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Full Length Research Paper

Host range, symbiotic effectiveness and nodulation competitiveness of some indigenous cowpea bradyrhizobia isolates from the transitional savanna zone of Ghana

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To identify indigenous rhizobia with potential as inoculants for increasing cowpea (*Vigna unguiculata*) yields, we have assessed the host range, symbiotic effectiveness and competitiveness for nodule occupancy among five (AII-2-1, AII-5-2, AI-4-3, AII-3-4 and BIII-2-2) indigenous cowpea bradyrhizobia isolates from the transitional savanna zone of Ghana. ERIC-PCR DNA fingerprinting patterns were used to identify the isolates occupying nodules. All the isolates nodulated cowpea, groundnut (*Arachis hypogeae*) and mungbean (*Vigna radiata*), but only AII-2-1, AII-3-4 and BIII-2-2 nodulated soybean (*Glycine max*). Apart from cowpea where all the isolates were effective, there were significant differences in the symbiotic effectiveness of the isolates on the other host legumes. Out of a total of about 250 cowpea nodules that were screened for each inoculum-mix, isolate AII-5-2 was the most competitive for nodule occupancy whilst AII-3-4 was the least. Isolate AII-5-2 occupied 71% of the nodules in an inoculum-mix consisting of equal proportions of AII-2-1, AII-5-2 and AI-4-3 (a 3-isolate-mix) and 60% of nodules in an inoculum-mix consisting of equal proportions of all the five isolates (a 5-isolate-mix). Therefore, among the isolates tested, AII-5-2 has the best potential for use as inoculant for maximizing cowpea yield in N_2- deficient agro-ecological zones of Ghana.

Key words: Competition, legumes, nitrogen fixation, rhizobia, symbiosis.

INTRODUCTION

Soil bacteria collectively called rhizobia can enter into a symbiotic association with legumes which leads to the formation of nitrogen fixing root nodules. This symbiotic interaction is of agronomic and ecological importance because of its significant amount of nitrogen to the total nitrogen budget in terrestrial ecosystems (Postgate, 1998). An important characteristic of this symbiotic interaction is host specificity, where defined species of rhizobia forms nodules on specific legumes. The rhizobia strains ability to form nodules with a wide range of legume hosts in an environment may contribute to their persistence (Bottomley, 1992).

Most West African soils are experiencing a decline in soil nitrogen status which is a major threat to food production (Sanginga, 2003). Biological nitrogen fixed via rhizobia-legume symbiosis has therefore been recommended for the sustenance of traditional agriculture (Peoples et al., 1995; Postgate, 1998). A common approach to improve symbiotic nitrogen fixation and legume productivity has been the reliance on superior or very effective exotic rhizobia strains as inoculants. This approach has, however, failed to achieve the desired responses in a lot of environments (Brockwell et al., 1995). The failure has largely been attributed to the poor nodulation competitiveness of the introduced rhizobia (Streeter, 1994). When introduced, the inoculant rhizobia must adapt to the prevailing soil conditions, multiply in the

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Table 1. Cowpea bradyrhizobia isolates used in this study.

Name of isolate	Relevant characteristics
AII-2-1	Slow growing[1]
AII-5-2	Slow growing[1]
AI-4-3	Slow growing[1]
AII-3-4	Fast growing[2]
BIII-2-2	Fast growing[2]
LBG 13	Fast growing[2*]

[1]Seven days to form visible colonies on yeast mannitol agar plates.
[2]Four days to form visible colonies on yeast mannitol agar plates.
*Originally isolated from bambara groundnut (*Vigna subterrenea*) and served as reference or positive control isolate.

soil and host rhizosphere and compete with the indigenous often ineffective rhizobia population for infection sites (Vlassak and Vanderleyden, 1997). But unfortunately, the inoculants often fail to occupy a significant proportion of the nodules (Dudman and Brockwell, 1968; Vlassak and Vanderleyden, 1997). A way of improving the success of inoculants can be to use native strains that are effective as well as competitive for nodulation as inoculants. Cowpea (*Vigna inguiculata*) is an important food legume that features prominently in many farming systems in Africa including Ghana (Fening and Danso, 2002). The legume is readily nodulated by the indigenous rhizobia population present in Ghanaian soils (Danso and Owiredu, 1988). A recent study on some 100 cowpea bradyrhizobia isolates in soils across the different ecological zones of Ghana indicated that a minority (26%) of the isolates are effective in fixing nitrogen with cowpea, a majority (68%) moderately effective and the remaining (6%) ineffective (Fening and Danso, 2002). It is also estimated that in Ghana the benefit of nodulated cowpea to soil nitrogen supply is 60 kg N ha^{-1} when residues from the crop are incorporated into the soil (Dakora et al., 1987). The potential for increasing cowpea yield as well as improving the soil nitrogen status by using very effective indigenous isolates as inoculants therefore exist. At present we lack adequate information on the diversity, symbiotic characteristics, as well as the competitiveness for nodule occupancy of this native population of rhizobia. As a step to increase cowpea yields in Ghana through effective symbiosis with these native rhizobia, we established a collection of indigenous cowpea bradyrhizobia isolates from the transitional savanna zone of Ghana. Five isolates forming effective N_2 fixing symbiosis with cowpea were selected for further study. In this study, we have determined the ability of these five individual isolates to nodulate and effectively fix nitrogen on groundnut (*Arachis hypogeae*), mungbean (*Vigna radiata*), soybean (*Glycine max*) and on cowpea as well. Their competitiveness for nodule occupancy on cowpea was also assessed.

