

## Effect of Horizontal DNA Transfer Between *Azotobacter* Strains on Protein Patterns of *Azotobacter* Transconjugants and Biochemical Traits in Bioinoculated Okra (*Abelmoschus Esculentus*, L.)

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**Abstract:** Four rhizobacterial strains belonging to the genus *Azotobacter* were genetically marked using different antibiotics and entered in five matings. Ten new recombinant isolates were used in this study, two from each mating, selected on the basis of their higher secretion of indolyacetic acid (IAA). These isolates were evaluated for their effects on growth and biochemical parameters in okra through field trial experiment conducted through the summer season of 2002. The wild type strain of *Azospirillum brasilense* was also used in biofertilizer combinations. The results obtained revealed that both transconjugants: Tr<sub>7</sub> and Tr<sub>8</sub> produced higher level of IAA compared to the mid parents. This reached 47% by Tr<sub>7</sub> from lactic acid and 90% by Tr<sub>8</sub> from ethanol intermediate. Biofertilizers exhibited significant effect on photosynthetic efficiency in growing, flowering and fruiting stages. Although, the response of plants inoculated with individual bacteria or in combination with the wild type strain of *Azospirillum* was inconsistent and varied from one genotype to another, bio-fertilizer combinations between *Azotobacter* strains (St<sub>1</sub>, St<sub>2</sub>, St<sub>4</sub>, Tr<sub>1</sub>, Tr<sub>3</sub> and Tr<sub>7</sub>) combined with *Azospirillum* exhibited more anthocyanin concentration in fruits over the recommended dose of nitrogen. Plants fertilized with *Azotobacter* transconjugants (AT) in combinations with *Azospirillum* produced appreciable marketable pod yield per collection. Plants inoculated with AT (Tr<sub>3</sub>, Tr<sub>4</sub>, Tr<sub>5</sub>, Tr<sub>6</sub>, Tr<sub>7</sub> and Tr<sub>8</sub>) *Azotobacter* biofertilizers in combination with *Azospirillum* revealed higher percentages of N and protein in plant tissue over that fertilized with the recommended dose of nitrogen. Most of protein bands appeared different molecular weights (MW) such as No. 18 has the same MW, No. 17 has two different MW, No. 15 has three different MW, No. 14 has four different MW. Although, the bands above the band No. 14 were differed in more than four molecular weights. In addition, higher level of similarity (0.78) was found between St<sub>3</sub> and St<sub>4</sub> than that obtained between other strains because they were related to the same species under the same genus. High level of similarity (above 0.60) was obtained between different transconjugants isolated from different matings because they are related to one parental strain. This implied that transconjugants were genetically different than the original sources.

**Key words :** *Azotobacter*, biofertilization, horizontal DNA transfer, transconjugants, okra.

### INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench) is mostly valued for its mucilaginous properties and it is one of the most popular all-year round local vegetables in Egypt. It is an important item in the local vegetable trade of the country, where it is grown exclusively by small scale farmers. The application of free-living nitrogen-fixing organisms such as *Azospirillum* and *Azotobacter*, could serve as efficient biofertilizers. These bacteria have been used in cereals with encouraging results (Meshram and Shende, 1982), but information about their effect on *Brassica* crops is limited (Agarwal, 1985). In *Brassica* crops, yield increases of 35-60% due to *Azotobacter* and *Klebsiella* have been reported under field conditions (Saha *et al.*, 1985). Members of the genus *Azotobacter* are heterotrophic, obligatory aerobic N<sub>2</sub>-fixing bacteria. The genetics of N<sub>2</sub> fixation in this genus indicated that *nif* and *fix*-like DNA is located in at least five different regions of the genome (Evans *et al.*, 1988). Associative or symbiotic microorganisms living in the rhizosphere of plants play an important role as a link between plant and soil and may contribute substantially to the productivity and longevity of man-made ecosystem (Diederichs and Manske, 1991). Bacterial inoculations may improve the nutrient and water uptake capacity in okra by stimulating the root growth. The improved nutrient uptake of the *Azotobacter* can

only partly explain the stimulation of root growth in the field trial. Cytokinins, known to be released by *Azotobacter*, may have played more important role under the relatively high N fertilization levels imposed by improving the nutrient (P) and water acquisition capacity of enhanced total root length (Martin *et al.*, 1989). The present investigation was, therefore, undertaken to induce genetic recombinations in *Azotobacter* strains through conjugation and testing their effects on the yield performance of okra for improved green pods.

## MATERIALS AND METHODS

### ***I. Genetic Materials and Growth Conditions of Bacterial Strains:***

Seeds of okra [ *Hibiscus esculentus* L. and/or *Abelmoschus esculentus*, amphidiploid (2n = 130)] kindly provided from Vegetable Research Department, Horticulture Research Institute, Agric. Res. Center, Giza, Egypt, through Dr. M.M. Abd El-Rahman, were used in this study. Four wild type strains of *Azotobacter* namely ; *Azotobacter beijerinckii* (ATCC 132) (St<sub>1</sub>), *Azotobacter vinelandii* (SMR 230) (St<sub>2</sub>), *Azotobacter chroococcum* (NRRL B 14346) (St<sub>3</sub>), and *Azotobacter chroococcum* (NRC Ru. 22) (St<sub>4</sub>), as well as, *Azospirillum brasilense* B-14647 strain were kindly provided from Microbiology Laboratory, Environmental, Water and Soil Res. Institute, Agric. Res. Center, Giza, Egypt. Six out of 25 antibiotics tested, ( Table 1) revealed variations in the genetic background of *Azotobacter* strains. Therefore, they were used in this work as a genetic markers . *Azotobacter* strains were grown in modified Ashby medium according to Rao (1984). However, *Azospirillum* was grown in nitrogen free bromothymol blue (NFB) medium as described by Dobereiner and Day (1976). All rhizobacterial strains were grown in nutrient broth at 28-30°C with shaking at 150 rpm for three days.

### ***II. Methodology:***

#### ***Antibiotic Susceptibility Assays:***

Antibiotic susceptibility was measured by plate diffusion method, according to Collins and Lyne (1985) with cultures grown to logarithmic growth phase in nutrient agar medium for each microbe. All antibiotics were used at a concentration of 400 mg/ml, according to Roth and Sonti (1989). The selectable markers were identified concerning antibiotic resistance and /or sensitive genes as listed in Table 1.

#### ***Conjugation:***

Nutrient broth cultures, in the late-exponential growth phase were used. Quantitative spot mating of conjugal transfer was carried out according to Lessel *et al.* (1993) by inoculating 10 ml samples of the donor culture 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 *Azotobacter* strains carrying the opposite genetic markers as shown in Table 1. From each of the five matings, two different isolates were selected (producing high amounts of IAA) to be applied in the field to test okra response to inoculation with *Azotobacter* transconjugants.

**Table 1:** Horizontal DNA transfer between *Azotobacter* strains having the opposite genetic markers through conjugation.

