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Enhancement Nitrogen Fixation via Inducing Recombinants in *Azospirillum*

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Abstract: This study aimed to induce recombinants between *Azospirillum* and *Azotobacter* inoculants. *Azotobacter* strains used as donors, however, *Azospirillum* strains were used as recipients, as well as, the reverse mating were done to induce recombinants from each microbe. Three *Azospirillum* strains and four *Azotobactor* strains were used in this study. The resulted isolates were evaluated for IAA production. The results revealed that eight transconjugants from four matings between *Azotobacter* as a donor and *Azospirillum* as a recipient produced significant values of IAA over the better parent. The mentioned transconjugants were selected to be applied in pots and field experiments at the absence and presence of 2,4-D. Some of transconjugants appeared significantly increase in some parameters over both the better parents and plants fertilized with the recommended dose. On the other hand, many transconjugants significantly increased some parameters over the better parent in response to the interaction between the addition of 2,4-D and biofertilizers. Although some transconjugants resulted from the reverse mating between *Azospirillum* as a donor and *Azotobacter* as a recipient produced significant amounts of IAA over the better parent This study indicated that *Azospirillum* was better recipient than *Azotobacter*.

Key words: 2,4-D, *Azospirillum*, *Azotobacter*, recombinants, wheat biofertilization.

INTRODUCTION

Wheat (*Triticum aestivum*) is the most important cereal crop worldwide, with a production of 585 x 10^6 metric tons of grain per year (Antle and Smith 1999). Moreover, it is a staple food for nearly 35% of the world population. The expected global demand for wheat was suggested previously (Rosegrant *et al*., 1995) that it would be 840 to 1050 million tons in 2002 and will be more in the future. Wheat is mainly consumed in the form of bread. It is also the major staple crop produced in Egypt, occupying about 32.6 percent of the total winter crop area.

Continuous use of high doses of chemical fertilizers has adversely affected soil health leading to a decline in productivity in many wheat growing areas in the world. In addition, there are large areas in Asia and Africa where wheat is grown under rainfed and/or limited water supply conditions. In these areas, soils are poor in mineral nutrients, organic carbon and rhizospheric activity. Due to economic reasons and associated risk factors, fertilizer application rates are far below the recommended doses. This contributes to low productivity of wheat in such areas. Therefore, to maximize production all over the world emphasis is being placed on the selection of high and low input efficient wheat genotypes responsive to bioinoculants and applied inorganic nutrients for different agro-climatic areas.

Bacteria of the genus Azospirillum are nitrogen-fixing bacteria which grow in close association with the roots of grasses without the formation of differentiated structures (Okon 1985). This organism may be found in root mucilages or within the root cortex of various agriculturally important plants (Elmerich 1984). *Azospirillum brasilense* has been observed to stimulate plant growth by producing plant growth hormones, enhancing the uptake of nutrients, and increasing the nitrogen content of plants (Okon 1985). In order to obtain these plant growth-promoting effects, good colonization of the plant roots by Azospirillum spp. appears to be important (Kapulnik *et al.* 1985). It has been shown that Azospirillum spp. can attach to root hairs (Elmerich 1984), and recently it has been communicated that A. brasilense can also attach to individual cells (Eyers *et al.* 1988). However, the nature of the association of Azospirillum spp. with plants remains unclear.

One of the most important interactions between microorganisms is the transfer of genetic material or horizontal gene transfer. Horizontal gene transfer is the direct transfer of genetic material from one organism to another. Horizontal gene transfer is a natural and ongoing process and has contributed to the genetic diversity and evaluation of all organisms. Transfer of DNA across kingdom boundaries is not uncommon in

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nature. In the past twenty years, humans have learned how to direct horizontal gene transfer to develop genetically modified organisms. There is currently an active debate in norway concerning the use of genetic technologies in agriculture. One of the main concerns with the use of genetically modified microorganisms is the transfer of genes from one strain to another. There are three main modes or methods of horizontal gene transfer including conjugation, transformation and transduction.

Conjugation is one of biotechnological techniques used in this study to induce genetically modified strains, it was including the exchange of DNA between two bacterial cells . Transferable plasmids can integrate into the chromosome and transfer chromosomal sequences. These plasmids often carry some selectable marker or a selective advantage for the cell that contains it.

The objective of this study aimed to develop recombinant inoculants from *Azospirillum* and *Azotobacter* strains via conjugation that can effectively compete with agri-chemical, to be used as a biofertilizers and phytostimulators with wheat plants treated with synthetic hormone 2,4-D (2,4-dichlorophenoxy acetic acid), this hormone was used to induce the paranodules in wheat plants to increase the nitrogen fixation.

MATERIALS AND METHODS

I. Materials:

Genetic Materials and Growth Conditions of Bacterial Strains:

Seeds of wheat Sakha 93 (2n = 42) kindly provided from Field Crop Research Institute, Agric. Res. Center, Giza, were used in this study. Three wild type strains of *Azospirillum*, as well as, four wild type strains of *Azotobacter* were used in this study as listed in Table 1.

Table 1: Bacterial strains used in this study and their designations.

Strains	Relevant genotypic markers	Designation	Source or references
Azospirillum brasilense NRRL B-14647	$Amp^- Erth^+ Ceph^- Rf^+ Str^-$	St_1	U.S Dept. of Agr. USDA Agr. Res. Service
Azospirillum lipoferum NRRL B-14654	$Amp^+ Erth^- Ceph^+ Rf^- Str^-$	St_2	U.S Dept. of Agr. USDA Agr. Res. Service
Azospirillum brasilense 12400T	$Amp^+ Erth^+ Ceph^+ Rf^+ Str^-$	St_3	IAM culture collection, Univ. of Tokyo, Japan.
Azotobacter vinelandii NRRL B-14644	$Amp^- Erth^- Ceph^+ Rf^- Str^-$	St_4	U.S Dept. of Agr. USDA Agr. Res. Service
Azotobacter vinelandii NRRL B-14641	$Amp^+ Erth^- Ceph^+ Rf^+ Str^+$	St_5	U.S Dept. of Agr. USDA Agr. Res. Service
Azotobacter chrocooccum NRRL B-14336	$Amp^- Erth^+ Ceph^+ Rf^- Str^-$	St_6	U.S Dept. of Agr. USDA Agr. Res. Service
Azotobacter vinelandii NRRL B-4204	$Amp^- Erth^- Ceph^+ Rf^- Str^+$	St_7	U.S Dept. of Agr. USDA Agr. Res. Service

Media and Growth Condition:

Azospirillum strains were grown in nitrogen free bromothymol blue (NFB) medium as described by Dobereiner and Day (1976). On the other hand, *Azotobacter* strains were grown in modified Ashby medium as described by Rao (1984). All rhizobacterial strains were grown at 28-30°C with shaking at 150 rpm for three days.

Genetic Marking:

Five antibiotics appeared variations out of 15 were presented in Table 2 concerning genetic marking of *Azospirillum* and *Azotobacter* strains. These were selected on the basis of genetic variations appeared in *Azospirillum* and *Azotobacter* strains, while the other antibiotics giving similar results in resistance and/or sensitivity among all *Azospirillum* and *Azotobacter* strains (data not presented here because of similarity), does not listed in the table

Table 2: Genetic variations in *Azospirillum* and *Azotobacter* strains.

Bacterial Strains	Antibiotic markers				
	Ampicilin	Erythromycin	Cephalexin	Rifampcin	Streptomycin
Azospirillum brasilense NRRL B-14647	-	+	-	+	-
Azospirillum lipoferum NRRL B-14654	+	-	+	-	-
Azospirillum brasilense 12400T	+	+	+	+	-
Azotobacter vinelandii NRRL B-14644	-	-	+	-	-
Azotobacter vinelandii NRRL B-14641	+	-	+	+	+
Azotobacter chrocooccum NRRL B-14336	-	+	+	-	-
Azotobacter vinelandii NRRL B-4204	-	-	+	-	+

+,- = Resistant and sensitive, respectively.

