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Research article

Isolation and characterization of nitrogen fixing bacteria that nodulate alien invasive plant species *Prosopis juliflora* (Swart) DC. in Marigat, Kenya

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Abstract: A total of 150 bacterial strains were isolated from the root nodules of *Prosopis juliflora* growing in soils collected from Marigat area of Kenya. Soil samples from representative colonized zones of *Tortilis*, Grass and *Prosopis* were used in trapping the microsymbionts. A physiological plate screening allowed the selection of 60 strains which were characterized based on morphological, cultural and biochemical characteristics. Tolerance to salinity, acid and alkaline pH and resistance to antibiotics were studied as phenotypic markers. Morphological characteristics allowed the description of a wide physiological diversity among tested isolates. Establishing mutualistic interactions in novel environments is important for the successful establishment of some non-native plant species. The associations may have negative impact on the interaction networks of the native species whereby non-native species becoming dominant. Our study suggests that *P. juliflora* may have led to the diversity of N-fixing microsymbionts observed in the study area. The study provides basis for further research on the phylogeny of rhozobial strains nodulating *P. juliflora*, as well as their use as inoculants to improve growth and nitrogen fixation in arid lands of Kenya. The data obtained in this study can be used for strain improvement and cross-inoculation experiments with different species when searching for well adapted and compatible partners.

Keywords: *Prosopis juliflora* - Rhizobia - Microsymbionts - Diversity.

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INTRODUCTION

Prosopis juliflora (Sw.) DC is an evergreen shrub but sometimes grows into a small tree in the family Fabaceae, sub-family *Mimosaidea* (Polhill *et al.* 1981). The species is native to South America, Central America and the Caribbean (Pasiecznik *et al*. 2001). In the United States, it is well known as mesquite. It is fast growing, nitrogen-fixing and tolerant to arid conditions and saline soils (El-Keblawy & Al-Rawai 2007). It is fast growing, nitrogen-fixing and tolerant to arid conditions and saline soils. The family leguminosae comprises approximately 650 genera, 1800 species and is the largest family of flowering plants (Polhill *et al.* 1981). Its species have worldwide distribution, are adverse, survive in a wide climatic condition and have a multiplicity of use. The leguminosae is divided into three sub families; *Caesalpinoidae, Mimosoidae* and *Papilionoidea*. The greatest occurrence of nodulation, 97% is in the Papilionaidae compared to 90% in the *Mimosoidae* and only 23% in the *Caesalpinidae* (Faria *et al.* 1989).

The current taxonomy has revealed a wide diversity of microsymbionts that can form N_2-fixing symbioses with legume roots in a manner that is similar to rhizobia at the genus, species and intra species level. Traditionally, rhizobia were exclusively members of the *Rhizobiaceae* family in the *Alphaproteobacteria* class of bacteria, which includes the genera *Allorhizobium, Azorhizobium, Bradyrhizobium Mesorhizobium, Rhizobium* and *Sinorhizobium* (Sprent 2008). The term rhizobia have been used for all the bacteria that can

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produce nodules and fix atmospheric nitrogen in legumes (Brewin 2004, Cheng 2008). Rhizobia are bacteria capable of entering their legume hosts through root hairs, sites of lateral root emergence, or directly through root epidermis (Sprent *et al.* 2013), where they can induce the development of nodules where biological nitrogen fixation takes place (Gage 2004). The formation of root nodules involves complex molecular signaling pathways between legumes and rhizobia (Stacey 2007). Within root nodules, organic forms of reduced atmospheric nitrogen produced by the bacteria are utilized by the host plant and ultimately enter the earth's food webs. In exchange, bacterial symbionts acquire carbohydrates from legumes. Surprisingly, rhizobia are not monophyletic and represent a diverse array of bacteria found in both the Alphaproteobacteria ('alpha rhizobia', *e.g.* genera *Rhizobium* and *Bradyrhizobium*) and Betaproteobacteria ('beta rhizobia', *e.g.* genera *Burkholderia* and *Cupriavidus*) classes (Gyaneshwar *et al.* 2011). This symbiosis plays a very important role in agriculture as it can relieve the requirements for nitrogenous fertilizers during the growth of leguminous crops. However, relatively little information is available regarding microsymbionts species associated with *P. juliflora* an emerging alien invasive plant species in Kenya.