Matings	Transconjugant Genotype ( AT )	Designation of transconjugants used in this study
St <sub>1</sub> (Osb <sup>S</sup> Tetra <sup>R</sup> ) x St <sub>2</sub> (Osb <sup>R</sup> Tetra <sup>S</sup> )	Osb <sup>R</sup> Tetra <sup>R</sup>	Tr <sub>1</sub> Tr <sub>2</sub>
St <sub>1</sub> (Epi <sup>R</sup> Strep <sup>S</sup> Osb <sup>S</sup> Tetra <sup>R</sup> ) x St <sub>2</sub> (Epi <sup>S</sup> Strep <sup>R</sup> Osb <sup>R</sup> Tetra <sup>S</sup> )	Epi <sup>R</sup> Strep <sup>R</sup> Osb <sup>R</sup> Tetra <sup>R</sup>	Tr <sub>3</sub> Tr <sub>4</sub>
St <sub>1</sub> (Neo <sup>S</sup> Osb <sup>S</sup> Amx <sup>S</sup> Tetra <sup>R</sup> ) x St <sub>4</sub> (Neo <sup>R</sup> Osb <sup>R</sup> Amx <sup>R</sup> Tetra <sup>S</sup> )	Neo <sup>R</sup> Osb <sup>R</sup> Amx <sup>R</sup> Tetra <sup>R</sup>	Tr <sub>5</sub> Tr <sub>6</sub>
St <sub>2</sub> (Epi <sup>R</sup> Strep <sup>S</sup> ) x St <sub>3</sub> (Epi <sup>S</sup> Strep <sup>R</sup> )	Epi <sup>R</sup> Strep <sup>R</sup>	Tr <sub>7</sub> Tr <sub>8</sub>
St <sub>3</sub> (Neo <sup>S</sup> Epi <sup>S</sup> Strep <sup>R</sup> Amx <sup>S</sup> ) x St <sub>4</sub> (Neo <sup>R</sup> Epi <sup>R</sup> Strep <sup>S</sup> Amx <sup>R</sup> )	Neo <sup>R</sup> Epi <sup>R</sup> Strep <sup>R</sup> Amx <sup>R</sup>	Tr <sub>9</sub> Tr <sub>10</sub>

#### ***IAA-detection with the Salkowski Colorimetric Technique:***

*Azotobacter* strains were grown overnight in LB broth at 30°C. Production of IAA in the culture supernatant was assayed by using the PC method, as described by Pilet and Chollet (1970). This method was shown to be the most sensitive and most specific Salkowski-based colorimetric technique (Glickmann and Dessaux, 1995). For the reaction, 1 ml of reagent R1 consisting of 12 g FeCl<sub>3</sub> per liter in 7.9 M H<sub>2</sub>SO<sub>4</sub>, was added to 1 ml of the sample supernatant, mixed well and left in the dark for 30 min at room temperature. Absorbance was measured at 530 nm. IAA concentration was calculated from the standard curve of ten concentrations of 3 indolylacetic acid (C<sub>10</sub> H<sub>9</sub> NO<sub>2</sub>) ranging between 2 up to 20 mg/ml using the regression equation.

**Microbial Inoculants:**

The plants were grown in El-Baramoon Farm, Agric. Experimental Res. Station, El-Dakhlia Governorate, through the summer season of 2002, both with and without inoculation. Single inoculations with *Azotobacter* strains, as well as dual (*Azotobacter* + *Azospirillum brasilense* B-14647 strain) co-inoculation were applied. Inoculation with the rhizobacteria was carried out three times after the plants emergence, with 5 ml of inoculum (approximately  $10^8$  ml<sup>-1</sup> CFU) per pot.

**Plant Growth:**

Field experiment was conducted in a randomized complete block design . Four seeds of okra were sown in each hill, rows were three meters long, 70 cm apart and 50 cm spacing between plants . The plants were thinned for two per hill after emergence. The plants were biofertilized with parental and *Azotobacter* transconjugants . The plants were fertilized with phosphorus (80 kg/feddan). Biofertilization (GMs) was conducted with half recommended dose of N in addition to the recommended dose of potassium and phosphorus . Recommended dose of N was used as a positive control.

**Growth Analysis:**

Plant growth rate was recorded at 55 days – plants – old . The plants were separated into leaves, stems and roots ; dried at 80°C and weighted.

**Leaf area / plant (L.A. / P):**

This trait was determined using the fresh weight method according to A.O.A.C. (1990) using leaves of 57 days plants old.

**Biochemical and Physiological Traits:**

**Chlorophyll Concentration:**

Chlorophyll was measured in leaves at 63 (I) and 96 (II) days plants old from sowing. Although fruit chlorophyll content was measured at 80 day - plants -old from sowing, according to Markinney (1941).

**Determination of Nitrogen Content:**

Nitrogen content in dried plant materials was determined by the wet digestion of dried and finely pulverized plant material using the macrokjeldahl method according to Jackson (1958 & 1973).

**Estimation of Total Anthocyanin:**

Extraction of anthocyanins was carried out with ethanolic HCl (95% ethanol 1-1.5 N HCl) and measurement of colour at the wavelength of maximum absorption. Determination of total anthocyanins in okra was conducted according to Ranganna (1979).

**Fruit Yield and its Components:**

Immature green pods were harvested at 60 days - plants - old when reached to maturity stage and continuously every other day starting in June 9 to the first day of September 2002, giving 42 collections.. The following traits were recorded :

**Average Weight of Fresh Pods Yield per Plant**

$$= \frac{\text{Total weight of fruits / plot / collection}}{\text{Number of plants / plot / collection}}$$

**Responses Percentage ( Yield % ) to Inoculation**

$$= \frac{\text{Fruit yield under inoculation} - \text{Fruit yield without inoculation}}{\text{Fruit yield without inoculation}} \times 100$$

**Electrophoresis of Proteins in Bacterial Strains and Their Transcojugants:**

This technique was conducted in the Genetic Engineering Research Center, Fac. of Agric., Ain Shams Univ. according to the methods described by Ried and Collmer (1985).

**Similarity Level of Fingerprints:**

Jaccard's similarity coefficient was used to determine the similarity level of fingerprints according to Patwary *et al.* (1993) from the following formula;  $J_{ij} = C_{ij} / (n_i + n_j - C_{ij})$

Where,  $C_{ij}$  is the number of positive matches between two individuals, while  $n_i$  and  $n_j$  is the total number of bands in individuals  $i$  and  $j$ , respectively.

**Statistical Analysis:**

The data were subjected to the analysis of variance of factorial arrangement in a randomized complete blocks design with the general linear model (GLM) procedure for repeated measures of SAS (1995).

**RESULTS AND DISCUSSION**

**Transconjugant Efficiency in IAA Production:**

Genetical techniques were used in this work to improve the performance of *Azotobacter* inoculants. Biofertilizer transconjugants induced in this work were applied in pots experiment to assess their efficiency and impact on the soil ecosystem. Most genetically modified *Azotobacter* strains revealed significant increase in IAA production over the mid parents using different precursors including tryptone, tryptophan, ethanol and lactic acid ( Table 2 ).

**Table. 2:** Transconjugants efficiency in IAA production by *Azotobacter* transconjugants resulted from the mating between *Azotobacter beijerinckii* ATCC 132 (St<sub>1</sub>) and *Azotobacter vinelandii* SMR 230 (St<sub>1</sub>) .

