International Scholars Journals

Advanced Journal of Environmental Science and Technology ISSN 2756-3251 Vol. 12 (1), pp. 001-009, January, 2021. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

A study of *Cordeauxia edulis* as an endangered leguminous shrub to ensure its successful cultivation

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Accepted 16 October, 2020

Cordeauxia edulis **is an endangered leguminous shrub native to semi-arid border between Ethiopia and Somalia. To enable its successful cultivation in new environment, we investigated the effect of its microbial residents on establishment and growth. Plant growth-affecting bacteria and arbuscular mycorrhizal fungi (AMF) were the focus of this study. Bacteria were isolated from rhizosphere soil and pre-germinated seeds of *C. edulis* and tested for *in vitro* inhibition of *Aspergillus niger*, *A. biciliate* and *A. versicolor*. The three aspergilli were found as frequent colonizers of seeds with deteriorating effect on emergence. Six bacteria were further evaluated in greenhouse experiments for their effect on plant growth. The isolates with beneficial effects seemed to belong to *Pseudomonas* sp., *Klebsiella* sp. and *Pantoea* sp. Occurrence and abundance of AMF was determined as spore density and % root colonization in the field samples. Effect of AMF on plant growth was also studied in greenhouse, on seedlings inoculated with its native population and the results compared with those inoculated with a commercial mix, Vaminoc or *in vitro* cultured *Glomus intraradices*. Our results show that, *C. edulis* harbours both AMF and bacteria with plant growth affecting potential and that it responds positively to microbial inoculations in foreign environments.**

Key words: Antagonism, arbuscular mycorrhizal fungi, *Aspergillus* spp, *Cordeauxia edulis*, beneficial bacteria.

INTRODUCTION

Cordeauxia edulis Hemsl. is a 2 to 3 m spreading leguminous shrub native to semi-arid central Somalia and the Somali Regional State of Ethiopia. It produces edible seeds and the leaves are infused to make tea and the leaf extracts are used locally for making fast insoluble dyes. Animals graze on it and the wood is a valuable source of building material. As an evergreen and palatable shrub, *C. edulis* is the mainstay of livestock in dry seasons (Zimsky, 1990). The seed also has an export potential due to its chestnut-like flavour and a high nutrient content namely, starch 37%, sugar 24%, protein 13%, fat 11% and various minerals (Gutale and Ahmed, 1984).

Cordeauxia edulis is important for soil conservation and mulching (Bekele-Tesema et al., 1993) but lately its survival is vulnerable due to over-exploitation during dry season (Vivero et al., 2005). In view of great danger of its extinction, different measures have been suggested to preserve *C. edulis* and improve its production. Protection of *C. edulis* shrubs with fences and to grow them in nurseries are examples of such measures. Furthermore, little progress has been achieved in introducing *C. edulis*

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outside of its natural habitat. Attempts have been made to grow the species in Kenya and Israel (Lucas and Synge, 1978; Nerd et al., 1990). Nerd et al. (1990) planted seedlings at four sites in the Negev Desert (Israel), but plants grew successfully at one site only. The reasons for failure at the other three sites according to the authors, may be unsuitable climate and/or biological factors. New strategies are therefore, needed to achieve successful cultivation of *C. edulis* specifically outside its natural habitat.