Invasive species are now considered as the second most important cause of the reduction in biodiversity in the world, after habitat loss. Loss of species biodiversity of the soil biota may be an acceptable consequence of the development and maintenance of agricultural systems. Habitat destruction at best erodes genetic diversity of the legume host and at worst leads to extinction, the subsequent fate of the rhizobial flora, through time, would be similar. A rich diversity of rhizobial species has been found in the tropics (Dreyfus *et al*. 1988, Odee *et al*. 1997). The need for conservation of both legume and host is therefore, paramount. There are numerous instances of woody legume introductions becoming weedy resulting (in some cases) in the need for expensive control programs. In particular, *P. juliflora* and *Leucaena leucocephala* have become noxious weeds in exotic environments (Fagg & Stewart 1994). Of the 45 species of *Prosopis* described by Allen & Allen (1981), 12 showed nodules, the efficiency of which varies among the species and among varieties within a species. Rhizobium strain isolated from *P. juliflora* nodualted peanut plants and was classified as belonging to the cowpea group (Subba Rao *et al.* 1982); the same occurred for rhizobium isolated from 5 species of the genera *Acacia* and *Albizzia* (Basak & Goyal 1975). *P. juliflora* nodules have apical meristem, with indeterminate growth, thus with standing harsher stress condition provoked by temperature, drought and salinity, than species with globose nodules (Felker & Clark 1980). Invasive trees in the Australian legume genus *Acacia* Mill. *sensu stricto* (Leguminosae subfam. Mimosoideae, formerly *Acacia* subgen. Phyllodineae DC; Maslin (2008), have received much research attention because of their invasion success and severe impacts on native ecosystems globally (Richardson *et al.* 2011). Acacias are known to form successful rhizobial interactions in their introduced ranges (Birnbaum *et al.* 2012, Wandrag *et al.* 2013) and have, in some instances, been co-introduced with their rhizobia (Crisóstomo *et al*. 2013, Ndlovu *et al.* 2013).

The unique partnership between legumes and rhizobia has been suggested as a major contributing factor to the success of some legumes as prominent invasive species in many parts of the world (Parker 2001) and that the ability to find 'compatible' rhizobia in introduced regions plays an important role in establishment success of legumes (Rodríguez-Echeverría *et al.* 2011). Variation in colonizing ability may be related to opportunities for symbionts acquisition from legume taxa that are indigenous to the invaded habitat (Richardson *et al.* 2000, Parker 2001). However, due to the variously restricted host ranges of rhizobia (Parker *et al*. 2004) only a subset of native legume taxa are likely to be potential sources of symbionts for any particular invasive legume. Most invaders will therefore encounter high spatial variability in symbionts availability owing to the heterogeneous distribution of native legumes across the landscape.

Under the right conditions, *P. juliflora* can produce a variety of valuable goods and services: construction materials, charcoal, soil conservation and rehabilitation of degraded and saline soils. Concern about deforestation, desertification and fuel wood shortages in the late 1970s and early 1980s prompted a wave of projects that introduced *P. juliflora* and other hardy tree species to new environments across the world. *P. juliflora* has survived where other tree species have failed and in many cases become a major nuisance. In 2004 it was rated one of the world's top 100 least wanted species (IUCN 2009). *P. juliflora* has invaded and continues to invade, millions of hectares of rangeland in South Africa, East Africa, Australia and coastal Asia (Pasiecznik *et al.* 2001). The tree species plays a leading role in the afforestation of arid lands. Their capability of growing on degraded land under arid conditions has made them especially suitable for this purpose. In general, mutualisms between invasive plants and rhizobia have been shown to increase plant biomass and to improve establishment success (Weir *et al*. 2004).

MATERIALS AND METHODS

Host Plant and Soil sampling

Soil samples in this study were collected from three zones within the experimental site. In each zone, soil samples were taken from six random sampling points, at a depth of 15–30 cm. The soil samples were pooled into a single sample and transferred to the laboratory for analysis and trapping experiment in the green house at KEFRI Muguga as described by Odee *et al*. (1997). Each soil was collected with aseptic precautions to avoid cross-contamination between soils from different zones. The soils were analyzed using the methods described in Anderson & Ingram (1989). The pH was measured in calcium chloride $CaCl_2$) as follows: 10 g of soil was suspended in 20 cm^{-3} of 10 $mol.m^{-3}$ $Cacl_2$ solution and measured using a Corning 240 pH meter.

