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RESPONSE OF FIVE VARIETIES OF WHEAT (*TRITICUM ESTIVUM* L.) TO LOCAL ISOLATES OF AZOSPIRILLUM BACTERIA (*AZOSPIRILLUM*)

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Abstract

This study included isolation four isolates of Azospirillum spp., that nitrogen fixation from rhizosphere soil and the roots of some cultivated crops in Al-Muthanna province, the species were identified by studying their biochemical, phenotypic and microscopic characteristics, the results showed that 3 isolates were of type A. lipoferum and one isolate of type A. brasilense, depending on the efficiency of the isolates in the nitrogen fixation and its negative impact to denitrification two isolates (A1, A2) were selected it belongs to A. lipoferum. They were used as inoculants to supplement the wheat requirements of nitrogen to study the response of wheat plant variety to inoculation with selected isolates was studied in the traits of growth, yield and some of its components for five varieties of wheat (Abu Ghraib, Bora, Sectira, Rasheed and IPA 99).The experiment included two treatments: the treatment of inoculants (A1, A2), while the second treatment included five varieties of wheat mentioned above. The field experiment was carried out by using completely randomized block design (CRBD) with three replications. The results showed the superiority of isolation A2 in all studied traits, while Abu Ghraib variety recorded superiority in most traits studied. The results showed a significant Interaction between the varieties and the bacterial inoculants. The combination (Abu Ghraib × A2) gave the highest mean and most of the studied traits showed the chlorophyll content, the leaf area of the flag and the grains yield and grains content of nitrogen, phosphorus and potassium (%) (Spad 58, 31.27 cm^2, 2.96 µg -1, 2.79%, 0.48% and 1.96%) respectively.

Key words : Bio-fertilization, *Azospirillum* spp., wheat.

Introduction

The wheat crop (*Triticum aestivum* L.) is one of the most important crops in terms of food and ranks first among cereals in the world and in Iraq in terms of importance and cultivated area, which is one of the most common crops (Shafshaq and Aldababi, 2008), wheat is also an essential source of human and animal nutrition and is a source of essential amino acids, minerals, vitamins, beneficial phytochemicals and fiber (Shewry, 2009). Due to the pollution of the environment, many countries in the world have resorted to the use of bio-fertilizers to increase crop production at the lowest possible cost. Bio-fertilizers include many microorganisms, such as Azospirillum, which live with the roots of the cereals plant as a bacterial inoculants to plant supplement the nutrients with some of their nitrogen requirements as well as it produces some hormones, such as: Indole acetic acid, Gebrilins and cytokines, which work on the growth and development of the root, which increases the absorption of water and nutrients, by the root thus reducing production costs and reducing environmental pollution. Azospirillum bacteria have been isolated from different geographical regions of the world as they are found in most of the Iraqi soils (Al- Sameria, 2007), it is the most efficient nitrogen fixation among nitrogen-fixing organisms. Which are associated with the roots of wheat plants.

Many studies have shown that the plant hormones (IAA), Indol-3-acetic acid, produced by Azospirillum, stimulate plant growth to stimulate root growth (Akbari

Table 1 : Some chemical and physical properties of soil before planting.

pH1: 1	Ece1 : 1 ds.m^{-1}	g Om. kg^{-1}	N mg.kg^{-1}	P mg.kg^{-1}	K g.kg^{-1}	Clays	Silt	Sand	Total bacteria cfug^{-1}
						g. kg^{-1}			
8.1	4.6	3	12.4	16.2	138.1	490	345	165	2.6×10^5
							Clay soil		

et al., 2007). It stimulates additional root hairs and lateral roots. The effect of Azospirillum on the root hairs and the lateral roots are not only attributable to the IAA, but also to the production of other hormones such as Gibberellins or cytokines.

Nitrogen-fixed has the ability to increase nutrient levels in the soil, improve water and nutrient uptake and help these organisms increase soil fertility, and resist agricultural environment problems.

The aim of this study is to isolate and identified Azospirillum bacteria present in the soil of the rhizosphere and the use of efficient isolates and the use of efficient isolates in the nitrogen fixation as inoculants for wheat plants to improve its growth and productivity and know the best variety of wheat in response to this agriculture which gives the highest yield.

