GROWTH AND YIELD OF ZUCCHINI TYPE SUMMER SQUASH (*CUCURBITA PEPO* L.) FERTILIZED BY COMBINED *AZOTOBACTER CHROOCOCUM* MUTANTS AND MINERAL N-FERTILIZATION

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Abstract

Summer squash (*Cucurbita pepo* L.) zucchini type cv "Eskandarany" grown in reclaimed sandy soil at the Horticulture Research Station, Arab El-Awammer, Assiut Governorate, Egypt was fertilized with nitrogen (0, 30, 60, 90 and 120 units / feddan) from ammonium nitrate (NH_4NO_3) after being either inoculated with one of *Azotobacter chroococum* strains (5 mutants and wild type strain) or without inoculation. *Azotobacter chroococum* mutant strains were induced by N- methyl N- nitro –N-nitrosogaunidine (NTG) treatment for suspension of the wild type cells in Microbial Genetics Lab. at the Department of Genetics, Minia University. Assessment of plant growth, development and yield indicated that application of 120N units/feddan gave the longest stem, greatest number of leaves, female flowers and immature fruits and early and total yield as compared to other sole N applications. Bio-fertilization using *Azotobacter* wild type strain resulted in a significant increment in stem length, number of female and male flowers, number and size of immature fruits and early and total yield under the conditions of N fertilizer application up to 120 units per feddan. Number of leaves and female flowers showed similar result all N fertilization levels but not 120 N units. In general, use of induced *Azotobacter* mutants resulted in an increase in all studied parameters comparing with the use of wild type strain. One of the *Azotobacter* mutant strains (denoted #7) seemed to be the best among the others for enhancing plant growth, development and yielding. This *Azotobacter* mutant strain combined with 90 mineral N units had 5.5% increase over the yield produced with sole 120 N units, thus saving one quarter of the mineral N amount added. It is concluded that combined utilization of *Azotobacter chroococum* and mineral N-fertilizer could enhance productivity of summer squash in new reclaimed sandy soil and mutation could be employed as potential approach to elevate the efficiency of this bacterium species as a bio-fertilizer.

INTRODUCTION

Squash (*Cucurbita pepo* L.) is one of the most popular vegetable crops grown in Egypt. In most monoecious cucurbit plants, the ratio of staminate to pistillate flowers greatly varies when the plants are grown under different environmental conditions, including photoperiod, temperature, nutrient availability, or exogenous treatment with

plant hormones (Lau and Stephenson, 1993, Swiader *et al.*, 1994, Yin and Quinn, 1995).

Biological fertilization by N_2 fixing bacteria has recently received a significant attention in production of crop plants. The efficient use of bio-fertilizers may be affected by different strain groups such as nitrogen fixer and nutrient mobilization microorganisms which help in increasing the availability of minerals and their forms in the composted materials and increase levels of extractable macro- or micronutrients (El-Karamany *et al.*, 2000).

Enhanced productivity of different crop plant in wheat , El-Metwaly, 1998, in pepper (*Capiscum annuum* L.) Abdalla *et al.*, 2001, in Cantaloupe(*Cucumis melo*) Adam *et al.* 2002) as result of bio-fertilizer application has been reported. Abd El-Fattah and Sorial (2000), on summer squash, indicated that bio-fertilizer treatment (Halex2) significantly enhanced the induction of female flowers, which was reflected afterward on the increase of fruit yield.

It is widely documented that a considerable number of bacterial species, mostly those associated with the rhizosphere, are able to exert a beneficial effect on plant growth. These bacteria include strains in the genera *Bacillus, Pseudomonas, Rhizobium* and others have been called 'plant growth regulator promoting rhizobacteria' (PGPR) (Bloemberg and Lugtenberg, 2001). PGPR bacteria can stimulate growth and yield of crop species including potato, radish, tomato, lettuce, beans, cucumber (De Silva *et al.*, 2000). All the monitored activities and formulation properties suggest an effective use of *Bacillus subtilis* as a plant –strengthening agent and/or bio-control of diseases.

Nowadays, bio-fertilizers are considered one of the top biotechnology applications for establishing productive non-polluting organic agriculture (Shehata, and El- Khawas, 2003). It can help to overcome the ecological problems resulting from the loss of plant nutrients and provide sustainable solutions for present and future agricultural practices (Rai, 2006). Completely fermented organic matters resulted in bio-fertilizers which improve the physical properties of soils leading to better aeration and water and nutrient retention capacity.

