INTERACTION BETWEEN SOME HIGHER PLANTS AND AZOTOBACTER CHROCOCCUM

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Abstract

The interaction between four higher plants and Azotobacter chroococcum was studied in soil extract solution under sterile conditions. Cell counts of the test organism increased in the solution and on the rhizoplane. Incoulation with bacteria increased the dry weight of the test plants and their length. In addition it increased the nitrogen content of these plants and that of soil extract solutions. The extent of nitrogen fixation depends on the plant type.

Introduction

The beneficial effects of Azotobacter chroococcum on plant growth in soil of low fertility are well documented since long time ago (Mishustin and Naumova 1962; Jackson et al., 1964; Rovira 1965 and Denarié and Blacheré 1966). Recent work by several authors (Monib et al, 1979; Mahmoud et al. 1984; Kumar et al. 1984 and Barbash and Lozhikina 1985) have shown the beneficial effects of associative symbiosis between Azotobacter and certain higher plants.

Some authors assumed that bacteria utilize root exudates and provide the plants with nitrogenous compounds they secrete during N_2-fixation. However, Clark (1969) showed that different sugars, organic acids and amino acids were detected in root secretion

ov various plants. The role of organic matter in the interaction of higher plants and microorganisms in artificial ecosystem was studied by Golovko et al. (1984) who found that root exudates of cabbage and stubble root remains produce an increased in the quantity of certain microbial groups and they also found that perlite cultivation results in the accumulation of amino acids (64.1-120 mg per 100 g of dry perlite).

Brown (1982) thouth that the beneficial effects result from absorption by the plant roots of growth regulator substances produced in the bacterial culture used as inoculum, and from production of such substances in rhizosphere rather than from nitrogen fixation by the bacteria in the soil.

The present work was carried out to test the ability of a strain of Azotobacter chroococcum to colonize on the root system of some higher plants (wheat, barley, lupine and fenugreek) grown in soil extract solution under sterile conditions. The effect of interaction between plants and the test organism was evaluated by recording the change in dry weight of plants, length and nitrogen content as well as nitrogen content in soil extract solution.

Material and Methods

Barley, wheat, lupine and fenugreek were grown in soil extract culture to examine their response to inoculation with

Azotobacter. For each type of plant, seeds were chosen similar in size and weight and surface sterilized with 95% alcohol and 0.1% HgCl$_2$ solution (Rovira 1956). Seeds were then placed in petridish on sterile cotton wool, impregnated with distilled water and kept at 25°C till root emergence. Soil extract solution was distributed after sterilization in conical flasks (100 ml capacity) at the rate of 50 ml, plugged with cotton wool and resterilized. A batch of conical flasks was prepared for each type of seed, two thirds of which were inoculated with 1 ml of Azotobacter suspension of known cell density, prepared by growing the organism on nitrogen-deficient agar plates for 5 days at 30°C, then suspended in sterile distilled water and the one-third was left without inoculation as a control. Three seedlings aseptically placed on sterile thin cloth and tightly wrapped with threads and fine holes in the centre to permit downward movement of the root. Another set of conical flasks, inoculated with Azotobacter but without plants, was used as a second control. Conical flasks were kept in a room, exposed to day light after wrapping the bottom with paper to protect the roots from light.

Examination were carried out at weekl, intervals. For each type of plant, three of the inoculated conical flasks as well as three of the uninoculated ones were taken at random to estimate plant length, dry weight and nitrogen content of plants and soil extract solution. Bacteriological analysis was made on some other three conical flasks. Soil extract solution was made up to the original volume with distilled water before analysis. Control conical flasks were treated similarly.

Bacteriological and chemical analysis. For the determination of Azotobacter viable counts on the rhizoplane, 50 ml of sterile water and glass beads were added to the bottle containing the roots, while in solution, glass beads were placed in the original cultures. After mechanical shaking for 5 min. 10-fold dilutions were prepared and five tubes of nitrogen-deficient agar medium were inoculated from each dilution each tube was poured in petridish and incubated at 30°C for 15 days, then counted.

