillum* and Gibberellin on Morpho-physiological properties, yield and yield components in corn (cultivar 370).

MATERIALS AND METHODS

To investigate the effects of *Azospirillum* bacteria and Gibberellin hormone on yield and yield components of corn, a factorial experimental based on RB design with three replications was carried out in the research farm of Miyandoab agricultural office, Urmia, West Azerbaijan, Iran that placed at longitude 46°36´, latitude 36°58´ and elevation 1143, during 2014 growing season. *Azospirillum* bacteria in three levels (control 25g *Azospirillum* applying with seed and soil) and Gibberellin hormone in three levels (control, 100 and 200 mg/Liter) were evaluated. In terms of the soil characteristics at the research farm, the soil texture was a lay-loam structure containing 1.11% of organic carbon and 0.11% of nitrogen, in addition to 7.22 p.p.m of phosphorus and 295 p.p.m of potassium. The soil acidity was indicated at a pH of 7.79 at a depth of 0-30 cm. In this study, corn seeds were inoculated with *Azospirillum* before planting and Gibberellin hormone was sprayed on plants before flowering. After preparing the farm on April 15 2014; the cultivation practices applied were those commonly used for this crop. Every plot contained 5 culture lines, each of which were 5 meters in length and placed 70 cm apart from each other. The total plot area was 21 m^2. To avoid marginal effects and to minimize error, plots were situated beside each other no closer than 50 cm. To enhance accuracy, margins were placed at the beginning and the end of plots as well as the 50 cm border between each plot. After planting, the farm was irrigated immediately and other irrigations were done every 10-14 days by attention to the moist of soil. Data were recorded for traits chlorophyll index, the height of the first ear from the ground, dry leaf weight, ear weight, seed weight, seed number per ear row, 1000 Grain weight, biological yield and seed yield. Data analysis was done using SAS ver. 9.2 and SPSS ver. 21 programs.

RESULTS AND DISCUSSION

A. Chlorophyll a

Variance analysis showed that effects of gibberellin hormones and *Azospirillum* × Gibberellin interaction on chlorophyll a were significant at the 1% probability level (Table 1). The results of the means comparison of gibberellin hormone on Chlorophyll a showed that treatment 200 mg/ gibberellin hormone had the highest amount of Chlorophyll a (1.73 mg/gr). The interaction no *Azospirillum* (control) with 100 mg/liter gibberellin hormone and no *Azospirillum* (control) no gibberellin hormone (control) with 2.1 and 0.48 mg/gr respectively, had the highest and the lowest Chlorophyll a content respectively (Table 4).

B. Chlorophyll b

According to the analysis of variance table (Table 1) effects of gibberellin hormones and *Azospirillum* × Gibberellin interaction on chlorophyll b were significant at the 1% probability level.

Table 1: Variance analysis of studied traits.

S. O. v	df	Ms									
		Chlorophyll$_a$	**Chlorophyll$_b$**	**ear Length**	**Ear weight**	**Cob weight**	**seed number per row**	**1000Grain weight**	**biological yield**	**grain yield**	**harvest index**
Replication	2	0.08^{ns}	0.02^{ns}	5.39^{*}	91.15^{ns}	43.69^{ns}	51.29^{*}	846.36^{ns}	7.92^{ns}	3.60^{**}	0.12^{ns}
Azosprilium	2	0.08^{ns}	0.15^{ns}	14.06^{**}	213.3^{*}	17.46^{**}	100.70^{**}	5087.24^{**}	56.48^{**}	15.18^{**}	0.35^{**}
Gibberellin	2	1.17^{**}	0.90^{**}	8.25^{**}	85.81^{ns}	46.83^{**}	146.81^{**}	4718.99^{**}	104.84^{**}	0.77^{ns}	0.90^{**}
Azosprilium × Gibberellin	4	0.98^{**}	0.99^{**}	1.56^{ns}	29.48^{ns}	15.65^{**}	74.20^{**}	521.27^{ns}	41.08^{**}	2.15^{*}	0.99^{**}
Error	16	0.18	0.14	0.51	63.46	2.49	15.88	544.01	10.7	0.55	0.14
CV (%)	-	23.31	16.57	12.20	16.70	15.07	11.35	13.61	8.39	14.33	6.57

Ns, * and **: no Significant, Significant at 5% and 1% probability levels

Table 2: Means comparison of *Azosprilium* Bactria on studied traits.