Free living N-fixing bacteria are associated with roots of many cereal crops and have beneficial effects on crops yield (Neyra and Dobereiner ,1977). A remarkable increase in the plant fresh and dry weight and nitrogen content in plant and soil has been achieved when used either *Azotobacter vinelndii* wild type strain or its mutants

(AbdelRaheem *et al.*, 1995). The present research study investigates nitrogen fixation utilizing *Azotobacter chroococum* induced biochemical mutants aiming to obtain more efficient strains for production of summer squash towards reducing mineral N-application in production of summer squash.

MATERIALS AND METHODS

Laboratory study

Azotobacter chroococum wild type strain was isolated from rhizosphere around roots of the squash plants cultivated in the farm of Agricultural Faculty, Minia University, Mania, Egypt (Dakhly and Abedel-Mageed, 1997). A complete media (CM) was used for *Azotobacter chroococum* culturing (Strandberg and Wilson 1968). Minimal medium (MM) was used to isolate the auxotrophic of *Azotobacter chroococum* (Mchenney and Melton, 1986). All media were autoclaved at 121° C for 20 min.

Cells of *Azotobacter chroococum* were treated with 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg N- methyl N- nitro –N-nitrosogaunidine (NTG) mg /ml of cell suspension for one and two h at 30 C°. *A. chroococum* wild type single colonies were subculture on slants of complete medium and incubated at 30° C for 3 days. One loop from subculture was added to 5 ml sterilized distilled water in a test tube. One ml sample of cell suspension were distributed on sterilized test tubes .The final concentrations of N-methyl N- nitro –N-nitrosogaunidine (NTG) were 0.0, 0.20, 0.40, 0.60, 0.80 and 1.0 mg /ml. The suitable dilutions from each concentration were plated on complete media using six plants for each concentration .The plates were incubated at 30° C for three days and the surviving colonies were counted. Single colonies were tested on MM and CM and incubated at 30° C for 5 days. Bacterial growth was compared on MM and CM and the mutants were selected. The selected mutants were test for their specific single or double requirements by applying Holliday (1960) system.

Field study

Field experiment was carried out during two successive growing seasons at the Farm of Agriculture experiments, Horticulture Research Station, Arab El-awammer, Assiut Governorate, Egypt. Summer squash (*Cucurbita pepo* L.) zucchini type cv "Eskandarany" seeds were sown on Sept.10 and 20 in 2008 and 2009, respectively. Five application rates of nitrogen fertilizer from ammonium nitrate (NH_4NO_3) (0, 30, 60, 90 and 120 units of nitrogen) were studied under conditions of plant inoculation

with either one of five *Azotobacter* mutants, wild type strain and without inoculation. Each N-level was divided into three equal doses, and applied during field preparation then after 20 and 40 days from seed sowing. The treatments were arranged in a split-plot design with three replicates. The N-fertilizer rate was in the main plot and the *Azotobacter* mutants in sub-plot. The plot area was 12.8 m^{22} and consisted of four rows each 4 m long and 0.8 m wide. Plants were spaced 40 cm apart on one side of the ridge. The cultural practices were done in accordance with those advised for summer squash production.

A random sample of three plants from each treatment was used for evaluating main stem length (cm, 45 days after sowing), number of leaves/plant, number of produced female and male flowers, average fruit length and diameter (cm) and average fruit weight (g). During the production season, fruits were harvested at two days intervals, counted and then weighed and number of fruits/plant was recorded. Early yield was determined from the early 4 harvests, whereas the average total yield was recorded during the whole harvesting period (Ton/Feddan). Data were statistically processed following the procedure of analysis of variance (Gomez and Gomez, 1984) and means were compared using "The Least Significant Difference Test" (LSD) at 0.05 probability level.

