

RESPONSE OF CUCUMBER AND OKRA TO COMBINED APPLICATION OF AZOTOBACTER TRANSFORMANTS AND CHEMICAL NITROGEN FERTILIZER

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ABSTRACT

The present investigation was carried out (Lab. study) in microbial genetics lab., Genetic Dept., Minia University and a field study was carried out at Sakha agriculture research station farm during the two successive summer seasons of 2004 and 2005.

DNA extract from *Bacillus sp.* (grown under 20% NaCl) was used to transform salt isolate to sensitive isolates of *Azotobacter vinelandii* Wild type. The producing transformants were used to infect cultivars of cucumber (hybrid prince) and Okra (Eskandrandi).

Results showed that the highest frequencies of transformants (330×10^{-6}) was obtained on 10% NaCl at 24 hours.

Results also indicated that there were significant differences in growth characteristics between the plants inoculated with the tested strains when compared with the control plants (non-inoculated) or the plants inoculated with the wild type strain. All transformants in our study had the best effect on most growth characters and yield as compared with wild type strain.

The best transformant was T-4 which had the highest percentage of increasing total yield with both crops. The data show that there were differences among both methods of incubation (Liquid or capsules) in their effects on the two crops in their response. The best method was liquid which had the highest values of all growth characters and total yield.

Finally, the present investigation recommends the importance of inoculating vegetable crops with transformants of *A. vinelandii* to improve their growth and yield with using the liquid method.

Keywords: *Okra, cucumber, (Cucumis sativus, L); Azotobacter vinelandii, Azotobacter transformants*

INTRODUCTION

The cucumber (*Cucumis sativus*) is a widely cultivated plant in the gourd family Cucurbitaceae. Though it technically is a fruit, cucumbers are widely considered vegetables. Many alternative health practitioners believe all disease begins in the colon. The okra fiber, absorbing water and ensuring bulk in stools, helps prevent and improve constipation. Fiber in general is helpful for this but okra is one of the best, along with ground flax seed and psyllium (Sylvia W. Zook, 2002).

Okra is commonly used in Southern, Creole, and Cajun cooking. This is due to the fact that it was initially introduced into the United States in its southern region. Since there is little frost in the southern region, okra grows well there. Okra is low in calories and is a good source of many nutrients including vitamin B₆, and L-Ascorbic acid (vitamin C), fiber, calcium, and folic acid. It is effective for the prevention of neural tube defects in developing

fetuses mainly due to its high content of vitamin B₆, calcium, fiber, and folic acid. United States imports a large volume of okra. According to the USDA report, in 2002, United States imported 21,022 metric tones (MT) of okra, in which, 97% of it came from Mexico (kochar@physiology.wisc.edu).

All members of the family Azotobacteraceae can fix, atmospheric nitrogen, Azotobacters are aerobic, mainly soil- dwelling organisms with allniquic array of metabolic capabilities in addition to nitrogen fixation. Different species and strains have various abilities to synthesize alginates, polyhydroxybutyrate (P-OHB) and plant hormones (Elessawy *et al.*, 1984; Gonzalez *et al.*, 1986 and Horan *et al.*, 1983).

Developments in molecular biology and methods available for genetic manipulation in Azotobacters have made possible the genetic analysis of nitrogen fixation and related aspects of nitrogen metabolism. Chromosome mobilization mediated by conjugative plasmids and transformation occurs in *A. vinelandii* and *A. beijerrink*.

The frequency of nif gene transfer from wild type *A. vinelandii* to *A. vinelandii* nif mutants is 10⁻⁴ - 10⁻⁵ per recipient using R.68 and 10⁻⁸ using Rp4.

Urea was applied at 50 kg N ha⁻¹ to supply adequate nitrogen to okra. Each of the pots was provided with two drainage holes and a saucer. The treatments were thoroughly mixed with the soil and moistened with water for one week for proper equilibration. The treatments were replicated three times in a completely randomized block design (CRBD) (Oluwatoyinbo *et al.*, (2005). The response of two okra varieties ('White velvet ' and 'NHAE 47-4 ') to fertilization was studied by Majanbu *et al.*, (2005) in northern Nigeria using four rates of nitrogen (0, 25, 50 and 100 kg ha⁻¹). Nitrogen application significantly increased green pod yield, pod diameter, number of fruits per plant, number of seeds per pod and pod weight. The two varieties responded to nitrogen application differentially with respect to green pod yield. For optimum green pod yield of 'White velvet ' 35 kg N ha⁻¹ is suggested while for variety 'NHAE 47-4 ', N fertilization can be increased to 70 kg ha⁻¹.

The aims of the present investigation were:-

1- Studying the role of *Azotobacter vinelandii* to reduce or eliminate the harmful effects of salinity cucumber and Okra plants. 2- To improve the potentially of the existing isolates of *Azotobacter vinelandii*, and 3- evaluating the enhancement of salt-tolerance of cucumber and Okra plants.

MATERIALS AND METHODS

This investigation was carried out at the Genetics Dept. Faculty of Agriculture, Minia University and a field experiment was carried out at Sakha Agriculture research station during the two successive summer seasons of 2004 and 2005.

I- Laboratory experiments:

A- Strains:-

1- *Azotobacter vinelandii* strain which was isolated in Genetics laboratory (Abdel-Rahem *et al.*, 1995).

