Effect of Seed Inoculation with *Azotobacter* and Soaking in Trace Element Solution on the Growth and Yield of Some Vegetable Crops

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ABSTRACT

In an attempt to improve growth and yield, seeds of cabbage, cauliflower, onion and lettuce were subjected before sowing to one of the following treatments: (a) soaking in a solution containing the trace elements B, Cu, Mn and Mo; (b) inoculation with isolates of *Azotobacter chroococcum* (Beijerinck), (c) soaking in the trace element solution and inoculation with *Azotobacter*. Seeds of these treatments as well as untreated seeds were planted in the field in randomized complete block design and growth of the plants was measured at the time of transplanting and at harvest.

Seed soaking depressed the growth of the 4 vegetable crops tested during the seedling stage, but this did not persist at later stages of growth, except for lettuce. Inoculation with *Azotobacter* significantly improved the growth of cabbage, cauliflower, and onion transplants and showed slight beneficial effect on lettuce. Growth improvement, however did not result in a significant increase in the yields of the tested crops.

In another experiment, inoculation of seed tubers of two potato cultivars before sowing with *Azotobacter* did not increase the yield obtained, number of tubers per plot, or mean weight of tuber.

INTRODUCTION

Seeds coated with dry materials containing trace elements have been commercially used in field crops to supply the essential elements under deficient environment. Recently, soaking the seeds before planting in diluted solution of the essential trace elements was used by several workers to supply the deficient nutrients. Such treatments were reported to eliminate deficiency symptoms, to increase rate of seed germination, to induce better plant development, and to result in higher yields (10,13,15).

Also, in attempts to improve growth and increase crop yield, seed inoculation with *Azotobacter* preperations has been tried. The results reported, however, were rather contradicting. Growth promotion and accelerated flowering of plants from inoculated seeds were frequently reported (4,6,12). However, only few experiments have been

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partially successful in increasing the yield or hasten maturity by inoculation (3,7). Responses in crop yield due to *Azotobacter* inoculation are more likely to be expected with greenhouse grown plants and with vegetable crops that are usually grown under controlled conditions (3).

Studies on the effect of inoculating seed potatoes by *Azotobacter* preparation before planting on yield have been reported. Pre-sowing treatment of potato seed tubers with ammonium molybdate solution followed by treatment with Azotobacterin increased tubers yield by 17.8 % (15). However, treatment of seed potato with *Azotobacter* gave no increase in yield of tubers (14). In a previous greenhouse experiment, under Tripoli conditions, inoculation with *Azotobacter chroococcum* isolates was found to improve the growth of wheat but had no effect on the growth of tomato plants (1).

The objective of the present investigation was to test the response of different vegetable crops to *Azotobacter* inoculation under the field conditions. The effect of seed soaking in a diluted solution of trace elements on the growth and yield of the vegetable crops grown in the calcareous sandy soil was also tested (the farm of Faculty of Agriculture, Tripoli).

MATERIALS AND METHODS

The experiments were conducted in the field during the period from December 1973 to June 1974. Cabbage (cultivar Bronzwick), cauliflower (cultivar Snowball A), onion (cultivar Texas Yellow Grano 502) lettuce (cultivar Great Lakes 118) and two potato cultivars (Alpha and Bintje) were used in these experiments.

Seed Treatments and Planting

In case of the first 4 crops, that need transplanting, the experiments consisted of 4 treatments in which the seeds were treated before sowing as follows: (1) soaked for 16 hrs in a trace elements solution; (2) inoculated with suspension of *Azotobacter chroococcum* isolates; (3) soaked for 16 hrs in the trace elements solution then inoculated with the azotobacter suspension; and (4) the control treatment. The trace elements solution used for soaking the seeds in treatments no. 1 and 3 was the same as that used by Nuzhnova (10) and had the following concentrations: 0.02 % H_3BO_3, 0.05 % $MnSO_4 \cdot 7H_2O$, 0.05 % $ZnSO_4$, 0.05 % $CuSO_4$ and 0.01 % $(NH_4)_2$ MoO_4. The seeds that were soaked in this solution were permitted to air dry for 15 minutes before sowing. Seeds were inoculated with azotobacter by soaking them for 30 minutes in a mixed suspension of agar-grown cultures of *A. chroococcum* isolates. After inoculation seeds were planted directly.