Materials and Methods

Four samples of soil were collected from four agricultural areas in Al-Muthanna Governorate. After dilution of the samples with sterile water to the fifth dilution, these samples were placed in Nfb container tubes containing blue bromothymol dye and incubated at 28°C for 24-48 hours, by studying some of the phenotypic traits and growth potential of these isolates in some agro-organisms and biochemical properties and their susceptibility to the consumption of certain carbohydrates as sources of carbon, when comparing these characteristics with the characteristics of the species of *Azospirillum* species, it can be concluded that isolation A1 belongs to A. brasilense and isolates A2, A3, A4 belong to type A. lipoferum, each possessing characteristics of the characteristics of that species.

Bacterial inoculants preparation and its use as bio-fertilizers

Two isolates of the Azospirillum bacteria, A. lipoferum, were selected for use in the field experiment, as they were the most efficient in the nitrogen fixation. In order to prepare a sufficient amount of inoculation for the field experiment, conical flasks of 250 ml containing (100 ml)of liquid culture media were prepared, after sterilization, each was Inoculated by adding 1 ml of the liquid media using sterilized pipettes. These flasks were incubated at a temperature incubator (30°C) for 2-3 days. The density of the bacteria was estimated using the method of the dilutions and counting according to Black (1965). The density of the used inoculation (2.5×10^7) was colony formation unit.ml^{-1}.

Inoculation of seeds

After the liquid inoculants was prepared and mixed with the peat moss, the five varieties of wheat plant were pollinated by inoculated Peat moss after its sterilization, and the addition of gum arabic with a concentration of 10%, to increase the adhesion of bacteria with wheat seeds, it was left for an hour and a half to ensure that all the seeds were contaminated with bacteria and a number of seeds were placed without inoculation.

Field experience

The field experiment was carried out to study the effect of inoculation of Azospirillum bacteria and their interaction with wheat varieties, the field experiment was carried out by using completely randomized block design (CRBD) with two replications in 2016/12/8, using two factors: bio-fertilization, including two isolates of the bacterium Azospirillum that coded (A1 and A2), the second factor included five varieties of bread wheat (Abu Ghraib, Bora, Sectira, Rasheed and IPA 99) that coded) M1, M2, M3, M4, M5, respectively which was sourced from the General Authority for Agricultural Research,. The experiment was carried out with three replicates of experimental units: 3 x 5 x 3 = 45 experimental units. Five varieties of wheat seeds of the inoculated and non-inoculated were planted within the experimental units with the seeds quantity of 120 kg.ha^{-1}, and phosphate fertilizer was added at a quantity of 120 kg P. ha^{-1} at one time of cultivation in the form of triple superphosphate fertilizer. The potassium fertilization process was carried out with 80 kg K. ha^{-1} in the form of potassium sulphate at one time at cultivation.

The studied traits of the wheat crop are growth characteristics (cm), chlorophyll content, flag leaf area (cm^2), weight of 1000 grains (g), grains yield (1 µg), protein in grains (%). Nitrogen (%), phosphorus (%) Potassium (%) in grains.

Results and Discussion

Chlorophyll content (SPAD)

The results of the statistical analysis of table 2 showed significant differences in the inoculation of Azospirillum isolates in the chlorophyll content, the isolates A1 and A2 were the most efficient, giving the highest means of 48.6 and 53.2 SPAD sequentially, in comparison to the measurement of A0, which gave the lowest mean of 40.0 SPAD. The reason of this increase is the use of bio-fertilizers, especially the use of Azospirillum bacteria for the production of hormones, absorption of nutrients and water during different stages of growth and the isolate of A2 on isolation A1 increased by 9.47%, also the isolate A1was superior to isolate A2 with an increase of 9.47%.

The results of table 2 indicate that there were significant differences between the varieties in the chlorophyll content and Abu Ghraib is superior to other varieties, gave the highest mean of the chlorophyll content of 52 SPAD, whereas the two varieties did not differ significantly in this trait, they recorded means of 49.6 and 47.67 SPAD sequentially, it may be due to the difference in the varieties in this trait to a difference in the nature of the growth of varieties and their response to the environmental conditions prevailing in the experiment area, this result was agreed with Al-Rifaei (2006), Al-Haidari (2009) and Mohammed (2011) results, about the different varieties of wheat among themselves in the chlorophyll content because of their differences in the genetic structure and its response to environmental conditions.