- 2- *Halobacterium sp.* Strain isolated from sea water as described by Dakhly (1993).
- 3- The members of this genus are able to grow over a wide salinity range from 10 to 0% total salts (Mullakhanbhal and Larsen, 1975). This isolate was used as a donor parent.

B- Media:-

- 1- Complete medium (CM), was used for *Azotobacter vinelandii* culturing (Strandberg and Wilson, 1968).
- 2- Nutrient agar medium was used to grow the isolate of *Halobacterium* (Dakhly 1993).

c. DNA extraction:-

DNA from strain (Halophilic bacteria) was isolated and purified according to Ausubel *et al.*, (1987).

D. Transformation procedure:-

According to Rifaat *et al.*, (1974)

Inocula preparation:-

1- Free cells inoculums:-

The most efficient *Azotobacter vinelandii* transformants were propagated singly in conical flasks 100 ml containing 500 ml. of the nutrient both medium inocula with a loop-full of 48 hours old culture. For preparation of heavy free call inoculate of field experiments, the efficient *Azotobacter vinelandii* transformant isolates were propagated singly on nutrient agar medium. The suspension was used for seedling inoculation. The heavy microbial cell inocula contained from 10⁸ to 10⁹ viable cells per ml. as determined by the plate count method.

2- Immobilized cells inoculums (capsules):-

An appropriate volume of the active *Azotobacter vinelandii* transformants free cell suspension was mixed with an equal volume of a sterilized cool sodium alginate (50% w/v). The final alginate concentration upon dilution with the active free-cells suspension was 2.5% (w/v). The mixture was extruded through a sterile. Pasteur pipette into a sterile 3% CaCl₂ (w/v). Beads of approximately 2 mm diameter were formed and were hardened in 3% CaCl₂ solution for 2 hours before washing.

The beads were washed with served rinses of sterile distilled water. Before use, beads were blotted dry on filter paper. Viable cell counts in the beads ranged from 10⁷ to 10⁸/g beads. The prepared heavy free cell suspensions of *Azotobacter* microorganisms were used to prepare heavy sodium alginate immobilized cell inocula as described above for field experiments. The obtained alginate capsules were used as seedling inocula. The viable cell counts in the beads before use were 10⁸ – 10⁹ gm. beads.

II- Field experiments:

Two vegetable crops i.e. cucumber (hybrid prince) and Okra cv. Eskandrani were used.

Three transformants of *Azotobacter vinelandii* addition to wild type strain were used for plant inoculation. Three rates of nitrogen fertilizers i.e. 0.0, 150, and 300 kg of ammonium sulphate were used as sources for chemical nitrogen fertilizer. Finally, two methods of inoculation: used

suspension for seed inoculation or put a capsule of isolates and transformant beside the plants.

The seeds of both cucumber and okra plants were inoculated with liquid transformants before sowing. The sowing data was at April 16th and April 18th in both seasons for cucumber and okra respectively.

The cucumber seeds were sowing on ridges 3 m long and 1 m width, while the distance between hills was 30 cm, while, the distance between hills was 60 cm for Okra plants.

The RCBD (randomized complete block design with three replicates) were used. Whereas, the three nitrogen rates were distributed in main plots and methods of inoculation (suspension or capsules) in subplots the three transformants addition to wild type strain and control plants were distributed in sub-sub plots.

At full blooming stage, ten plants were randomly taken from each experimental unit and the number of fruits/plant, weight of fruit/plant, stem lengths (cm) and number of leaves/plants were measured.

Statistical analysis:

Data were subjected statistically to analysis of variance according to Streel and Torric (1960). Least significant difference (LSD) was calculated to compare between treatment means others than the control.

RESULTS

Number of recipient cells/ml and number of transformants as well as the percentages of transformants after 5 days of incubation at 30°C were presented in Table (1).

Table (1): Number and percentage of *Azotobacter vinelandii* transformants grown on complete media supplemented with 10 or 15% of NaCl.

Treatments	12 hrs		24 hrs	
	10%NaCl	15%NaCl	10%NaCl	15%NaCl
No. control (recipient cells)	*(1)122000 100%	122000 100%	122000 100%	122000 100%
On complete media %				
No. control (recipient cells)	*(2)0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00
On selective media %				
No. transformants %	32.00 00.26x10 ⁻³	4.0 0.32x10 ⁻⁶	40.0 0.33x10 ⁻³	6.0 0.19x10 ⁻⁶

*(1)-*Azotobacter vinelandii* (recipient cells) when grown on complete media without salts.

*(2)-*Azotobacter vinelandii* (recipient cells) when grown on complete media with salts (10 or 15% NaCl).

Data in Table (1) show that the extracts DNA from *Bacillus spp* gave the NaCl sensitive cells of *Azotobacter vinelandii* the ability to be salt tolerant. It can be observed also that percentages of transformants were 260x10⁻⁶ on 10% NaCl and 32x10⁻⁶ on 15% at 12 hours but 330 x 10⁻⁶ on 105 NaCl and 49x10⁻⁶ on 15% at 24 hours. In this respect, Gabor (1965) Orgara and Duncan (1973) Heumann and Springer (1977) and Abdel Halim and Ali

(1990) studied transformation of "OSm" genes from *Bacillus spp.* To *R. leguminosarum* and *R. lupine*. They found that the mean frequency of transformants was 21×10^{-5} for *R. leguminosarum* while it was 15×10^{-5} for *R. lupine*.

