Response of Tomato and Two Other Vegetable Crops to Inoculation with Azotobacter

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ABSTRACT

The abundance of Azotobacter in the rhizospheres of inoculated and non-inoculated tomato, chard, and beet plants was determined to test the effect of inoculation, plant species, and age of plant on the Azotobacter population established in the rhizosphere. Greenhouse and field experiments were also conducted to test the effect of Azotobacter inoculation on growth, flowering and yield of tomato plants.

Inoculation increased the abundance of Azotobacter in rhizospheres of all tested plants and the highest established population was in tomato rhizosphere which showed highly significant improvement in growth by the treatment.

Under greenhouse conditions treating of tomato with Azotobacter, by seed inoculation before sowing by root inoculation of the seedlings before transplanting or by both, resulted in highly significant improvement in growth and earliness of flowering of the plants. Inoculation of the seeds was more effective than inoculation at the seedling stage.

Under the field conditions, inoculation of tomato resulted in better growth of the plants, early fruit production, and highly significant increases in total yield of fruits.

INTRODUCTION

Growth improvement and earlier flowering by Azotobacter seed inoculation was reported by several investigators on wheat, tomato, and maize (4,8,12). Under Libyan conditions, seed inoculation with Azotobacter gave highly significant stimulation in seedling growth of cabbage, cauliflower, and onion; whereas lettuce showed no significant response. The improved growth obtained at the seedling stage, which also persisted after transplanting, did not result in significant increase in the yield of the tested speceis at harvest (3). The variability of plant species in response to Azotobacter inoculation was also reported by other investigators (5,13).

The establishment and multiplication of Azotobacter in the rhizosphere of inoculated plants seems to be an important factor if improvement of growth is to be expected. Brown *et al.* (4,5) obtained good establishment of Azotobacter in the rhizosphere of cabbage and cereals by inoculation of seeds, roots, or soil; whereas with best few Azotobacter were established in the rhizosphere of young plants and then disappeared by

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harvest. Inoculation of wheat affected plant growth and sometimes the yield, provided that there were at least 10^4–10^6 Azotobacter per gram of rhizosphere soil. This number was readily attained in greenhouse experiments than in the field.

An important factor affecting the numbers of Azotobacter established in the rhizosphere is the age of the plant. Its effect comes through controlling the amount of root excretions provided for Azotobacter proliferation (12), and through its effect on the abundance of other microorganisms which may antagonize the growth and multiplication of Azotobacter (11). The highest numbers of Azotobacter in spring wheat rhizosphere were found before tiller formation and the lowest at flowering and ear formation (5). Similar results were reported by other investigators, obtaining increased Azotobacter population in the rhizosphere of inoculated young plants and subsequent decrease in numbers with age (4,10).

The aim of the present investigation was to test the effect of seed inoculation on the abundance of Azotobacter in the rhizosphere of three plant species at different stages of seedling growth; and to study the effect of seed or seedling inoculation with Azotobacter on the growth and yield of tomato.

MATERIALS AND METHODS

The greenhouse and field experiments of this investigation were conducted at the farm of the Faculty of Agriculture, Alfateh University during the period from October, 1975 to August, 1976. The soil used in these experiments was sandy loam having a pH of 8.0 and containing less than 0.5% organic matter.

Cultures of Azotobacter and Methods of Inoculation

Azotobacter chroococcum strains, 1, 2, 5 and 6, locally isolated (1), were separately cultivated on modified Burk's medium. Each strain was grown in 100 ml aliquot of the medium in 500 ml capacity Erlenmeyer flask. The cultures were incubated for 2 weeks at 28–30°C, and just before inoculation the four grown cultures were mixed together and used for inoculating the seeds or roots.

Seed inoculation was carried out by soaking the seeds in the inoculum for one hour before sowing. Inoculation of tomato seedlings was done by dipping the roots in the inoculum for 30 min. before transplanting.