While the interaction showed the superiority of the combination (Abu Ghraib × A2) significantly on all other combinations and gave an mean of 58 SPAD, did not differ significantly with the combination (Bora × A2), with a mean of 55 SPAD as shown in table 2, while the combination (IPA 99 × control treatment) gave the lowest mean of 35 SPAD, this may be due to variability in the response of varieties to the previous yield and soil nutrient content (Bakht, 2009).

Flag leaf area (cm^2)

The results of table 3 showed that inoculation with the bio-fertilizer of Azospirillum had a significant effect on the increase in the flag leaf area compared to the non-addition of bio-fertilizer (control treatment), also the results of the table indicated the superiority of isolate of A2 was higher, with the highest rate in of the flag leaf area of 26.23 cm^2, with an increase of 49.12% was recorded higher than the control treatment that recorded 17.59 cm^2, while isolate A1 recorded an increase rate of 37.35% was recorded higher than the control treatment.

Table 2 : Effect of inoculation with local isolates of Azospirillum bacteria and varieties of wheat and their interaction in chlorophyll content of wheat plant (SPAD).

N / A	M1	M2	M3	M4	M5	Mean
A0	45	41	41	38	35	40.0
A1	53	51	49	47	43	48.6
A2	58	55	53	51	49	53.2
Mean	52.00	49.00	47.67	45.33	42.33	

LSD0.05 A = 1.885 M = 2.434, A*M = 4.215.

Table 3 : Effect of inoculation with local isolates of Azospirillum bacteria and varieties of wheat and their interaction in flag leaf area (cm^2).

N / A	M1	M2	M3	M4	M5	Mean
A0	26.67	18.17	19.40	26.10	21.63	17.59
A1	28.80	19.97	21.17	29.13	21.73	24.16
A2	31.27	22.50	23.43	30.40	23.57	26.23
Mean	28.91	20.21	21.33	28.54	22.31	

LSD 0.05 A = 0.183 M = 0.237, A*M = 0.410.

Table 4 : Effect of inoculation with local isolates of Azospirillum bacteria and varieties of wheat and their interaction in weight of 1000 grains (g).

N / A	M1	M2	M3	M4	M5	Mean
A0	27.66	29.27	30.31	32.26	33.65	30.65
A1	29.72	31.53	32.86	34.38	32.78	32.78
A2	32.52	34.65	35.72	37.23	35.70	35.70
Mean	29.97	31.82	34.62	34.62	35.84	

LSD 0.05 A = 0.243 M = 0.0.315, A*M = 0.543.

The superiority of bio-fertilizer may be attributed not only to increase its nutrient supply, but also to obtain a plant has no disease and is resistant to it (Bashan and de-Bashan, 2005) pointed out that bio-fertilization leads to increased resistance of plants to diseases and activate the role of biological control through the production of antibiotics, which have a role in the resistance to diseases, and the mechanisms of biological control of diseases is the ability of these inoculants to analyze some of the spin-offs that are harmful to the plant,so the use of microbial inoculants as natural enemies of some of the pathogens that affect the plant, which reflected positively on the various growth features, including the flag leaf area.

Abu Ghraib variety gave the highest mean of the flag leaf area of 28.91 cm^2 (table 3), which did not differ significantly from the variety Rasheed, which gave a mean of 28.54 cm^2, while the variety Bora was recorded of 20.21 cm^2, perhaps the reason for the superiority of both Abu Ghraib and Rasheed varieties in this capacity is that they took advantage of their genetic and physiological abilities to achieve the requirements of growth better than the other varieties reflected in the increase in the flag leaf area, this result agreed with the results of both Kadum (2006), Al-Muhammdy (2010) and Al-Jumeily (2011), who found in their study to different varieties of wheat in the flag leaf area.

The results (table 3) showed that there was a significant effect of the interaction between the varieties and the biological fertilization, the combination (Abu Ghraib × A2) gave the highest mean of this trait of 31.27 cm2, while the combination (Bora × control treatment) gave the lowest mean of the flag leaf area of 18.17 cm^2.

