

Effect of *Rhizobium* Strains on Growth of Two *Sesbania* species

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Abstract

The objective of this study was to evaluate the effectiveness of *Rhizobium* strains on *Sesbania* (*S. aculeata* and *S. grandifolia*) growth. In this two-factor factorial pot experiment, inocula of ten *Rhizobium* strains were applied on two *Sesbania* species. The effect of inocula, *Sesbania* species and their interaction on growth parameters was statistically significant. *Sesbania* treatments with different inocula showed higher growth than uninoculated plants (control) and the treatment with N addition. In *S. grandifolia*, the mixture of strains responded better in all parameters, however in *S. aculeata*, no specific *Rhizobium* strain showed extraordinary performance. *Rhizobium* inoculation exhibited 3-fold increase in *S. grandifolia* and 4-fold in *S. aculeata* shoot dry matter, nodule dry matter and nitrogen contents.

Key words: Effect, *Rhizobium* strains, Growth, *Sesbania*

Introduction

Soil health is critical for producing high quality and sustainable crop production. Leguminous plants proved excellent tool for this purpose by improving soil fertility through regenerative means. Leguminous plants in association with *Rhizobium* can fix significant amount of atmospheric nitrogen from air which contributes to the soil nitrogen pool (Jefing *et al.*, 1992). On a global level, annual contribution of biological nitrogen fixation has been estimated about 172 million tons. Legumes contribute about 25% (35 m tons) of biologically fixed nitrogen, which is slightly less than that supplied to agro-ecosystems through chemical fertilizers (Azam, 2001; Lshizuka, 1992). *Sesbania* being rich in nitrogen fixing bacteria is extensively cultivated in Pakistan (Fazal, 1994). In symbiosis with *Rhizobium*, it can fix upto 542 kg N ha⁻¹ (FAO, 1984). Its inoculation with superior rhizobial strains is essentially required to increase the yield of legumes through nitrogen fixation (Athar, 1998).

The *Rhizobium*-legume symbiosis can increase yield with subsequent decrease in pollution (Freiberg *et al.*, 1997). However, rhizobial isolates vary in their nitrogen fixation potential and in improving the vegetative and reproductive growth of different crops under varying environmental conditions (Chatel *et al.*, 1973; Shishidu and Pepper, 1990; Gopalakrishnan and Grish, 1999). So effective nitrogen fixing strains of rhizobia are being developed as inoculant for various legumes (Hardarson, 1993; Brockwell and Bottomely, 1995; Shah *et al.*, 2000).

To fully exploit biological nitrogen phenomenon, determination of the effect of indigenous rhizobial isolates on the growth of *Sesbania* was felt necessary.

Materials and Methods

To investigate the effect of ten rhizobial strains on growth of two *Sesbania* species i.e. *Sesbania grandifolia* and *Sesbania aculeata*, a pot experiment was carried out in growth room at 30°C day and 25°C night temperature with 12 hours photoperiod. The two *Sesbania* species were sown in pots having sterilized sand (pH 7) and was laid out in completely randomized design (CRD) with two-factor factorial arrangement and three replications.

Eleven treatments either as a single strain or in combination i.e. T₁ (Sa-1), T₂ (Sa-2), T₃ (Sa-3), T₄ (Sa-4), T₅ (Sa-5), T₆ (Sa-6), T₇ (Sa-7), T₈ (Sg-1), T₉ (Sg-2), T₁₀ (Sg-3), T₁₁ (Sa-1+Sa-2+Sg-1+Sg-2), one treatment with nitrogen @ 2 mmole l⁻¹ and a control treatment were tested both on two *Sesbania*.

Inoculum of rhizobial isolates prepared in yeast mannitol broth was applied @ 2 ml/seedling one week after transplantation. Hoagland solution (1/4 strength) was applied once in a week to fertilize the plants and autoclaved distilled water was used according to their requirements. Data regarding shoot, root and nodule dry weights and plant height were collected five weeks after inoculation and subjected to analysis of variance technique and least significant difference (LSD) test at 5% probability level.

Results and Discussions

Growth Parameters

The inoculation of rhizobial strains significantly improved growth of both *Sesbania* species. The effect of inoculation in term of shoot and root dry weights and plant height was non-significant between the two species.