Strains	Precursor of IAA											
	Tryptone			Tryptophan			Ethanol			Lactic acid		
	µg / ml	Yield %	Efficiency%	µg / ml	Yield %	Efficiency%	µg / ml	Yield %	Efficiency%	µg / ml	Yield %	Efficiency%
St 1	6.39	100		30.20	100		0.89	100		0.570	100	
str 2	7.77	100		7.77	100		0.83	100		0.733	100	
Tr 1	6.29	89	-11	18.30	96	-4	1.42	166	+65	0.893	137	+37
tr2	8.05	114	+14	4.57	24	-76	2.14	249	+148	0.597	92	-8
tr3	7.77	110	+10	25.78	136	+36	1.68	196	+95	1.093	168	+68
tr4	8.30	117	+17	2.00	11	-89	0.84	98	-2	0.720	110	+10
tr5	8.81	125	+25	33.93	179	+79	0.58	67	-33	0.510	78	-22
tr6	8.40	119	+19	28.60	151	+51	0.91	106	+6	1.140	175	+75
tr7	7.91	112	+12	26.30	139	+38	1.71	199	+99	0.977	150	+50
tr8	6.46	91	-9	28.64	151	+51	2.20	256	+156	1.090	167	+67
tr9	8.13	115	+15	27.98	147	+47	1.87	217	+117	0.547	84	-16
F-test	**	**	**	**	**	**	**	**	**	**	**	**
L.S.D 5%	0.72	9	10	4.78	25	28	0.43	46	52	0.226	33	38
L.S.D 1%	0.98	12	14	6.52	34	39	0.59	63	72	0.308	45	52

Note: Tr<sub>5</sub> and Tr<sub>9</sub> were selected to be used in the field trial and renamed Tr<sub>1</sub> and Tr<sub>2</sub>, respectively

The results obtained herein indicate that microbial transconjugants which in significantly produced high amounts of IAA from more than two precursors, were affected to significantly increase total chlorophyll concentrations (Data not shown). Because of this, plant growth promoting rhizobacteria (PGPR) are of increasing importance as inoculants for bio-fertilization and bio-stimulation in sustainable agriculture. Bio-fertilization, the ability of rhizobacteria to fix atmospheric nitrogen, accounts for a substantial nitrogen supply to crops (Bloemberg and Lugtenberg, 2001). Nitrogen is an essential plant nutrient, while reduced forms of nitrogen (NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>) are essential for the synthesis of many important biological molecules including amino acids (Proteins) and nucleic acids (DNA and RNA). The results confirmed that IAA was secreted from different substrate pathways (Prinsen *et al.*, 1993). Furthermore, *Azotobacter* transconjugants harboring 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). Together, these results confirm the important role of IAA produced by *Azotobacter* in altering root morphology, increasing leaf chlorophyll concentrations and illustrating the beneficial effect of genetic techniques in improving the mechanism of *Azotobacter* – plant interaction. In this report, different *Azotobacter* transconjugants that have extra copy of DNA may have different IAA biosynthesis pathways.

As shown in Table 3, the indole-3-acetic acid was found to significantly increase by some GM strains in the presence of ethanol and lactic acid, while the other precursors did not. In addition, transconjugants-Tr<sub>5</sub> and Tr<sub>7</sub> revealed superiority in IAA production, which reached to 47% (lactic acid) and 90% (ethanol) increase over their mid-parents, respectively. Transconjugant-Tr<sub>7</sub> revealed significant increase in IAA production using ethanol and lactic acid precursors, as well as, appeared a significant increase in total chlorophyll concentration and

plant dry weight (Data not shown). The higher production of plant growth substances (IAA) by *Azotobacter* transconjugants has often been proposed as one of the key factors responsible for the observed plant growth promotion and also the improvement of biochemical traits (Jain and Patriquin, 1985). These bacterial transconjugants are beneficial for plant growth in a direct way, because the direct enhancement of plant growth and biochemical traits can be achieved by plant hormones such as auxins secreted by these strains of rhizobacteria.

**Table. 3:** Transconjugants efficiency in IAA production by *Azotobacter* transconjugants resulted from conjugation between *Azotobacter vinelandii* SMR 230 (St<sub>1</sub>) and *Azotobacter chroococcum* NRRL B-14346 (St<sub>2</sub>).

Strains	Precursor of IAA											
	Tryptone			Tryptophan			Ethanol			Lactic acid		
	ug / ml	Yield %	Efficiency%	ug / ml	Yield %	Efficiency%	ug / ml	Yield %	Efficiency%	ug / ml	Yield %	Efficiency%
St 2	7.77	100		7.77	100		0.83	100		0.73	100	
St 3	7.46	100		28.95	100		1.47	100		0.78	100	
tr 1	7.67	101	+1	6.66	36	-64	1.83	159	59	0.35	46	-54
tr2	7.59	100	0	5.68	31	-69	0.74	64	-36	0.19	25	-75
tr3	8.43	111	+11	5.40	29	-71	1.68	146	+46	0.86	114	+14
tr4	5.87	77	-23	6.20	34	-66	1.51	131	+31	1.03	137	+37
tr5	5.69	75	-25	7.71	42	-58	0.72	62	-38	1.11	147	+47
tr6	6.91	91	-9	8.58	47	-53	1.78	155	+55	1.07	141	+41
tr7	7.79	102	+2	6.53	36	-64	2.19	190	+90	1.03	137	+37
tr8	8.68	114	+14	8.76	48	-52	1.27	111	+11	0.43	57	-43
tr9	6.61	87	-13	9.54	52	-48	1.19	104	+4	0.53	70	-30
tr10	6.46	85	-15	3.02	16	-84	0.79	69	-31	0.42	56	-45
tr11	8.07	106	+6	9.43	51	-49	1.10	96	-5	0.43	57	-43
F-ttest	**	**	**	**	**	**	**	**	**	**	**	**
L.S.D 5%	1.24	15	15	1.88	10	10	0.44	27	27	0.27	35	35
L.S.D 1%	1.63	20	21	2.48	13	13	0.57	35	37	0.35	45	47

Note: Tr<sub>5</sub> and Tr<sub>7</sub> were selected to be used in the field trial renamed Tr<sub>7</sub> and Tr<sub>8</sub>, respectively

**Table. 4:** Transconjugants efficiency in IAA production by *Azotobacter* transconjugants resulted from conjugation between *Azotobacter chroococcum* NRRL B-14346 (St<sub>1</sub>) and *Azotobacter chroococcum* ARC Ru22 (St<sub>2</sub>).

Strains	Precursor of IAA											
	Tryptone			Tryptophan			Ethanol			Lactic acid		
	ug / ml	Yield %	Efficiency%	ug / ml	Yield %	Efficiency%	ug / ml	Yield %	Efficiency%	ug / ml	Yield %	Efficiency%
St 3	7.46	100		28.95	100		1.47	100		0.780	100	
St 4	8.05	100		8.05	100		0.99	100		0.317	100	
tr1	6.88	89	-11	8.99	49	-51	2.16	175	+75	0.173	32	-68
tr2	5.57	72	-28	4.35	23	-77	1.68	136	+36	0.313	57	-43
tr3	6.93	89	-11	10.21	55	-45	0.82	67	-33	0.140	26	-74
tr4	7.39	95	-5	11.25	61	-39	1.01	82	-18	0.557	102	+2
tr5	5.29	68	-32	20.39	110	+10	1.15	94	-6	0.803	147	+47
tr6	8.84	114	+14	18.43	100	0	2.16	175	+75	0.323	59	-41
tr7	10.54	136	+36	17.83	96	-4	0.79	64	-36	0.120	22	-78
tr8	6.53	84	-16	15.37	83	-17	1.67	135	+35	0.597	109	+9
tr9	1.67	22	-78	14.72	80	-20	1.33	108	+8	0.560	102	+2
tr10	1.72	22	-78	11.18	60	-40	1.16	94	-6	0.570	104	+4
tr11	7.66	99	-1	14.44	78	-22	1.97	160	+60	0.487	89	-11
tr12	6.55	85	-15	18.03	97	-3	0.88	72	-28	0.280	51	-49
F-ttest	**	**	**	**	**	**	**	**	**	**	**	**
L.S.D 5%	0.97	11	11	3.40	18	18	0.53	37	37	0.183	33	33
L.S.D 1%	1.32	15	15	4.59	24	25	0.71	50	50	0.247	44	45

Note: Tr<sub>6</sub> and Tr<sub>11</sub> were selected to be used in the field trail which renamed Tr<sub>9</sub> and Tr<sub>10</sub>, respectively

As shown in Table 4, transconjugant-Tr<sub>6</sub> produced significant amounts of IAA using tryptone and tryptophan precursors. Both transconjugants-Tr<sub>7</sub> and Tr<sub>11</sub> produced significantly amounts of IAA using tryptone and ethanol precursors, respectively, and both of them significantly increased the total chlorophyll concentrations. Higher efficiency in IAA production over the mid-parents was reached, 36% by Tr<sub>7</sub>, 75% by Tr<sub>6</sub>, 47% by Tr<sub>5</sub>, using tryptone, ethanol and lactic acid precursor, respectively.

