

THE ENHANCEMENT OF WHEAT PRODUCTIVITY BY INOCULATION WITH ASSOCIATIVE DIAZOTROPHS AND FOLIAR APPLICATION OF METHANOL UNDER DIFFERENT N-FERTILIZER LEVELS.

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Abstract: Two field trials were conducted in clay soil at the Experimental Farm of Faculty of Agriculture, Cairo University at Giza during the two seasons of (98-99 & 99-2000) to study the effect of inoculation with associative diazotroph bacteria, foliar application of methanol and different levels of N-fertilizer on soil microflora, growth and yield of wheat (*Triticum aestivum* L.) CV. Giza 164. Composite inoculum was used containing mixed culture (1:1:1) from, *Azospirillum barsilenes*, *Azotobacter chroococcum* and *Bacillus polymyxa*, at time of planting. Methanol solution (20% v:v) was sprayed twice, 60 and 80 days after planting. N-fertilizer was applied at the rates of 0,35 and 70 kg N fed⁻¹. Results obtained of 90 days old plant indicated that inoculated treatment recorded higher values of total counts of bacteria, actinomycetes

and fungi in rhizosphere soil compared with those treated with foliar methanol or N-fertilized. During the vegetative growth period, plant height, dry biomass and plant N-content of wheat were particularly improved by inoculation in combination with N-fertilization at rates of 70 kg and 35 kg N fed⁻¹. The increases in plant dry biomass and N-content due to inoculation in combination with the two N-levels amounted to 241% and 283%, respectively. Foliar application of methanol in combination with the two N-fertilizer levels also imposed significant increases that ranged from 119 to 148% for biomass and 102 to 203% for plant N-content. At harvest, inoculation with composite inoculum in combination with N-fertilization at either levels scored the highest values in all yield parameters tested.

Introduction

Wheat is a very important grain crop in Egypt and represents a major source of food. Any effect to increase

wheat yield is of prime concern. Nitrogen requirement for wheat is estimated by 220kg Nha⁻¹. The efforts to decrease chemical fertilizer by using biofertilizers might improve

economy and reduce environmental pollution. The effect of associative microorganisms in increasing yield and N_2 - fixation has been reported by many research workers (Bala and Kundu, 1988; Hegazi, 1988 and Rashid et al, 1993). The association between free living nitrogen fixing bacteria (*Azospirillum* spp, *Azotobacter* spp. and *Bacillus* spp.) and wheat, sorghum and maize has been demonstrated to cause significant increases in yield and total nitrogen content. Inoculation of wheat with nitrogen fixing bacteria under different nitrogen fertilizer levels increased grain yield and protein content. It has been reported that the highest grain yield and protein in three wheat cultivars were obtained by inoculation with *Bacillus polymyxa* and 180kgNha^{-1} (Omar et al, 1996). Combined inoculation with *Azospirillum barsilense* and *Azotobacter chroococcum* and the application of 140 Kg N ha^{-1} caused wheat plants to derive 24% of their nitrogen requirement from atmospheric nitrogen (Soliman et al, 1995) . Wheat plants can obtain more than 20-50% of their nitrogen from biological nitrogen fixation (Hegazi et al 1998).

It is well known that the productivity of C_3 crops is lower than that of C_4 crops. This may be due to the very high level of photorespiration, which is a common

physiological aspect in the C_3 plants compared to C_4 plants (Cheng and Kung 1994). Many attempts were carried out to control photorespiration , such as increasing CO_2 concentration in the atmosphere surrounding the plants as well as culture of crops . Recently, Nonomura and Benson (1992a) reported that a spray treatment of plant with methanol was more effective to inhibit photorespiration in C_3 plants when it was applied for long period of light intensity of sunlight. From practical point of view, methanol was used by some investigators to improve growth and economic yield of C_3 crops. (Cossins, 1964; Allen et al, 1991; Nonomura and Benson 1992a, b and 1993 Li et al, 1995 and Manderscheid and Weigel 1995); They stated that foliar application of aqueous (10-50%) methanol increased growth , yield and development of C_3 plants in arid environments. They recorded growth improvement estimated by 50% for tomato and cotton and by 60% for eggplant, whereas wheat grain and fruit yield of melon were increased by 100 and 36% , respectively. The single application of 25% (v:v) of methanol increased seed weight, seed yield plant^{-1} and pod number plant^{-1} of soybean by 10, 14.6 and 8.2% respectively as compared to untreated ones.

