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The Efficiency of Irrigation System and Nitrogen Fixation for inoculated Soybeans by using N15 Tracer Techniques

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***Abstract*—**Repeated additions of the unfertilized bacteria led to increase the activity of Nitrogen-fixing bacteria in the root zone with drip irrigation system compared to traditional manual vaccination to increase the proportion of Nitrogen from 29% to 64%, and the efficiency of adding Nitrogen fertilizer did not exceed 9.5% while dropped to 4%, due to the amount of fertilizer added was not exceed 20kg N/h, and the second was the existence of a large amount of available Nitrogen in the soil by fixation, while the efficiency of irrigation system between 2.08 to 2.26 kg/m3.

***Keywords*—**Drip irrigation system, Nitrogen Biological Fixation, Neutron Probe, N-15 Tracer Techniques

I. Introduction

NITROGEN macro-element - as known- is very essential for plant growth. In spite of Nitrogen (N_2) constitutes ~ 79% of the atmosphere, but it is not used directly by the plant, except some types of Microorganisms coexist with legume plants within the root zone (rhizosphere) to convert atmospheric Nitrogen to ammonia by vital complex processes called Biological Nitrogen Fixation. These processes were subjected to multi studies aimed to identifying organisms that coexist with the host plant and to quantify the amount of fixing Nitrogen [1], [2], [3], [4], [5]. As a result of significant rise in the human population growth and the increasing demand for food and high costs of energy and mineral fertilizers and for sustainable agriculture, these things and others made a responsibility on scientific researchers in this field to study and understand the mechanism of Biological Nitrogen-fixation process to increase the effectiveness of this mechanism and, therefore, securing adequate amounts of Nitrogen. The process of coexistence between leguminous plants and bacteria (rhizobia) Nitrogen-fixing air within the root nodes (modules) on the roots by exchange energy components of the plant to the bacteria while bacteria release fixing nitrogen to the plant legumes which coexist with it. In the seventies attentions were focused on obtaining energy and their costs and the manufacture costs of mineral fertilizers, while in the nineties, attentions focused on the environmental matters and pollutions caused by excessive use of these fertilizers, which push scientific researches to increase the efficiency of bacterial strains which fixing nitrogen- in order to increase the effectiveness biogenetic with economic legumes crops. The introduction of effective Rhizobium to the soil system by traditional cross-pollinated process is an important but had not been engaged an important role for many legume crops, because these crops have become adapted to local active breeds, but there is a legume plants that plays an important role in securing good source of protein for human and animal such as Soybean. The inoculated bacterial also an important factor in terms of preparation of effective strains in order to get the best production of the crop as a result of their coexistence with leguminous.

Important question is when and where preparing inoculated bacterial and what is concentration of inoculated bacteria should added to the seeds or soil to confirm the formation of effective colonies on the root system of the plant host [6], [7], [8], and therefore it is very necessary to subject pollinator species of bacteria to the good conditions of preparation, isolation, timing, place and the mechanism addition, especially if the target pollination entrance recently to our area and there is no effective strains in the soil of that region such as soybean crop. Also, the environmental and climatic conditions and the scarcity of rainfall were surrounding imposed additional conditions on the cultivation of all irrigated crops, to rationalize the use of irrigation water and fertilizer, Hence, the aims and goal of this research to answer some questions including:

1 – Did the repeated cross-pollination by irrigation system increasing the rate of fixing Nitrogen.

2 – Which the best traditional pollination by inoculated Packaging seeds manually before planting or cross-pollination by irrigation water?

3 – Is there any relationship between increasing the efficiency of Nitrogen fixation and the efficiency of irrigation system?

II. Materials and Method

The experiment was carried out during the growing summer season of 2008 in Agriculture research station, Soil samples were taken to determine some chemical and physical properties of the site. Samples were collected before planting to a depth of 45 cm. Soil reaction (pH), conductivity (EC), organic matter (O.M) were measured, available phosphorus measured according to Olsen method, total & available Nitrogen (NO_3, NH_4) were estimated, also samples were collected direct after harvest and tested for total & available Nitrogen (Table 1). Soybean was planted in lines (20*75 cm) by rate of 80 Kg/ha, while the Corn (as a reference crop) was

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planted (15* 75 cm) by rate 30 Kg/ha. In the center of the experiment a selected area (1*2 m) was used for adding labeled N-15. The experiment was designed according to randomize complete block design (RCBD), 6 treatments with 6 replications did in 36 plots, and density of plant 66600 plants / ha for soybeans and 88800 plant/ ha for the Corn, statistical analysis was done by Analysis of variance (ANOVA) with a Duncan multiple domains (DMRT) at a confidence level 95%. Strains of bacteria known (Brady Rhizobium Japonium) selected, which has highly efficiency with the roots of soybeans.