For chemical analysis, plants were dried at 100°C to constant weight and pulverised. Total nitrogen in plants as well as in soil extract solution was determined using the kjeldahl method (Jackson 1958).

Results

Effect of bacterization on plant growth

The effect of inoculating plants with Azotobacter was assessed by the determination of plant length and dry weight, nitrogen content of plant and soil extract solution. The favourable effect of bacterization was noticed after two weeks where inoculated plants were heavier and longer than uninoculated ones, but the response to bacterization varied from one plant to another (Tables 1, 2 and 3). At the end of the experiment, the percentage increase resulting from inoculation was varied from 16.00 to 25.68 for dry weight of shoot, from 12.50 to 33.33 for dry weight of root and from 14.28 to 20.38 for height of shoot of the tested plants (Table 4). The

effect of inoculation is also indicated by the increase in nitrogen content in plant tissue and in soil extract solution. Big amounts of nitrogen were found due to the interaction between plants and Azotobacter but type of plant plays an important role in that respect, barley and wheat gave higher amounts, while lupine and fenugreek gave lesser amounts during the five weeks of experiment. At the same time soil extract solution was found to contain big amount of nitrogen which had fixed by the bacteria. It was found higher in barley and wheat soil extract solution and lower in lupine and fenugreek (Table 5). It is evident that no significant changes in nitrogen were noticed in system devoid of Azotobacter, indicating that no nitrogenous compounds e.g. NH_3 were introduced in the system during the period of the experiment. As a result of the association between the tested plants and the N_2-fixer microorganisms all systems invariably showed positive results.

Effect of plant type on the development of Azotobacter.

It is clear from (Table 6) that all root system harboured population of Azotobacter within the first week ranging from 1.0 - 2.9 x 10^4 /root. Changing in microbial density thereafter depended on the plant type. In case of barley and wheat progressive increase was noticed at the end of the experiment while in case of lupine and fenugreek slight increase was noticed. Counts of graminaous plants were ranging from 4.6 - 6.2 x 10^4/root, while of leguminous plants, counts were ranging from 3.0 - 3.9 x 10^4/root.

Regarding the changing in bacterial counts of soil extract solution, it is clear that Azotobacter could survive for relatively long periods in soil extract solution lacking carbon source since the change in counts in the control was remain more or less constant, no change in nitrogen content of soil extract was recorded. Azotobacter counts ranging from 2.0 - 3.9 x 10^4/flask within the first week for the tested plants reaching from 5.9 - 6.1 x 10^4/flask for graminaous plants and from 4.6 - 5.9 x 10^4/flask for leguminous plants at the end of the experiment. Since the product of root exudates are not identical for different plants, the effect on the propagation of Azotobacter is vary according to the plant involved. Wheat followed by barley seemed to be the most favourable plant in that respect. Fenugreek and lupine followed wheat and barley. Bacterial count of graminaous plants increased 10-12 fold in whole system, while leguminous plants increased 8-9 fold at the end of the experiment comparable to the initial bacterial count at the begining of the experiment.

Discussion

The beneficial association between higher plants and Azotobacter has been extensively studied from different points of view. This relationship which could not be regarded as symbiosis is still obscure in its nature. Since many years ago several authors had suggested that Azotobacter probably influene the development of plants by producing growth regulating substance (Burger and Bukatsch 1958;

Brakel and Hilger 1965; Vancura and Macura 1960; Burlingham 1964; Hennquin and Blacheré 1966 and Brown and Barlingham 1968). While others have reported that beneficial effects are due to root secretion (Clark 1969). Moreover Berestetskii and Kravchenko (1980) showed that volatile organic compound which found in germinating wheat, corn, pea and lupine seeds were used as energy source for soil microorganisms.