Seeds of *P. juliflora* were obtained from Kenya Forestry seed Center Muguga. The seeds of uniform size were selected and nipped using a blade and surface sterilized by immersion in 90% alcohol for 30s, followed by 3% sodium hypochlorite (NaOCl) solution for 3 min and rinsing several times in sterile water. Seeds were then germinated on 1% water agar plates and incubated at 26^{o}C (Vincent 1970). The experiment was established in a greenhouse with temperature ranging from 19^{o}C to 30^{o}C, using complete randomized blocks design (CRBD) with 10 replications, in PVC pots. Four seeds were planted which were later thinned out to two per pot. The tree plants were then harvested after 90 days.

Nodulation Assessment and Rhizobial Isolation

The plants were cut at the level of the growth media to separate the shoot and the root. Shoot height was recorded in centimeters using a meter ruler. Nodules were detached from the root, counted and fresh weight also recorded at the same time. The different plant parts were then put in brown paper bags and then oven dried at 60^{o}C for 2 days. The dry weights in mg of the plants were then taken separately using an electronic weighing balance. Ten nodules were picked per pot giving a total of 100 nodules per trap host per zone. Fifty of those nodules were selected at randomly for isolation and the rest were kept in McCartney bottles containing silica gel. Fresh nodules were transferred into 95% ethanol for 5–10 s and then in 1% NaOCl for 6 min. Each nodule was then successively rinsed in sterile distilled water (at least 6 changes) to remove traces of the sterilant, each time sterilizing the forceps by dipping it into 95% alcohol followed by flaming with Bunsen burner. The surface sterilized nodule was then placed in a drop of sterile petri-dish and then crushed with a blunt tipped forceps. A loopful of the crushed nodule was then picked and streaked across the surface of yeast extract mannitol agar (YMA) plates. The composition of YMA was according to Vincent (1970). The streaked plates were incubated at 28^{o}C until colonies appeared. Different types of isolates were re-isolated on diagnostic YMA media before being transferred to YMA. The isolates were then stored at -70^{o}C for further work.

Intrinsic antibiotic resistance (IAR)

Stock solution of the following antibiotic were prepared; Ampicillin (Sigma) 20 $\mu g.ml^{-1}$, Streptomycin (Sigma) 200 $\mu g.ml^{-1}$, Kanamycin (Sigma) 100 $\mu g.ml^{-1}$, Tetracycline (Sigma) 50 μgml^{-1} Rifamycin (Sigma) 50 $\mu g.ml^{-1}$, Nalicidixic acid (Sigma) 20 $\mu g.ml^{-1}$. The solutions were filter-sterilized using 0.22 micro ml Millipore filters and stored in one use aliquots at -20^{o}C at each agar plate preparation, an aliquot of each antibiotic was thawed at room temperature and added aseptically to freshly prepared sterile molten YEMA (Yeast extract and Agar from Difco Labaoratories) at 50^{o}C to 60^{o}C according to Vincent (1970) to give the desired final concentration. Rhizobial isolates were grown in yeast Manitol broth to late exponential phase and diluted accordingly before being used to inoculate the agar plates with a Denley multipoint inoculator. There were two replicates for each antibiotic concentration combination. Growth on antibiotic plates was compared with control (antibiotic free) plates and scored as follows, (+) good growth same as control, (+/-) growth less or weak than control and (–) no growth.

Salt and pH tolerance level determination

The level of tolerance to sodium chloride (NaCl) was determined on YMA plates containing the following levels of NaCl concentration in $mol.m^{-3}$; 0, 2, 4, 6, 9, 10. Rhizobia isolates were grown and inoculated as for IAR. Growth was compared with YMA plates with the normal concentration and scored as follows, (+) good growth same as control, (+/-) growth less or weak than control and (–) no growth. The same was done for pH tolerance level determination.

Statistical analysis

The data obtained from the ten replicates were subjected to statistical analysis by using Genstat version 12.0

computer program. Morphological characterization data was converted into an absence/presence binary matrix (0, 1) using the method described by (Rohlf 1993).