The objective of this investigation was to study the effect of inoculation with associative diazotrophs, and foliar application of methanol under different N-fertilizer levels on soil microflora and growth and yield of wheat plants.

Materials and Methods

Two field experiments were laid out at the Experimental Farm of Faculty of Agriculture, Cairo University, Giza during the two seasons of 98-99 and 99 - 2000 to study the enhancement of wheat productivity by inoculation with associative N₂-fixing bacteria and foliar application of methanol under different N-fertilizer levels. Before planting soil samples were collected from the two experimental sites and analysed for some physical and chemical properties according to Black et al, 1965 (Table 1).

Super phosphate (15.5% P₂O₅) and potassium sulphate (50% K₂O) were added during seed bed preparation at rates of 200 and 100kg fed.⁻¹, respectively. Wheat grains, cultivar Giza 164, were planted in rows 30 cm in between at seed rate of 60 kg fed.⁻¹. Seven treatments were included as follows: 1) untreated (control), 2) inoculated + 35 kg N fed.⁻¹, 3) inoculated + 70kg N fed.⁻¹, 4) uninoculated + 35 kg N fed.⁻¹, with foliar application of methanol., 5) uninoculated + 70 kg N fed.⁻¹,

with foliar application of methanol, 6) uninoculated + 35kg N fed.⁻¹, without foliar application of methanol, and 7) uninoculated + 70 kg N fed.⁻¹, without foliar application of methanol. The plot area was 3x3.5m (1/400 fed.) and the experimental design was randomized complete block (RCB) with four replications.

Wheat grains were inoculated with commercial composite inoculum containing *Azospirillum barsilenes*, *Azotobacter chroococcum* and *Bacillus polymyxa* (1:1:1) which was kindly obtained from the Agriculture Microbiology Dept., Soil-Water and Environ. Res, Instit., ARC. The inoculum was added to the wheat grains at rate of 1g./1Kg grains. Methanol solution (20% v.v) was applied at the a rate of 250L fed.⁻¹ at two times, 80 days after planting and 20 days later. Nitrogen fertilizer, as ammonium sulphate (20.5 % N), was applied in two equal doses, 21 and 35 days after planting .

Total counts of bacteria, actinomycetes and fungi were estimated three times; before planting, at 90 days old plants (growth period) and after harvest in soil rhizosphere samples according to Wollum (1982). spike length, number of grains spike⁻¹, grains weight (g / spike) and weight of 1000 grains (seed index).

Table(1):Soil analysis of samples from the two experimental sites in the two seasons.

Analysis	season.	
	S1	S2
Mechanical analysis	%	%
Coarse sand	8.69	8.96
Fine sand	23.48	25.46
Silt	29.87	27.99
Clay	37.96	37.59
Texture	Clay loam	Clay loam
Chemical analysis		
Organic matter %	2.1	2.4
Total nitrogen %	0.12	0.14
Water holding capacity %	54.32	56.37
CaCO ₃ %	2.75	2.81
PH	7.8	8.0
Concentration of Anions and Cations (1:1 soil suspenson)		
Ec. (ds/ m)	3.1	3.3
		meqL⁻¹
Bicarbonate (HCO ₃ ⁻)	8.4	8.11
Chloride (Cl ⁻)	11.71	10.57
Sulphate (SO ₄ ²⁻)	16.43	18.58
Calcium (Ca ⁺⁺)	9.53	8.61
Magnesium (Mg ⁺⁺)	2.57	2.60
Sodium (Na ⁺)	22.93	25.71

Also, straw and grain yields (ton fed⁻¹) were recorded after harvesting from the whole plots. Total nitrogen contents of soil samples, plant tissues, straw and grains were estimated by using the semi-microkjeldahl methods (Black et al, 1965). Statistical analysis were done according to Snedecor and Cochran (1967).