Plants and microorganisms exist together in natural ecosystems. Some plant-associated bacteria and fungi (including arbuscular mycorrhizal fungi, AMF) are known to exert beneficial effects on germination, growth and yield (Vessey, 2003; Regvar et al., 2003; Madhaiyan et al., 2005; Nezarat and Gholami, 2009). Among these, plant growth-promoting bacteria (PGPB) are increasingly recognized for their importance in crop productivity during the last three decades. In a study by Karlidag et al. (2007) growth, yield and nutrition of apple trees increased after inoculation with Bacillus M3 and/or OSU-142 and/or Microbacterium FS01 in combination. PGPB are also known for their antagonistic ability towards different pathogens. For example, an isolate B579 or *Bacillus subtilis* caused vacuolation, swelling and lysis of hyphae of *Fusarium oxysporum* f. sp. Cucumerinum, which is a destructive pathogen on *Cucumis sativus* L. (Chen et al., 2009). Raupach and Kloepper (1998) showed that three-way mixture of INR7, ME1 and GBO3 as a seed treatment resulted in an intensive plant growth promotion and disease reduction of multiple cucumber pathogens to a level statistically equivalent to the synthetic elicitor Actigard applied as a spray. In addition to the disease reduction, PGPB have also been demonstrated to enhance defense responses in plants to various diseases. This could be achieved through inhibition of pathogens. The *in vitro* study of Dey et al. (2004) confirmed that *A. niger*, *A. flavus* and *Sclerotium rolfisii* were inhibited by some of the PGPB isolates obtained from the rhizosphere of *Arachis hypogaea* L.

PGPB are known to affect plant growth by various mechanisms. Examples of mechanisms are direct growth promotion due to enhanced nutrient uptake, phyto-hormone production, competition for substrate and site, niche exclusion, and/or indirect due to induced systemic resistance, detoxification of surrounding soil and through bio-control of plant pathogens (Mahaffee and Kloepper, 1994; Höflich et al., 1997; Elo et al., 2000; Bais et al., 2004).

Another group of focus in this study is AMF, that connects the living plant roots with their surrounding soil microhabitats (Toro et al., 1997). The benefits of AMF include changes in the root architecture (Miller et al., 1997), increased yield, nutrient accumulation, and/or successful propagation (Stanley et al., 1993; Cho et al., 2009). Tarafdar and Rao (1997) found that, AMF inoculation resulted in improved grain yield of arid-zone legumes by 15 to 22%. The improved yield was attributed to the positive interactions between nitrogen fixing Rhizobium and AMF. Pre-conditioning of legume crops with AMF increased the vegetative growth and seed yield and improved nodulation of the root system in studies carried out by Lambert and Weidensaul (1991) and Mathur and Vyas (2000). In another study, Hamel et al. (1991) found that, the growth of both maize and soybean plants was greatly enhanced, as a result of inoculation with *G. intraradices*. Although more nitrogen appeared to be transferred from soybean to maize in AMF-inoculated plants, they attributed the growth enhancement mainly to a better phosphorus uptake in the presence of added AMF.

Knowledge on what microorganisms colonize and live with *C. edulis* and if they have any function in its establishment and growth is largely lacking. Though it is a leguminous shrub, we hypothesize that *C. edulis* plants harbour micro-organisms other than nitrogen-fixing bacteria that are important for its growth, survival and development. Thus, the inoculation of *C. edulis* with selected microorganisms is expected to improve its emergence, seedling establishment and growth independent of its native environment.

This is the first study to report on (1) associations of AMF with *C. edulis* (2) response of *C. edulis* to AMF inoculation and (3) occurrence of bacteria in *C. edulis* environment with the ability to improve its emergence and growth in a foreign environ-ment. Bacteria with potential to confer beneficial effects were further characterized for some of their functional traits in order to understand the underlying mechanisms.

MATERIALS AND METHODS

Soils, plant material and growth conditions

Soils from different *C. edulis* environments were analyzed for studies on occurrence of AMF with *C. edulis* plants. The soils were sampled from Tony farm, Dire Dawa located in Eastern Ethiopia, from Bokh in Somali regional state of Ethiopia and from greenhouse cultivations maintained at Ultuna, Sweden. At Ultuna, the plants were grown in Bokh soil mixed with Ultuna field soil (0.05:1 v/v). The plants grown in Tony farm were 8 to 19 months old and at Ultuna greenhouse they were about two years old before they were used for the AMF study. The characteristics of the different soils are summarized in Table 1. Occurrence and abundance of AMF in these soils were analyzed in terms of spore density and % root colonization using the standard procedures described as follows:

Pre-germinated seeds of *C. edulis* were used for bacterial inoculation experiment in greenhouse. The seeds were collected as nuts from natural stands near Bokh and stored at temperature 5 ± 2°C and relative humidity 60% until used. Seeds without pericarp were pre-germinated by sowing them in shallow pots containing moist sterile sand and incubating at the temperature regime of 25°C/15°C day/night. The light was supplied for 12 h by using Philips high-pressure sodium and mercury lamps (HPIT, 400W, Belgium) (Philips and Hayman, 1970).

