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Review

Potential use of rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops

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Rhizobia form root nodules that fix nitrogen (N_2) in symbiotic legumes. Extending the ability of these bacteria to fix N_2 in non-legumes such as cereals would be a useful technology for increased crop yields among resource-poor farmers. Although some inoculation attempts have resulted in nodule formation in cereal plants, there was no evidence of N_2 fixation. However, because rhizobia naturally produce molecules (auxins, cytokinins, abscicic acids, lumichrome, rhiboflavin, lipo-chito-oligosaccharides and vitamins) that promote plant growth, their colonization and infection of cereal roots would be expected to increase plant development, and grain yield. We have used light, scanning, and transmission electron microscopy to show that roots of sorghum and millet landraces from Africa were easily infected by rhizobial isolates from five unrelated legume genera. With sorghum, in particular, plant growth and phosphorus (P) uptake were significantly increased by rhizobial inoculation, suggesting that field selection of suitable rhizobia/cereal combinations could increase yields and produce fodder for livestock production.

Key words: Rhizobia Rhizobia, N2 fixation, natural endophytes, non-legume infection, cereal crops, landraces, sorghum, millet.

INTRODUCTION

Efforts at extending N_2-fixing ability to important non-leguminous crops such as cereals has long been a major goal of workers in the field of biological nitrogen fixation. Making cereals self-sufficient in N nutrition would be of great benefit to resource-poor farmers in Africa. One approach for achieving this goal has involved the isolation and characterization of N_2-fixing bacteria from a variety of wild and cultivated cereal crops (Stoltzfuz et al., 1997), an exercise which produced a wide array of diazotrophs from plant organs including roots and stems. Some of those microbes so far identified in non-legumes include *Gluconoacetobacter diazotrophicus* (formerly *Acetobacter diazotrophicus*) from sugarcane (Cavalcante and Dobereiner, 1988; Gillis et al., 1989; Fuentes-Ramirez et al., 1993; Caballero-Mellado, 1994; James et al., 1994; Sevilla et al., 1998, 2001; Reis et al., 2001; Riggs et al., 2001; Muthukumarasamy et al., 2002). Strains of *G. diazotrophicus* have also been isolated from roots and stems of coffee (Jimenez-Salgado et al., 1997). *Azospirillum* is another much studied diazotroph, especially the species *lipoferum* and *brasilense,* which have been shown to infect a number of cereal plants including wheat, maize and sorghum (Reynders and Vlassak, 1982; Pacovsky et al., 1985; Dobereiner and Boddey, 1981; Christansen and Vanderleyden, 1993, Fallik and Okon, 1996, Mallik et al., 1997; Kapulnik et al., 1983; Weber et al., 1999; Dobbelaere et al., 2001) Other

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known diazotrophs include *Herbaspirillum seropedicae* (Dobereiner et al., 1993; Weber et al., 1999; Riggs et al., 2001), *Klebsiella pneumoniae* and *Panotoea agglomerans* (Riggs et al., 2001), *Enterobacter sp*, *Klebsiella oxytoca*, *Azotobacter, Arthrobacter*, *Azoarcus*, *Bacillus* and *Zooglea* (Mirza et al., 2001).

Information on N_2-fixation by these associative diazotrophs is rather scanty and amounts fixed disappointingly low, except for *G. diazotrophicus* which fixes ecnomical amounts of N_2 in sugarcane (Boddey et al., 1988; Dobereiner et al., 1993; Sevilla et al., 1998; 2001). However, growth promotion has been observed with many of these diazotrophs even where N_2-fixation could not be demonstrated. In general, these diazotrophs are reported to improve root growth and function, often leading to increased uptake of water and mineral nutrients. Plant inoculation with *Azospirilum brasilense,* for example, promoted greater uptake of NO_3^-, K^+, and $H_2PO_4^-$ in corn, sorghum, wheat and setaria (Lin et al., 1983; Okon and Kapulnik, 1986; Murty and Ladha, 1988; Zavalin et al., 1998; Saubidet et al., 2000), leading to higher crop yields. Because rhizobia also produce various metabolites such as auxins, cytokinins, riboflavin and vitamins (Phillips and Torrey, 1970; Dakora, 2003), their invasion of legume and non- legume plant roots should promote an increase in plant growth. This review examines rhizobial bacteria as natural endophytes of cereal and their potential for increasing yields of major cereal crops in Africa.