MATERIALS AND METHODS

Bacterial isolates

The cowpea bradyrhizobia isolates used in this study are listed in Table 1. The isolates were stored as frozen stock cultures in yeast mannitol liquid media containing 15% glycerol at -80°C (Manniatis et al., 1982). Strain LBG 13, a local fast growing strain, obtained from Professor Frank Kumaga (Crop Science Department, University of Ghana, Legon) served as a reference strain. LBG 13 was originally isolated from bambara groundnut (*Vigna subterranea*). It also nodulates cowpea and is thought to be promiscuous (Frank Kumaga, personal communication). Each isolate was streaked on yeast mannitol agar (YMA) plates containing Congo red (Hahn, 1966; Somasegaran and Hoben, 1994) for control of purity. Single colonies were re-streaked onto trypton yeast (TY) plate to reduce the slimy nature of the strains and prepare cell suspensions for PCR fingerprinting. The fast growers formed visible colonies after four days and the slow growers formed visible colonies after seven days. The isolates were identified based on their unique ERIC-DNA PCR fingerprint (de Bruijn, 1992; Versalovic et al., 1991) patterns (Figure 1), but their taxonomic affiliation is yet to be established.

Legume genotype, seed sterilization and growth conditions

Legumes used in this study were cowpea cv. Asontem (Asafo-Adjei et al., 2005), groundnut cv. Ex-kumawu, mungbean cv. Berken and soybean cv. Tropical Glycine cross (TGx) 1842-9E (Pulver et al., 1985). The cowpea and groundnut cultivars are local varieties from Ghana and were obtained from Professor Frank Kumaga (Crop Science Department, University of Ghana, Legon). The TG x 1842-9E soybean cultivar is among the new soybean cultivars specifically developed for Africa and was also obtained from Professor Frank Kumaga. The mungbean cultivar was obtained from Professor N. Kent Peters (Highview Ave, Silversprings, USA). The legume seeds were surface sterilized according to the method described by Somasegaran and Hoben (1994) and germinated on 1.5% water agar in Petri dishes. The Petri dishes with the sterile seeds were wrapped in an aluminum foil and incubated for two days at 25°C in darkness for germination. Groundnut was incubated for 6 days due to its slow rate of germination. The germinated seedlings were further planted in a sterile support medium consisting of sand and perlite mixture (1:3 ratio v/v) in Magenta® vessels (Sigma Chemical Company, St Louis, USA) and supplied with half-strength Broughton and Dilworth nitrogen-free nutrient solution (Broughton and Dilworth, 1970). The seedlings were grown at the Phytotron, University of Tromsø at a temperature of 25°C with a light intensity of 400 µmol (14 h light/10 h dark) and 85% relative humidity.

Host range and symbiotic effectiveness of the isolates

To determine the host range and symbiotic effectiveness of the individual isolates, a 10 ml inoculum consisting of 1 x 10^8 cells of each of the isolates was transferred to the root system of 2-day old sterile seedlings of each legume genotype established in the Magenta® vessels. Ten replicates were used for each legume host genotype. Cowpea served as a positive control legume for the isolates since they were originally isolated from cowpea nodules. Strain LBG 13 served as a reference strain for the nodulation test. Non-inoculated plants from each legume species served as control to check for cross contamination. Inoculants were prepared by suspending rhizobia cells collected from the surface of the YMA plate in de-ionized water to an optical density (OD_{600}) of approximately 0.5. The optical densities were measured using the Spectra Max 250 Molecular Device (Global Medical Instrumentation Inc,