Young plants of *C. edulis* were also used for another inoculation experiment in greenhouse. The plants were grown, using regular fertilization and irrigation, in sterilized sand for eight months before

Table 1. Physiochemical characteristics of soils used in the study.

Parameter	Ultuna	Greenhouse soil	Ulleråker
Texture	Sandy–loam	Peat based	Sandy –loam,
pH	6.6	5.5-6.5	6.4
Nitrogen, total	0.12 %	130 g/kg	0.022 g/kg
Phosphorus (P-AL) g/kg	20.2	100	24.6
Potassium (K-AL) g/kg	7.2	160	9.1
Mg (Mg-AL) (g/kg)	5.1	260	8

*Ultuna and Ulleråker soils were dry.

inoculation with AMF.

Estimation of AMF content

Occurrence of AMF content in terms of spore density and % root colonization was estimated in the different soils. For spore extraction, 10 g of soil from each sample was suspended in fresh and aerated tap water. The soils were wet-sieved, decanted according to Brundrett et al. (1999). Fine roots and spores were collected from the sieves for further analysis. Spores were counted in stereo microscope and data transformed to density per 10 g dry soil.

AM colonization of *C. edulis* roots was estimated using a method as described by Phillips and Hayman (1970) and modified by Bharadwaj et al. (2007). Briefly, roots were washed free from soil, cleared in 10% KOH and stained with acid fuchsin and/or trypan blue. Each root system was examined microscopically and scored for the presence of AM-fungal hyphal penetration points, vesicles, arbuscules or internal hyphae, if any.

Isolation of *C. edulis* associated bacteria

Both rhizosphere soil samples and surface decontaminated *C. edulis* seeds were used for this study. For isolation of bacteria from the rhizosphere soils, 5 g of Bokh soil was suspended in 20 ml sterile distilled water. The soil suspension was appropriately diluted using 0.02 M of $MgSO_4$ and spread on diluted tryptic soy broth agar (TSA10, 10 g tryptic soy broth, 15 g agar, Oxoid) and N-free nutrient agar (beef extract 1 g, yeast extract 2 g, Peptone 5 g, NaCl 5 g, Agar 15 g, pH 7.4). These two different nutrient media were used to enable isolation of as many diverse types of bacteria as possible. For isolation of bacteria from seeds, the pre-germinated seeds (5 seeds per 100 ml) of *C. edulis* were crushed in 0.1 M of MgSO4. The seed suspensions were appropriately diluted and spread on TSA10, as described above for the rhizosphere soil. After incubation for two days at 21°C, morphologically different colonies of bacteria were selected. In total, 71 bacterial isolates were obtained that were purified before further studies were conducted.

***In vitro* characterization of bacteria**

All bacterial isolates were screened for their ability to inhibit *C. edulis* pathogens *in vitro*. The pathogens chosen for the bioassay were seed-borne aspergilli from *C. edulis* itself. Three different aspergilli were commonly found colonizing the seeds of *C. edulis* in this study. These were isolated and identified on basis of morphology as *A. niger*, *A. biciliate* and *A. versicolor* (Singh et al., 1991). For *in vitro* inhibition assay, the aspergilli were prepared as suspensions from their fresh cultures grown on sterile diluted potato dextrose agar (PDA, 15 g agar and 15 g potato dextrose broth in 1 L distilled water). The suspensions were mixed with half strength of potato dextrose broth (PDB, 15 g potato dextrose broth in 1 L distilled water) that was maintained at 45°C. The pathogen inoculated PDB was then poured in sterile Petri plates and left to solidify before the bacteria were inoculated at 4 equidistant spots /plate. All the plates were incubated at 21°C. Two replicates were prepared for each combination. Inhibition zone, if any, was assessed a week after bacterial inoculation.

