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RESPONSE OF PEARL MILLET CULTIVARS TO INOCULATION WITH NITROGEN-FIXING BACTERIA†

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SUMMARY

The results of field experiments conducted with millet cultivars inoculated with different nitrogen-fixing bacteria at the ICRISAT Centre, Hyderabad, India are described. Significant interactions were observed between host cultivars and bacterial strains, but some cultivars showed consistently increased grain and dry matter yields, suggesting the possibility of exploiting suitable plant and nitrogen-fixing bacterial associations for increasing grain yield. Inoculation also resulted in increased nitrogen uptake up to 14.9 kg ha^{-1}, and larger grain nitrogen contents.

INTRODUCTION

The association between cereal plants and nitrogen-fixing bacteria, as shown by increased nitrogenase activity, is well established (Dart and Wani, 1982; Wani *et al.*, 1983, 1984) and *Azospirillum* and *Azotobacter* either singly or in combination have been used to study inoculation responses with various cereals (Wani *et al.*, 1976; Baltensperger *et al.*, 1978; Bouton *et al.*, 1979; Kapulnik *et al.*, 1981). In a few experiments cultures of nitrogen-fixing enterobacteriaceae were used to study inoculation responses (Wood *et al.*, 1981; Wright and Weaver, 1982). Inoculation with *Azospirillum* increased yield and nitrogen content in several crop species in the field (Smith *et al.*, 1976; Kapulnik *et al.*, 1981; Subba Rao *et al.*, 1982). Such effects are of particular interest with pearl millet which is grown on nutritionally poor soils in the semi-arid tropics, often without added mineral nitrogen. In this paper we report the response of field grown pearl millet (*Pennisetum americanum* (L.) Leeke) cultivars to inoculation with different nitrogen-fixing bacteria.

MATERIALS AND METHODS

Millet cultivars

Two supposedly high nitrogenase stimulating millet cultivars, cv. IP 2787 and cv. ICMS 7819, a synthetic variety developed from an Ex-Bornu population, were selected for Experiment I. Millet cv. ICMS 7819 did not respond to inoculation in this experiment and was replaced in Experiment II by two

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ICRISAT cvs, WC C 75 and ICMS 7703. Experiment III was conducted in the same season as Experiment II with three hybrid cultivars: BJ 104 and MBH 110, commonly cultivated in India, and MEBH 23/81 which had yielded well in initial trials. These three cultivars were also used in Experiment IV.

Bacterial cultures

The cultures of *Azospirillum lipoferum* (1) and *A. brasilense* (SP 7) ATCC No. 29145 were obtained from Dr J. Balandreau, CNRS, Nancy, France and Dr J. Dobereiner, Brazil, respectively. *A. lipoferum* (ICM 1001) was isolated at ICRISAT from the rhizosphere of sorghum cv. CSH 1, grown in vermiculite and inoculated with Napier bajra root extract (NBRE) culture. *Azotobacter chroococcum* (ICM 2001) was isolated from the rhizosphere of *Cenchrus ciliaris* plants grown in the field. NBRE was a mixed culture prepared by macerating 25 g of roots and the rhizosphere soil of field grown forage grass, Napier bajra hybrid 21 (*Pennisetum americanum* x *P. purpureum*), in 500 ml nitrogen-free sucrose medium using a mechanical blender. The macerate in a sucrose medium was incubated for 24 h at room temperature (28 ± 2°C) on a rotary shaker with a speed of 150 rpm.

Preparation of inoculants

The cultures of azospirilla and *Azotobacter* were multiplied in nitrogen-free malate and sucrose broth media, respectively, containing 100 mg yeast extract per litre. After inoculation, the media were incubated at 33°C for three days on an incubator-cum-rotary shaker at 150 rpm. Peat inoculants were prepared by injecting 30 ml of culture broth, with a count of 10^8 cells ml^{-1} (except NBRE which had 10^9 cells ml^{-1}), into a packet containing 40 g gamma irradiated peat (supplied by Agriculture Laboratories Pty Ltd, Australia). Inoculated peat packets were cured for 10–12 days at 33°C in an incubator. At the time of field inoculation all the inoculants had 10^8 bacterial cells g^{-1} of peat, except the NBRE-peat culture which had 10^9 cells g^{-1} peat. For control treatments, the peat packets were inoculated with sterile N-free sucrose medium. Peat inoculants of pure bacterial cultures were free from contamination at 10^{-5} dilution.