The results summarized in Table 5 revealed that transconjugants; Tr<sub>1</sub>, Tr<sub>3</sub> and Tr<sub>6</sub> produced significant amounts of IAA over the mid-parents as shown from Tr<sub>1</sub> using lactic acid, and Tr<sub>3</sub>, Tr<sub>6</sub> from ethanol pathways. However, transconjugant-Tr<sub>11</sub> produced significant amounts of IAA using tryptone and ethanol precursors, as consequently significantly increased chlorophyll concentration over the mid-parents (Data not shown). This

study indicated that the genetical techniques used are able to improve the performance of microbial inoculants through their efficacy in phytohormones producing over their mid-parents. The results obtained herein are in accordance with Ivanov *et al.* (1992), who found that inoculation of yellow lupins with *Arthrobacter* or *Azospirillum* increased root development and active absorbing surface, leaf chlorophyll content and respiration rate. The results indicated that lactic acid pathway in IAA biosynthesis was efficient in GM *Azotobacter* – Tr<sub>1</sub>, while ethanol pathway was efficient in transconjugants; Tr<sub>3</sub>, Tr<sub>6</sub> and Tr<sub>11</sub>. Though, transconjugant-Tr<sub>11</sub> was efficient in the two biosynthesis pathways of tryptone and ethanol. Expression analysis of the *ipdC* (indole-3-pyruvic acid decarboxylase) gene revealed a strict regulation (Dobbelaere *et al.*, 1999).

**Table. 5:** Transconjugants efficiency in IAA produced by *Azotobacter* transconjugants resulted from the conjugation between *Azotobacter beijerinckii* ATCC 132 (St<sub>1</sub>) and *Azotobacter chroococcum* NRRL B-14346 (St<sub>2</sub>).

Strains	Precursor of IAA											
	Tryptone			Tryptophan			Ethanol			Lactic acid		
	ug / ml	Yield %	Efficiency%	ug / ml	Yield %	Efficiency%	ug / ml	Yield %	Efficiency%	ug / ml	Yield %	Efficiency%
St 1	6.39	100		30.20	100		0.89	100		0.57	100	
St 3	7.46	100		28.95	100		1.47	100		0.78	100	
tr 1	5.39	78	-22	19.86	67	-33	1.51	128	+28	0.92	136	+36
tr2	7.25	105	+5	2.41	8	-92	0.86	73	-27	0.60	88	-12
tr3	3.75	54	-46	9.96	34	-66	2.00	170	+70	0.72	107	+7
tr4	6.45	93	-7	10.28	35	-65	0.73	62	-38	0.54	80	-20
tr5	6.73	97	-3	15.01	51	-49	1.25	106	+6	0.63	93	-7
tr6	6.42	93	-7	8.50	29	-71	1.70	144	+44	0.35	52	-48
tr7	7.01	101	+1	0.45	2	-98	1.02	86	-14	0.44	65	-35
tr8	3.20	46	-54	24.74	84	-16	0.76	65	-35	0.49	72	-28
tr9	3.33	48	-52	10.00	34	-66	1.15	98	-2	0.42	62	-38
tr10	5.65	82	-18	9.38	32	-68	0.64	54	-46	0.39	58	-42
tr11	8.36	121	+21	17.73	60	-40	1.71	145	+45	0.63	93	-7
F-test	**	**	**	**	**	**	**	**	**	**	**	**
L.S.D 5%	0.92	13	15	3.34	11	12	0.47	35	39	0.17	24	26
L.S.D 1%	1.21	17	20	4.39	14	16	0.61	46	53	0.22	31	36

Note: Tr<sub>10</sub> and Tr<sub>11</sub> were selected to be used in the field trial which named Tr<sub>3</sub> and Tr<sub>4</sub>, respectively .

The results illustrated in Table 6 demonstrated that only transconjugant-Tr<sub>1</sub> produced significant amounts of IAA compared to the mid-parents, indicating that IAA biosynthesis from tryptone pathway was only effective in this isolate. However, transconjugant-Tr<sub>2</sub> significantly enhanced IAA biosynthesis from lactic acid pathway. In addition, ethanol precursor was suitable for IAA biosynthesis pathways from different *Azotobacter* transconjugants including Tr<sub>2</sub>, Tr<sub>3</sub>, Tr<sub>4</sub>, Tr<sub>6</sub>, and Tr<sub>7</sub>. Higher efficiency in IAA biosynthesis over the mid-parents was achieved by Tr<sub>1</sub> (31%) from tryptone, Tr<sub>3</sub> (174%) from ethanol and Tr<sub>2</sub> (57%) from lactic acid pathway. These phytostimulation enhance plant growth in a direct way.

Recently, it has been shown that the pathway of IAA biosynthesis is differently regulated by catabolite repression (Carrena-Lopez *et al.*, 2000) and that IAA synthesis is regulated by the autoinduction of IAA (Vande Broek *et al.*, 1999). In addition, the two efficient transconjugants resulted from each conjugation were selected for application in field trial experiment. The application of these plant-growth-promoting rhizobacteria (PGPRs) would increase crop yield, thereby helping to feed the growing world population, because they are environmentally friendly alternative to chemical fertilizers. The discovery of many traits and genes that are involved in the beneficial effect of PGPRs has resulted in a better understanding of the performance of bioinoculants in the field, and provides the opportunity to enhance the beneficial effects of PGPR strains by genetic modification.

#### **Effect of Biofertilization with GM Strains on Biological and Biochemical Traits:**

As shown from the results presented in Table 7, plant dry weight, crop growth rate, and leaf area were significantly affected by biofertilization with *Azotobacter* strains and their transconjugants. In addition, leaf area was also significantly affected by double biofertilization (*Azotobacter* with /or without *Azospirillum*). The results obtained here are in agreement with those obtained by Singh and Bhargava (1994), who found that inoculation of *Brassica napus* cv. ISN-129 with *Azotobacter chroococcum* following the application of different amounts of nitrogen produced the greatest increase in seed yield and total dry matter when no external nitrogen had been applied. The significant effect in response to inoculation with *Azotobacter* could be attributed to their effects on the number of primary branches and pods, associated with a higher leaf area and a faster crop growth rate.