II. Methodology:

Antibiotic Susceptibility Assays:

Antibiotic susceptibility was measured by plate diffusion method, according to Collins and Lyne (1985). The diameter of resulting zones of inhibition was measured according to Toda *et al.*, (1989). All antibiotics were produced by Hoechst Orient S.A.E., Cairo, Egypt, they were used with the concentration of 400 mg/ml, according to Roth and Sonti (1989).

Conjugation:

Nutrient broth cultures, in the late-exponential growth phase were used in conjugation experiment. Quantitative spot mating of conjugal transfer was carried out according to Lessel *et al.* (1993) by inoculating 10 ml samples of the donor cultures onto the surface of selective medium, previously seeded with 100 ml of the recipient culture. A single colony of transconjugants was picked up and transferred to slant nutrient agar medium. Conjugation was carried out between *Asospirillum* against *Azotobacter* strains as shown in Table 3. Two different isolates from each mating were selected to be applied in the field, on the basis of higher production of IAA.

Table 3: Horizontal DNA transfer between *Azospirillum* and *Azotobacter* strains that having the opposite genetic markers.

Matings	Transconjugant genotype	Designation of transconjugants
St_4 ($Erth^-$ $Ceph^+$) x St_1 ($Erth^+$ $Ceph^-$)	$Erth^+$ $Ceph^+$	Tr_1 Tr_2
St_5 ($Erth^-$ $Strep^+$) x St_3 ($Erth^+$ $Strep^-$)	$Erth^+$ $Strep^+$	Tr_3 Tr_4
St_6 ($Ceph^+$ Amp^-) x St_1 ($Ceph^-$ Amp^+)	$Ceph^+$ Amp^+	Tr_5 Tr_6
St_7 ($Erth^+$ $Ceph^-$) x St_1 ($Erth^+$ $Ceph^-$)	$Erth^+$ $Ceph^+$	Tr_7 Tr_8
St_3 ($Erth^+$ $Strep^-$) x St_5 ($Erth^-$ $Strep^+$)	$Erth^+$ $Strep^+$	Tr_9 Tr_{10}

IAA-Detection with the Salkowski Colorimetric Technique:

Azospirillum and *Azotobacter* strains were grown overnight in NFB and modified Ashby media, respectively at 30°C. Production of IAA in the culture supernatant was assayed as described by Pilet and Chollet (1970). Absorbance was measured at 530 nm. IAA concentrations was calculated from the following formula of regression (Fig. 1) as follows :

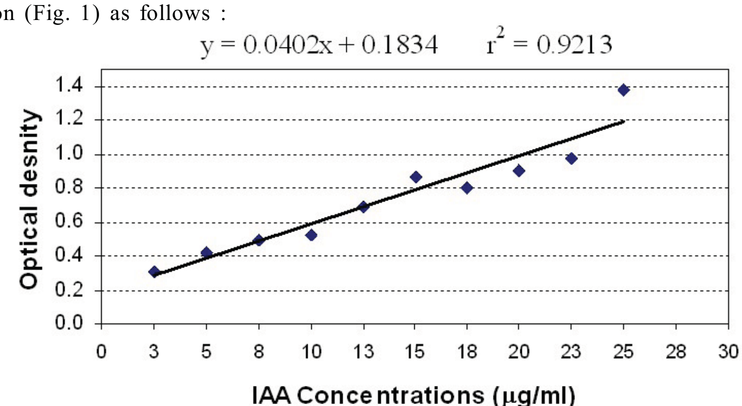

Fig. 1: Standard curve of IAA.

$$x = \frac{y - a}{b}$$

Where: y = Optical density at 530 nm, x = Concentration of IAA, b = Regression = 0.0402, a = Absorbance at 530 nm when the concentration of IAA equal zero = 0.1834

Pots and Field Trials:

The plants were grown in the farm of Tag El-Ez Agriculture Research Station, through the winter season of 2007 with and without the addition of 2,4-D. Inoculation with rhizobacteria was carried out three times after the plants emergence, with 5 ml of each inoculum (approximately 10^8 ml^{-1} CFU) per pot, each containing four plant. Plants are treated with 0.7 ug ml^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D) followed by inoculation.

Field Trials:

Seeds of wheat were sown, rows three meters long, 50 cm apart and 20 cm spacing between hills. The plants were grown under biofertilization of *Azospirillum* and *Azotobacter* in December 2007 in a split plot design. The plants were watered to the field capacity with Nile river water as needed until harvest in May

2008. The plants were fertilized with phosphorus 37% (40 kg/feddan). Biofertilization was conducted as shown in Table 4 depending on plant density of 200000 plant/feddan represent 40000 hills.

Leaf Area / Plant (L.A. / P):

It was determined in the leaves of 120 days plant-old using the flag leaf area method according to Stickler (1964) using the following formula :

Flag leaf area (cm^2) = Maximum length (cm) x Maximum width (cm) x 0.75

Table 4: Biofertilizer inoculants and chemical fertilization applied to wheat plants .

Time of inoculation after sowing	Treatment No. 1 (25% N / hill)	Treatment No. 2 (recommended dose of N / hill)	Biofertilization + 25% N / hill
Before sowing	0.21 g urea	0.85 g urea	As the same in treatment No. 1 + *Azospirillum or Azotobacter* isolates with or without 2,4-D
After month	0.42 g urea	1.69 g urea	As the same in treatment No. 1 + *Azospirillum or Azotobacter* isolates with or without 2,4-D
After two months	0.42 g urea	1.69 g urea	As the same in treatment No. 1 + *Azospirillum or Azotobacter* isolates with or without 2,4-D

Biochemical and Physiological Traits:

Chlorophyll Concentration:

One gram of three leaves collected from different plants in each inoculant at 65 days plant-old was well mixed in 10 ml of 80% methanol and kept overnight in the dark. Read the absorbance of this solution at 650 and 665 nm against the solvent blank (80% methanol). Calculate the amount of chlorophyll presented in the extract (mg chlorophyll per g tissue) using the following equations according to Markinney (1941) ;

$$\text{mg Chl. (a) / g tissue} = 16.5\ A\ 665 - 8.3\ A\ 650 \times \frac{V}{1000 \times W}$$

$$\text{mg Chl. (b) / g tissue} = 33.8\ A\ 650 - 12.5\ A\ 665 \times \frac{V}{1000 \times W}$$

$$\text{mg Total Chl. / g tissue} = 25.5\ A\ 650 - 4.0\ A\ 665 \times \frac{V}{1000 \times W}$$

Where: A = Absorbance at specific wavelengths, V = Final volume of chlorophyll extract in 80% methanol, W = Fresh weight of tissue extracted.

Determining the Concentration of Nitrogen:

Determination of nitrogen in the shoot samples was carried out according to (APHA, 1992) using a standard curve of colorimetric technique. Nitrogen concentrations were calculated from the linear regression equation as follows:

$$x = \frac{y - a}{b}$$

Where: y = Optical density at 425 nm, x = Concentration of nitrogen, b = Regression = 0.1167, a = Absorbance at 425 nm when the concentration of N equal zero = 0.051, Total nitrogen content (mg / plant) = N_2 x dry weight of plant x 100, Crude protein in grain (%) = Grain N percentage x 5.7, (Sharma, 2001).

Grain Yield and its Components:

Dry wheat grains were harvested at 150 days plant-old when reached to suitable maturity stage at the first of May 2008. Yield was calculated as the weight of harvested grains measured in the sample of four randomly mature plants.

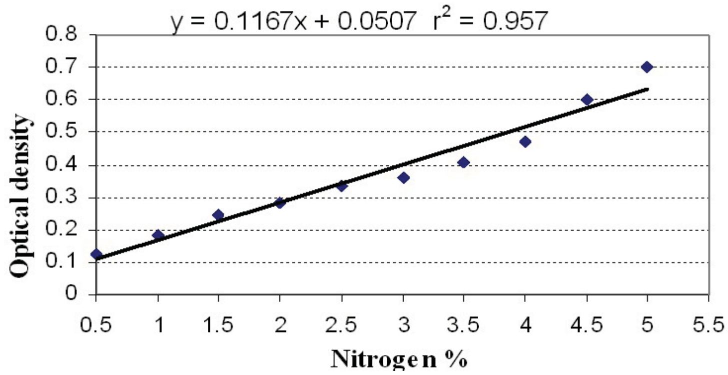

Fig. 2: Standard curve used in calculation nitrogen percentage produced by parental strains and their recombinant isolates.

