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Rhizobial infection of African landraces of sorghum (*Sorghum bicolor* L.) and finger millet (*Eleucine coracana* L.) promotes plant growth and alters tissue nutrient concentration under axenic conditions

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Abstract
Seven strains of infective root-nodule bacteria, ("rhizobia"), namely *Rhizobium* GHR2, *Bradyrhizobium japonicum* Tal 110, *Sinorhizobium meliloti* strain 1, *Rhizobium leguminosarum* bv. *viciae* Cn6, *R. leguminosarum* bv. *viciae* strain 30, *Rhizobium* NGR234 and *Azorhizobium caulinodans* ORS571, were used to study the effects on growth of sorghum and finger millet seedlings cultured aseptically in Leonard jars with 1/2 strength Hoagland nutrient solution containing 1 mM KNO_3. The use of scanning electron microscopy on 10-d-old plants revealed the presence of all 6 test strains on root epidermal surfaces as well as inside the tissues of inoculated, but not uninoculated, sorghum and millet roots. Applying root macerate prepared from surface-sterilized inoculated sorghum plant material successfully induced nodule formation and N_2 fixation in soybean seedlings, thus authenticating these internally located root tissue bacteria as rhizobia. Inoculating sorghum seedlings with 4 rhizobial strains (i.e. *B. japonicum* Tal 110, *S. meliloti* strain 1, *R. l.* bv *viciae* Cn6 and R.l. bv. viciae strain 30) significantly ($P<0.05$) promoted sorghum shoot growth by 11–51% on fresh weight basis and 8–55% on dry weight basis. There was also 21–32% increase in root length of inoculated sorghum plants compared to uninoculated control. Additionally, root tissue concentrations of P and K were markedly ($P<0.05$) increased by 17–250% in inoculated sorghum roots relative to uninoculated plants, while in shoots Zn and Cu were significantly ($P<0.05$) decreased. Bioassays of the test strains for indole acetic acid (IAA) showed that they produced biologically active concentrations of this growth-promoting molecule, which ranged from 0.18 to 2.26 µg IAA per ml culture filtrate. These findings suggest that rhizobial infection of cerals such as sorghum and finger millet can promote an increase in plant growth via improved P and K nutrition and possibly the release of metabolites such as IAA.

Keywords: Growth promotion, IAA, inoculation, microscopy, finger millet and sorghum, mineral nutrition, soybean nodulation

1. Introduction

Rhizosphere interactions between plants and microbes play a vital role in plant development, with benefits ranging from nutrient acquisition to hormonal stimulation of growth. Recent studies have started to explore the use of rhizobia as plant growth-promoting rhizobacteria in agriculturally-important crops such as wheat, maize, rice, potato, lettuce, radish, canola, and oilseed rape (Al-Mallah et al., 1989, 1990; Chabot et al., 1996; Law and Strijdom, 1989; Reddy et al., 1997; Spencer et al., 1994; Yanni et al., 1997; Antoun et al., 1998; Noel et al., 1996; Schloter et al., 1997; Chaintreuil et al., 2000; Yanni et al., 2001). Many of these crop species have presumably been bred for specific traits that could affect their rhizosphere response to rhizobial interaction. Although several studies have been conducted on rhizobia on aspects relating to growth stimulation which go beyond the traditional role of these microbes as N_2 fixers, only a few have been done on African cereal crops (Matiru and Dakora, 2004). Because a number of studies (McInroy and Kloepper, 1995; Yanni et

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al., 1997; Chaintreuil et al., 2000; Yanni et al., 2001) have identified rhizobia as natural endophytes of agronomically-important crops such as cotton, sweet corn and rice, a wider use of unbred plant material such as landraces is likely to provide new insights into the benefits of rhizobial inoculation with various non-legume crop species. Unfortunately, no such studies have yet been done with the major cereal crops in Africa, except for the report by Matiru and Dakora (2004).

Like other soil bacteria, rhizobia are a source of phytohormones and other growth-promoting rhizosphere signal molecules (Law and Strijdom, 1989; Loper and Schroth, 1986; Lynch and Clark, 1984; Phillips et al., 1999; Matiru and Dakora, 2005a,b) and can potentially influence plant development. Law and Strijdom (1989) have reported the ability of *Bradyrhizobium* strain CB756 to promote root growth in seedlings of cowpea (a legume) and wheat (a cereal) when cultured in Leonard jars or agar medium. In contrast, they also found that two local isolates of *Bradyrhizobium* sp., which produced higher concentrations of indole acetic acid (IAA), inhibited root development in the two plant species, indicating strain differences in root growth stimulation. A number of studies have also shown that rhizobia can infect roots of non-legume crop plants (de Bruijn et al., 1995; Reddy et al., 1997; Spencer et al., 1994; Webster et al., 1997) and invade intercellular spaces (Gough et al., 1997; Reddy et al., 1997) or xylem vessels (O'Callaghan et al., 1997). Some recent reports in North Africa (Yanni et al., 1997, 2001) have suggested that clover rhizobia do occur as natural endophytes of rice plants grown in the Nile delta because of rice rotation with clover for over 1000 years. In West Africa, photosynthetic bradyrhizobia from *Aeschinomene sensitiva* are also reported to occur as natural endophytes of the African wild rice *Oryza breviligulata* (Chaintreuil et al., 2000). These findings suggest that rhizobia probably influence plant growth in more ways than just N supply via N_2 fixation in nodules.