Azosprilium	**Ear weight (gr)**	**Ear length (cm)**	**Cob weight gr**	**Seed number per row**	**1000 Grain weight (gr)**	**biological yield (t/he)**	**grain yield (t/he)**	**harvest index**
Control	364.3b	20.94b	29.58b	292.33b	143.92b	35.54b	15.81c	42.85b
Applied with Seed	368.12b	23.33a	32.23a	297.11a	185.22a	40.51a	17.34b	42.50b
Mixed with soil	392.51a	22.77a	31.65a	298.78a	184.91a	37.45ab	18.39a	48.64a

*: Treatments with the same letter have not significant difference

The means comparison results of gibberellin hormone showed that treatment 100 and 200 mg/liter gibberellin hormone by average of 2.25 and 2.59 mg/gr respectively achieved highest amount of chlorophyll b in compare with control treatments. Between *Azospirillum* × Gibberellin interaction, no *Azospirillum* (control) with 200 mg/liter gibberellins hormone by average of 2.05 and no *Azospirillum* (control) no gibberellin hormone (control) with 1.6 mg/gr had the highest and the lowest Chlorophyll b content respectively (Table 4).

(Jeffrey and Gyles, 2003) reported, gibberellin hormone by increasing the synthesis of chlorophyll, cause to increased photosynthesis in plant.(Saidi *et al.*, 2009) reported that, growth stimulating substances such as gibberellin and Cytokinin increased the amount of and photosynthesis in wheat. In this research between Chlorophyll a and Chlorophyll b positive and significant correlation (r = 0.82**) was seen.

C. Ear weight

The results of variance analysis showed that the *Azospirillum* treatments (at the 5% level) had the significant effects on ear weight. The results of means comparison cleared that treatments *Azospirillum* applied with soil and control had the largest and lowest weight of ear by average of 392.5 and 364.3gr, respectively. In represent research organic fertilizer increased the weight of the corn cob this effect can resulting in increased nutrient uptake and better growth of the plant. In addition to nitrogen-fixing *Azospirillum* bacteria could produce a variety of growth factors, such as indole acetic acid, gibberellins and vitamins which can stimulate the growth of plants. (Zahir *et al.*, 2004) reported that the Ear weight of corn increased 18% due to use of *Azospirillum*, also (Hamidi *et al.*, 2011) found that application of *Azospirillum* increased ear weight compared with the control.

D. Ear length

Analysis of variance revealed that the *Azospirillum* treatments and gibberellin hormone had significant effects on ear length at the 1% probability level (Table 1). The results of means comparison also cleared that treatments *Azospirillum* applied with seed and control had the largest and lowest length of ear (23.33 and 20.94 cm, respectively). In this study, it confirmed that the using of *Azospirillum* applied with seed or mixed with soil in compared with control increased 10.30 and 8.23% ear length, respectively (Table 2). On the other hand, the results of means comparison showed that gibberellin hormone had an increasing effect on ear length (Table 3), as treatment 100 mg/liter gibberellin hormone in compared with control and treatment 200 mg/liter gibberellin hormone increased ear length 3 and 5 and 8perccnt respectively. In this study, it was cleared that trait ear length with traits seed number per row (r = 50**), and biological yield (r = 0.37*) had significant and positive correlations.

Table 3: Means comparison of Gibberellin hormone on studied traits.

Gibberellin (mg/lit)	$Chlorophyll_a$ (mg/gr)	$Chlorophyll_b$ (mg/gr)	ear Length (cm)	Cob weight (gr)	seed number per row	1000 Grain weight (gr)	biological yield (t/he)	harvest index
0	1.01b	1.96b	21.50b	28.66b	291.56b	155.60b	39.38a	43.12b
100	1.73a	2.35a	22.16ab	33.14a	297.33a	160.7b	33.85b	45.31ab
200	1.4ab	2.59a	23.38a	31.66a	299.33a	197.63a	40.26a	46.21a

*: Treatments with the same letter have not significant difference

Table 4: Means comparison of interaction effects *Azesprilium* Bactria × Gibberellin hormone on studied traits.

Azosprilium	Gibberellin	Chlorophyll$_a$ (mg/gr)	Chlorophyll$_b$ (mg/gr)	Cob weight (gr)	seed number per row	biological yield (t/he)	grain yield (t/he)	harvest index
control	0	1.6c	1.6d	25.52c	282.11c	32.03c	15.09e	47.11a
	100	2.7a	2.7 ab	31.23b	297.60ab	35.66bcd	15.91de	44.58b
	200	3.05a	3.05 a	31.85b	297.3ab	38.93b	16.01cd	42.10cb
Applied with Seed	0	1.96bc	1.96d	32.01b	289.61b	35.08bcd	17.1bcd	48.57a
	100	2.12ab	2.12bcd	36.33a	297.63ab	37.12bcd	16.96 bcd	43.24b
	200	2.77a	2.77ab	29.33b	296.21ab	40.33b	17.97b	42.50cb
Mixed with soil	0	2.59a	2.59abc	31.14b	297.11ab	44.13a	17.62bc	38.63c
	100	2.23ab	2.23bcd	31.93b	297.51ab	38.9b	18.18ab	36.47c
	200	1.96bc	1.96cd	31.95b	303.1a	44.66a	19.38a	43.18cb