Experimental Design and Statistical Analysis:

Field experiment was done in split-plot design involves assigning the treatments of one factor 2,4-D treated plants in the main plots arranged in a completely random, where biofertilization was assigned to subplots within each main plot. The data was subjected to the analysis of variance of split-plot design according to Snedecor and Cochran (1955).

The data of indole acetic acid were subjected to the analysis of variance. Least significant difference (LSD value) was used to compare between the means if the F-test was significant.

RESULTS AND DISCUSSION

Production of Indole Acetic Acid:

Diverse soil microorganisms including bacteria are capable of producing physiologically active quantities of auxins, which may exert pronounced effects on plant growth and establishment.

The resulted isolates from the matings between *Azotobacter* and *Azospirillum* strains were evaluated for IAA production as shown in Table 5. The results revealed that transconjugants Tr_2 and Tr_5 resulted from the mating between St_4 x St_1 appeared significant increase in IAA production over the mid parents, whereas, Tr_2 and Tr_3 resulted from the mating between St_5 x St_3 revealed significant increase in IAA production over the mid parents. Together, transconjugant Tr_2 and Tr_5 resulted from the mating between St_6 x St_1 revealed the same trend in IAA production. In addition, Tr_1 and Tr_4 resulted from the mating between St_7 x St_1 revealed significant increase in IAA production over the mid parents. These results indicated that some recombinants induced higher amounts of IAA from tryptophan. The mentioned transconjugants were selected to be applied in pots and field experiments.

Table 5: Means of IAA concentrations produced by *Azospirllum* and *Azotobacter* strains and their transconjugants:

Matings	Donor (*Azotobacter*)	Recipient (*Azospirillum*)	Mid-parents	Tr1	Tr2	Tr3	Tr4	Tr5	F-test	LSD 0.05	LSD 0.01
St_4 x St_1	10.6	38.8	24.7	25.5	**56.8**	36.3	47.1	**52.3**	**	7.3	10.2
St_5 x St_1	19.4	38.9	29.1	11.3	11.7	12.5	10.9	7.3	**	2.4	3.4
St_5 x St_3	19.4	37.2	28.3	39.5	**45.0**	**44.5**	40.8	41.6	**	4.6	6.5
St_6 x St_3	3.2	37.9	20.6	7.5	5.9	7.3	4.2	9.5	**	3.5	4.9
St_6 x St_1	3.2	38.8	21.0	40.4	**44.9**	41.1	36.8	**45.3**	**	5.4	7.5
St_7 x St_1	1.1	38.9	20.0	**42.6**	40.3	39.4	**42.4**	39.1	**	3.2	4.5

Table 5: Continued:

Matings	Donor (*Azotobacter*)	Recipient (*Azospirillum*)	Mid-parents	Tr1	Tr2	Tr3	Tr4	Tr5	F-test	LSD 0.05	LSD 0.01
St_1 x St_5	38.9	19.4	29.1	34.6	32.9	10.1	2.7	0.5	**	2.5	3.5
St_3 x St_5	37.2	19.4	28.3	5.5	36.8	33.9	**50.4**	**49.6**	**	2.3	3.2
St_3 x St_6	37.9	3.2	20.6	0.7	0.3	1.3	0.8	0.7	**	0.4	0.5
St_1 x St_6	38.8	3.2	21.0	6.9	8.1	4.9	0.6	1.0	**	0.9	1.2
St_1 x St_7	38.9	1.1	20.0	1.3	0.2	4.9	5.5	1.0	**	1.1	1.6

The phytohormones are essential in diverse aspects of plant growth and development. The indole-3-acetic acid (IAA), is known to regulate diverse aspects of plant growth and development, including cell division, cell extension, and cell differentiation and play a crucial roles in root initiation, apical dominance, tropisms, and senescence (Hagen and Guilfoyle 2002).

Furthermore, bioinoculant recombinants (extra copy of DNA) may produce high amounts of IAA than their parental strains, because it may have extra copy of *ipdC* gene, which encodes a key enzyme in the indole pathway of IAA synthesis (Dobbelaere *et al*., 1999). Matings between *Azospirillum* as a donor and *Azotobacter* as a recipient did not demonstrate any transconjugants produced IAA significantly over the mid parents or over the better parent, except for, Tr4 and Tr5 resulted from the mating between St_3 x St_5 exhibited significant heterosis (related to the mid parents) and significant hybrid vigor (related to the better parent) in IAA produced from tryptophan pathway. This indicated that these *Azospirillum* transconjugants may carry extra copy of *ipd* C gene, which encodes a key enzyme in the IPYA pathway of IAA synthesis by *Azospirillum* (Dobbelaere *et al.* 1999).

Evaluate of Recombinants in Pots Experiment:

As shown from the data summarized in Table 6 that the means of chlorophyll content (chlorophyll a , chlorophyll b and total chlorophyll) were significantly higher (0.677, 3.89, 4.57 mg/g, respectively) in plants treated with 2,4-D over the untreated plants. This may be due to enhancement auxiliary photoprotective photosynthetic pigments, because 2,4-D treatments acted as auxin stimulated and increased bacterial colonization of the nodular structures modified from the lateral roots of wheat plants associated with *Azospirillum* and *Azotobacter* (Biabani 2008). Consequently, the performance of chlorophyll concentrations in leaves giving healthy plants and stimulated plant growth. Whereas the means of plant height and shoot dry weight in plants treated with 2,4-D were significantly increased over the untreated plants (Narula *et al.* 2006). As shown from the results presented in Table 7, transconjugant Tr_6 increased chlorophyll a (0.842 mg/g) significantly over both the better parent and recommended dose of nitrogen. In addition, transconjugants; Tr_1, Tr_2, Tr_3 and Tr_4 improved chlorophyll a significantly over their better parents. However, transconjugant Tr_8 was the only one that improved chlorophyll b significantly over the better parent and also over the plants fertilized with recommended dose. On the other hand, transconjugants Tr_6, and Tr_8 increased total chlorophyll significantly over their better parent and over the plants fertilized with recommended dose.

Table 6: Means of different growth parameters affected by all bacterial strains under the effect of 2,4-D.

Parameters	-2,4-D	+2,4-D	F-test	L.S.D	
				0.05	0.01
Chl a (mg/g)	0.621	0.677	0	0.047	0.109
Chl b (mg/g)	3.63	3.89	**	0.03	0.06
Total Chl (mg/g)	4.25	4.57	**	0.07	0.17
Plant height (cm)	49	52	**	2	4
Shoot DW (g)	0.412	0.430	0	0.008	0.019

Transconjugant Tr_6 increased significantly plant height over the better parent. In addition, transconjugants Tr_2 and Tr_6 significantly increased dry weight over their better parents and the plants fertilized with recommended dose. Meanwhile, transconjugants Tr_3 and Tr_4 significantly increased shoot dry weight over their better parent.

Most transconjugants except Tr_9 and Tr_{10} for significantly increased plant height, shoot dry weight, chlorophyll a, chlorophyll b, and total chlorophyll over the plants fertilized with 25% of recommended dose. This are due to the effect of *Azospirillum* recombinants which enhanced the nutrient uptake, adequate nutrition synthesis, synthesized more chlorophyll concentrations, more carbohydrates, as well as, proteins and finally protoplasm as it adapted well in the soil. Chlorophyll concentration was the best measure of the differential response of wheat cultivars to inoculation with *Azospirillum*. Bacterization with *Azospirillum* recombinants enhanced the yield of chlorophyll concentrations than that affected by their parental strains. This leads us to speculate that a possible biostimulation was achieved in the yield of chlorophyll concentration exists within the tissues of the *Azospirillum* inoculated plants whether above plants fertilized with 25% of recommended dose of nitrogen or above that within the plants tissues inoculated with their parental strains. Promotion of chlorophyll concentration following inoculation with *Azospirillum* recombinants was derived mainly from a general effect on root growth and function (Tsimilli-Michael *et al.* 2000). This means that *Azospirillum sp.* frequently resulted healthier and larger (Bashan *et al*. 2004) and sometimes greener (Swedrzynska and Sawicka 2000) plants, suggesting enhanced photosynthesis (Amir *et al.* 2001).