RESULTS AND DISCUSSION

Soil analysis

The soil pH of the zones varied from 7.48 *Tortilis* zone, 7.56 *Prosopis* zone and 8.32 Grass zone in water. In $CaCl^2$ soil pH varied from 6.85 *Tortilis* zone, 7.08 *Prosopis* zone and 7.41 Grass zone in. Electrical conductivity ranged from 0.043 mS.cm-1 *Tortilis* zone, 0.053 $mS.cm^{-1}$ *Prosopis* zone and 0.064 $mS.cm^{-1}$ Grass zone. Available ammonia varied from 1.78 ppm Grass zone, 2.37 ppm *Prosopis* zone and 2.11 ppm *Tortilis* zone. Available nitrates varied from 1.12 ppm Grass zone, 2.21 ppm *Prosopis* zone and 1.18 ppm Acacia zone.

Height, fresh, nodulation and dry weight determination

Tortilis zone showed significance difference in terms of plants height compared to the other zones. *Prosopis* zone was the best in terms of nodulation. The other two zones did not show significance difference in terms of nodulation. In terms of nodule fresh weight, *Prosopis* zone had higher significant difference compared to the other two zones which did not show significance difference. Dry shoot matter was highest in *Prosopis* zone and *Acacia* zone but there was no significance difference. Grass zone was poor in terms of shoot dry matter compared to the other two zones.

Isolation and Identification of Microsymbionts

A total of 150 microsymbionts were successfully isolated from the root nodules of the host plants. The isolates were differentiated by their growth rate into either fast-growing (3 days) or slow-growing 5 days. Most of the fast growing bacteria were observed as acid producers, while the slow growers were alkaline producers based on the changes of pH in YEMA incorporated with BTB. Most of the strains failed to absorb the red colour from Congo red.

Physiological characteristic

Table 1. Differentiating physiological traits of the rhizobial isolates recovered from *Prosopis juliflora* using soil collected from grass zone (GZPJ).

Isolate	pH tolerance					NaCl tolerance (%)						Intrinsic antibiotic resistance (IAR)					
	4	6	8	9	10	0	1	2	3	4	5	AMP	STR	KAN	RIF	TET	NAL
GZPJ-1	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-
GZPJ-2	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-
GZPJ-3	+	+	+	+	+	+	+	-	+	+	-	+	-	-	-	-	-
GZPJ-4	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
GZPJ-5	+	+	+	+	+	+	-	-	-	+	+	-	-	-	-	+	-
GZPJ-6	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-
GZPJ-7	-	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-
GZPJ-8	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-
GZPJ-9	-	+	+	+	+	+	+	-	-	+	+	+	-	-	-	-	-
GZPJ-10	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-
GZPJ-11	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-
GZPJ-12	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-
GZPJ-13	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-
GZPJ-14	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-
GZPJ-15	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-
GZPJ-16	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	+	-
GZPJ-17	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	+	-
GZPJ-18	+	+	+	+	+	+	+	-	-	+	-	+	-	-	-	+	-
GZPJ-19	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-
GZPJ-20	-	+	+	+	+	+	+	-	-	+	-	+	-	-	-	-	-

Note: +, -, strains were positive and negative, respectively.

As shown in tables (1–3), the isolates tested showed a wide diversity in their pH tolerance. From 90% to 100% of the isolates grew in lightly acid and neutral pH. A few of the isolates were unable to withstand either very low or very high pH across the zones. *Prosopis* zone had over 60% isolates susceptible to pH4. Over 70% of the isolates grew well in pH 6 showing a neutral and base-tolerant tendency. This tendency might be related to the basic pH that characterizes most of the origin soil from which the isolates were recovered. As shown in

(Table 2), the isolates exhibited a wide diversity in their salt tolerance. The salt inhibitory concentration varied among strains. There was tolerance to low salt levels but the percentage of tolerant strains decreased rapidly and only a few of the strains showed moderate growth at pH 5. All the isolates performed well across the zones in

Table 2. Differentiating physiological traits of the rhizobial isolates recovered from *Prosopis juliflora* using soil collected from tortilis zone (TZPJ).