RHIZOBIA AS NATURAL ENDOPHYTES OF THE LEGUMINOSAE

Rhizobia (species of *Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, and *Sinorhizobium*) form intimate symbiotic relationships with legumes by responding chemotactically to flavonoid molecules released as signals by the legume host. These plant compounds induce the expression of nodulation (*nod*) genes in rhizobia, which in turn produce lipo-chito-oligosaccharide (LCO) signals that trigger mitotic cell division in roots, leading to nodule formation (Dakora 1995; Lhuissier et al., 2001).

Nitrogen is required for cellular synthesis of enzymes, proteins, chlorophyll, DNA and RNA, and is therefore important in plant growth and the production of food and feed. For nodulating legumes, nitrogen is provided through symbiotic fixation of atmospheric N_2 by nitrogenase in rhizobial bacteroids. This process of biological nitrogen fixation (BNF) accounts for 65% of the nitrogen currently utilized in agriculture, and will continue to be important in future crop productivity, especially in sustainable systems. In Africa, grain legumes fix about 15-210 kg N ha^{-1} seasonally, while tree legumes fix about 43-581 kg N ha^{-1} y^{-1} (Dakora and Keya, 1997). Leaf prunings of trees and the foliage of annual legumes are thus an important component of sustainability in fallow, agroforestry and tropical cropping systems. The N fixed by rhizobia in legumes can also benefit associated non-legumes via direct transfer of biologically-fixed N to cereals growing in intercrops (Englesham et al., 1981), or to subsequent crops rotated with symbiotic legumes (Dakora and Keya, 1997). In many low input grassland systems, the grasses depend on the N fixed by their legume counterparts for their N nutrition and protein synthesis, which is much needed for forage quality in livestock production (Paynel et al., 2001). In addition to N_2 fixation in legumes, rhizobia are also capable of contributing to growth promotion in non-legume species.

RHIZOBIAL INFECTION OF NON-LEGUMES UNDER EXPERIMENTAL CONDITIONS

Although rhizobia naturally infect legumes as host plants, some *Rhizobium* strains can form symbiotic relationships with non-legume species such as *Parasponia* (Trinick, 1979). Effective nodulation has also been observed in *Parasponia andersonii*, following *Bradyrhizobium* inoculation of plantlets regenerated from calii (Davey et al., 1993). The nodulation of *Parasponia* by both *Rhizobium* and *Bradyrhizobium* strains provides further encouragement that rhizobial infection and nodule formation in non-legume crops is a possibility in the future. Thus the ability of rhizobia and bradyrhizobia to infect and nodulate *Parasponia* has increased the prospect and search for rhizobial nodulation of non-legume plants such as cereals.

A number of workers have experimentally demonstrated the ability of rhizobia to colonize roots of non-legumes and localize themselves internally in tissues, including the xylem (Spencer et al., 1994). Following that success, several attempts have been made to extend nodulation and N_2-fixing ability to non-legume crops (Al-Mallah et al., 1990; Gough et al., 1997a; Antoun et al., 1998; Stone, 2001). Some of these early experiments successfully induced nodulation in oilseed rape, though only after treating the seedling roots with enzymes followed by inoculation with rhizobia (Al-Mallah et al., 1990). Applying *Bradyrhizobium japonicum* to radish significantly increased plant dry matter by 15% but without nodulation (Antoun et al., 1998). *Azorhizobium caulinodans* ORS571, which induces stem and root nodules in the tropical legume *Sesbania rostrata,* has also been shown to colonize the internal tissues of *Arabidopsis thaliana* through cracks at points of lateral root emergence (Gough et al., 1997a: 1997b). The co-application of *A. caulinodans* and flavonoids such as naringenin and daidzein, even at very low concentrations (5×10^{-5} M) significantly enhanced microsymbiont colonization of roots and promoted localization in the xylem of *A. thaliana* (Stone, 2001). Other studies have similarly demonstrated the ability of

Rhizobium leguminosarum bv. *phaseoli* to colonize roots of lettuce plants (Chabot et al., 1996).