Six antagonistic bacterial isolates from the *in vitro* study above were further characterized for the functional traits that have sometimes been suggested to be involved in the bacteria mediated plant growth effects (Alström, 2001). These traits were production of cellulases, phosphatases, chitinases, siderophores and hydrogen cyanide (HCN). Production of chitinases and cellulases by the bacteria was tested qualitatively on nutrient agar amended with chitin and cellulose respectively (Alström, 2001; Arora et al., 2005). Phosphate-solubilizing activity was estimated on Pikovskaya agar (Pikovskaya, 1948). Appearance of a clearing zone around the bacterial isolates after 7 days of incubation at 22°C was considered as presence of enzymatic activity. Production of siderophores was determined by the method of Schwyn and Neilands (1987), using the CAS reagent (chrome azurol S; Fluka Chemika, Buchs, Switzerland). Isolates were grown on CAS agar plates supplemented with 2% glucose, 0.5% L-glutamic acid and 5 mgL-1 biotin and incubated for up to 7 days.

Decolourization of the blue-coloured ferric-CAS complex, resulting in a yellow–orange halo around colonies, was considered a positive indication of siderophore production. Production of HCN was detected by the method used by Alström and Burns (1989). Production of cyanide was determined by a colour shift from yellow to orange in picrate/Na_2CO_3 filter paper. The ability to produce fluorescent pigment was tested by culturing them on King's medium B agar (KBA King et al., 1954). The plates were inspected for fluorescence under UV light (366 nm) after incubation at 21°C for 48 h.

Greenhouse experiments

Two different experiments were conducted in greenhouse to study the influence of AMF and bacteria respectively on *C. edulis* growth. The effect on *C. edulis* growth was recorded as shoot length, number of leaves, shoot and root dry weight and nutrient content in the leaves.

Experiment 1: Effect of AMF inoculation on *C. edulis*

Three different AMF inocula were applied to the young *C. edulis* plants: 1) *C. edulis* AMF inoculum, 2) Vaminoc, (Becker

Table 2. AMF content expressed as spore density per 10 g soil and % colonized root/ cm in three different soils cultivated with *C. edulis*.

Sampling site	Age of seedlings (Months)	Spore density*	% colonization in roots *
Dire Dawa , Ethiopia	8	20 bc	37.5 ab
Dire Dawa , Ethiopia	19	15 c	42.8 a
Ultuna, Sweden	8	43 a	25 b
Bokh, Ethiopia	20	27 ab	0 c
None (sterile sand)	8	0 d	0 c

* Means followed by the same letter in the same column are not significantly different at P = 0.05.

Underwood, MicroBio, UK) and 3) *G. intraradices* (Ginco, Belgium). The spores obtained from the extraction above constituted *C. edulis* AMF inoculum. Approximately 250 spores of each inoculum were applied per plant.

Commercially available soil (Hasselfors AB, Sweden) for greenhouse cultures, field soil from Ulleråker and sand were used for this experiment. These soils (Table 1) were thoroughly mixed at 4:1:2 v/v respectively. The mixture was sterilized twice at +80°C for 24 h at an interval of 24 h and left for one week for stabilization before use. Eight months old *C. edulis* plants with mean height of 12 cm were transferred to pots (14 cm x 13.5 cm x 18.5 cm) containing this mixture as growth substrate.

Plants were fertilized regularly with Hoagland nutrient solution (Hoagland and Arnon, 1938) modified to half phosphorus content. The control plants were treated as above but without AMF. Each treatment was replicated four times with one plant per pot. Rotation of the pots was done regularly to minimize any border effect. At the end of the experiment, which lasted for 11 months, AMF content was estimated according to the procedures described earlier.

Experiment 2: Effect of bacterial inoculation on *C. edulis*

This study aimed at investigating the effect of bacteria on seed germination and on plant growth in non-sterile conditions. The seeds used were not surface sterilized before inoculation due to non-sterile nature of the experiment and in order to mimic the practical conditions. For the germination test, the seeds of *C. edulis* were inoculated with each of 71 bacterial isolates. Fresh cultures of bacteria were suspended in 0.1% sterile bacteriological peptone solution.

