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NITROGEN FIXATION ASSOCIATED WITH SORGHUM AND PEARL MILLET

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Sorghum and pearl millet are often grown in poor fertility conditions where estimates of the N balance in crop production indicate a very efficient uptake of soil nitrate and/or an input from biological N_2-fixation. This is being examined in long term, nitrogen-balance field experiments at ICRISAT. In the second season as much as 72 KgN/ha was removed by one sorghum cultivar, grown with no N fertiliser addition in a low fertility soil (c 0.03% in the top 30 cm).

The roots of field grown sorghum and millet plants stimulate nitrogenase activity. Assays of field, pot and tube grown plants indicate differences between lines in the level of activity, but there is often much variability between plants. Activity was increased in a core assay by reducing mechanical disturbance during transportation, and injecting C_2H_2 gas directly after cutting off the plant tops. Plants grown in iron cores in the field and then assayed had significantly higher activity than plants cored at the time of assay. There was diurnal variation in N_2-ase activity which did not seem to be related to temperature, with most activity at the end of the photoperiod. Activity was correlated with the ontogenetic development of the host plant, being most at flowering. Nitrogenase activity was positively correlated with soil moisture content, and also varied substantially between fields.

An intact plant assay system was developed for seedlings grown in tube culture, and plants grown through their life cycle in pots, where the tops of the plants were sealed from the assay chamber and remained in the ambient atmosphere. N_2-ase activity was larger and less variable in such systems than in soil core or excised root assays. Cutting off the tops of plants reduced activity. Seventy six-day-old, intact sorghum plants averaged 191 nmol C_2H_4/plant/h and decapitated plants only 11 nmol. Three sorghum lines had activity ranging from 1.6 to 3.6 µmol C_2H_4/plant/h *cf* soil alone with 13 nmol. A sand:farmyard manure medium (97:3 w/w) supported best plant growth and N_2-ase activity.

We exposed sorghum seedlings to $^{15}N_2$ and demonstrated that nitrogen is fixed in the root zone and rapidly transferred to the tops of the plants. CSH-5 seedlings were grown in a sand:farmyard manure (97:3 w/w) mixture in 25-x 200 mm test tubes with an attached side tube. When exposed to $^{15}N_2$, the tops of the plants were sealed from the root system by a Suba Seal and silicone rubber sealant, and gas in the root medium in the test tubes was exchanged by water displacement. The oxygen content of the root zone was monitored and maintained at 20%. After exposing 20-day-old CSH-5 seedling to $^{15}N_2$ for 3 days, ^{15}N was detected in the growth medium (0.005 ^{15}N atom % excess). Seven days after the labeled gas was removed, the ^{15}N atom % excess in the plants increased considerably with 0.029 atom % excess in the roots and

0.019 atom % excess in the shoots. Seedlings were also grown for 24 days in a device developed at Rothamsted for gassing 10 plants at once. The gas in the root chamber was replaced by flushing with CO_2 then absorbing out the CO_2 over soda lime and allowing a N_2:O_2 mixture to be drawn in. Table 1 shows that ^{15}N was incorporated in the shoot system by the end of the 3-day exposure period; ^{15}N incorporated in the leaves had further increased 9 days later.

We found significant differences in amounts and patterns of organic carbon exuded into culture media by seedling roots of 6 sorghum cultivars grown in axenic liquid culture. Sorghum genotypes that exuded the most organic carbon supported the most N_2-ase activity by an Azospirillum lipoferum inoculum.

Monitoring growth and nitrogenase activity of 5 bacterial strains in semisolid synthetic media, containing root exudates from 3 sorghum genotypes as the sole organic carbon source, showed significant ($P<0.05$) differences in growth and activity between bacteria and between exudate media. The bacteria x genotype interaction was significant, indicating qualitative differences in root exudates, which may be important in selecting bacteria for inoculation trials.

A field trial was conducted during the 1982 rainy season on an Alfisol with 3 sorghum hybrids inoculated with different nitrogen-fixing bacteria. Inoculating with Azospirillum lipoferum and a root extract from Napier bajra (Pennisetum americanum x P. purpureum) increased dry-matter production ($P<0.10$). The trend toward increased grain yield from inoculation was not statistically significant.