The following treatments tested in the experiment were as:

T1: injection of bacterial inoculants with the irrigation water one time at planting. .

T2: injection of bacterial inoculants twice with irrigation, the first at planting, and the second when the fourth leaf was grown.

T3: as in T2, plus a third time at flowering stage.

T4: as in T3, plus a fourth injection after flowering stage.

C1: Control, only one traditional way inoculants by mixing bacterial inoculants with the seed. .

C2: Control without inoculation. .

Irrigation was scheduled by using a Neutron probe, when soil moisture at 75% of field capacity with the effective depth 25 cm at first stage from planting to flowering, and 50 cm from the flowering until weaning. Axis tubes of neutron probe were fixed in three replications to follow the situation moisture of the soil in order to schedule the irrigation, the total amount of irrigation water added in 20 times about 7736 m3/ ha. Collected plant samples for normal isotopic analysis and account for the experimental plots marked by N-15, where, two plants picked from reference crop Corn and legume crop Soybean from the center line at the stage of fully physiological, wet weight determined and then dried on 65Cº more than 72 hrs, and tests were undergone to determining the theoretical ratio of N-15 by a spectrum (150 Jasco), total Nitrogen by a digestion wet (Kjeldahl), dry matter and total Nitrogen uptake (kg N/h) content in plant tissue, in addition to Nitrogen derived from Soil (Ndfs), Nitrogen derived from fertilizer (Ndff), Nitrogen derived from air for legume crop (Ndfa), and the amount of total irrigation was measured to calculate the efficiency of irrigation water (Ef) on the basis of the production of dry matter only (Efd) or on the basis of grain production (Efy).

III. Results and Discussion

The production of dry matter in shoot (vegetative part) between 4.4 T/ha for T1 and 5.2 T/ha for C2, while the production of dry matter to the same portion of plant 4.7, 4.9, 4.5, and 4.8 T/ha for treatments C1, T4, T3, and T2 respectively, also the grain production 3.4 T/ha for C1, 3.8 T/ha for T3, T4, and T1. (Table 2). Although, there are some minor differences in grain production which indicating a positive result for injection technology which did not lead to reduce crop production, but keeping soybean production was the same compared to traditional inoculation. This result, despite of positive but remains questionable, because of the high concentration of available Nitrogen in the soil which made the crop response governed by the impact of remaining Nitrogen in the soil, and not due to tested treatment, however the increasing of available Nitrogen concentration inhibiting the action of responsible bacteria which fixing Nitrogen. But, the fact remains positive results especially if the ideal situation of available Nitrogen in the soil was low.

The production of dry matter of shoot for reference crop (Corn) ranged between 7.2 T/ha for T2, and 8.1 T/ha for T1, while the production of dry matter for the same part of plant 7.4, 7.3, 8.0 and 7.8 T/ha for C2, C1, T4 and T3respectively (Table 3), these results indicate that the reference crop did not reach maturation stage when the sampling shoots and grain, due to early harvest for fear of losing a large part of the grain by birds.