Results and Discussion

I: Microbial status:

A large number of different microorganisms are commonly found in soil including bacteria, actinomycetes and fungi. Data in table (2) show that, irrespective of N-fertilizer application, the inoculation with N₂-fixing bacteria or foliar application of methanol during vegetative period growth (90 days old plant) had stimulated the total counts of bacteria, actinomycetes and fungi in wheat rhizosphere soil. The addition of N-fertilizer alone led to percentage increases ranged between 0-25%, 25-60% and 27-33% in numbers of fungi, actinomycetes and bacteria, respectively, compared with their counts in soil before planting in the S1 and S2, respectively. Foliar application of methanol scored higher percentage increases which ranged from 25 to 50%, 120 to 129% and 90 to 100% for the three microbial groups, respectively. The corresponding values for the inoculation treatments were 50-75%

, 229-260% and 188-218%. It is obvious that inoculation with the associative diazotrophs imposed higher stimulation values compared with those of foliar application of methanol. At the end of the second season soil microflora in experimental sites scored higher counts that increased by about 25, 28.5 and 36.4% for number of fungi, actinomycetes and total bacteria compared with the numbers recorded before planting. These higher percentage increases in soil microflora may be due to the enhancement of plant growth and root exudates. These data are in agreement with those of Pondey et al, (1998) and Mona et al, (2001), who reported that inoculation with diazotrophic bacteria had an activation effect on the population of both bacteria and actinomycetes and increased its numbers by more than 50% at the end of experimental time as compared with the numbers recorded before planting.

II: Plant growth:

Table (3) presents mean plant height, biomass and plant N-content after 90 days from sowing. The results show that the untreated control recorded the lowest values of all tested parameters in the two seasons compared with the treated ones. In general, all treatments recorded significant increases ranging from 8.8% to 28%

Table (2):Microbial Status of soil and wheat rhizosphere soil in the first (S₁) and second (S₂) seasons as affected by inoculation, foliar application of methanol and N- fertilization.

Parameters Time of determination	No. of fungi 10 ³ CFU* g soil ⁻¹		No. of actinomyces 10 ⁴ CFU*g soil ⁻¹		No. of total bacteria 10 ⁶ CFU* g soil ⁻¹	
	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂
1- Before planting	4	4	5	7	9	11
2 - During growth period (90days)**						
A- Treated with N- fertilization	4	5	8	9	12	14
B- Treated with foliar application of methanol	5	6	11	16	18	21
C- Treated with composite inoculum	6	7	18	23	26	35
3- At harvest **	5	5	6	9	11	15

* Colony Forming Unit

** Average of all the experimental plots.

as compared with the untreated control in the two seasons.

Also, plant dry biomass and plant N-content showed even higher significant increases due to inoculation with diazotrophs, foliar application of methanol and N-fertilization compared with the untreated plants (Table 3). The percentage increases over the control due to N-fertilization (35 or 70 kg N fed⁻¹), foliar application of methanol or/and inoculation ranged from 39-241% in case of plant dry biomass, and from 53-283% in plant N-content in the two seasons. The data also show that the highest biomass of 12.94 g plant⁻¹ was recorded for inoculated treatment that received 70kg N fed⁻¹ Table (3). This

treatment also scored the highest plant N-content; respectively 324.9 and 367.7 mg/plant in the two seasons S₁,S₂. The data presented in Table (3) indicate that inoculation or foliar application of methanol in combination with different levels of N-fertilizer had significantly positive effect on growth of wheat plants. The improvements in plant growth and N-content were particularly pronounced in the inoculated treatments than with methanol. These data are in accordance with those reported by Zamber, et al (1984); Rashid, et al, (1998); Hegazi et al (1998) and Mona et al, (2001) who reported that inoculation with associative diazotrophs bacteria led to increases