For rhizobacteria to exert physiological effects on plant growth the bacteria must first effectively colonize the root surface, Douglas et al (1985). It was found in this investigation that all root systems harboured population of Aotobacter witin the first week, while microbialdensities changed thereafter depending on the plant type It was higher in presence of wheat and barley and lower in presence of lupine and fenugreek. The results reported herein were in full agreement with those obtained by Monib et al (1979) who found that microbial propagation depended on the type of plant being much higher in presence of wheat, followed by barley, maize, broad bean and cotton, while in presence of fenugreek and lentil lower rates of multiplication were recorded.

Microbial propagation depends not only on the type of plant but also on the variety of the same plant, however kumar et al. (1984) found that the rhizosphere of cotton variety H14 harboured significantly higher population of bacteria, actinomycetes, fungi and Azotobacter than variety P 5-10. Contrary, Mahmoud et al. (1984) noticed that total microbial flora of tomato and common

bean plants decreased slightly during the growth season in soil and rhizosphere. The R/S ratio were positive depending on plant species and ages. There was destinct relation between stages of plant development and the densities of total microbial flora. Densities of Azotobacter seemed to be contstant in their low count in the rhizosphere.

Inoculation of plants with bacteria increased their dry weight length and nitrogen content in plants and in soil extract solution. However, Monib et al. (1979) found that dry weight of plants increased by 5-12% and length by 3-18% in addition to increase nitrogen content of plants and nutrient solution.

Recent work by Azcon et al. (1978); Bagyaraj and Menge (1978) and Carr (1981) has shown that if an inoculum of Azotobacter chroococcum is added with the endophyte, plants grow better than if inoculated with endophyte only. Azotobacter chroococcum alone has been used for many years as a bacterial inoculant to improve plant growth, but only occasionally yields have been increased significantly (Brown 1974). Thus dual inoculation may prove to be more benificial in soil of low fertility than inoculation with either organism alone (Brown and Carr 1984). In this respect further studies were needed using dual inoculation for increasing soil fertility and crop yield.

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Table 1: Dry weight of shoot (mg) at different periods (weeks) (mean of three shoots) .

Plants		1	2	3	4	5
Barley	A	39	39	42	51	67
	B	46	48	55	62	82
Wheat	A	28	29	32	45	87
	B	48	50	50	54	103
Lupine	A	437	484	521	535	545
	B	516	521	530	547	685
Fenugreek	A	27	28	42	60	100
	B	59	61	74	78	116

A uninoculated with Azotobacter
B inoculated with Azotobacter

Table 2 : Dry weight of root (mg) at different periods (weeks) (mean of three roots) .

Plants		1	2	3	4	5
Barley	A	12	19	22	26	32
	B	14	22	24	29	36
Wheat	A	11	13	24	35	47
	B	13	16	30	39	50
Lupine	A	54	[illegible]7	75	82	88
	B	68	[illegible]	84	95	107
Fenugreek	A	9	[illegible]	[illegible]	17	27
	B	11	[illegible]	[illegible]	21	36

A uninoculated with [illegible]

B inoculated with Azotobacter

Table 3 : Height of shoot (cm) at different periods (weeks) (mean of three shoots).

Plants		1	2	3	4	5
Barley	A	6.55	7.00	8.11	8.16	8.20
	B	6.91	7.83	9.27	9.35	9.50
Wheet	A	5.22	5.44	5.75	6.44	7.00
	B	6.55	6.77	7.00	7.30	8.00
Lupine	A	12.33	13.96	15.00	16.77	15.50
	B	12.55	15.05	15.16	16.83	18.66
Fenugreek	A	2. 77	2. 77	3. 66	6. 00	11.66
	B	3. 05	3.72	5. 77	8. 50	13.33

A uninoculated with Azotobacter
B inoculated with Azotobacter

Table 4 : Percentage increase over control of dry weight (mg) of shoot, root and height of shoot (cm) after five weeks (mean of three readings)

Plants	Dry Weight of shoot			Dry weight of root			Height of shoot		
	A	B	%	A	B	%	A	B	%
Barley	67	82	22.30	39	36	12.5	8.20	9.50	15.8
Wheat	87	103	18.30	47	50	27.6	7.00	8.00	14.28
Lupine	545	685	25.68	88	107	21.5	15.50	18.66	20.38
Fenugreek	100	116	16.00	27	36	33.33	11.66	13.33	14.32