Field characters (cucumber):

1-Number o fruits/plants:-

The data presented in Table (2) showed that there is increasing values of the plants inoculated with four transformants of *Azotobacter vinelandii* in number of fruits/plant compared with uninoculated plants in both seasons. Two methods of inoculation differed significantly each from other in both seasons. However, liquid showed significantly higher values compared to the capsules method. Increasing N-rate increased number of fruits/plant. The yield increases, possible earlier maturity, and a higher total N in the tops may have been stimulated by bacterium produced phytohormones Tien *et al.*, (1979) which suggested improve plant growth by their direct effects on metabolic processes. The interactions among tree treatments showed significant effects on this trait.

2- Weight of fruits:-

Weight of total fruits/plant (Table 3) was also increased significantly after inoculation with *Azotobacter* transformants compared with (control) plants (without nitrogen fertilizers). T2 was the best transformant which showed the highest values of weight of total fruits/ plant in both seasons. Also, weight of fruits/plant was increased significantly as rate of chemical nitrogen increased as shown in Table (3). The interaction between chemical nitrogen and methods of inoculation were significant in first season but non-significant in second season for this trait. Also, the interaction between transformants and nitrogen closes were significant in both season for weight of fruits/plant.

The interaction between chemical nitrogen, methods of inoculation and transformants were significant in both seasons. This is in agreement with the results obtained by Tien *et al.*, (1979) suggested improve plant growth by their direct effects on metabolic processes. The yield increases, possible earlier maturity, and a higher total N may have been stimulated by bacterium produced phytohormones.

3- Growth characters of cucumber (steam length after 60 days and number of leaves after 45 and 60 days)

Data presented in Table (4) showed that both of stem length and leaves number/plant of cucumber plant were significant affected by both nitrogen rates and inoculation with different transformants of *Azotobacter* while the methods of inoculation had insignificant. Cucumber plant length and number of leaves were significantly increased with inoculation by transformants. T4 was the best transformant showed the highest plant (137.3 ± 4.9 and 161.6 ± 7.84) in the first and second seasons, respectively. The number of leaves were increase after inoculation with T4 (61.17 ± 2.14 and 58.78 ± 14.39) in the first and second seasons respectively. These results were in the same trend with those obtained by Barea and Brown (1974) El-Shehaby (1981).

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T2-3

T4

b-Okra:

1- Growth characters:-

Data presented in Table (5) showed that the inoculation Okra plants with transformants of *Azotobacter vinelandii* increased plant length compared with uninoculated plants. Chemical nitrogen rates were different significantly in plant length. Also, data presented in Table (3) showed that number of levels of Okra plants was affected by inoculation with *Azotobacter vinelandii* transformants in both seasons. On the other hand, the chemical fertilizer rates showed significant effect on these traits the interaction effects showed significant differences on these traits. The highest values of these traits were obtained with fertilized okra plants with highest rate of fertilizer (300 kg N/ed.) and inoculation these plants with (T1). These results are in partial agreement with those obtained by Osman *et al.*, (1990) that increasing N levels had increased leave weights.

2-Yield and yield components:-

Data presented in Table (6) showed that the inoculation process of okra plant with *A. vinelandii* transformants had significant effects on number of fruits/plant and weight of fruits/plant in both season. On the other hand the high rates of nitrogen fertilizer showed significant increased in number of fruits/ plant and weight of fruits/plant in both seasons compared to the lower rate as shown in Table (3).

Regarding to the interaction, effects data show that significant differences, the highest number of fruits/plant (974.7 ± 58.40 and 755.3 ± 26.10) were obtained offer inoculation with (T1) in the first and second season respectively and fertilization with 300 kg N/Fed. of chemical fertilizer (Table 7). These results are in similar with those obtained by Foly *et al.*, (2002), Dakhly and Abdel-Mageed, (1997) and Dakhly (1993).

Prabhjeet *et al.*, (1994) inoculated Rape cv. ISN-129 grown at New Delhi in 1988/89 with *A. chroococcum* by coating the seeds with bacteria-containing peat and was given 0, 20 or 40 kg N/ha. Both inoculation and applied N increased seed yield. The response to *A. chroococcum* was greatest in the absence of N fertilizer, while the highest overall seed yield was given by 40 kg N plus inoculation. The yield increase in response to inoculation was attributed to a greater number of primary branches and pods, associated with a higher LAI, particularly at the pod-filling stage, and a faster CGR. Yield component data is tabulated.

DISCUSSION

The major problem facing the farmer is the high cost of chemical fertilizers. Furthermore, chemical fertilizer production and utilization are considered to be an air, soil and water polluting agent. Therefore, the utilization of transformants, biostimulants and biofertilizers are considered today by many scientists as a promising alternative, particularly for developing countries. The importance of use of bio-manure or mineral fertilizers in tropical agriculture and in increasing world food production had been thoroughly discussed (Abad *et al.*, 1997).