RESULTS AND DISCUSSION

Laboratory study

Data in Table (1) show the effect of different N- methyl N- nitro –N-nitrosogaunidine concentrations (0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg /ml of cell suspension) for 1 and 2 h at 30° C on *Azotobacter chroococum*. The percentage of wild type survival tended to decline (100, 70, 55, 42, 30 and 19) with increasing concentration of mutagenic agent (0.0, 0. 2, 0.4, 0.6, 0.8 and 1.0 mg /ml) for 1 h exposure time. A sharper decline (100, 60, 40, 18.7.and 0.2 %) was observed in percent survival when subjected to mutagenic agent for two h. For 1 h subjection time, 25 (1.56%) out of 1643 tested colonies proved to be mutants. They were identified as four alanineless, two valeneless, two adenineless argenineless, three histidineless, one threonineless, two methionineless, two prolineless, two treptophanelss and six reucrtant. For 2 h subjection time, 34 (2.34 %) out of 1489 were identified as mutants, three alanineless, three valeneless, two adenineless, two

argenineless, three histidineless, three threonineless, two methionineless, three prolineless, four treptophanelss, three phenylalanineless, three tyrosineless and three revertant mutants. It can be observed that 0.8 and 1.0 mg (NTG)/ ml induced an elevated frequency of mutation whether *Azotobacter chroococum* cells were subjected for 1 or 2 hours. Similar results were found in *Azotobacter* by Bishop *et al* (1980), Abdel-Raheem *et al.* (1995), Dakhly *et al.*, (1998), Kumar and Norula (1999), Mahmoud, (2000) and Hassan *et al.* (2000).

Field study

The effect of mineral nitrogen fertilizer and bio-fertilization with *Azotobacter chroococum* wild and mutant strains on stem length, number of leaves, male and female flower and immature fruits per plant, early and total yield per plant, and fruit length, diameter and weight of zucchini type summer squash cv. "Eskandarany" are presented in Tables (2, 3 and 4). Plants received 30, 60, 90 and 120 units/fed. mineral nitrogen fertilizer had longer stem, increased number of leaves, and produced greater number of female flowers (Table 2) and immature fruits (Table 3) compared with untreated plants (plants grown without mineral or bio-fertilizers, negative control). While we practiced harvest at regular intervals, there were an increase in fruit length, diameter and weight (Table 4). It is noticeable, that squash plants in this study showed a significant increase in number of male flowers at 30 and 60 N units (Table 2) reflecting the overall growth vigor. However, it decreased then stabilized afterwards at 90 and 120 N units as the plant requirements of N were balanced. Availability of male flowers is regarded useful as to provide pollination to set fruit i[illegible] female ones. Indeed those plants produced higher early and total yield (Table 3).

Besides day length and temperature, it is well documented that summer squash plants cv. "Eskandrany" positively respond to availability of N in terms of enhanced growth, female flowers development and fruit yield (Lau and Stephenson, 1993, Swiader *et al.*, 1994, Yin and Quinn, 1995, Abd El-Fattah and Sorial, 2000, Refai and Mohamed, 2009). This is especially true in low fertility medium such as reclaimed soil used in this study (Abd El-Fattah and Sorial (2000). Obviously, the longest stem, greatest number of leaves, female flowers and immature fruits and early and total yield were obtained here when plants were fertilized with 120N units/feddan. In agreement with other studies conducted using zucchini type summer squash cv. "Eskandrany", femininity leading to increased harvested immature fruits is a major

component of fruit early and total yield (Goicoechea *et al.*, 1995, Mohamed, 1996, Noel *et al.*, 1996, Refai and Mohamed, 2009).

Bio-fertilization using *Azotobacter* wild type strain for squash plants that received no mineral N fertilizer resulted in a significant increment in stem length, number of leaves, female flowers and immature fruits, early and total yield of larger fruits (length, diameter and weight) but reduced number of male flowers. Such result existed also for stem length, number of female and male flowers, number and size of immature fruits and early and total yield under the conditions of N fertilizer application up to 120 units per feddan. Number of leaves and female flowers showed similar response under conditions of N fertilization in the range from 30 to 90 N units but not 120 N units per feddan. There were significant differences among the *Azotobacter* bio-fertilizer treatments. With few exceptions, use of induced *Azotobacter* mutants in this study resulted in longer stem, increased number of leaves, female flowers and immature fruits and reduced number of male flowers and elevated early and total yield comparing with the use of wild type strain. Obviously, *Azotobacter* mutant strain 7 seemed to be the best among the other mutants for enhancing summer squash plant growth, development and yielding. Utilization of *Azotobacter* wild strain combined with 60 N and 90 units produced 40.7% and 15.8% reduction, respectively, in fruit yield compared to using sole 120 N units. Worthwhile to mention, that this reduction was narrowed to 15.8% when used *Azotobacter* mutant strain 7 combined with 60 N units. An increase of 5.5% over the yield produced with sole 120 N units was obtained when used *Azotobacter* mutant strain 7 combined with 90 N units. This shows that *Azotobacter* mutant strain 7 can save one quarter of the mineral N amount while realizing higher yielding. Such reduction in mineral N application means saving a quarter of the energy need in N fertilizer industry besides conserving our living environment and reducing negative effects leading to undesirable climatic changes.

