Root exudate of tobacco (*Nicotiana tabacum* L.) as chemoattractant for *Azospirillum*

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The nitrogen-fixing bacterium *Azospirillum* occurs in fairly large numbers in the rhizosphere of tobacco (*Nicotiana tabacum* L.). Root exudate from axennically grown tobacco plants (var. Bagyalakshmi) induced positive chemotactic response in *Azospirillum brasilense*. We suggest that the sugars and amino acids in the root exudate serve as chemoattractants to the bacteria.

IT has been well documented that motile bacteria sense and respond to changes in the concentration of chemicals in the environment and change their direction of movement[1,2]. Bacteria swim towards chemicals that serve as attractants and move away from compounds that are repellants. This behavioural response, called chemotaxis, has been studied in detail in enteric bacteria like *E. coli* and *Salmonella*[3]. In plant root-associated nitrogen-fixing bacteria like *Azospirillum*, chemotaxis plays a significant role. Positive chemotaxis has been considered to be an expression of the nutritional requirements of the organism. Recently, Okon *et al*[4] demonstrated positive chemotaxis of *Azospirillum* cells towards various carbon compounds. In this communication we report the chemotactic response of *Azospirillum brasilense* (ATCC 31459) isolate Sp. 7 and tobacco strains of *Azospirillum brasilense* (Az.Nt.5) to root exudates of tobacco (*Nicotiana tabacum*).

A popular chewing variety of tobacco, Bagyalakshmi, was chosen for the study. Root exudate was collected following the method of Lee and Gaskins[5]. Fifteen-day-old tobacco seedlings raised in pots were transferred to pre-sterilized 500-ml-capacity wide-mouthed bottles containing 100 g of acid-washed sand moistened with half-strength Fähraeus solution[6]. The mouth of the bottle was covered with paper to avoid contamination. The assembly was left undisturbed for three weeks, in about 4 h of sunshine per day; when required, the sand was moistened with Fähraeus solution. After three weeks the seedlings were gently removed and root exudate collected in the sand bed was extracted with 80% cold ethyl alcohol. After condensation, the exudate was finally passed through a Seitz filter.

The root exudate was analysed for total and reducing sugars[7], and amino nitrogen[8]. Qualitative paper chromatograhy was used for identification of amino acids[9].

Azospirillum brasilense Sp. 7 and Az.Nt.5 were grown in 25 ml yeast extract glucose broth for 48 h at 30°C on a shaker (110 strokes per minute) and the cells were harvested by centrifugation. Washed cells were suspended in sterile physiological saline and finally suspended in 100 ml of chemotactic buffer (pH 7.2), which contained 10 mM potassium phosphate, 1 mM magnesium chloride and 0.1 mM EDTA in distilled water.

Adler's capillary tube method[10] and paper disc method[4] were used in the chemotaxis assay. *Azospirillum* cells were exposed to the root exudate taken in a capillary tube by placing the open end of the tube into the glass chamber containing the cell suspension. After 60 and 120 min of incubation at 30°C, the tubes were carefully removed and rinsed with 10 ml of sterile buffer. The number of bacteria in the capillary tube was determined by serial dilution on yeast extract glucose agar. Control tubes contained only the chemotactic buffer.

In the paper disc assay[11], 100 μl of root exudate was allowed to be absorbed into a 10 mm sterile filter paper disc and the disc was air-dried. Petri plates containing yeast extract glucose agar seeded with the two isolates of *Azospirillum* were prepared and the paper discs were placed on the surface. The plates were growth examined after incubation for growth of bacterial colonies around the paper disc. Control discs carried only chemotaxis buffer.

Root exudate of tobacco induced a positive chemotactic response in both isolates of *Azospirillum*, although the response of isolate Az.Nt.5 was greater (Table 1). Analysis of the root exudate showed that it

Table 1. Chemotactic response of *Azospirillum* towards root exudates of tobacco.

Treatment	Isolate	Cell count (10^5 ml^{-1}) 24 h	48 h
Sterile buffer	Sp. 7	19.0	24.0
	Az.Nt.5	12.0	18.0
Root exudate	Sp. 7	69.0	92.0
		(263)	(283)
	Az.Nt.5	86.0	148.0
		(616)	(722)

Data are mean of three determinations. Figures in parentheses are per cent increase over control.

Table 2. Composition of root exudates of tobacco (var. Bagyalakshmi).