Weight of 1000 grains (g)

The results of the statistical analysis of table 4 showed significant differences in the weight of 1000 grains. The isolates of Azospirillum, A2, A1 of 35.70 g and 32.78 g respectively and recorded an increase of 16.48 and 6.95% higher than the control treatment, the reason of this increase is due to the positive effect of Azospirillum and their production to growth regulators, especially oxytens, which play an important role in increasing various growth indicators, and the yield components by increasing the division of cells and their extension and then the expansion of the root hairs, which in turn will increase the surface area of the roots and the absorption of nutrients is greater than reflected positively on the increase of various indicators of growth and vegetation composition of dry matter and then increase the fullness of grains and high weight (Conejos *et al*., 2009) and these are agreed with results of Poureidi *et al*. (2015) and Zaied *et al*. (2003), also the isolate A2 was superiority on the isolate A1.

The results in table 4 showed the difference in wheat varieties significantly in the weight of 1000 grains, given that the variety IPA 99 the highest mean of this trait of 35.84 g, significantly superior to all varieties while Abu Ghraib variety recorded lowest mean of 29.97 g. This result was consistent with what was found by Al-Kaiar (2005) and Al-Azzawi (2005) by different wheat varieties among them in the weight of 1000 grains.

Results of table 4 indicated that there was a significant interaction between the varieties and the bio fertilization of the Azospirillum bacteria, Rasheed cultivar with isolate A2 gave the highest mean of 37.23 g while the Abu Ghraib cultivar gave a lowest mean of 27.66 g.

Grains yield ($Mg.ha^{-1}$)

The results of table 5 showed the effect of the biological inoculants of the bacteria in the grains yield, the isolates A2 and A1 superior to the control treatment with asignificantly increase rate of 36.13% and 24.08% sequentially, this increase may be due to the ability of Azospirillum bacteria to nitrogen fixation for the plant as well as the use of bio-fertilizers releases nutrients slowly and reduces the loss by washing, especially nitrogen (Muneshwar *et al*., 2001), which increased the nitrogen content of the plant, which in turn increased the biological processes is one of the components of enzymes and proteins and chlorophyll and it enters into processes and enzymatic reactions. The results in table 5 showed that wheat varieties were significantly different in grain yield, with Abu Ghraib cultivar giving the highest mean of 2.61Mg.ha-^{1} thus significantly superior all varieties, also Bora variety gave a mean of 2.41 $Mg.ha^{-1}$. This result is agreed with the findings of Al-Mohammadi (2010), Al-Ghurairi (2011) and Al-Aaajeebi (2014). The results of table 5 showed a significant interaction between the varieties and the bio-fertilization of the Azospirillum bacteria in the grain content. The combination (Abu Ghraib × A2) gave the highest mean of this trait of 2.95 $Mg.ha^{-1}$ and significantly superior most of the combinations, while the combination (IPA 99 × control treatment) gave a lowest mean of 1.55 $Mg.ha^{-1}$.

Concentration of nitrogen in grains (%)

The results of table 6 showed that the addition of bio-fertilizer to Azospirillum bacteria significantly affected in nitrogen concentration of wheat grains, the results showed that the A2 and A1 isolates were superior to the control treatment. The isolate A2 recorded the highest mean of 2.58%, with a 40.22% increase of the control treatment, while isolate A1 recorded of 27.17% increase in the same treatment, possibly due to bacteria's ability to produce: Gibberillins and materials of quasi cytokinins (Piccoli and Bottini, 1994), which increases the growth of the root, which in turn increases the uptake of nitrogen and other nutrients from the soil and thus increases its concentration within the plant, this is agreed with Askary *et al*. (2009), which indicated that wheat inoculant with Azospirillum gave an increase in grain as well as a increase in nitrogen concentration in grain amounted 22.8% compared with the control treatment.The results showed also in table 6 the difference in wheat varieties significantly in the concentration of nitrogen in the grain of wheat plant, Abu Ghraib gave the highest mean of 2.79% and significantly superior on most varieties except

Table 5 : Effect of inoculation with local isolates of Azospirillum bacteria and varieties of wheat and their interaction in grains yield ($Mg.ha^{-1}$).

