Proc. Indian Acad. Sci. (Plant Sci.), Vol. 97, No. 1, February 1987, pp. 33–37.

Nitrogenase activity of pearl millet—*Azospirillum* association in relation to the availability of organic carbon in the root exudates

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MS received 30 May 1986; revised 28 January 1987

Abstract. There were significant differences in the amount of organic carbon exuded by the roots of different pearl millet varieties. The nitrogenase activity of the axenically grown pearl millet varieties inoculated with *Azospirillum* was related to the quantity of organic carbon released by their roots. However, the amount of exudate was found to be inadequate for the optimum expression of N_2-ase activity which was considerably enhanced following the addition of carbon source. The stimulation of activity varied with the type of a carbon source and was related to preference shown by the organism for its growth in vitro. There was no significant change in the total plant dry weight following the addition of carbon to the root zone but there was a marginal reduction in the root growth and the root/shoot ratio.

Keywords. Root exudates; organic carbon; N_2-ase activity; pearl millet; *Azospirillum*.

1. Introduction

Plant roots release a wide range of organic substances in the rhizosphere supporting the growth and activity of different microorganisms (Rovira 1965; Peters and Alexander 1966). In recent years, the nitrogen fixing bacterium, *Azospirillum* has received considerable attention because of its capacity to form nitrogen fixing associations with a wide range of host plants (Dobereiner and Day 1976; Rao and Venkateswarlu 1982) resulting in enhanced dry matter production and yield. Von Bulow and Döbereiner (1975), Neyra and Döbereiner (1977) and Vlassak and Reynders (1978) reported a large variation in nitrogenase activity among different varieties of maize, pearl millet and wheat. A number of mutual interactions between the roots of the host plant and microsymbiont have been observed by Glatzle and Martin (1981) competing for O_2 between respiring roots and the bacteria and by Hubbel *et al* (1981) for the role of auxins in plant growth response with *A. brasilense*. But no information is available on the role of organic carbon in the root exudates for successful establishment of the associative symbiosis in pearl millet. However, Lee and Gaskins (1982) with sorghum grown in nutrient solution and Beck and Gilmour (1983) with wheat reported enhanced exudation of organic carbon upon inoculation with N_2-fixing bacteria. The present paper deals with the exudation of organic carbon by different varieties of pearl millet and its relationship with the expression of N_2-ase activity associated with plants inoculated with *Azospirillum*. The effect of adding different carbon sources to the root zone on N_2-ase activity was also investigated.

2. Materials and methods

2.1 *Collection and analysis of root exudates*

Plants were grown aseptically in 200 × 25 mm test tubes containing acid washed and sterilized sand. Surface sterilized (0·1% w/v $HgCl_2$ followed by thorough washing in sterile water) seeds were pre-germinated in a sterile petri-dish containing moist filter paper and 3 seedlings were transferred to each tube. The tubes were arranged in wooden racks and kept on a small bench top in an artificially illuminated growth room (30°C/25°C day/night temperatures and a 14 h photoperiod with 12,000 lux light intensity). Five ml of Hoagland's N-free nutrient solution supplemented with 50 ppm nitrogen as NH_4NO_3 was added to each tube to maintain the plants.

After verifying the sterility at the end of 12 days root exudates were collected by carefully removing the plants and rinsing the root system and sand from each tube in 10 ml distilled water. All the exudates from one treatment were pooled, centrifuged, desalted and concentrated in a rotary vacuum evaporator to represent 1 ml of final exudate for every 10 seedlings. The amount of organic carbon in the exudates was estimated by the Walkley and Black rapid titration method (Jackson 1958).

2.2 *Assay of the N_2-ase activity*

Plants were grown from surface sterilized seeds in 200 × 25 mm test tubes (1 per tube) with 30 g sterile sand + vermiculite mixture (1 : 1) as the supporting medium with half strength Hoagland's N-free nutrient solution. Three day old plants were inoculated with 1·0 ml of pure cell suspension of 4-day old *A. brasilense* (absorbance 0·38) per plant. On the 5th day 2 ml of 2% w/v solutions of different carbon sources were dissolved in plant nutrient solution and added to the root zone. Control tubes received the nutrient solution only. Five replicate tubes were maintained for each treatment. N_2-ase activity of the intact plants was estimated after 3 weeks without pre-incubation by measuring C_2H_4 produced after 24 h of incubation with 10% C_2H_2 by gas chromatography (Rao and Venkateswarlu 1982). Appropriate control tubes were included in the assay. Uninoculated tubes having plants did not show any C_2H_2 reduction while tubes inoculated with *A. brasilense* in the absence of plants did show traces of activity, which was taken into account in the final calculations. Following the assay the plants were harvested and the dry weight of shoot and roots were recorded separately. At every point sterility was checked and the contaminated tubes were rejected.

3. Results and discussion

There were significant differences in the organic carbon and reducing sugar content of the root exudates among the pearl millet varieties (table 1). BJ–104 exuded highest amounts of organic carbon and reducing sugars followed by MBH–110, MP–15 and MBH–118. However no significant differences were observed in total and amino-N. The total plant dry matter also differed significantly among varieties with the highest in MBH–118 (table 2). However, there was an inverse relationship between the plant dry weight and root exudation. But the seed size did not influence root exudation.

Table 1. Composition of root exudates of pearl millet varieties.

		Exudate (μg/10 seedlings)		
Variety	Organic-C	Reducing sugars	Amino-N	Total N
BJ-104	510 ± 12·7	58 ± 2·4	4·8 ± 0·3	72 ± 2·4
MP-15	380 ± 11·3	42 ± 2·8	5·0 ± 0·3	67 ± 1·4
MBH-110	470 ± 10·5	49 ± 1·6	4·2 ± 0·2	63 ± 1·8
MBH-118	330 ± 8·5	39 ± 1·2	4·7 ± 0·4	75 ± 2·7

Mean ± standard deviation.