Table 7: Means of different growth parameters for each bacterial strain affected by 2,4-D.

Biofertilizers		Chl a (mg/g)	Chl b (mg/g)	Total Chl (mg/g)	Plant height(cm)	Shoot DW (g)
Recommended dose		0.717	3.92	4.63	58.2	0.448
25% of RD		0.561	3.28	3.85	47.3	0.360
St_4 †		0.537	4.18	4.72	48.2	0.377
St_1 ††		0.603	3.75	4.36	50.0	0.445
Mid parents		0.570	3.97	4.54	49.1	0.411
Tr_1		0.732	3.74	4.47	55.0	0.392
Tr_2		0.745	3.63	4.38	51.5	0.490
St_5 †		0.591	3.57	4.16	47.0	0.401
St_3 ††		0.490	4.10	4.59	47.7	0.398
Mid parents		0.540	3.84	4.38	47.3	0.400
Tr_3		0.788	3.95	4.74	52.3	0.427
Tr_4		0.687	3.74	4.42	53.3	0.453
St_6 †		0.713	3.82	4.54	51.0	0.438
St_1 ††		0.603	3.75	4.36	50.0	0.445
Mid parents		0.658	3.79	4.45	50.5	0.441
Tr_5		0.601	3.83	4.43	51.2	0.450
Tr_6		0.842	4.05	4.89	52.8	0.486
St_7 †		0.678	3.64	4.32	50.3	0.409
St_1 ††		0.603	3.75	4.36	50.0	0.445
Mid parents		0.641	3.70	4.34	50.2	0.427
Tr_7		0.737	3.48	4.21	48.3	0.441
Tr_8		0.609	4.36	4.97	50.3	0.434
St_3 †		0.490	4.10	4.59	47.7	0.398
St_5 ††		0.591	3.57	4.16	47.0	0.401
Mid parents		0.540	3.84	4.38	47.3	0.400
Tr_9		0.468	3.20	3.66	48.0	0.362
Tr_{10}		0.581	3.42	4.00	46.7	0.369
F-test		**	**	**	**	**
LSD	0.05	0.117	0.24	0.25	5.3	0.025
	0.01	0.155	0.32	0.34	7.0	0.033

† , †† = Donor and recipient , respectively. RD = Recommended dose

As shown from the results presented in Table 8, the mean squares of different growth parameters from split plot analysis demonstrated that 2,4-D was significantly affected on biochemical traits ; chlorophyll a, chlorophyll b and total chlorophyll (mg/g), as well as, plant height and shoot dry weight (g). In addition, the mean squares of all mentioned traits were significantly affected by biofertilizers. Increased chlorophyll content and consequently enhanced photosynthesis, were due to the response of plant to inoculation with *Azospirillum*, *Azotobacter* and their transconjugants (Sharma *et al.* 2003). It was assumed that increased production of photosynthesis enhanced plant growth and yield (Alam *et al.* 2001).

Table 8: Mean squares of different growth parameters from split plot analysis.

S.V.	D.F	Plant height	Shoot D.W	Chl a	Chl b	Total Chl
Main plot						
Rep.	2	0.0335	0.1712	0.0580	2.815	0.0008
Doses	1	0.0823*	1.8842**	2.7541**	381.565**	0.0079*
Error		0.0032	0.0011	0.0080	3.370	0.0001
Sub plot						
Biofertilizers	17	0.0648**	0.5718**	0.7091**	56.205**	0.0095**
Doses x Bio	17	0.0144*	0.0541	0.0529	13.173	0.0001
Error	68	0.0103	0.0446	0.0483	21.053	0.0005

***Inoculants Evaluated in the Field*:**

As shown from the data presented in Table 9 the means of chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll) were significantly higher in plants treated with 2,4-D over the untreated ones. This may be due to enhancement auxiliary the pigments within the wheat plants, because 2,4-D treatments acted as auxin stimulated and increased bacterial colonization of the nodular structures modified from the lateral roots of wheat plants via inducing a formation of paranodules on roots as a result of crack entry invasion (Narula *et al.* 2006). However, Hubbell *et al.* (1979) stated that the performance of chlorophyll concentrations in leaves giving concomitant changes result in healthy plants and stimulated plant growth whereas the means of plant height and shoot dry weight in plants treated with 2,4-D were significantly increased over the untreated ones. On the other hand, studies of the mode of action of *Azospirillum* on plants commonly focus on a single mechanism, such as hormonal effect, N_2 fixation, proton extrusion and mineral uptake (Bashan *et al.* 2004). However, the evidence suggested by Bashan and Dubrovsky (1996) supported the hypothesis that inoculation affects the whole plant .

Table 9: Means of different growth parameters at 65 days plant-old affected by all bacterial inoculants under the effect of 2,4-D.

Parameters	-2,4-D	+2,4-D	F-test	L.S.D	
				0.05	0.01
Chl a (mg)	0.633	0.713	0	0.062	0.143
Chl b (mg)	3.08	3.45	0	0.33	0.77
Total Chl (mg)	3.71	4.16	0	0.27	0.63
Plant height (cm)	57.3	60.6	0	2.6	6.0
Shoot DW (g)	0.876	0.925	0	0.031	0.072

Data presented in Table 10 showed that transconjugants; Tr_2, Tr_3, Tr_4, Tr_7, Tr_8 were significantly increased chl a over their better parents in response to the interaction between the addition of 2,4-D and biofertilizer inoculants. In addition, Tr_7 was significantly improved total chl over the plants fertilized with nitrogen recommended dose at the absence of 2,4-D, as well as, the better parent and plants fertilized with recommended dose at the presence of 2,4-D. However, Tr_6 and Tr_8 under the effect of 2,4-D were significantly increased shoot dry weight over their better parent, as well as, Tr_4 appeared the same trend over the plants fertilized with recommended dose. The results obtained herein are in agreement with Kennedy and Tchan (1992), who found that treatment with auxins increased the colonization of roots by soil bacteria e.g., *Azospirillum* point Reports suggested that the colonization of these bacteria is caused by factors like N_2 fixation, siderophores, ammonia excretion, phytohormones (Lakshminarayana 1993) and antifungal properties etc. (Verma *et al*. 2001), collectively enhancing the root proliferation, increase in the lateral roots and root hair formation.

Table 10: Means of different growth parameters at 65 days plant-old resulted from the interaction between treatments of 2,4-D and bacterial strains.

Inoculants	Chl a (mg/g)		Chl b (mg/g)		Total Chl (mg/g)	
	- 2,4-D	+2,4-D	- 2,4-D	+2,4-D	- 2,4-D	+2,4-D
Recommended dose	0.798	0.842	3.41	3.61	4.21	4.45
25% of RD	0.578	0.494	2.78	2.78	3.36	3.27
St_4 †	0.636	0.588	3.09	3.19	3.72	3.78
St_1 ††	0.699	0.708	3.55	3.72	4.25	4.43
Mid parents	0.668	0.648	3.32	3.46	3.99	4.10
Tr_1	0.653	0.634	3.61	3.82	4.26	4.45
Tr_2	0.577	0.846	2.77	3.21	3.35	4.06
St_5 †	0.487	0.566	2.70	3.29	3.19	3.86
St_3 ††	0.570	0.638	3.01	3.33	3.58	3.97
Mid parents	0.528	0.602	2.85	3.31	3.38	3.91
Tr_3	0.752	0.777	3.02	3.68	3.78	4.45
Tr_4	0.654	0.781	3.14	3.76	3.79	4.54
St_6 †	0.680	0.721	3.28	3.55	3.96	4.27
St_1 ††	0.699	0.708	3.55	3.72	4.25	4.43
Mid parents	0.689	0.715	3.42	3.64	4.11	4.35
Tr_5	0.622	0.769	3.03	3.37	3.65	4.14
Tr_6	0.742	0.841	3.20	3.42	3.94	4.26
St_7 †	0.516	0.683	2.66	3.34	3.17	4.03
St_1 ††	0.699	0.708	3.55	3.72	4.25	4.43
Mid parents	0.607	0.696	3.11	3.53	3.71	4.23
Tr_7	0.796	0.869	3.82	4.55	4.62	5.41
Tr_8	0.621	0.982	3.18	3.75	3.80	4.74
St_3 †	0.570	0.638	3.01	3.33	3.58	3.97
St_5 ††	0.487	0.566	2.70	3.29	3.19	3.86
Mid parents	0.528	0.602	2.85	3.31	3.38	3.91
Tr_9	0.556	0.563	2.51	2.80	3.06	3.36
Tr_{10}	0.454	0.525	2.61	2.87	3.06	3.39
F-test	**		NS		*	
LSD 0.05	0.127				0.40	
0.01	0.169				0.53	

Table 10: Continued.