Component	Concentration (μg ml^{-1})
Total soluble sugars*	186.0
Reducing sugars*	2.8
Amino nitrogen†	86.6
Amino acids (qualitative determination) Aspartic acid, arginine, alanine, cystine, histidine, threonine, tyrosine, tryptophan, phenylalanine and leucine.	

*In glucose equivalents.
†In glutamic acid equivalents.

was fairly rich in amino nitrogen and soluble sugars (Table 2). Chromatographic analysis indicated the presence of several amino acids. Barak *et al.*[11] demonstrated a positive response of *A. brasilense* towards several organic acids, sugars and amino acids. In recent years even strain-specific chemotaxis has been demonstrated in *Azospirillum*[12]. Maize and kallar grass (C4 plants) isolates of *Azospirillum* showed more chemotactic response to malic acid while isolates from C3 plants were attracted by amino acids and sugars.

The results indicate that root secretions of tobacco can not only serve as a source of energy for micro-organisms but, perhaps more importantly, attract the diazotrophic bacterium *Azospirillum*. At present we do not know the chemotactic behaviour of the other three species of *Azospirillum*, *A. lipoferum*, *A. serapedica* and *A. amazonense*.

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Accumulation of choline and glycinebetaine in salt-stressed wheat seedlings

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Choline and glycinebetaine accumulation in 16-day-old salt-resistant wheat cv. Sonalica grown in medium containing 0 to 200 mM NaCl increased with increasing salinity in both shoots and roots. Per cent increase in accumulation was linearly correlated with salinity. The results are consistent with the hypothesis that choline and glycinebetaine are compatible cytoplasmic solutes involved in osmotic adjustment to salinity stress. We discuss the physiological significance of these quaternary ammonium compounds.

ALIPHATIC quaternary ammonium compounds, in particular glycinebetaine and choline, are found in a large number of plants, although their concentrations vary widely in different species[1], in different organs[2] and with age of the plant[3]. Glycinebetaine concentration usually exceeds that of choline and may even form a large percentage of the total plant nitrogen. Yet, the physiological function of quaternary ammonium compounds has received scanty attention. It has been suggested that these compounds may act as a major cytoplasmic osmoticum under conditions of low intracellular osmotic potential[4]. In previous reports, we have examined the relationship between salt tolerance and the endogenous concentration of glycinebetaine and total quaternary ammonium compounds in shoot and root systems during different stages of growth in rice[5,6]. We have found that the salt-tolerant cultivars of rice are highly efficient in maintaining high levels of glycinebetaine and total quaternary ammonium compounds than the salt-sensitive cultivars. In view of the findings concerning cellular and physiological processes involving glycinebetaine and choline that underlie salt tolerance of rice[5,6], wheat[7,8] and other plants[1], we did experiments to find the relationship, if any, between salinity levels of growth medium and concentrations of choline and glycinebetaine in shoots and roots of seedlings of a salt-resistant wheat cultivar, Sonalika.

Seeds of wheat (*Triticum aestivum* L.) cv. Sonalika (salt-resistant) were obtained from the Gujarat Agricultural University, Vijapur. Seeds of uniform size were surface-sterilized with 0.1% mercuric chloride for three minutes, and washed thoroughly with distilled water and blotted dry. These seeds were transferred to sterilized petri dishes containing Whatman No. 1 filter paper circles moistened with distilled water. The seeds were kept for germination for 5 days. Six-day-old seedlings were supplied unaerated nutrient solution[8] containing 0.025, 0.050, 0.075, 0.100, 0.125, 0.150, 0.175 or 0.200 M NaCl. NaCl was omitted from control nutrient solution. The nutrient solution was renewed twice a week. All plants were grown under conditions of 16 h light (with six 100 W incandescent lamps) and 8 h dark at $26 \pm 1^{\circ}C$. Shoots and roots were harvested 16 days after germination for estimation of choline and glycinebetaine. Choline and glycinebetaine were determined by the periodide-dichloroethane method[7], with modifications in the preparation of periodide reagent[9]. The data were statistically analysed for linear correlation[10].

Figure 1 shows that choline and glycinebetaine increased with increasing level of NaCl salinity in both shoots and roots. Even though choline and glycinebetaine are synthesized from the same precursor ethanolamine[11], choline content of shoots and roots always exceeded glycinebetaine at all levels of salt treatment (Figure 1*a*, *b*). Per cent increase in accumulation of these two compounds in both shoot and root were correlated linearly ($r = 0.945$ to 0.998) with salinity of growth medium (Figure 2).