Initial number of Azotobacter per seed was determined by the most probable number technique using samples of seeds from tomato, chard and beet soaked for one hour in the inoculum. Since the commercial seeds of chard and beet are not true seeds but are rather dry fruits containing 2 to 4 seeds each (3 on the average), the number of Azotobacter cells loaded on each seed ball of chard and beet was divided by 3 to obtain the initial number of Azotobacter potentially loaded per developing seedling.

A. Greenhouse experiments

Experiment I. Effect of seed inoculation on growth of seedlings and abundance of azotobacter in the rhizosphere Three different plants were used in this experiment; tomato (cultivar Roma VF), chard (cultivar Blond A Carde Blanche) and beet (cultivar Detroit Dark Red). Inoculated and non-inoculated seeds of the tested species were planted in greenhouse benches filled with soil to a depth of 25 cm. A separate bench was used for each treatment, and the seeds were sown in rows 15 cm long and 40 cm a part. Two replicate rows were sown for each treatment.

Rhizosphere Sampling and Azotobacter Counting

The abundance of Azotobacter was determined in soil samples taken from the rhizospheres of inoculated and non-inoculated seedlings. Samples were taken 4 times during the period of plant growth. The count was made in one composite rhizosphere soil sample taken from each replicate of the treated and non-treated plants. A rhizosphere soil sample was collected by removing from 3 different sites along the plant row, a total of 10 to 30 seedlings, depending on the plant age, with their roots and adjacent soil. After vigorous shaking of the roots to remove superfluous soil, the soil particles adhering to the roots were carefully removed with a fine spatula. One gram of the collected rhizosphere soil was then mixed with a series of sterile water blanks for preparing the necessary dilutions, and count of Azotobacter was estimated by the most probable number technique using mannitol nitrogen-free broth medium for cultivation.

Growth Measurements

At the age of 70 days a sample of 10 seedlings, randomly selected, was taken from each replicate of the inoculated and non-inoculated test plants for growth measurements. The length, and the fresh and dry weights of the seedlings were determined, and the data obtained were subjected to statistical analysis and the L.S.D. between means were calculated (14).

Experiment II. Effect of inoculation at transplanting time on growth and flowering of tomato This experiment includes 4 treatments: a) seedlings from non-inoculated seeds, b) seedlings from inoculated seeds, c) seedlings from non-inoculated seeds, inoculated at transplanting, and d) seedlings from inoculated seeds, reinoculated at transplanting. The seedlings were 60 days-old, taken from the first experiment, and were transplanted into pots 20 cm in diameter, filled with the farm sandy loam soil. Five grams of superphosphate were added to each pot and one seedling was planted per pot. From each treatment six replicate pots were planted, and the pots were kept in the greenhouse for 45 days during which the plants were watered daily and examined for the start of flowering. The first flower truss was recorded for each replicate plant, and at the end of the experiment the height of the plants was determined.

B. Field experiment. Effect of seed and seedling inoculation on growth and yield of tomato

Inoculated and non-inoculated seeds were planted in well prepared seedbeds in the open field in rows 65 cm apart. After 60 days from sowing, seedlings of the following treatments were transplanted: a) seedlings from non-inoculated seeds, b) seedlings from inoculated seeds, c) seedlings from inoculated seeds reinoculated with Azotobacter before transplanting.

The seedlings were planted in rows 75 cm apart and spaced at 30 cm within the row. The plot consisted of one row 5 meters long and thus containing 17 plants. Each treatment was replicated 6 times and the plots were laid out in the field in a randomized complete block design. Complete fertilizer (12–24–12) was added to all plots at the rate of 600 Kg/hectar and was applied in two doses, the first 15 days after transplanting and the second 30 days thereafter. Recommended practices for irrigation and weed and pest control were applied equally to all plots.

After 45 days from transplanting when the plants of all treatments were in bloom, the number of plants bearing early fruits in each plot was recorded, and the length of main stem of plants in each plot was measured. Fruits that showed start of ripening were

picked and the weight and number of harvested fruits were recorded for each plot. The average weight of fruit was calculated by dividing the total weight of fruits by the number of fruits. Harvesting was carried out twice a week and 7 harvests were taken. Fruits picked during the first two weeks of the harvesting period were considered as early yield. Weight of early yield as well as total yield of fruits per plot were determined and all data obtained were subjected to statistical analysis (14).