T5

t7

In most cases, single applications of these organic manure or mineral fertilizers are carried out (Akanbi and Togun, 2002). There is little attention on combine effect of organic and mineral fertilizers. The little available information on the crop response to joint application of chemical fertilizer and organic manure centered on the agronomy of production (Akanbi and Togun, 2002). With the current move towards increased food production in Egypt, it has become necessary to study how yield of okra is affected by time of application of organic and inorganic fertilizers so as to allow a good economic comparison between the two fertilizer types and their time of application also, method o application of biofertilizers.

Oluwatoyinbo *et al.*, (2005) concluded that for optimum performance of okra, on acid soil application of lime and phosphorus fertilizer at 500 kg ha¹ and 10 kg ha¹, respectively is beneficial. They added that the presence of lime ameliorates soil acidity and adequate lime reduces the amount of fertilizer phosphorus required for good crop growth and fruit yield. This, it does through its ability to neutralize soil acidity and the subsequent effects of increasing available P and Ca.

Akanbi *et al.*, (2006) concluded that based on the yield response and economic indicators, it pays better to apply combination of 2 ton /ha compost + 30kg. N /ha for okra fertilization in the humid forest zone of Nigeria. This treatment produced a favourable 1. 8:1 benefit: cost ratio and had an increased in net returns of 59 and 47% above the 4t. /ha compost and use of recommended NPK mineral fertilizer treatment, respectively.

Finally, the optimum conditions for Okra and cucumber need more extensive studies in next ten years. The efficiency of the conjunctive application of the organic and inorganic fertilizer could be improved by split application at planting and 3 weeks after planting.

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استجابة الخيار والبامية للتطبيق المشترك من متحولات الازوتوباكتر والسماذ النترجيني الكيماوى

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أجرى هذا البحث فى معمل الوراثة الميكروبية بقسم الوراثة - كلية الزراعة - جامعة المنيا وتجربة حقلية أجريت فى مزرعة محطة البحوث الزراعية بسخا خلال الموسمين الصيفيين الناجحين ٢٠٠٤ ، ٢٠٠٥ . استخلص DNA من البكتريا الباسيلس (التى تحتوى على ٢٠ % كلوريد صوديوم) المستخدم فى الحصول على عزلة لاستخدامها فى التحول الوارثى لعزلات حساسة من الازوتوباكتر فلندباى (طراز برى) الطراز البرى والتحولات تستخدم فى تلقيح أصناف الخيار (هجين برنس) وصنف (أسكندرانى) من البامية . تشير النتائج أن أعلى تكرارات متحولات (١٠x٣٣٠^{-٦}) قد حصل عليها من ١٠ % كلوريد صوديوم على ساعة تحصيل . النتائج تدل على وجود اختلافات معنوية فى صفات النمو بين النباتات الملقحة بالسلالات المختبرة عندما قورنت الكنترول (الغير ملقحة) أى النباتات الملقحة بالطراز البرى . أيضاً أحسن متحول كان المتحول رقم ٤ حيث أعطيت أعلى بمتوسط فى زيادة المحصول مع كلا المحصولين . النتائج أظهرت اختلافات جوهرية بين الطريقتين المستخدمتين فى التلقيح (السائلة والكبسولات) فى تأثيرهما على كلا المحصولين والاستجابة لهم . وكانت أحسن طريقة فى التلقيح هى السائلة حيث أدت إلى زيادة معنوية فى أغلب صفات النمو والصفات المحصولية وفى النهاية فإن الدراسة الحالية توصى (أهمية التلقيح لمحاصيل الخضر بالمتحولات من الازوتوباكتر فلندباى وذلك لتحسين النمو والمحصول

Table (2): Effects of Azotobacter transformants and chemical nitrogen rates on growth characters and yield (gm/plant) of cucumber seasons 2004 and 2005.

N	M	S	No. of fruits/plant		Weight of fruits		Stem length (cm)		No. of leaves/plant		Stem length (60d)		No. of leaves/plant(60d)	
			2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
N 1	1	1	285.9	256.4	29.04	21.91	71.67	99.63	26.42	32.54	156.3	176.1	73.33	66.67
N 2	1	1	177.3	216.5	18.09	18.32	64.71	84.00	24.21	24.38	125.1	159.9	45.58	48.17
N 0	1	1	133.9	188.0	13.27	15.85	71.08	75.38	20.88	19.45	111.1	128.1	42.96	39.50
F test			**	**	**	**	n.s	**	**	**	**	**	**	**
L.S.D. 0.05			19.26	24.48	1.99	1.60	-	2.84	1.90	1.23	3.6	14.3	3.84	3.84
1	M 1	1	208.1	218.2	20.86	18.79	73.72	93.00	24.75	25.31	130.6	155.6	52.89	50.39
1	M 2	1	189.9	222.4	19.41	18.61	64.58	79.67	22.92	25.60	131.0	153.9	55.03	52.50
F test			**	n.s	**	n.s	*	**	*	n.s	n.s	n.s	n.s	n.s
1	1	S 1	189.3	212.4	19.91	18.80	69.56	84.67	22.56	24.58	128.8	146.7	48.06	47.61
1	1	S 2	187.6	206.5	20.82	18.89	68.39	86.33	24.17	25.19	125.1	153.6	51.72	48.83
1	1	S 3	204.1	234.8	19.65	18.86	65.56	86.11	22.94	24.33	132.0	157.1	54.89	50.56
1	1	S 4	215.1	227.5	20.15	18.24	73.11	88.22	25.67	27.71	137.3	161.6	61.17	58.78
F test			**	**	n.s	n.s	n.s	n.s	**	**	**	**	**	**
L.S.D. 0.05			9.99	9.38	-	-	-	-	1.50	1.52	4.4	7.84	2.14	4.34

N1, N2, N3 are nitrogen levels; S1, S2, S3, S4 are Azotobacter transformants; M1 and M2 are inoculation methods

Table (3): Effects of Azotobacter transformants and chemical nitrogen rates on growth characters and yield (gm/plant) of Okra seasons 2004 and 2005.