The aim of this study was 1) to assess the effects of rhizobial inoculation on plant growth and mineral nutrition of a sorghum landrace, 2) to study rhizobial colonization of roots of African sorghum and millet landraces, and 3) to establish, using Koch's postulates, whether the bacteria visualized on the inside of cereal roots with SEM are indeed N_2-fixing rhizobia.

2. Material and Methods

Studies of millet and sorghum root infection by 6 rhizobial strains

Plant culture

All plant culture experiments were arranged in a completely randomized design (CRD). Seeds of African landraces of finger millet (*Eleucine coracana* L.) and sorghum (*Sorghum bicolor* L.) obtained from Ruiru market, Kenya, were used in this study. The seeds were surface-sterilized by soaking in 75% ethanol for 2 min, in 10% sodium hypochlorite (bleach) solution for 10 min, and rinsing 5 times with sterile de-ionised water. Autoclaved Leonard jars were then sown to the surface-sterilized seed of millet and sorghum, and seedlings thinned out to 2 plants per jar at 10 d after planting. Each Leonard jar consists of a one-half beer bottle open at both ends and stuck with cotton wool at the neck to support a wettable wig and filled with acid-washed sand. The bottle with sand was inverted neck-down into a jar containing modified Hoagland nutrient solution and assembled as described by Vincent (1970). The Hoagland nutrient solution consisted of 246.5 g $MgSO_4$, 110.0 g $CaCl_2$, 87.1 g K_2SO_4, 87.1 g K_2HPO_4, 68 g KH_2PO_4, 18.7 g sequestrene (138 Fe), 724.0 mg $MnCl_2.4H_2O$, 110.0 mg $ZnCl_2$, 70.0 mg $CuCl_2.2H_2O$, 25.0 mg $NaMoO_4.2H_2O$, 60.0 mg $CoCl_2.6H_2O$ and 5.7 mg H_3BO_3 per 1,000 ml of deionized water. This molar concentration was adjusted to 1/2 strength nutrient solution containing 1 mM KNO_3.

The rhizobia used in this study included *Bradyrhizobium japonicum* Tal 110, *Sinorhizobium meliloti* strain 1, *Rhizobium leguminosarum* bv. *viciae* strain 30, *R. leguminosarum* bv. *viciae* strain Cn6, *Bradyrhizobium* CB756, as well as strains with broad host range such as *Rhizobium* NGR234 and *Rhizobium* GHR2. Broth cultures were prepared from each of the 6 rhizobial strains by growing the stain in yeast extract mannitol agar medium (Vincent, 1970) for 72 h, followed by measurement of the optical density at OD_{600}. Cell turbidity was adjusted to 0.2 OD_{600} units, and 3 ml of the broth culture of each strain used to inoculate seedlings of sorghum and millet. Uninoculated control plants received 3 ml of sterile yeast mannitol agar medium without rhizobia. The Leonard jars were then covered with sterile non-wettable cotton wool as anti-contamination mulch, and the plants placed in a growth chamber with 16 h light per day, 70% relative humidity, and 28°C/16°C day/night cycle. For each plant species, 4 replicate Leonard jars were used per rhizobial strain.

Microscopic studies of sorghum and millet root infection by rhizobia

At 10 d after planting, root material (1 g fresh weight) of inoculated and uninoculated plants were harvested per replicate to study rhizobial colonization and infection of sorghum and millet roots using scanning electron microscopy (SEM). The 1 g root tissues from each replicate jar was chopped into small pieces (2–3 mm) and fixed in 2.5% gluteraldehyde in 10 mM phosphate buffer saline solution (PBS), pH 7.4, for 16 h. After washing in PBS, the specimens were post-fixed in 1% osmium tetroxide (OsO4) for 1 h, washed again in PBS, and the samples dehydrated by passing through a series of increasing concentration of ethanol (30–100%). The samples were then