In the present study, we partitioned mineral N application to several sequential doses to maximize uptake via reducing its possible leach. Interestingly, however, *Azotobacter* tend to show an enhancing effects on growth, development and fruit yielding of summer squash at relatively high level of mineral N applications. A similar simulative effect for this bacterium on growth and development were previously reported by others (Bochow and Dolej, 1999 and Adam *et al.* (2002)). Such simulative effect may be due to its added N fixation for the growing plants (AbdelRaheem *et al.* (1995). In such regard, *Azotobacter* would be beneficial in terms of reducing further

mineral N application that may not be desired as far as the human health and environment conservation is concerned. However, it is suggested (De Silva *et al.*, 2000, Sudhakar *et al.*, 2000) that the mechanism seem also to be based on hormons due to releasing exogenous bacterial metabolites having precursors of auxin (indole-3-pyruvic acid), or inducers (GA3 fraction) for auxin synthesis. Many other investigators showed that such bacterial inoculation of seeds or roots leads to changes in plant growth which is caused by growth regulating substances especially those of gibberelin, cytokinin and IAA (Goicoechea *et al.*, 1995, Noel *et al.*, 1996). The bacterial bio-fertilizer application might then promote the crop growth by increasing root number and root length. Subsequently, root system can absorb more water and nutrients from soil including the applied N. Thus, N lose hazards to the environment is reduce, especially, in reclaimed/sandy soil.

Overall results of this study suggest that combined utilization of *Azotobacter chroococum* and mineral N-fertilizer could enhance productivity of summer squash in new reclaimed sandy soil. Mutation could be a potential approach to elevate the efficiency of this bacterium species as a bio-fertilizer.

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Table 1. Mutagenic effect of N- methyl N- nitro –N-nitrosogaunidine on *Azotobacter chroococum* wild type.

Exposure time	Mutagen Conc. mg/ml	Survivals		No .of colonies testing	No. of mutants	Mutation frequency %	Requirements of single mutants											
		No.	%				Ala⁻	Val⁻	Ad⁻	Arg⁻	His	Thr⁻	Meth+-	Pro⁻	Trep⁻	Phoala⁻	Tyr⁻	Rever⁻
1 hour	0.00	100000	100	250	0.00	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.20	70000	70.0	300	4.00	1.33	1	0.0	0.0	0.0	1	0.0	0.0	0.0	0.0	0.0	0.0	2
	0.40	55000	55.0	280	3.00	1.07	0.0	1	0.0	0.0	0.0	1	0.0	0.0	1	0.0	0.0	0.0
	0.60	42000	42.0	320	5.00	1.56	0.0	0.0	2	0.0	1	0.0	0.0	0.0	1	0.0	0.0	1
	0.80	30000	30.0	220	7.00	3.18	2	0.0	0.0	1	0.0	0.0	2	1	0.0	0.0	0.0	1
	1.00	19000	19.0	273	6.00	2.20	1	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	0.0	2
	Total			1643	25	1.56	4	2	2	1	3	0.0	2	2	2	0.0	0.0	6
2 hour	0.00	100000	100	270	1.00	0.37	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.20	60000	60.0	279	7.00	2.50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1
	0.40	40000	40.0	245	6.00	2.45	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1	0.0	0.0	0.0
	0.60	18000	18.0	295	8.00	2.71	0.0	0.0	1	0.0	1	1	1	1	1	0.0	0.0	2.0
	0.80	7000	7.0	300	9.00	3.00	1	1	0.0	1	0.0	2	0.0	1	1	1	1	0.0
	1.00	200	0.2	1oo	3.00	3.00	0.0	0.0	1	0.0	1	0.0	0.0	0.0	1	0.0	0.0	0.0
	Total			1489	34	2.34	3	3	2	2	3	3	2	3	4	3	3	3

Table 2. Effect of six of *Azotobacter chroococum* strains combined with mineral N-fertilizer on growth and development of zucchini type summer squash (*Cucurbita pepo* L.) cv "Eskandarany" plants grown in 2008 and 2009 ..