*: Treatments with the same letter have not significant difference

E. Cob weight

According to analysis of variance table *Azospirillum* treatments, gibberellin hormone and their interactions had significant effects on the cob weight at 1% probability level (Table 1). Between *Azospirillum* and gibberellin interaction compare means, *Azospirillum* mixed with seed × 100 mg/liter gibberellins by average of 36.33 gr and control of both treatments (no *Azospirillum*, no gibberellin hormone) by average of 25.52 gr allocated highest and lowest value in respect of cob weight. In represent study cob weight showed a positive and significant correlation (r = 0.37*) only with Chlorophyll a (Table 5).

F. Seed number in row

The results of variance analysis showed that *Azospirillum* treatments, gibberellins hormone and their interactions had significant effects on the seed number in row at 1% probability level (Table 1). The results of means comparison showed interaction effects *Azospirillum* mixed with soil × 200 mg/liter gibberellins by average of 303 gr and control of both treatments (no *Azospirillum*, no gibberellin hormone) by average of 282 grhad the best and the lowest value in respect of seed number in row respectively. It can be noted; these two treatments provided better environment condition in terms of nutrition and biochemical, so they increased the production of flowers and pollen and subsequently increased the seed number in row.

In the other hand, *Azospirillum* not only increased nitrogen availability due to nitrogen fixation, but also synthesized growth hormones and provided water and nutrients by changing in root physiology and increased root system.(Hassanzadeh *et al.*, 2010) reported that organic fertilizers, especially bacteria that facility the absorption of phosphorus increased the seed number in barley.

(Khaleghi *et al.,* 2012) reported level combine of gibberellin with bio-fertilizer significantly increased number of grains per ear. According to results of correlation table (Table 5) between seed number in row and ear Length (r = 0.50**) and cob weight (r = 0.61**) positive and significant correlation was seen (Table 5).

G. 1000 kernel weight

Based on the analysis of variance table (Table 1) *Azospirillum* treatments and gibberellin had significant effects on 1000 kernel weight. Result of compare mean showed only significant difference was seen between *Azospirillum* application treatments with control (no *Azospirillum*) in terms of 1000 kernel weight. In this research *Azospirillum* mixed with soil and seed by average of 184.95 and 185.20 respectively, achieved highest 1000 kernel weight in compare with control treatments (no *Azospirillum*c) by average of 143.90. (Eidizade *et al.,* 2012) reported that, use of bio-fertilizers mixed with seed allocated the highest 1000 kernel weight compared with other treatments.

The results of the means comparison of gibberellin hormone on 1000 kernel weight showed that treatment 200 mg/liter gibberellin had the highest 1000 kernel weight by average of 197.62. The results also cleared, this treatment in compared with control and 100 mg/liter Cytokinin hormone increased 1000 kernel weight 21.31 and 18.79% respectively (Table 2). Increased seed weight on the effects of gibberellins is due to the strength of the sink,with the use of these hormones may increase the rate of cell division in seed and increased seed storage cell; In addition gibberellin hormones increased seed storage cell (Taiz and Zeiger, 2006). (Saeidi *et al.,* 2007) reported that plant hormone increased seed number and 1000 seed weight in wheat, that these results confirmed our results. The results of correlation between traits (Table 5) revealed 1000 seed weigh showed negative and significant correlation with seed number per row (r = -0.54**).

H. Biological Yield

Analysis of variance showed that *Azospirillum* treatments, gibberellin and their interactions had significant effects on biological yield at 1% probability level (Table 1). The means comparison results revealed that *Azospirillum* mixed with soil in combine with 200 mg/liter gibberellin hormone by average of 44.66 t/ha achieved highest amount of biological yield. It should be noted that in this research combine of two control treatments (no *Azospirillum*, no gibberellin hormone) by average of 32.03 t/ha had the lowest value in respect of biological yield. Bacteria that increased the growth such as *Azospirillum*, have an ability to build biologically active substances such as nicotinic acid, Pentonic acid, biotin, B vitamin, Auxin and gibberellin which have an effective and useful role in promoting root growth (Kader *et al.,* 2002). Also bio-fertilizers can convert main elements from unavailable to available form by biological processes as well as develop root system, nutrient absorption and transport, and finally increase the growth and biomass of plant. (Nanda et al., 1995) stated that inoculation of corn seeds with *Azospirillum* bio-fertilizers increased the biological yield significantly (Table 2). In represent study biological yield showed a positive and significant correlation with ear weight (r = 0.41**) and ear length (r = 0.37**).