Inoculants		Plant height (cm)		Shoot DW (g)	
		- 2,4-D	+2,4-D	- 2,4-D	+2,4-D
Recommended dose		66.7	70.3	0.943	1.010
25% of RD		49.7	51.7	0.818	0.850
St_4 †		52.7	57.3	0.839	0.938
St_1 ††		60.7	64.7	0.811	0.900
Mid parents		56.7	61.0	0.825	0.919
Tr_1		66.7	64.0	0.929	0.921
Tr_2		62.7	66.0	0.869	0.930
St_5 †		54.3	60.0	0.816	0.812
St_3 ††		52.7	56.3	0.875	0.911
Mid parents		53.5	58.2	0.845	0.862
Tr_3		58.7	61.7	0.815	0.867
Tr_4		63.0	68.0	1.046	1.080
St_6 †		64.7	68.3	0.905	0.946
St_1 ††		60.7	64.7	0.811	0.900
Mid parents		62.7	66.5	0.858	0.923
Tr_5		53.7	56.3	0.908	0.978
Tr_6		69.3	71.3	0.969	0.991
St_7 †		48.0	54.7	0.907	0.928
St_1 ††		60.7	64.7	0.811	0.900
Mid parents		54.3	59.7	0.859	0.914
Tr_7		56.3	59.7	0.810	0.946
Tr_8		56.3	60.3	0.888	0.996
St_3 †		52.7	56.3	0.875	0.911
St_5 ††		54.3	60.0	0.816	0.812
Mid parents		53.5	58.2	0.845	0.862
Tr_9		47.7	49.3	0.814	0.834
Tr_{10}		47.3	51.7	0.815	0.804
F-test		NS		*	
LSD	0.05			0.40	
	0.01			0.53	

† , †† = Donor and recipient, respectively RD = Recommended dose NS, *, ** = Insignificant, significant at 0.05 and 0.01 probability levels, respectively.

As shown from the results presented in Table 11, the mean squares of different growth parameters demonstrated that chlorophyll a, chlorophyll b and total chlorophyll, as well as, plant height and shoot dry weight were significantly affected by the doses of 2,4-D. In addition, the mean squares of all mentioned traits were significantly affected by biofertilizer inoculants. Furthermore, the mean squares of both parameters chl a and total chl were significantly affected by the interaction between the doses of 2,4-D and biofertilizer inoculants. This agreed with Sriskandarajah *et al.* (1993), who reported that the change of morphology of roots to form paranodules was attributed to the addition of auxin analogues.

Table 11: Mean squares obtained from split plot analysis for different growth parameters at 65 days plant-old.

S.V.	D.F	Chl a	Chl b	Total Chl	Plant height	Shoot D.W
Main plot						
Rep.	2	0.0027	0.0388	0.0232	77.009	0.0008
Doses	1	0.1722*	3.7089*	5.4793*	306.704*	0.0625*
Error		0.0056	0.1633	0.1092	9.731	0.0014
Sub plot						
Biofertilizers	17	0.0682**	0.8761**	1.3158**	277.521**	0.0268**
Doses x Bio	17	0.0176**	0.0725	0.1306*	5.841	0.0025
Error	68	0.0061	0.0522	0.0600	48.714	0.0019

In addition, Kennedy *et al.* (1997) reported that 10^7 azospirilla in wheat seedlings are active in nitrogen fixation. Sabry *et al.* (1997) showed that the wheat grown in pots and inoculated repeatedly with *A. caulinodans* colonized tissues at the point of emergences of lateral roots and appeared to contribute significant amounts of fixed nitrogen to the plant.

The results obtained herein are agreement with Bashan *et al.* (1995), who found that inoculation of wheat seedlings with plant growth-promoting bacterium *Azospirillum brasilense* significantly increased the quantity of several phytosynthetic pigments, such as chlorophyll a, chlorophyll b and the auxiliary photoprotective pigments.

As shown from the results presented in Table 12, both transconjugant Tr_3 and Tr_4 increased plant height significantly over their better parent. In addition, transconjugant Tr_8 significantly increased plant height over both the better parent and the plants fertilized with recommended dose of N. Transconjugant Tr_1 and Tr_2 increased leaf area significantly over the better parent. Furthermore Tr_4 was significantly increased leaf area over the better parent and the plants fertilized with recommended dose. In addition Transconjugant Tr_8 was significantly increased the number of tillers/plant and spike height over the better parent. Meanwhile, Tr_5 was significantly increased the number of tillers/plant over the better parent and the plants fertilized with recommended dose. Transconjugant Tr_1 was significantly increased spike height over the better parent. The results obtained herein agreed with Saubidet *et al.* (2002), who demonstrated that inoculated wheat plants showed a higher biomass, grain yield, N content and a higher grain protein concentration than the uninoculated ones.

Table 12: Means of different yield parameters for each bacterial strain affected by treatments of 2,4-D.

Biofertilizers		Plant height (cm)	Leaf area (cm^2)	No. of tillers / plant	Spike Height (cm)
Recommended dose		108.3	62.01	6.7	17.8
25% of RD		93.7	46.21	5.8	14.7
St_4 †		99.7	47.93	5.5	14.8
St_1 ††		105.5	53.78	5.8	16.7
Mid parents		102.6	50.85	5.7	15.7
Tr_1		107.7	57.54	7.0	18.3
Tr_2		101.2	63.53	7.0	16.9
St_5 †		102.8	50.30	6.0	14.6
St_3 ††		101.2	58.08	6.2	17.6
Mid parents		102.0	54.19	6.1	16.1
Tr_3		110.3	55.83	6.8	16.1
Tr_4		111.2	64.70	6.2	15.2
St_6 †		101.5	48.95	5.7	17.7
St_1 ††		105.5	53.78	5.8	16.7
Mid parents		103.5	51.36	5.8	17.2
Tr_5		103.7	48.39	8.2	17.0
Tr_6		110.7	52.28	6.2	15.9
St_7 †		100.8	63.83	6.2	15.4
St_1 ††		105.5	53.78	5.8	16.7
Mid parents		103.2	58.80	6.0	16.0
Tr_7		104.8	57.34	7.2	17.3
Tr_8		114.3	49.83	7.5	19.3
St_3 †		101.2	58.08	6.2	17.6
St_5 ††		102.8	50.30	6.0	14.6
Mid parents		102.0	54.19	6.1	16.1
Tr_9		96.7	46.80	6.3	14.3
Tr_{10}		100.0	46.91	5.8	14.4
F-test		**	**	0	**
LSD	0.05	5.5	2.67	1.3	1.3
	0.01	7.3	3.55	1.8	1.8

† , †† = Donor and recipient, respectively RD = Recommended dose *, ** = Significant at 0.05 and 0.01 probability levels, respectively.

The results are also in harmony with Boddey *et al.* (1986), who found that field inoculation of wheat plants show in many cases significant yield increases. It has also been stated that the establishment of *Azospirillum* recombinants in the roots is a critical step towards an effective plant growth promotion (Bashan and Holguin, 1997).

