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Effects of *Azospirillum* bacteria and cytokinin hormone on morphology, yield and yield components of corn (*Zea mays* L.)

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Abstract

To investigate the effects of Azospirillum bacteria and cytokinin hormone on yield and yield components of corn, a factorial experimental based on RB design was carried out in the research farm of Miandoab agricultural office during 2014 growing season. Azospirillum bacteria in three levels (control 25g Azospirillum applying with seed and soil) and Cytokinin hormone in three levels (control, 100 and 200 mg/Liter) were evaluated. Variance analysis showed that the effect of Azospirillum bio-fertilize on Grain number and biological yield and the effect of Cytokinin hormone on all traits except stem diameter and grain yield were significant. Also, the interaction Azospirillum × Cytokinin had significant effects on plant height, dry stem weight, row number per ear, Grain number per row, 1000 grain weight and grain yield. Means comparison revealed that Azospirillum applied with grain had the highest number of grain in row, it also cleared that 100 mg/liter Cytokinin hormone had the best value in respect of traits dry stem weight, ear number per plant, row number per ear and 1000 grain weight. Means comparison of interaction effects also showed that azospirillum mixed with soil × 100 mg/liter Cytokinin hormone in compared with the other interactions had the highest value in traits stem diameter, dry stem weight, row number per ear, Grain number per row and grain yield.

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Introduction

Maize (*Zea mays L.*) have second rank after wheat in respect of production, and third rank after wheat and rice in respect of cultivation area (Fateh *et al.,* 2006). Iran have an appropriate weather condition, so this plant grows very good. In recent decades, because of the continuous increasing in demand and prices, farmers were forced to use intensive management methods that its aim is the increasing of crop production (Borgard *et al.,* 2008). In this agricultural system for the lack of nutrients in the soil, it used the overuse of chemical fertilizers that it not only increase the cost, but also destroy the balance of the nutrient, so the yield of crops would reduce (Siddiqui and Pichtel, 2008). Understanding the different methods of plant nutrition in order to optimize the using of climatic factors, farm management and agricultural inputs have an effective help to increase quality and quantity production and also reduce the using of chemical fertilizers. By selecting the correct methods of plant nutrition can not only protect the environment but also prevent the water pollution, soil erosion and conserve from biodiversity, as well as increase the efficiency of inputs (Beauchamp, 1986). Bio-fertilizers for its high compatibility with the environment and reduce the appling of chemical fertilizers not only reduce the negative effects of these fertilizers, but the using of Bio-fertilizers before, during and after environmental stresses, can moderate stress effects on plant and subsequently increase crop yield (Yar Mahmoodi *et al.,* 2010. Zahir *et al.* 2004) reported that the grain 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 grain 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. At last these researches reported that the bio-fertilizers had dramatically significant effects on all traits except harvest index. These researches suggested that the mix effect of Azotobactera and Azospirillum in compared with the control had the maximum number of days to ear emergence, days to maturity, leaf number above the ear, dry plant weight, ear length, plant height, stem diameter, row number per ear, grain number of row, biological and grain yield.

The recent years is done many efforts to improve conditions for germination and vigor of grains 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 (Faghi nabi, 2007). Plant growth regulators, chemical compounds that are used in small rate, cause that the plant growth and development be better (Muniralzaman, 2000). These materials used or seed treatment either leaves spraying. Plant growth regulators including internal and external components can regulate plant growth in different pathways. On the other hand, increasing or decreasing of plants growth related to plant growth regulators (Muniralzaman, 2000). Cytokinin, as a main hormone in the plant growth and development, effects on cell division, chloroplast development, differentiation of bud and root, stem meristem initiation, stress tolerance and aging of plant (Kuroha *et al.,* 2009). This hormone produced in some parts of plant which are meristem and cell division occurs still actively in them (Lahoti *et al.,* 2003). Shahba *et al,* (2008) studied the effects of different treatments on seed germination of Sahgrass plant and reported that concentrations 0.5 and 1 micro molar of Kinitin hormone increased the seed germination. Vaziri and Tavakoli (2013) observed an increasing in the number of grain and 1000 grain weight of spring Safflower plant due to use spraying Cytokinin hormone. Esa Zade *et al.* (2013) also found that the appling of Cytokinin hormone would increase grain number, 1000 grain weight and grain yield in safflower plant.

