



Full Length Article

The Symbiotic Efficiency of Legume Tree *Rhizobia* for Host Range Legumes in Central Saudi Arabia

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Abstract

Pot experiments were conducted under greenhouse conditions to evaluate the symbiotic efficiency and host range of several *Rhizobium* isolates on woody tree legumes and cross-nodulation between local *Acacia* trees grown in Central Saudi Arabia. The results indicated that *Acacia* trees varied in their ability to form symbiotic relationships with the *Rhizobium* isolates. Depending on their nodulation responses to isolates, three groups of *Acacia* were recognized. The first group presented nodulation with more than one of the isolates and formed real nodules with most of the *Rhizobium* species. The second was composed of trees with ineffective nodules with most of the isolates. The third group failed to form nodules with the tested *Rhizobium*. *Acacia saligna* and *A. tortillis* were the most tree species, having effective nodules with all isolates. *Rhizobium* isolates KSA6, KSA8 and KSA9 were able to cross-nodulate *A. tortillis*, *A. saligna* and *A. gerrardii* as well as their own hosts. Seedlings of *A. tortillis* and *A. saligna* inoculated with isolates of KSA6 and KSA8 showed levels of growth and biomass more than those of inoculated ones with isolates of KSA9. The *Rhizobium* isolates KSA6 and KSA8 can be utilized to enhance productivity in afforestation programs in the central region of Saudi Arabia. © 2016 Friends Science Publishers

Keywords: *Acacia* sp.; Cross-nodulation; Host range; Symbiotic efficiency; Saudi Arabia

Introduction

Many legumes can start a symbiotic partnership with specific bacteria called *Rhizobia*. These bacteria possess an enzymatic complex termed dinitrogenase that enables them to capture atmospheric nitrogen, reduce it to ammonia and include it into nitrogenous forms that can be incorporated by the host plant.

Rhizobium specificity is known in terms of nodulation and nitrogen fixing efficiency on a host legume trees. The specificity helps as a basis for forecasting the need to inoculate plants, for choosing the species for infection, and for making suitable *Rhizobium* inoculants for a variety of legume species. Rhizobia are recognized for their talent to carry out symbiotic nitrogen fixation by creating nodules on the roots of legumes. As a result of this ability, leguminous plants can be found in infertile soils as pioneer plants that stabilize and enhance soil fertility, thereby preventing soil erosion and desertification (Howieson *et al.*, 2008; Sprent and Gehlot, 2010). For successful nodulation and N₂-fixation with the target host, inoculation remains a 'numbers game' in that methodologies for inoculation must provide high numbers of selected living *Rhizobia* at the time of root emergence (Lupwayi *et al.*, 2000; Graham, 2005). Rhizobia can stay in the soil for 9–15 months before they contact and infect their host. They can supply 103–104 different species

of *Rhizobium* per hectare, thus overcoming the negative effects of suboptimal placement of rhizobia or mismatches with non-hosts (Stephens and Rask, 2000; Catroux *et al.*, 2001; Herridge, 2008).

Burton (1979) mentioned that, efficiency groups of legumes are subsets of cross-inoculation groups that reply likewise to a set of rhizobium strains and, have parallel *Rhizobium* specificity for nodulation and effectiveness. Efficiency groups are helpful mainly as guidelines for inoculant preparation, but can also serve as the source for expecting the need to inoculate certain plants. Also, Trinick (1982) indicated that trees nodulating with *Rhizobium* have specific rhizobial requirements compared with those nodulating with *Bradyrhizobium*.

Saudi Arabia encompassed a number of leguminous tree species, amongst, which neither they possess nodulation ability nor unknown the associated nodule bacteria. *Acacia* populations play a major role in the productivity and stability of the desert environment, but they are threatened, because of their low levels of genetic diversity, restricted geographical range, small population sizes and low densities as well as the irrational cutting of trees and the extreme environmental conditions wherever they are found. Information regarding the symbiosis with woody legume species in natural ecosystems and the distribution of root nodule bacteria under these conditions is limited. The aims

of this study were to evaluate the symbiotic efficiency and the host range of *Rhizobia* of some leguminous trees and the cross-nodulation patterns with local *Acacia* trees grown in the central region of Saudi Arabia.