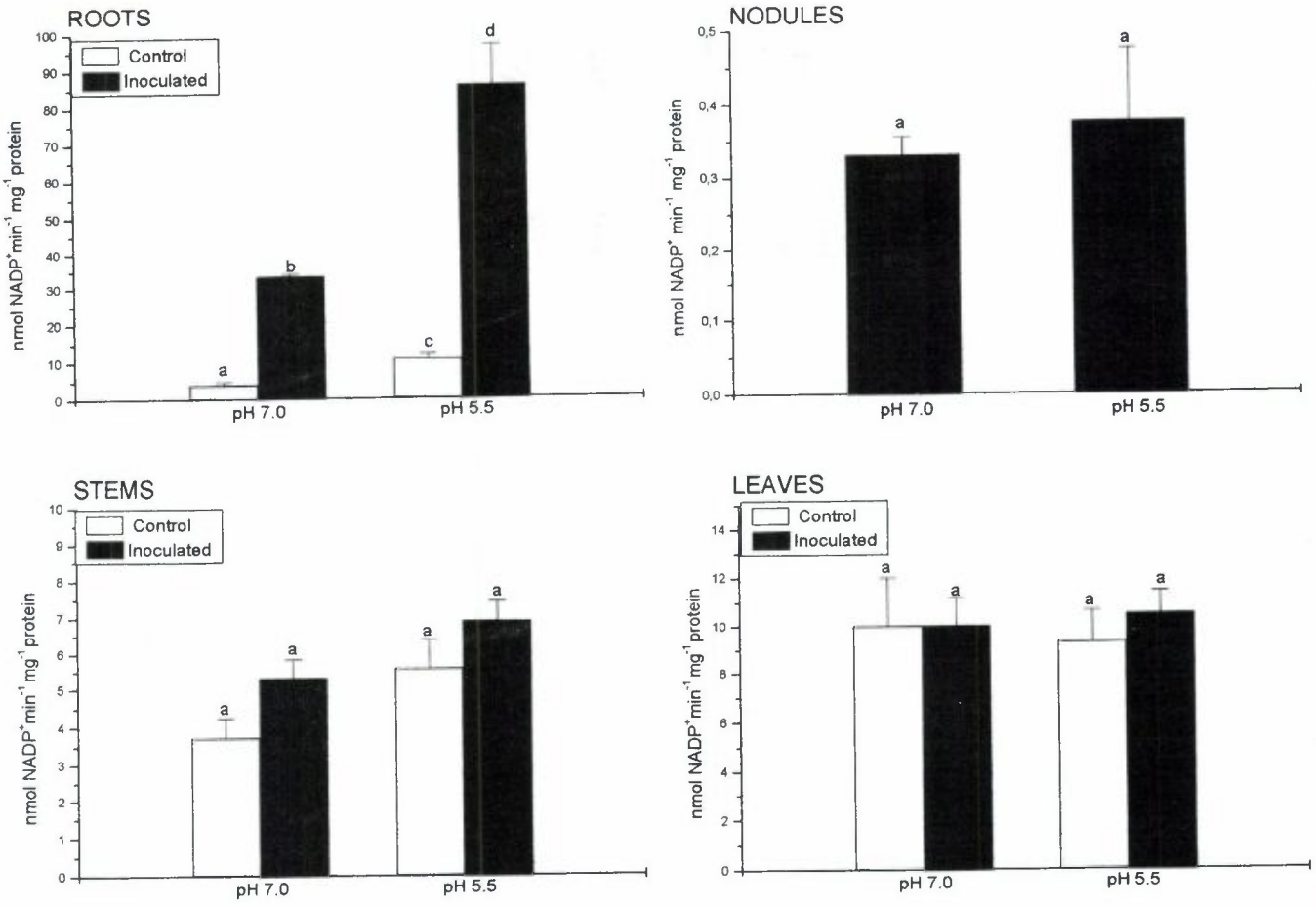

Figure 2. Glutamate synthase specific activity of roots, stems, leaves and nodules of peanut plants. Data are means ± S.E of four independent determinations. Different letters in each column indicate significant differences ($P<0.05$), according to the Duncan's test.

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dried in a Balzer's critical point dryer (Model CPD020, Leichtenstein, Germany), mounted on aluminium stubs, gold-palladium coated, and viewed for bacterial colonization of root surface using a scanning electron microscope (Leica Stereoscan Model 440, Cambridge, UK). The root specimens were also sectioned and the inner tissues viewed for colonization by rhizobia using SEM techniques.

Leonard jar experiments on rhizobial inoculation of sorghum and millet and its effects on plant growth and mineral nutrition.

Plant culture and growth analysis in Leonard jars with rhizobial inoculation

One inoculation experiment was conducted with sorghum and millet using Leonard jars in growth chambers. As described above, seedlings of sorghum and millet landraces were raised in Leonard jars from surface-sterilized seed material, and inoculated with 10 ml of the broth cultures of each of the 4 rhizobial strains, namely *B. japonicum* Tal 110, *S. meliloti* strain 1, *R. l.* bv. *viciae* Cn6 and R. l. bv. *viciae* strain 30. Uninoculated controls received equal volume of sterile yeast extract mannitol agar broth medium without rhizobia. Prior to sterilization, the nutrient solution in Leonard jars was adjusted to contain 1.0 mM NO_3 for meeting the N requirements of the two cereal plants. In all, 4 replicate jars were used for each rhizobial strain. After inoculation, the plants were left to grow in a growth chamber under similar light and temperature conditions as indicated previously and harvested for growth analysis at 94 d after planting. Root length was measured with a ruler, and the plants separated into shoots and roots for fresh weight determination. All samples were then oven-dried at 80°C for 48 h, weighed and ground into very fine powder for nutrient analysis.