N	M	S	No. of fruits/plant		Weight of fruits		No. of leaves/plant (45d)		No. of leaves/plant (60d)		Stem length (45 d)		Stem length (60 d)	
			2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
N 1	1	1	820.8	662.7	7.627	6.297	25.63	25.54	17.50	19.00	94.83	93.83	129.8	125.9
N 2	1	1	691.9	599.9	6.454	5.628	25.42	23.50	16.63	14.92	93.33	94.21	132.8	135.4
N 0	1	1	643.0	602.6	5.953	5.419	17.40	15.17	23.29	16.38	80.54	77.04	110.9	111.7
F test			**	**	**	**	**	**	**	**	**	**	**	**
L.S.D. 0.05			29.6	36.6	0.506	0.290	0.99	1.64	3.43	3.14	2.28	5.69	6.52	5.15
1	M 1	1	736.4	634.9	7.046	6.028	23.25	22.53	21.72	19.83	94.67	91.83	128.9	123.2
1	M 2	1	700.8	608.6	6.310	5.535	22.38	20.28	16.56	13.69	84.47	84.89	120.1	125.5
F test			**	n.s	**	**	n.s	n.s	**	**	**	**	**	n.s
1	1	S 1	770.8	672.2	7.057	6.079	21.22	21.06	22.39	17.39	90.78	86.28	123.6	123.3
1	1	S 2	715.3	620.9	6.576	5.839	22.06	20.00	18.56	17.28	95.72	93.17	126.4	123.5
1	1	S 3	688.4	600.1	6.427	5.530	22.78	20.17	17.50	15.94	84.44	84.28	123.1	123.5
1	1	S 4	699.7	593.8	6.651	5.677	25.19	24.39	18.11	16.44	87.33	89.72	124.9	126.7
F test			**	**	**	**	*	**	**	n.s	**	**	n.s	n.s
L.S.D. 0.05			23.9	30.6	0.300	0.283	2.13	2.37	1.80	-	4.75	4.92	-	-

N1, N2, N3 are nitrogen levels; S1, S2, S3, S4 are Azotobacter transformants; M1 and M2 are inoculation methods

Table (4): Effects of *Azotobacter* transformants and chemical nitrogen rates on growth characters and yield (gm/plant) of cucumber seasons 2004 and 2005.

N	M	S	No. of fruits/plant		Weight of fruits		No. of leaves/plant (45d)		No. of leaves/plant (60d)		Stem length (45 d)		Stem length (60 d)	
			2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
N 1	M 1	1	290.5	267.9	29.49	22.94	78.50	102.92	27.42	35.08	158.0	172.3	69.75	66.17
N 1	M 2	1	281.3	244.9	28.58	20.88	64.83	96.33	25.42	30.00	154.5	180.0	76.92	67.17
N 2	M 1	1	191.6	206.1	19.36	18.26	60.33	91.08	25.33	21.17	123.7	171.9	45.67	45.75
N 2	M 2	1	162.9	226.8	16.82	18.38	69.08	76.92	23.08	27.58	126.5	147.9	45.50	50.58
N 0	M 1	1	142.4	180.5	13.72	15.16	82.33	85.00	21.50	19.68	110.2	122.5	43.25	39.25
N 0	M 2	1	125.4	195.5	12.83	16.57	59.83	65.75	20.25	19.21	112.0	133.8	42.67	39.75
F test			n.s	**	n.s	n.s	*	n.s	n.s	**	n.s	**	*	n.s
L.S.D. 0.05			-	13.2	-	-	14.23	-	-	3.55	-	14.53	4.60	-
N 1	M 1	S 1	258.1	245.6	27.98	23.84	65.33	96.67	23.17	31.83	148.8	166.7	61.17	59.00
N 1	M 2	S 2	250.5	244.7	29.95	21.71	71.00	95.00	27.33	33.50	156.0	177.5	63.00	57.50
N 1	M 1	S 3	301.7	259.9	28.10	21.08	68.33	96.83	25.33	29.00	152.7	176.2	79.00	68.50
N 1	M 2	S 4	333.3	275.6	30.11	21.00	82.00	110.00	29.83	35.83	167.5	184.2	90.17	81.67
N 2	M 1	S 1	174.5	210.0	17.90	17.60	70.00	85.50	24.83	23.83	127.3	145.8	42.50	44.67
N 2	M 2	S 2	180.0	200.5	18.73	19.27	68.17	89.00	24.50	23.50	113.7	145.8	49.33	49.33
N 2	M 1	S 3	181.8	234.2	18.53	17.98	54.00	78.50	21.67	23.67	132.7	172.5	45.17	46.83
N 2	M 2	S 4	172.3	221.2	17.20	18.43	66.67	83.00	25.83	26.50	126.7	166.5	45.33	51.83
N 3	M 1	S 1	135.3	181.6	13.85	14.96	73.33	71.83	19.67	18.08	110.2	127.5	40.50	39.17
N 3	M 2	S 2	132.3	174.4	13.78	15.69	66.00	75.00	20.67	18.58	105.7	128.3	42.83	39.67
N 3	M 1	S 3	128.8	210.2	12.32	17.52	74.33	83.00	21.83	20.33	110.7	122.5	40.50	36.33
N 3	M 2	S 4	139.5	185.7	13.13	15.29	70.67	71.67	21.33	20.78	117.8	134.2	48.00	42.83
F test			**	*	*	n.s	**	**	**	*	**	n.s	**	**
L.S.D. 0.05			17.31	16.2	1.54	-	9.46	5.02	2.53	2.62	8.93	-	3.72	7.60
1	M 1	S 1	202.4	200.0	21.42	18.49	74.78	91.56	23.00	24.67	129.8	149.4	48.33	47.11
1	M 1	S 2	199.8	213.2	20.76	20.30	73.89	91.33	25.89	22.83	130.8	157.7	47.89	46.00
1	M 1	S 3	221.1	226.3	20.75	18.66	67.78	92.78	22.44	25.00	127.6	155.2	49.67	47.56
1	M 1	S 4	209.3	233.2	20.49	17.70	78.44	96.33	27.67	28.74	134.3	159.9	65.67	60.89
1	M 2	S 1	176.2	224.8	18.40	19.11	64.33	77.78	22.11	24.50	127.8	143.9	47.78	48.11
1	M 2	S 2	175.4	199.8	20.89	17.48	62.89	81.33	22.44	27.56	119.4	149.4	55.56	51.67
1	M 2	S 3	187.1	243.2	18.55	19.06	63.33	79.44	23.44	23.67	136.4	158.9	60.11	53.56
1	M 2	S 4	220.8	221.7	19.80	18.78	67.78	89.11	23.67	26.67	140.3	163.3	56.67	56.67
F test			**	**	n.s	n.s	n.s	n.s	**	**	**	**	**	**
L.S.D. 0.05			9.99	9.38	-	-	-	-	1.50	1.52	4.4	7.84	2.14	4.34