Seeds of the 4 treatments were planted in well prepared seed beds in the open field in rows in a randomized complete block design with 4 replicates for each treatment. Samples from each replicate of cauliflower, cabbage and lettuce were taken at the age of 45 days, while in case of onion, samples were taken after 60 days from sowing. For each sample the following measurements were taken: length of seedlings, number of leaves per seedling, and fresh and dry weights of 10 seedlings.

Microbiological Tests

Rhizosphere soil samples from inoculated and uninoculated transplants of cabbage and lettuce were analyzed for total microflora, by the dilution plate method on nutrient agar, and numbers of *Azotobacter* were estimated by the most probable number technique on nitrogen-free sucrose medium. The determinations were carried out in duplicates.

Tests on Mature Plants

Transplants taken from the four above treatments were planted for testing any further effect of seed treatment on later stages of growth and the yield of these crops. The transplants were planted in plots in a randomized complete block design with 5 replicates. In case of cabbage, cauliflower, and lettuce each plot consisted of 18 plants 30 cm apart in one row, while in onion the plot contained 36 plants 15 cm apart. Two weeks after transplanting, 12–24–12 NPK fertilizer at the rate of 400 kg/ha was applied; sprinkler irrigation was applied when necessary. The best 15 plants of each plot were harvested at the mature stage and the weight of the vegetative growth was recorded. The average weight of plants in each plot was then calculated. In case of onion, the whole plot was harvested and the average weight of plant was calculated.

In the potato experiment, seed tubers weighing about 50 gm each were taken from the two cultivars Alpha and Bintje and were soaked in the *Azotobacter* suspension for 30 minutes. Treated and untreated seed tubers of both cultivars were planted in a randomized complete block design in 4 replications. Each plot consisted of one row 3 metres long; the rows were 60 cm apart. In each row 10 tubers were planted at a distance of 30 cm from each other. Proper cultural practices were followed until maturity then the plants were dug out and yield as well as number of tubers per plot were recorded.

RESULTS

Response at Seedling Stage

Azotobacter inoculation and seed soaking in the trace element solution greatly affected the vegetative growth of plants during the seedling stage. The data obtained on average length of seedlings, number of leaves per plant, fresh and dry weights in the four studied crops are shown in Table 1.

Seed inoculation with *Azotobacter* improved the growth of the plants during the seedling stage. The increases in fresh weight, dry weight and length of seedlings due to seed inoculation were highly significant in cabbage, onion, and cauliflower. In lettuce there was a significant increase in dry weight, but no significant increase in length or fresh weight. On the other hand, seed soaking in the nutrient solution used has an adverse effect on the vegetative growth of all crops tested. The reductions in fresh weight, dry weight and length of seedlings at the age of 45 to 60 days due to seed soaking were highly significant. The effect was especially pronounced on the dry weight and length of seedlings. It should also be noted that, under the conditions of these experiments, the control plants did not show any deficiency symptoms to any of the trace elements used.

In the soaked and inoculated seeds the reduction in vegetative growth of lettuce and onion induced by seed soaking was not balanced by *Azotobacter* inoculation. The seedlings from this treatment were significantly lower in fresh and dry weight than the untreated control. In cabbage and cauliflower, however, the improvement in growth of seedlings by inoculation did overcome the reduction caused by soaking, especially in cabbage where significant increases over the control were noted in fresh and dry weights. Photographs of seedlings from the 4 treatments for the vegetable crops tested are shown in Figures 1, 2, 3 and 4. The differences in size of seedlings due to seed treatment are

Table 1 Effect of seed soaking and *Azotobacter* inoculation on seedling growth of some vegetable crops.

