INFLUENCE OF ROOT EXCRETIONS AND GERMINATING SEEDS ON NITROGEN-FIXATION BY AZOTOBACTER

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In a previous paper (Uppal *et al.*, 1939), it was shown that *Azotobacter* from cultivated rice soils was stimulated to greater activity in fixing atmospheric nitrogen when grown in pure culture in association with growing roots of rice plants. This increased activity on the part of the micro-organism was further shown to be due to a change in reaction of the culture medium brought about by rice seedlings growing in it. In the experiments to be described below, the influence of the presence of growing roots of wheat, *jowar* (*Sorghum vulgare* L.) and radish on the fixation of nitrogen by *Azotobacter* in Ashby's cultures, has been investigated with a view to determining whether the living roots of these plants also stimulate the development of the micro-organism and thus enhance its nitrogen-fixing efficiency.

Experimental Methods and Results

For the purpose of inoculation, isolate III of *Azotobacter*, which has previously been shown to be an efficient nitrogen-fixer (Uppal *et al.*, 1939), was grown on Ashby's agar, and scrapings from a two days' growth of this micro-organism were used to inoculate the culture bottles containing 50 ml. of Ashby's solution.

Seeds from which experimental seedlings originated, were disinfected in a one in 500 solution of corrosive sublimate for 15 minutes. These seeds were then washed in sterile water and aseptically removed to sterilised glass chambers lined inside with sterile, damp filter-paper. When the seedlings had made growths of 2 to 3 inches in length, 9 seedlings of each kind were transferred to culture bottles.

Four sets of culture bottles each containing 50 ml. of Ashby's solution were prepared for each kind of plant and were treated in the following manner:—

(1) Inoculated with Is. III,

(2) Nine seedlings of each kind were grown in each culture bottle in this set,

(3) Same as in (2) above but each culture bottle was inoculated with Is. III, and

(4) Same as in (3) above, and in addition one gram of sterilised soil was added to each culture bottle.

At the conclusion of the experiment after 20 days, the total nitrogen was determined from duplicate samples of each culture bottle including the seedlings and soil. The results are given in Table I.

TABLE I

Influence of growing plant roots on nitrogen-fixation by Azotobacter

(Total nitrogen in mg.)

Ashby's solution containing	Radish		Rice		Wheat		Jowar	
	Individual bottle	Average	Individual bottle	Average	Individual bottle	Average	Individual bottle	Average
9 seedlings ..	4·31	4·27	1·66	1·51	7·71	8·03	4·20	3·93
	4·23		1·35		8·36		3·66	
9 seedlings+ ..	6·89	6·37	2·27	2·35	8·71	8·48	4·39	4·34
Is. III ..	5·85		2·44		8·26		4·30	
9 seedlings+ ..	9·43	9·02	5·06	4·88	13·23	13·44	7·23	7·31
Is. III+soil ..	8·61		4·70		13·66		7·39	
Net nitrogen fixed by Is. III in presence of seedlings and soil	3·17		1·79		3·83		1·80	

N.B.—Ashby's solution and soil in these experiments contained 0·06 mg. N and 1·58 mg. N respectively. Is. III alone fixed 0·48 mg. N.

Results in the above table show that, as reported earlier (Uppal *et al.*, 1939), *Azotobacter*, in association with living roots of seedlings alone or in combination with soil, was able to fix larger amounts of nitrogen than in the absence of such association. The organism was aroused to greatest activity when wheat seedlings were used, and fixed 3·83 mg. {13·44—(8·03—1·58)} nitrogen, followed closely by radish (3·17 mg. N). Rice and *jowar* were poor in this respect and exerted an almost equal degree of stimulation. It may be noted, however, that, when wheat seedlings were used alone without the soil, the organism did not fix, in such association, as much nitrogen as when radish was used. The significance of these results is not quite clear at present.