Millet cultivars IP 2787 and ICMS 7819 were grown with irrigation in the 1982 summer season, in an Alfisol soil containing 0.04% total N in the top 15 cm layer. The cultivars' responses to incoulation differed in both dry-matter production and grain yield. Cultivar ICMS 7819 did not respond to inoculation but Azospirillum lipoferum increased IP 2787's grain yield 17% (Table 2). In a further field experiment during the rainy season with 3 millet cultivars and 5 inoculum strains, and an inoculum-free control, inoculations again produced definite effects, with indications of strain x cultivar interaction (Table 3). Inoculation with either A. lipoferum or Azotobacter chroococcum increased yields of cultivars WC-C75 and IP 2787 - with a mean increase of 27% for A. lipoferum and 19% for A. chroococcum - but cv. ICMS 7703 was not responsive to inoculants. However, inoculation with A. brazilense decreased the yield of cultivar WC-C75, but not that of IP 2787.

Soil samples from around the roots of millet plants grown in traditional cultivation areas in northwest India were examined for N_2-fixing bacteria. Using C_2H_2 reduction activity in soil dilutions inoculated into small vials containing N-free media we found the MPN of N_2-fixing bacteria ranged from 10^2 to 10^5 per g soil depending on soil type and location. Forty-two percent of the 3700 isolates picked from N-free medium showed N_2-ase activity and 95% were able to grow on MacConkey bile salt medium. Many of the isolates were mixed cultures which lost their N_2-ase activity when purified to a single type of bacteria. Colony morphology and biochemical tests suggested that many of the N_2-ase positive, pure cultures were Enterobacter spp., a few were Azospirillum, while others were Pseudomonas spp.

Table 1 $^{15}N_2$ incorporation by sorghum hybrid CSH-5 seedlings.

	Time of harvest after exposure to $^{15}N_2$			
	0 days		9 days	
	Shoot	Root	Shoot	Root
Dry weight (mg/plant)	264	246	400	673
^{15}N atom % excess	0.056	0.059	0.102	0.073
^{15}N incorporated (μg/plant)	15.9		25.5	

Average of 3 replications; plants grown in 35-× 295-mm plastic tubes filled with sand:FYM (97:3 w/w); 24-day-old seedlings exposed to $^{15}N_2$ (40 atom % excess) for 3 days. Data from collaborative project with Rothamsted Experimental Station funded by the UK Overseas Development Administration.

Table 2. Effect of inoculation with nitrogen-fixing bacteria on dry-matter production and grain yield of pearl millet with irrigation, ICRISAT Center, summer season 1982.[1]

	Plant dry matter (kg/ha)			Grain yield (kg ha)		
Inoculum	IP 2787	ICMS 7819	Mean	IP 2787	ICMS 7819	Mean
Azospirillum lipoferum[2]	1740	1810	1770	930	1360	1140
A. braziliense	1410	1930	1670	760	1240	1000
NBRE	1630	1910	1770	880	1230	1050
Not inoculated (control)	1550	1880	1670	830	1310	1070
SE	±113		±80	±29		±21
Mean	1580	1880		850	1280	
SE	±56			±3.6		
CV (%)	16			16		

1. 12 replications (net plot size 16.9 m²). No nitrogen fertilizer applied. Each plot inoculated twice, once at sowing and 30 days later with 2.5 liters of liquid inoculum prepared by suspending in 25 liters of water, a 70-g peat culture with viable bacterial count of 10^8/g peat.
2. Strain 4 ABL obtained from Dr. J. Balandreau.

Table 3. Effect of inoculation with different nitrogen-fixing bacteria on grain yield of pearl millet, rainy season 1982.

	Grain yield (kg/ha)[1]			
Inoculum	IP 2787	ICMS 7703	WC-C75	Mean
Azospirillum lipoferum	2090	2110	2560	2250
Azospirillum lipoferum(1)[2]	2060	2010	2310	2130
Azotobacter chroococcum	2140	2110	2110	2120
NBRE	1870	1980	2070	1970
Azospirillum braziliense	1720	1760	1450	1650
Control (not inoculated)	1690	2030	1870	1860
SE		±203		±98
Mean	1930	2000	2060	
SE		±52		
CV (%)		22		

1. Average of five replications (net plot size 13.5m²). No nitrogen fertilizer applied. Each plot inoculated twice, once at sowing and 30 days later with 2.5 liters of liquid inoculum prepared by suspending a 70-g peat culture packet with viable bacterial count of 10^8 g peat, in 25 liters of water.
2. Strain 4 ABL obtained from Dr. J. Balandreau.