Naturally-occurring rhizobia, isolated from nodules of *Parasponia* and some tropical legumes, have also been shown to infect roots of many agricultural species such as rice, wheat and maize via cracks made by emerging lateral roots (Webster et al., 1997). Inoculation of rice and wheat with *A. caulinodans* strain ORS571 carrying a *lac*Z reporter gene showed that a high proportion of the internal plant colonization occurred from lateral root cracks. Supplying the flavonone naringenin at 10^{-4} or 10^{-5} M concentration increased rhizobial entry via cracks and promoted intercellular localization in wheat roots (Webster, 1997; 1998). But this endophytic establishment of *A. caulinodans* in wheat roots is nevertheless possible without the addition of flavonones (Sabry et al., 1997). What is however most intriguiging is the report that the *nod* D1 gene product of *Rhizobium* strain NGR234 responds to activation by phenolic compounds isolated from wheat extracts (le Strange et al., 1990). Whether the role of flavonoids in non-legume root infection is accidental or symbiotically-related, remains to be determined.

In a study with maize, Chabot et al. (1996) used bioluminescence from *R. leguminosarum* bv. *phaseoli* strains harbouring *lux* genes to visualize *in situ* colonization of roots by rhizobia, as well as to assess the efficiency with which these bacteria infected maize roots. Their observations were consistent with findings on maize root colonization and infection by rhizobia reported by Schloter et al. (1997) and Yanni et al. (2001).

As a major food crop, rice has probably attracted more dollar investment in nodulation studies with rhizobia than any other non-legume species (Chaintreuil et al., 2000; Yanni et al., 2001). Al-Mallah et al. (1989) were the first to successfully induce nodular structures on rice roots after treating 2-d-old seedling roots with a cell wall-degrading enzyme mixture followed by rhizobial inoculation in the presence of polyethylene glycol. In a later study, Weber et al. (1999) detected an increase in rhizobial infection of rice roots with the application of low concentrations of naringenin(10^{-4} and 10^{-5} M). Interestingly, the same flavonone was shown to enhance the colonization of rice roots and internal localization in xylem by *Azorhizobium caulinodans* strain ORS571 (Gopalaswamy et al., 2000). Similar rice-rhizobial interactions have been reported by de Bruijn (1995) and Ladha (1997). However, it is only recently that the isolation of rhizobia as natural endophytes of cereals or other non-legume plant species has began.

RHIZOBIA AS NATURAL ENDOPHYTES OF NON-LEGUME CROPS

The success with which laboratory studies infected cereal roots with rhizobia led to the hypothesis that during legume-cereal rotations and/or mixed intercropping rhizobia are brought into closer contact with cereal roots, and this probably results in non-legume root infection by native rhizobial populations in soil. With that background, attempts have been made to determine if rhizobia naturally infect roots of cereals and other major food plants. The study by Yanni et al. (1997) was the first to isolate *R. leguminsarum* bv. *trifolii* as a natural endophyte from roots of rice in the Nile delta. Because rice has been grown in rotation with berseem clover for about 7 centuries in the Nile delta, this practice probably promoted closer rhizobial affinity for this cereal as a “host plant”. This hypothesis is re-inforced by the fact that the clover-nodulating rhizobia isolated from rice could occur up to 2.5×10^{7} cells g^{-1} fresh weight of root, concentrations similar to those obtained for bacteroids in legume root nodules. Chaintreiul et al. (2000) similarly isolated photosynthetic bradyrhizobia from roots of the African brown rice, *Oryza glaberrima*, which generally grows in the same wetlands as *Aeschynomene sensitive*, a stem-nodulated legume associated with photosynthetic strains of *Bradyrhizobium*. Again, this may well suggest co-evolution of *Aeschynomene* bradyrhizobia and wild genotypes of African brown rice. But whether these bradyrhizobia affect growth of *O*. *glaberrima* plants, has not been determined.

Besides rice, rhizobia have also been isolated as natural endophytes from roots of other non-legumes species such as cotton, sweet corn (McInroy and Kloepper, 1995), maize (Martinez-Romero et al., 2000), wheat (Biederbeck et al., 2000) and canola (Lupwayi et al., 2000) either grown in rotation with legumes or in a mixed cropping system involving symbiotic legumes. Because intercropping of symbiotic legumes with cereals, vegetables and tuber crops is a common feature of African agriculture, a programme of field isolation is likely to discover rhizobia as natural endophytes in roots of the non-legume components of these cropping system.

