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ROOT EXUDATION OF SORGHUM AND UTILIZATION OF EXUDATES BY NITROGEN-FIXING BACTERIA

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Summary—Qualitative differences in the soluble exudates of seedlings of different sorghum (*Sorghum bicolor*) genotypes were demonstrated by variation in growth of diazotrophic bacteria in exudate-containing media. Isolates varied in their ability to utilize the exudate from a given genotype. Variation in the amounts of soluble carbon exuded by axenically grown seedlings of the different genotypes was also observed. However, the variation in plant-associated acetylene reduction activity of one strain, *Azospirillum lipoferum* 4ABL, did not reflect the variation in exudation of soluble organic carbon, nor the variation in ability of the isolate to utilize the exudates in semi-solid media.

INTRODUCTION

There are numerous reports of plant responses to inoculation with nitrogen-fixing bacteria (Okon, 1984). Whether these responses result from a direct improvement of nitrogen nutrition through fixation, from the effects of plant hormones produced by the bacteria, or from the effects of the inoculum on other rhizosphere organisms, such as the inhibition of minor pathogens, is still uncertain. The survival and competitive ability of introduced microorganisms in the plant rhizosphere are dependent not only on numerous abiotic factors such as temperature, pH, moisture and oxygen tension; but also on the types and numbers of other organisms present and the quality and quantity of root exudates and plant debris available as carbon and energy sources.

In inoculation experiments host plant-bacterial strain interactions have been observed by a number of workers and in a few cases been related to root exudates. Neal and Larson (1976) reported the stimulation of a N_2-fixing *Bacillus* sp. in the rhizosphere of certain wheat lines; and there are differences in the establishment of *Azospirillum* strains in wheat roots (Baldani *et al.*, 1983) and in the rhizosphere of *Zea mays* (O'Hara *et al.*, 1981). *Azospirillum*, as compared to *Azotobacter*, *Klebsiella*, *Rhizobium*, *Pseudomonas* and *Escherichia*, is preferentially absorbed to pearl millet and guinea grass roots (Umali-Garcia *et al.*, 1980). Substances in the root exudates bind to *Azospirillum* and promote this absorption.

Differences in associative N_2 fixation between genotypes of various grasses and cereals have been demonstrated using C_2H_2 reduction (Neyra and Dobereiner, 1977; Dart and Wani, 1982). Cultivars of sorghum have demonstrated this variability at the seedling stage when grown in tubes, in pots in the glasshouse, and in field experiments (ICRISAT, 1983). In this study we have examined the exudation of soluble carbon from seedlings of 6 sorghum genotypes and compared exudation, plant growth and plant associated nitrogenase activity in microbiologically defined conditions. As host cultivar-bacterial strain interactions have been observed in field inoculation experiments we have also examined the utilization of soluble root exudates by different N_2-fixing bacteria.

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MATERIALS AND METHODS

Exudate collection and concentration

Seeds of four *Sorghum bicolor* (L.) Moench lines (IS 3003, CSH 5, IS 2333, IS 15165) were surface sterilized as follows: they were first washed (on a rotary shaker) in 3.5% H_2O_2 (v/v with H_2O) for 1.5 h, rinsed in sterile water, washed for 2 h in 2% chlorox (v/v H_2O), and then rinsed again in sterile water. The seeds were put on nutrient agar plates at 33°C to germinate and to check for contamination. After 48 h, seedlings were transferred (4/tube) onto stainless steel wire mesh supports in cotton-plugged, sterile tubes (25 × 200 mm) containing 20 ml distilled water. Plants were held in a glasshouse with the temperature ranging from 21°C at night to 37°C around noon. Every 2 days for 8 days, the tubes were shaken, the medium decanted and fresh sterile water added. Non-contaminated media samples were frozen and then lyophilized to concentrate to about 0.05 (5%) the volume.