in plant dry matter and plant N-content. Also, Bernard (1995) suggested that free living bacteria may be considered as plant growth-promoting rhizobacteria (PGPR) and could benefit plants growth through different mechanisms of action, including: a) the production of secondary metabolites such as antibiotics, cyanide and hormone like

sub-stances, b) the production of siderophores, c) antagonism to soil borne root pathogens, d) phosphate solubilization and e) dinitrogen fixation. The increases in plant growth by foliar application of aqueous methanol was also reported by Cheng and Kung (1994) and Kandil and Mahrous, (1996) for C₃ plants in arid environments.

Table(3):Effect of N-fertilizer level, foliar application of methanol and inoculation with associative diazotrophs on mean plant height, biomass and plant N-content of wheat plants (90 days old) in the season 1998/99 (S₁) and 1999/2000 (S₂).

Parameters			Plant height (cm.)				Plant dry biomass (g plant ⁻¹)				Plant N-content (mg plant ⁻¹)			
Treatments			S ₁		S ₂		S ₁		S ₂		S ₁		S ₂	
c.*	ME* *	N-Fer Kg fed ⁻¹	S ₁	%	S ₂	%	S ₁	%	S ₂	%	S ₁	%	S ₂	%
-	-	-	87.31	-	99.33	-	3.79	-	3.99	-	93.17	-	96.0	-
-	-	35	95.0	8.8	114.0	15	5.26	39	5.99	50	142.70	53	157.67	64
+	+	35	102.34	17	116.34	17	6.61	74	7.56	89	188.17	102	206.57	115
-	-	35	105.70	21	120.0	21	8.29	119	8.88	123	247.83	166	254.57	165
-	-	70	103.67	19	118.70	20	7.40	95	8.17	105	203.07	118	215.10	124
+	+	70	108.0	24	126.33	27	9.31	146	9.90	148	270.57	190	291.27	203
-	-	70	112.0	28	121.30	22	12.94	241	12.92	223	324.93	249	367.77	283
L.S.D.0.05			7.50	-	12.56	-	2.44	-	2.76	-	86.60	-	130.62	-

* Inoculation

** Foliar application of methanol

III: Yield and yield components:

Data in table (4 and 5) indicated that at harvest stage in both seasons (S_1 and S_2), the untreated control recorded the lowest values of all yield parameters and components tested followed by the treatment that received 35 Kg N fed^{-1} only. The full dose of N-fertilizer (70 Kg N fed^{-1}) alone or in combination with inoculation or foliar application of methanol caused significant increases in yield parameters and determined yield components. Table (5) presents the calculated percentage increases in some yield parameters in the two seasons i.e grain weight (g spike^{-1}), grain index (weight of 1000 grains g), and grain yield (ton fed^{-1}), due to the different treatments compared with the untreated control. N-fertilization alone at rate of 70 kg N fed^{-1} induced percentage increases ranged from 26 to 81%, 3 to 4% and from 39-47, respectively in grain yield (ton fed^{-1}), grain weight and grain index (g spike^{-1}). Further increases in all determined parameters were recorded by foliar application of methanol in the N-fertilized plants, especially with the full dose (70 kg N fed^{-1}). These data are in agreement with Nonomura and Benson (1992a,b) who reported that foliar application of aqueous methanol (10-50%) increased wheat grain yield by 100%. Similarly, Cheng and Kung (1994), Li et al, (1995); Kandil and Mahrous, (1996) and

Mona et al, (2001) also, reported that applied of foliar methanol increased growth and yield of C_3 plants in arid environments.