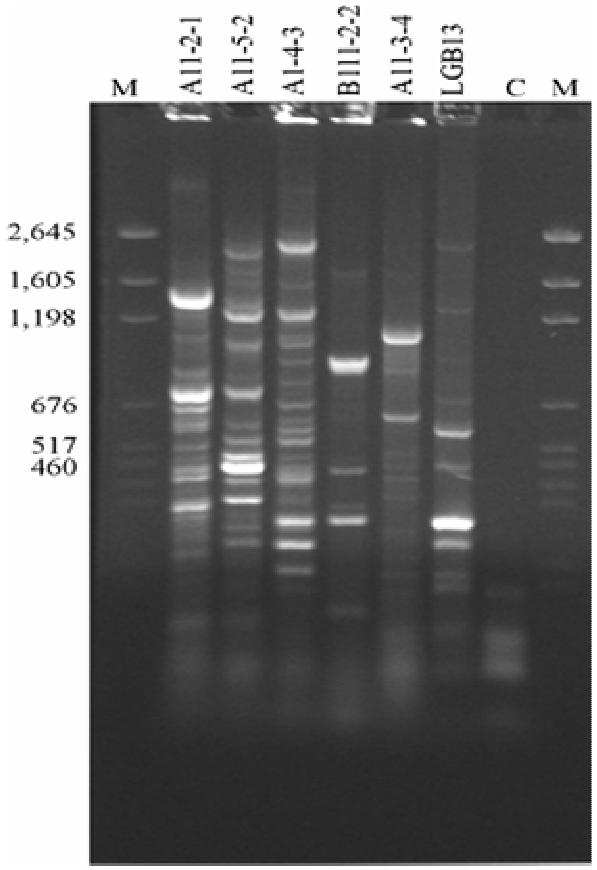

Figure 1. ERIC-DNA PCR fingerprint patterns of the five different cowpea bradyrhizobia isolates including the reference or the positive control isolate LBG 13. M, molecular size marker pGEM (Promega); C, negative control.

Albertville, Minnesota, USA). The bacterial cell number for each isolate at this optical density was determined by dilution and counting of colonies on YMA plates after 4 days and 7 days for the fast and slow growers, respectively.

Host range was assessed by scoring for the presence (or absence) of nodules on the root system five weeks (35 days) after inoculation. Symbiotic efficiency of the isolates was determined by measuring the dry weight of roots, shoots and nodules of test plants (Buttery et al., 1997; Somasegaran and Hoben, 1994; Wynne et al., 1980). Comparison of the symbiotic efficiency among the isolates was done using Analysis of variance (ANOVA) and Tukey`s test. Correlation between shoot dry weight and nodule dry weight was assessed where applicable, to support the symbiotic efficiency analysis (Wynne et al., 1980).

Competition for nodule occupancy on cowpea

Competition for nodule occupancy among the isolates was carried out on cowpea as the host legume. Two separate competition experiments were set up. The first experiment involved inoculant mixture of the three slow growers AII-2-1, AII-5-2 and AI-4-3, known hereafter as 3-isolate-mix, whilst the second mixture included all the five isolates (the three slow growers above, as well AII-3-4 and BIII-2-2) and known hereafter as 5-isolate-mix. Inoculants were prepared by suspending each isolate in sterile water to an optical density (OD_{600}) of 0.5. The isolates were mixed in equal proportions and used to inoculate five sterile cowpea seedlings already established in Magenta® vessels. The actual proportion of each isolate present in the inoculants was also determined by plating out serial dilutions of each isolate on YMA and counting the colonies. The amounts of nitrogen fixed by the mixed isolates under competitive nodulation were determined as described previously for experiments with the single isolates. Five weeks after inoculation, the plants were harvested by cutting the shoot portion from the root system. The whole root system was washed in a gentle stream of tap water to get rid of sand and perlite particles, and then surface sterilized for 10 s in 70% ethanol followed by immersing in 6% H_2O_2 for 10 min (Svenning et al., 2001). A total of 252 nodules for the 3 isolate mix and 250 for the 5 isolate mix, were randomly harvested from the roots and each nodule crushed in 50 µl of sterile water in a 1.5 ml eppendorf safe lock tubes (Eppendorf AG, Hamburg, Germany). The nodule suspension was streaked on YMA plates containing Congo red (Hahn, 1966) and further re-streaked onto trypton yeast (TY) agar plates for PCR identification.

Identification of isolates present in nodules by ERIC PCR fingerprinting

The identity of each nodule re-isolate was determined based on the ERIC-PCR DNA fingerprint patterns previously established for the five cowpea bradyrhizobia isolates (Figure 1).

Crushed nodules were screened to identify the isolate present in a nodule. To achieve this, a loopful (1 µl) of bacterial cells from the TY plates were suspended in 50 µl of sterile water and one microlitre of this suspension directly used in the PCR reaction as described by Svenning et al. (2001). The PCR reaction consisted of the following; 18.5 µl of sterile MilliQ water, 2.5 µl of 10 x reaction buffer for DyNAzyme polymerase (Finnzymes, Oy, Espoo, Finland), 1.0 µl of 50 pmol each of ERIC 1 and ERIC 2 primers (Eurogentec, Seraing, Belgium), 0.5 µl of 10 mM dNTP mix F-560L and 1.0 µl of DyNAzyme II DNA polymerase (2 U/µl) F-501 (Finnzymes). PCR conditions for these primers were in accordance with de Bruijn (1992). Twelve microlitres of the PCR amplified DNA products were loaded onto 1.5% Seakem®LE agarose gels (FMC BioProducts, Rockland, Me., USA) and run at room temperature in Tris-borate-EDTA (TBE) buffer at 80 V for 3 h. The gels were stained in ethidium bromide and photographed using the Gel Doc™2000 Documentation System (Biorad, Hercules, California, USA). ERIC-PCR DNA fingerprints of cell suspensions of original isolates in the inoculum-mix were included in each gel run to facilitate the visual identification of the isolates in nodule suspensions (Figure 2).