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Table 1: Effect of *Rhizobial* strains on growth of *Sesbania* species

Treatments	Shoot dry wt. (g)		Root dry wt. (g)		Plant height (cm)		Nodule dry wt. (mg)		Shoot N (mg)		ARA (m mole hr ⁻¹ g ⁻¹)	
	<i>Sesbania grandifolia</i>	<i>Sesbania aculeata</i>	<i>Sesbania grandifolia</i>	<i>Sesbania aculeata</i>	<i>Sesbania grandifolia</i>	<i>Sesbania aculeata</i>	<i>Sesbania grandifolia</i>	<i>Sesbania aculeata</i>	<i>Sesbania grandifolia</i>	<i>Sesbania aculeata</i>	<i>Sesbania grandifolia</i>	<i>Sesbania aculeata</i>
T ₀ (No Inoculation)	0.09 e	0.05 ab	0.022 b	0.025 ab	8.6 e	8.3 b	-	-	1.21 c	0.87 c	-	-
T ₁ (Sa-1)	0.27 abc	0.14 a	0.050 ab	0.030 ab	18.3 a	13.1 a	18.3 b	12.4	8.41 ab	4.30 bc	0.27 ab	0.24 ab
T ₂ (Sa-2)	0.20 b-e	0.23 ab	0.059 ab	0.063 ab	17.5 ab	14.4 a	22.0 b	21.3	6.15 a-d	7.39 ab	0.28 ab	0.26 ab
T ₃ (Sa-3)	0.17 b-e	0.15 a	0.030 ab	0.044 ab	10.1 e	15.1 a	9.7 b	16.0	3.35 cde	4.53 bc	0.21 ab	0.59 a
T ₄ (Sa-4)	0.11 de	0.27 a	0.040 ab	0.040 ab	11.8 de	16.8 a	13.9 b	23.7	3.10 cde	9.41 a	0.23 ab	0.03 b
T ₅ (Sa-5)	0.28 ab	0.24 a	0.024 b	0.090 a	10.1 e	13.0 a	11.7 b	16.7	7.65 abc	7.56 ab	0.06 b	0.54 a
T ₆ (Sa-6)	0.17 b-e	0.24 a	0.036 ab	0.044 ab	12.6 cde	15.3 a	20.0 b	18.0	3.68 cde	7.79 ab	0.19 b	0.46 ab
T ₇ (Sa-7)	0.26 a-d	0.16 ab	0.085 a	0.034 ab	16.2 a-d	14.6 a	22.7 b	16.7	6.67 a-d	3.91 bc	0.31 ab	0.25 ab
T ₈ (Sg-1)	0.13 cde	0.16 ab	0.030 ab	0.024 b	13.4 b-e	13.8 a	15.7 b	16.0	2.78 de	2.73 c	0.18 ab	0.14 ab
T ₉ (Sg-2)	0.10 e	0.23 a	0.036 ab	0.045 ab	12.4 cde	16.3 a	12.3 b	24.7	2.42 de	7.80 ab	0.07 b	0.29 ab
T ₁₀ (Sg-3)	0.17 b-e	0.18 ab	0.035 ab	0.053 ab	16.2 a-d	14.3 a	15.5 b	19.0	3.98 b-e	3.79 bc	0.11 ab	0.28 ab
T ₁₁ (Sa-1+ Sa2 + Sg-1 + Sg-2)	0.38 a	0.21 a	0.087 a	0.044 ab	17.2 a-c	15.8 a	38.0 a	20.3	9.99 a	7.80 ab	0.53 a	0.24 ab
T _N (N)	0.14 b-e	0.17 ab	0.033ab	0.044 ab	10.2 e	14.0 a	-	-	2.51 de	2.48 c	-	-

Mixture of strains performed better than individual strain treatments in *S. grandifolia*. Upto four times higher shoot dry weight was recorded in inoculated treatment of T₁₁ (mixture of strains) *S. grandifolia* plants. In *Sesbania aculeata*, mixture of strains and T₉ (Sg-2 strain inoculation) performed better and produced 4-5 times more shoot dry matter than control. No specific host affinity was observed regarding species-strain specificity.