The inoculation with the diazotrophs had a pronounced influence on plant growth and yield (Table 4 and 5). Data in table (5) indicate that the significant increases in all yield parameters were particularly pronounced in the inoculated treatments fertilized with 70 kg N fed^{-1} compared with foliar application of methanol. This inoculated treatment scored the following percentage increases: 106 to 121%, 4 to 9% and 82 to 155% respectively in grain weight, grain index and grain yield compared with the untreated control in the two seasons. These data are in agreement with Zambre et al, (1984); Omar et al (1991); Zaki et al (1992), and Rashid et al, (1998), who reported that grain yield and yield component of wheat inoculated with a mixture of diazotrophs microorganisms were significantly greater than the uninoculated treatment. They added that inoculation of field grown wheat generally increased the total yield from 10 to 70%.

Table (6) shows the calculated protein contents in grain and straw yields and percentage increases due to the different treatments compared with the untreated control. The

highest increases amounted to 167% in straw protein and 183% in grain protein. In this respect, Hegazi et al (1998) reported that wheat plants can obtain from 20 - 50% of their nitrogen from inoculation with N₂-fixing bacteria.

concluded that inoculation of moderately N-fertilized wheat plants with associative diazotrophic microorganisms or their treatment with foliar aqueous methanol significantly increases grain yield and total N-yield.

In conclusion, from the above mentioned results, it could be

Table (4):Effect of inoculation, foliar application of methanol and N-fertilization on plant height and some yield components of wheat plants at harvest

Parameters / Treatments			Plant height (cm)		Spike length (cm)		No. of grains (Spike ⁻¹)	
Inoc.*	ME**	N-Fer. Kg fed ⁻¹	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂
-	-	-	103.6	108.8	11.3	11.8	23.3	23.2
-	-	35	111.2	111.3	12.3	12.4	26.8	29.4
-	+	35	115.4	113.9	12.9	12.9	30.3	32.8
+	-	35	119.7	117.3	14.7	14.4	33.9	38.5
-	-	70	117.9	116.1	13.6	13.4	32.4	33.8
-	+	70	120.8	122.5	15.1	15.3	39.2	42.1
+	-	70	122.4	126.7	16.3	24.3	43.3	44.9
L.S.D. 0.05			8.0	5.5	0.99	1.03	5.2	5.40

* Inoculation

** Foliar application of methanol.

Table(5) Effect of inoculation, foliar application of methanol and N-fertilization on yield and some yield components of wheat plants at harvest in the two seasons (S₁) and (S₂).

Parameters			Grains weight (g spike ⁻¹)				Weight of 1000 grains (g)				Straw yield (Ton fed ⁻¹)				Grain yield (Ton fed ⁻¹)			
treatments			S ₁		S ₂		S ₁		S ₂		S ₁		S ₂		S ₁		S ₂	
Inoc*	ME*	N-Fer Kg fed ⁻¹	S ₁	%	S ₂	%	S ₁	%	S ₂	%	S ₁	%	S ₂	%	S ₁	%	S ₂	%
-	-	-	1.09	-	1.11	-	42.8	-	43.0	-	1.77	-	1.96	-	0.84	-	1.23	-
-	-	35	1.26	16	1.24	12	43.6	2	43.7	2	2.07	17	2.29	17	1.20	43	1.36	11
-	+	35	1.37	26	1.51	36	43.9	3	44.2	3	2.38	34	2.64	35	1.37	63	1.47	20
+	-	35	1.62	49	1.83	65	44.9	5	44.9	4	2.81	58	3.12	59	1.83	118	1.71	39
-	-	70	1.52	39	1.63	47	44.3	4	44.5	3	2.43	37	2.84	45	1.52	81	1.55	26
-	+	70	1.97	81	2.02	82	45.6	7	45.7	6	2.56	45	3.26	66	1.92	129	2.02	64
+	-	70	2.24	106	2.45	121	46.5	9	46.7	9	2.94	66	3.81	94	2.14	155	2.24	82
L.S.D.0.05			0.23	-	0.24	-	1.2	-	1.3	-	0.40	-	0.37	-	0.18	-	0.19	-

* Inoculation

** Foliar application of methanol

Table (6):Effect of inoculation, foliar application of methanol and N-fertilization on protein content in straw and grains of wheat at harvest in the two seasons (S₁) and (S₂).