Materials and Methods

Tree Species and Rhizobial Isolates

Tree species were selected for their prominence in the afforestation and reforestation in the central region of Saudi Arabia. Seeds of each species were obtained from the Range and Forestry Applied Research Unit, College of Food and Agricultural Sciences, King Saud University and the Forestry and Wood Technology nursery, Faculty of Agriculture, Alexandria University. *Rhizobium* isolates were isolated from each tree species and leguminous plants (Table 1).

Isolation of Nodule Bacteria

Nodules were collected from the lateral roots of trees and legumes growing at the Experimental Station of the Food and Agricultural College at Dirab during the growth seasons of 2013 and 2014. Bacteria were isolated from surface-sterilized nodules using the standard procedure (Vincent, 1970) and cultured in yeast extract–mannitol agar (YMA) medium. Single colonies were picked and checked for purity by repeatedly streaking on YMA medium (Ruiz-Díez *et al.*, 2009) and verified by colony morphology, absorption of Congo red (25 mg mL^{-1}) and Gram reaction. Nine isolates were sharply identified. All the isolates were subculture and incubated on YMA medium in slants at 28°C and kept at 4°C and storage in 20% glycerol at -80°C for long-standing storage.

Seedling Preparation and Inoculation Assay

Seeds were soaked in hot water (100°C) for 15 min to break seed coat dormancy and in cool water for 24 h and then sown in sterilized medium (autoclaved at 121°C for one hour) having a mixture of vermiculite and sand (1:1 v/v). After germination, the seedlings were transplanted into pots (20 cm diameter) with sterilized mixture of sand and vermiculite (2:1). Pots were organized in a complete randomized design in a greenhouse with a temperature regime of $24^\circ/17^\circ\text{C}$ day/night, 13/11 h light/dark period relative humidity of $65\pm 5\%$ and with a photon flux density of about $400\text{--}500 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Seedlings were inoculated with 8 days old *Rhizobium* by applying 10 ml of rhizobia broth culture having approximately $10^9\text{--}10^{10}$ rhizobia mL^{-1} within 48 h after planting and then splashed with 30 mL of sterile water. Plants were maintained in the greenhouse during the experiment, watered every other day with distilled water daily and received weekly with standard Hoagland's solution minus N. The host range and effectiveness of inoculating tree seedlings with *Rhizobium*

was tested. *Rhizobium* isolates were verified for their capability to procedure nodules with their original hosts and with other leguminous hosts using inoculation assays. Each isolate was tested on triplicate plants. The control plants were not inoculated. All test plants and controls were maintained in pots for two months. Seedlings were recorded for nodulation depending on the existence and color of the nodules when at least two seedlings had nodulated. The results were scored as positive and effective nodulation when the nodules were healthy and pink to light brown (E), and as ineffective when the nodules were dark brown or black. The nodulation ability and the symbiotic efficiency of the isolates were tested by seedling inoculation assays

Effectiveness Assessment and Seedling Harvest

Two months after the inoculation with *Rhizobium* isolates, the seedlings were harvested and three seedlings per inoculation treatment were randomly selected for effectiveness assessment. Seedlings were counted for nodulation and efficiency depending on appearance of nodules, nodule color and growth characteristics, including seedling length (cm), seedling diameter (mm), number of leaves and nodules per seedling. Seedlings were scored as symbiotically active (E) when seedlings were healthy and bigger than the uninoculated seedlings (control) and the nodule color was light brown or rose. Seedlings were evaluated or assessed as an ineffective (I) when the seedlings have nodules and healthy than the control seedlings and the nodules were dark brown or black, (2) unnodulated (O) when no nodules were found on the seedling roots. For biomass estimation, different seedling parts, viz., stem, root and nodules, were separated and oven-dried at 70°C for 72 h. Total nitrogen was estimated in the seedlings using the Kjeldhal method.