N1, N2, N3 are nitrogen levels; S1, S2, S3, S4 are *Azotobacter* transformants; M1 and M2 are inoculation methods

Table (5): Effects of *Azotobacter* transformants and chemical nitrogen rates on growth characters and yield (gm/plant) of Okra seasons 2004 and 2005.

N	M	S	No. of fruits/plant		Weight of fruits		No. of leaves/plant (45d)		No. of leaves/plant (60d)		Stem length (45 d)		Stem length (60 d)	
			2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
N 1	M 1	1	768.8	652.4	7.429	6.183	25.92	26.50	19.08	22.00	96.83	95.00	131.6	126.8
N 1	M 2	1	872.8	672.9	7.825	6.410	25.33	24.58	15.92	16.00	92.83	92.67	128.1	125.0
N 2	M 1	1	753.8	582.3	7.341	6.133	24.58	25.33	16.17	17.83	97.58	95.83	140.8	131.9
N 2	M 2	1	629.9	634.7	5.567	5.123	26.25	21.67	17.08	12.00	89.08	92.58	124.7	138.9
N 0	M 1	1	686.4	570.6	6.368	5.767	19.25	15.75	29.92	19.67	71.50	84.67	114.3	110.8
N 0	M 2	1	599.7		5.538	5.072	15.54	14.58	16.67	13.08		69.42	107.6	112.6
F test			**	n.s	**	**	*	n.s	**	n.s	**	**	n.s	n.s
L.S.D. 0.05			32.1	-	0.503	0.484	2.66	-	3.70	-	4.18	3.44	-	-
N 1	M 1	S 1	920.7	729.0	8.492	6.48	22.33	24.17	25.33	21.50	90.83	85.83	134.5	126.7
N 1	M 2	S 2	784.5	633.0	7.295	6.463	24.50	23.50	16.83	20.17	99.33	98.33	125.3	123.5
N 1	M 1	S 3	801.2	636.8	7.437	5.985	26.83	25.00	14.50	17.76	94.00	92.00	127.3	126.3
N 1	M 2	S 4	776.8	651.8	7.285	6.330	28.83	29.50	13.33	16.67	95.17	99.17	132.2	127.2
N 2	M 1	S 1	704.8	679.2	6.665	6.483	24.00	22.17	18.83	14.67	101.50	96.67	123.0	126.2
N 2	M 2	S 2	735.0	631.2	6.413	5.63	22.17	22.17	15.50	15.50	92.76	95.00	133.5	132.7
N 2	M 1	S 3	629.2	536.0	6.073	5.125	25.50	21.83	18.17	14.83	86.83	88.17	140.7	140.0
N 2	M 2	S 4	698.5	553.3	6.663	5.238	30.00	27.83	14.00	14.67	92.33	97.00	133.8	142.8
N 3	M 1	S 1	687.0	608.3	6.015	5.347	17.33	16.83	23.00	16.00	80.00	76.33	113.3	117.0
N 3	M 2	S 2	626.5	598.5	6.018	5.388	19.50	14.33	23.33	16.17	95.17	86.17	120.5	115.7
N 3	M 1	S 3	634.8	627.3	5.772	5.480	16.00	12.67	19.83	15.33	72.50	72.67	101.2	104.2
N 3	M 2	S 4	623.8	576.3	6.005	5.462	16.75	15.83	27.00	18.00	74.50	73.00	108.7	110.0
F test			**	**	**	**	**	n-s	**	n-s	**	*	**	*
L.S.D. 0.05			23.4	53.0	0.520	0.491	3.09	-	3.12	-	8.23	5.51	4.74	9.80
1	M 1	S 1	786.3	685.0	7.421	6.223	22.33	23.67	25.67	20.89	97.89	91.67	128.6	126.8
1	M 1	S 2	711.6	633.0	6.524	5.992	24.22	22.78	22.89	20.33	102.56	99.56	124.1	120.9
1	M 1	S 3	724.6	627.0	7.023	5.967	21.11	18.89	18.11	19.67	83.89	84.56	128.4	119.9
1	M 1	S 4	723.0	594.4	7.214	5.928	25.33	24.78	20.22	18.44	94.33	91.56	134.4	125.2
1	M 2	S 1	755.3	659.3	6.693	5.936	20.11	18.44	19.11	13.89	83.67	80.89	118.7	119.8
1	M 2	S 2	719.1	608.8	6.627	5.686	19.89	17.22	14.22	14.22	88.89	86.78	128.8	127.0
1	M 2	S 3	652.2	573.1	5.831	5.093	24.44	21.44	16.89	12.22	85.00	84.00	117.7	127.1
1	M 2	S 4	676.4	593.2	6.088	5.426	25.06	24.00	16.00	14.44	80.33	87.89	115.3	128.1
F test			*	n.s	**	n.s	**	**	**	n.s	**	*	**	n.s
L.S.D. 0.05			33.7	-	0.425	-	3.01	2.37	2.55	-	6.72	6.45	7.44	-