A uninoculated with Azotobacter

B inoculated with Azotobacter

Table 5 : Nitrogen content of plants and soil extract solution of the different periods

Plants		In plants(mg / 100 mg dry weight)					In solution (mg /100 ml)				
		Weeks					Weeks				
		1	2	3	4	5	1	2	3	4	5
Barley	A	1.80	2.30	2.80	2.80	2.90	1.2	1.2	1.3	1.4	1.60
	B	1.90	2.30	2.60	3.50	3.70	1.60	5.00	8.40	11.00	13.10
Wheat	A	1.90	2.50	2.90	3.10	3.30	3.30	3.40	3.50	3.60	3.60
	B	2.70	3.00	3.40	4.50	4.30	3.4	5.40	8.80	11.5	14.60
Lupine	A	6.4	7.6	7.70	8.60	8.90	4.60	4.60	4.40	4.20	4.20
	B	7.6	8.4	9.6	9.60	10.6	3.60	3.60	5.80	6.40	7.20
Fenugreek	A	1.80	3.10	4.00	4.40	4.50	2.60	2.81	3.70	3.75	3.83
	B	3.40	3.40	3.50	4.40	5.50	3.8	4.1	4.28	4.92	5.50

A uninoculated with Azotobacter
B inoculated with Azotobacter

extract solution :

Plants	*Azotobacter* counts in rhizoplane x 10^4					*Azotobacter* counts in soil extract solution x 10^4				
	Weeks					Weeks				
	1	2	3	4	5	1	2	3	4	5
Barley	1.0	1.2	1.8	3.1	4.6	2.0	2.3	4.2	5.0	5.9
Wheat	2.9	3.1	3.3	5.3	6.2	3.9	5.0	5.1	5.5	6.1
Lupine	1.9	2.5	2.8	3.4	3.9	3.9	4.0	4.1	4.3	4.6
Fenugreek	1.4	1.5	1.7	2.1	3.0	3.0	3.3	4.9	5.7	5.9

Intial *Azotobacter* count was 1.5×10^4 in whole system .

العلاقة بين بعض النباتات الراقيه وبين نوع معين من الميكروبات الدقيقة

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ملخص

يهدف هذا البحث الى معرفة علاقة النباتات الراقيه بنوع معين من الميكروبات الدقيقه هو الازوتوباكتر كروكوكم فى مستخلص التربه المعقم على مدى خمس اسابيع من بداية التجربة . لذلك تم تعيين كل من الوزن الجاف للساق والجذر وطول الساق والمحتوى النيتروجينى لكل من النبات ومستخلص التربه وكذلك عد البكتيريا الموجوده على الجذر والموجوده فى محلول مستخلص التربه هذا وقد اوضحت النتائج ما يلى :

١— كانت هناك زيادة محسوسه فى الوزن الجاف لكل من الساق والجذر وكذلك زيادة فى طول الساق وذلك نتيجة للعلاقة التى نشأت بين النبات الراقى وبين الكائن الدقيق حيث كانت هذه الزيادة تختلف باختلاف نوع النبات .

٢— النباتات النجيلية كانت اكثر استجابة لهذه العلاقة عن النباتات البقولية .

٣— زاد المحتوى النيتروجينى لنبات القمح والشعير بنسبة اكبر من نبات الترمس والحلبه وزاد المحتوى النيتروجينى فى مستخلص التربه للقمح والشعير عنه فى الترمس والحلبه .

٤— اعداد البكتريا على جذر نبات القمح والشعير كانت اكبر من اعدادها على جذر نبات الترمس والحلبه واعداد البكتريا فى مستخلص التربه كان اعلى فى النباتات النجيلية عنه فى النباتات البقولية . بينما كانت اعداد البكتريا على الجذور عموما اقل من اعدادها فى مستخلص التربه .