Statistical analysis

All statistical computations were done with the MINITAB statistical program (Minitab Inc., Coventry, UK). Possible effects of rhizobia isolate on plant production were tested using one-way analysis of variance (ANOVA). In cases of significant differences, a Tukey post hoc test was performed in order to reveal where differences occurred. A possibility level of $p < 0.05$ was considered significant.

RESULTS

Host range and symbiotic effectiveness of isolates

The ability of the five cowpea bradyrhizobia isolates to form nodules and effectively fix nitrogen on cowpea, groundnut, mungbean and soybean was determined. Re-

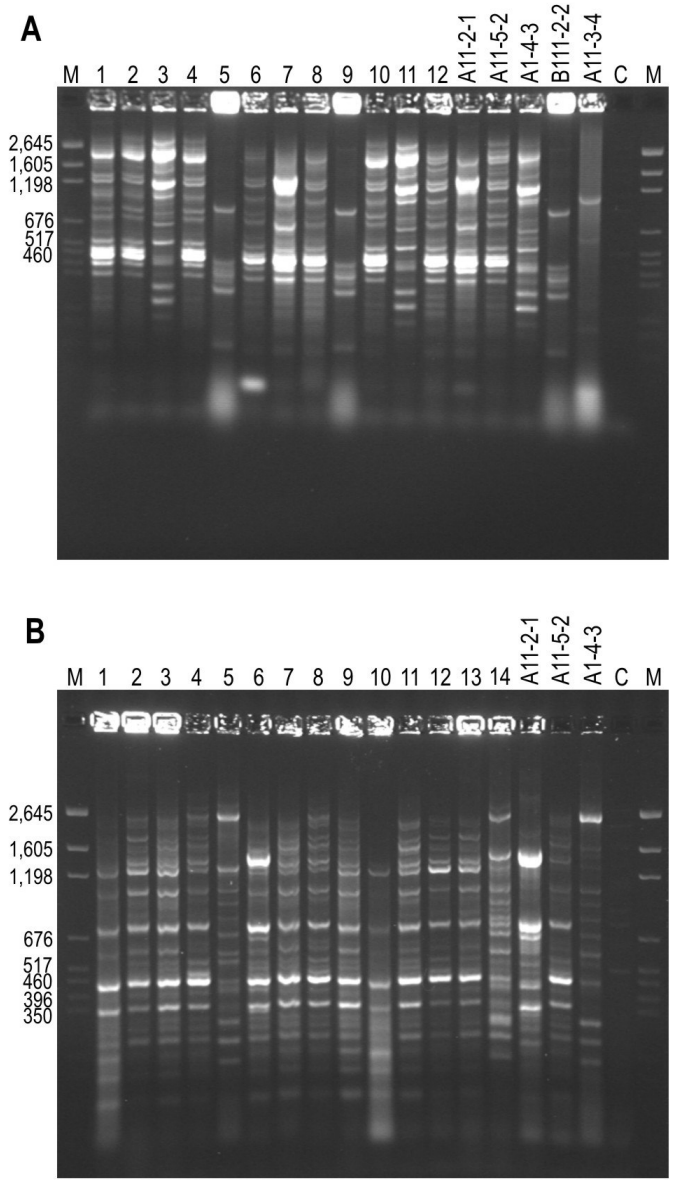

Figure 2. ERIC-DNA PCR fingerprint patterns of nodule isolates from the experiment with a 5-isolate mix (A) and a 3-isolate mix (B). Lanes 1-12 and lanes 1-14 show fingerprint patterns of nodule isolates in A and B, respectively. ERIC-DNA PCR fingerprint patterns of the individual isolates used for inoculation are included in both gels. M, molecular size marker pGEM (Promega); C, negative control.

sults indicated that all the isolates including the reference strain LBG 13 formed nodules on cowpea, groundnut and mungbean with significant differences (P <0.05) in the average number of nodules formed per host plant (Table 2). With the exception of isolate AII-2-1, the numbers of nodules formed on groundnut by the remaining isolates were estimated because they were very tiny and difficult to count (Table 2). A rough count indicated that there were more than 150 nodules on each root. With soybean as host, only isolates AII-2-I, AII-3-4 and BIII-2-2 formed nodules while the isolates AI-4-3, AII-5-2 and the reference strain LBG 13, did not form nodules. There was a significant difference (P <0.05) in the number of nodules formed by the isolates that nodulated soybean (Table 2).