Treatments[2]		Number of leaves		Stem length		Number of Female flower		Number of male flower	
		2008	2009	2008	2009	2008	2009	2008	2009
Nitrogen units (kg/Feddan)	0	21.37	20.47	30.07	29.83	6.93	6.74	12.76	12.36
	30	23.79	23.04	37.61	36.64	8.75	8.44	14.14	13.41
	60	26.25	26.58	43.90	43.06	11.18	10.95	15.11	14.49
	90	28.90	29.36	52.66	52.14	13.64	13.21	15.14	14.30
	120	31.30	30.97	61.54	60.60	15.47	15.36	14.97	14.59
L.S.D. 0.05		0.02	0.07	0.03	0.04	0.26	0.01	0.09	0.11
Azotobacter Strains	Control⁻	25.16	25.00	40.36	39.58	9.84	9.53	15.50	15.01
	Control⁺	25.92	25.51	44.46	43.58	10.55	10.40	14.56	14.04
	3	26.16	25.85	44.96	43.98	10.82	10.70	14.40	13.76
	4	26.40	26.21	45.34	44.74	11.25	11.02	14.30	13.65
	5	26.58	26.45	46.54	45.88	11.55	11.28	14.20	13.52
	6	26.87	26.73	47.02	46.26	11.95	11.60	14.16	13.47
	7	27.16	26.85	47.42	47.16	12.40	12.05	13.86	13.36
L.S.D. 0.05		0.023	0.076	0.071	0.052	0.260	0.009	0.089	0.104
0 Nitrogen units	Control⁻	19.50	19.00	25.00	24.70	5.00	4.75	14.00	13.27
	Control⁺	21.00	20.25	31.00	30.30	6.50	6.25	13.00	12.60
	3	21.30	20.25	30.00	29.60	6.50	6.35	13.00	12.50
	4	21.75	20.75	30.00	30.20	7.25	7.25	12.50	12.00
	5	21.80	20.75	31.00	30.70	7.50	7.35	12.50	12.30
	6	22.00	21.03	31.50	31.00	7.75	7.50	12.30	12.03
	7	22.25	21.25	32.00	32.30	8.00	7.75	12.00	11.80
30 Nitrogen units	Control⁻	22.80	22.00	33.00	31.70	7.00	6.50	15.00	14.50
	Control⁺	23.50	22.25	36.00	34.60	8.00	7.75	14.50	13.80
	3	23.70	22.70	36.60	35.30	8.00	7.90	14.00	13.50
	4	23.75	23.00	36.90	36.00	8.75	8.60	14.00	13.30
	5	24.00	23.50	40.00	39.30	9.00	8.80	14.00	13.00
	6	24.25	23.80	40.30	39.70	10.00	9.50	14.00	13.00
	7	24.50	24.00	40.50	39.90	10.50	10.00	13.50	12.80
60 Nitrogen units	Control⁻	25.25	25.00	39.00	38.50	9.50	9.40	16.50	16.40
	Control⁺	25.80	25.53	43.50	42.70	11.00	10.50	15.00	14.50
	3	26.00	26.50	44.30	43.20	11.00	11.00	15.00	14.30
	4	26.25	26.80	44.70	43.70	11.25	11.00	15.00	14.20
	5	26.33	27.00	44.90	43.90	11.50	11.25	15.00	14.00
	6	26.80	27.75	45.30	44.60	11.50	11.50	15.00	14.00
	7	27.30	27.50	45.60	44.80	12.50	12.00	14.30	14.00
90 Nitrogen units	Control⁻	27.50	28.50	46.80	45.70	12.50	12.00	16.00	15.50
	Control⁺	28.50	29.00	51.50	50.30	13.00	12.50	15.00	14.30
	3	28.80	29.00	52.50	51.30	13.25	13.00	15.00	14.00
	4	29.00	29.50	53.30	52.60	13.50	13.00	15.50	14.30
	5	29.25	29.75	54.50	54.00	14.00	13.50	14.50	14.00
	6	29.50	29.80	54.80	54.20	14.50	14.00	15.00	14.00
	7	29.75	30.00	55.20	56.90	14.75	14.50	15.00	14.00
120 Nitrogen units	Control⁻	30.75	30.50	58.00	57.30	15.20	15.00	16.00	15.40
	Control⁺	30.80	30.50	60.30	60.00	14.25	15.00	15.30	15.00
	3	31.00	30.80	61.40	60.50	15.35	15.25	15.00	14.50
	4	31.24	31.00	61.80	61.20	15.50	15.25	14.50	14.43
	5	31.50	31.25	62.30	61.50	15.75	15.50	15.00	14.30
	6	31.80	31.25	63.20	61.80	16.00	15.50	14.50	14.30
	7	32.00	31.50	63.80	61.90	16.25	16.00	14.50	14.20
L.S.D. 0.05		0.05	0.17	0.16	0.12	0.58	0.02	0.20	0.23