Six seeds per treatment were soaked in each bacterial suspension (approx.10^9 viable cells/ml) for two hours. The soaked seeds were sown in pots (4 cm x 16.5 cm x 18 cm) containing moist sterile vermiculite. The pots were covered to maintain moisture and incubated at 25°C /15°C day and night, respectively, and 12 h light/day. Germination was recorded after one week. Qualitative assessment of seed surface coverage by aspergilli was made visually, using a scale 0% to 100%, where 0 marked the absence and 100, when the whole surface was covered by any of the *Aspergillus* sp.

The above germination test in combination of the *in vitro* inhibition assay led to the selection of six bacterial isolates for further study on their effect on *C. edulis* growth. This study was also conducted in non-sterile conditions using bacterial suspensions from above .The roots of 14 days old *C. edulis* seedlings were dipped in each bacterial suspension for about 30 min before planting in pots (26.5 cm x 16.5 cm x 13 cm) containing Ultuna field soil. Control seedlings were treated with water only. Five replicates were prepared for each combination with one seedling per pot. The plants were harvested after 10 months and the growth parameters and mineral nutrients were measured for each plant.

Statistical analysis

All data were analyzed by analysis of variance, using the general linear model of Minitab Statistical Software. The effect of bacterial isolates on the number of survived plants was analyzed using the R software v. 2.10.1. A logit analysis was performed, assuming binomially distributed data and a logit link function.

RESULTS

Occurrence and abundance of AMF in *C. edulis* soils

The results from AMF content of the investigated soils are summarized in Table 2. AMF was present in all the four soils analyzed but the content varied depending on the soil source. *C. edulis* in Dire Dawa soil showed low spore density (15 to 20 /10 g soil) and moderate root colonization (42.8%); while Ultuna soil harboured higher spore density (43 /10 g soil) and comparatively lower root colonization (25%). There seems to be no correlation between the spore density per gram soil and % root colonization. Samples from control plants grown in sterile sand were marked by the absence of AMF (Table 2).

Influence of AMF on *C. edulis* growth

The results showed that *C. edulis* got colonized by all the three types of inocula; *C. edulis* -AMF, Vaminoc and *G. intraradices* but the degree of colonization depended on the inoculum source. Estimated root colonization in Vaminoc-inoculated plants was significantly higher (50.8 %) than the other two (18 to 39%, Table 3); however, the spore number was significantly higher after inoculation with *C. edulis* -AMF (Table 3). Nutrient content of shoots as a result of AMF inoculation increased in some cases as indicated by the data in Table 4. *C. edulis* -AMF seemed to enhance the level of Ca, Mg and Na content in the shoots but this increase was not always statistically significant.

Among the three AMF inocula, *G. intraradices* and Vaminoc inoculated plants contained a higher P content in the shoot, which was not the case in *C. edulis* -AMF inoculated plants when compared with that in the control plants (Table 4); however, it is not statistically significant.

Table 3. Average % root colonization and recovered spore density of AMF per 10 g dry soil from plants inoculated with *C. edulis*_AMF, Vaminoc, *G. intraradices* in greenhouse experiment.

Source	Spore density	% colonization in roots
Yeheb	0.85 a	18.4 c
Vaminoc	0.74 c	50.8 a
G. intraradices	0.78 b	39 b
Control	0.7 d	0.33c

* Means followed by the same letter in the same column are not significantly different at P= 0.01. See text for details of the experiment.

Influence of *C. edulis* associated bacteria

Majority of the 71 tested isolates (90%, 63 from seeds and 8 from rhizosphere soil) showed some degree of growth inhibition towards at least one of the three aspergilli, or inhibition in sporulation or both (data not shown). Inoculation with one isolate, YES 72, improved survival of four out of 5 plants; however, it is not statistically significant. This isolate inhibited not only the growth of mycelium but also suppressed the ability of *A. biciliate* to sporulate. Certain isolates inhibited two (YES 62) or all three (e.g. YES 33) aspergilli tested (Table 5) and caused delay in sporulation of aspergilli.