Cultural practices

The experiments were conducted on alfisols both during the rainy and dry season (Table 1) using factorial randomized block designs (RBD) with 5–13 replications at the ICRISAT Centre, Hyderabad, India (16.5° N, 78.5° E, 545 m altitude). Experiments conducted during the dry season were irrigated as required by furrow irrigation, except in Experiment IV where plots were irrigated lightly with sprinklers after each inoculation.

Liquid inoculum was prepared by thoroughly mixing the peat inoculum in unchlorinated tap water (2.3 g l^{-1}). Each plot was inoculated at sowing and after thinning the plants 20–25 days after sowing, except in Experiment IV. At each inoculation 100–105 ml culture suspension was added per metre row

Table 1. *Details of pearl millet inoculation trials*

Experiment	Year	Season	Soil pH	Total nitrogen in soil (ppm) 0–15 cm	Total nitrogen in soil (ppm) 16–30 cm	E.C. (m.mhos cm^{-1})	Gross plot size (m^2)	Harvest area (m^2)
I	1982	Dry	6.5	470	445	0.60	31.5	16.9
II	1982	Rainy	8.3	479	453	0.29	18	9
III	1982	Rainy	6.2	418	490	0.06	12	9
IV	1983	Dry	7.7	489	464	0.35	18	9

length, except in Experiment I where each metre of row length received 70–75 ml culture suspension. The inoculum was poured below the seed in the planting furrow when inoculation was done at the time of sowing. Otherwise, a small furrow was opened by the side of the seedlings, inoculum added and the furrow closed.

Plant to plant spacing of 10 cm was maintained by thinning the plants 16–20 days after sowing. Weeding and inter-row cultivations were carried out as and when required. Plant parts above ground level were harvested from the net plot area and the ears separated and threshed; the plant matter was then chopped, dried at 70°C for three days, and the grain and plant dry matter weights recorded. Total nitrogen contents of powdered grain and plant dry matter samples from each treatment were estimated by a micro-Kjeldahl digestion method and a Technicon Auto-analyser.

Experiment I

A basal dose of 40 kg P_2O_5 ha^{-1} as single superphosphate was applied. The crop was sown manually on ridges spaced 75 cm apart on 31 December 1981. Each treatment was replicated 12 times.

Experiment II

The crop was sown manually on ridges spaced 75 cm apart on 25 June 1982. A basal dose of 40 kg P_2O_5 ha^{-1} as single superphosphate was applied. Each treatment was replicated 5 times.

Experiment III

A basal dose of 20 kg N ha^{-1} as urea and 20 kg P_2O_5 ha^{-1} as single super phosphate was applied. The crop was sown manually in rows spaced 50 cm apart on 19 June 1982. The experiment consisted of 15 treatments replicated 7 times.

Experiment IV

The field was fertilized with 40 kg P_2O_5 ha^{-1} as single superphosphate. The crop was sown on 13 January 1983 on ridges spaced 75 cm apart. Each treatment was replicated 13 times. The crop was inoculated 7 and 28 days after planting and on each occasion irrigated lightly the following day. It was top-dressed with 16 kg N ha^{-1} as urea 25 days after sowing.

RESULTS

Experiment I

Inoculation with *Azospirillum lipoferum* (1) and NBRE increased grain yield of cv. IP 2787 by 11.7% and 6%, respectively, over the uninoculated control but cv. ICMS 7819 did not respond to inoculation with any of the cultures used (Table 2). Inoculation of both the cultivars with *A. brasilense* resulted in reduced grain yield. There was significant interaction between host cultivar and bacterial strain for plant dry matter yield. Inoculation also increased the total dry matter of both cultivars except when cv. IP 2787 was inoculated with *A. brasilense* (SP 7).

There were no significant changes in the nitrogen content of grain or plant tissues due to inoculation with nitrogen-fixing bacteria but inoculation of cv. IP 2787 resulted in a marginally increased grain nitrogen percentage.

Experiment II

The mean yields of cultivars inoculated with *A. lipoferum* (1) and ICM 1001 were 14 and 21% greater, respectively, than that of the uninoculated control but inoculation with *A. brasilense* reduced yield by 12% (Table 3). The dry matter yields showed a similar response to inoculation as grain yield. Inoculation of cvs IP 2787 and WC C 75 with nitrogen-fixing bacteria increased the grain yields up to 26.5% and 36.8%, respectively, over uninoculated controls but cv. ICMS 7703 did not respond favourably to inoculation.