Growth of bacteria in exudate media

Semi-solid (0.175% agar w/v) salts medium was prepared as follows: 0.5 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, 2 mg $Na_2MoO_4 \cdot 2H_2O$, 10 mg $MnSO_2 \cdot 2H_2O$, 4 ml of 1.64% aqueous Fe·EDTA, 20 mg $CaCl_2$, water to 1 l. After autoclaving and cooling concentrated exudates from the different sorghum lines were added to the semi-solid medium to give a final concentration of 0.4 g organic-

Table 1. Organic carbon exuded into the culture medium from day 2 to day 9, and plant root and shoot dry weights on day 9, of axenic seedlings of 6 sorghum genotypes

Genotype	Organic C (mg/tube)	Root wt (mg/tube)	Shoot wt (mg/tube)
IS 3003	9.5 (0.42)	29 (0.7)	52 (1.8)
IS 2333	9.4 (0.40)	23 (0.6)	32 (0.8)
IS 15165	8.4 (0.42)	37 (0.8)	80 (1.3)
CSV-5	6.9 (0.46)	25 (0.5)	37 (1.3)
CSH-5	5.9 (0.34)	24 (0.6)	42 (1.3)
IS 889	5.2 (0.30)	15 (0.6)	31 (0.7)

Means of 15–28 replications (SE).
Linear correlation coefficients:
organic C vs root wt $r = 0.60$ NS at $P = 0.05$;
organic C vs shoot wt $r = 0.38$.

DISCUSSION

Genotypes of sorghum have been shown to support different levels of associated nitrogenase activity, and in inoculation experiments bacterium by cultivar interactions were observed (ICRISAT, 1983). This study was undertaken to determine whether quantitative or qualitative differences in root exudation could be detected which might be responsible for these findings. Variation in the composition of soluble exudate was demonstrated. Diazotrophic bacteria are able to utilize a variety of compounds as carbon and energy sources, but whether the observed differences in this study resulted from variation in the relative amounts of these compounds or to the presence of promoter or inhibitory substances is yet to be determined. By 48 h, C_2H_2 reduction activity had decreased in all cultures and growth had usually ceased indicating substrate deficiency or poor ability of bacteria to utilize some of the exudate. Bacterial cultures 27155, 7ATZ and 480 had very low C_2H_2 reduction activity at both 24 h and 48 h in IS 2333 exudate media; however, significant growth of these cultures did occur during this period. There must have been either a brief undetected spurt of nitrogenase activity, or these cultures were able to scavenge some nitrogen from the medium. Bacteria utilized the exudate from a given genotype differentially. Some specificity has been observed both between diazotrophic species and between strains of a given species, in their ability to colonize the roots or rhizosphere of a given cereal. This may be a reflection, at least in part, of the observed differences in ability to utilize the soluble exudates present. Baldani and Dobereiner (1980) proposed some specificity of C4 plants for *A. lipoferum* over *A. brasilense*. In our experiments *A. lipoferum* strains exhibited the most growth but some of the *A. brasilense* strains also grew well on the exudate media. The non-*Azospirillum* isolates did not grow well. This was not simply due to an inability to use malate, a possible significant component of the exudates, as the four ICRISAT isolates grew well and exhibited high C_2H_2 reduction activity in both sucrose and malate containing media.

This work was restricted to exudates of seedlings but while recognizing that exudates change with plant age and are affected by many factors, seedling studies were thought to be meaningful as sorghum cultivar differences in associated nitrogenase activity (ICRISAT, 1983) and $^{15}N_2$ incorporation into plant tops (Giller *et al.*, 1984) have been observed at the seedling stage. Quantitative differences between genotypes in the exudation of soluble organic compounds were consistently observed (Fig. 2). There was no correlation between the amount of exudate and root or shoot weight (Table 1). Even with replicates of a given cultivar there was little relation ($r < 0.50$) between root weight and exudate. This is contrary to the observations of Prikryl and Vancura (1980) with wheat root exudation. The quantitative measures of exudates under these conditions are most likely an underestimate of those available from seedlings in soil where mechanical forces (Barber and Gunn, 1974), and soil microorganisms (Prikryl and Vancura, 1980; Lee and Gaskins, 1982; Barber and Martin, 1976) play an important role. There is a need for radio-tracer studies, similar to those of Whipps and Lynch (1983) and Beck and Gilmour (1983), using tropical (rather than temperate) grown cereals, to

