



### Effect of bio-fertilization and Nitrogen level on yield, chemical constituents and Nitrogen use efficiency on two wheat cultivars grown under calcareous soil conditions

<sup>1</sup>Elham, A. Badr\*, M.H.M. Afifi<sup>1</sup> and Camilia ,Y.EL-Dewiny<sup>2</sup>

1.Field Crops Research Dept., National Research Centre, Dokki, Giza, Egypt.

2 .Soil and Water Use Dept., National Research Centre, Dokki, Giza, Egypt.

**Abstract :** Field experiment was carried out at the Experimental Farm of Apis second area village in Alexandria, during (2013/2014)and (2014/2015) winter season. The aim of the present work was to evaluate the influence of N-fertilizer levels (zero,50, 100 and 150 kg N/fed) applied alone or with bio fertilization (cerilaline) on plant growth of two wheat cultivars in calcareous soil(caco<sub>3</sub>22%). The obtained results showed that: Gemeiza 7 cultivar surpassed Sakhai93 in plant height (cm),flag leaf area(cm), dry matter (g/ m<sup>2</sup>),no. of spikes/m<sup>2</sup>(grain, straw, biological yield(ton/fed),weight of 1000 grain (gm)and chemical analysis of grain (N,P,K and protein (%))excepted for harvest index(%).Application of mineral fertilizer up to increase 100kg/fed increase wheat parameters yield characters and macronutrient concentration in grains. Application of bio fertilization led to increase the studied parameters in comparison with non- inoculation treatments. Using of the bio fertilizer enhanced Plant height (cm), Flag leaf area (cm<sup>2</sup>), Dry matter (g/m<sup>2</sup>) and No. of spikes (m<sup>2</sup>).Inoculation with bio fertilizer high significantly increased wheat grain yield from (1.45 to 1.55) ton/ fed. For Gemeiza 7 and from( 1.34 to 1.43) ton /fed for Sakha 93.The analysis of grain revealed that N, P ,K and protein contents were increased when inoculated with bio fertilization. It could be concluded from this study that. Wheat bio fertilization with cerialine under calcareous conditions caused marginal increases in growth or yield parameters when higher N levels adopted. Also, Wheat cultivars differed in their ability to use N unites and Sakha-93 cultivar possessed greater ability to produce greater units of grains and biological (grain +straw) per N unite used compared with Gemeiza 7 - cultivar

**Key Words:** Fertilization- *Azospirillum lipoferum* - *Bacillus polymxa* - protein content - Wheat (*Triticum aestivum* L.).

## Introduction

Wheat (*Triticum aestivum*, L.) is one of the most important cereal crops in Egypt and over the world used in human food and animal feed. Wheat provides 37 % of the total calories for the people and 40 % of the protein in the Egyptian diet. Recently, a great attention of several investigators has been directed to increase the productivity of wheat to minimize the gap between the Egyptian production and consumption by increasing wheat production through increasing unit land area productivity and increasing cultivated area. Increasing wheat yield per unit area can be achieved by breeding high yielding varieties and applying the optimum cultural practices such as organic fertilization which reduce pollution and sustain soil fertility through their effect on the physical, chemical and biological properties of soil, but its use alone is not sufficient to meet the requirement of nutrients. The yield of wheat is a function of many factors among them the cultivars and elements fertilization being the most ones. One of the most important elements which are necessary for organic matter synthesis in

wheat is nitrogen. If wheat as a non legume plant species could fix nitrogen, it will be make possibility from unlimited source of this element from air, make accessible nitrogen from in soil and make possible that its alone provide nitrogen nutrition<sup>1</sup>. So, using nitrogen fixation bacteria such as (*Azospirillum*) assume a greater importance. Therefore it can fix atmospheric nitrogen to supply plants with part of nitrogen – requirement to reduce the amount of N-chemical fertilizer, production costs and environmental pollution. Several reports emphasized the role of symbiotic N- fixing bacteria in increasing yield and improving nutrient uptake of field crops<sup>2</sup>. Combination of inorganic and bio-fertilizer showed the best growth and yield components of cereals<sup>3</sup>. Application of new methods as is nodulation of wheat ,transfer gene to the crop plants<sup>4</sup>, using higher number of investigation genotypes and bio-fertilizer.