Parameters			Protein content in straw Kg fed ⁻¹				Protein content in grains Kg fed ⁻¹			
treatments										
Inoc. *	ME**	N-Fer Kg fed ⁻¹	S ₁	%	S ₂	%	S ₁	%	S ₂	%
-	-	-	0.98	-	1.16	-	8.8	-	12.6	-
-	-	35	1.13	15	1.14	-1	14.7	67	16.8	33
-	+	35	1.46	49	1.65	42	16.9	92	18.3	45
+	-	35	1.87	91	1.95	68	21.0	139	22.7	80
-	-	70	1.69	72	1.81	56	18.8	114	18.9	50
-	+	70	2.11	115	2.20	90	22.2	152	23.5	87
+	-	70	2.62	167	2.77	139	24.9	183	27.0	114
L.S.D.0.05			0.17	-	0.29	-	2.50	-	3.50	-

* Inoculation

** Foliar application of methanol

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تحسين إنتاجية محصول القمح بالتلقيح البكتيري بالبكتريا المثبتة لنيتروجين الجوى بصورة حرة و الرش بمحلول الميثانول تحت مستويات مختلفة من التسميد النيتروجينى .

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الجيزة - مصر
** قسم النبات - المركز القومي للبحوث - الجيزة - مصر
*** قسم المحاصيل - كلية الزراعة - جامعة القاهرة - الجيزة - مصر

تم إجراء تجربتين فى أراضى طينية بالمزرعة التجريبية لكلية الزراعة - جامعة القاهرة
بالجيزة خلال موسمي ٩٨ - ٩٩ / - ٢٠٠٠ وذلك لدراسة تأثير التلقيح بالبكتريا المثبتة
للأزوت الجوى بصورة حرة و الرش بمحلول الميثانول تحت مستويات مختلفة من التسميد
النيتروجينى على كل من المحتوى الميكروبي للتربة ونمو النباتات ومحصول القمح صنف جيزة
١٦٤ واللقاح البكتيرى المستخدم عبارة عن خليط من سلالات الأروسيبريليم بيرازيلنس و
الأزوتو بكر كروكوم و الباسيلس بولى ميكسا بنسب (١ : ١ : ١) وقت الزراعة وتم الرش
بمحلول الميثانول بتركيز ٢٠% (حجم : حجم) مرتين فى عمر ٦٠ و ٨٠ يوم من الزراعة
وكانت مستويات التسميد النيتروجينى صفر و ٣٥ و ٧٠ كجم ن / فدان و أظهرت النتائج
المتحصل عليها من العينة النباتية عمر ٩٠ يوم أن المعاملة باللقاح البكتيرى أدى إلى زيادة
المحتوى الميكروبي للتربة (بكتريا _ اكتينوميسيس _ فطريات) مقارنة بتلك المتحصل عليها
من معاملات التسميد النيتروجينى أو الرش بالميثانول . وكان لمعاملة اللقاح البكتيرى مع كل من
مستوي التسميد النيتروجينى ٣٥ و ٧٠ كجم ن / فدان تأثير منشط معنوى وصل إلى ٢٤١% لوزن
النبات الجاف و ٢٨٢% بالنسبة لمحتوى النبات من النيتروجين . بينما أدى الرش بمحلول
الميثانول فى وجود السماد النيتروجينى إلى زيادات تراوحت بين ١١٩% إلى ١٤٨% بالنسبة
لوزن النبات الجاف بينما كانت ١٠٢% إلى ٢٠٣% لمحتوى النبات من النيتروجين . و فى
مرحلة الحصاد كانت أعلى الزيادات مسجلة فى وجود معاملات التلقيح البكتيرى مع التسميد
النيتروجينى عند أى من المستويين حيث أعطت أعلى زيادة فى جميع قياسات المحصول الناتج و
فى المحتوى البروتينى للمحصول .