RESULTS

Effect of inoculation on the abundance of Azotobacter

The number of Azotobacter loaded per one true seed after soaking in the inoculum for one hour (initial numbers) were 21, 14 and 65 thousands, respectively, for tomato, chard and beet. This indicates that the initial numbers on chard and tomato seeds were close to each other at the time of sowing, but the number of Azotobacter was about 3 to 4 times higher in case of beet. This difference may be due to the size and the physical structure of the seed surface.

Table 1 shows the numbers of Azotobacter in rhizospheres of seedlings from inoculated seeds. It is obvious that the numbers of Azotobacter in the rhizospheres of non-inoculated plants were low, and that seed inoculation greatly increased the numbers in rhizosphere soils of the three tested plants. The high numbers of Azotobacter attained by seed inoculation were maintained in the rhizosphere of inoculated plants throughout the period of the experiment. The numbers found at the age of 40 days were higher than those found at 10 days. The rate of increase in number of Azotobacter during the period from 10 to 40 days was higher in chard than in the other two crops. These results indicate the establishment and multiplication of Azotobacter in the rhizosphere of the tested plants by seed inoculation.

During the experimental period, the rhizosphere of inoculated tomato contained higher counts of Azotobacter than chard and beet rhizospheres (about 10 to 50 times higher). The lowest Azotobacter population was in beet rhizosphere. This shows that the plant species have a great effect on rhizosphere microflora.

Table 1 Abundance of Azotobacter in rhizospheres of inoculated and non-inoculated plants.

Plants	Age of plants at sampling (day)	Average No. of Azotobacter/g oven dry soil	
		Non-inoculated	Inoculated
Tomato	10	8.04×10^2	1.8×10^6
	40	17.9×10^2	16.8×10^6
	60	15.9×10^3	16.7×10^6
	80	0.61×10^2	0.36×10^6
Chard	10	8.04×10^2	1.6×10^5
	40	1.7×10^2	17.0×10^6
	60	4.4×10^2	18.1×10^5
	80	0.41×10^2	16.4×10^4
Beet	10	5.6×10^2	2.2×10^4
	40	0.42×10^2	42.2×10^4
	60	1.5×10^2	10.4×10^5
	80	20.5×10^3	17.5×10^4

Effect of seed inoculation on seedling growth

Growth responses of the tested plants to Azotobacter inoculation are shown in Table 2. The highest growth response of the three kinds of plants was obtained in tomato. Seed inoculation caused highly significant increases in length, fresh and dry weights of tomato seedlings; the increases in these growth measurements amounted to 125%, 123% and 127% respectively. Growth of chard was stimulated by inoculation especially with regard to fresh and dry weights of seedlings, but the increases did not reach the significant level. The growth of beet did not show any response to inoculation.

Effect of inoculation on growth and flowering of tomato plants

The response of tomato growth and time of flowering to seed inoculation, seedling inoculation or both is shown in Table 3. The improvement in growth of tomato plants obtained by the inoculation treatments is also illustrated by Figure 1.

Each of the three inoculation treatments, compared with the untreated control, resulted in highly significant improvement in plant growth and earliness of flowering. The highest response was obtained by the seed plus seedling inoculation treatment. The differences in response between the seed plus seedling inoculations and the seed inoculation alone were not significant in either plant height or time of flowering.

The improved growth and flowering induced by seed inoculation was much more than that obtained by the seedling inoculation. The difference between these two treatments was highly significant in plant growth and significant in the time of flowering. This indicates that inoculation of the seeds with Azotobacter before sowing is more effective for better growth and earlier flowering than inoculation at the seedling stage. This is also noted from the results obtained in the seed plus seedling inoculation treatment, showing only slight improvement in growth over the seed inoculation treatment (Table 3).

Table 2 Effect of seed inoculation with Azotobacter on seedling growth.