Statistical Analysis

The statistical analysis was done with the SAS software package (version 9.22, 1995). The trial was laid out in split plot in completely randomized design in three replicates. The data were analyzed using analysis of variance (ANOVA), with least significant difference (LSD) analysis ($P < 0.05$). The data pertaining to counts, viz. the number of leaves and nodules were subjected to square root transformation before applying ANOVA.

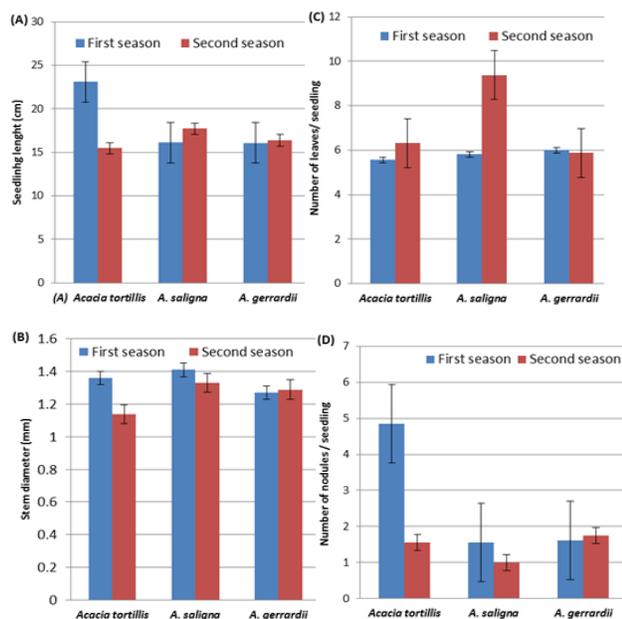
Results

Host Range and Effectiveness of *Rhizobium* Isolates

The host range and effectiveness of inoculating tree seedlings with *Rhizobium* was tested in pots in a green house. *Rhizobium* isolates were evaluated for their capability to procedure nodules in roots of their original hosts and with other leguminous hosts using inoculation

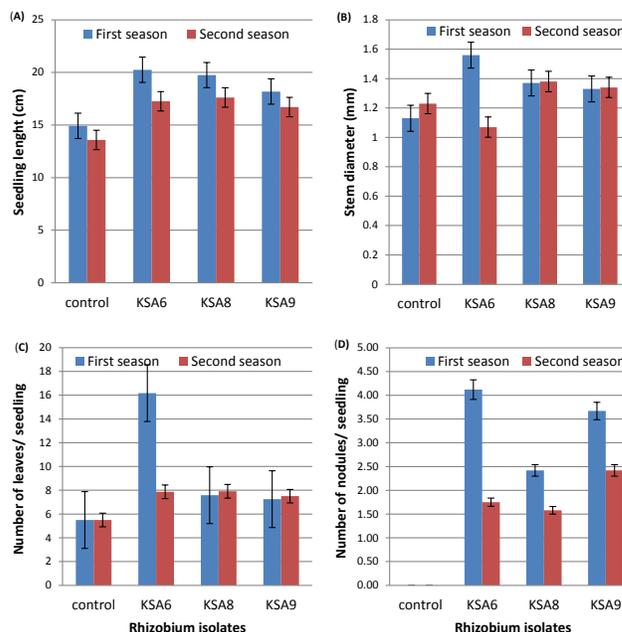
Table 1: The original hosts of the *Rhizobium* isolates used in the study