Genetically modified strains (Tr<sub>2</sub>, Tr<sub>4</sub>, Tr<sub>5</sub>, Tr<sub>6</sub> and Tr<sub>7</sub>) in combinations with *Azospirillum* significantly produced total chlorophyll I concentration than their mid-parents and the recommended dose of nitrogen. The combinations of these biofertilizers, (GM *Azotobacter* + *Azospirillum*) improved the yield of total chlorophyll II concentration over *Azotobacter* strains. In addition, transconjugant 8 individually, and also the combinations of GM *Azotobacter* strains (Tr<sub>1</sub>, Tr<sub>5</sub> and Tr<sub>7</sub>) + *Azospirillum* significantly improved the yield of total chlorophyll II over their mid-parents. Furthermore, transconjugant 8 individually and the combinations of *Azotobacter* transconjugants (St<sub>4</sub>, Tr<sub>1</sub>, Tr<sub>5</sub>, Tr<sub>6</sub> and Tr<sub>7</sub>) with *Azospirillum* significantly improved total concentration of chlorophyll II over that in plants fertilized with the recommended dose of nitrogen. The results obtained herein are in agreement with Das *et al.* (1990), who found that field application of *Azotobacter* and *Azospirillum* provide an opportunity to reduce the need for chemical nitrogen fertilizer by 50% without adverse effect on mulberry leaf yield and quality. Individual biofertilizers of *Azotobacter* (St<sub>1</sub>, St<sub>2</sub>, Tr<sub>2</sub>, Tr<sub>3</sub> and Tr<sub>7</sub>) and their combinations (St<sub>3</sub>, Tr<sub>2</sub>, Tr<sub>3</sub> and Tr<sub>4</sub>) with *Azospirillum* significantly increased the yield of total chlorophyll I/II concentrations over that in the plants fertilized with recommended dose of nitrogen.

**Table 6:** Transconjugants efficiency in IAA production by *Azotobacter* transconjugants resulted from the conjugation between *Azotobacter beijerinckii* ATCC 132 (St<sub>1</sub>) and *Azotobacter chroococcum* ARC Ru22 (St<sub>1</sub>).

Strains	Precursor of IAA											
	Tryptone			Tryptophan			Ethanol			Lactic acid		
	µg / ml	Yield %	Efficiency%	µg / ml	Yield %	Efficiency%	µg / ml	Yield %	Efficiency%	µg / ml	Yield %	Efficiency%
St 1	6.39	100		30.20	100		0.89	100		0.570	100	
St 4	8.05	100		8.05	100		0.99	100		0.317	100	
tr 1	9.43	131	+31	2.50	13	-87	1.31	139	+39	0.467	105	+5
tr2	5.67	79	-21	1.62	8	-92	2.17	230	+130	0.693	157	+57
tr3	7.74	107	+7	3.02	16	-84	2.58	274	+174	0.337	76	-24
tr4	7.07	98	-2	2.41	13	-87	1.32	140	+40	0.290	65	-35
tr5	7.76	107	+7	0.57	3	-97	0.86	91	-9	0.430	97	-3
tr6	6.10	84	-16	16.17	85	-15	1.42	151	+51	0.370	84	-16
tr7	4.58	63	-37	1.53	8	-92	1.66	176	+76	0.350	79	-21
F-test	**	**	**	**	**	**	**	**	**	**	**	**
L.S.D 5%	1.28	16	19	1.74	8	9	0.36	35	41	0.203	42	50
L.S.D 1%	1.77	23	27	2.40	11	13	0.50	48	58	0.279	58	71

Note: Tr<sub>3</sub> and Tr<sub>6</sub> were selected to be used in the field trial which renamed Tr<sub>5</sub> and Tr<sub>6</sub>, respectively

**Table 7:** Analysis of variance (mean squares) of vegetative and biochemical traits responded to inoculation with *Azotobacter* transconjugants.

S.V.	D.F.	Plant dry weight 55 DAP	Crop growth rate(g/day)	Leaf area (cm <sup>2</sup> )	Total Chl. I in leaves	Total Chl. II in leaves	Total Chl. at 80 DAP in fruits	Anthocyanin at 85 DAP in fruits
Replication	2	10.91 <sup>NS</sup>	0.0036 <sup>NS</sup>	26578 <sup>NS</sup>	0.0289 <sup>NS</sup>	0.0013 <sup>s</sup>	0.0457*	0.000003 <sup>NS</sup>
Biofertilization ( <i>Azotobacter</i> )	13	52.33**	0.0173**	44042*	0.7564**	2.0054**	0.1453**	0.000008**
Double biofertilization (with and without <i>Azotobacter</i> )	1	40.88 <sup>NS</sup>	0.0134 <sup>s</sup>	533726**	12.1106**	18.7724**	0.0050 <sup>NS</sup>	0.00001**
Bio x Double	13	10.04 <sup>NS</sup>	0.0033 <sup>NS</sup>	12932 <sup>NS</sup>	0.5449**	1.1005**	0.0798**	0.00002**
Error	54	13.33	0.0044	20635	0.1499	0.2189	0.0137	0.000001

The results obtained herein indicated that biofertilizers provide nutrients to okra plants, which is reflected mainly in increased leaf chlorophyll concentrations over the mid-parents and the recommended dose of nitrogen and thus may increase quality of fruits in okra. The results also demonstrated that application of biofertilizers especially *Azotobacter* and *Azospirillum* could safely be used with half of the normal recommended dose of chemical nitrogen fertilizer to improve the growth, chemical and physiological traits in okra. The combination of *Azotobacter* with *Azospirillum* has further potential for the improvement of chlorophyll concentration in okra. Since biofertilizers are cheaper than chemical fertilizers, their use can reduce the input cost for fruit production in okra.

In addition, total Chl. I ( at 63 days -plant - old ) and total Chl. II ( 96 days - plant - old ) were more affected by GM *Azotobacter* strains, double biofertilizers and the interaction between both of them. This indicated that the application of biofertilizers enhanced the performance of chlorophyll concentrations in leaves of okra affecting on green pod yield, pod weight and number of fruits per plant. The results revealed that biofertilizers exhibited significant effect on photosynthetic efficiency at growing, flowering and fruiting stages. This may be attributed to adequate availability of nutrients at these stages. The positive effect of biofertilizers in increasing growth and physiological traits in plants and fruits was in accordance with the findings of Thakur and Panwar (1997).

The economic importance of this study was aimed to develop microbial inoculants that can effectively compete with agrichemicals, for use as biofertilizers and phytostimulators and to assess the impact of new inoculants on indigenous bacterial populations in soils. This because certain micro-organisms found in the rhizosphere are known to improve soil fertility and consequently plant health and growth. These micro-organisms supply nutrients to plants by degrading organic matter, convert atmospheric nitrogen into a usable form, protect plants from disease, and stimulate plant growth directly through the production of phytostimulating compounds. The increased use of microorganisms is now seen as an advantageous alternative to chemical treatment and will contribute substantially to the goal of environmental friendly, sustainable agriculture.

Concerning physiological traits in fruits, the GM *Azotobacter* biofertilization and their interaction with double biofertilization with and without *Azospirillum* were more effective on stimulating the formation of total chlorophyll concentration at 80 days plant-old and anthocyanin concentration in fruits at 85 days plant-old. Although, double biofertilization had more effect on anthocyanin concentrations in fruits. PGPR can fix atmospheric nitrogen and supply it to plants; they synthesize siderophores that can solubilize and sequester iron from the soil and provide it to plant cells, they synthesize several different phytohormones that can act to enhance various stages of plant growth; they may have mechanisms for the solubilization of minerals such as phosphorus that then become more readily available for plant growth; and they may synthesize some less well characterized low molecular mass compounds or enzymes that can modulate plant growth and development (Glick *et al.*, 1994a & b). A particular PGPR may significantly affect on fruits physiological traits and development by using any one, or more, of these mechanisms.

The results represented in Tables 8 and 9 illustrate that the collection number 11 is giving the maximum yield of fruits among all treatments. In addition, *Azotobacter* was found to be effective in improving the okra crop and this agrees with earlier reports (Das *et al.*, 1990). The significant increase above the half recommended dose of nitrogen was affected by some biofertilizers beginning from the collection No. 6 up to No. 11.