The purpose of this study was to evaluate the effects of azospirillum and Cytokinin on yield and yield components in corn (cultivar 370).

Materials and methods

View test

To investigate the effects of Azospirillum bacteria and Cytokinin 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 Miandoab 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 Cytokinin hormone in three levels (control, 100 and 200 mg/Liter) were evaluated.

Soil analysis

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 ppm of phosphorus and 295 ppm of potassium. The soil acidity was indicated at a pH of 7.79 at a depth of 0-30 cm.

Treatments

In this study, corn seeds were inoculated with azospirillum before planting and Cytokinin 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 m2. 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.

Traits yield and yield component

Data were recorded for traits chlorophyll index, the height of the first ear from the ground, dry leaf weight, ear weight, grain weight, Grain number per ear row, 1000 grain weight, biological yield and grain yield. Data analysis was done using SAS ver. 9.2 and SPSS ver. 21 programs.

Results and discussion

Plant height

Variance analysis showed that the effects of Cytokinin hormones and azospirillum & Cytokinin interaction on plant height were significant at the 1% probability level (Table 1). The results of the means comparison of cytokinin hormone on plant height showed that treatment 200 mg/liter cytokinin hormone had the highest height (220.48 cm). The results also cleared, this treatment in compared with control and 100 mg/liter cytokinin hormone increased the plant height 15.4 and 13.6% respectively (Table 2). These results were predictable, because the cytokinin hormone stimulates cell growth and development and subsequently increases the plant height. The interaction effects Azospirillum applied with seed × 200 mg/liter cytokinin hormone and no azospirillum (control) × no cytokinin hormone (control) with 224.86 and 177.8 cm respectively, had the highest and the lowest plant height, respectively (Table 4). In this study, it was cleared that the using of azospirillum was cussed that the growth of root and uptake of nitrogen and phosphorus would increase. Also the increasing of cell development and synthesis of growth hormones especially IAA were due to application of cytokinin hormone. So we can predict that the plant height will increase when we use azospirillum and cytokinin hormone with together. Schoenwitz and Ziegler (1986) reported that there was a direct relationship between the frequencies of Azetobacter in the Rhizosphere with the root growth of maize. In this condition, these bacteria produced Auxin, gibberellin and Cytokinin hormones two times more than normal condition.

Dry stem weight

The results of variance analysis showed that cytokinin hormone (at the 1% level) and interaction

effect azospirillum × cytokinin (at the 5% level) had the significant effects on dry stem weight. The means comparison results of cytokinin hormone showed that treatment 100 mg/liter cytokinin hormone had the highest weight (131.40 cm). The results also cleared, this treatment in compared with control and 200 mg/liter cytokinin hormone increased dry stem weight 14 and 9.4%, respectively (Table 2). On the other hand, the results showed that the interaction effects no azospirillum (control) × 100 mg/liter cytokinin and no azospirillum (control) × no cytokinin hormone (control) with 159.46 and 119.52 gr, had the highest and the lowest dry stem weight, respectively. According to the means comparison of interaction effects, it confirmed that the using of Azospirillum and cytokinin with together could not have a positive effect on dry stem weight.

Table 1. Variance analysis of studied traits.

