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Response of Phenological, Morphological Traits and Crop Production to Apply Different Level of *Azospirillum* and *Azotobacter* of Maize Genotypes

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RESEARCH ARTICLE

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ABSTRACT

BACKGROUND: Organic farming has emerged as an important priority area globally in view of the growing demand for safe and healthy food and long term sustainability and concerns on environmental pollution associated with indiscriminate use of agrochemicals.
OBJECTIVES: This study was done to determine effect of different level of biofertilizer on phenological, morphological and seed yield characteristics of corn genotypes.
METHODS: Current research was conducted as split plot in randomized complete bock design with three replications in Payamnoor university of Ilam in 2009-2010. Treatments were cultivar (SC604, SC704 and SC807) as main plots and rhizobacteria (non- inoculation, inoculation with *Azetobacter*, *Azospirillum* and dual inoculation of *Azotobacter* and *Azospirillum*) as subplots.
RESULT: The effect of cultivar on day to maturity, plant height, stem diameter, dry to matter and grain yield was significant. SC 704 had the highest, day to maturity, plant height, dry to matter and grain yield. The effect of plant growth promoting rhizobacteria on all traits was significant. Dual inoculation of *Azotobacter* and *Azospirillum* had the highest ear initiation day to ear, day to maturity, plant height, stem diameter, dry to matter and grain yield. Obtained results show that double- inoculation was the most effective treatment in all cultivars. Other effective treatment were *Azotobacter* and *Azospirillum* inoculation, separately This is indicated that using growth prompting fertilizers as double- inoculation caused to increasing maize yield through synergistic effects by improving growth prompting hormones, controlling pathogenesis and growth reducing agents due to producing fungicide antibiotics and compounds (antagonistic effect) and also air molecular nitrogen fixing and also producing growth prompting hormones such as oxine, cytokenine and giberlines and solving mineral compound.
CONCLUSION: The highest and lowest grain yield obtains from SC 704 with dual inoculation of *Azotobacter* and *Azospirillum* and SC 604 with non-inoculation treatment, respectively. Also studied cultivars showed different responded to most parameters.

KEYWORDS: *Biofertilizer, Corn, Dry weight, Grain yield, Nutrition.*

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1. BACKGROUND

Chemical fertilizers have several negative impacts on environment and sustainable agriculture. Therefore, bio-fertilizers are recommended in these conditions and growth prompting bacteria uses as a replacement of chemical fertilizers (Wu *et al*., 2005). Chemical fertilizers are significant to succor nutrients in soil. Heavy doses of chemical fertilizers and pesticides are commonly used in order to enhance corn yields. Excessive nitrogen content in soil causes an inappropriate high uptake of this macronutrient by plants, which may result in inadequate growth and development due to the accumulation of nitrogen compounds in plant tissue (Szulc, 2013). In organic agriculture, one management goal is to increase and maintain soil quality with a high biological activity. Organic cropping system often has to deal with a scarcity of readily available nutrients in contrast to high input cropping system which relies widely available on soluble fertilizers (Soleimanzadeh and Ghooshchi, 2013). Zhang *et al*. (2019) reported Organic fertilizer application significantly increased soil organic carbon content and enzyme activities, root length density down the soil profile, plant biomass and nutrient uptake compared to the lime treatment and the control. Growth promoting bacteria induced increasing plant yield as clone in plants root (Gholami *et al*., 2009). Growth prompting bacteria are including *Azotobacter*, *Azospirillum* and *Pseudomonas* (Zahir *et al*., 2004; Turan *et al*., 2006). Tilak (1992) reported positive effects of double- inoculation of *Azotobacter* and *Azospirillum* on dry matter of maize and sorghum. Nouraki *et al*. (2016) reported bacteria have positive role in the production of bio-fertilizers and hormones which play a significant role in regulating plant growth while mixing them with chemical fertilizers as a supplement the level and depth of the roots. This combination also increases the rate of water and nutrient absorbance which raise the rate of growth and photosynthesis. These combination also increase the grain yield, yield components, and biological function, it has been found that bio-fertilizers can be combined with chemical fertilizers in a complementary way to reduce the excessive amount of chemical fertilizers used to grow corn. It was shown that the mixing of biological fertilizers with chemical fertilizers could reduce the needs of chemical fertilizers up to 25% and these results are comparable to the application of 100% chemical fertilizers. Therefore, the best hybrid maze is the single cross 704 that has good yield potential when the chemical fertilizer is used at either 25% or 50% of the current application when mixed with the bio-fertilizer. Rai and Caur (1998) studied *Azotobacter* and *Azospirillum* and double-inoculation and alone inoculation effects on wheat growth and yield. Double- inoculation of *Azotobacter* and *Azospirillum* had positive effects on plant height, spike length, grain yield, biological yield and harvest index in various wheat genotypes. It is proved that hormones such as oxine, giberline and cytokenine are synthesized by many *Azotobacter* spp (Sing *et al*., 2004). In-

