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EFFECT OF DIFFERENT RHIZOBIAL STRAINS ON THE GROWTH AND NITROGEN UPTAKE IN ACACIAS UNDER SALINE SOIL

UBAIDULLAH SHIRAZI, R. ANSARI, MUKHTIAR ALI, S.M. ALAM, BARKAT KHANZADA, AISHA SHREEN, M.A. KHAN, S.M. MUJTABA AND MUHAMMAD ALI

Plant Physiology Division,
Nuclear Institute of Agriculture, Tandojam, Pakistan.

Abstract

A field trial was conducted at NIA experimental farm, Tandojam, Pakistan to study the effect of different Rhizobial strains on the survival and growth of two Australian *Acacias* viz., *Acacia ampliceps* and *Acacia stenophylla* under saline condition. Inoculation of tree seedlings improved survival and growth of both *Acacia* species as compared to uninoculated ones. *A. stenophylla* showed higher survival as compared to *A. ampliceps*, whereas the over all performance of *A. ampliceps* was much better. *A. ampliceps* inoculated with rhizobial strain no. 63/1 showed greater plant height, stem diameter ,and plant canopy followed by 403/1 and 399/1. Performance of tree seedlings with the application of 100 mg N/kg of soil alone were better as compared to two strains of Rhizobia. *A. stenophylla*, inoculated with strain no. 403/1 proved better for its symbiotic partner followed by 63/1. The uptake of nitrogen by plant also increased in inoculated plants but the values for N-content in seedlings without N application were higher.

Introduction

The Australian *Acacias* viz., *A. ampliceps* and *A. stenophylla* have showed a great potential to grow under saline lands of Pakistan (Ansari *et al.*, 1997). The successful plantation and better growth of these species are mainly associated with balanced supply of plant nutrients especially nitrogen since saline soils are mostly low in nitrogen with low rates of nitrogen mineralization. The presence of high concentration of sodium salts reduces the growth and activity of microbial population and hence its symbiotic partner also suffers, resulting in low growth performance due to limited supply of nutrients. Inoculation with effective Rhizobia has great potential for increasing productivity of *Acacias* (Kang & Li, 1997). It is therefore necessary that these species should be inoculated with effective Rhizobial strains in order to optimize nitrogen fixation and resulting in higher crop productivity. The present study was conducted to examine the effect of different Rhizobial strains on the growth and nitrogen uptake of *Acacia ampliceps* and *A. stenophylla* growing under saline condition.

Materials and Methods

Seeds of *Acacia ampliceps* (Wolfe Creek, Western Australia) and *Acacia stenophylla* (Cow Creek, W.A.) supplied by CSIRO Australia, were pretreated with boiling water for 10 minutes and sown directly in plastics bags filled with river sand. Three strains of *Rhizobium japonicum* (399/1, 403/1, 63/1), also supplied by CSIRO were grown in yeast mannitol broth (YMB) at 30°C for one week. These culture suspensions were subsequently applied to three months old seedling @ 1 ml/seedling containing approximately 10^7 cfu/ml. Two uninoculation treatments with and without nitrogen were kept as control.

The trial was conducted at the experimental farm of Nuclear Institute of Agriculture, Tandojam. The soil was saline, where salinity ranged between (2-10 mS/cm) and (1.5-5 mS/cm) in 1:25 soil water extract at surface (0-30 cm) and subsurface (30-60 cm), respectively. The soil was medium to heavy in texture, neutral in reaction and low in nitrogen and phosphorus, while potassium was sufficient. Salinity was mostly associated with neutral salt (NaCl) dominated at surface layer which declined with depth.

Six months old seedlings of two *Acacias*, already inoculated with different Rhizobial strains, were transplanted in the field in auger holes along the side of the ridges. The experiment was laid according to Randomized Complete Block Design (RCBD). Growth observations were recorded in terms of survival percentage, plant height, stem diameter and plant canopy.

Composite leaf samples from newly expanded leaves were collected from each treatment from 24 months old plantation. The samples were dried at 80°C, ground by Retsch grinder and total nitrogen determined by Kjeldahl method (Jackson, 1962). The data was subjected to analysis of variance (Steel & Torrie, 1980).

Results

Seeding survival: Both the *Acacia* sp., showed better survival (Table 1). *A. stenophylla* had comparatively higher population density than *A. amplicpes* but the latter species had high seedling stability from the initial stages. *A. ampliceps* inoculated with Rhizobium strain no. 63/1 showed maximum survival i.e. 87% followed by strain no. 403/1 and 399/1 while similar values (87%) were gained by other *Acacia* inoculated with strain no. 403/1 followed by 63/1 and 399/1. In the control treatment with positive nitrogen, the seedling survival in case of *A. ampliceps* improved, while the survival percentage of *A. stenophylla* was comparatively better under both +ve and –ve Nitrogen control treatments.

Table 1. Effect of different Rhizobial strains on seedlings survival (%) of *Acacia* species at different growth intervals.

Species	Treatments	2 months	4 months	8 months	12 months
		Seedling survival %			
	T1=399/1	54.2	54.2	54.2	54.2
	T2=403/1	56.3	56.3	56.3	56.3
Acacia ampliceps	T3=63/1	87.5	87.5	87.5	87.5
	T4=+ve N	56.3	56.3	56.3	56.3
	T5=-ve N	37.5	37.5	37.5	37.5
	T1=399/1	79.0	68.8	68.8	68.8
	T2=403/1	87.5	87.5	87.5	87.5
Acacia stenophylla	T3=63/1	83.3	83.3	81.3	81.3
	T4=+ve N	83.3	83.3	77.1	77.1
	T5=-ve N	81.3	81.3	75.0	64.6

Plant growth: The growth performance recorded in terms of plant height, stem diameter and plant canopy (crown diameter) showed that at 24 months *A. ampliceps* were much better than *A. stenophylla* (Table 2) with an average plant height of 555 and 430 cm, respectively. Maximum height in *A. ampliceps* was observed in plants inoculated with strain no. 63/1 followed by 403/1 and 399/1 treated seedlings, while the seedlings of *A. stenophylla* showed maximum height in 403/1 inoculated plants followed by 63/1 and 399/1. The response of nitrogen application in control showed that seedlings of *A. ampliceps* scored next to the treatment having maximum plant height as compared to *A. stenophylla* seedlings under this treatment with lowest plant height.