Plant height, leaf number, shoot and root dry weights increased in presence of all six tested bacterial isolates (Table 6). The degree of the increases was isolate dependent. Three isolates, YES 33, YES 40 and YES 62 increased the plant dry weight, plant height and number of leaves per plant. But, the best growth was recorded as a result of inoculation with YES 72.

The six bacterial isolates also increased the nutrient contents in the shoot (Table 7).

Particularly, YES 62 and YES 72 increased shoot contents of Ca, P, Mg, K, S and N. The mixed inoculum enhanced the plant growth but not to the same extent as the single bacterial inoculations. However, the nutrient content in the plant shoots was comparable in magnitude as the single bacterial treatments (Tables 6 and 7). With respect to the functional characteristics, all the six bacteria tested in the greenhouse experiments were shown to produce cellulases and siderophores. None of them were fluorescent or produced HCN, chitinases or phosphatases.

DISCUSSION

This is the first report providing evidence for presence and colonization of AMF and bacteria exhibiting beneficial potential in *C. edulis*. The plant harbours not only AMF but also bacteria antagonistic towards *A. niger*, *A. biciliate* and *A. versicolor*. The antagonistic bacteria in particular, seem to have potential to affect both seed germination and plant growth. *C. edulis* has been considered difficult to cultivate outside its native environment, which may be partly due to lack of availability of certified seeds and partly due to a low population of beneficial microorganisms. Our experiments with the inoculation of *C. edulis* in greenhouse indicate that, this species could be grown outside its native environment, if provided with inoculation of beneficial micro-organisms. Our results show potential both with respect to improvement of the viability of seeds and plant establishment.

Occurrence of AMF has earlier been reported in the fine roots of some endangered plant species such as *Amorpha crenulata* Rydb and *Jacquemontia reclinata* House ex Small (Fisher and Jayachandran, 2002). In our study, inoculation of *C. edulis* plants with its own AMF or commercial Vaminoc or *in vitro* cultured *G. intraradices*, increased the nutrient content of the plants significantly. AMF has been shown to significantly increase the dry weight and total P content of *A. crenulata* and *J. reclinata* seedlings, grown in native soil (Fisher and Jayachandran, 2002). Similarly, Tüfenkçi et al. (2005) reported that there was significant effect of inoculation of *G. intraradices* on phosphorous, potassium, calcium, zinc and copper contents in shoots of chickpea. In another study, Taylor and Harrier (2001) confirmed that Mn and Mg concentrations were significantly increased within the shoot tissue of strawberry colonized by different isolates of AM fungi. Utilization of AMF is, therefore, vital role for successful survival and establishment of endangered plant species (Koske and Gemma, 2006) including *C. edulis*.

Root colonization and spore density were not found to be significantly correlated. In a study by Moriera et al. (2006), % root colonization and spore numbers were found inversely related to each other. In spite of a low spore density and % root colonization, influence of native AMF on *C. edulis* seedling growth and increase in the nutrient content in the shoot was at least as good as that gained by Vaminoc and *G. intraradices*. Vaminoc resulted though in higher colonization than the pure culture of *G. intraradices*, which may be due to the synergism between different AMF present in Vaminoc. Maja (2009) also indicated the low AMF spore density and partly root colonization of *C. edulis* by AMF. The results from our study are only partly consistent with other studies, in which a range of plant growth responses was found greatest when using native AMF (Klironomos, 2003; Rowe, 2007). It is possible that the viability of the AMF in the native *C. edulis* inoculum used in our study, differed significantly from that in Vaminoc and *G. intraradices*.

In our study, plant growth increased in the presence of most bacterial isolates, namely YES 33, YES 40, YES 62 and YES 72, confirming their status as PGPB. Askary et al. (2009) showed that the application of *A. brasilense* and *R. meliloti* affected grain yield and N, P, K content of *Triticum aestivum*. Similarly, growth and P content of *Santalum album* L was improved after inoculation with *Bacillus coagulans* in a study by Mamatha et al. (2002).