Measurement of macro- and micro-nutrients in organs of inoculated sorghum plants in Leonard jars

The preparation of plant samples for the determination of macro-nutrient (P, P, Ca, Mg and Na) and micro-nutrients (Fe, Cu, Zn, Mn and B) in organs was done by dry-ashing, followed by acid digestion. A weighed amount (1 g dry matter) of plant material from each of the 4 replicates was ashed overnight in a crucible at 550°C in a muffle furnace, and the ash digested in 5 ml of 6 M HCl at 50°C for 30 minutes and filtered. The concentrations of nutrient elements were then determined after appropriate dilution, by direct aspiration on a calibrated simultaneous ICP spectrophotometer (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusetts, USA).

Bioassay for indole acetic acid (IAA) production by rhizobial strains

The rhizobial strains used in these experiments were analyzed for IAA production. For rapid quantitative estimation in broth culture, the colorimetric method of Gordon and Weber (1951) was used. The cultures were grown in the dark for 7 d, centrifuged at 15,000 x g for 10 min, and IAA assayed in duplicate supernatant samples. The presence of IAA in each supernatant was measured colorimetrically by adding two parts of 0.01 M $FeCl_3$ in 35% $HClO_4$ to one part supernatant, followed by reading the optical density at 530 nm after 25 min. The recorded absorbances were read off a standard curve prepared from pure indole acetic acid. Three separate assays were performed, and their average used for estimating IAA formation.

Nodulation bioassay of soybean seedlings using sterile sorghum root macerate

In order to confirm that the bacteria observed microscopically inside sorghum roots were indeed rhizobia, root tissue from sorghum plants inoculated with *B. japonicum* Tal 110 was surface-sterilized, and 1 g of material macerated under aseptic conditions for testing Koch's postulates. In each case, 15 ml of sterile distilled water was added to the macerate, thoroughly mixed, and 5 ml of the homogenate used to inoculate 5-d-old soybean seedlings grown aseptically in Leonard jars. Three replicate jars were used for each treatment, including the control. The treatments used consisted of uninoculated soybean control (minus sorghum root macerate), uninoculated soybean (plus macerate of uninoculated sorghum root), and inoculated soybean (plus macerate of surface-sterilized inoculated sorghum root). The surface of each jar was covered with sterile cotton wool as anti-contamination mulch and maintained in the glasshouse. At 4 weeks after inoculation, the plants were harvested and checked for nodulation. The soybean plants from each Leonard jar were then separated into nodules, roots and shoots and oven-dried at 65°C for determination of dry matter. Ground plant samples were used for N analysis and fixed-N calculated as the difference between nodulated and non-nodulated control.

Statistical analysis

The effects of the different inoculation treatments on plant growth and mineral nutrition were determined statistically using a one-way analysis of variance (ANOVA) from STATISTICA software package. Means were compared using Duncan test.

3. Results

Microscopic evidence of rhizobial infection of sorghum and millet roots

Scanning electron microscopy of sorghum roots showed a large number of bacterial cells on the surface of both main and lateral roots of inoculated sorghum plants (Figs. 1a and b), but not on those of uninoculated controls. Millet

