TARC Notes

Nitrogen fixation by the roots of *Pennisetum purpureum* and some other C_4 grass species

A number of reports have shown the existence and operation of nitrogen fixing process in grass-bacteria associations. Nitrogen fixing activity has been estimated in the rhizosphere of various grass species including maize, sorghum, sugarcane, rice, wheat and several kinds of forage grasses. Dominant N_2-fixer in each association has also been investigated[1].

Although the activities obtained from grass-bacteria associations were less stable and lower than that obtained from legume-rhizobium symbiosis, enhancing the nitrogen fixing activity should be further studied because of its possible value for tropical agriculture.

Pennisetum purpureum (Napiergrass) is one of the most productive forage crops with the efficient C_4 photosynthesis, and reported to have a considerable nitrogenase activity in its root[2].

The present paper reports the factors regulating the nitrogen fixation by *Pennisetum* root and nitrogenase activity detected in the roots of some other C_4 grasses grown in our experimental field located in humid subtropics. The pH of soil was about 4.8.

Plants used in the present study included *Pennisetum purpureum* and 7 strains of *Panicum* species and were planted in the field with basic fertilizer dressings of 20 kg N, 80 kg P, and 80 kg K per ha. Also 1 kg of sodium molybdate was sprayed as a diluted solution. From 6 to 10 months after planting, root systems were separated from plant tops and washed with sterilized water for the assay.

A method used for measuring nitrogenase activity was similar to that reported by Abrantes et al.[3] with slight modifications. Before the assay, exised root samples were washed again with sterilized water and placed into vessels shown in Fig. 1. The vessels were evacuated three times to 80 mm Hg and re-

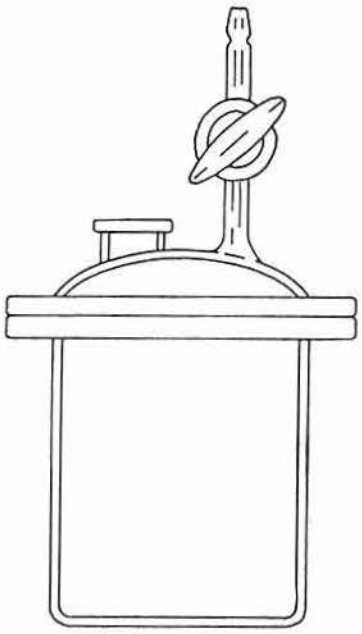

Fig. 1. A vessel used for acetylene reduction assay

filled with desired gas mixtures. Acetylene reduction was carried out with 10% acetylene for scheduled periods at a room temperature (25–27°C). Ethylene produced was measured on 1 ml gas samples collected through a rubber cap with a Yanaco G-80 hydrogen-flame ionizing gaschromatograph fitted with a 2 m×3 mm Porapak R column using N_2 as a carrier gas at 50°C.

Fig. 2 shows the nitrogenase activity in *Pennisetum* root as affected by pO_2. Fig. 2a shows its time-course characteristics without preincubation, while Fig. 2b shows result after preincubated for 17 hrs. The pO_2 of 0.02 maximized the acetylene reduction by the root, and the preincubation doubled the rate. As expected, 20% of O_2 severely inhibited the rate to about one-fifth.

This result initiated further experiments under the pO_2 of 0.02 after preincubated for 17 hrs.

Nitrogenase activity changed remarkably with the age of the root used (Table 1). The younger the root, the lower the activity. Uniformalizing the root age may minimize the fluctuation of acetylene reducing activity. Similar result might be obtained when the

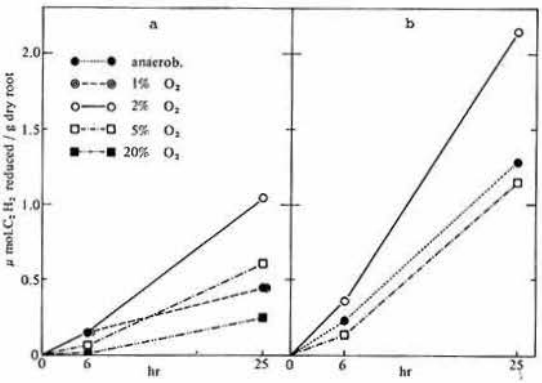

Fig. 2. Time courses of acetylene reduction by *Pennisetum* roots incubated at various O_2 concentrations. a) without preincubation b) C_2H_2 was introduced after preincubated for 17 hrs at each pO_2.

Table 1. Nitrogenase activity in *Pennisetum* roots developed from various stalks

Positions of roots developed	nmol. C_2H_2reduced/ hr.g dry root
Original cutting & primary stalks	28*±3**
Secondary stalks	19 ±3
Tertiary stalks	1 ±0.3
Fourth stalks & tertiary stalks without tillering	1 ±0.3

* means of three replicates
** standard error

root samples were fractionated according to their color gradient, because the surface color is considered to reflect root aging[1].

In order to assume the effective carbon sources for acetylene reduction by *Pennisetum* root, 5 ml of various carbon source solutions were applied to ca. lg of root (dry wt.) at the beginning of preincubation. Concentration of carbon source solutions was 50 mM. The pH of organic acid solutions was adjusted to 6.0 with NaOH.

As shown in Table 2, sugars such as glucose and mannit markedly enhanced the capacity for reducing acetylene in the root, while malic acid slightly enhanced. This result is in sharp contrast to that obtained by Van Berkum et al.[5] They reported that addition of malate and bicarbonate doubled the nitrogen fixation by isolated sorghum root while glucose had no effect. The above disagreement might be due to the difference in dominant nitrogen-fixing organisms involved, because the requirement for carbon sources differs among bacteria. Although the data are not shown here, semi-solid glucose medium recovered more number of nitrogen-fixing bacteria than malate medium from *Pennisetum* root by MPN method.

Table 2. Enhancement effect of exogenous carbon sources on nitrogenase activity in *Pennisetum* root

Carbon source	nmol. C_2H_2reduced/ hr.g dry root
H_2O as control	40*±10**
Glucose	105 ± 3
Mannit	167 ±17
Malic acid	52 ± 8
Succinic acid	95 ±20
Pyruvic acid	79 ±16

*, ** same as Table 1
Concentration of carbon source solutions was 50mM, and PH of organic acid solutions was adjusted to 6.0 with NaOH.

These results suggest that the content of sugars in the root is one of the important factors regulating the nitrogen fixation by *Pennisetum* root.

Acetylene reduction by *Panicum* roots was shown in Table 3. The highest activity was detected in the root of *Panicum coloratum* (var. Kabulabula). Interspecific and varietal differences were recognized regarding nitro-

Table 3. Nitrogenase activity in the roots of *Panicum* spp.

Species (variety)	nmol. C_2H_2reduced/ hr.g dry root
Panicum maximum (M 70/81, 12)	11*±1**
Panicum maximum (Hamill)	2 ±0.3
Panicum maximum (Gatton)	5 ±0.7
Panicum coloratum (Burnett)	16 ±3.2
Panicum coloratum (Kabulabula)	26 ±4.0
Panicum coloratum (Solai)	23 ±5.0
Panicum macrophyllum	13 ±0.8

* means of four replicates
** same as Table 1

genase activity. Dissimilar to the results reported by Day et al.[2], *Panicum maximum* showed lower activity. In addition to the acidic condition of the soil, inadequate environmental factors might limit the rate.

Since the differences of environmental conditions might result in the dissimilarity of microflora in grass-rhizosphere, effective associations between grass and bacteria would vary in different regions.

Bacteria/plant combination test is now in progress at our laboratory.

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