As shown from data presented in Table 13, transconjugants Tr_1, Tr_2 and Tr_4 were significantly increased leaf area over their better parents in response to the interaction between the addition of 2,4-D and biofertilizer recombinants, as well. In addition, Tr_1 and Tr_8 were significantly increased spike height over their better parents.

As shown from the results presented in Table 14, the mean squares of different parameters demonstrated that the doses of 2,4-D, as well as, biofertilizer inoculants were significantly affected on all mentioned parameters; plant height, leaf area, number of tillers/plant and spike height. Furthermore, the mean squares of both parameters; leaf area and spike height were significant in response to the interaction between the doses of 2,4-D and biofertilizer isolates .

Although, leaf area and spike height were significantly affected by the interaction between the doses of 2,4-D and inoculation of biofertilizer isolates. It was assumed that increased production of different yield traits enhanced wheat growth and yield .

Table 13: Means of different parameters affected by the interaction between 2,4-D and biofertilizer inoculants.

Inoculants		Plant height (cm)		Leaf area (cm^2)		No. of tillers/plant		Spike height (cm)	
		- 2,4-D	+2,4-D	- 2,4-D	+2,4-D	- 2,4-D	+2,4-D	- 2,4-D	+2,4-D
Recommended dose		106.0	110.7	56.90	67.13	6.3	7.0	16.3	19.2
25% of RD		89.3	98.0	45.43	47.00	5.7	6.0	14.7	14.7
St_4 †		94.3	105.0	45.63	50.23	5.0	6.0	14.7	14.8
St_1 ††		103.3	107.7	50.40	57.15	5.3	6.3	15.5	17.8
Mid parents		98.8	106.3	48.01	53.69	5.2	6.2	15.1	16.3
Tr_1		106.3	109.0	52.43	62.65	6.7	7.3	16.8	19.7
Tr_2		93.0	109.3	62.33	64.73	6.0	8.0	17.0	16.8
St_5 †		102.3	103.3	47.55	53.05	5.3	6.7	14.7	14.5
St_3 ††		98.3	104.0	53.20	62.95	5.7	6.7	16.3	18.8
Mid parents		100.3	103.7	50.38	58.00	5.5	6.7	15.5	16.7
Tr_3		109.0	111.7	51.45	60.20	6.3	7.3	15.0	17.2
Tr_4		105.0	117.3	61.80	67.60	5.7	6.7	15.2	15.2
St_6 †		99.0	104.0	47.10	50.80	5.3	6.0	16.7	18.7
St_1 ††		103.3	107.7	50.40	57.15	5.3	6.3	15.5	17.8
Mid parents		101.2	105.8	48.75	53.98	5.3	6.2	16.1	18.3
Tr_5		95.7	111.7	45.58	51.20	8.0	8.3	15.3	18.7
Tr_6		108.0	113.3	47.45	57.10	5.3	7.0	15.2	16.7
St_7 †		96.7	105.0	59.40	68.25	5.7	6.7	14.5	16.3
St_1 ††		103.3	107.7	50.40	57.15	5.3	6.3	15.5	17.8
Mid parents		100.0	106.3	54.90	62.70	5.5	6.5	15.0	17.1
Tr_7		96.7	113.0	53.78	60.90	6.7	7.7	16.2	18.5
Tr_8		110.7	118.0	45.90	53.75	7.3	7.7	18.8	19.8
St_3 †		98.3	104.0	53.20	62.95	5.7	6.7	16.3	18.8
St_5 ††		102.3	103.3	47.55	53.05	5.3	6.7	14.7	14.5
Mid parents		100.3	103.7	50.38	58.00	5.5	6.7	15.5	16.7
Tr_9		93.7	99.7	45.20	48.40	6.0	6.7	14.7	14.0
Tr_{10}		98.0	102.0	44.93	48.90	5.3	6.3	14.3	14.5
F-test		*		*		NS		*	
LSD	0.05	7.8		3.78				1.9	
	0.01	10.3		5.02				2.5	

†, †† = Donor and recipient, respectively RD = Recommended dose NS, * = Insignificant and significant at 0.05 probability level, respectively.

Table 14: Mean squares of different parameters obtained from split plot analysis.

S.V.	D.F	Plant height	leaf area (cm^2)	No. of tillers/ plant	Spike height (cm)
Main plot					
Rep.	2	57.694	107.374	3.250	0.176
Doses	1	1571.704*	1112.650*	23.148*	48.000*
Error		62.509	19.280	0.731	0.778
Sub plot					
Biofertilizers	17	178.863**	247.073**	3.020*	13.544**
Doses x Bio	17	35.233	11.737*	0.285	2.485*
Error	68	22.896	5.396	1.344	1.313

Response of Yield Components to Bioinoculants:

The results presented in Table 15 indicated that 2,4-D was significantly affected on the only one parameter (weight of spikes/plant). In addition, the weight of spike (g) and weight of spikes (g/plant) were significantly affected by biofertilizers, although, the number of spikes/m^2 and the number of grains/spike were insignificant affected.

The mean squares of all mentioned parameters did not show any significant effects on the yield in response to the interaction between the addition of 2,4-D and biofertilizer isolates. This agreed with Bashan *et al.* (1995), who are stated that responses to *Azospirillum* inoculation in non-leguminous plants are still difficult to estimate. The results inconsistency in inoculation experiments was related to the inoculation techniques, inoculation rate, low survival of inoculated strains, physical and chemical characterization of the soil, physiological state of the bacteria, improper strain, plant genotype, presence of high number of native microorganisms and pesticides influence. All of these criteria affected on plant response to inoculation (Bashan *et al.* 1995).

As shown from the results presented in Table 16 that transconjugants Tr_5 and Tr_7 were significantly increased shoot dry weight/plant over their better parents. In addition, transconjugant Tr_4 is the only one that was significantly increased straw dry weight/tiller over the better parent. However, transconjugants Tr_5 and Tr_7 significantly increased the weight of straw/plant over the better parents. This indicated that inoculation with

Table 15: Mean squares of yield components from split plot analysis.

S.V.	D.f	No. of spikes /m^2	No. of grains /spike	Weight of spik (g)	Weight of spikes (g/plant)
Main plot					
Rep.	2	58.028	16.898	0.005	40.097
Doses	1	2045.370	2.676	0.816	482.294**
Error		623.676	37.398	0.055	4.660
Sub plot					
Biofertilizers	17	278.412	48.571	0.209**	53.681**
Doses x Bio	17	264.331	35.186	0.065	4.488
Error	68	262.362	27.785	0.039	16.509

Table 16: Means of different yield parameters for each bacterial strain under the effect of 2,4-D.

Biofertilizers	Shoot DW (g/tiller)	Shoot DW (g/plant)	Straw weight (g/tiller)	Straw weight (g/plant)
Recommended dose	1.89	12.45	2.97	19.78
25% of RD	1.41	8.21	2.38	13.84
St_4 †	1.87	9.98	2.95	16.10
St_1 ††	1.81	10.74	2.82	16.59
Mid parents	1.84	10.36	2.89	16.34
Tr_1	1.90	13.20	2.92	20.42
Tr_2	1.77	12.54	2.88	20.24
St_5 †	1.85	11.44	2.73	16.40
St_3 ††	1.78	10.93	2.80	17.15
Mid parents	1.82	11.19	2.76	16.78
Tr_3	1.87	12.77	2.91	19.86
Tr_4	1.89	11.69	3.09	19.14
St_6 †	1.82	10.36	2.81	16.00
St_1 ††	1.81	10.74	2.82	16.59
Mid parents	1.81	10.55	2.82	16.29
Tr_5	1.83	15.03	2.82	23.10
Tr_6	1.69	10.39	2.74	16.97
St_7 †	1.79	10.98	2.95	18.20
St_1 ††	1.81	10.74	2.82	16.59
Mid parents	1.80	10.86	2.88	17.39
Tr_7	1.94	13.99	3.15	22.60
Tr_8	1.79	13.52	2.87	21.57
St_3 †	1.78	10.93	2.80	17.15
St_5 ††	1.85	11.44	2.73	16.40
Mid parents	1.82	11.19	2.76	16.78
Tr_9	1.56	10.01	2.52	16.00
Tr_{10}	1.62	9.24	2.62	15.11
F-test	NS	**	**	**
LSD 0.05		2.99	0.23	3.87
0.01		3.97	0.31	5.14

†, †† = Donor and recipient, respectively RD = Recommended dose NS, ** = Insignificant and significant at 0.01 probability level, respectively.

recombinants leads to significantly increased shoot dry weight/plant, straw dry weight/tiller and weight of straw/plant over the uninoculated plants fertilized with 25% recommended dose of nitrogen. This agreed with Rai and Gaur (1988), who found significant increases in the yield of wheat grains and uptake of nitrogen by inoculated crop over the uninoculated ones when seeds were inoculated either with *Azospirillum* spp. or *Azotobacter* spp. or the combination of both inoculants.