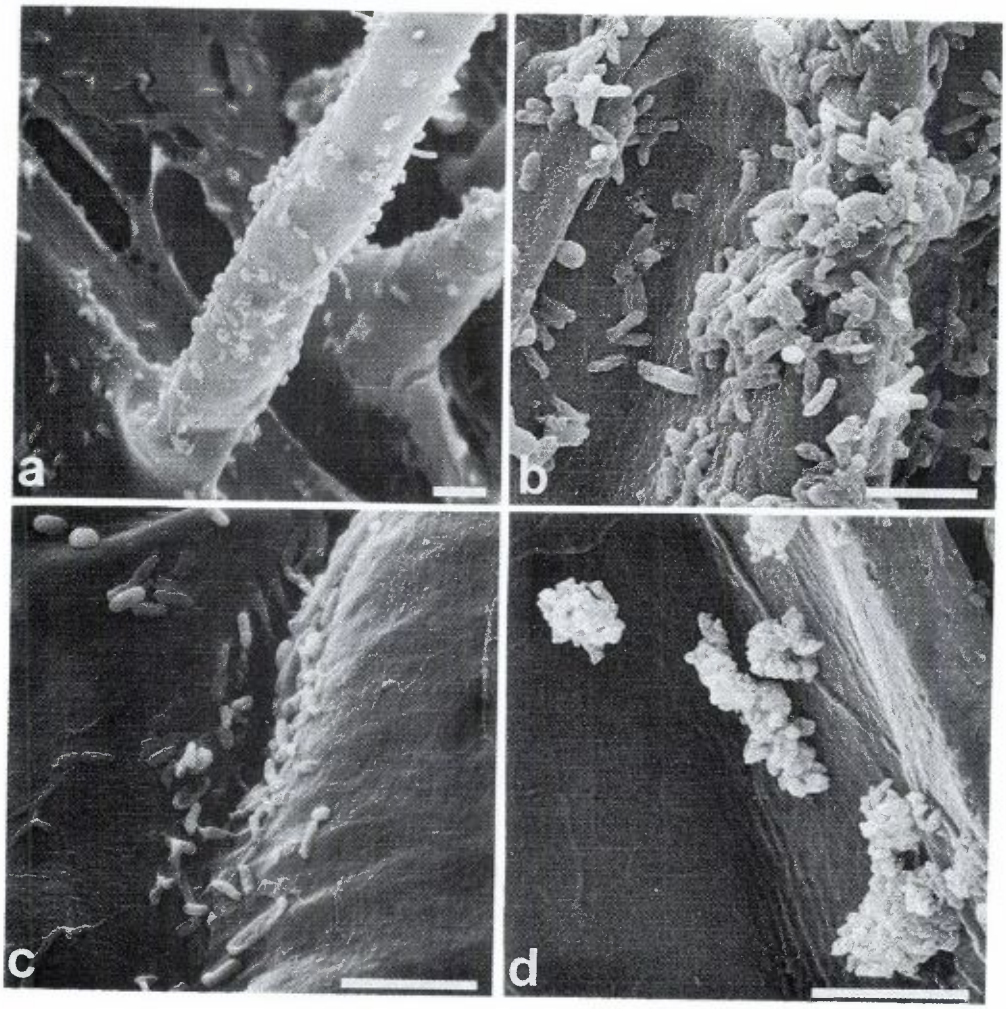

Figure 1. Scanning electron micrographs showing rhizobial cells on 10-d-old sorghum plant roots. a) *Azorhizobium caulinodans* ORS571, b) *Bradyrhizobium japonicum* Tal 110, c) *Rhizobium* GHR2, and d) *Rhizobium leguminosarum* bv. *viciae* Cn6 Lentil. Bar = 3 μm.

showed similar results. Attempts to locate rhizobial cells on the inside part of sectioned root tissue using SEM showed clumps of *B. japonicum* Tal 110 and single cells of *Rhizobium* GHR2 in the root (Figs. 2a and b). Other rhizobial strains including *Azorhizobium caulinodans* ORS571, *Rhizobium* NGR234 and *Rhizobium* GHR2 which were applied to sorghum and millet plants, could be similarly observed inside root tissue using SEM techniques. Sectioned root tissue prepared specifically for TEM also showed the presence of bacteria in the root's interior tissue (data not shown).

Plant growth response to rhizobial inoculation in Leonard jars

With sorghum, shoot growth, measured as dry matter, increased significantly (Table 1) by 8–54%. All four rhizobial strains used in this study caused a significant ($P<0.05$) increase in the shoot dry matter of sorghum (Table 1). Although sorghum root dry matter was unchanged with rhizobial inoculation, total biomass of plants inoculated with *Rhizobium leguminosarum* bv. *viciae* 30 and *R. leguminosarum* bv. *viciae* Cn 6 were significantly increased ($P<0.05$) at whole-plant level relative to control (data not shown). Fresh weights showed a similar pattern, and were significantly ($P<0.05$) increased by 11–51% with rhizobial inoculation (Table 1). However, the shoot and root biomass of millet plants were unaltered by rhizobial inoculation (data not shown).

The root lengths of sorghum plants were measured and found to increase significantly ($P<0.05$) with inoculation relative to uninoculated control. All the four rhizobial strains used in this study stimulated a significant ($P<0.05$) increase in root length of sorghum plants (Table 2). However, the root lengths of millet plants were unchanged by rhizobial inoculation (Table 2).