SIGNIFICANCE OF RHIZOBIAL INFECTION OF CEREALS AND ITS POTENTIAL FOR INCREASED YIELDS IN AFRICA

Of the studies so far described, only two are of relevance to the African situation, namely the isolation of rhizobia as natural endophytes of *Oryza sativa* in Egypt, and *Oryza glaberrima* in Senegal and Guinea (Yanni et al., 2001; Chantreuil, 2000). However, studies currently conducted in South Africa using African landraces of sorghum, millet and Sudan grass from Kenya have shown the ability of *Bradyrhizobium japonicum* TAL 110, *Azorhizobium caulinodan* ORS571, *Rhizobium* NGR234, *Rhizobium* GHR2, *Sinorhizobium meliloti* strain1, *Rhizobium leguminosarum* bv. *viceae* Cn6, and *R. leguminosarum* bv. *viceae* strain 30 to infect roots of African landraces of sorghum and millet. Because rhizobia produce

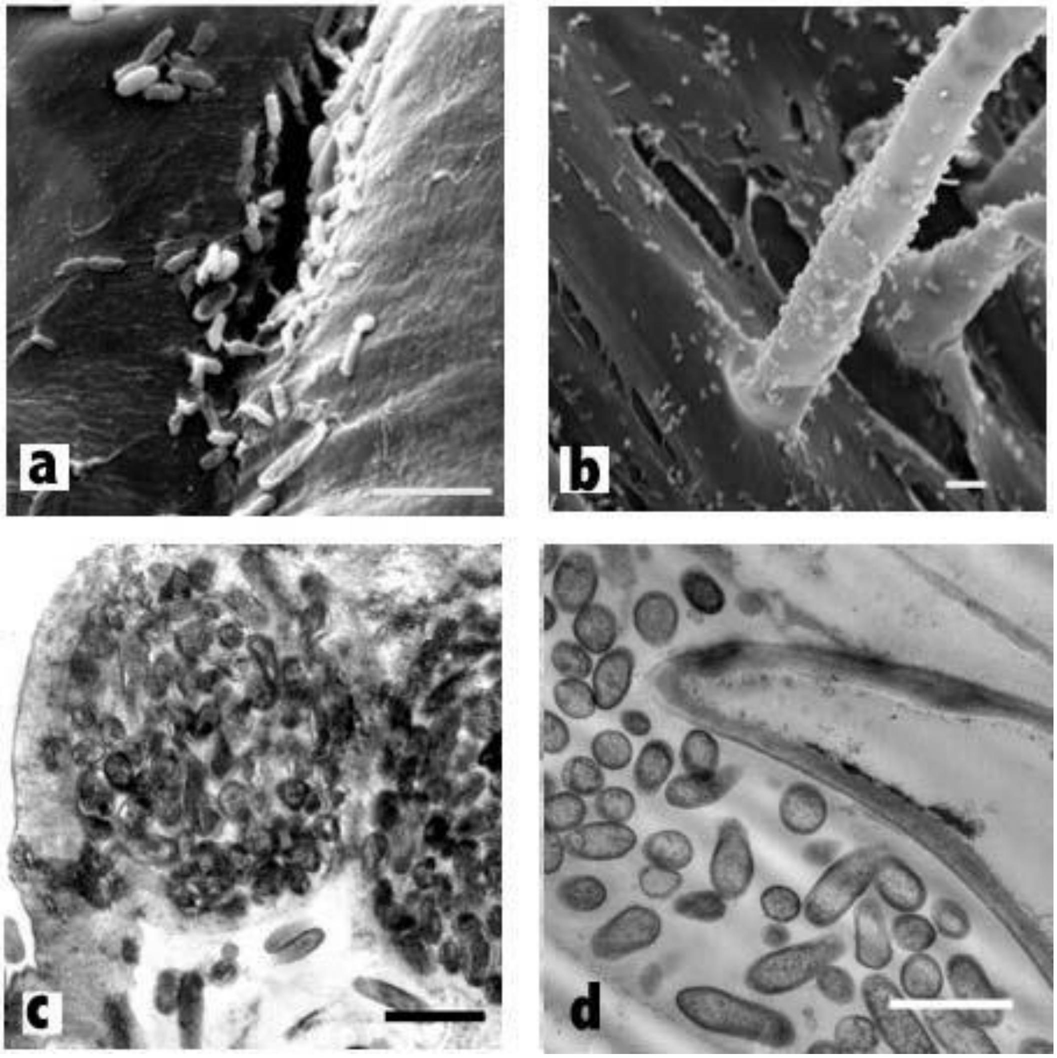

Figure 1. (a) Scanning electron micrograph showing *Rhizobium* GRH2 at the crack of sorghum root. Bar = 3 µm. (b) Scanning electron micrograph showing *Azorhizobium caulinodans* ORS571 on the outer surface of 94-d-old sorghum plant. Bar = 3µm. (c) Transmission electron micrograph of *Bradyrhizobium japonicum* TAL 110 inside sorghum root tissue. Bar = 20 µm. (d) Transmission electron micrograph of *Bradyrhizobium japonicum* TAL 110 inside millet root tissue. Bar = 20µm (V.N. Matiru and F.D. Dakora, unpubl. data).