The mean total nitrogen uptake was increased following inoculation with *A. lipoferum*, *A. chroococcum* and NBRE but reduced following inoculation with *A. brasilense*. There was a significant variety x bacterial culture interaction for grain nitrogen percentage. This largely reflected differences in grain yield, as in the case of WC C 75 inoculated with *A. brasilense*, but high nitrogen percentages

Table 2. *Grain yield and plant dry matter (t ha^{-1}) of pearl millet cultivars inoculated with nitrogen-fixing bacteria in Experiment I*

	A. lipoferum 1	*A. brasilense* SP 7	NBRE	Uninoculated control
		Grain yield		
IP 2787	0.92	0.76	0.88	0.83
ICMS 7819	1.36	1.24	1.23	1.31
Mean	1.14	1.00	1.05	1.07
		Plant dry matter		
IP 2787	1.74	1.41	1.63	1.55
ICMS 7819	1.81	1.94	1.92	1.80
Mean	1.78	1.67	1.77	1.68

* $P < 0.05$.

Table 3. *Grain yield (t ha^{-1}), total nitrogen uptake (kg ha^{-1}) and grain nitrogen percentage of pearl millet cultivars inoculated with nitrogen-fixing bacteria in Experiment II*

	A. lipoferum 1	*A. lipoferum* ICM 1001	*A. lipoferum* NBRE	*A. chroococcum* ICM 2001	*A. brasilense* SP 7	Uninoculated control	SE±
				Grain yield			
IP 2787	2.06	2.09	1.87	2.14	1.72	1.69	
ICMS 7703	2.01	2.11	1.98	2.11	1.76	2.03	0.20
WC C 75	2.31	2.56	2.07	2.11	1.45	1.87	
Mean	**2.13**	**2.25**	**1.97**	**2.12**	**1.64**	**1.86**	**0.12***
				Nitrogen uptake			
IP 2787	**42.8**	**41.3**	**38.0**	**48.4**	**36.8**	**35.6**	
ICMS 7703	**39.8**	**45.6**	**43.5**	**45.1**	**33.4**	**38.9**	**4.85**
WC C 75	**41.1**	**53.4**	**41.8**	**39.3**	**29.8**	**38.5**	
Mean	**41.3**	**46.8**	**41.1**	**44.3**	**33.3**	**37.0**	**2.80***
				Grain nitrogen %			
IP 2787	**1.30**	**1.22**	**1.25**	**1.32**	**1.29**	**1.27**	
ICMS 7703	**1.23**	**1.27**	**1.30**	**1.23**	**1.19**	**1.16**	**0.040***
WC C 75	**1.21**	**1.33**	**1.21**	**1.21**	**1.31**	**1.26**	
Mean	**1.25**	**1.27**	**1.25**	**1.25**	**1.26**	**1.23**	**0.023**

* $P < 0.05$.

were observed when IP 2787 was inoculated with either *A. chroococcum* or *A. lipoferum* (1) and when WC C 75 was inoculated with *A. lipoferum* (ICM 1001).

Experiments III and IV

The grain yields of the three varieties were not significantly influenced by nitrogen-fixing bacteria in either of these experiments though the inoculated treatments showed a trend towards larger yields (Table 4). The biggest response was shown by cv. BJ 104, which was the lowest yielding cultivar amongst the uninoculated controls in both experiments. Inoculation of BJ 104 with nitrogen-fixing bacteria increased grain yield by 12–26% and 7–19% over the uninoculated control during rainy and dry seasons, respectively. There was a trend ($P \leq 0.1$) towards a variety x bacterial culture interaction for grain nitrogen percentage in Experiment III. A larger grain nitrogen content of 1.68% in BJ 104 inoculated with *A. lipoferum* (ICM 1001) was observed. Other treatments in Experiment III and all the inoculated treatments in Experiment IV showed only a marginal increase in grain nitrogen percentage over the respective uninoculated controls. Plant tissue nitrogen contents of millet cultivars in Experiment III were not influenced by inoculation; however, in Experiment IV, plant tissue nitrogen contents of all the cultivars were reduced by inoculation with *A. lipoferum* (ICM 1001) and *A. chroococcum* (ICM 2001). Inoculation with

Table 4. *Grain yield (t ha^{-1}) and nitrogen percentage of pearl millet cultivars inoculated with nitrogen-fixing bacteria in Experiments III and IV*