Table 2. Acetylene reduction activity of *Azospirillum lipoferum* 4ABL associated with seedlings of 6 sorghum genotypes

Genotype	Experiment 1 Activity[a] (nmol C_2H_4 tube^{-1} h^{-1})	Experiment 2 Activity[b] (nmol C_2H_4 tube^{-1} h^{-1})	Root wt[c] (mg tube^{-1})	Shoot wt[c] (mg tube^{-1})
IS 3003	37 (5.2)	20	29 (2.6)	45 (3.4)
IS 2333	10 (1.7)	15	22 (0.8)	26 (1.1)
IS 15165	53 (9.0)	32	58 (1.8)	83 (2.1)
CSV-5	20 (2.3)	15	27 (1.1)	35 (2.1)
CSH-5	4 (3.2)	7	32 (1.1)	40 (4.3)
IS 889	13 (4.9)	8	15 (0.6)	25 (1.4)

[a]Assayed on day 9. Means of 18 replications (SE).
[b]6–10 replicates were assayed on days 4, 6, and 9. Means of the 3 days are given.
[c]Plant weights on day 9. Means of 6–10 replications (SE).
Linear correlation coefficients:
activity (expt 1) vs root wt $r = 0.85$ significant $P < 0.05$;
activity (expt 1) vs shoot wt $r = 0.93$ significant $P < 0.01$;
activity (expt 2) vs root wt $r = 0.83$ significant $P < 0.05$;
activity (expt 2) vs shoot wt $r = 0.86$ significant $P < 0.05$;
activity (expt 1) vs organic C $r = 0.45$ NS;
activity (expt 2) vs organic C $r = 0.65$ NS;
activity (expt 1) vs seed wt lost $r = 0.95$ significant $P < 0.01$.

estimate the potential amounts of substrate available for associative nitrogen fixation. For although there is evidence that some nitrogen-fixing bacteria are present within roots the numbers are small when compared to the rhizosphere populations that utilize exudate and cell debris. (McClung *et al.*, 1983; Giller and Day, 1985).

Sorghum genotypes varied in their ability to support nitrogen fixation by *A. lipoferum* 4ABL, but there was no correlation between activity and the amount of soluble exudate measured in axenic systems. The ranking of genotypes for plant associated activity also differed from that for the growth and activity of 4ABL in semi-solid exudate media. For example, IS 15165 and IS 2333 both exhibited high rates of exudation. C_2H_2 reduction activity associated with IS 15165 seedlings was high but that associated with IS 2333 seedlings was low, while in exudate media (containing equal organic carbon) the opposite ranking was observed. One possible explanation is that 4ABL alters the exudation pattern of the two cultivars differentially. In support of this there was little difference in the root weights of IS 2333 grown axenically and in the presence of 4ABL, while the root weight of IS 15165 was greater in the presence of 4ABL suggesting that the bacterium had stimulated carbon flow to and through the root system. However, for uninoculated plants, no correlation was observed between root weight of different cultivars and the amount of soluble exudate. A second explanation for the different rankings is that the non-soluble, non-diffusible exudates are more important in determining plant associated growth and C_2H_2 reduction activity. However, in the absence of other organisms which might at least partially break down the complex polysaccharides it is uncertain whether much of the non-diffusible material would be accessible to 4ABL.

One implication of the different genotype rankings between synthetic media and plant culture systems is that there may be little profit in screening bacterial isolates for potential rhizosphere activity by measuring their growth and C_2H_2 reduction activity in synthetic media (even root exudate containing media). It would be interesting to compare the relationship between root exudates and root extracts in supporting bacterial growth.

Varying inoculation responses of crops to diazotrophic bacteria have been observed by many workers; however before consistent beneficial effects can be expected a greater understanding of the plant rhizosphere nitrogen-fixing bacteria association is required. Our study has clearly demonstrated variation in amounts and composition of root exudates, but the relationship between exudation and bacterial growth and C_2H_2 reduction activity is not simple and needs further investigation.

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