	Length of seedling cm	no, of leaves/ seedling	Fresh wt of 10 seedlings gm	Dry wt of 10 seed-lings gm
Cabbage				
1. Control	13.0	3.9	15.0	1.32
2. Soaked	10.6	3.7	10.9	0.87
3. Inoculated	16.3	4.7	28.1	3.5
4. Soaked & Inoculated	13.7	4.5	19.2	2.07
L.S.D. at 5%	1.08	—	3.8	0.09
L.S.D. at 1%	1.56		5.46	0.129
Cauliflower				
1. Control	12.8	3.7	14.4	1.9
2. Soaked	11.0	3.5	13.3	1.2
3. Inoculated	14.6	4.1	20.5	2.0
4. Soaked & Inoculated	12.2	3.8	13.5	1.4
L.S.D. at 5%	1.14	—	3.89	0.192
L.S.D. at 1%	1.64	—	5.59	0.276
Onion				
1. Control	18.4	–	5.6	0.6
2. Soaked	15.6	–	2.7	0.31
3. Inoculated	25.8	–	8.0	0.95
4. Soaked & Inoculated	18.0	–	2.9	0.4
L.S.D. at 5%	2.19	–	0.85	0.074
L.S.D. at 1%	3.15	–	1.03	0.107
Lettuce				
1. Control	11.0	3.8	11.4	0.71
2. Soaked	8.5	3.6	5.4	0.3
3. Inoculated	11.9	4.0	14.4	1.0
4. Soaked & Inoculated	8.2	4.0	8.3	0.45
L.S.D. at 5%	1.28	–	4.0	0.113
L.S.D. at 1%	1.85	–	5.75	0.162

quite obvious especially in case of cabbage and onion. A better growth of root system was also noted in transplants taken from the inoculated treatment.

The effect of seed inoculation on the numbers of *Azotobacter* in the rhizosphere of cabbage and lettuce was studied. Numbers of total microflora and *Azotobacter* in the rhizosphere soil of 8 weeks old plants from inoculated and uninoculated seeds are shown in Table 2. The results obtained show that the rhizosphere soil of inoculated plants contained higher numbers of *Azotobacter* than rhizosphere of uninoculated plants. Counts of *Azotobacter* in rhizosphere of inoculated cabbage and lettuce were, respectively, four and two times higher than the uninoculated plants. Numbers of total microflora were not greatly affected by seed inoculation.

Response at Harvest Stage

The results obtained on average weight of plants at harvest time are shown in Table 3. In cauliflower, cabbage, and onion, the observed adverse effect of seed soaking in the

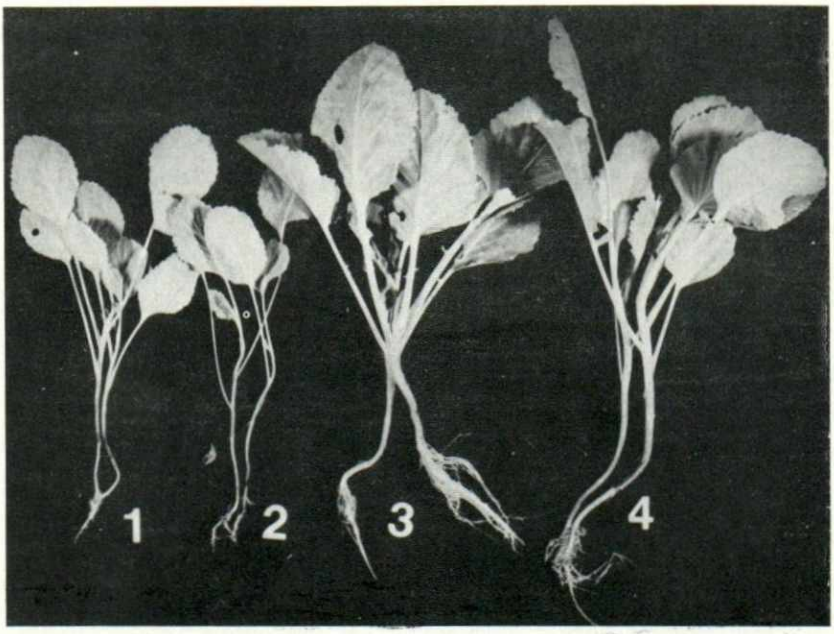

Fig. 1. Cabbage transplants from untreated and treated seeds: (1) control from untreated seeds; (2) transplants from seeds soaked in the trace element solution; (3) transplants from *Azotobacter* inoculated seeds; (4) transplants from seeds soaked and inoculated.