Effect of Recombinants Bioinoculation on Nitrogen and Protein Percentage in Wheat:

The data summarized in Table 17 appeared that the means of nitrogen and protein percentage were significantly increased in plants treated with 2,4-D concerning shoots and grains over the untreated plants. This agreed with Kennedy and Tchan (1992), who reported that treatment with concentrations of auxins has been shown to increase colonization and N_2 fixation of azospirilla in wheat by supporting larger acetylene reduction (nitrogenase activity) than plants treated only with bacteria, which consequently increases nitrogen and protein percentage. This also agreed with Zaied *et al*. (2007), who found that the association between *Azospirillum* and 2,4-D revealed significant increase in Chlorophyll b and grain yield/plant in kanola. The treatment with 2,4-D induces nodular outgrowth on the roots of wheat leading more associate with the roots of wheat plants and more nitrogen was fixed. This agreed with Perigio *et al.* (1993), who demonstrated that 2,4-D-inoculated wheat induced p-nodules modified lateral roots, the structure of which is enhanced by rhizobial inoculation.

As shown from the results presented in Table 18 that some recombinant inoculants appeared significant increases in nitrogen and protein percentage in the shoots of plants grown in pots, shoot at 65 days plant-old,

Table 17: Means of nitrogen and protein percentage in plants grown in pots and field..

Parameters		-2,4-D	+2,4-D	F-test	L.S.D	
					0.05	0.01
Shoot (pots)	N_2 %	2.36	2.49	0	0.03	0.08
	Pro %	13.43	14.20	**	0.19	0.44
Shoot at 65 days plant-old	N_2 %	1.99	2.16	**	0.05	0.12
	Pro %	11.35	12.34	**	0.31	0.71
Shoot at harvest time (field)	N_2 %	1.88	2.04	0	0.11	0.26
	Pro %	10.72	11.61	0	0.63	1.46
Grains (field)	N_2 %	2.10	2.39	**	0.06	0.14
	Pro %	11.96	13.65	**	0.34	0.77

Table 18: Means of nitrogen percentage (N%) and protein percentage (P%) affected by each bacterial strain under the effect of 2,4-D.

Biofertilizers		Shoot in pots		Shoot at 65 days old-plant		Shoot at harvest time		Grains	
		N%	P%	N%	P%	N%	P%	N%	P%
Recommended dose		2.60	14.80	2.40	13.69	2.20	12.55	2.38	13.55
25% of RD		2.24	12.79	1.85	10.53	1.74	9.91	1.95	11.13
St_4 †		2.37	13.51	2.01	11.48	1.89	10.80	2.09	11.90
St_1 ††		2.41	13.76	1.97	11.20	1.81	10.31	2.11	12.03
Mid parents		2.39	13.64	1.99	11.34	1.85	10.55	2.10	11.96
Tr_1		2.31	13.16	2.22	12.66	2.18	12.44	2.24	12.74
Tr_2		2.71	15.43	2.04	11.63	1.94	11.04	2.44	13.92
St_5 †		2.30	13.14	1.98	11.26	1.93	11.01	2.20	12.56
St_3 ††		2.39	13.64	1.88	10.74	1.88	10.73	2.18	12.41
Mid parents		2.35	13.39	1.93	11.00	1.91	10.87	2.19	12.49
Tr_3		2.65	15.08	2.08	11.85	1.97	11.25	2.30	13.12
Tr_4		2.48	14.16	2.28	12.98	2.07	11.80	2.27	12.91
St_6 †		2.48	14.12	1.97	11.25	1.85	10.54	2.10	11.96
St_1 ††		2.41	13.76	1.97	11.20	1.81	10.31	2.11	12.03
Mid parents		2.45	13.94	1.97	11.23	1.83	10.42	2.10	11.99
Tr_5		2.38	13.55	2.23	12.71	2.16	12.33	2.49	14.22
Tr_6		2.63	14.97	2.24	12.78	2.03	11.60	2.45	13.94
St_7 †		2.45	13.99	2.01	11.46	1.83	10.41	2.13	12.13
St_1 ††		2.41	13.76	1.97	11.20	1.81	10.31	2.11	12.03
Mid parents		2.43	13.88	1.99	11.33	1.82	10.36	2.12	12.08
Tr_7		2.52	14.37	2.07	11.81	2.00	11.39	2.45	13.97
Tr_8		2.28	12.98	2.31	13.16	2.14	12.20	2.31	13.17
St_3 †		2.39	13.64	1.88	10.74	1.88	10.73	2.18	12.41
St_5 ††		2.30	13.14	1.98	11.26	1.93	11.01	2.20	12.56
Mid parents		2.35	13.39	1.93	11.00	1.91	10.87	2.19	12.49
Tr_9		2.23	12.74	1.92	10.93	1.78	10.13	2.24	12.78
Tr_{10}		2.20	12.57	1.94	11.08	1.85	10.56	2.11	12.01
F-test		**	**	**	**	**	**	**	**
LSD	0.05	0.13	0.76	0.25	1.42	0.26	1.48	0.27	1.54
	0.01	0.18	1.00	0.33	1.88	0.35	1.97	0.36	2.05

† , †† = Donor and recipient, respectively RD, ** = Recommended dose and significant at 0.01 probability level, respectively.

shoot at harvest time and grains over the mid parents. This agreed with Rennie (1980), who reported an 18% increase in plant dry mater and total nitrogen in maize inoculated with *A. brasilense* and 12.6% of plant N derived from nitrogen fixation. Furthermore Rennie *et al.* (1983) reported that inoculation with *A. brasilense* caused contributions of N via biological nitrogen fixation to various wheat cultivars as determined by the ^{15}N isotope dilution technique.

The shoots of plants grown in pots were more affected by transconjugants Tr_2, Tr_3 and Tr_6, which were significantly increased nitrogen percentage over their better parents, as well as, these transconjugants were significantly increased protein percentage over their better parent. In addition, nitrogen percentage in the shoots of plants grown in the field at 65 days plant-old was more affected by Tr_4, Tr_5, Tr_6 and Tr_8, which significantly increased nitrogen percentage over their better parent. This also leading to significant increase in protein percentage over their better parent.

Nitrogen percentage, as well as protein percentage was significantly increased in the shoots of plants at the harvest time in response to inoculation with Tr_1, Tr_5 and Tr_8 over the better parent.

Transconjugants Tr_2, Tr_5, Tr_6 and Tr_7 significantly increased nitrogen percentage, as well as, protein percentage in grains over their better parents.

As shown from the data presented in Table 19 ; Tr_2 and Tr_3 were affected to significantly increase nitrogen and protein percentage at the presence and the absence of 2,4-D in plants grown in pots. However, Tr6 appeared the same trend only at the absence of 2,4-D.

Table 19: Means of nitrogen and protein percentages for each bacterial strain affected by 2,4-D.