In general, there is a lake of information concerning the response of wheat genotypes to bio-N fertilization. Therefore, the present investigation was designed to study the ability of bio-N fertilization treatment namely cerialine combined with three rates of chemical N fertilizer for covering N requirements of two wheat Egyptian cultivars .

## Materials and Methods

Field experiments were conducted in the experimental Farm of Apis second area village in Alexandria during 2013/2014 and 2014/2015 winter season to evaluate the effect of fertilization with different inorganic nitrogen levels of (0, 50, 100 and 150 kg N/fed.), and alone or combined with (*Azospirillum lipoferum* and *Bacillus polymxa*) as bio fertilizer on the growth characters and Yield, as well as N, P and K percentage and protein content in grains of wheat (*Triticum aestivan L.*) cultivars (Gemeiza 7 and Sakha 93).

Analysis of chemical and physical properties of the experimental soil (0 to 30 cm) are shown in Table (1). The determination of soil physical and chemical analysis were carried out according to the methods reported by<sup>5</sup>

**Table (1): Some physical and chemical properties of the experimental soil (average of two seasons).**

Particle size distribution, (%)			Soil texture	pH*	EC* dS/m	Total caCO <sub>3</sub> %	Total N (%)	Available P mg/kg <sub>s</sub>	O.M. %
Sand	Silt	Clay							
38.0	19.5	42.35	Sandy Clay loam	7.9	2.3	22%	0.09	7.25	0.75

The experimental design was split- split plot with three replicates. Wheat cultivars (Gemeiza 7 and Sakha 93) were used and randomly distributed in the main plots. Four nitrogen fertilization levels (0, 50, 100 and 150 kg N/fed. as ammonium nitrate 33.5%) were used and randomly distributed in the sup plots, while sub-sub plots were devoted to tow bio fertilization treatments, i.e. untreated seeds (control), treated seeds with cerialine. Wheat grains, just before planting, were inoculated with a mixture of a non-symbiotic N fixing (*Azospirillum lipoferum* and *Bacillus polymxa*) under the commercial name cerialine Coating of wheat grains was conducted as recommended by the Ministry of Agriculture, Giza, Egypt. Wheat Cultivars (Gemeiza 7 and Sakha 93), were planted at the rate of 60 kg/fed. at 18 and 15 November 2013/2014 and 2014/2015 seasons, Wheat Cultivars (Gemeiza 7 and Sakha 93), were planted at the rate of 60 kg/fed. at 18 and 15 November 2013/2014 and 2014/2015 seasons, respectively Wheat grains were sown in rows 20 cm apart. Nitrogen fertilizer levels were applied after the 1st irrigation. Phosphorus fertilizer was applied before planting as superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>) at a rate of 50 kg P<sub>2</sub>O<sub>5</sub> /fad. The Experimental unit was (10.5 m<sup>2</sup>) (3m x 3.5m). During the season a sample was taken at 90days and plant height (cm), dry matter production per m<sup>2</sup> and flag leaf area were determined. At the end of the experiment wheat plants were harvested to evaluate the effect of the applied treatments on plant-growth. Grain and straw yields were determined using a quadrat (3 x 3.5 m). The plants were threshed to obtain yield per plot and yield (ton/fd<sup>-1</sup>). Straw yield (ton/ fd<sup>-1</sup>), biological yield and 1000-grains weight were determined and harvest index . Chemical analysis was carried out on dried and ground samples. Nitrogen was determined by micro-Kjeldahl according to<sup>5</sup> After wet digestion of the samples, according to<sup>6</sup> P was determined by spectrophotometry, K by flame photometer, N-fertilizer use efficiency (NFUE). The efficiency of N-fertilizer (NFUE) at N<sub>i</sub> the change in grain or biological yield per unit change in N was determined according to (Sowers et al 1994). was determined according to (Sowers et al

1994). While protein content was calculated by multiplying total N protein content was calculated by multiplying total N concentration by the factor 5.75.