phytohormones such as auxins, cytokinins, gibberllins and abscicic acid, it is likely that their release into cropping systems promotes plant growth and possibly increases yield even though no N_2 fixation by rhizobia has been detected in these non-legumes. This is in addition to the role of microbial metabolites in making nutrients available to plants (Dakora and Phillips, 2002). Rhizobial release of nodulation signals such as lipo-chito-oligosaccharides (LCOs) is also known to stimulate seed germination in a wide range of plant species by a still unknown mechanism. For example, recent findings show that lumichrome and LCOs released by rhizobia stimulate growth of crop plants (Phillips et al., 1999; Zhang et al., 2002; Dakora 2003). Large increases in plant growth were observed in sorghum, soybean and cowpea genotypes when supplied with 5 nM concentrations of lumichrome (Dakora et al., 2002). This suggests that *in planta* release of lumichrome by rhizobial endophytes could be a factor in stimulating cereal growth following rhizobial inoculation. From our studies, it is clear that where rhizobial infection was easily demonstrated for sorghum using light, scanning and transmission electron microscopy, plant growth was increased in response to inoculation; however, where it was difficult to establish rhizobial presence in millet roots, plant growth was unaffected by rhizobial inoculation (V. N. Matiru and F.D. Dakora, unpublished data). Additionally, because P uptake by sorghum increased with inoculation, it would seem that the bacteria induced some changes in phosphate transporter activity of sorghum root plasma membrane, leading to improved P nutrition.

The data presented in Figure 1 have clearly demonstrated the ability of various laboratory strains of rhizobia to colonize and infect roots of major cereal crops in Africa. It is important to note that the rhizobial strains used to infect sorghum and millet (Figure 1) in our study originated from different legumes, e.g. *Bradyrhizobium japonicum* from soybean*, Azorhizobium caulinodan* from *Sesbania rostrata*, *Rhizobium* NGR234 from *Lablab purpureus, Sinorhizobium meliloti* from *Medicago sativa*, *Rhizobium leguminosarum* bv. *viceae* Cn6, and *R. leguminosarum* bv. *viceae* strain 30 from *Vicia faba* . The fact that such a diverse group of rhizobia isolated from different legume genera could each infect African landraces of sorghum and millet as host plants, suggests that rhizobial infection of non-legumes is probably more widespread in nature than previously thought. Although these rhizobial endophytes have not been evaluated for N_2 fixation in these landraces, their benefit as a source of growth-promoting molecules to the host plant is more likely to be marked than a possible supply of N by nitrogenase. Not only do rhizobial metabolites such as lumichrome enhance drought tolerance via stomatal control in plants, they also promote tillering in sorghum (V.N. Matiru and F.D. Dakora, unpubl. data), leading to increaseed growth and possibly grain yield. A combined approach of screening cereal roots from farmers' fields for their rhizobial status and conducting field trials on rhizobial inoculation of cereals has great prospects for identifying cereal genotypes with affinity for rhizobial stimulation of plant growth. In such a study, the unbred African landraces of sorghum, millet, rice and maize would be better candidate material for selection of superior strain/host plant combinations as conventional breeding sometimes results in loss of useful genetic traits.

CONCLUSION

More research is needed on the interaction between indigenous African cereal crops and rhizobia as the latter are known to confer protection against pathogens and drought while promoting growth of the host plant (Dakora, 2003). Of particular importance would be the screening of several landraces for rhizobial infection of roots, followed by an assessment of the environmental factors that favour (or discourage) rhizobial infection of these non-legume crops. Such a screening programme may well discover that rhizobial infection of plant roots is a common feature of both legume and non-legume species. It is only then that these plant/bacterial associations involving rhizobia and non-legumes can be genetically manipulated for increased plant growth and possibly grain yield. Rhizobial promotion of sorghum growth as feed could, have marked effect on livestock production just as rhizobially-increased grain yield could enhance the food security of rural African communities.

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