I. Grain Yield

Based on the analysis of variance table, *Azospirillum* treatments (at 1% probability level) and *Azospirillum* × Gibberellin interaction (at 5% probability level) had significant effect on grain yield (Table 1). The result of means comparison showed that *Azospirillum* mixed with seed with average of 18.39 t/ha had the highest seed yield between studied treatments and increased grain yield 5.5 and 16.5 percent compare with *Azospirillum* mixed with seed and control (no *Azospirillum*). Micro-organisms such as *Azospirillum* increase the absorption of nutrients as well as accelerate plant growth. Bacteria Azotobacter genus and *Azospirillum* are the most important bacteria which stimulate plant growth by biological nitrogen fixation and production of soluble phosphorus in the soil as well as product hormones that stimulate plant growth and subsequently effect on improving crop yield.

Among interaction effects, *Azospirillum* mixed with soil × 200 mg/liter gibberellin with 19.38 t/ha and combine of two control treatments (no *Azospirillum*, no gibberellin hormone) by average of 15.09 t/ha had the highest and the lowest grain yield, respectively. The production of growth regulators such as Auxin and gibberellin by *Azospirillum* bacteria (Fallik *et al.,* 1989) is an important mechanism to increase corn yield. In this study, corn seeds that had been treated with *Azospirillum* due to synthesis more growth hormones, had more growth and development. Plant hormone that had positive effects on cell deviation and sink physiological (Zhang *et al.,* 2005; Yang *et al.,* 2003) due to increase physiological sink size, synthesis photosynthesis components such as chlorophyll and solution protein and growth hormones such as IAA, had positive effects on photosynthesis potential of plant. On the other hand, the using of bio-fertilizers *Azospirillum* provided good conditions by converting nutrients such as nitrogen and phosphorus to available form for root growth and development.

Table 5: Correlation Coffenetic of studied traits.

	Chlorophyll$_a$	**Chlorophyll$_b$**	**Ear weight**	**Ear Length**	**Cob weight**	**seed number per row**	**1000 Grain weight**	**biological yield**	**grain yield**	**harvest index**
Chlorophyll a	1									
Chlorophyll b	0.82^{**}	1								
Ear weight	0.01^{ns}	0.12^{ns}	1							
Ear Length	0.20^{ns}	0.23^{ns}	0.19^{ns}	1						
Cob weight	0.37^{**}	0.29^{ns}	0.31^{ns}	0.21^{ns}	1					
seed number per row	0.30^{ns}	0.33^{ns}	0.29^{ns}	0.50^{**}	0.61^{**}	1				
1000 seed weight	0.06^{ns}	0.13^{ns}	0.37^{*}	-0.07^{ns}	0.26^{ns}	-0.54^{**}	1			
biological yield	0.31^{ns}	0.18^{ns}	0.37^{*}	0.37^{*}	0.06^{ns}	0.02^{ns}	0.33^{ns}	1		
grain yield	0.13^{ns}	0.18^{ns}	0.33^{ns}	0.23^{ns}	0.32^{ns}	0.50^{**}	0.61^{**}	0.25^{ns}	1	
harvest index	0.22^{ns}	0.05^{ns}	0.18^{ns}	0.19^{ns}	0.21^{ns}	0.29^{ns}	0.05^{ns}	-0.87^{**}	0.37^{*}	1

Ns, * and **: no Significant, Significant at 5% and 1% probability levels

So when we used *Azospirillum* Bactria with 200 mg/liter gibberellin hormone, 1000 seed weight, seed number per ear and seed yield were increased significantly and dramatically. (Khaleghi *et al.,* 2012) observed an increasing 1000 Grain weight, number of grains per ear, biological yield and grain yield in corn due to use gibberellin hormone.

The results of correlation coefficients showed that seed yield had significant and positive correlations with seed number per row (r = 0.50**) and 1000 seed weight (r = 0.61**). Since traits seed number and 1000 seed weight are the most components of cereal seed yield, so these results were expectable.

J. Harvest index

Harvest index is economic performance ratio to total dry matter production an indicator of the ability of plants to allocate resources between vegetative and reproductive structures (Carruthers, 2000).In this study, it was cleared thatinteraction effect *Azospirillum* × gibberellin had a significant effect on Harvest index at 1% probability level (Table 1). The results of means comparison showed that interaction effect of *Azospirillum* mixed with soil × no gibberellins (control) hormone had the highest harvest index (48.57%), while interaction effects of *Azospirillum* mixed with soil × 100mg/liter gibberellin by average 40.14percent had the lowest harvest index (Table 4). In this research between harvests index showed negative and significant correlation (r= -0.87**) with biological yield and positive and significant correlation (r = 0.37**) with grain yield (Table 5).

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