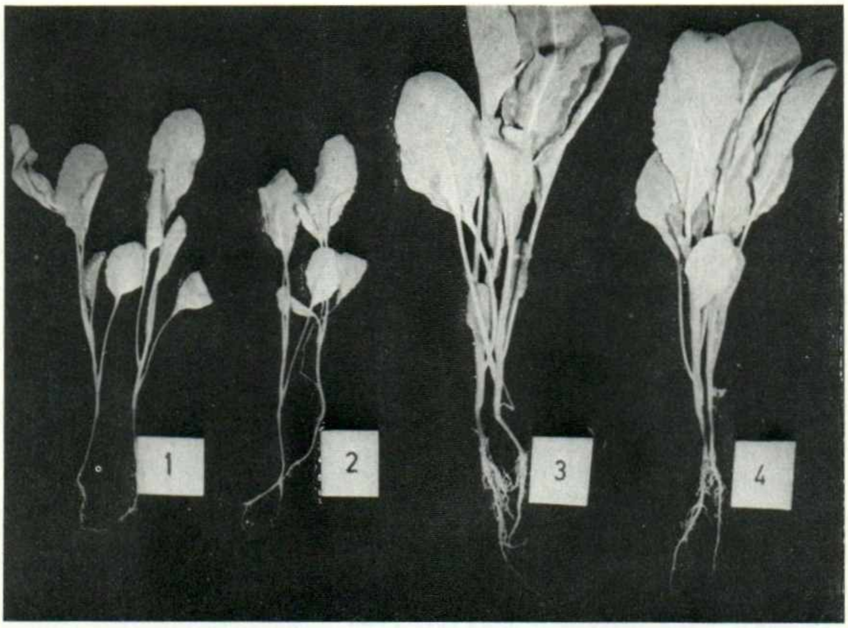

Fig. 2. Cauliflower transplants from untreated and treated seeds: (1) control from untreated seeds; (2) transplants from seeds soaked in the trace element solution; (3) transplants from *Azotobacter* inoculated seeds; (4) transplants from seeds soaked and inoculated.

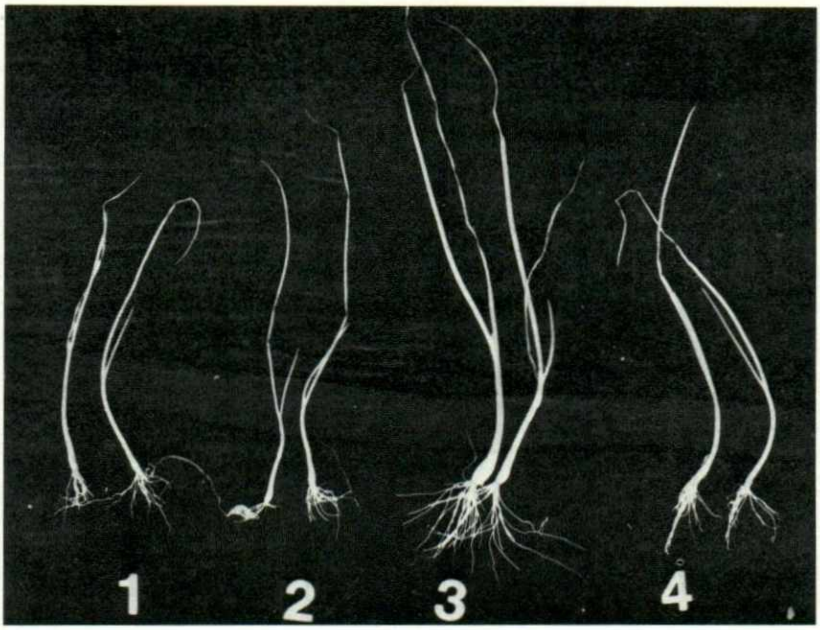

Fig. 3. Onion transplants from untreated and treated seeds: (1) control from untreated seeds; (2) transplants from seeds soaked in the trace element solution; (3) transplants from *Azotobacter* inoculated seeds; (4) transplants from seeds soaked and inoculated.