Except for cowpea where all the isolates were symbiotically effective, there was variation in the symbiotic effectiveness (nitrogen fixation) of the isolates among the different legume genotypes tested. With soybean the isolate AII-3-4 produced a significantly higher (P <0.05) mean shoot/total dry weight than the remaining isolates, being the most effective on this host (Figure 3). This was followed by isolate BIII-2-2 which also produced a significantly higher (P <0.05) mean shoot/total dry weight on soybean than isolate AII-2-1 and the isolates AI-4-3, AII-5-2 and the reference strain LBG 13 which did not form nodules on soybean. Isolate BIII-2-2 was moderately effective. In spite of the ability of isolate AII-2-1 to nodulate soybean, its performance was similar (P <0.05) to the non-infective isolates and the non-inoculated control (Figure 3). A strong positive correlation (r = 0.961, p <0.001) between shoot dry weight and nodule dry weight was observed which indicated that the isolates that produced high shoot/total dry weights on soybean were more effective and fixed more nitrogen on this legume host.

Even though all the isolates formed nodules on groundnut the symbiosis with AII-2-I produced the highest mean shoot/total dry weight which was significantly higher (P <0.05) than the symbiosis with the other isolates. But the remaining isolates were not significantly different (P <0.05) from the non-inoculated control in terms of mean shoot/total dry weight produced which indicated that they formed ineffective symbiosis with groundnut as host plant (Figure 4). Correlation between shoot dry weight and nodule dry weight produced by the isolates on groundnut could not be determined because the nodules formed by all the isolates, except AII-2-1, were very difficult to detach from the root system.

There were also marked differences in the symbiotic effectiveness among the isolates on mungbean. Isolate AI-4-3 produced the highest mean shoot/total dry weight on this host plant and was followed by AII-3-4, LBG 13, BIII-2-2, AII-5-2 and AII-2-1 in a decreasing order (Figure 5). The performance of isolate AI-4-3 was not significantly different (P <0.05) from AII-3-4 in terms of the mean shoot/total dry weight produced even though it was significantly higher (P <0.05) than the other isolates tested. Isolate AII-3-4 performed significantly better than isolates AII-2-1, AII-5-2 and the non-inoculated control and was therefore moderately effective. Isolates BIII-2-2, AII-5-2 and the reference strain LBG 13 produced values that were not significantly different (P <0.05) from each other or from the non-inoculated controls and therefore ineffective on mungbean. A strong positive correlation (r = 0.800, p <0.001) between shoot dry weight and nodule dry weight supporting this data was observed.

The ANOVA test revealed a significant effect (P <0.05) of treatment (inoculum) on shoot/total dry weight produced on

Table 2. Number of nodules formed by the five cowpea bradyrhizobia isolates and the reference strain on the different legume host genotypes.

Isolate	Number of nodules/ plant[#]			
	Soybean	Groundnut	Mungbean	Cowpea
Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0
LBG 13	0 ± 0c	*> 150	30 ± 18ab	52 ± 13bc
AII-2-1	5 ± 3b	124 ± 25	1 ± 1b	90 ± 18a
AII-3-4	40 ± 19a	*>150	27 ± 13ab	26 ± 8c
BIII-2-2	27 ± 9a	*>150	10 ± 7b	50 ± 30dc
AI-4-3	0 ± 0c	*>150	63 ± 48a	63 ± 14ab
AII-5-2	0 ± 0c	*>150	4 ± 3b	64 ± 22ab

[#]Values represents means of ten plants ± standard deviation.
*Small white nodules difficult to detach from the roots and presented as estimates.

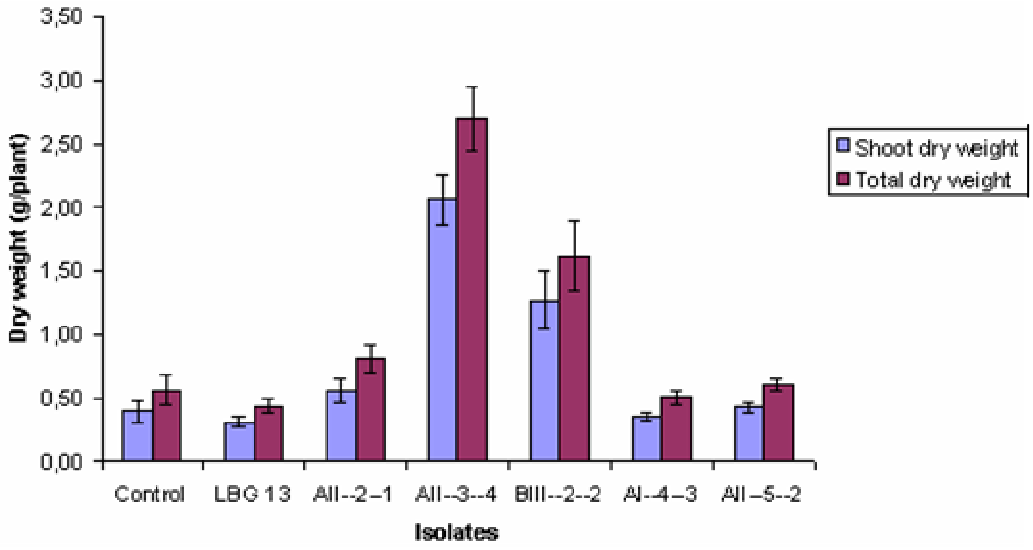

Figure 3. Shoot/total dry weight as a measure of the symbiotic effectiveness of the different indigenous cowpea bradyrhizobia isolates on soybean. Values are means of 10 plants. Bars represent standard errors.