#### Statistical analysis:

All the obtained data were statistically analyzed using Costat Software<sup>7</sup>

## Results and Discussion

### A-Growth parameters:

Data presented in Tables (2) indicated that Gemeiza-7 cultivar surpassed Sakha93 in plant height (cm), flag leaf area (cm<sup>2</sup>), dry matter (g/m<sup>2</sup>) and no of spikes (m<sup>2</sup>) compared to Sakha93 cultivar. Such varietal differences between wheat cultivars may be due to the genetically differences between cultivars and genotypic differences concerning partition of dry matter. The superiority of Gemeiza 7 cultivar may be due to the increase in the efficiency to photosynthetic<sup>8</sup> which reflected on increasing the production of more sizeable organs. The results of varietal differences in growth parameters in this study are in agreement with those obtained by<sup>9,10</sup>. Regarding the effect of nitrogen levels the analysis of variance showed that growth characters of wheat plants as plant height (cm), flag leaf area, number of spikes /m<sup>2</sup> and total dry matter were significantly influenced by N rates Table(2). The highest plant height and the maximum enhancement were exerted in plants treated with N at the recommended level (100N/fed) or 150 kg ) without significant differences among these treatments. Similar results were obtained by<sup>11,12</sup>. The highest flag leaf area (cm<sup>2</sup>), dry matter (g/ m<sup>2</sup>) and spike number/m<sup>2</sup> were obtained with the highest N level, i.e. 100 kg N/fed. Such results clarified that N is essential for cell division and elongation as well as dry matter content of wheat plants this is consistent with the results in the other papers<sup>12,13</sup>. Regarding the effect of bio-fertilization on growth parameters, the results presented in Table(2) show the effect of bio fertilization treatments on growth parameters indicated that treated wheat grains with bio fertilization (cerilaline) caused a significant increase in all growth characters, as plant height (cm), flag leaf area, number of spikes /m<sup>2</sup>, and total dry matter (g/m<sup>2</sup>). The highest mean values were recorded under application of bio fertilizers comparing with non application ones. Results of this investigation point out that the plant height depended on investigated N- fixer (bio fertilizer). The shortest plants were under control treatment. The difference between control and inoculation of wheat seeds with N<sub>2</sub>-fixer (cerilaline), was statistical highly significant. The flag leaf area in the inoculation plants was larger than that in control Table(2), Inoculation with cerilaline increased flag leaf area (cm<sup>2</sup>) compared with control. The significant increase in flag leaf area could also contribute to the increased total photosynthetic and could be also attributed to the stimulation of the root elongation and enlargement of root surface area as has been reported previously.<sup>13</sup>

### B. Yield and its components:

The results presented in Table (3) indicated that two both cultivars (Gemeiza7 and Sakha 93) significantly differed in yield and its components i.e., grain, straw and biological yield (ton/fed) and weight of 1000 grain (gm) of wheat. Gemeiza 7 was significantly greater in grain, straw and biological yield (ton/fed) compared with the other cultivar. For harvest index date in Table (3) show in significant effect between the two studied cultivars. This results agree with those obtained by<sup>8</sup>. 1000 grains weight was Gemeiza7 variety . The 1000 grains weight and grain yield (g) (ton/fed) has highly significant, and have positive genetic and phenotypic<sup>14,15</sup>. This might be a reflection to the higher values recorded in number of spike/m<sup>2</sup>, and 1000- grain weight Tables( 2 and 3). Several researchers reported significant varied differences among different wheat cultivars in grain yield/ha<sup>8,13,16</sup>. The mean values of grain, straw and biological yield, harvest index and weight of 1000 grain (gm) of wheat as affected by N- fertilizer application are presented in Table (3). Grain yield is the most important characteristic of wheat cultivar. Nitrogen has positive influence on increasing of grain yield<sup>8,17</sup>.