Injection inoculated bacteria method with frequency impact on the production of total dry matter and total Nitrogen uptake and content in the plant full of Nitrogen and Nitrogen derived from the soil for soybeans crop (Table 4), from the results that the total dry matter of all studied treatments were close, there were no significant differences between treatments, the quantity of dry matter produced 8.7, 8.1, 8.6, 8.3, 8.4 and 82 T/ha for C2, C1, T4, T3, T2 and T1 respectively, while it was noted significant in the total Nitrogen index in the plant tissues, where T1 recorded clearly superior on control C2. There are no significant differences between the averages followed by the same letter within a column according to Duncan multiple tests at a confidence level 95%. Whereas no any significant differences between the rest treatments with noted trend in the increase of Nitrogen concentration in plant tissues for T3, T2, and T1 compared to control C2, although the behavior of total nitrogen index derived from the soil similar to an index of dry matter in terms of there were not significant differences between tested treatments, but observed that a trend to increase the amount of Nitrogen uptake for repeated vaccination treatments with the highest amount of Nitrogen uptake for T1, and the lowest for the C2, and the quantity of total Nitrogen uptake were 157, 160, 165, 181, 169 and 192 kg N/ ha for C2, C1, T4, T3, T2 and T1 respectively, due to the amount of Nitrogen biological fixation and Nitrogen derived from the soil, so the percentage of Nitrogen derived from the soil 75, 70, 43, 36 and 35% of the total Nitrogen uptake for C2, C1, T4, T3, T2 and T1 respectively. Increase in the Nitrogen derived from the soil for C1 and C2 refer to the ineffectiveness of the existing strains of Rhizobium, also the presence of available Nitrogen in the form NH4 in the soil before planting, which reflected on the amount of Nitrogen derived from air were (37, 47, 100, 108, 107, and 122 kg N/ha) for treatments C2, C1, T4, T3, T2, and T1 respectively. From the results there were no significant differences between T4, T3, T2, and T1, while there were significant differences between (T4, T3, T2, and T1) and control treatments (C1 and C2). An Important thing was the variance in the amount of biological Nitrogen fixation between (T4, T3, T2, and T1) and C1, which reached these differences, for example, 86 kg N /

TABLE I
CHEMICAL AND PHYSICAL PROPERTIES OF SOIL SITE

Soil depth cm	Soil Nitrogen						Available Phosphorus ppm	EC Ds/m	pH	O.M %	Texture
	Total %		NH4 ppm		NO3 ppm						
	B.A[1]	A.A[2]	B.A[1]	A.A[2]	B.A[1]	A.A[2]					
0-15	0.09	0.08	182	21	6.0	1.1	7.2	0.31	8.0	0.71	Sandy clay Loam
15-30	0.08	0.08	180	26	7.0	1.0	5.4	0.39	8.0	0.56	
30-45	0.09	0.07	188	19	8.0	0.7	5.3	0.42	7.9	0.77	

[1] Before Agriculture
[2] After Agriculture

TABLE II
PRODUCTION OF DRY MATTER, PERCENTAGE OF TOTAL NITROGEN AND NITROGEN UPTAKE IN VEGETATIVE AND GRAIN PARTS OF SOYBEAN

Soybean				
Treatment / Plant Parts	Vegetative Part		Seed Production	
	T/h	%	T/h	%
T1	4.4a	54	3.8a	46
T2	4.8a	57	3.6a	43
T3	4.5a	54	3.8a	46
T4	4.9a	56	3.8a	44
C1	4.7a	58	3.4a	42
C2	5.2a	59	3.6a	41
Total Nitrogen N%				
	Vegetative Part N%		Seed Production N%	
T1	1.01a		3.85a	
T2	0.77a		3.66a	
T3	0.92a		3.6a	
T4	0.72a		3.41a	
C1	0.99a		3.73a	
C2	0.86a		3.00a	
Nitrogen Uptake Kg N/ha				
	Vegetative Part		Seed Production	
	Kg N/ha	%	Kg N/ha	%
T1	45a	23	147a	77
T2	37a	22	132a	78
T3	41a	23	140a	77
T4	35a	21	130a	79
C1	47a	29	113a	71
C2	45a	29	111a	71

TABLE III
PRODUCTION OF DRY MATTER, PERCENTAGE OF TOTAL NITROGEN AND NITROGEN UPTAKE IN VEGETATIVE AND GRAIN PARTS OF CORN

Sorghum Maize				
Treatment / Plant Parts	Vegetative Part		Seed Production	
	T/h	%	T/h	%
T1	8.1a	66	4.1b	34
T2	7.2a	63	4.3b	37
T3	7.8a	60	5.1ab	40
T4	8.0a	56	6.4a	44
C1	7.3a	61	4.7ab	39
C2	7.4a	58	5.4ab	42
Total Nitrogen N%				
	Vegetative Part N%		Grain Production N%	
T1	1.03a		1.65a	
T2	0.90a		1.48a	
T3	0.94a		1.63a	
T4	0.81a		1.56a	
C1	0.88a		1.67a	
C2	0.87a		1.68a	
Nitrogen Uptake Kg N/ha				
	Vegetative Part		Grain Production	
	Kg N/ha	%	Kg N/ha	%
T1	83a	55	68ab	45
T2	67a	52	63a	48
T3	74a	47	84ab	53
T4	65a	40	99a	60
C1	65a	44	84ab	56
C2	64a	42	90ab	58