Original host plant	Seed source	Isolated code
<i>Acacia ampliceps</i>	Dirab	KSA5
<i>A. ehrenbergiana</i>	Dirab	KSA9
<i>A. gerrardii</i>	Riyadh	KSA12
<i>A. origena</i>	Abha	KSA13
<i>A. saligna</i>	Egypt	KSA3
<i>A. seyal</i>	Dirab	KSA1
<i>A. tortillis</i>	Baha	KSA6
<i>Leucaena leucocephala</i>	Egypt	KSA8
<i>Medicago sativa</i>	Al-Madinah	KSA10
<i>Vicia faba</i>	Dereiaa	KSA2
<i>V. faba</i>	Dereiaa	KSA11


Fig. 1: The mean effect of the tree species on the growth properties of the host trees. (A) Seedling length (B) Stem diameter, (C) Number of leaves and (D), Number of nodules/seedling ($n=16 \pm SE$)

assays. Each isolate was tested on triplicate plants. The control plants were not inoculated. All test plants and controls were maintained in pots for two months. Seedlings were assessed for nodulation depending on the existence and color of the nodules when at least two seedlings had nodulated. The results were scored as positive and effective nodulation when the nodules were healthy, turgid and pink to light brown (E), and as ineffective when the nodules were dark brown or black, (I).

The tested tree species differed in their capability to nodulate by the *Rhizobium* isolates. Table (2) showed that each strain produced nodules on the roots of their host, and all control seedlings were without nodules. The host range was reported for a hereditarily diverse group of *Rhizobium* isolates from the nodules of *Vicia faba* (KSA2), *Acacia ampliceps* (KSA5), *A. tortillis* (KSA6), *Leucaena leucocephala* (KSA8) and *A. gerrardii* (KSA12).


Fig. 2: The mean effect of the *Rhizobium* isolates on the growth properties of the host trees

KSA6, *Rhizobium* isolated from *A. tortillis* seedlings; KSA8, *Rhizobium* isolated from *Leucaena leucocephala* seedlings; and KSA9, *Rhizobium* isolated from *A. ehrenbergiana* seedlings. (A) Seedling length, (B) Stem diameter, (C), Number of leaves and (D), Number of nodules/seedling ($n=12 \pm SE$)

Depending on their nodulation responses to the isolates, three groups of trees were identified (Table 3). The first group showed nodulation with more than one of the tested isolates. This group comprised *A. saligna*, *A. gerrardii*, *A. tortillis*, *L. leucocephala*, *A. ampliceps*, *A. ehrenbergiana*, *A. origena*, *A. seyal*, *Medicago sativa* and *V. faba*. Members of this group developed nodules typical by most the *Rhizobium* isolates. The second group was included the plant and tree species that produced unproductive nodules by one or more of the tested isolates, which comprised *A. gerrardii*, *A. origena*, *A. tortillis*, *A. seyal*, *M. sativa* and *V. faba*. Group three contained the species that failed to form nodules with any of the tested *Rhizobium*, and comprised three tree species namely, *A. asak*, *A. etabica* and *A. vectoria*.

Symbiotic Properties

Based on the results in Table (3), three tree species from group one, namely, *A. gerrardii* (Ag), *A. saligna* (As) and *A. tortillis* (At), were selected for their symbiotic properties and because of their ability to form nodules with more than one *Rhizobium* isolate. Symbiotic properties included seedling length, stem diameter, number of leaves and number of nodules as well as the dry biomass of stem, root and nodules and the total nitrogen (%) per seedling. Table (4) shows the analysis of variance of species, strains and their interaction.

Table 2: Effectiveness of Rhizobium isolates on their hosts of isolation and other legumes