		Mean square								
S. O. V	df	Plant height	Stem diameter	dry stem weight	ear Length	row number per ear	Grain number per row	1000 grain weight	biological yield	grain yield
Replication	2	423.03	0.06	23.06	0.39	0.69	0.83	1581.3	39.28	5.02
Azesprilium	2	181.6^{ns}	0.01^{ns}	31.41^{ns}	14.06**	0.85^{ns}	7.81*	476.30^{ns}	16.03*	2.96^{ns}
Cytokinin	2	3113.8**	0.01^{ns}	1277.5^{ns}	8.25**	1.35^{ns}	6.28*	9992.2**	104.84**	2.30^{ns}
Azesprilium×Cytokinin	4	421.9^{ns}	0.06**	360.98^{ns}	11.56**	1.84*	4.95*	627.27^{ns}	6.72^{ns}	3.33*
Error	16	169.7	0.01^{ns}	171.51	0.51	0.54	1.17	535.43	4.88	0.88
(%)CV	-	6.54	5.28	9.33	12.20	4.78	2.85	13.28	8.79	4.33

^{ns}, * and **: no Significant, Significant at 5% and 1% probability levels.

Stem diameter

In this study, it was cleared that interaction effect azospirillum × cytokinin had a significant effect on stem diameter at 1% probability level (Table 1). The results of means comparison showed that interaction effect azospirillum mixed with soil × 200 mg/liter cytokinin hormone had the highest stem diameter (2.64 cm), while interaction effects no azospirillum (control) × no cytokinin hormone (control) and azospirillum applied with seed × no cytokinin hormone (control) with 2.32 and 2.29 cm, respectively, had the lowest stem diameter (Table 4).

Table 2. Means comparison of Azesprilium Bactria on studied traits.

Azesprilium	Plant height (cm)	Stem diameter (cm)	dry stem weight (gr)	ear Length (cm)	Grain number (per row)	1000 grain weight	biological yield (t/ha)	grain yield (t/ha)
control	204.30a	2.48a	141.75a	20.94b	45.72b	177.25	37.75ab	21.25
Applied with Grain	196.8a	2.41a	140.94a	23.33a	47.16a	179.45	36.34b	21.42
Mixed with soil	196.27a	2.41a	138.19a	22.77a	47.72a	165.90	40.63a	22.32

*: Treatments with the same letter have not significant difference.

Ear length

The results of variance analysis showed that the treatments azospirillum, cytokinin hormone and their interactions 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 cytokinin hormone had an increasing effect on ear length (Table 3), as treatment 100 mg/liter cytokinin in compared with control and treatment 200 mg/liter cytokinin in compared with 100 mg/liter cytokinin and control, the ear length increased 3 and 5 and 8%.

The results of interaction means comparison also confirmed that treatment azospirillum mixed with siol with ear length 24.5 cm and treatment no azospirillum (control) × no cytokinin hormone (control) with ear length 18.2 cm had the largest and lowest value, respectively. In this study, it was cleared that trait ear length with traits plant height, leaf number, stem diameter, dry stem and leaf weight had significant and positive correlations.

Table 3. Means comparison of Cytokinin hormone on studied traits.

Cytokinin (mg/lit)	Plant height (cm)	Stem diameter (cm)	ear Length (cm)	Grain number per row	Grain number per row	1000 grain weight	biological yield (t/ha)	grain yield (t/ha)
0	186.48b	131.40b	21.50b	15.06b	46.58a	210.69a	39.83a	20.12
100	190.41b	153.80a	22.16ab	15.84a	45.92b	165.38b	33.74b	21.39
200	220.48a	145.66ab	23.38a	15.45ab	46.85a	145.97b	41.15a	22.11

*: Treatments with the same letter have not significant difference.

Row number per ear

In this study cleared that there were significant differences between cytokinin levels and interaction effects of azospirillum × cytokinin in respect of trait row number per ear at the 5% probability level. In between cytokinin levels, treatment 100 mg/liter with 15.84 had the highest row number per ear. So we can conclude this treatment provide better condition for more growth and using of environment condition. On the other hand, interaction effect no azospirillum (control) × 100 mg/liter cytokinin with 16.57 and no azospirillum (control) × no cytokinin hormone (control) with 14.05 introduced as the best and the worst treatments in respect of row number per ear (Table 4).

Table 4. Means comparison of interaction effects Azesprilium Bactria × Citoky nin hormone on studied traits.