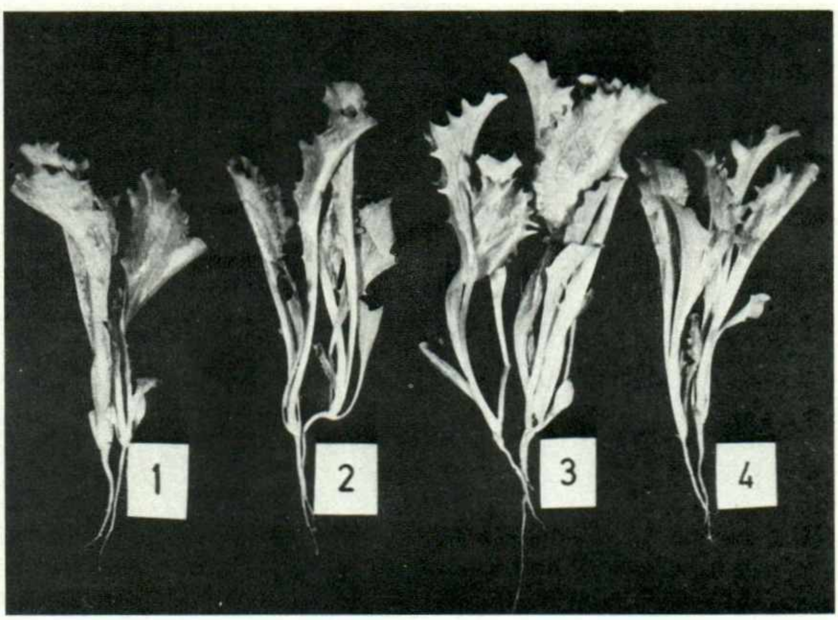

Fig. 4. Lettuce transplants from untreated and treated seeds: (1) control from untreated seeds; (2) transplants from seeds soaked in the trace element solution; (3) transplants from *Azotobacter* inoculated seeds; (4) transplants from seeds soaked and inoculated.

Table 2 Numbers[1] of total microflora and *Azotobacter* in rhizosphere soil of transplants from inoculated and uninoculated seeds.

Treatment	no. of total micro-organisms per gram dry soil $\times 10^6$	no. of Azotobacers per gram dry soil $\times 10^3$
Cabbage		
Uninoculated	23.2	34.6
Inoculated	47.5	136.8
Lettuce		
Uninoculated	38.1	66.0
Inoculated	39.2	130.1

[1]Average of 2 replicates.

Table 3 The effect of seed soaking and Azotobacter inoculation on subsequent vegetative growth.

Seed pre-treatment	Mean weight of plants at harvest time kg			
	Cauliflower	Cabbage	Lettuce	Onion
1. Untreated(control)	0.517	0.755	0.648	0.133
2. Soaked	0.551	0.845	0.535	0.118
3. Inoculated	0.595	1.079	0.660	0.198
4. Soaked & inoculated	0.559	0.907	0.561	0.114
L.S.D 5% level	N.S.	N.S.	0.102	N.S.

trace element solution on growth of plants during the seedling stage disappeared at the later stages of growth. At harvest, weight of plants from the soaked treatment were not significantly different from those of the untreated control plants. In lettuce, the adverse effect of seed soaking persisted until harvest time. Lettuce plants from soaked seeds were significantly less in weight than the control. The adverse effect of soaking was also pronounced in the soaked and inoculated treatment.

Cabbage, cauliflower, and onion from the *Azotobacter* inoculated treatment continued to show improved growth over the untreated control at harvest. The mean weight increase over the control amounted to 42.9%, 9% and 48.8%, respectively; however, these differences were below the significant level. On the other hand, lettuce plants from inoculated treatment did not show any growth improvement at harvest time.

Effect of Azotobacter Inoculation on Potato Yield

The results obtained on average yield and number of tubers per plot from the inoculated and uninoculated treatments are shown in Table 4. *Azotobacter* inoculation of seed tuber at the time of sowing had no significant effect on the yield obtained, number of tubers per plot or mean weight of tubers.