Rhizobium code	Original host plant	Aa	Am	Aeh	Aet	Ag	Ao	At	As	Ase	Av	Ll	Ms	Vf
KSA1	<i>Acacia seyal</i>	O	O	O	O	O	O	O	O	E	O	O	O	O
KSA2	<i>Vicia faba</i>	O	O	O	O	E	E	E	E	E	O	E	O	E
KSA3	<i>A. saligna</i>	O	O	O	O	O	O	O	E	O	O	O	O	I
KSA5	<i>A. ampliceps</i>	O	E	E	O	E	I	E	E	I	O	E	I	I
KSA6	<i>A. tortillis</i>	O	E	O	O	E	O	E	E	O	O	E	I	E
KSA8	<i>Leucaena leucocephala</i>	O	O	O	O	E	E	O	E	O	O	E	E	E
KSA9	<i>A. ehrenbergiana</i>	O	E	E	O	E	O	E	E	O	O	E	O	O
KSA10	<i>Medicago sativa</i>	O	O	O	O	O	O	O	E	E	O	O	E	O
KSA11	<i>V. faba</i>	O	O	O	O	E	E	E	E	E	O	E	O	E
KSA12	<i>A. gerrardii</i>	O	O	O	O	I	I	I	E	I	O	O	O	O
KSA13	<i>A. origina</i>	O	O	O	O	O	O	O	O	O	O	O	O	O

Aa, *Acacia asak*; Am, *A. ampliceps*; Aeh, *A. ehrenbergiana*; Av, *A. vectoria*; As, *A. saligna*; At, *A. tortillis*; Vf, *Vicia faba*; Ll, *Leucaena leucocephala*; Ag, *A. gerrardii*; Ao, *A. origina*; Ase, *A. seyal*; Aet, *A. etabca*; Ms, *Medicago sativa*. (E) Positive Nodulation; (I) Ineffective Nodulation; (O) No Nodulation

Table 3: The three main groups of tree legumes species based on their ability to form nodules with the *Rhizobium* isolates tested

Group 1	Group 2	Group 3
Tree species that formed effective nodules with one or more <i>Rhizobium</i> strains	Tree species that formed ineffective nodules with one or more <i>Rhizobium</i> strains	Tree species unable to form nodules with <i>Rhizobium</i> strains
<i>Acacia ampliceps</i>	<i>A. gerrardii</i>	<i>A. asak</i>
<i>A. ehrenbergiana</i>	<i>A. origina</i>	<i>A. etabica</i>
<i>A. gerrardii</i>	<i>A. seyal</i>	<i>A. vectoria</i>
<i>A. origina</i>	<i>A. tortillis</i>	
<i>A. saligna</i>	<i>M. sativa</i>	
<i>A. seyal</i>	<i>V. faba</i>	
<i>A. tortillis</i>		
<i>Leucaena leucocephala</i>		
<i>Medicago sativa</i>		
<i>Vicia faba</i>		

Table 4: Analysis of variance (ANOVA) of species and strains and their interaction and growth properties

Dependent variables	Independent variables					
	First season			Second season		
	Species	Strains	Sp. x St.	Sp.	St.	Sp. x St.
Seedling length	259.02**	70.38**	11.35	15.04	45.40**	7.69
Stem diameter	0.08	0.36*	0.38**	0.152	0.23**	0.56**
Number of leaves	497.43	274.47	261.91	58.19**	15.58**	9.91**
Number of nodules	57.44**	39.85**	8.10	0.002**	0.001*	0.003**

*: Significant at 0.05 probability level; **: significant at 0.001 probability level

Table 5: Interaction between tree species and *Rhizobium* isolates on growth properties of the host trees