Table 2. Effect of different Rhizobial strains on different growth parameters after 24 months under saline conditions.

	Acacia ampliceps				*Acacia stenophylla*			
Treatments	**Plant height (cm)**	**Stem diameter (D10) (cm)**	**Stem diameter (DBH) (cm)**	**Plant canopy (m)**	**Plant height (cm)**	**Stem diameter (D10) (cm)**	**Stem diameter (DBH) (cm)**	**Plant canopy (m)**
T1	531	14.58	9.60	9.02	413	11.65	6.30	6.15
T2	548	15.60	11.31	12.42	457	11.91	7.05	8.86
T3	597	15.30	12.05	8.20	453	11.51	7.70	8.97
T4	590	16.13	8.60	7.84	385	13.09	6.30	7.60
T5	510	15.40	10.89	9.47	444	11.60	6.55	9.61
Mean	555	15.20	10.49	9.38	430	11.95	6.78	8.24

Stem diameter: Like plant height, *A. ampliceps* also showed higher values of stem diameter of 15.2 and 10.5 cm, at the base and at breast height respectively as compared to *A. stenophylla* with 11.95 and 6.8 cm. The seedling treated with different Rhizobia showed maximum diameter at base (D10) of plants inoculated with strains 63/1 and 403/1 in *A. ampliceps* and *A. stenophylla*, respectively. In control treatments the response of nitrogen was more with maximum D10 values for both the species. Stem diameter at breast height (DBH) in both the species was high where seedlings were treated with strain no. 63/1. The values within the individual species ranged between 12.1-8.60 and 7.7-6.30 in *A. ampliceps* and *A. stenophylla*, respectively. The response of nitrogen application was almost nil, as was evident from the lowest DBH values in both *Acacias*. The statistical difference for both the parameters in the two acacias was significant but was non-significant within the individual treatments.

Plant canopy: Like other characters, the canopy of *A. ampliceps* was also high as compared to *A. stenophylla*. Canopy within the individual treatment ranged between 12.42-7.84 and 9.61-6.15 m with average values of 9.38 and 8.24 in *A. ampliceps* and *A. stenophylla*, respectively. Maximum plant canopy in *A. ampliceps* was recorded in plants treated with strain no. 403/1, whereas in *A. stenophylla*, none of the inoculated seedling group exceeded with uninoculated seedlings. The differences within the species as well as within the treatment were non-significant.

Leaf nitrogen content: Salinity redueed nitrogen content in leaves in both the species (Table 3). Nitrogen content in leaves of plant ranged between 1.2-1.3% and 1.1 - 1.5% in *A. ampliceps* and *A. stenophylla,* respectively. The seedlings inoculated with different Rhizobial strains showed very little increase in nitrogen uptake in both the species. However, the strains 399/1 and 403/1 were comparatively better than 63/1 in both the *Acacias*. In uninoculated trees where N was applied @ of 100 mg N/kg of soil showed lowest nitrogen values. On the other hand, tree seedlings without N-application showed highest nitrogen content i.e., 1.3 and 1.5% in case of *A. ampliceps* and *A. stenophylla*, respectively.

Table 3. Leaf nitrogen content (%) of Acacias inoculated by different Rhizobial strains.

Treatments	*Acacia ampliceps*	*Acacia stenophylla*
T1 = 399/1	1.35	1.39
T2 = 403/1	1.31	1.32
T3 = 63/1	1.27	1.37
T4 = (+ve N)	1.21	1.12
T5 = (-ve N)	1.31	1.50

Discussion

Inoculation with different Rhizobial strains showed an increase in seedling survival of both the *Acacias* under saline conditions. However, the magnitude of growth response varied within the species and between the strains. The strain 63/1 proved most suitable in *A. ampliceps*, while the association of *A. stenophylla* and strain no. 403/1 was much better. Differences between Rhizobial strains in the extent of growth response and amount and rate of nitrogen fixation have also been reported by Kang & Li (1998).

Seedling survival and growth in two control treatments (uninoculated with 100 mg N/kg soil and uninoculated without N-application) also varied. Survival percent in both the *Acacias* species was high in +ve N plant as compared to –ve N controls. It was also interesting that seedlings of N-applied controls not only have good survival and growth as compared to unapplied nitrogen but also from some inoculated ones which might be due to un-matching of *Acacia* species with these strains as in case of strain no 399/1 in both *Acacia* species. Effect of nitrogen in N-fixing seedlings was also observed by Cobbia (1991), who found that in situation with low soil N-supplies, N-fixing trees often benefit from N-fertilization. On the other hand, it was against the findings of Umali-Garcia *et al.*, (1988), who found that N-fertilization of potted *Albizzia falcataria* with equivalent of 30 to 100 mg N/ha reduced biomass by 10-60%, although nodule activity and hence N fixation was not examined.

The values for N-content in uninoculated seedling without nitrogen were higher as compared to N-applied control as well as inoculated seedlings. This might be due to presence of native occurring Rhizobia as was also reported by Kang & Li (1998). The present study would suggest that inoculation of *Acacia* species with suitable strains of Rhizobia not only improved the percent survival, but also growth and nitrogen content in tree seedlings under saline soil.

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