TABLE IV
METHOD IMPACT AND REPEATED INOCULATED INJECTION ON THE PRODUCTION OF DRY MATTER, NITROGEN UPTAKE, AND BIOLOGICAL NITROGEN FIXATION FOR SOYBEAN

Treatment	Dry matter	Total N content	Nitrogen Uptake	Nitrogen Withdrawn From Soil	Nitrogen Withdrawn From Fertilizer	Nitrogen Withdrawn From Air		Efficiency of added Nitrogen
	T/ ha	%	Kg N/ ha	%	%	%	Kg N/ ha	%
T1	8.2a	2.31a	192a	35	0.6	64a	122a	6.0
T2	8.4a	2.01ab	196a	36	0.6	63a	107a	5.5
T3	8.3a	2.18ab	181a	39	0.7	60a	108a	6.0
T4	8.6a	1.91ab	165a	38.5	0.5	61a	100a	4.0
C1	8.1a	1.98ab	160a	70	0.8	29b	47bc	6.5
C2	8.7a	1.71b	157a	75	1.2	24b	37c	9.5
LSD	2.3	0.51	70	-	-	15	50	-

-There is no significant difference between averages followed by the same letter within a Column according to multiple Duncan test at 95%.

TABLE V
DRY MATTER PRODUCTION, NITROGEN UPTAKE, AND NITROGEN WITHDRAWN FROM SOIL FOR SORGHUM MAIZE UNDER DIFFERENT ADDITION OF INOCULATION TREATMENT

Treatment	Dry matter	Total Nitrogen	Nitrogen uptake	Nitrogen withdrawn from soil		Efficiency of added Nitrogen
	T/ha	%	Kg N/ha	%	Kg N/ha	%
T1	12.2a	1.24a	152a	98a	149a	12.5
T2	11.5a	1.10a	130a	99a	128a	11.0
T3	13.0a	1.20a	157a	99a	155a	12.5
T4	14.4a	1.20a	164a	99a	162a	11.0
C1	12.0a	1.20a	149a	99a	148a	08.5
C2	12.8a	1.20a	155a	99a	152a	12.5
LSD	2.6	0.22	49	0.90	49	-

-There is no significant difference between averages followed by the same letter within a Column according to multiple Duncan test at 95%.

TABLE VI
NITROGEN FIXING PERCENTAGE AND IT'S DISTRIBUTE IN PLANT TISSUES

Treatment	Amount of N uptake from fertilizer Kg N/ ha	Amount of N uptake from Soil Kg N/ ha	Fruit System		Root System		Total fixed N/ha
			%	Kg N/ha	%	Kg N/ha	
T1	1.2	69.0	82	100	18	22	122 a
T2	1.3	61.0	81	87	19	20	107 a
T3	1.3	71.7	82	89	18	18	108 a
T4	1.0	64.0	85	85	15	15	100 b
C1	1.3	109.0	93	44	7	3	47 bc
C2	2.0	118.0	81	30	19	7	037 c

TABLE VII
EFFICIENCY OF IRRIGATION SYSTEM ACCORDING TO TESTED TREATMENT

Treatment	Soybean Kg/m^3			Sorghum Corn Kg/m^3		
	Grain E_{fy}	Vegetative System E_{fd}	Whole Plant E_f	Grain E_{fy}	Vegetative System E_{fd}	Whole Plant E_f
T1	0.98	1.14	2.12	1.04	2.1	3.14
T2	0.92	1.16	2.08	1.14	1.86	3.00
T3	0.98	1.16	2.14	1.32	2.02	3.34
T4	0.98	1.26	2.24	1.64	2.06	3.70
C1	0.86	1.22	2.08	1.22	1.88	3.10
C2	0.92	1.34	2.26	1.38	1.90	3.28