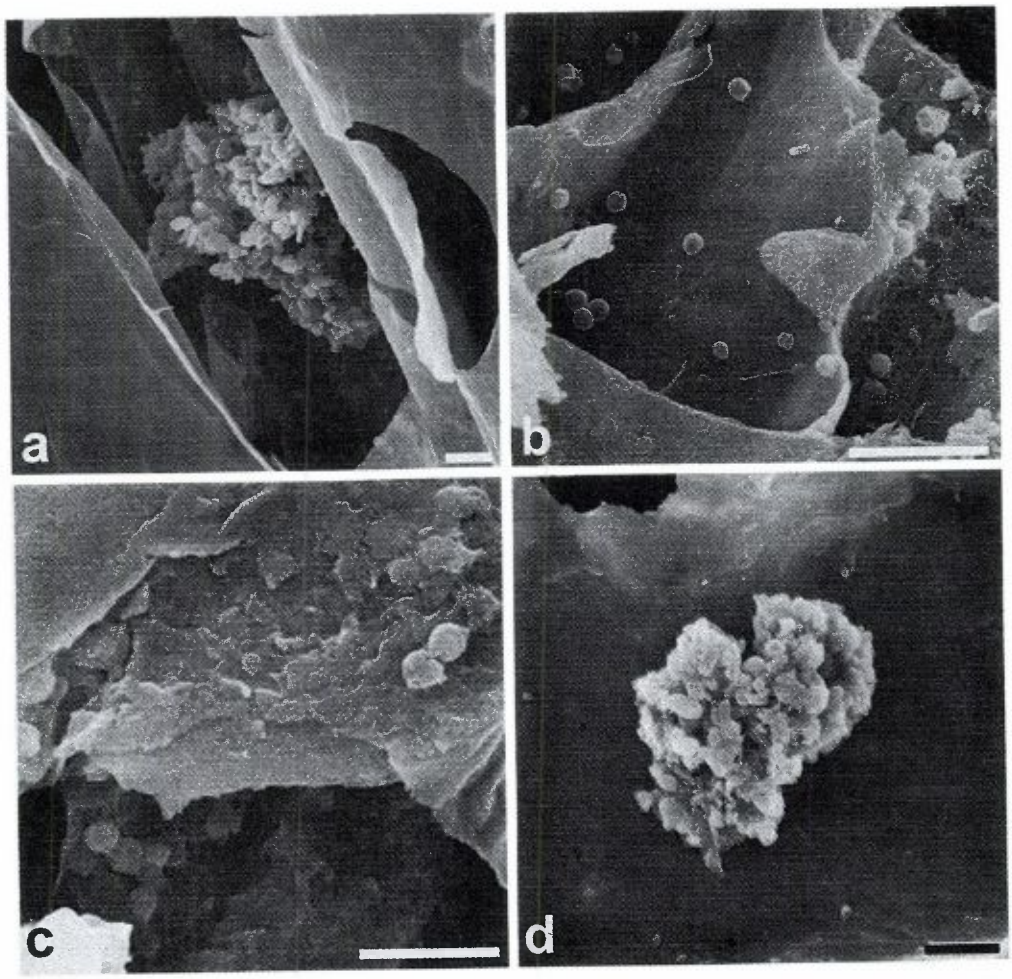

Figure 2. Scanning electron micrograph showing rhizobial association within 10-d-old sorghum and millet plant roots. a) *Bradyrhizobium japonicum* Tal 110 inside sorghum roots, b) *Rhizobium* GHR2 inside sorghum roots, c) *Azorhizobium caulinodans* ORS571 inside millet roots, and d) *Rhizobium leguminosarum* bv. *viciae* Cn6 Lentil inside millet roots. Roots were sectioned and the insides scanned. Bar = 3 μm.

Effects of rhizobial inoculation on the concentration of mineral nutrients in organs of sorghum plants

Because sorghum showed a marked growth response to rhizobial inoculation, the mechanism of growth promotion was studied through analysis of mineral nutrients in tissues. Inoculation of sorghum with *Sinorhizobium meliloti* strain 1 and *R. leguminosarum* bv. *viciae* strain Cn6 significantly ($P<0.05$) decreased N concentration in shoots (Table 3). Both *R. leguminosarum* bv. *viciae* strain 30 and strain Cn6 also decreased shoot Na levels (Table 3). With micronutrients, however, all test strains markedly ($P<0.05$) reduced the levels of Zn and Cu in shoot relative to control (Table 3). The shoot concentration of Mn and Al were also decreased by inoculation of sorghum with *R. leguminosarum* bv. *viciae* strain 30 and strain Cn6, respectively (Table 3). The data for sorghum roots were equally exciting, in that P and K concentrations were significantly ($P<0.05$) increased by rhizobial inoculation relative to control (Table 4). Except for *B. japonicum* Tal 110, inoculating sorghum with all the other test strains markedly ($P<0.05$) decreased Na in roots (Table 4). Of the micronutrients, Al concentration in roots was markedly reduced by rhizobial inoculation of sorghum relative to uninoculated control (Table 4).

Soybean nodulation by sterile sorghum root macerate

The application of 5 ml macerate prepared from sterile roots of sorghum plants which had been inoculated with *B. japonicum* Tal 110 produced nodules on soybean plants. However, the uninoculated controls as well as soybean plants treated with the macerate of uninoculated sterile sorghum root showed no evidence of nodulation. Because the soybean plants were effectively nodulated by sterile sorghum root macerate, the root, shoot and total dry matter

Table 1. Fresh and dry matter yield of shoots and roots of sorghum grown aseptically with 1 mM NO_3 and inoculated with different rhizobial strains. Values followed by dissimilar letters in a column are significantly different at $P<0.05$ (one-way ANOVA). Data are presented as Mean±S.E. (n=4).