Hiltner, as quoted by Waksman (1931), found that "non-symbiotic nitrogen-fixation is stimulated by growing plant roots; the higher plants use up the available nitrogen in the soil and thus create a nitrogen-hunger

for the non-symbiotic nitrogen-fixing bacteria. The plants supply the bacteria with available energy, in the form of rotting root, hairs, root tips, etc." Waksman and Starkey (1931) came to a similar conclusion—" in the neighbourhood of growing roots of plants there is an excretion of soluble carbohydrates and addition of other residues to the soil which may serve as food for bacteria. Plants rapidly consume most of the available combined nitrogen from this portion of the soil. These two factors, namely, the presence of available sources of energy and a nitrogen minimum, would favour the rapid development of *Azotobacter* and *Clostridium* and lead to nitrogen fixation ". Vyas (1934), working with maize seedlings, also noted that the maize roots excreted some stimulative product which enabled the non-symbiotic nitrogen-fixing micro-organisms to fix larger amounts of nitrogen. Viswa Nath reported (1939) that the gain in the nitrogen-content of the soil under field conditions may be partly due to the stimulating action of root excretions on the nitrogen-fixing bacteria.

Whatever may be the contributing factors, it is obvious that the association of growing roots of plants with *Azotobacter* stimulates the development of the latter and leads to an enhanced activity on its part in fixing atmospheric nitrogen, although it may be noted that the beneficial effect so exerted on the micro-organism varies with the type of associated plant. It is claimed, however, by some workers that sprouting seeds and living plants themselves have the power of fixing atmospheric nitrogen during germination and the subsequent growth of the seedling. Lipman and Taylor (1924) found that wheat and barley plants grown in culture solutions fixed nitrogen, but it may be noted that no attempt was made in these experiments to maintain sterile conditions and that tap water was used for making culture solutions. Burk (1937), in controlled experiments with peas, did not obtain any evidence of nitrogen-fixation during germination of pea seeds.

Sen (1929) has suggested the possibility that nitrogen-fixing micro-organisms may live symbiotically in the roots of rice plants, whilst Viswa Nath (1932) holds the view that the rice plant itself has the power of fixing atmospheric nitrogen. The latter (1940) has also reported that maize seed, when germinated in a known volume of air devoid of all combined nitrogen, absorbed atmospheric nitrogen during germination of the seed and the subsequent growth of the seedlings. Jamieson, as quoted by Winters (1924), claimed that all green plants possess the power of fixing nitrogen. On the other hand, Krassilnikov, as quoted by Lochhead (1940), has shown that *Azotobacter* was unable to grow in the rhizosphere of wheat, *i.e.*, the subterranean part of the plant system, and was severely suppressed in that of maize. He attributed this to the toxic effect of root secretions.

In view of the conflicting evidence on the ability of growing plants to fix atmospheric nitrogen, an experiment was done to determine whether seeds absorbed nitrogen during germination and the subsequent growth of seedlings. Seeds of wheat, *jowar* (*Sorghum vulgare* L.) radish and pea, which were previously disinfected in a one in 500 solution of corrosive sublimate for 15 minutes, were germinated on damp filter-paper in moist, sterilised chambers at room temperature. Fifty seeds and an equal number of 4- and 20-day old seedlings of each kind of plant were analysed for total nitrogen. Results are presented in Table II and show that germinating seeds and young seedlings do not possess the power of fixing elemental nitrogen when tested under aseptic conditions.

TABLE II

Nitrogen-fixation by germinating seeds and seedlings

(Total Nitrogen in gm.)

Kind of plant	Seeds		4-day old seedlings		20-day old seedlings	
	50 seeds	Average	50 seedlings	Average	50 seedlings	Average
Radish ..	0·026 0·024	0·025	0·023 0·027	0·025	0·029 0·027	0·028
Rice ..	0·007 0·007	0·007	0·008 0·008	0·008	0·007 0·007	0·007
Wheat ..	0·049 0·053	0·051	0·048 0·048	0·048	0·044 0·036	0·040
Jowar ..	0·020 0·017	0·018	0·020 0·018	0·019	0·019 0·016	0·018
Pea* ..	0·197 0·174	0·185	0·184 0·185	0·184	0·187 0·177	0·182

* In the case of peas, 25 seeds or seedlings were used.

SUMMARY

In the presence of growing roots of wheat, radish, rice and *jowar* in Ashby's cultures, *Azotobacter* fixed larger amounts of atmospheric nitrogen than in their absence. The stimulating effect, however, varied with the kind of plant used, wheat exerting the greatest beneficial effect followed closely by radish. Rice and *jowar* were poor in this respect and exerted an almost equal degree of stimulation.

None of the seeds tested possessed any power of fixing elemental nitrogen during germination and the subsequent growth of the seedlings.

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