cowpea (Figure 6). However, no significant differences could be found between treatments when these were tested pair wise with the post hoc test.

Nodule occupancy of isolates on cowpea

Competition for nodule occupancy was studied with cowpea as host plant. Nodule occupancy experiments were conducted among the three slow growing isolates (AII-2-1, AII-5-2 and AI-4-3) and also with mixed inocula containing all the five isolates present in equal proportions (5-isolate-mix). Viable plate counts confirmed that all the isolates were equally represented in the inoculants prepared. The ERIC-PCR DNA fingerprint patterns of rhizobia recovered from a total of 252 nodules from the 3-isolate-mix experiment showed that isolate AII-5-2 occupied 71% of the nodules. Isolate AII-5-2 was therefore the most competitive isolate for nodule occupancy on cowpea in the 3-isolate-mix inoculum. Isolates AI-4-3 and AII-2-1 occupied, respectively, 15 and 14% of the total nodules analyzed.

With the 5-isolate-mix, the dominance of isolate AII-5-2 was also observed. It occupied about 60% out of a total of 250 nodules screened. The isolates AI-4-3 and BIII-2-2 both occupied 16% of the total nodules each, whilst isolates AII-2-1 and AII-3-4 occupied 8 and 1% of the nodules, respectively.

DISCUSSION

The efficient exploitation of biological nitrogen fixation to improve agricultural productivity requires that the popula-

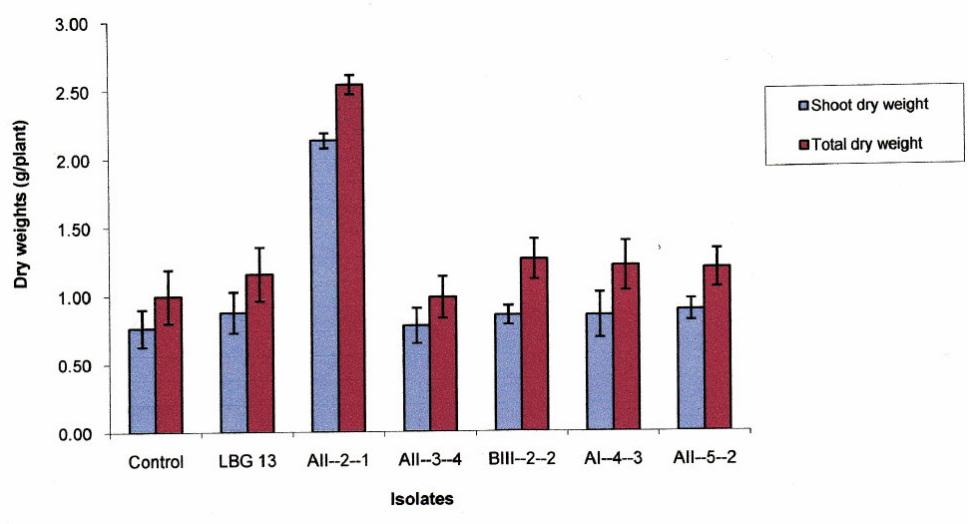

Figure 4. Shoot/total dry weight as a measure of the symbiotic effectiveness of the different indigenous cowpea bradyrhizobia isolates on groundnut. Values are means of 10 plants. Bars represent standard errors.