Table (6): Effects of *Azotobacter* transformants and chemical nitrogen rates on growth characters and yield (gm/plant) of cucumber seasons 2004 and 2005.

N	M	S	No. of fruits/plant		Weight of fruits/plant		Stem length (45 d)		No. of leaves/plant (45d)		Stem length (60 d)		No. of leaves/plant (60d)	
			2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
N 1	M 1	S 1	284.5	241.6	29.57	24.68	73.67	100.33	26.33	34.33	153.7	168.3	85.00	58.00
N 1	M 1	S 2	261.0	255.4	29.60	21.53	75.00	101.00	24.67	33.00	170.0	183.3	57.00	55.00
N 1	M 1	S 3	311.7	265.0	29.81	21.40	17.33	98.33	24.33	32.00	146.7	162.3	70.00	65.00
N 1	M 1	S 4	304.7	309.7	28.98	24.16	94.00	112.00	34.33	14.00	161.7	175.0	94.00	86.67
N 1	M 2	S 1	231.7	249.6	26.40	23.00	57.00	93.00	20.00	29.33	144.0	165.0	64.33	60.00
N 1	M 2	S 2	240.0	233.9	30.30	21.89	67.00	89.00	30.00	34.00	142.0	171.7	69.00	60.00
N 1	M 2	S 3	291.7	254.8	26.40	20.76	65.33	95.33	26.33	26.00	158.7	190.0	88.00	72.00
N 1	M 2	S 4	362.0	241.4	31.23	17.58	70.00	108.00	25.33	30.67	173.3	193.3	86.33	76.67
N 2	M 1	S 1	194.3	195.0	20.60	17.04	65.33	85.00	23.33	21.00	128.0	160.0	44.00	43.33
N 2	M 1	S 2	200.7	202.1	20.13	21.49	66.33	97.00	30.00	18.33	115.7	169.7	46.67	44.67
N 2	M 1	S 3	189.0	220.1	17.94	18.82	44.67	92.00	20.33	21.33	127.0	180.0	39.00	41.00
N 2	M 1	S 4	182.3	207.3	18.77	15.69	65.00	90.33	27.67	24.00	124.0	178.0	53.00	54.00
N 2	M 2	S 1	155.3	225.0	15.20	18.17	74.67	86.00	26.33	26.67	126.7	131.7	41.00	46.00
N 2	M 2	S 2	159.3	198.9	17.33	17.04	70.00	81.00	19.00	28.67	111.7	140.0	52.00	54.00
N 2	M 2	S 3	174.7	248.2	19.11	17.14	63.33	65.00	23.00	26.00	138.3	165.0	51.33	52.67
N 2	M 2	S 4	162.3	235.0	15.63	21.18	68.33	75.67	24.00	29.00	129.3	155.0	37.67	49.67
N B	M 1	S 1	128.3	163.4	14.10	13.75	85.33	89.33	19.33	18.67	107.7	120.0	43.00	40.00
N B	M 1	S 2	137.7	182.1	12.53	17.87	80.33	76.00	23.00	17.17	106.7	120.0	40.00	38.33
N B	M 1	S 3	162.5	193.8	14.51	18.75	87.33	88.00	22.67	21.67	109.0	123.3	40.00	36.67
N B	M 1	S 4	141.0	182.7	13.73	13.25	76.33	86.67	21.00	21.23	117.3	126.7	50.00	24.00
N B	M 2	S 1	141.7	199.8	13.60	16.16	61.33	54.33	20.00	17.50	112.7	135.0	38.00	38.33
N B	M 2	S 2	127.0	166.7	15.03	13.51	51.67	74.00	18.33	20.00	104.7	136.7	45.67	41.00
N B	M 2	S 3	95.0	226.6	10.14	19.29	61.33	78.00	21.00	19.00	112.3	121.7	41.00	36.00
N B	M 2	S 4	138.0	188.8	12.53	17.33	65.00	56.67	21.67	20.33	118.3	141.7	46.00	43.67
F test			**	*	**	**	n.s	**	**	n.s	*	n.s	**	n.s
L.S.D. 0.05			24.5	32.0	2.17	3.36	-	11.34	3.56	-	12.1	-	5.25	-