[1] Control⁻ and Control⁺ = without inoculation or inoculated with *Azotobacter* wild strains, 3 to 7 are induce *Azotobacter chroococum* mutants

[2] Interaction of nitrogen application rate and Azotobacter strains was significant.

Table 3. Effect of six of Azotobacter strains combined with mineral N-fertilizer on number of fruits, eqarly and total yield zucchini type summer squash (Cucurbita pepo L.) cv "Eskandarany" plants grown in 2008 and 2009

Treatments		Number of fruits		Early yield		Total yield (ton/Feddan)	
		2008	2009	2008	2009	2008	2009
Nitrogen units (kg/feddan)	0	3.300	3.164	1.121	1.108	3.383	3.234
	30	4.021	3.893	1.504	1.476	4.627	4.481
	60	5.129	5.000	2.071	2.034	6.055	5.899
	90	6.071	5.950	2.671	2.591	7.771	7.587
	120	6.436	6.343	2.819	2.720	8.587	8.424
L.S.D. $_{0.05}$		0.02	0.01	0.001	0.021	0.031	0.004
Azotobacter Strains	Control$^-$	4.180	4.070	1.450	1.415	4.856	4.742
	Control$^+$	4.760	4.640	1.870	1.834	5.713	5.555
	3	4.920	4.790	1.950	1.884	5.921	5.768
	4	5.040	4.930	2.046	2.005	6.151	6.001
	5	5.170	5.040	2.160	2.100	6.352	6.175
	6	5.330	5.200	2.330	2.269	6.630	6.444
	7	5.540	5.420	2.456	2.393	6.969	6.789
L.S.D. $_{0.05}$		0.018	0.007	0.001	0.021	0.030	0.004
0 Nitrogen units	Control$^-$	2.100	2.000	0.300	0.295	1.739	1.650
	Control$^+$	3.000	2.850	1.000	0.995	2.999	2.836
	3	3.250	3.200	1.150	1.090	3.311	3.245
	4	3.500	3.300	1.200	1.192	3.596	3.383
	5	3.500	3.350	1.300	1.330	3.649	3.476
	6	3.750	3.600	1.400	1.389	4.013	3.834
	7	4.000	3.850	1.500	1.465	4.375	4.211
30 Nitrogen units	Control$^-$	3.200	3.000	1.150	1.100	3.296	3.186
	Control$^+$	3.500	3.400	1.400	1.382	3.938	3.810
	3	4.000	3.850	1.350	1.341	4.605	4.430
	4	4.000	3.900	1.550	1.535	4.625	4.483
	5	4.250	4.100	1.450	1.440	4.968	4.780
	6	4.400	4.250	1.700	1.685	5.198	5.000
	7	4.800	4.750	1.930	1.850	5.760	5.676
60 Nitrogen units	Control$^-$	4.200	4.100	1.500	1.480	4.735	4.610
	Control$^+$	5.100	5.000	1.850	1.810	5.941	5.813
	3	5.000	4.800	1.950	1.920	5.733	5.588
	4	5.100	5.000	1.900	1.880	5.993	5.863
	5	5.300	5.150	2.250	2.210	6.261	6.070
	6	5.500	5.350	2.450	2.385	6.669	6.460
	7	5.700	5.600	2.600	2.550	7.054	6.888
90 Nitrogen units	Control$^-$	5.200	5.150	1.750	1.700	6.370	6.290
	Control$^+$	5.900	5.750	2.450	2.395	7.339	7.130
	3	6.000	5.800	2.550	2.420	7.500	7.229
	4	6.200	6.100	2.800	2.730	7.983	7.824
	5	6.300	6.200	2.950	2.810	8.190	8.029
	6	6.400	6.300	3.050	2.985	8.400	8.230
	7	6.500	6.350	3.150	3.100	8.613	8.375
120 Nitrogen units	Control$^-$	6.200	6.100	2.550	2.500	8.138	7.976
	Control$^+$	6.300	6.200	2.650	2.590	8.348	8.184
	3	6.350	6.300	2.750	2.650	8.454	8.348
	4	6.400	6.350	2.780	2.690	8.560	8.454
	5	6.500	6.400	2.850	2.710	8.694	8.520
	6	6.600	6.500	3.050	2.900	8.869	8.694
	7	6.700	6.550	3.100	3.000	9.045	8.794
L.S.D. $_{0.05}$		0.04	0.02	0.002	0.046	0.068	0.010