As shown from the results represent in Table 10 that the significant increase in fruit yielding per collection above the plants fertilized with the half recommended dose of nitrogen beginning from the collection No. 4 (affected by isolates resulted from the mating between  $St_1 \times St_2$ ) and the collection No. 5 in Table 11 (affected by isolates resulted from the mating between  $St_1 \times St_3$ ) up to collection No. 14 when *Azospirillum* was one of the biofertilizer component. A similar results concerning an increase in fruit yielding variations affected by the combination of biofertilizers have been reported before by Bashan and Levany (1990), who found that an increase in the foliar dry matter and accumulation of minerals in stem and leaves of *Brassica napus* due to inoculation with *Azotobacter* during the reproductive phase could be transferred to the pods and seeds.

The results in Tables 12 and 13 illustrated significant increase in fruit yielding affected by some biofertilizers above the plants fertilized with half recommended dose which was shown beginning from the collection No. 6 up to the collection No. 13. It is clear from the present investigation that the biofertilizers improved the biochemical traits and quality of fruits as shown in the previous parts of this study, while the biofertilizers had no such adverse effect when compared with the recommended dose of nitrogen.

The results summarized in Tables 14 and 15 reveals that combined application of each of the *Azotobacter* strains with the wild type strain of *Azospirillum* following the application of the half recommended dose of nitrogen produced significant increase in fruit yield beginning from the collection No. 5 ( Table 14 ) and the collection No. 6 ( Table 15 ) up to the collection No. 13. Significant increase shown in fruit yielding due to some treatments above the plants fertilized with the half and full recommended dose of N restricted the rising cost of nitrogen fertilizers and the possibility of subsequent environmental pollution on their extensive usage both in developing and developed countries. The results obtained herein agree with that obtained by Sudhakar *et al.* (2000), who found that a combination of nitrogen fixing bacteria (NFBs) where *Azotobacter* was one of the biofertilizer components improved leaf yield over single NFB treatments.

The results in Table 16 revealed significant increase in fruit yielding above the plants fertilized with the half recommended dose of nitrogen as shown by some *Azotobacter* strains beginning from the collection No. 6 up to No. 13. The results indicated that biofertilization was found to produce greatest fruit yielding above the half recommended dose of N. The yield increase in response to inoculation could be attributed to a greater number of primary branches and pods, associated with a higher leaf area index and a faster crop growth rate. The effects of *Azospirillum* inoculation in combination with the different strains of *Azotobacter* resulted from the mating between  $St_3 \times St_4$  ( Table 17 ) demonstrated that the mixture of biofertilizers exhibited increase in fruit yielding beginning from the collection No. 6 up to the collection No. 13 as shown before ( Table 16 ) in individual treatments with *Azotobacter* biofertilizers.

The results obtained in this study agreed with those obtained by Renato de Freitas (2000), who reported that plants inoculated with a mixture of four bacteria tended to exhibit a higher yield response than plants inoculated with individual bacteria. However, plant responses to the various inoculants were not consistent in fruit yielding from one stage to another. Use of the rhizobacteria in new agriculture may have limited nitrogen fertilizers and the possibility of subsequent environmental pollution, for this biofertilization was environment-friendly.

**Table 8:** Effect of inoculation with *Azotobacter* transconjugants resulted from the matings between  $St_1 \times St_2$  on fruit fresh weight of okra per collection .

No. of Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	14
50%	6.50	10.96	7.86	9.86	13.24	13.13	26.66	26.75	17.56	23.51	32.11	21.25	13.59	3.97
Full dose	2.56	5.51	7.87	11.66	19.91	21.81	29.71	23.04	34.40	27.44	38.64	28.35	17.04	3.74
st 1	6.08	6.19	6.58	12.22	12.09	23.57	31.31	26.43	21.19	25.35	32.10	21.91	16.18	3.46
st 2	3.15	4.97	5.17	13.75	10.22	21.40	20.73	34.11	28.20	26.19	31.01	24.34	14.69	3.97
Tr1	4.83	6.16	9.00	11.43	15.28	17.47	32.47	32.31	22.54	28.47	32.28	24.11	21.24	5.48
Tr2	4.86	7.74	7.50	10.01	13.57	23.26	32.11	32.24	31.66	24.88	42.41	20.49	15.18	4.06

**Table 9:** Effect of inoculation with *Azotobacter* transconjugants resulted from the matings between  $St_1 \times St_3$  on fruit fresh weight of okra per collection .

No. of Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	14
50%	6.50	10.96	7.86	9.86	13.24	13.13	26.66	26.75	17.56	23.51	32.11	21.25	13.59	3.97
Full dose	2.56	5.51	7.87	11.66	19.91	21.81	29.71	23.04	34.40	27.44	38.64	28.35	17.04	3.74
st 1	6.08	6.19	6.58	12.22	12.09	23.57	31.31	26.43	21.19	25.35	32.10	21.91	16.18	3.46
st 3	6.06	7.89	6.19	11.55	13.34	20.92	27.98	26.72	29.36	23.27	38.81	21.19	21.19	3.14
Tr3	6.82	8.42	7.00	12.38	11.66	14.81	27.85	35.39	24.48	32.83	38.10	29.41	14.24	4.44
Tr4	6.14	7.87	8.22	17.33	19.50	24.72	33.55	26.00	25.50	23.11	39.52	23.10	23.66	5.06

**Table 10:** Effect of biofertilizer with *Azotobacter* transconjugants resulted from the matings between  $St_1 \times St_2$  in combination with wild type strain of *Azospirillum* on fruit fresh weight of okra per collection .

No. of Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	14
50%	6.50	10.96	7.86	9.86	13.24	13.13	26.66	26.75	17.56	23.51	32.11	21.25	13.59	3.97
Full dose	2.56	5.51	7.87	11.66	19.91	21.81	29.71	23.04	34.40	27.44	38.64	28.35	17.04	3.74
st 1 + Azos	6.08	6.19	6.58	12.22	12.09	23.57	31.31	26.43	21.19	25.35	32.10	21.91	16.18	3.46
st 4 + Azos	7.01	7.86	7.65	14.99	11.85	18.47	31.91	31.17	24.57	34.97	41.45	26.76	17.01	4.01
Tr5 + Azos	6.66	8.10	7.41	14.90	14.79	28.55	37.74	34.72	24.97	30.23	38.41	28.62	17.61	3.25
Tr6 + Azos	8.23	8.58	8.40	12.10	14.68	18.00	34.07	38.47	26.97	33.20	40.04	30.13	18.86	4.81

**Table 11:** Effect of biofertilizer with *Azotobacter* transconjugants resulted from the matings between  $St_1 \times St_3$  in combination with wild type strain of *Azospirillum* on fruit fresh weight of okra per collection .

No. of Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	14
50%	6.50	10.96	7.86	9.86	13.24	13.13	26.66	26.75	17.56	23.51	32.11	21.25	13.59	3.97
Full dose	2.56	5.51	7.87	11.66	19.91	21.81	29.71	23.04	34.40	27.44	38.64	28.35	17.04	3.74
st 2 + Azos	4.28	8.09	9.00	13.18	22.59	23.03	35.15	37.12	30.51	40.39	43.15	31.21	20.07	4.54
st 3 + Azos	5.16	7.29	8.40	18.64	19.63	22.94	32.32	36.46	31.22	35.80	46.58	32.61	21.13	6.33
Tr7 + Azos	6.83	11.75	11.41	14.79	21.03	22.42	41.40	34.95	23.38	25.21	35.34	23.52	18.31	5.03
Tr8 + Azos	4.50	8.02	6.56	10.84	15.94	20.76	41.25	36.95	26.45	28.54	40.94	28.05	22.80	4.40

**Table 12:** Effect of biofertilizer with *Azotobacter* transconjugants resulted from the matings between  $St_1 \times St_4$  on fruit fresh weight of okra per collection .