deed, improving plant growth due to *Azotobacter* inoculation is contributed to produce hormones by this bacteria and improving root growth (Zaied *et al*., 2003). Nieto and Frankenberger (1991) also observed being 5 times dry weight of aerial part of maize plant using *Azotobacter* bacteria.

2. OBJECTIVES

This study was done to determine effect of different level of biofertilizer on phenological, morphological and seed yield characteristics of corn genotypes.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

In order to investigate the effect of *Azotobacter* and *Azospirillium* on morphological traits and grain yield of maize cultivars, an agricultural experiment was conducted in 2013 in Ilam Technical and Vocational Training Center as split plots in a randomized complete block design with 3 replications. Experimental treatments including three single cross corn cultivars 704, 604 and 804 in main plots and biological fertilizers at four levels including inoculation with *Azospirillum*, inoculation with *Azotobacter*, inoculation with mixed bacteria of Azotobacter and Azospirillum and without inoculation (control) it was on the subplots.

3.2. Farm Management

The test site was 33 degrees and 47 minutes north latitude and 46 degrees and 36 minutes east longitude with an altitude of 975 meters above sea level. The study area has an average annual rainfall of 452 mm, average annual temperature of 18.9 °C, maximum and minimum absolute annual temperature of 42.2 and -8.6 °C, respectively. The average monthly temperature, rainfall and relative humidity in the experimental years are presented in table 1. The physical and chemical properties of the soil at the test site are presented in table 2. Each experimental plot consisted of 6 rows at a distance of 75 cm and a length of 8 m.

Table 1. Monthly mean value of precipitation and temperature in, Ilam 2013

Parameter	Apr.	May	Jun.	Jul.	Aug.	Sep.
Precipitation (mm)	98.56	77.44	0	0	10.11	-
Temperature (◦C)	12	15.3	25.8	27.2	25.1	-

Table 2. Soil physical and chemical properties of experimental area

Soil texture	Available P (mg kg^{-1})	Available K (mg kg^{-1})	Total N (%)	Organic Carbon (%)	EC ($dS.m^{-1}$)	pH
clay loam	1.09	220	0.08	0.66	1.09	7.05

Seed sowing operations were carried out on May 30, 2013. The amount of nitrogen and phosphorus chemical fertilizers based on soil test for all treatments in the field was 300 k .ha^{-1}, 150 kg of superphosphate and 100 kg of potash sulfate. For inoculation of seeds, the amount of seven grams of inoculum, each gram of which contained 108 live and active bacteria, was moistened with

sugar water at a concentration of 20% and in the ratio of 2 kg of inoculum per 100 kg of seeds with seeds and was used. After soaking the seeds with *Azospirillum* and *Azotobacter*, the container was rotated for a few minutes so that the inoculum with the help of sugar water solution well covered the surface of the seeds (inoculation as seed). The treated seeds were placed in the shade on a clean surface for ten minutes to dry and were ready for planting.

3.3. Measured Traits

During the period, phenological traits such as number of days to emergence, number of days to emergence of ear, number of days to maturity were recorded. The final harvest area of each plot was 1.5 m^2. Seed yield, its components and qualitative traits were estimated after the physiological maturity. After separating seed from selected plants and weighing them, seed yield was calculated based on 14% moisture. To estimate 100 seed weigh, 10 samples of seed containing 10 seed were separated and means was calculated.

3.4. Statistical Analysis

Analysis of variance and mean comparisons were done via MSTAT-C software and Duncan multiple range test at 5% probability level.