DISCUSSION

To supply the essential nutrients under deficient environments, seed soaking in nutrient solutions containing the trace elements have been used by several investigators for field crops and some vegetable crops (10,13,15). Under the conditions of the present

Table 4 Effect of tuber inoculation with *Azotobacter* on average yield of potato.

Treatment	Yield of tubers per plot kg	Number of tubers per plot	Mean weight of tuber gm
Alpha Cultivar			
Uninoculated	7.59	136.2	56.7
Inoculated	7.62	118.5	65.4
Bintje Cultivar			
Uninoculated	6.79	121	56.2
Inoculated	7.71	141.2	54.8
L.S.D. at 5%	N.S.	N.S.	N.S.

investigation, soaking the seeds of cabbage, cauliflower, onion and lettuce in the trace elements solution had adverse effect on plant growth.

The reason for the depressive effect of seed soaking is not known. It is probable that the relatively high concentration of trace elements may inhibit the enzymes activity in young tissues (5). The non persisting detrimental effect of soaking at further growth stage support this explanation. Except for lettuce, plants from soaked seeds, at harvest, showed equal or slightly improved growth than plants from untreated seeds. Variations in the extent of the detrimental effect of seed soaking were noted on the four vegetable crops tested. Lettuce plants and to some extent, onion were more sensitive to the adverse effect of soaking in the trace element solution than cabbage and cauliflower. This could be due to that lettuce and onion tissues are more tender than cabbage and cauliflower and therefore had less ability to tolerate the harmful effect observed with soaking in the trace element solution. Nieuwhof (9) have indicated that most cabbage varieties are moderately tolerant to salt level in the soil.

The stimulation of growth during the seedling stage due to *Azotobacter* inoculation supports previous results on wheat, tomato, and maize (1,3,6,12). Although this observed stimulation in growth during the seedling stage was highly significant in three of the crops tested it did not result in significant increases in the yield. Yet, inoculated cabbage and onion plants were still showing improved growth over the untreated plants. Significant increases in yield of cabbage by *Azotobacter* inoculation were obtained by Brown *et al.* (3) and Lehri and Mehrotra (7).

According to Brown and co-workers (3,4) the stimulation in growth induced by *Azotobacter* inoculation does not seem to be a result of nitrogen fixation or to eliminating a harmful effect, but through the production of growth promoting substances in the rhizosphere of inoculated plants. The extent and persistance of the stimulating effect will depend on the amount of promoting substances produced. Therefore, the establishment and multiplication of *Azotobacter* in the rhizosphere of inoculated plants become a prerequisite for production of the promoting substances and growth stimulation of plants.

In the present investigation the vegetable crops tested showed variation in their response to inoculation. Cabbage and onion were the two crops highly stimulated, whereas lettuce did not show any response even during the seedling stage. Variation in response to *Azotobacter* inoculation were also reported by other investigators (3,12). The variability in response to inoculation could be due to variation between the different plants to support *Azotobacter* growth and multiplication in the rhizosphere.

Brown *et al.* (2) reported the establishment of high numbers of *Azotobacter* in the rhizosphere of different crop plants by inoculation, while *Azotobacter* could not be established in the rhizosphere of sugar beet. Also, rhizospheres of crucifer plants were reported to support high counts of *Azotobacter* (8,16).

The establishment and multiplication of *Azotobacter* in rhizosphere soil depend upon the kind and amount of organic carbon compounds secreted by the roots, which could be used by the inoculated organisms as carbon/energy source. The difference in the kind and amount of these compounds in root excretions of the different plants could probably be the reason for the variation in response to inoculation expressed by the different plants. Also, the variation in the amount and constituents of root excretions along the different stages of plant growth (11) may explain the reason for the pronounced growth stimulation during the seedling stage and the decrease in response at harvest noted in the present investigation. Jackson *et al.* (6) also reported the decrease in stimulation of tomato plants, caused by *Azotobacter* inoculation, after flowering.

The nonsignificant effect of inoculating seed potatoes with *Azotobacter* is in agreement with the result of Timonin (14) who obtained no significant response of potato yield to *Azotobacter* inoculation.

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