No. of Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	14
50%	6.50	10.96	7.86	9.86	13.24	13.13	26.66	26.75	17.56	23.51	32.11	21.25	13.59	3.97
Full dose	2.56	5.51	7.87	11.66	19.91	21.81	29.71	23.04	34.40	27.44	38.64	28.35	17.04	3.74
st 1	6.08	6.19	6.58	12.22	12.09	23.57	31.31	26.43	21.19	25.35	32.10	21.91	16.18	3.46
st 4	7.01	7.86	7.65	14.99	11.85	18.47	31.91	31.17	24.57	34.97	41.45	26.76	17.01	4.01
Tr5	6.66	8.10	7.41	14.90	14.79	28.55	37.74	34.72	24.97	30.23	38.41	28.62	17.61	3.25
Tr6	8.23	8.58	8.40	12.10	14.68	18.00	34.07	38.47	26.97	33.20	40.04	30.13	18.86	4.81

**Table 13:** Effect of biofertilizer with *Azotobacter* transconjugants resulted from the matings between  $St_2 \times St_3$  on fruit fresh weight of okra per collection .

No. of Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	14
50%	6.50	10.96	7.86	9.86	13.24	13.13	26.66	26.75	17.56	23.51	32.11	21.25	13.59	3.97
Full dose	2.56	5.51	7.87	11.66	19.91	21.81	29.71	23.04	34.40	27.44	38.64	28.35	17.04	3.74
st 2	3.15	4.97	5.17	13.75	10.22	21.40	20.73	34.11	28.20	26.19	31.01	24.34	14.69	3.97
st 3	6.06	7.89	6.19	11.55	13.34	20.92	27.98	26.72	29.36	23.27	38.81	21.19	21.19	3.14
Tr7	7.02	7.98	8.95	11.88	13.12	18.62	28.23	29.42	31.79	27.77	40.61	22.96	17.30	3.52
Tr8	4.44	7.98	9.85	15.49	15.32	20.69	36.81	34.40	24.90	27.44	35.22	27.23	20.99	2.84

**Table 14:** Effect of biofertilizer with *Azotobacter* transconjugants resulted from the matings between St<sub>1</sub> x St<sub>4</sub> in combination with wild type strain of *Azospirillum* on fruit fresh weight of okra per collection.

No. of Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	14
50%	6.50	10.96	7.86	9.86	13.24	13.13	26.66	26.75	17.56	23.51	32.11	21.25	13.59	3.97
Full dose	2.56	5.51	7.87	11.66	19.91	21.81	29.71	23.04	34.40	27.44	38.64	28.35	17.04	3.74
st 1 + Azos	7.13	5.25	7.95	16.14	21.53	27.69	34.26	34.22	33.39	30.88	45.33	25.04	19.98	4.97
st 2 + Azos	4.28	8.09	9.00	13.18	22.59	23.03	35.15	37.12	30.51	40.39	43.15	31.21	20.07	4.54
Tr1 + Azos	5.57	10.06	7.74	12.13	19.66	20.70	35.69	28.09	34.16	29.76	40.65	28.60	19.66	6.44
Tr2 + Azos	5.46	6.81	7.11	13.96	23.19	26.67	44.81	38.75	24.54	33.26	36.61	25.42	21.44	9.67

**Table 15:** Effect of biofertilizer with *Azotobacter* transconjugants resulted from the matings between St<sub>2</sub> x St<sub>3</sub> in combination with wild type strain of *Azospirillum* on fruit fresh weight of okra per collection .

No. of Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	14
50%	6.50	10.96	7.86	9.86	13.24	13.13	26.66	26.75	17.56	23.51	32.11	21.25	13.59	3.97
Full dose	2.56	5.51	7.87	11.66	19.91	21.81	29.71	23.04	34.40	27.44	38.64	28.35	17.04	3.74
st 1 + Azos	7.13	5.25	7.95	16.14	21.53	27.69	34.26	34.22	33.39	30.88	45.33	25.04	19.98	4.97
st 3 + Azos	5.16	7.29	8.40	18.64	19.63	22.94	32.32	36.46	31.22	35.80	46.58	32.61	21.13	6.33
Tr3 + Azos	4.79	7.69	9.68	17.05	19.29	27.30	41.26	35.68	26.63	28.65	42.42	28.25	23.05	4.47
Tr4 + Azos	6.23	9.03	10.06	14.63	18.66	26.25	42.49	28.88	28.38	24.99	34.67	21.96	17.38	8.77

**Table 16:** Effect of biofertilization with *Azotobacter* transconjugants resulted from the mating between St<sub>3</sub> x St<sub>4</sub> on fruit fresh weight per collection.

No. of Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	14
50%	6.50	10.96	7.86	9.86	13.24	13.13	26.66	26.75	17.56	23.51	32.11	21.25	13.59	3.97
Full dose	2.56	5.51	7.87	11.66	19.91	21.81	29.71	23.04	34.40	27.44	38.64	28.35	17.04	3.74
st 3	6.06	7.89	6.19	11.55	13.34	20.92	27.98	26.72	29.36	23.27	38.81	21.19	21.19	3.14
st 4	7.01	7.86	7.65	14.99	11.85	18.47	31.91	31.17	24.57	34.97	41.45	26.76	17.01	4.01
Tr9	6.00	7.10	6.42	10.82	16.56	22.55	34.73	33.04	28.77	28.22	37.87	23.35	16.06	3.11
Tr10	5.63	8.58	7.00	11.01	18.08	21.01	32.59	32.43	27.21	28.07	37.30	27.26	15.88	2.09

**Table 17:** Effect of biofertilizer with *Azotobacter* transconjugants resulted from the mating between St<sub>3</sub> x St<sub>4</sub> combined with wild type strain of *Azospirillum* on fruit fresh weight per collection.

No. of Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	14
50%	6.50	10.96	7.86	9.86	13.24	13.13	26.66	26.75	17.56	23.51	32.11	21.25	13.59	3.97
Full dose	2.56	5.51	7.87	11.66	19.91	21.81	29.71	23.04	34.40	27.44	38.64	28.35	17.04	3.74
st 3 + Azos	5.16	7.29	8.40	18.64	19.63	22.94	32.32	36.46	31.22	35.80	46.58	32.61	21.13	6.33
st 3 + Azos	5.36	7.56	6.71	13.80	20.33	21.56	40.89	38.69	29.99	43.35	37.60	27.57	20.85	4.05
Tr9 + Azos	7.17	10.23	9.70	13.33	20.45	24.54	39.76	38.90	24.98	23.31	30.86	20.75	19.76	4.50
Tr10 + Azos	7.04	10.74	7.37	9.13	17.32	19.91	39.67	36.90	29.16	25.45	34.58	23.38	17.25	4.86

**Genetic Analysis of Protein Patterns in *Azotobacter* Strains and Their Transconjugants:**

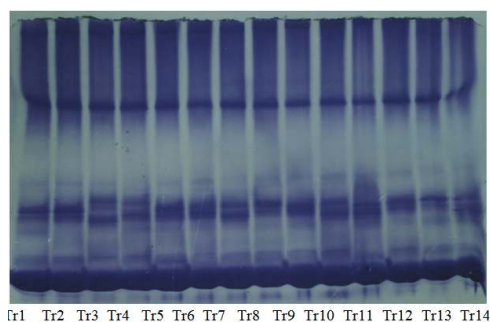
It can be stated that protein fingerprinting is probably advisable as a phylogenetic tool at an interspecific level, especially since other techniques are available which are more suitable for such purpose (RFLP analysis, DNA sequencing). However, if cautiously interpreted, fingerprint data may well serve as one criterion among others to evaluate genetic relationships at an intraspecific level or between closely related species. Hybridization-generated fingerprint bands are inherited to the offspring according to the Mendelian rules.