	Growth properties								
	First season				Second season				
	SD	RD	Nod D	TN	SD	RD	Nod D	TN	
At	Cont	0.15±0.05 ^{bc}	0.065±0.01 ^{ab}	0.00 ^d	8.41±1.17 ^c	0.173±0.05 ^{ab}	0.067±0.01 ^{ab}	0.00 ^c	10.6±2.19 ^{ab}
	KSA6	0.18±0.02 ^{ab}	0.057±0.01 ^b	0.24±0.02 ^{ab}	9.59±0.22 ^{ab}	0.08±0.02 ^{bc}	0.027±0.01 ^{bc}	0.10±0.02 ^{bc}	11.9±1.23 ^{ab}
	KSA8	0.15±0.01 ^b	0.057±0.02 ^{bc}	0.21±0.02 ^b	8.69±0.39 ^b	0.07±0.02 ^{bc}	0.022±0.01 ^c	0.09±0.02 ^{bc}	7.03±0.81 ^c
	KSA9	0.22±0.03 ^a	0.07±0.01 ^{ab}	0.29±0.04 ^a	9.24±0.35 ^{bc}	0.08±0.01 ^{bc}	0.03±0.01 ^{bc}	0.11±0.01 ^{bc}	12.2±1.54 ^a
As	Cont	0.11±0.08 ^{cd}	0.045±0.02 ^c	0.00 ^d	9.58±0.22 ^{ab}	0.08±0.02 ^{bc}	0.033±0.02 ^{bc}	0.00 ^c	9.11±1.06 ^{ab}
	KSA6	0.14±0.01 ^c	0.07±0.02 ^{ab}	0.21±0.02 ^{bc}	12.44±1.44 ^a	0.193±0.05 ^a	0.072±0.02 ^{ab}	0.27±0.07 ^a	8.34±0.78 ^{bc}
	KSA8	0.18±0.02 ^{ab}	0.073±0.01 ^a	0.25±0.03 ^{ab}	12.0±1.80 ^a	0.17±0.01 ^{ab}	0.073±0.01 ^a	0.24±0.08 ^{ab}	7.91±0.56 ^c
	KSA9	0.14±0.09 ^{bc}	0.053±0.03 ^{bc}	0.02±0.12 ^{bc}	9.54±0.81 ^{bc}	0.103±0.05 ^b	0.04±0.02 ^b	0.14±0.07 ^b	11.1±2.75 ^{ab}
Ag	Cont	0.06±0.01 ^d	0.018±0.01 ^d	0.00 ^d	5.91±1.81 ^c	0.06±0.02 ^c	0.02±0.01 ^c	0.00 ^c	11.9±1.22 ^{ab}
	KSA6	0.18±0.06 ^{ab}	0.07±0.02 ^{ab}	0.24±0.08 ^{ab}	6.91±0.85 ^c	0.153±0.03 ^{ab}	0.072±0.02 ^{ab}	0.23±0.04 ^{ab}	9.5±0.30 ^{bc}
	KSA8	0.15±0.03 ^{bc}	0.07±0.02 ^{ab}	0.23±0.04 ^{ab}	6.89±0.46 ^c	0.16±0.03 ^{ab}	0.07±0.01 ^{ab}	0.23±0.04 ^{ab}	9.4±0.34 ^{bc}
	KSA9	0.07±0.01 ^d	0.023±0.01 ^d	0.09±0.01 ^c	6.61±1.0 ^c	0.175±0.06 ^{ab}	0.07±0.02 ^{ab}	0.24±0.08 ^{ab}	9.8±1.37 ^b

Lowercase letters show significant differences between isolates (in Colum); values with the same letter are statistically similar and those with different letters are significantly different at (P > 0.05). (n= 4 ±SD). KSA6, *Rhizobium* isolated from *Acacia tortillis* seedlings; KSA8, *Rhizobium* isolated from *Leucaena leucocephala* seedlings; and KSA9, *Rhizobium* isolated from *A. ehrenbergiana* seedlings. SD, stem dry weight (g); RD, root dry weight (g); Nod D, nodular dry weight (g); TN, total nitrogen /seedling (ppm). Ag, *A. gerrardii*; As, *A. saligna*; and At, *A. tortillis*

The data indicate that the growth characteristics of the tree species and *Rhizobium* isolates varied in both two seasons. In the first season, highly significant differences were noted for the species and strains in terms of, seedling length, number of nodules and stem diameter. No significant differences were found between species in the number of nodules, strains and the interactions of seedling length, number of leaves and nodules. However, significant differences were found in the stem diameter among strains tested. In the second season, the interaction between species and strains showed highly significant differences in stem diameter, number of leaves and nodules per seedling, whereas the seedling length did not differ significantly (Table 4).