### C. Chemical analysis of grain:

Both varieties differed significantly in N P K content in grain wheat, Gemeiza 7 possessed significantly greater values than did the other cultivars in Table(3). Also, the trend of variation in protein content was similar to nitrogen content. In relation to total nitrogen content of the grains, it was observed that the nitrogen chemical supply was essential for a larger accumulation in the grains. Also, by increasing nitrogen rate the mean values of N% was increased up to 150 kg N/fed. application, These results are in a great harmony

with those obtained by<sup>18,19,20</sup>. N and protein content was significantly increased, the increase was more pronounced with fertilization with Cerilaine compared with control. Inoculation of wheat grain with N<sub>2</sub>-fixers (cerilaine) was responsible for the statistically increments in content of studied nutrients as compared to the inoculated plants. Under application of bio fertilizers the mean values of N was higher (2.23%) than those obtained under non-inoculated (2.19%) treatment. This promoting effect could be related to the N-supplementary effect of non-symbiotic N<sub>2</sub>-fixing bacteria (used as bio-N-fertilizer) to plants due to their ability to fix free molecular atmospheric nitrogen as well as the role of these bacteria in improving the availability of soil elements, through secreting chelator substances (such as organic acids) which are important for solubilizing sparingly soluble inorganic compounds to make easy forms available for plants uptake<sup>11,17</sup>. Moreover, the hormonal exudates (such as indole acetic acid, gibberellins and cytokinins) of these micro-organisms can modify root growth (morphology and physiology) resulting in more efficient absorption of available nutrients from the soil, N and protein content was significantly increased, the increase was more pronounced with fertilization with cerilaine compared with control<sup>20,21,22</sup> (Table 4). Inoculation of wheat seeds with N<sub>2</sub>-fixers (Cerilaine) was responsible for the statistically increments in nutrient concentrations as compared to the un inoculated plants. Under application of bio-fertilizers the mean values of N was higher (2.22%) than those obtained under non-application (2.16%) treatment. This promoting effect could be related to the N-supplementary effect of non-symbiotic N<sub>2</sub>-fixing bacteria (used as bio-N-fertilizer) to plants due to their ability to fix free molecular atmospheric nitrogen as well as the role of these bacteria in improving the availability of soil elements, through secreting chelator substances (such as organic acids) which are important for solubilizing sparingly soluble inorganic compounds to make easy forms available for plants uptake. Moreover, the hormonal exudates (such as indole acetic acid, gibberillin and cytokinins) of these micro-organisms can modify root growth (morphology and/ or physiology) resulting in more efficient absorption of available nutrients from the soil<sup>23,24,25</sup>.

#### D .Interaction between wheat cultivars, nitrogen fertilizer levels and bio fertilizer

The interaction between cultivars and nitrogen levels had a significant effect on all growth parameters of wheat, except flag leaf area. Also, the interaction had a significant effect on grain yield Table(5,6). The effect of nitrogen levels up to 100 kg N/fed on grain yield was more obvious in both cultivars compared with the control treatment. The interaction between cultivars and the bio-fertilizer inoculation had a significant effect on plant height (cm), number of spikes /m<sup>2</sup> and dry matter (g/ m<sup>2</sup>). On the other hand, the interaction between the two factors studied was insignificant on harvest index. The interaction among the three factors studied were significant on plant height, flag leaf area, dry matter (g/ m<sup>2</sup>) and number of spikes /m<sup>2</sup>). The grain yield increases, caused by the inoculation with bio-fertilizer are not just related with the capacity that these bacteria present to fix the atmospheric nitrogen but also, due to the production of plant growth promoting substances<sup>26,27,28</sup>. The phyto-hormones promote the root growth of plants, consequently increasing nutrients and water absorption areas<sup>29,30</sup>. Data in Table (5) show that the dual application of N with bacterial inoculation (Cerilaine) caused significant increase in N and K content in grains at harvest and grain protein content compared to the other treatments. The possible mechanisms leading to higher nitrogen content were explained by<sup>31,32</sup>. Also, The triple interaction had a significant effect on yield components and nutrient concentration except K content<sup>33,34,35</sup>.

#### E-Nitrogen use Efficiency

Data presented in Table(7) s clearly show significant differences in the ability of both cultivars to produce higher grain or biological yields from nitrogen unit used under different N rates tested. In general the data show that Sakha-93 cultivar possessed greater ability to produce greater units of grains and biological (grain +straw) per N unite used compared with Gemeiza 7 - cultivar. Gradual increases in NUE were reported as Nitrogen rates applied decreased in both cultivars<sup>23,24,25</sup>.