N1, N2, N3 are nitrogen levels; S1, S2, S3, S4 are *Azotobacter* transformants; M1 and M2 are inoculation methods

Table (7): Effects of *Azotobacter* transformants and chemical nitrogen rates on growth characters and yield (gm/plant) of Okra seasons 2004 and 2005.

N	M	S	No. of fruits/plant		Weight of fruits/plant		Stem length (45 d)		No. of leaves/plant (45d)		Stem length (60 d)		No. of leaves/plant (60d)	
			2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
N 1	M 1	S 1	866.7	755.3	8.477	6.493	27.00	26.67	27.33	26.33	98.67	91.67	144.7	139.7
N 1	M 1	S 2	632.3	635.3	5.970	6.140	27.67	27.00	22.00	22.33	102.00	103.33	117.3	121.7
N 1	M 1	S 3	796.0	610.3	7.700	6.043	20.67	23.33	13.33	23.00	93.00	90.00	133.7	123.3
N 1	M 1	S 4	780.3	608.7	7.570	6.057	28.33	29.00	13.67	16.33	39.67	95.00	130.7	122.7
N 1	M 2	S 1	974.7	702.7	5.507	6.323	17.67	21.67	23.33	16.67	83.00	80.00	124.3	113.7
N 1	M 2	S 2	936.7	630.7	8.620	6.787	21.33	20.00	11.67	18.00	96.67	93.33	133.3	125.3
N 1	M 2	S 3	806.3	663.3	7.173	5.927	33.00	26.67	15.67	12.33	95.00	94.00	121.0	129.3
N 1	M 2	S 4	773.3	695.0	7.000	6.603	29.33	33.00	13.00	17.00	96.67	103.33	133.7	131.7
N 2	M 1	S 1	747.3	669.0	7.357	6.753	22.67	25.00	18.33	16.33	102.67	96.67	117.7	114.0
N 2	M 1	S 2	327.3	645.0	7.470	6.207	22.33	26.00	15.00	19.33	107.33	102.00	133.0	126.0
N 2	M 1	S 3	707.0	564.0	7.323	5.380	22.67	21.00	16.33	18.00	73.67	81.67	150.0	139.7
N 2	M 1	S 4	733.7	592.0	7.213	5.740	33.67	29.33	15.00	17.67	106.67	103.00	162.7	148.0
N 2	M 2	S 1	662.3	689.3	5.973	6.213	25.33	19.33	19.33	13.00	100.33	96.67	128.3	138.3
N 2	M 2	S 2	642.7	617.3	5.357	5.123	22.00	18.33	16.00	11.67	78.00	88.00	134.0	139.3
N 2	M 2	S 3	551.3	508.0	4.823	4.420	28.33	22.67	20.00	11.67	100.00	94.67	131.3	140.3
N 2	M 2	S 4	663.3	514.7	6.113	4.737	29.33	26.33	13.00	11.67	78.00	91.00	105.0	137.7
N B	M 1	S 1	745.0	630.7	6.430	5.423	17.33	19.33	31.33	20.00	92.33	86.67	123.3	126.7
N B	M 1	S 2	675.0	618.7	6.133	5.630	22.67	15.33	31.67	19.33	98.33	93.33	122.0	115.0
N B	M 1	S 3	670.7	706.7	6.047	6.027	20.00	12.33	24.67	18.00	85.00	82.00	101.7	96.7
N B	M 1	S 4	655.0	582.7	6.860	5.987	17.00	16.00	32.00	21.33	82.67	76.67	110.0	105.0
N B	M 2	S 1	629.0	586.0	5.600	5.270	17.33	14.33	14.67	12.00	67.67	66.00	103.3	107.3
N B	M 2	S 2	578.0	578.3	5.903	5.147	16.33	13.33	15.00	13.00	92.00	79.00	119.0	116.3
N B	M 2	S 3	599.0	548.0	5.497	4.933	12.00	15.00	15.00	12.67	60.00	63.33	100.7	111.7
N B	M 2	S 4	592.7	570.0	5.150	4.937	16.50	15.67	22.00	14.67	66.33	69.33	107.3	115.0
F test			*	*	**	n.s	**	n.s	n.s	*	**	*	**	**
L.S.D. 0.05			58.4	26.1	0.735	-	1.28	-	-	4.50	11.64	12.04	13.8	16.1

N1, N2, N3 are nitrogen levels; S1, S2, S3, S4 are *Azotobacter* transformants; M1 and M2 are inoculation methods