Table 4. Effect of AMF from three different sources on the growth parameters and shoot nutrients content of *C. edulis* in greenhouse (n = 4).

*Source	Number of leaves/plant	Shoot height (cm)	Shoot dry wt (g/plant)	Shoot nutrient content (g/kg)						
				Ca	P	Mg	K	Na	S	N
C. edulis	17.5a	15.6a	3.9a	32.10a	3.8a	3.3a	14.5a	2.2b	2.3a	21.1a
Vaminoc	19.3a	13.52a	2.5a	22.98b	4.4a	2.9a	12.3a	2.3a	1.88a	21.6a
G. intraradices	19a	14.6a	3.6a	28.9ab	4.5a	2.8a	14.7a	0.9b	2.05a	20.9a
None	15.8a	14.28a	3.6a	27.7b	4.03a	2.4a	15.3a	0.9b	2.08a	24.2a

Means followed by the same letter in the same column are not significantly different at P= 0.05.

Table 5. *In vitro* effect of *C. edulis* seed associated bacteria on the growth of three *Aspergilli* species on potato dextrose agar (n = 3).

*Isolate	*A. niger*	*A. biciliate*	*A. versicolor*
YES 23	-	+	+
YES 33	+	+	+
YES 40	-	-	+
YES 57	+	-	-
YES 62	+	-	+1)
YES 72	-	+ 1)	+

* + and - means presence and absence of inhibition 1) sporulation inhibited.

Likewise, the enhancement of rice growth was influenced by the PGPB collected from the rhizosphere soil of rice field (Ashrafuzzaman et al., 2009). In a study by Paul et al. (2005), *P. fluorescens* was shown to enhance nutrient mobilization in the rhizosphere of black pepper, which resulted in enhanced plant vigour. Studies by Puente et al. (2004) revealed that, inoculation of rhizoplane bacteria isolated from rock-growing cacti, promoted growth of a cactus species possibly by supplying essential minerals for a prolonged period of time.

Furthermore, Yoav and Holguin (2002) studied effect of inoculation of seeds with a mixture of *Pseudomonas syringae* and *Azospirillum brasilense*. These authors found reduction of the pathogen population in the rhizosphere, an increase in the *A. brasilense* population, the prevention of bacterial speck disease development and improved plant growth. Fatima et al. (2009) were able to isolate PGPB strains that positively affected the germination of wheat, increased biomass and root shoot length by inhibiting *R. solani* growth. In a study by Ziedan (2006), isolates of *B. subtilis* and *P. fluorescens* significantly increased fresh weight, number of pods per plant, pod yield of peanut and showed inhibition to the pathogens; *A. niger* and *F. oxysporum*. In our study, growth of *C. edulis* increased due to bacterial isolates probably by reducing the detrimental effects of *Aspergillus* spp. and/or by enhancing the plant defense responses.

The aim of this study was not to identify AMF and the bacteria associated with *C. edulis*. Our preliminary observations from spore morphology indicate presence of *Glomus* spp. as the main genus. Uhlmann et al. (2006) reported presence of *Glomus aggregatum* being the dominant spore type among twelve plant species investigated. Likewise, our preliminary observations indicate that the six bacterial isolates differ from each other and possibly belong to the genera Pseudomonas, Pantoea and Klebsiella. Further studies will only confirm the identity and efficiency of these micro-organisms in *C. edulis* in natural conditions.