Table 2. Organic carbon of root exudates in relation to plant dry weight in pearl millet varieties.

Variety	Seed size (wt of 10 seeds) (mg)	Organic carbon[a] (μg)	Dry wt of 10 seedlings (mg)	Organic carbon as % of plant dry weight
BJ-104	55·1	510 ± 12·7[b]	34·2 ± 0·65	1·49
MP-15	69·5	380 ± 11·3	60·3 ± 0·98	0·63
MBH-110	99·2	470 ± 10·5	54·5 ± 0·84	0·86
MBH-118	106·7	330 ± 8·5	63·8 ± 1·13	0·52

[a]Amount exuded by 10 seedlings.
[b]Mean ± standard deviation.

Table 3. Nitrogenase activity of inoculated pearl millet as influenced by the addition of sucrose.

	N_2-ase activity (n mol C_2H_4 plant^{-1}h^{-1})*	
Variety	Control	With sucrose
BJ-104	21·0 ± 4·2	49·0 ± 4·5 (133)
MP-15	14·5 ± 3·1	32·7 ± 6·2 (125)
MBH-110	17·5 ± 2·8	45·0 ± 3·9 (157)
MBH-118	12·5 ± 3·5	46·5 ± 5·5 (272)

*Mean ± standard deviation.
Figures in parantheses indicate per cent increase over control (without sucrose addition).

The amount of organic carbon in the exudates as percentage of total plant dry weight varied from 0·51–1·49%. This is relatively low compared to the observations of Barber and Gunn (1974) who reported about 4–5% of dry weight of plants as organic carbon in the root exudates of wheat grown in unsterile soil. While, Haller and Stolp (1985) observed 7% of net photosynthates in the root exudates of 4-week old maize plants growing in quartz sand and Lee and Gaskins (1982) indicated that 25% of total 14C fixed in the plant was exuded by the roots.

The nitrogenase activity of the intact plants also showed marked differences among the varieties (table 3). Maximum activity was recorded in var. BJ-104 (21 nmol C_2H_4 plant^{-1} h^{-1}), the exudates of which also contained highest amount of organic carbon. The activity among other varieties was also related to the relative quantities of organic carbon in the exudates suggesting that exudate carbon influences the growth of the microsymbiont in the root zone and its N_2-ase activity. From the correlations it was found that N_2-ase activity was directly proportional to the organic carbon content of the root exudate (r = 0·79). Difference in root exudate carbon might then be an important factor for the varietal variation in the N_2-ase activity as also suggested by Vlassak and Reynders (1978) in wheat and Van Bulow and Dobereiner (1975) in maize. However, the amount of carbon available in the exudates appears to be inadequate for the optimum activity of the organism as revealed by an increase in the activity upon providing an extraneous carbon source. The increase in the activity ranged from 125–272% in different varieties. The existence of similar latent activity which could only be induced upon providing an external carbon source was also reported by Hess and Kiefer (1981) and Lethbridge and Davidson (1983) in sorghum, wheat and maize.

The extent of this stimulation of N_2-ase activity varied with the type of carbon source added (figure 1). The maximum stimulation of activity was found with succinate followed by fructose and the least with glucose and 2-ketoglutaric acid. Interestingly these later substrates are poorly utilized by *Azospirillum* in pure culture compared to succinate (Venkateswarlu and Rao 1985), thus establishing a link between the utilization of these compounds in the root zone and the induction of N_2-ase activity.

Root dry weight marginally declined in the plants supplemented with external carbon source without any significant change in the total plant dry weight. The root/shoot ratio decreased with fructose, malate and succinate while with other sugars it was not significantly affected. These results conform to those reported by Glatzle and Martin (1981) and could result from competition between respiring roots and the bacteria. But there was no increase in the total plant dry matter with the addition of any of the carbon sources.

From the study it may be concluded that root exudates of pearl millet regulate the activities of *Azospirillum* in the rhizosphere and the induction of N_2-ase activity, but

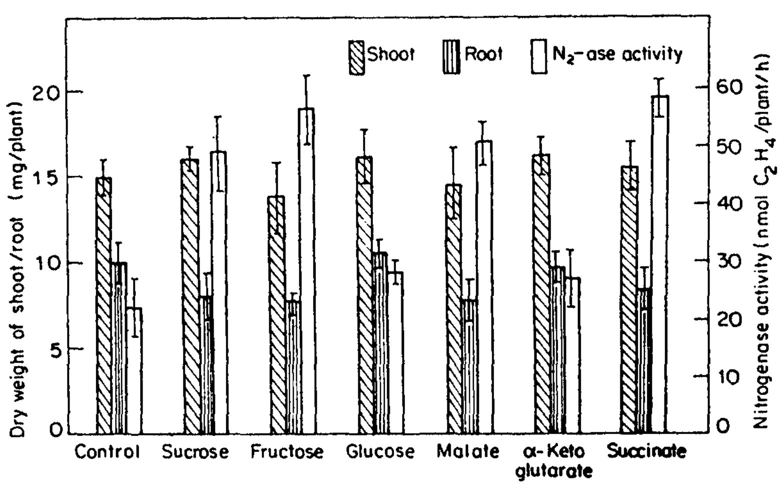

Figure 1. N_2-ase activity and dry matter production of inoculated pearl millet (var. BJ-104) as influenced by the addition of different carbon sources to the root zone.

the organic carbon exuded by the roots at the seedling stage is not adequate for the optimum expression of the symbiosis.

Acknowledgement

The authors wish to thank Dr A N Lahiri for his keen interest.

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