4. RESULT AND DISCUSSION

The results of analysis of variance for the studied traits in this experiment showed that the effect of genotypes on all traits (except the number of days to emergence and the number of days to ear emergence) had a significant effect. Also effect of Bio-fertilizer on all measured traits was significant. But interaction effect of genotypes and Bio-fertilizer only on days to ear emergence and grain yield was significant at 5% and 1% probability level, respectively (Table 3). Comparing the mean genotypes for the number of days to maturity, it was observed that single cross cultivar 704 had the highest number of days to maturity (Table 4). Comparison of the mean effects of rhizobacterial fertilizers on the number of days to emergence showed that the lowest number of days to emergence belonged to inoculated bacteria and its value relative to the number of days to emergence in control treatment (no inoculation) 4 days are reduced (Table 5). Bacteria used in this experiment accelerated seedling emergence in the field by producing growth-promoting hormones, secreting indole-3-acetic acid, and biocontrolling seedling diseases, thereby reducing the number of days to emergence of corn genotypes. Hafeez *et al*. (2004) also reported faster growth of seedlings of cotton cultivars due to inoculation of seeds with various growth-promoting bacteria. As can be seen in table 5, the inoculation treatment of both bacteria with 115 days has the highest number of days to maturity.

Table 3. Analysis variance of measured parameters

S.O.V	df	Days to emergences	Days to ear emergence	Days to maturity	Plant height	Stem diameter	Plant dry weight	Grain yield
Replication	2	53.08	4.33	32.86	2377	0.34	5952	1595782
Genotypes (G)	2	5.25 ^{ns}	0.75 ^{ns}	114.6**	1105.05**	0.743**	29038**	2462452**
Error a	4	3.33	6.08	6.48	107.16	0.037	2909.6	1272157
Bio-fertilizer (B)	3	20.47**	40.17*	47.2**	2936.2**	0.834**	6206**	6158113**
G×B	6	1.58 ^{ns}	3.56*	3.4 ^{ns}	95.2 ^{ns}	0.062 ^{ns}	514.1 ^{ns}	373479**
Error b	18	0.47	0.46	2.8	54.7	0.042	439.1	39994
CV%	-	12.08	7.16	7.14	6.9	9.16	11.89	16.8

^{ns}, * and ** represent not significant and significant at 5% and 1% probability levels, respectively.

Table 4. Effect of cultivar on mean of phonological and morphological traits

Genotypes	Days to emergences	Days to ear emergence	Days to maturity	Plant height (cm)	Stem diameter (cm)	Plant dry weight (g.m^{-2})	Grain yield (kg.ha^{-1})
SC704	9.6 a*	68.5 a	115.3 a	201.1 a	1.95 b	259.5 a	10850a
SC604	10.6 a	67.7 a	109.3 b	184.5 b	2.33 a	181.8 ab	8323 b
SC804	10.9 a	70 a	133.3 ab	183.4 b	2.42 a	168.4 b	8417 b

*Mean which have at least once common letter are nit significant different at the 5%level using (DMRT)

Therefore, it can be said that inoculation of corn seeds with Azospirillum and Azotobacter in combination with the production of plant growth stimulating hormones and increase the efficiency of root absorption improves plant development and ultimately prolongs the maturation period of the plant. The results of comparing the mean of cultivars showed that single cross 704 had the highest plant height (Table 4). It seems that the single cross 704 has been able to gain more height due to its longer growth period. Comparison of the mean stem diameter for growth rhizobacterial fertilizers shows that the maximum stem diameter in simultaneous inoculation of two type bacteria is 2.57 cm (Table 5). Among the studied genotypes, the highest plant dry weight belongs to single cross cultivar 704 (Table 4). Therefore, it can be said that single cross 704 with higher dry weight production capacity compared to other genotypes, had a higher photosynthetic potential. Therefore, higher dry weight can be a guarantee to increase grain yield, because the photosynthetic material produced is transferred to the grains. Also Bahamin *et al.* (2014) showed that when seeds were in inculcation by Nitroxin biologic fertilizer seed yield reached 3840 kg per hectare, showing 28% increase compared to non-inculcation treatment. Seed yield of cereals is determined by two main components, seed number per unit area and mean seed weight. Seed yield is usually strongly associated with the number of seeds per unit area (Azimi *et al.,* 2013). Examination of the average plant dry weight at different levels of rhizobacterial fertilizers showed that seed inoculation treatment with bacteria of both sex-

es *Azotobacter* and *Azospirillum* produced the highest amount of plant dry weight, which is 25% higher than the lowest plant dry weight (treatment without inoculation) (Table 5). The results show that co-inoculation can enhance the synergistic relationship between Azotobacter and Azospirillum bacteria.