The three different aspergilli were frequently found to colonize the *C. edulis* seeds. They were isolated, purified and identified based on morphology as *A. niger*, *A. biciliate* and *A. vesicolor*. They are well known post-harvest pathogens (Narayanasamy, 2006) but the pathogenicity of the isolated aspergilli needs to be confirmed on certified healthy seeds. The different bacterial isolates inhibited the growth of at least one species of aspergilli. Sadeghi et al. (2006) reported that the *in vitro* antagonism by the two Streptomyces isolates towards *Rhizoctonia solani* Ag-4 was through production of siderophore and chitinases. PGPB have been found to colonize the rhizosphere by production of bacterial allelochemicals, including iron–chelating siderophores, antibiotics, biocidal volatiles, lytic enzymes and detoxification enzymes (Glick, 1995; Sturz and Christie, 2003; Bais et al., 2004). They enhance plant growth either directly by producing phytohormones or indirectly by inhibiting pathogens through the synthesis of different compounds (Benizri et al., 2001). Nwachukwu (2003) showed that biocontrol agents, *Chaetomium globosum*, *Trichoderma harzianum*, *T. viride* and *P. fluorescens* reduced the fungal incidence of A. niger, *A. flavus*, *Fusarium moniliforme* and *Bortyodiplodia theobromate* and increased seed germination and seedling growth. Their biocontrol agents were more effective than benomyl. Inhibition of *Aspergillus* spp. by *C. edulis* bacteria is probably due to nutrient competition mediated by iron–chelating siderophores and cellulases that are essential for rhizosphere colonization.

Conclusion

In conclusion, our study shows that micro-organisms

Table 6. Effect of *C.edulis* seed-associated bacteria alone and their mixture on the growth parameters of *C. edulis* (n = 5).

Bacterial isolates	Number of leaves per plant	Shoot height (cm)	Shoot dry weight (g per plant)	Root dry weight (g per plant)
YES 23	2.4±2.4*	1.14±1.14*	0.32±0.32*	0.30±0.30*
YES 33	4.8± 2.06	2.96±1.25	0.574±0.25	0.51±0.22
YES 40	4.2± 2.58	2.66±1.65	0.52±0.32	0.51±0.31
YES 57	2.2 ±2.20*	1.22±1.22*	0.28±0.28*	0.27±0.27*
YES 62	5.8 ±2.42	2.52±1.08	0.55±0.26	0.35±0.18
YES 72	8.2± 2.15	4.3±1.20	0.90±0.28	0.53± 0.14
Mix	3.6± 1.47	1.88±0.82	0.36±0.20	0.36±0.23
None	1.4± 1.40*	0.94±0.94*	0.12±0.12*	0.24±0.24*

* Only one surviving plant.

Table 7. Effect of *C. edulis* seed associated bacteria on shoot nutrient contents (g/ kg) (SE, n = 5).

Isolate	Ca	P	Mg	K	S	N
YES 23	4.6±4.60*	0.48±0.20*	0.12±0.12*	1.84±1.84*	0.3±0.3*	5.62±5.62*
YES 33	11.4±4.90	1.56±0.73	0.32±0.13	5.08±2.13	1.34±0.63	16.1±6.93
YES 40	8.02± 4.98	1.3±0.80	0.36±0.23	4.42±2.80	0.88±0.55	9.72±5.96
YES 57	3± 3.00*	0.54±0.54*	0.14±0.14*	2.28±2.28*	0.44±0.44*	5.56±5.56*
YES 62	10.62±4.53	1.48±0.61	0.36±0.15	4.49±2.13	1.14±0.47	14.54±5.98
YES 72	11.2±3.09	1.54±0.36	0.52±0.15	6.88±1.91	1.28±0.33	18.4±4.85
Mix	11.06±4.69	1.5±0.64	0.54±0.22	5.88±2.44	1.12±0.47	19.02±8.29
None	1.3±1.30*	0.24±0.24*	0.08±0.08*	1.4±1.40*	0.24±0.24*	4.76±4.76*

* Only one surviving plant.

inhabiting *C. edulis*, have an important role in germination, seedling establishment and growth and uptake of nutrient by the host plant. These results are promising but further studies using certified *C. edulis* seeds would confirm the beneficial role of microbial residents of *C. edulis*.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Swedish International Development Cooperation Agency (Sida) for the financial support for the project, through its Department for Research Co-operation (SAREC) in collaboration with the Haramaya University, Ethiopia. Thanks are due to Dr. Lars Andersson for timely advice and critical reading of the manuscript and Dr. D.P. Bharadwaj for technical advice on the AMF part of the study.

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