(1) Control$^-$ and Control$^+$= without inoculation or inoculated with *Azotobacter* wild strains, 3 to 7 are induce *Azotobacter chroococum* mutants

(2) Interaction of nitrogen application rate and Azotobacter strains was significant.

Table 4. Effect of six of Azotobacter strains combined with mineral N-fertilizer on fruit Length, diameter and weight of zucchini type summer squash (Cucurbita pepo L.) cv "Eskandarany" plants grown in 2008 and 2009.

Treatments		Fruit length (cm)		Fruit diameter (cm)		Fruit weight (g)	
		2008	2009	2008	2009	2008	2009
Nitrogen units (kg/feddan)	0	7.759	7.657	2.025	1.943	80.93	80.65
	30	8.937	8.861	2.514	2.469	91.57	91.30
	60	9.714	9.650	2.829	2.804	94.50	94.17
	90	11.802	11.739	3.385	3.359	102.21	101.83
	120	13.195	13.119	3.929	3.904	106.71	106.23
L.S.D. $_{0.05}$		0.070	0.062	0.013	0.006	0.07	0.09
Azotobacter Strains	Control$^-$	9.153	9.130	2.536	2.512	88.38	88.11
	Control$^+$	9.860	9.766	2.780	2.742	93.74	93.42
	3	9.996	9.920	2.820	2.778	94.74	94.40
	4	10.356	10.320	2.981	2.946	95.74	95.47
	5	10.490	10.430	3.057	3.006	96.47	96.14
	6	10.962	10.843	3.136	3.092	97.92	97.48
	7	11.153	11.026	3.244	3.194	99.30	98.84
L.S.D. $_{0.05}$		0.048	0.028	0.008	0.006	0.066	0.082
0 Nitrogen units	Control$^-$	6.700	6.800	1.800	1.750	66.30	66.00
	Control$^+$	7.700	7.500	1.950	1.850	80.00	79.60
	3	7.730	7.700	1.980	1.900	81.50	81.20
	4	7.780	7.750	2.000	1.980	82.20	82.27
	5	7.900	7.850	2.097	2.000	83.40	83.00
	6	8.200	8.000	2.150	2.050	85.60	85.20
	7	8.300	8.000	2.200	2.070	87.50	87.30
30 Nitrogen units	Control$^-$	7.800	7.750	2.000	2.000	82.40	82.23
	Control$^+$	8.800	8.780	2.400	2.350	90.00	89.70
	3	8.900	8.800	2.450	2.370	92.10	92.00
	4	8.950	8.900	2.500	2.470	92.50	92.20
	5	9.100	9.000	2.700	2.600	93.50	93.30
	6	9.310	9.167	2.750	2.710	94.50	94.10
	7	9.700	9.630	2.800	2.780	96.00	95.60
60 Nitrogen units	Control$^-$	8.900	8.850	2.430	2.410	90.20	90.00
	Control$^+$	9.100	9.000	2.700	2.700	93.20	93.00
	3	9.150	9.100	2.710	2.700	93.60	93.10
	4	9.500	9.450	2.800	2.750	94.00	93.80
	5	9.550	9.500	2.850	2.820	94.50	94.30
	6	10.800	10.750	3.010	3.000	97.00	96.60
	7	11.000	10.900	3.300	3.250	99.00	98.40
90 Nitrogen units	Control$^-$	10.300	10.250	2.900	2.900	98.00	97.70
	Control$^+$	10.700	10.600	2.950	2.930	99.50	99.20
	3	11.000	10.900	3.020	3.000	100.00	99.70
	4	12.250	12.250	3.603	3.550	103.00	102.60
	5	12.400	12.370	3.620	3.610	104.00	103.60
	6	12.900	12.800	3.750	3.700	105.00	104.50
	7	13.067	13.000	3.850	3.820	106.00	105.50
120 Nitrogen units	Control$^-$	12.067	12.000	3.550	3.500	105.00	104.60
	Control$^+$	13.000	12.950	3.900	3.880	106.00	105.60
	3	13.200	13.100	3.940	3.920	106.50	106.00
	4	13.300	13.250	4.000	3.980	107.00	106.50
	5	13.500	13.430	4.020	4.000	106.97	106.50
	6	13.600	13.500	4.020	4.000	107.50	107.00
	7	13.700	13.600	4.070	4.050	108.00	107.40
L.S.D. $_{0.05}$		0.108	0.062	0.019	0.013	0.15	0.18