Azesprilium	Cytokinin (mg/lit)	Plant height (cm)	ear Length (cm)	Stem diameter (cm)	dry stem weight (gr)	1000 grain weight	biological yield	grain yield (t/ha)
	0	177.86d		2.32b	119.52b	199.81ab	35.73ce	20.56cd
Control	100	200.7bc	18.02e	2.51ab	159.46a	168.70bc	37b	21.96ac
	200	186.2cd	22.16c	2.41ab	143.8ab	163.24be	40.53be	20.01d
	0	215.8ab	22.66bc	2.29b	132.7ab	221.72a	10.10be	21.43bd
Applied with Grain	100	210.7ab	23.34ac	2.48ab	156.7ab	164.25bc	31.66e	21.52bd
	200	224.86a	23.34ab	2.47ab	135.7ab	152.39cd	37.26	21.30bd
	0	177.8d	23bc	2.43ab	141.9ab	211.33a	43.66ab	22.62ab
Mixed with soil	100	192.6bs	23.17bc	2.41ab	145.2ab	160.07bc	32.56de	23.18a
	200	194bd	20.65d	2.60a	127.4ab	122.29d	45.66a	22.38ab

*: Treatments with the same letter have not significant difference.

Grain number in row

The results of variance analysis showed that treatments azospirillum, cytokinin and their interactions had significant effects on the Grain number in row at 5% probability level (Table 1). The results of means comparison showed that treatment azospirillum applied with seed and mixed with soil with 47.16 and 47.72, respectively and control with 45.72 had the best and the lowest value in respect of Grain number in row (Table 2). This result emphasized that the application of cytokinin effected on Grain number in row, not how to use it. On the other hand, azospirillum enzyme 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.,* (1388) reported that organic fertilizers, especially bacteria that facility the absorption of phosphorus, increased the Grain number in barley. The results of means

comparison showed interaction effects azospirillum mixed with soil × 100 and 200 mg/liter cytokinin with 48.9 and 48.52 had the highest Grain number respectively. On the other hand treatment no azospirillum (control) × 200 mg/liter cytokinin had the lowest Grain number in row (44.8). 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 Grain number in row. In this study, trait Grain number in row had significant positive correlations with traits plant height and leaf number. A higher corn plant had a longer ear, so we state that there is a significant and positive relationship between Grain number and 1000 grain weight.

1000 grain weight

The results of analysis variance showed that treatments cytokinin (at 1% probability level) and azospirillum × cytokinin had significant effects on 1000 grain weight (Table 1). In this study, 100 mg/liter cytokinin with 210.69 gr and control with 145.97 gr had the height and the lowest 1000 grain weight, respectively. On the other hand, there wasn't a significant difference between 100 and 200 mg/liter cytokinin levels in respect of this trait, but these treatments in compared with control increased 1000 grain weight, 30 and 21% respectively (table 1). Cytokinin hormone by increasing the rate of cell division in the seed caused that seed storage cells would increase and subsequently 1000 grain weight would improve (Taiz and Zeiger., 2006). Saeidi *et al* (1385) reported that cytokinin hormone increased Grain number and 1000 grain weight in wheat, that these results confirmed our results. The results of means comparison showed that interaction treatment azospirillum applied with seed × 100 mg/liter cytokinin hormone had the height 1000 grain weight (221.72 gr). On the other hand interaction treatment azospirillum mixed with soil × 200 mg/liter cytokinin hormone had the lowest 1000 grain weight (122.29 gr) (Table 4). It was notable in this study that interaction effect 100 mg/liter Cytokinin hormone with all levels of azospirillum had more 1000 grain weight in compared with other treatments. So we can conclude these treatments have provided good conditions in respect of the absorption of water and nutrients, synthesized growth hormones and translate photosynthesis materials, and subsequently improved 1000 grain weight. In this study, 1000 grain weight had significant positive relationships with traits plant height and leaf number and significant negative relationships with traits ears number per plant, row number per ear and Grain number in row. On the other hand, it was cleared that the corn plants with more height had more storage photosynthetic materials in their stems, so these plant can have more remobilization of photosynthetic materials to seed and subsequently improve 1000 grain weight, especially in the bad conditions and environment stress (heat and water stress) at the end of growth season. Typically, with increasing in traits the ear number per plant, row number per ear and Grain number per row, the competition for absorption photosynthetic materials in tanks will increase and subsequently would decrease 1000 grain weight.