The mean effect of the tree species on the growth properties of the seedlings varied. *A. tortillis* seedling had the greatest length, number of leaves and nodules per seedling compared with those of *A. saligna* and *A. gerrardii* in the first season. In the second season, *A. saligna* showed significant increase in its growth properties compared with the other two species studied (Fig. 1).

The *Rhizobium* isolate, KSA6 was the most effective on all the tree species and had the greatest positive effect on the growth properties of the seedlings, followed by KSA8, whereas KSA9 had less ability to form a symbiosis with tree species compared with the other two isolates. In the second season, KSA8 was more effective than KSA6 or KSA9 (Fig. 2).

The significant differences in the interactions between tree species and *Rhizobium* isolates varied greatly between the two seasons. The responses of the host legume trees to inoculation with different *Rhizobium* isolates varied greatly in the dry matter produced (Table 5). For the stem dry weight, *A. tortillis* gave the largest value when it's inoculated with *Rhizobium* KSA9 in the first season, whereas *A. saligna* had the highest value with *Rhizobium* KSA6 in the second season. As the root dry weight, *Rhizobium* KSA8 was most effective on *A. saligna* and gave the highest value compared with those of KSA6 and KSA9 during the two seasons. The same trend was found for the nodule dry weight. *Rhizobium* KSA9 gave the highest value with *A. tortillis* in the first season, but in the second season, *A. saligna* inoculated with *Rhizobium* KSA6 gave the highest value compared with the control and other *Rhizobium* isolates (Table 5). The same trend was found for the total nitrogen (%) per seedling. *A. saligna* inoculated with *Rhizobium* KSA6 had the highest total nitrogen per seedling compared with *A. tortillis* and *A. gerrardii* in the first season, whereas *A. tortillis* inoculated with *Rhizobium* KSA9 had the highest value in the second season (Table 5).

Discussion

The host range and effectiveness of inoculating tree seedlings with *Rhizobium* was tested in pots in a green house. *Rhizobium* isolates were evaluated and ranked for their capability to procedure nodules with their original

hosts and with other leguminous hosts using inoculation assays. The results demonstrated that *Acacia* trees varied in their ability to form symbioses with the *Rhizobium* isolates. Among the tree species that formed effective nodules, *A. saligna*, which was the most species, created nodules with the 9 tested *Rhizobium* isolates compared with *A. gerrardii* and *Leucaena leucocephala* (6 isolates). By contrast, there were some woody legume trees that could not form nodules with any of the isolates. These trees may have a requirement for a specific strain of *Rhizobium*, possibly an endogenous strain. These results were consistent with the findings of Ndoye *et al.* (1990), Bala and Giller (2001) and Cummings *et al.* (2009), but not with the result of Ali *et al.* (2010), who reported that *Acacia* species could not form nodules with any of the *Rhizobium* isolates from *L. leucocephala* and *Sesbania sesban* (Table 2). Woldemeskel and Sinclair (1998) reported the specificity, in relations of both nodulation and efficiency, of some Australian *Acacia* species. Turk and Keyser (1992) indicated that *Leucaena leucocephala* and *Gliricidia sepium* belong to an effectiveness group. We can add *A. saligna*, *A. gerrardii*, *A. tortillis*, *A. ampliceps*, *A. ehrenbergiana*, *A. origena* and *A. seyal* to this group. *Acacia* spp. were capable of forming nitrogen fixing nodules with a wide variety of *Rhizobium*. The results we obtained are in agreement with the finding of Mytton *et al.* (1988). Similar results were reported by Mahmood and Akthar (2008) when *Vigna mungo* was successfully cross-inoculated with the *Rhizobium* isolates from tree legumes. The *Rhizobium* isolates species that failed to nodulate in those acacias might be explained by the loss of a symbiotic plasmid (sym plasmid) or a lateral gene transfer or changes in the host specific *nod* gene (Haukka *et al.*, 1996; Kucuk *et al.*, 2006; Han *et al.*, 2008). Environmental conditions, especially water deficiency, may play a crucial role in nodulation and may be responsible for some non-specific nodulation (Ben Ramdhane *et al.*, 2009; Fitouri *et al.*, 2012). Based on our observations, it is that the environmental conditions and Nod factors played an important role in the nodulation process. Further studies are required to explain why some woody *Acacia* grown in Saudi Arabia are unable to nodulate their own *Rhizobium* strains?