(1) Control$^-$ and Control$^+$= without inoculation or inoculated with *Azotobacter* wild strains, 3 to 7 are induce *Azotobacter chroococum* mutants

(2) Interaction of nitrogen application rate and Azotobacter strains was significant.

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نمو ومحصول الكوسة (كيوكربيتا بيبو) طراز زوكينى المخصبه باستخدام توليف من طفرات الازوتوباكتر والتسميد الازوتى المعدنى

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أجريت هذه الدراسة خلال موسمين شتويين متتالين لعامي ٢٠٠٨ و ٢٠٠٩ بغرض دراسة استجابة الكوسة صنف اسكندرانى لتوليفة من الازوت المعدني والتسميد الحيوي باستخدام طفرات جديدة من الازوتوباكتر كروكوكم و التعرف على كفاءة هذه الطفرات من خلال تثبيت النتروجين الجوى لتقليل إضافة التسميد الازوتى المعدني . وكانت معاملات التسميد الازوتى المعدني {صفر (بدون تسميد) ، ٣٠، ٦٠ ، ٩٠ ، ١٢٠ وحدة من النتروجين) للفدان والسلالات الازوتوباكتر المستخدمة تضمنت خمسة طفرات مستحدثة بالإضافة إلى السلالة الأصلية (البرية) ولقد تم الحصول على هذه الطفرات باستخدام مركب ان ميثيل–ان نترو–ان نتروزجوادين (NTG) فى معامل الوراثة الميكروبية بقسم الوراثة جامعة المنيا . وقد اجريت التجربة الحقلية فى محطة البحوث الزراعية بأسيوط . ولقد أظهرت الدراسة فيما يتعلق باستخدام التسميد الازوتى منفردا أفضل نمو (طول الساق–عدد الأوراق) وإنتاج الإزهار المؤنثة و المحصول (المحصول الكلى و المبكر) كان عند استخدام ١٢٠ وحدة أزوت معدني للفدان وانه عند استخدام السلالة البرية الأصلية فقد كانت هناك زيادة فى النمو و المحصول مع إضافة التسميد الازوتى المعدني حتى مستوى ١٢٠ وحدة نتروجين للفدان إما فيما يتعلق بالطفرات فقد كانت عموما تعطى زيادة فى كل القياسات السابقة متفوقة على السلالة الاصلية من الازوتوباكتر وكان واضحا وجود سلالة من طفرات الازوتوباكتر أعطيت رقم (٧) متفوقة على الأخريات وأمكن باستخدام هذه الطفرة توفير ٢٥% من السماد المعدنى مع زيادة ٥.٥% فى المحصول مما جعلنا نستنتج ان مدخل استخدام الطفرات فى انتاج سلالات يمكن إن يؤدى إلى إنتاج سلالات جديدة ذات كفاءة أعلى في التسميد الحيوي. وتوضح هذه الدراسة إمكانية الاستفادة منها فى تطوير وإنتاج أسمدة حيوية لتقليل استخدام التسميد الازوتى المعدني بما يتواءم مع الاتجاهات الحديثة فى الحفاظ على البيئة و المصادر الطبيعية للطاقة وتقليل التلوث البيئي نحو إبطاء التغيرات المناخية فى الاتجاه الغير مرغوب وإضرارها على بيئة الكائنات الحية.