Treatment	Average fresh wt. of plant g	Average dry wt. of plant g	Average length of plant cm
Tomato			
Non-inoculated	1.82	0.131	10.5
Inoculated	4.07	0.298	23.7
L.S.D. at 5%	1.347	0.086	4.81
L.S.D. at 1%	1.937	0.123	6.9
Chard			
Non-inoculated	6.64	0.456	21.6
Inoculated	13.69	0.841	24.2
L.S.D. at 5%	7.5	0.479	6.75
L.S.D. at 1%	10.7	0.689	9.7
Beet			
Non-inoculated	6.38	0.44	20.6
Inoculated	5.66	0.388	18.4
L.S.D. at 5%	4.9	1.24	8.8
L.S.D. at 1%	8.14	2.06	14.6

Table 3 Effect of seed and seedling inoculation with Azotobacter on growth and time of flowering of tomato.

Treatment	Av. height of plant cm	Av. age of plants in days at the start of flowering
Non-inoculated control	17	104.6
Seed inoculated	27.8	88.3
Seedling inoculated	22.3	94.8
Seed and seedling inoculated	28.3	81.8
L.S.D. at 5%	2.77	6.92
L.S.D. at 1%	3.83	9.57

Response of tomato to inoculation under field conditions

Field observations indicated that the stimulation of growth in the inoculated plants compared with the non-treated control plants was quite obvious during the seedling stage. After transplanting however, the differences between the treatments in stem length of plants decreased gradually. The average stem length of plants, measured at the beginning of harvesting time is shown in Table 4. There was no significant difference between the non-treated control and the seed inoculated treatment, but the plants of the seed plus seedling inoculation treatment were significantly higher in stem length.

The results on the percentage of fruiting plants at the beginning of harvest, early yield, and total yield of fruits obtained under the different inoculation treatments are given in Table 4. The data on percentage of fruiting plants and early yield of fruits indicate that the inoculation resulted in early fruiting and fruit ripening. At the beginning of harvesting time, the percentage of fruiting plants in the inoculated treatments were

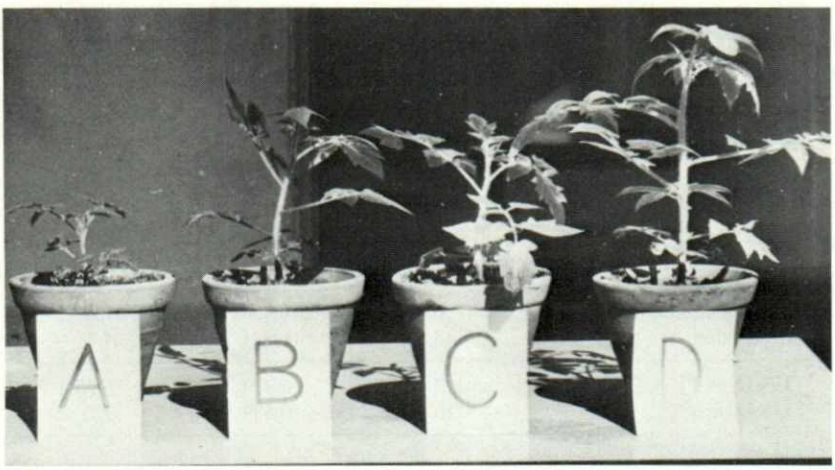

Fig. 1. Tomato plants from the different inoculation treatments, after 2 weeks from transplanting: (a) non-treated control (b) inoculated at the seedling stage before transplanting (c) inoculated at the seed stage. (d) inoculated at the seed stage the seedling reinoculated before transplanting.

Table 4 Effect of seed and seedling inoculation with Azotobacter on growth and yield of tomato (Roma VF).

Treatment	Stem length cm	% of fruiting plants at the start of harvest	Early yield per plot Kg	Total yield per plot Kg	Total No. of fruits per plot	Average wt. of fruit g
Non-inoculated (control)	27.8	1.9	0.716	7.975	194.5	43.3
Seed inoculated	29.3	12.0	1.583	18.250	373.5	45.8
Seed and seedling inoculated	33.1	23.0	3.413	20.123	444.0	45.0
L.S.D. at 5%	5.32	13.49	1.187	6.617	115.76	7.21
L.S.D. at 1%	—	18.94	1.689	9.411	164.44	—

higher than that in the non-inoculated control; and the difference between the seed plus seedling treatment and the control was highly significant. Also, the increase in early yield of tomatoes induced by the seed plus seedling inoculation treatment over the control was highly significant. Seed inoculation alone increased both the percentage of fruiting plants at the beginning of harvest and the early yield of fruits, but the increases did not reach the significant level.