Rhizobial strain	Fresh weight (g/plant)		Dry weight (g/plant)	
	Shoot	Root	Shoot	Root
No inoculation	3.7±0.32a	4.2±0.65a	1.28±0.13a	0.74±0.11a
Bradyrhizobium japonicum Tal 110	4.5±0.15b	4.4±0.30a	1.63±0.09b	0.76±0.09a
Sinorhizobium meliloti strain 1	4.1±0.22b	5.3±0.40a	1.43±0.09b	0.88±0.07a
Rhizobium leguminosarum bv. *viciae* strain 30	5.0±0.17b	4.9±0.33a	1.65±0.08b	0.85±0.78a
R. leguminosarum bv. *viciae* strain Cn6	5.6±0.32b	4.9±0.54a	1.98±0.13c	1.01±0.09a

Table 2. IAA production and effects of rhizobial inoculation on root length of sorghum and millet plants grown aseptically with 1 mM NO_3. Values followed by dissimilar letters in a column are significantly different at $P<0.05$ (one-way ANOVA). Data presented are Mean± S.E. (n=4). Each IAA value is an average of three separate assays. ND = not determined.

Rhizobial strain	IAA produced (μg ml^{-1})	Root length (cm)	
		Sorghum	Millet
Uninoculated control	–	63.73±2.89a	71.30±3.83a
Bradyrhizobium japonicum Tal 110	0.95	81.15±1.54b	75.97±2.09a
Sinorhizobium meliloti strain 1	1.66	84.08±2.46b	69.70±2.68a
Rhizobium leguminosarum bv. *viciae* strain 30	0.68	80.15±7.94b	61.98±1.87a
R. leguminosarum bv. *viciae* strain Cn6	0.18	76.85±2.93b	64.17±2.30a
Bradyrhizobium CB756	2.26	ND	ND
Rhizobium GHR2	0.45	ND	ND
Azorhizobium caulinodans ORS571	0.28	ND	ND
Rhizobium NGR 234	0.63	ND	ND

of these soybean plants were significantly greater than those of non-nodulated controls, and led to a measurable amount of fixed-N in the nodulated plants.

4. Discussion

Rhizobial colonization and infection of sorghum and millet roots

All the rhizobial strains tested in this study successfully colonized the roots of both sorghum and millet plants. TEM data revealed the presence of high numbers of bacteria on the surface and inside of main and lateral roots of inoculated sorghum and finger millet plants (Figs. 1 and 2), but not on uninoculated controls (data not shown). Under the SEM, small rod-shaped bacteria, typical of rhizobial cells, were seen as colonies around cracks on the root epidermal surfaces of the sorghum and millet plants. Because cracks serve as entry points for rhizobial invasion of non-legume hosts (de Bruijn et al., 1995; Spencer et al., 1994; Yanni et al., 1997), the swamp of bacteria found around the cracks of the sorghum and finger millet roots could suggest initiation of the infection process (Fig. 2). However, rhizobia are also known to invade their hosts via enzymatic action on plant cell walls (Mateos et al., 1992; Jimenez-Zurdo et al., 1996).

The bacteria observed on the surface and inside of sorghum and millet roots were assumed to be the applied rhizobial strains because uninoculated plants showed no evidence of bacteria on the root epidermis or in interior tissues. This assumption was validated when root macerate prepared from sterile inoculated sorghum plants successfully induced nodulation and N_2 fixation in soybean seedlings. No nodules were however found on soybean plants inoculated with plant homogenate prepared from surface-sterilized roots of uninoculated sorghum plants, suggesting that the bacteria observed microscopically inside sorghum root tissues were indeed the authentic N_2-fixing rhizobia applied to cereal plants. The detection of bacteria inside roots of inoculated sorghum and millet under axenic conditions in this study, is consistent with other reports (Gough et al., 1997; James et al., 1994; O'Callaghan et al., 1997; Reddy et al., 1997; Reis et al., 1999; Schloter et al., 1997; Yanni et al., 1997; Chaintreuil et al., 2000; Yanni et al., 2001), which showed the presence of various bacteria inside root tissue of their host-plants. As the test plants in these studies are non-legumes, it is still not known whether they benefited otherwise from the rhizobial infection of roots.

Table 3. Concentration of macro-and micro-nutrients in shoots of sorghum plants grown aseptically with 1 mM NO_3 and inoculated with different rhizobial strains. Values followed by dissimilar letters in a column are significantly different at P<0.05 (one-way ANOVA). Data are presented as Mean±S.E. (n=4). The concentrations of P, K, Ca, Fe and B were unaffected by the treatments.