N / A	M1	M2	M3	M4	M5	Mean
A0	2.16	1.98	1.94	1.87	1.55	1.91
A1	2.71	2.49	2.41	2.25	1.97	2.37
A2	2.95	2.77	2.62	2.49	2.11	2.59
Mean	2.61	2.41	2.32	2.20	1.88	

LSD 0.05 A = 0.053 M = 0.069, A*M = 0.119.

Table 6 : Effect of inoculation with local isolates of Azospirillum bacteria and varieties of wheat and their interaction in nitrogen concentration in the grains (%).

N / A	M1	M2	M3	M4	M5	Mean
A0	2.33	2.07	1.92	1.55	1.31	1.84
A1	2.83	2.65	2.58	1.97	1.68	2.34
A2	3.22	2.89	2.71	2.34	1.75	2.58
Mean	2.79	2.54	2.40	1.95	1.58	

LSD 0.05 A = 0.198 M = 0.255, A*M = 0.442.

Table 7 : Effect of inoculation with local isolates of Azospirillum bacteria and varieties of wheat and their interaction in potassium concentration in the grains (%).

N / A	M1	M2	M3	M4	M5	Mean
A0	1.83	1.80	1.80	1.76	1.75	1.79
A1	1.94	1.88	1.84	1.81	1.78	1.85
A2	1.96	1.91	1.85	1.83	1.81	1.87
Mean	1.91	1.86	1.83	1.80	1.78	

LSD 0.05 A = 0.194 M =0.250, A*M = 0.433.

Bora, which recorded an mean of 2.54%, while the variety IPA 99 recorded a lowest mean of 1.58%. The results of table 6 showed that there was an interaction between the varieties and biological fertilization. The combination (Abu Ghraib × A2) gave the highest mean of 3.22%, while the combination (IPA 99 x control treatment) gave the lowest mean of 1.31%.

Potassium concentration in grains (%)

The results of the statistical analysis of able 7 showed that there was no significant effect on the potassium concentration in the grains, when inoculation with Azospirillum. The results also indicate that there is no significant effect in the varieties as well as for the interaction between the class and the biological fertilization of Azospirillum bacteria in the potassium concentration in wheat grain.

Table 8 : Effect of inoculation with local isolates of Azospirillum bacteria and varieties of wheat and their interaction in phosphorus concentration in the grains (%).

N / A	M1	M2	M3	M4	M5	Mean
A0	0.32	0.30	0.28	0.27	0.23	0.28
A1	0.43	0.37	0.35	0.32	0.28	0.35
A2	0.48	0.45	0.39	0.35	0.31	0.40
Mean	0.41	0.37	0.34	0.31	0.27	

LSD = 0.05 A = 0.0280 M = 0.0361, A*M= 0.0626.

Concentration of phosphorus in grains (%)

The results of the statistical analysis of able 8 showed that there was no significant effect on the phosphorus concentration in the grains, when inoculation with Azospirillum, as the results showed that the superiority of isolate A2, it recorded the highest rate of 0.40% and an increase of 42.86% compared the control treatment A0, which recorded a rate of 0.28%, the reason to the increase may be due to the ability of Azospirillum bacteria to the production of enzymes to the analysis of pectin, it works to decompose the middle lamella of the cortical cells that are colonized by these bacteria without causing weakness of the cell, thus increasing the absorption of water and nutrients such as nitrogen, phosphorus, potassium and other elements by root cells. This is consistent with Lin *et al*. (1983) and Al-Sultani (2012). The result of table 8 showed that there are significant differences between the varieties in this trait. Abu Ghraib variety gave the highest mean of 0.41%, which significantly superior of all the varieties in this study. Bora also significantly superior of all the varieties with a mean of 0.37%. When IPA 99 variety recorded a lowest mean of 0.27%, this result was consistent with what Rifai (2006) found in different wheat varieties in the phosphorus content of the plant. The results of table 8 showed significant interaction between cultivars and bio-fertilization in this trait. The interaction of Abu Ghraib cultivar with isolate A2 gave the highest mean of 0.48%, significantly superior most of the interactions except the interaction between Abu Ghraib and isolate A1, with isolate A2 and the interaction between Bora cultivar and isolate A2, which recorded means of 0.43% and 0.45% sequentially while the interaction between the cultivar IPA 99 with the control treatment was the lowest mean of 0.23%.

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