The results obtained also show that both the seed inoculation and the seed plus seedling inoculation treatments resulted in highly significant increases in the total yield of tomato. The increases in yield of the two treatments over the non-treated control, amounted to 128% and 152% respectively. As shown in Table 4, these increases in total yield were due to increases in number of fruits and not to average weight of fruits.

DISCUSSION

In agreement with the results of other investigators (4,13), seed inoculation was found to increase the population of Azotobacter in rhizosphere soil of the inoculated plants; the abundance of the established Azotobacter population was dependent on the species of plant. The results (Tables 1 and 2) also show that there seems to be some relation between the abundance of Azotobacter population established in the rhizosphere of inoculated plants and the magnitude of growth improvement obtained by inoculation. The highest established population was in tomato rhizosphere and it was the only plant among the three tested that showed highly significant growth stimulation by inoculation. Lower Azotobacter population was maintained in chard rhizosphere and the growth of the plants was slightly stimulated. The lowest population was found in beet rhizosphere which did not show any growth improvement. These results indicate that the inoculum as such is not the main cause of growth stimulation, and points to the importance of the Azotobacter population established in the rhizosphere after inoculation. Brown *et al.* (5) have also noticed that the lack of response in wheat yield in some years accompanied the failure of Azotobacter to get established in the rhizosphere of inoculated plants due to environmental factors.

The established Azotobacter population probably determines the magnitude of growth regulating substances produced in the rhizosphere and consequently account for the major portion of growth improvement induced by inoculation. Evidence has now accumulated that the accelerated growth and flowering induced by Azotobacter

inoculation is through the production of growth promoting substances (6). B-indole acetic acid and gibberellin-like substances have been detected in the cultures of *Azotobacter chroococcum* (7,8,15,16). However, the amount of these substances in the inoculum was found to be small enough to account for the accelerated growth obtained by Azotobacter inoculation, and it is believed that these growth regulating substances may have continued to be synthesized for a period after roots were being colonized with the inoculated Azotobacter (6,7).

The results obtained show that inoculation of tomato seeds was more effective in growth stimulation as well as inducing earlier and more abundant flowering than inoculation at the seedling stage. Reinoculation of the seedlings at transplanting had non-significant additive stimulating effect on growth and earliness of flowering over seed inoculation alone. These results point to the importance of time of inoculation on the crop response expected from Azotobacter inoculation. Beside the fact that the abundance of Azotobacter population established in the rhizosphere after inoculation is dependent on the age of the plant, the uptake of the growth substances produced must associate with the critical stage of plant development during which time roots are formed and the vegetative and reproductive primordia are differentiated. In case of tomato plants, this stage starts immediately after two weeks from the expansion of the cotyledonary leaves (17). The effect produced by inoculation during this stage of plant development determines the response observed during subsequent stages of plant development and the yield obtained. Inoculation at a later stage of plant development or after this critical stage had started either would result in no effect on growth or have slight stimulating effect (9). Inoculation of tomato transplants (cultivar Homstead) with Azotobacter at the age of 60 days resulted in no significant increase in the vegetative growth of the plants (2). This supports the importance of time of inoculation for obtaining stimulation of growth in tomato and may also indicate varietal differences in response to inoculation.

The results of the field experiment agree with those of the greenhouse experiment. These results show that the improvement induced by seed inoculation continued until later stages of plant development and was reflected in highly significant increase in the total yield over the untreated control. The increase in total yield by reinoculation at transplanting was not significant. However, the early yield obtained under the reinoculation treatment (seed plus seedling inoculation was significantly higher than that obtained under the seed inoculation alone. This may be due to the effect of reinoculation on early fruiting and fruit ripening.

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