#### Conclusion

It could be concluded from this study that Nitrogen fertilization for wheat under calcareous soil conditions is essential with moderate levels up to 100kg/fed. Wheat bio-fertilization with cerilaine under calcareous conditions caused marginal increases.

**Table (2) effect of nitrogen fertilizer levels and bio fertilizer on some growth characters of wheat varieties(combined analysis of two seasons)**

Treatment	Plant height(cm)	Flag leaf area(cm)	Dry matter (g/m <sup>2</sup> )	No of spikes/m <sup>2</sup>
varieties				
Gemeiza7	89.29	37.23	345.62	407.08
Sakha93	83.54	29.49	328.28	338.46
L.S.D	2.69	0.65	14.32	2.4
N-Levels				
Zero kg/fed	70.75	25.76	305.10	290.00
50kg/fed	86.25	32.24	341.13	384.5
100kg/fed	94.75	39.56	356.18	409.67
150kg/fed	93.92	35.88	345.41	406.92
L.S.D	1.53	0.95	8.17	1.28
Biofertilizers				
Uniculation	82.75	31.55	327.73	364.67
Cryaline	90.08	35.17	346.18	380.88
L.S.D	0.86	0.57	6.51	0.72

**Table (3) effect of nitrogen fertilizer levels and bio fertilizer on some yield characters of wheat varieties(combined analysis of two seasons)**

Treatment	Yield (ton/fed)			Harvest Index (%)	1000 grain weight(gm)	%				
	Grain	Straw	Biological			N	P	K	protein	
varieties										
Gemeiza7	2.68	5.15	7.83	34.22	74.08	2.31	0.28	0.139	13.28	
Sakha93	2.67	4.94	7.61	35.08	58.08	2.11	0.26	0.129	12.13	
L.S.D	0.03	0.07	0.03	0.57	2.09	0.03	0.002	0.001	0.17	
N-Levels										
Zero kg/fed	1.48	4.44	5.92	25	60.33	2.04	0.22	0.106	11.73	
50kg/fed	2.83	4.61	7.44	38.03	66.49	2.16	0.25	0.131	12.42	
100kg/fed	2.98	5.67	8.65	34.45	68.98	2.31	0.28	0.148	13.28	
150kg/fed	2.94	5.45	8.39	35.04	68.52	2.32	0.31	0.149	13.34	
L.S.D	0.01	0.02	0.03	0.13	1.55	0.01	0.01	0.002	0.07	
Biofertilizers										
Uniculation	2.62	5.00	7.62	34.38	64.47	2.19	0.26	0.135	12.59	
Cryaline	2.67	5.08	7.75	34.45	67.68	2.23	0.27	0.132	12.82	
L.S.D	0.01	0.01	0.02	0.07	1.22	0.01	0.01	0.001	0.06	

**Table (4) Effect of nitrogen and bio-fertilizer on N,P,K and protein uptake of wheat grains (combined analysis of two seasons)**

Varieties	Treatment	Nutrient up take (kg/fed)			
		N	P	K	Protein yield
Gimeiza 7	Control +cerilaline	33.94	3.61	1.79	195.14
	50kgN/fed+cerilaline	59.89	7.15	3.57	344.23
	100kgN/fed+cerilaline	67.48	8.26	4.31	387.8
	150kgN/fed+cerilaline	67.79	9.32	4.32	389.76
L.S.D 5%		1.70	0.31	0.10	11.03
Sakha 93	Control +cerilaline	28.88	3.22	1.47	166.02
	50kgN/fed+cerilaline	55.18	6.47	3.51	317.06
	100kgN/fed+cerilaline	60.72	8.25	3.97	349.14
	150kgN/fed+cerilaline	60.87	7.59	3.93	349.98
L.S.D 5%		1.55	0.29	0.09	10.09

**Table (5) Effect the interaction between nitrogen fertilizer levels and bio fertilizer on some growth characters of wheat varieties (combined analysis of two seasons)**