Bacterial strain	N (%)	Mg (%)	Na (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Al (mg/kg)
No inoculation	0.51± 0.04a	0.17±0.01a	636.50±103.82a	5.81±0.53a	81.63±25.93a	32.10±1.71a	359.25±78.94a
B. japonicum Tal 110	0.41± 0.01ab	0.14±0.00a	503.75±29.47a	4.50±0.07b	14.53±0.75b	32.00±1.06a	247.00±10.79a
S. meliloti strain 1	0.42±0.02ab	0.18±0.06ab	512.75±45.88a	4.47±0.37b	14.63±1.73b	39.08±6.36a	301.00±57.46a
R. leguminosarum bv. *viciae* strain 30	0.53±0.06a	0.14±0.00a	354.25±27.23b	4.13±0.11b	10.73±0.71b	24.83±1.18ab	225.75±13.35a
R. leguminosarum bv. *viciae* strain Cn6	0.39±0.02b	0.15±0.01a	376.50±50.85b	4.73±0.28b	11.70±0.52b	27.70±5.76a	182.00±23.77b

Table 4. Concentration of macronutrients in roots of sorghum plants grown aseptically with 1 mM NO_3 and inoculated with different rhizobial strains. Values followed by dissimilar letters in a column are significantly different at P<0.05 (one-way ANOVA). Data are presented as Mean±S.E. (n=4). The concentrations of N, Ca, mg, Cu, Zn, Mu, Fe and B were not affected by treatments.

Bacterial strain	P (%)	K (%)	Na (mg/kg)	Al (mg/kg)
No inoculation	0.06±0.02a	0.40±0.00a	1,313.75±169.43a	1,172.75±207.73a
Bradyrhizobium japonicum Tal 110	0.07±0.02ab	0.40±0.00a	907.25±63.66b	792.25±134.44b
Sinorhizobium meliloti strain 1	0.18±0.03b	1.17±0.43b	722.75±96.85b	699.50±96.97b
Rhizobium leguminosarum bv. *viciae* strain 30	0.09±0.09b	0.97±0.32b	765.75±59.21b	702.50±70.72b
Rhizobium leguminosarum bv. *viciae* strain Cn6	0.21±0.01b	1.17±0.44b	520.75±35.52c	492.75±44.07b

Rhizobial stimulation of plant growth and enhanced mineral nutrition in sorghum

The inoculation of asepically-grown seedlings of an African landrace of sorghum with infective cells of rhizobial strains promoted shoot growth by 11–51% on fresh weight basis, and 8–54% on dry weight basis. In fact, all the inoculated plants showed significantly (P<0.05) greater shoot fresh weight and dry matter compared with uninoculated control, but their root masses remained unaffected (Table 1). The two strains of *R. l.* bv. *viciae* were numerically more effective in their stimulation of shoot growth relative to the other strains (Table 1). There was also a significant 21–32% increase in root length with rhizobial inoculation of sorghum (Table 2), thus suggesting the possible release of root-growth-promoting molecule by the test strains. In that regard, *Bradyrhizobium* strain CB756 is known to have stimulated an increase in root length of wheat with inoculation (Law and Strijdom, 1989). Out of 5 *Bradyrhizobium* species tested, only strain CB756 could consistently induce an increase in root length through the release of an unknown stimulatory substance present in both cell suspensions and cell-free culture filtrates (Law and Strijdom, 1989). Recent studies (Dakora, 2003; Dakora et al., 2002; Phillips et al., 1999; Smith et al., 2002; Matiru and Dakora, 2005a,b) have shown that rhizobial metobolites such as lumichrome and lipo-chito-oligosuccharide Nod factors can stimulate an increase in plant growth among legume and non-legume species. For example, applying 5 nM lumichrome to the roots of cowpea, soybean, sorghum, finger millet and maize plants was found to increase its translocation in xylem to shoots, which then induced early initiation of trifoliate leaf development, increased expansion in unifoliate and trifoliate leaves and promoted stem elongation, leading to increased total biomass (Matiru and Dakora, 2005a,b).

IAA is a phytohormone that also stimulates root development in plants, and has been shown to be the common cause of corn growth promotion following inoculation with *Azospirillum* (Okon et al., 1995). An assay for the production of this molecule in the presence of tryptophan (Gordon and Weber, 1951) revealed the ability of the test strains to form IAA from tryptophan oxidation. Although the method (Gordon and Weber, 1951) used here is less sensitive, in that it detects down to only 0.5 μg ml^{-1}, the test strains were found to produce biologically significant amounts of IAA (Table 2). It is however not known whether it is the IAA or some other active molecule that stimulated root elongation in sorghum plants relative to uninoculated control (Table 2). Shoot concentration of N

and Na were decreased by some rhizobial strains such as *S. meliloti* strain 1, *R. leguminosarum* bv. *viciae* strain 30 and *R. leguminosarum* bv. *viciae* strain Cn6 (Table 3). Rhizobial inoculation however, increased P and K concentrations in sorghum roots, but not in shoots, possibly due to partitioning to organs and/or the developmental stage of the plant. To our knowledge, this is the first report of plant growth promotion via enhanced P and K nutrition in landrace of a major African cereal crop with rhizobial inoculation. Future studies using a wider collection of diverse landraces of sorghum and finger millet are likely to indicate greater benefits of rhizobial inoculation of these important African food crop species (Matiru and Dakora, 2004).

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