TABLE VIII
GRAIN AND VEGETATIVE PRODUCTION OF SOYBEAN AND CORN

Treatment	Fruit Parts		Vegetative Parts		Seeds Vegetation Ratio SVR	
	Soybean	Corn	Soybean	Corn	Soybean	Corn
T1	3.8	4.1	4.4	8.1	0.86	0.51
T2	3.6	4.3	4.8	7.2	0.75	0.60
T3	3.8	5.1	4.5	7.8	0.84	0.65
T4	3.8	6.4	4.4	8.0	0.78	0.80
C1	3.4	4.7	4.7	7.3	0.72	0.64
C2	3.6	5.4	5.2	7.4	0.69	0.73

ha between T1 and C1 and arrived at a minimum 53 kg N /ha C1 and T4, and gap increased when compared repeated inoculation T4, T3, T2, and T1 with C2. So, the repeated additions of inoculated bacteria led to increase the activity of bacteria of the atmospheric Nitrogen in the root zone led to increase the biological Nitrogen fixation from 29% to 64%. The efficiency of adding nitrogen fertilizer was low for all treatments, and did not exceed 9.5% for C1, and dropped to 4.0% for T4. This result was expected due to:
1- The amount of Nitrogen fertilizer added was relatively low which equal 20 kg N/ha added once as starter.
2- The second was the presence of relatively large amounts of available Nitrogen in the soil in addition to Nitrogen fixing from air.

Reference crop (Sorghum Corn) observed similar indicators of total dry matter and total Nitrogen uptake when compared with the same indicators for Soybean, but the situation was slightly different when compared Nitrogen in the tissues did not show in the Corn tissues any differences in the total of Nitrogen content and all tested treatment, (Table 5), the explanation of this result that the Corn crop cannot fixing atmospheric Nitrogen, on the other hand the presence of relatively large amounts of available Nitrogen in the soil which provide the needs of Nitrogen, and therefore did not show significant differences in the content of these tissues from Nitrogen.

The amount of fixing atmospheric Nitrogen in the plant tissues for whole plant at physiological maturity stage between 37 kg N/ ha for C2 and 122 kg N/ha for T1 (Table 6). The absence of significant differences in the amount of Nitrogen that was in the whole plant treated by T4, T3, T2, and T1 and had been reflected on the amount of fixing atmospheric Nitrogen in studied plant parts, including fruit system (grains) and shoot system (plant part above the soil surface), which ranged for grain 85% for T4, while for T1, T2, and T3 very close between 81% for T2 and 82% for T3 and T1. In comparing with the results of the atmospheric Nitrogen distribution in studied plant tissue with C1 and C2 found that 93% of the fixed total Nitrogen transferred to seed for C1, and 81% for C2. Tested amount of total Nitrogen derived from soil and fertilizer (kg N / ha) from the soil for C2 was (118 kg N / ha), followed by C1 was (109 kg N / ha). Whereas the amount of total Nitrogen derived from the soil for injections inoculated treatment with irrigation system ranged between 64 and 72 kg N / ha, So reasons for Soybean fully un depended on soil Nitrogen in T4, T3, T2 and T1 compared to C1 and C2 due to increasing amount of biological Nitrogen fixation for these treatments compared to C1 and C2, and thus secured a large part of its needs from an atmospheric Nitrogen. Efficiency of irrigation system Index was calculated (Ef) based on the total dry matter produced including grains to the amount of irrigation water actually added (kg /m3), also the efficiency of irrigation system for grain production (Efy) and shoot (Efd) to both the crops (Table 7), where the amount of water added to Soybeans was 3868 m3/ha and is the same quantity to the Corn, thus field efficiency of irrigation water was calculated on this basis, the values of irrigation efficiency for Soybeans ranged between 2:08 Kg/m3 for T2 and C1, and 2.26 kg / m3 for C2. From the results a clear similarity in the values between tested treatments [9], which means that the addition of inoculants with irrigation water did not have a negative impact compared to traditional method. Seeds Vegetation Ratio (SVR) was estimated (Table 8), for Soybeans ranged between 0.69 for C2 and 0.86 for T1, while for the Corn SVR ranged between 0.51 for T1 and 0.80 for T4. Thus there were not negative impact on the values of this index for both crops to inject the bacteria, but there were some positive results to increase with repetitive or injections frequency.

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