Biofertilizers	Shoot in pots				Shoot at 65 days old-plant			
	N%		P%		N%		P%	
	-2,4-D	+2,4-D	-2,4-D	+2,4-D	-2,4-D	+2,4-D	-2,4-D	+2,4-D
Recommended dose	2.63	2.56	15.01	14.58	2.37	2.44	13.48	13.89
25% of RD	2.19	2.30	12.48	13.09	1.80	1.90	10.23	10.82
St_4 †	2.20	2.54	12.57	14.45	1.93	2.10	11.01	11.95
St_1 ††	2.30	2.53	13.10	14.42	1.89	2.04	10.77	11.64
Mid parents	2.25	2.53	12.84	14.44	1.91	2.07	10.89	11.79
Tr_1	2.21	2.40	12.61	13.71	2.11	2.34	12.01	13.31
Tr_2	2.58	2.83	14.73	16.12	1.96	2.12	11.18	12.08
St_5 †	2.25	2.36	12.84	13.44	1.89	2.06	10.77	11.75
St_3 ††	2.29	2.50	13.03	14.24	1.81	1.96	10.30	11.18
Mid parents	2.27	2.43	12.93	13.84	1.85	2.01	10.54	11.46
Tr_3	2.50	2.79	14.24	15.93	1.98	2.18	11.29	12.41
Tr_4	2.43	2.54	13.88	14.45	2.14	2.41	12.20	13.75
St_6 †	2.36	2.59	13.45	14.79	1.91	2.04	10.88	11.61
St_1 ††	2.30	2.53	13.10	14.42	1.89	2.04	10.77	11.64
Mid parents	2.33	2.56	13.28	14.61	1.90	2.04	10.82	11.63
Tr_5	2.44	2.31	13.90	13.19	2.11	2.35	12.01	13.41
Tr_6	2.57	2.69	14.63	15.31	2.12	2.36	12.10	13.45
St_7 †	2.38	2.53	13.54	14.44	1.92	2.10	10.95	11.96
St_1 ††	2.30	2.53	13.10	14.42	1.89	2.04	10.77	11.64
Mid parents	2.34	2.53	13.32	14.43	1.91	2.07	10.86	11.80
Tr_7	2.43	2.61	13.85	14.89	1.96	2.18	11.19	12.43
Tr_8	2.21	2.35	12.60	13.37	2.17	2.45	12.36	13.96
St_3 †	2.29	2.50	13.03	14.24	1.81	1.96	10.30	11.18
St_5 ††	2.25	2.36	12.84	13.44	1.89	2.06	10.77	11.75
Mid parents	2.27	2.43	12.93	13.84	1.85	2.01	10.54	11.46
Tr_9	2.28	2.19	13.02	12.46	1.88	1.96	10.70	11.16
Tr_{10}	2.16	2.25	12.33	12.81	1.91	1.98	10.88	11.29
F-test	*		*		NS		NS	
LSD 0.05	0.19		1.07					
0.01	0.25		1.42					

Table 19: Continued

Biofertilizers	Shoot at harvest time				Grains			
	N%		P%		N%		P%	
	-2,4-D	+2,4-D	-2,4-D	+2,4-D	-2,4-D	+2,4-D	-2,4-D	+2,4-D
Recommended dose	2.14	2.26	12.20	12.91	2.33	2.42	13.29	13.81
25% of RD	1.70	1.78	9.69	10.14	1.91	2.00	10.88	11.39
St_4 †	1.71	2.08	9.76	11.84	1.99	2.18	11.36	12.44
St_1 ††	1.74	1.87	9.94	10.68	1.99	2.23	11.36	12.69
Mid parents	1.73	1.98	9.85	11.26	1.99	2.20	11.36	12.57
Tr_1	2.08	2.29	11.85	13.03	2.11	2.37	12.01	13.48
Tr_2	1.87	2.01	10.66	11.43	2.33	2.56	13.26	14.58
St_5 †	1.79	2.07	10.22	11.81	1.92	2.49	10.94	14.18
St_3 ††	1.83	1.94	10.40	11.06	2.00	2.35	11.42	13.41
Mid parents	1.81	2.01	10.31	11.44	1.96	2.42	11.18	13.80
Tr_3	1.85	2.10	10.53	11.96	2.08	2.52	11.88	14.35
Tr_4	2.03	2.11	11.60	12.01	2.09	2.44	11.94	13.89
St_6 †	1.80	1.90	10.25	10.82	1.90	2.30	10.82	13.09
St_1 ††	1.74	1.87	9.94	10.68	1.99	2.23	11.36	12.69
Mid parents	1.77	1.89	10.09	10.75	1.95	2.26	11.09	12.89
Tr_5	2.12	2.20	12.09	12.57	2.38	2.61	13.54	14.90
Tr_6	1.91	2.16	10.90	12.30	2.24	2.65	12.78	15.10
St_7 †	1.73	1.92	9.88	10.94	1.96	2.30	11.18	13.09
St_1 ††	1.74	1.87	9.94	10.68	1.99	2.23	11.36	12.69
Mid parents	1.74	1.90	9.91	10.81	1.98	2.26	11.27	12.89
Tr_7	1.96	2.03	11.18	11.60	2.29	2.61	13.07	14.86
Tr_8	2.11	2.18	12.01	12.40	2.08	2.55	11.84	14.51
St_3 †	1.83	1.94	10.40	11.06	2.00	2.35	11.42	13.41
St_5 ††	1.79	2.07	10.22	11.81	1.92	2.49	10.94	14.18
Mid parents	1.81	2.01	10.31	11.44	1.96	2.42	11.18	13.80
Tr_9	1.69	1.87	9.62	10.64	2.13	2.36	12.13	13.43
Tr_{10}	1.80	1.90	10.28	10.85	2.02	2.19	11.53	12.50
F-test	NS		NS		NS		NS	

†, †† =Donor and recipient, respectively RD=Recommended dose NS, * =Insignificant and significant at 0.05 probability level, respectively.

On the other hand, all inoculants did not show any significant differences in nitrogen and protein percentages in the shoots of plants grown in the field at 65 days plant-old, as well as, shoots at the harvest time and grains at the absence and the presence of 2,4-D under the effect of biofertilizer inoculants.

As shown in Table 20 the mean squares of different parameters from split plot analysis appeared that the nitrogen and protein percentages in shoots and grains were significantly affected by the addition of 2,4-D, as well as, by biofertilizer isolates. On the other hand, nitrogen and protein percentage in the shoot of plants grown in pots were significantly affected by the interaction between the doses of 2,4-D and biofertilizer inoculants. These results agreed with Subba Rao *et al*. (1985), who reported that increased N supply through N_2-fixation, higher nutrient uptake and higher photosynthesis rate and stomatal conductance in inoculated plants might have contributed to the increase in grown yield. On the contrary, this interaction did not show any significant effect on nitrogen or protein percentage of the shoots at 65 days plant-old, shoot at the harvest time and grains.

Table 20: Mean squares of nitrogen and protein percentages from split plot analysis.

S.V.	D.f	Shoot (pots)		Shoot at 65 days plant-old	
		N%	P%	N%	P%
Main plot					
Rep.	2	0.0021	0.0695	0.0016	0.0504
Doses	1	0.4928*	16.0121*	0.8086**	26.2725**
Error		0.0016	0.0525	0.0042	0.1373
Sub plot					
Biofertilizers	17	0.1372**	4.4570**	0.1573**	5.1114**
Doses x Bio	17	0.0247*	0.8037*	0.0065	0.2107
Error	68	0.0132	0.4301	0.0468	1.5194

Table 20: Continued

S.V.	D.f	Shoot at harvest time		Grains	
		N%	P%	N%	P%
Main plot					
Rep.	2	0.0106	0.3442	0.0804	2.6121
Doses	1	0.6530*	21.2172*	2.3849**	77.4845**
Error		0.0179	0.5819	0.0050	0.1637
Sub plot					
Biofertilizers	17	0.1271**	4.1288**	0.1401**	4.5530**
Doses x Bio	17	0.0108	0.3510	0.0249	0.8099
Error	68	0.0512	1.6634	0.0553	1.7965

In conclusion, inoculation with recombinants between *Azospirillum* and *Azotobacter* strains was more efficient and significantly increased many of growth and yield parameters in plants treated with 2,4-D over the untreated ones, as well as, increased nitrogen fixation and consequently reduced the amounts of chemical fertilizers. In addition, transconjugants significantly increased the yield components and growth traits over the better parent and sometimes over the plants fertilized with the recommended dose at the absence and presence of 2,4-D.

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