Based on the results in Table (3), three tree species from group one (*A. gerrardii*, *A. saligna* and *A. tortillis*) were selected for their symbiotic properties and because of their ability to form nodules with more than one *Rhizobium* isolate. The symbiotic properties included seedling length, stem diameter, number of leaves and number of nodules as well as the dry biomass of stem, root and nodules and the total nitrogen (%) per seedling. We found that the response of the host trees to the inoculation with the tested *Rhizobium* isolates varied greatly. Seedlings of *A. tortillis* and *A. saligna* inoculated with isolates KSA6 or KSA8 had higher biomasses than those inoculated with isolate KSA9 or uninoculated seedlings. In all three hosts, the *Rhizobium* isolate KSA9 induced the largest amount of dry matter and

total nitrogen (%) of their hosts, was isolated from the roots of *A. tortillis* seedlings, followed by that isolated from *L. leucocephala* ones. These results are in accordance with those of the earlier investigations of Somasegaran *et al.* (1990); Turk *et al.* (1993); Bala and Giller (2001); Kaur *et al.* (2005) and Elkoca *et al.* (2008) who reported that there is significant influence of rhizobial isolates on growth, biomass and total nitrogen content in plants. Inoculation with appropriate *Rhizobium* isolates ideally suited to the species and environment in terms of nitrogen fixation and assimilation enhanced the productivity of forests and plantations (Kaur *et al.*, 2013).

The results recommend that specific inoculants must be used with some or same trees, including *A. gerrardii* and *A. origina*, whereas common inoculants could be developed for *A. saligna*, *A. tortillis* and *L. leucocephala*. In addition, a specific inoculant might be developed for use with some tree species that are uninhibited for efficiency and nodulation. This suggestion is consistent with the findings of Somasegaran *et al.* (1989); Turk and Keyser (1992) and Ali *et al.* (2010).

It is possible that the *Rhizobium* isolates KSA6 and KSA8, which induced the maximum length, stem diameter and biomass of seedlings of *A. tortillis*, *A. saligna* and *A. gerrardii* can be exploited in forest plantations intended to be used for fuel, fodder and timber. By contrast, the isolates KSA8 and KSA9, which caused the maximum specific and total nitrogen per plant, can be utilized for afforestation and reforestation on the low nitrogen content lands in the central region of Saudi Arabia. Further studies are required to assess the practicability of our conclusions.

Conclusion

In deduction, the results of the current study have shown that *Acacia* trees are vary in their ability to develop symbioses with *Rhizobium* isolates. Three groups of trees were identified based on their nodulation responses to the test isolates. In the first group, the trees made actual nodules with most of the *Rhizobium* isolates. The second group was finished up of tree species that made fruitless, inactive nodules with one or more of the tested strains. The third group contained the species that failed to form nodules with any of the tested *Rhizobia*. Among the tree species that formed effective nodules, *A. saligna* and *A. tortillis* were the most promiscuous species, forming nodules with more of the tested *Rhizobium* isolates than *A. gerrardii* and *L. leucocephala*. The failure of some woody legume trees to form nodules with any of the isolates might be due to a requirement for a specific *Rhizobium*, possibly only the endogenous strain. *Rhizobium* isolates of *A. tortillis*, *L. leucocephala* and *A. ehrenbergiana* seedlings were able effectively to cross-nodulate *A. tortillis*, *A. saligna* and *A. gerrardii*, as well as their own host trees. Field testing is required to assess the practicality of our results and conclusions.

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