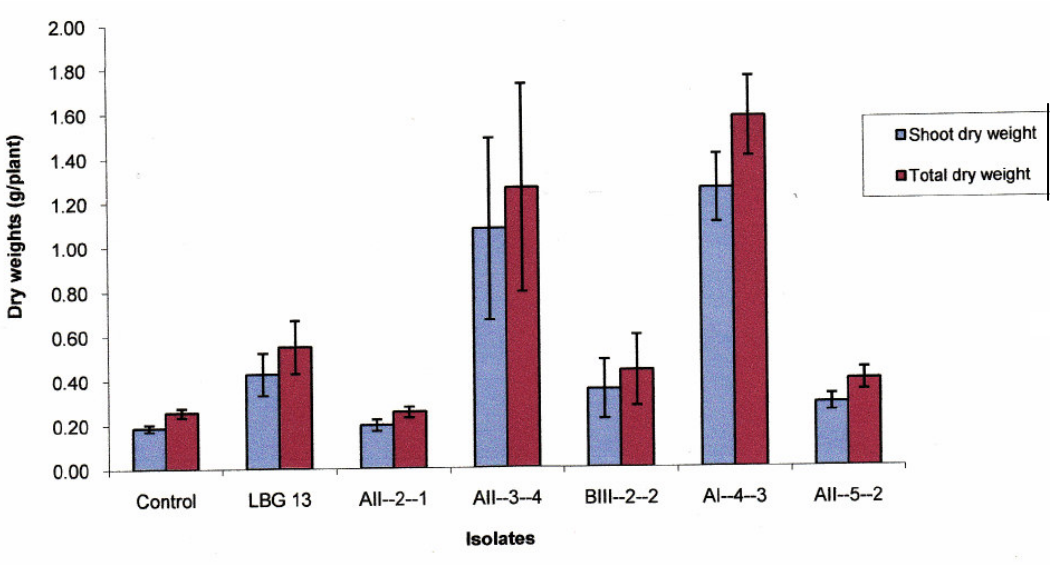

Figure 5. Shoot/total dry weight as a measure of the symbiotic effectiveness of the different indigenous cowpea bradyrhizobia isolates on mungbean. Values are means of 10 plants. Bars represent standard errors.

tions of indigenous rhizobia are adequately characterized. At present, there is a general lack of information about the diversity and symbiotic properties or performance of the indigenous rhizobia nodulating the different legume species in Africa despite some recent efforts to address this limitation (Diabate et al., 2005; Mpepereki et al., 1996). Rhizobia strains that nodulate cowpea in Africa have generally been described as promiscuous with varying degrees of effectiveness on cowpea and other compatible hosts (Allen and Allen, 1981; Fening and Danso, 2002; Singleton et al., 1992). Data from this study validate this concept. All the five selected cowpea bradyrhizobia isolates were generally promiscuous in nodulating all the legume hosts tested, with the exception of soybean which did not nodulate with AI-4-3 and AII-5-2. Eaglesham et al. (1984) have also reported the nodulation of groundnut, mungbean and soybean cultivars by indigenous rhizobia isolated from cowpea in Nigeria. However, a contrasting result was reported by Doku (1969) from an earlier study in Ghana. He observed that

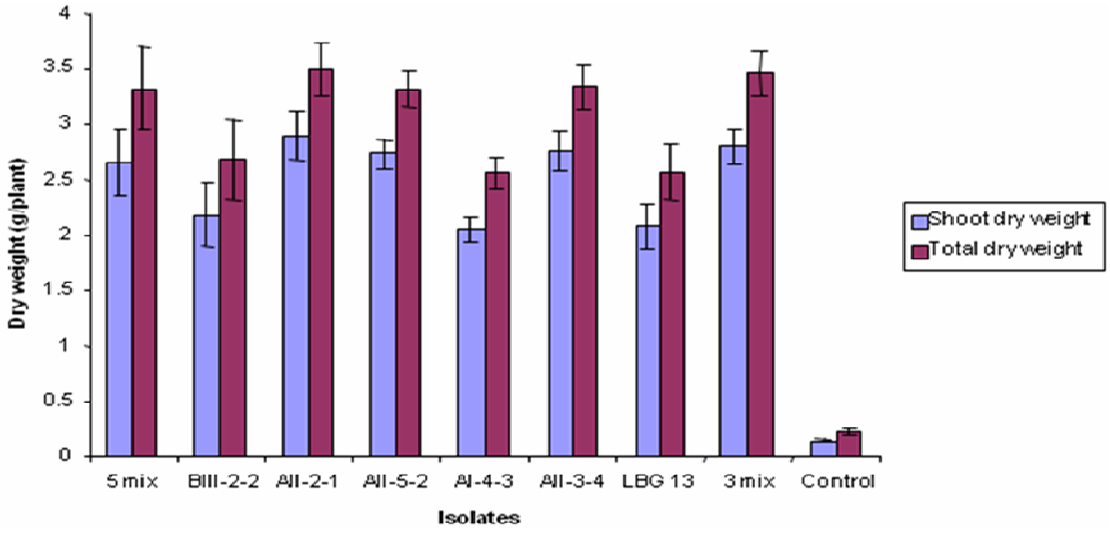

Figure 6. Shoot/total dry weight as a measure of the symbiotic effectiveness of the different indigenous cowpea bradyrhizobia isolates and the mix inoculants on cowpea. Values are means of 10 plants. Bars represent standard errors.

Table 3. Symbiotic effectiveness of the five different cowpea bradyrhizobia isolates on soybean cv. TGx 1842-9E, groundnut cv. Ex-kumawu, mungbean cv. Berken and cowpea cv. Asontem.