Varieties	N-levels	Biofertilizers	Plant height (cm)	Flag leaf area (cm <sup>2</sup> )	Dry matter g/m <sup>2</sup>	No. of spikes (m <sup>-2</sup> )
Gemeiza 7	0	Uniculation	64.67	24.79	300.0	312
		Cerilaline	82.67	33.92	355.6	393
	50	Uniculation	85.67	35.46	356.6	397
		Cerilaline	89.67	38.11	357.8	412
	100	Uniculation	97.67	41.36	363.6	437
		Cerilaline	99.33	43.41	364.6	534
	150	Uniculation	97.00	38.50	358.9	433
		Cerilaline	97.67	40.66	362.2	438
Sakha 93	0	Uniculation	54.67	20.66	297.2	222
		Cerilaline	81.00	24.68	314.2	233
	50	Uniculation	83.67	26.62	325.0	363
		Cerilaline	86.00	28.78	325.1	366
	100	Uniculation	89.67	35.05	345.0	381
		Cerilaline	91.33	36.74	351.4	386
	150	Uniculation	89.00	30.95	325.5	375
		Cerilaline	93.00	33.40	342.8	382
L.S.D 5%			7.70	N.S	27.85	28.11

**Table (6) Effect the interaction between nitrogen fertilizer levels and bio fertilizer on some yield characters of wheat varieties (combined analysis of two seasons)**

varieties	N-levels	Biofertilizers	Yield (ton/fed)			Harvest index	1000-grain Weight (gm)	%			
			Grain	Straw	Biological			N	P	K	Protein
Gemeiza 7	0	Uniculation	1.45	4.44	5.89	24.62	57.50	2.13	0.222	0.112	12.24
		Cerilaline	1.55	4.56	6.11	25.37	70.10	2.19	0.233	0.116	12.59
	50	Uniculation	2.64	4.60	7.24	36.46	73.27	2.22	0.252	0.133	12.76
		Cerilaline	2.65	4.66	7.31	36.22	77.53	2.26	0.270	0.135	12.99
	100	Uniculation	2.78	6.71	9.49	29.29	78.33	2.31	0.282	0.153	13.28
		Cerilaline	2.80	6.75	9.55	29.31	79.43	2.41	0.295	0.154	13.85
	150	Uniculation	2.76	5.72	8.48	32.54	77.10	2.35	0.325	0.153	13.51
		Cerilaline	2.79	5.72	8.51	32.78	79.37	2.43	0.334	0.155	13.97
Sakha 93	0	Uniculation	1.34	4.32	5.66	23.67	56.17	1.81	0.210	0.093	10.40
		Cerilaline	1.43	4.43	5.86	24.40	57.53	2.02	0.225	0.103	11.61
	50	Uniculation	2.53	4.55	7.08	35.73	56.77	2.08	0.235	0.125	11.96
		Cerilaline	2.64	4.62	7.26	36.36	58.40	2.09	0.245	0.133	12.01
	100	Uniculation	2.67	5.55	8.22	32.48	58.77	2.15	0.283	0.143	12.36
		Cerilaline	2.76	5.67	8.43	32.74	58.83	2.20	0.299	0.144	12.65
	150	Uniculation	2.66	5.13	7.79	34.14	57.80	2.22	0.265	0.143	12.76
		Cerilaline	2.73	5.24	7.97	34.25	60.33	2.23	0.278	0.144	12.82
L.S.D 5%			0.66	0.71	0.87	1.40	1.60	0.48	0.066	0.004	0.99

**Table (7) Nitrogen use efficiency(NUE)of grain and biological yield on two varieties in wheat (combined analysis of two seasons)**

N-Level kg/fed	NUE grain(kg grain /N fertilizer)		NUE biological(kg bio/N fertilizer)	
	Gemeiza 7	Sakha 93	Gemeiza 7	Sakha 93
50	118.58	127.75	346.66	364.11
100	111.20	121.36	324.89	345.91
150	110.29	119.73	322.22	341.25
L.S.D 5 %	2.84	2.92	12.52	13.06

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