Isolate	pH tolerance					NaCl tolerance (%)						Intrinsic antibiotic resistance (IAR)					
	4	6	8	9	10	0	1	2	3	4	5	AMP	STR	KAN	RIF	TET	NAL
TZPJ-1	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-
TZPJ-2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-
TZPJ-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TZPJ-4	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-
TZPJ-5	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-
TZPJ-6	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-
TZPJ-7	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TZPJ-8	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-
TZPJ-9	-	+	+	+	+	+	+	-	-	+	+	+	-	-	-	+	-
TZPJ-10	-	+	+	+	+	+	+	-	-	+	+	-	-	-	-	+	-
TZPJ-11	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-
TZPJ-12	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	+	-
TZPJ-13	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-
TZPJ-14	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-
TZPJ-15	-	+	+	+	+	+	+	-	+	+	+	-	-	-	-	+	-
TZPJ-16	-	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-
TZPJ-17	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
TZPJ-18	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TZPJ-19	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-
TZPJ-20	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-

Note: +,-, strains were positive and negative, respectively.

Table 3. Differentiating physiological traits of the rhizobial isolates recovered from *Prosopis juliflora* using soil collected from prosopis zone (PZPJ).

Isolate	pH tolerance					NaCl tolerance (%)						Intrinsic antibiotic resistance (IAR)					
	4	6	8	9	10	0	1	2	3	4	5	AMP	STR	KAN	RIF	TET	NAL
PZPJ-1	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
PZPJ-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PZPJ-3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PZPJ-4	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+
PZPJ-5	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
PZPJ-6	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
PZPJ-7	+	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-
PZPJ-8	-	+	+	+	+	+	+	-	-	+	-	-	+	-	-	+	-
PZPJ-9	-	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-
PZPJ-10	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
PZPJ-11	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+
PZPJ-12	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
PZPJ-13	-	+	+	+	+	+	+	-	-	+	+	+	-	-	-	-	-
PZPJ-14	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-
PZPJ-15	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-
PZPJ-16	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	+	-
PZPJ-17	+	+	+	+	+	+	+	+	-	+	+	+	-	+	-	-	-
PZPJ-18	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-
PZPJ-19	-	+	+	+	+	+	+	-	-	+	+	+	-	-	-	+	-
PZPJ-20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

Note: +,-, strains were positive and negative, respectively

terms of tolerance to salt. This result confirms a selection pressure for tolerance to salinity. The evaluation of intrinsic resistance to antibiotics (IAR) showed that most of the isolates (67%) exhibited high resistance to tetracycline (50 µg.ml^{-1}) and (50%) to ampicillin (20 µg.ml^{-1}) table 3. In the presence of nalidixic acid (20 µg.ml^{-1}) kanamycin (100 µg.ml^{-1}), rifamycin (50 µg.ml^{-1}) and streptomycin (200 µg.ml^{-1}), only 10% to 40% of

the isolates were resistant. All isolates from Grass zone were susceptible to nalidixic acid (20 µg.ml^{-1}) and kanamycin (100 µg.ml^{-1}) except a few which were isolated from *Prosopis* zone and *Tortilis* zone (Table 3). In terms of zones *Prosopis* had the highest number (38%) of strains which showed resistance to most of the antibiotics followed by *Tortilis* zone (23%) and Grass zone was (18%). Cluster analysis grouped the 60 strains by genomic similarity and resulted in the dendrogram as shown in (Figs. 1–3). The dendrogram are further divided into sub-clusters of 6 in the *Prosopis* zone, 5 in *Tortilis* zone and 5 in grass zone and some isolates without any grouping thus indicating that these isolates are probably unique and can be identified based on 16SrRNA gene sequencing technique.