Isolate	Legume host			
	Soybean	Groundnut	Mungbean	Cowpea
LBG 13	Non-nodulating	Ineffective	Ineffective	Effective
AII-2-1	Ineffective	Effective	Ineffective	Effective
AII-3-4	Effective	Ineffective	Moderately effective	Effective
BIII-2-2	Moderately effective	Ineffective	Ineffective	Effective
AI-4-3	Non-nodulating	Ineffective	Effective	Effective
AII-5-2	Non-nodulating	Ineffective	Ineffective	Effective

rhizobia isolated from cowpea could not nodulate soybean, groundnut or bambara groundnut (*V. subterranea*) even though cowpea nodulated with rhizobia isolated from these legumes (Doku, 1969). His conclusion was that the cowpea legume plant is a universal recipient of rhizobia. Nevertheless, results on host range studies reflect the promiscuity of the tested isolates and differences between these findings may be due to the particular isolate(s) tested.

It is well recognized that strains within a rhizobia population show great variation in their symbiotic effectiveness on their hosts. It is, therefore, not surprising to observe similar results in this study whereby apart from cowpea in which all the isolates were effective in fixing nitrogen, they exhibited some variations in interaction with other host plants (Table 3). Groundnut which is an important legume in Ghana in terms of both production and consumption (Ofori, 1993) was effectively nodulated only by isolate AII-2-I despite being a compatible host with all the isolates. The remaining isolates were ineffective and produced mean shoot/total dry weights which were not significantly different from the non-inoculated plants. In Ghana, it is estimated that groundnut may receive about 79% of its total nitrogen requirements from symbiotic association with rhizobia (Dakora et al., 1987). But the majority of the isolates we tested on groundnut were just ineffective. It may likely be that what was observed is not a true reflection of the entire population of groundnut nodulating rhizobia in the transitional savanna zone of Ghana. Notwithstanding, this observation needs to be investigated critically because of the possible impact on groundnut yields. Mungbean is not a popular food legume in Ghana in terms of cultivation and consumption, but native rhizobia are present that can effectively nodulate with it. The importance of soybean as a grain legume is gradually growing and gaining popularity in terms of cultivation and consumption in Ghana (Ofori, 1993). The soybean genotype TG x 1842-9E which was used in this study is among the new soybean cultivars specifically developed for Africa (Pulver et al., 1985). Earlier studies have shown that not all these cultivars are readily nodulated by the indigenous rhizobia

in some soils where trials have been carried out (Abaidoo et al., 2000; Okereke and Eaglesham, 1992). It was, therefore, not too surprising that among all the legumes tested, soybean was the only legume that did not nodulate with all the isolates.

Most superior isolates or inoculants have failed to produce the desired results of increased nitrogen fixation/yields maybe due to issues relating to their competitiveness for nodule occupancy. It is therefore relevant that rhizobia strains selected as inoculants are very competitive in forming nodules on the intended host legume and also be well adapted to the destined environment. Indigenous rhizobia are more adapted to the local environment and should be the first choice as inoculants. In most nodule occupancy experiments, competition between mostly two strains is assessed. But in the natural soil environment the normal situation is more likely to consist of several strains or isolates competing for infection and nodulation. In this study, experiments were conducted with inoculum mix of three or five isolates, respectively, which also represents an experimental approach different from the natural rhizosphere habitat. Nodule occupancy was determined using the ERIC-PCR DNA fingerprinting technique which is a reliable method that has been used by other workers to identify nodule occupants in competitive nodule occupancy studies (Labes et al., 1996; Niernann et al., 1997; Svenning et al., 2001). This study clearly demonstrated that isolate AII-5-2 was the most competitive among the isolates and occupied 71 and 60% of the total nodules in the 3-isolate-mix and 5-isolate-mix experiments, respectively. The remaining isolates which were relatively less competitive, formed less than expected proportion of nodules on cowpea, with isolate AII-3-4 being the least competitive since it formed less than 1% of the total nodules in the 5-isolate-mix experiment. The determinants of competitiveness for nodule occupancy by rhizobia are not fully understood. However, some factors such as genotype of both the host and the competing rhizobia strains (Cregan et al., 1989; Deoliveira and Graham, 1990; Josephson et al., 1991; McDermott and Graham, 1990; Vlassak and Vanderleyden, 1997), and adaptation to stress during early symbiotic interactions (Jensen et al., 2005; Ma et al., 2004) have been shown to influence the outcome. It is therefore likely one of these factors or a combination of these factors may have played a role in the superior competitiveness of isolate AII-5-2. This study would, therefore, form the basis for further experiments in the future to map out the determinant(s) for the superior competitiveness of isolate AII-5-2.

In conclusion, it has been demonstrated that the five indigenous cowpea bradyrhizobia isolates tested have a potential for a broader host range but with varying degrees of effectiveness on their hosts. Isolate AII-5-2 stands out as the most competitive on cowpea and therefore possesses a better potential for use as inoculant for increased cowpea production in Ghana.

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