	A. lipoferum		*A. chroococcum*		Uninoculated	
	1	ICM 1001	ICM 2001	NBRE	control	SE±
Experiment III						
			Grain yield			
BJ 104	2.89	2.91	2.72	3.06	2.45	
MBH 110	2.61	2.86	3.08	3.04	2.87	0.19
MBH 23/81	3.12	2.89	2.86	3.10	2.89	
Mean	2.87	2.89	2.89	3.07	2.73	0.11
			Grain nitrogen %			
BJ 104	1.43	1.48	1.45	1.49	1.30	
MBH 110	1.37	1.40	1.43	1.47	1.38	0.05
MBH 23/81	1.40	1.36	1.31	1.35	1.32	
	1.40	1.48	1.40	1.43	1.40	0.03
		1.37	1.25			
110	1.56	1.62	1.50	1.52	1.65	0.07
23/81	1.56	1.64	1.67			
		1.54	1.47			
			dry			
BJ 104	0.74					
MBH 110	0.80					
MBH 23/81	0.89					
			0.89	0.89	0.87	0.02**

* $P < 0.05$. ** $P < 0.01$.

A. lipoferum (ICM 1001) and NBRE resulted in increased total nitrogen uptake of 71 and 65 kg ha^{-1}, respectively, as against 56 kg ha^{-1} in uninoculated plants III.

DISCUSSION

Inoculation of pearl millet with nitrogen-fixing bacteria caused increased biological and grain yield of some millet cultivars grown under field conditions during the rainy and dry seasons but there was an interaction between the host cultivar and bacterial strain used. All four trials were conducted in fields where millet had been grown several times before. Cultivars IP 2787 and BJ 104 showed consistently increased grain and plant dry matter yields in trials but others, such as MBH 110, did not show a consistent response to inoculation.

The large increases in yield sometimes seen following inoculation indicate the possibility of exploiting suitable plant x nitrogen-fixing bacterial associations for increased grain and plant dry matter yields of millet cultivars in the semi-arid tropics.

Significant increases in grain yield of millet plants grown in soil in pot culture have also been reported by Venkateswarlu and Rao (1983) and of field grown cv. BJ 104 inoculated with *A. brasilense* by Subba Rao *et al.* (1982). Increased yields of field grown *Zea mays*, *Sorghum bicolor*, *Setaria italica* and *Panicum miliaceum*, and of forage grasses, following inoculation with *Azospirillum*, have been reported by Kapulnik *et al.* (1981), Smith *et al.* (1976), and Taylor (1979). The different responses to inoculation by different cultivars indicate the role played by the host plant in controlling response. This active role of the host plant genotype in supporting associative symbiosis has been previously demonstrated by Dobereiner (1976), Bouton *et al.* (1979), and Dart and Wani (1982).

The contrast between the results obtained in Experiments III and IV may be due to seasonal differences or to the fact that Experiment IV was conducted in a field which had previously been used three times for inoculation trials using the same cultures; this suggests that as the inoculated cultures become established the benefits from further inoculations with the same cultures may decline. The reduction in yield following inoculation with *A. brasilense* (SP 7) may arise because this particular strain is NO_3^- reductase positive, resulting in the loss of soil NO_3^--N by denitrification (Neyra *et al.*, 1977).

The interaction between host cultivars and bacterial strains in yield response was paralleled by a similar interaction in grain nitrogen percentage. Similar increases in nitrogen content in the plant tissues of millet cultivars inoculated with azospirilla and harvested before grain formation have been reported by Bouton *et al.* (1979), and in *Zea mays* and *Setaria italica* by Cohen *et al.* (1980). The increased total nitrogen uptake by inoculated millet cultivars was the result both of increased yields and an increased nitrogen percentage in the grains. A similar increased nitrogen uptake of 28.2 kg ha^{-1} was observed by Subba Rao *et al.* (1982), when cv. BJ 104, grown with 10 kg N ha^{-1}, was inoculated with *A. brasilense*.

Earlier reports have shown that nitrogen-fixing azospirilla and *Azotobacter* benefit the inoculated plants by fixing atmospheric nitrogen (Cohen *et al.*, 1980; Dart and Wani, 1982; Okon, 1982) and also by enhancing root hair formation and thus root uptake by the growth hormones secreted (Brown, 1974; Tien *et al.*, 1979).

Increased grain and plant dry matter yields of field grown millet plants with nitrogen-fixing bacteria and the possibility of an interaction between host cultivar and bacterial strains emphasize the need for screening large numbers of bacterial strains in association with common cultivars so as to select the best combinations of cultivars and strains. There are numerous reports indicating possible mechanisms by which crop plants may derive benefit from inoculation

with nitrogen-fixing bacteria. The extent to which each of these various processes contributes to increased yield of inoculated millet plants remains to be assessed.

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