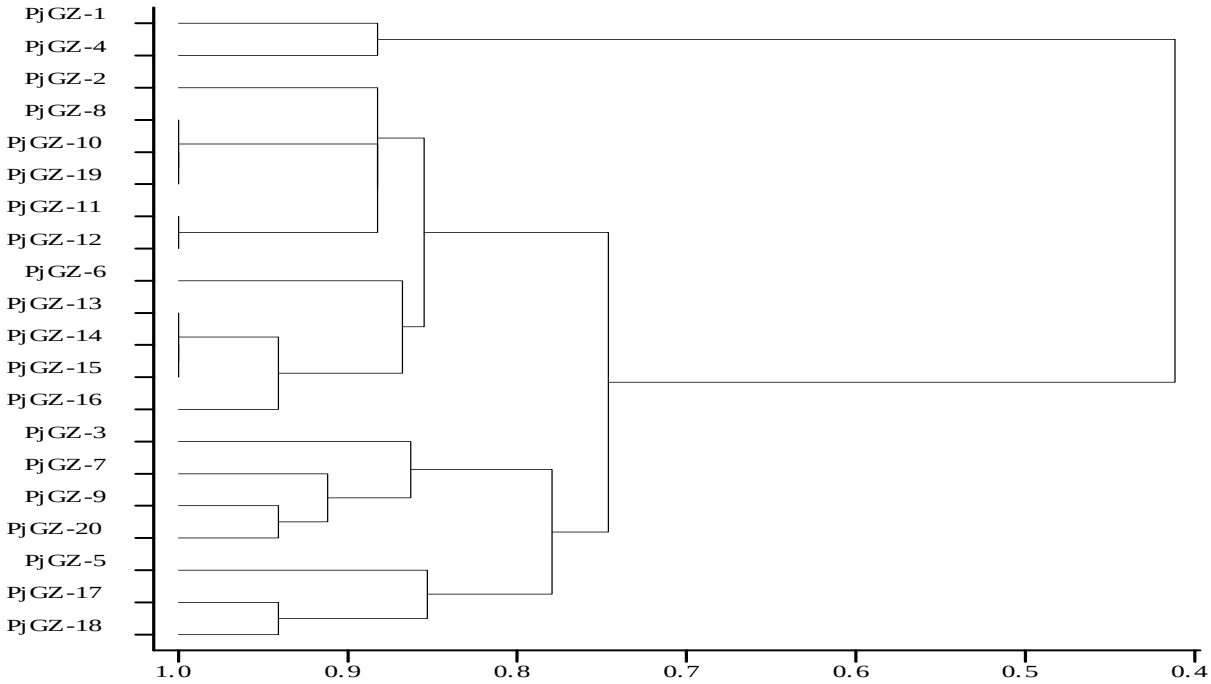

Figure 1. Dendrogram showing phenotypic relatedness based on Intrinsic Antibiotic resistance (IAR), pH and salt tolerance among 20 rhizobial isolates from Grass zone (PjGZ). [Cluster analysis was performed using the Unweighted Paired Group with Arithmetic Average (UPGMA)]

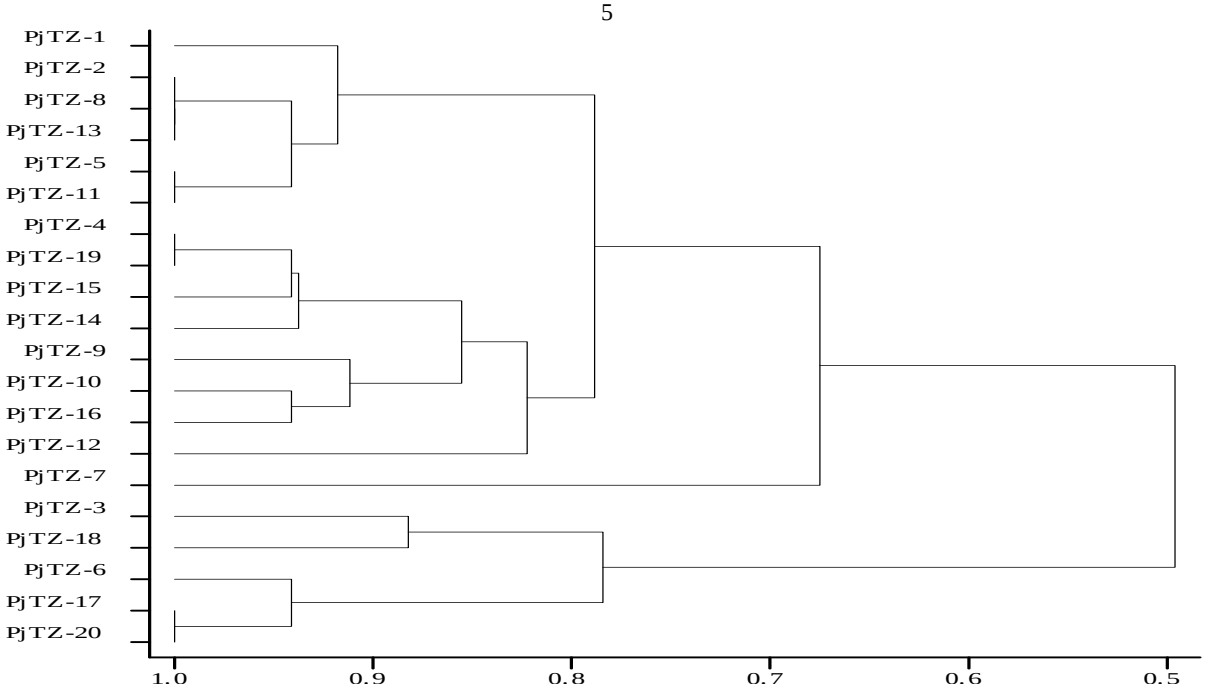

Figure 2. Dendrogram showing phenotypic relatedness based on Intrinsic Antibiotic resistance (IAR), pH and salt tolerance among 20 rhizobial isolates from *Tortilis* zone (PjTZ). [Cluster analysis was performed using the Unweighted Paired Group with Arithmetic Average (UPGMA)]