As shown from the results presented in Fig. 1, St<sub>1</sub> having the bands numbered 2 up to 15, while St<sub>2</sub>, St<sub>3</sub> and Tr<sub>7</sub> containing the maximum number of bands numbered from one up to 18. Although, Tr<sub>1</sub>, Tr<sub>2</sub>, Tr<sub>3</sub> and Tr<sub>9</sub> contained the bands number 1 up to 17. In addition, Tr<sub>4</sub> indicating the first 14 protein bands only. Although, St<sub>4</sub>, Tr<sub>5</sub>, Tr<sub>6</sub>, Tr<sub>8</sub> and Tr<sub>10</sub> have the first 15 protein bands. This indicated that there were levels of polymorphism between strains and their resulted transconjugants. On the other hand, both strains St<sub>2</sub> and St<sub>3</sub> have the similar number of bands (18 bands), while St<sub>1</sub> and St<sub>4</sub> indicating 14 and 15 protein bands, respectively. The optical density of the same protein band was differed among the parental strains and their transconjugants. This indicated some levels of polymorphism between strains and their transconjugants .

The results presented in Table 18 summarize the molecular weight ( kDa) of different protein bands in different *Azotobacter* strains and their transconjugants. The results indicated that the band No. 18 has similar molecular weight (2 kDa) in St<sub>2</sub>, St<sub>3</sub> and Tr<sub>7</sub>. Although, the band No. 17 has two different molecular weights, which equal 3 kDa (St<sub>2</sub>, St<sub>3</sub>, Tr<sub>7</sub>) and 2 kDa (Tr<sub>1</sub>, Tr<sub>2</sub>, Tr<sub>3</sub>, Tr<sub>9</sub>).

In addition, the band No. 16 also has two different molecular weights equals 4 kDa (St<sub>2</sub>, St<sub>3</sub>, Tr<sub>7</sub>) and 3 kDa (Tr<sub>1</sub>, Tr<sub>2</sub>, Tr<sub>3</sub>, Tr<sub>9</sub>). The band No. 15 has three different molecular weights equals; 2 kDa (St<sub>1</sub>, St<sub>4</sub>, Tr<sub>5</sub>, Tr<sub>6</sub>, Tr<sub>8</sub>, Tr<sub>10</sub>), 6 kDa (St<sub>2</sub>, St<sub>3</sub>, Tr<sub>7</sub>) and 4 kDa (Tr<sub>1</sub>, Tr<sub>2</sub>, Tr<sub>3</sub>, Tr<sub>9</sub>). However, the band No. 14 has four different molecular weights equals; 3 kDa (St<sub>1</sub>, St<sub>4</sub>, Tr<sub>5</sub>, Tr<sub>6</sub>, Tr<sub>8</sub>, Tr<sub>10</sub>), 11 kDa (St<sub>2</sub>, St<sub>3</sub>, Tr<sub>7</sub>), 6 kDa (Tr<sub>1</sub>, Tr<sub>2</sub>, Tr<sub>3</sub>,

Tr<sub>9</sub>), and 2 kDa (Tr<sub>4</sub>). Although, the bands above the band No. 14 were differed in more than four molecular weights. The results obtained herein are in agreement with Tegelström and Essen (1996), who reported that individuals originating from different breeders are more dissimilar than those from the same breeder.



**Fig. 1:** Protein fingerprints of four *Azotobacter* strains and their 10 transconjugants resulted from different five matings numbered from left to right (marker protein, St<sub>1</sub> - St<sub>2</sub>, ..., Tr<sub>1</sub> - Tr<sub>10</sub>)

**Table 18:** Molecular weight of protein bands among *Azotobacter* strains and their transconjugants using SDS-PAGE protein pattern .

No.of bands	Strains														
	M	St <sub>1</sub>	St <sub>2</sub>	St <sub>3</sub>	St <sub>4</sub>	Tr	Tr	Tr	Tr	Tr	Tr <sub>6</sub>	Tr	Tr	Tr	Tr <sub>0</sub>
1	205.0		178.5	180.5	180.7	182.2	154.7	182.0	128.9	127.8	128.2	180.5	127.8	154.7	128.2
2	116.0	97.8	154.1	157.4	126.7	154.7	183.9	158.7	96.1	90.1	90.2	157.4	90.1	183.9	90.2
3	66.0	74.2	129.8	127.5	94.7	126.5	128.9	126.2	75.1	73.5	75.0	127.5	73.5	128.9	75.0
4	45.0	64.4	91.6	92.2	72.7	92.5	94.4	97.0	54.9	63.4	63.7	92.2	63.4	94.4	63.7
5	29.0	56.9	73.2	73.1	55.6	74.9	74.1	76.0	48.9	55.1	56.4	73.1	55.1	74.1	56.4
6		48.9	64.3	63.9	47.9	63.1	63.7	63.5	43.5	49.6	49.4	63.9	49.6	63.7	49.4
7		45.2	56.5	56.2	43.8	54.9	54.7	55.0	35.8	43.5	45.8	56.2	43.5	54.7	45.8
8		34.7	48.6	48.2	34.3	48.3	48.1	49.1	26.1	35.8	37.8	48.2	35.8	48.1	37.8
9		22.7	44.7	44.9	23.7	43.5	43.5	45.1	20.1	26.8	26.7	44.9	26.8	43.5	26.7
10		19.2	33.0	33.3	19.6	33.5	35.9	36.4	11.1	19.8	19.9	33.3	19.8	35.9	19.9
11		12.2	25.1	25.3	11.5	24.2	25.2	24.8	6.7	10.9	6.3	25.3	10.9	25.2	6.3
12		6.6	22.5	23.0	6.5	19.9	19.4	19.4	4.8	6.6	11.1	23.0	6.6	19.4	11.1
13		5.1	18.3	19.8	4.7	11.4	11.1	11.1	3.6	5.1	5.3	19.8	5.1	11.1	5.3
14		3.8	11.6	11.6	3.8	6.6	6.4	6.8	2.6	3.8	3.9	11.6	3.8	6.4	3.9
15		2.5	6.2	6.6	2.6	4.9	4.8	4.9		2.7	2.9	6.6	2.7	4.8	2.9
16			4.9	4.8		3.7	3.8	3.9				4.8		3.8	
17			3.6	3.7		2.5	2.5	2.6				3.7		2.5	
18			2.5	2.4								2.4			

M = Marker protein.

As shown in this study, suitable software systems allow the interactive editing of the primary image on the computer screen, including background reduction, band sizing with the help of (in-lane or external) molecular weight standards, band matching, and comparison across gels. Primary images, as well as, processed data can be stored in the computer and used for later comparisons. Most importantly, image analysis allows us to set intensity thresholds for the bands to be scored and mobility thresholds for recognizing a match between two bands.

It is concluded that good productivity of okra over the recommended dose of nitrogen can be achieved in biofertilizer combinations between *Azotobacter* transconjugants with *Azospirillum*. However, the observed increase in green pod yield that resulted from biofertilization could be explained on the basis of increased number of pods picked per plant and fresh pod weight. Fruit load may have physiological implications on the overall productivity of okra in terms of green pod yield. The increase in number of pods due to biofertilization observed in the present study may be attributed to better development of fruits from the flower.

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