Biological Yield

The result of variance analysis showed that Azospirillum had a significant effect on biological yield at 5% and 1% probability levels (Table 1). The means comparison results also cleared that 25 g Azospirillum with 40.63 t/ha and 8 gr Azospirillum with 36.34 t/ha biological yield had the highest and the lowest biological yield, respectively (Table 1). 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. In this study, it was cleared treatments 200 and 100 mg/liter Cytokinin hormone

with 45.41 and 33.74 t/ha, respectively, had the highest and lowest levels of the biological yield (Table 2).

Grain Yield

When we are speaking about quantitative traits, grain yield is the most important trait. Several factors may effect on grain yield which between them environmental conditions and nutrition are the most important factors. If there is no coordination between dietary nutrients, with increasing in using of nutrients, grain yield not only does not improve but also reduces (rule *et al.,* 1386). The results of variance analysis showed that treatments Azospirillum, cytokinin and their interaction effect at 1%, 5% and 1% probability level respectively, had significant effects on grain yield (Table 1). The result of means comparison showed that treatment Azospirillum mixed with soil with 22.32 t/ha had the highest grain yield between studied treatments, while it didn't have significant difference with treatment Azospirillum applied with seed. Between these treatments, The lowest yield (21.25 t/ha) belonged to control (no Azospirillum, Table 2). 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. In between levels of cytokinin hormone, the highest and lowest grain yield belonged to 100 mg/liter cytokinin (22.22 t/ha) and control (21.13 t/ha), respectively (Table 3). Riou-Khamlichi *et al.,* (1999) believed that cytokinin hormone by increasing of the expression of genes which product saikelin tape d and subsequently stimulate and accelerate cellular division, increased yield and yield components. Also, the cytokinin hormone with increasing to translate sugars from phloem to apoplast (Ehneß and Roitsch, 1997) and stimulate stored sugar in vacuoles (Ying *et al.,* 1999) caused to provide more photosynthesis substances for the seed which growing, and subsequently increase Grain number and grain weight and finally grain yield. Vaziri and Tavakoli (2012) studied effect of foliar application of cytokinin hormone on grain yield in safflower. These researchers reported that the foliar application of cytokinin hormone in compared with control increased grain yield significantly. On the other hand, Saeidi *et al.,* (2006) with evaluation of the foliar application of abscisic acid and Cytokinins at different stages of wheat growth reported that the spraying of Cytokinin hormone can increase grain yield significantly. Among interaction effects, azospirillum mixed with soil × 100 mg/liter cytokinin with 23.18 t/ha and no azospirillum (control) × 200 mg/liter cytokinin cytokinin with 20.01 t/ha had the highest and the lowest grain yield, respectively. The production of growth regulators such as auxin and Cytokinin 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. It was notable that the high levels of Cytokinin hormone can also play a role in plant growth inhibition. But when we used azospirillum mixed with soil, it was provided less biological fertilizer for corn plant, and subsequently the synthesis of these hormones reduced in plant. At these conditions, the use of Cytokinin hormone stimulated plant growth and development significantly and dramatically. In this study, it was cleared that interaction effects 100 mg/liter Cytokinin hormone with all levels of azospirillum in compared with the other treatments had the higher grain yield. Cytokinin hormone that had positive effects on cell deviation and physiological tank (Zhang *et al.,* 2005; Yang *et al.,* 2003) due to increase physiological tank 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. So when we used Azospirillum Bactria with 100 and 200 mg/liter

Cytokinin hormone, 1000 grain weight, Grain number per ear and grain yield were increased significantly and dramatically. The results of correlation coefficients showed that grain yield had significant and positive correlations with traits row number per ear, Grain number per row, stem diameter, 1000 grain weight and dry stem weight. Since traits Grain number and 1000 grain weight are the most components of cereal grain yield, so these results were expectable.

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