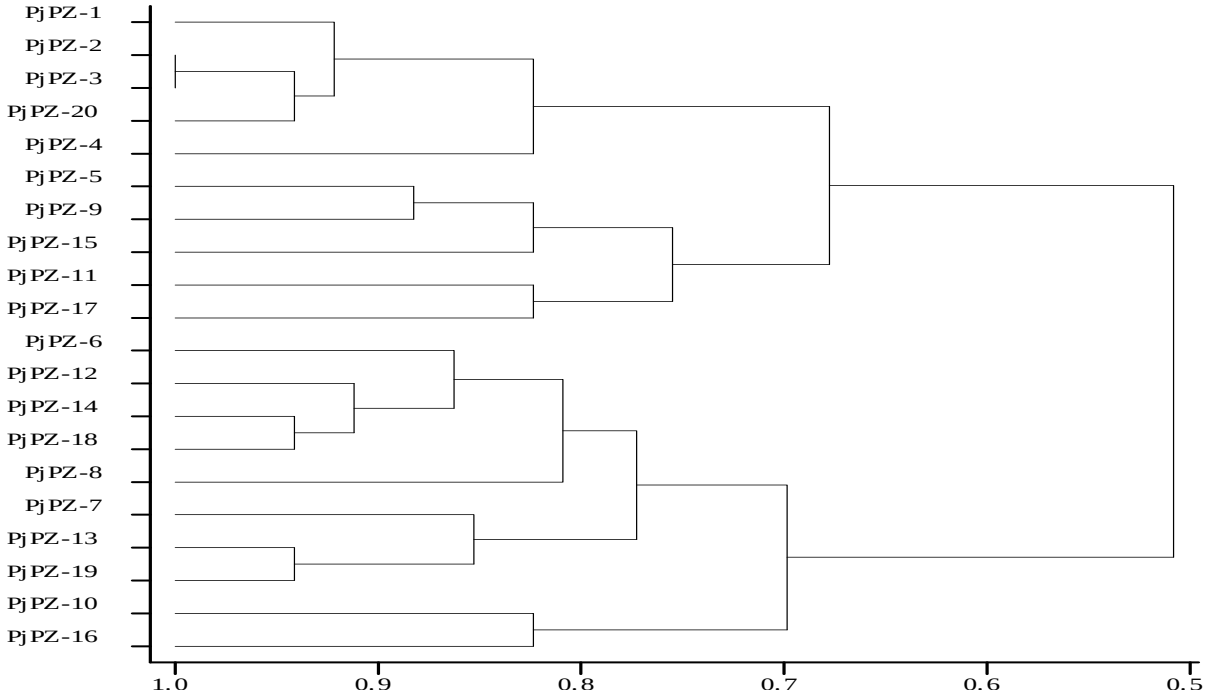

Figure 3. Dendrogram showing phenotypic relatedness based on Intrinsic Antibiotic resistance (IAR), pH and salt tolerance among 20 rhizobial isolates from *Prosopis* zone (PjPZ). [Cluster analysis was performed using the Unweighted Paired Group with Arithmetic Average (UPGMA)]

CONCLUSION

Our findings indicate that, like many other plant-mutualism interactions, native legume rhizobium interactions are impacted by the presence of invasive species in communities. This study, therefore, provides the basis for further research on the phylogeny of rhizobium strains nodulating *Prosopis juliflora* as well as their use as inoculants to improve growth and nitrogen fixation in arid lands. Since no inoculation studies have been carried out to experimentally measure the extent to which invasive legumes may be symbionts limited in different sites within their natural range. Continued efforts should be made to understand the complex association between the invasive plant species and the symbiotic partners and ultimately to employ efficient strains in the sustainable agricultural practice.

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