Table 5. Effect of Azospirillum and Azotobacter on mean phenological, morphological and grain yield traits

Bio-fertilizer	Days to emergences	Days to ear emergence	Days to maturity	Plant height (cm)	Stem diameter (cm)	Plant dry weight ($g.m^{-2}$)	Grain yield ($kg.ha^{-1}$)
Azotobacter	9.5 c*	69.2 b	112.9 b	185.6 b	2.35 ab	196.6 b	9448 b
Azospirillum	11.2 b	68.4 b	112.2 bc	193.9 b	2.15 bc	197.2 b	8907 c
Azot+ Azos	7.6 d	71.2 a	115.4 a	212.4 a	2.57 a	240.4 a	10190 a
Non-inoculation	13.2 a	66.1 c	109.9 c	168.6 c	1.86 c	178.7 b	8240 d

*Mean which have at least once common letter are nit significant different at the 5%level using (DMRT)

The production of acetic acid by Azotobacter bacteria as well as the production of auxins and gibberellic acids by azospirillum can justify the increase in dry weight of the corn plant. As can be seen in Table 2, the highest grain yield belonged to single cross 704. It seems that high grain yield and morphological traits in single cross cultivar 704 are due to the long phenological period of this genotype and the optimal use of environmental factors. Comparison of means between rhizobacterial fertilizers for grain yield showed that simultaneous inoculation treatment of both bacteria with a yield of 8894 $kg.ha^{-1}$ is at the highest level that increased the inoculation treatment of Azotobacter and Azospirillum compared to the control (no inoculation) 20 shows the percentage (Table 5). An important reason that can increase the impact of bio-fertilizers for Plant height this is that use of fertilizers leading to increased internodes length and it can stimulate the production of plant hormones produced by these fertilizers (Hassanpour *et al*., 2011). Inoculation with two bacteria, Azotobacter and Azospirillum, was more effective. Examination of the average interaction of cultivar in rhizobacterial fertilizers revealed that single cross 704 cultivar under seed inoculation treatment with bisexual bacteria produced the highest grain yield with 12320 $kg.ha^{-1}$ (Fig. 1). Due to producing plant hormones, bio-fertilizer, through stimulating cell division, increase the reservoir capacity in plant and develop the root and provide conditions for nutrients uptake lead to increase of photosynthesis, when plant approaches to maturity stage, it transfers assimilates into reproductive seeds (Jorfi, 2014). Some Researcher suggested integrated nutrient management strategies involving chemical fertilizers and bio-fertilizers enhance sustainability of crop production. Integrated plant nutrient management is the combined use of mineral fertilizers with organic resources such as cattle manures, crop residues, urban/rural wastes, composts,

green manures and bio-fertilizers (Kemal and Abera, 2015).

5. CONCLUSION

Obtained results show that double-inoculation was the most effective treatment in all cultivars. Other effective treatment were *Azotobacter* and *Azospirillum* inoculation, separately is indicated that using growth prompting fertilizers as double- inoculation caused to increasing maize yield through synergistic effects by improving growth prompting hormones, controlling pathogenesis and growth reducing agents due to producing fungicide antibiotics and compounds and also air molecular nitrogen fixing and also producing growth prompting hormones such as oxine, cytokenine and giberlines and solving mineral compound. Also studied genotypes showed different responded to most parameters. SC704 genotype obtained highest yield.

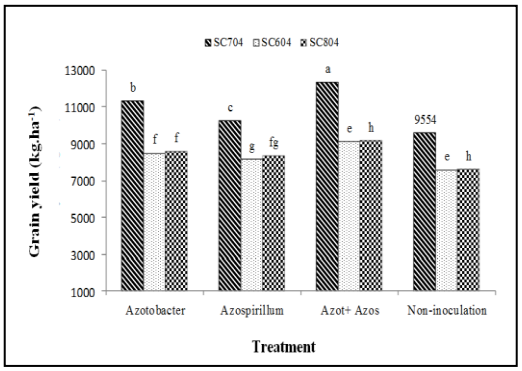

Fig 1. Interaction effects *Azotobacter*, *Azospirillum* inoculation and genotypes on grain yield via Duncan test at 5% probability level.

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