Mix culture response by *S. grandifolia* and *S. aculeata* was in accordance with Podder (1994), who found the mixed inoculation of *Rhizobium* in lentil was superior to a single strain for all the plant characteristics measured. However, it contradicts results of Nambiar *et al.* (1984) who reported that single *Rhizobium* culture performed better than mixed *Rhizobium* culture. Application of N also didn't cause significant difference as compared to control treatment. Root dry weight ranged from 0.02 g plant⁻¹ (T₀) to 0.09 (T₁₁) in *S. grandifolia* while it ranged from 0.02 (T₈) to 0.09 (T₅) g plant⁻¹ in *S. aculeata*.

Similar results were also seen regarding plant height which confirms the previous results that the treatment where mixture of strains is used performs better than the control and the treatment where single strain inoculation was done.

Symbiotic Traits

The parameters which were studied were nodule dry weight (mg plant⁻¹) and acetylene reduction assay (ARA, m mole hour⁻¹ g⁻¹). In *S. grandifolia*, nodules dry weight ranged from 9.7 to 38.00 mg and minimum was in treatment where *S. aculeata* native inoculation was applied while maximum was recorded for mixture of strains (T₁₁). In *S. aculeata*, it ranged from 12.4 to 24.7 mg in various treatments, which were statistically non-significant due to large variation among replications. In *S. grandifolia* maximum ARA was in treatment where mixture of strain was applied, while in *S. aculeata* single strain inoculation showed maximum ARA (m mol h⁻¹ g⁻¹).

Plant Nitrogen Contents

In *Sesbania grandifolia* maximum shoot N contents were found in T₁₁ (mixture of strains) which were five-fold when compared with control. This again confirms the previous results. However, in *Sesbania aculeata* maximum shoot nitrogen contents were observed in T₄ (a strain native to *S. aculeata*).

In conclusion, the results showed that *Rhizobium* inoculation enhanced the yield traits of *Sesbania* species. Both the two *Sesbania* species differed non-significantly regarding all the parameters. However, both performed differently to the inoculum application. *Sesbania grandifolia* responded better to the mixture of different rhizobial strain inoculum while *S. aculeata* responded better to inoculation of individual strains. All the treatments performed better than the control treatments. It is also obvious that a plant species can respond to the same extent to

inoculums which is not native to that when compared to a plant species with a native inoculums.

References

- Athar, M. Drought tolerance of lentil rhizobia (*Rhizobium leguminosarum*) from arid and semi arid areas of Pakistan. Lett. Appl. Microbiol., 1998. 26: 38-42.
- Azam, F. Legume-bacterium (*Rhizobium*) association symbiosis, a marriage of convenience, necessary evil or bacterium taken hostage by the legume. Pakistan J. Biol. Sci., 2001. 4: 757-761.
- Brockwell, J. and Bottomol, R.S. Recent advances in inoculant technology and prospects for the future. Soil Biol. Biochem., 1995. 27: 683-697.
- Chatel, D.L., Shipton, W.P. and C.A. Parker. Establishment and persistence of *Rhizobium trifolii* in Western Australia Soils. Soil Bio. Biochem., 1973. 5: 815-825.
- FAO, FAO Year Book, Vol. 51: 1984.
- Fazil, H.T. Miscellaneous crops. In Crop Production Ed. Bashir E. and Bantel, R. National Book Foundation, Islamabad, Pakistan. 1994. pp: 489-490.
- Freiberg, C.K. Fellay, A. Bairoch, W.J. Broughton, A. Rosenthal and X. Perret. Molecular basis of symbiosis between *Rhizobium* and legumes. Nature, 1997. 387: 394-401.
- Gopalakrishnan, S. and Grish, A.G. Physiological and biochemical characteristics of fast growing rhizobia of *Sesbania bispinosa* in the in vitro condition. Legume Res., 1999. 22(4): 227-232.
- Hardarson, G. Method for enhancing symbiotic nitrogen fixation. Plant and Soil, 1993. 152: 1-17.
- Jefing, Y., Herridge, D.F., Peoples, M.B. and Rerkasem, B. Effects of N fertilization on N₂ fixation and N balance of soybean grown after lowland rice. Plant and Soil, 1992. 147: 235-242.
- Lshizuka, J. Trends in biological nitrogen fixation research and application. Plant Soil, 1992. 141: 197-209.
- Nambiar, P.T.C., Rao, B.S. and Anjaiah, A. Studies on competition, persistence and method of application of a peanut *Rhizobium* strain NC. 92, Peanut Sci., 1984. 11: 83-87.
- Podder, A.K. Performance of single and mixed rhizobial inocula for nodulation and growth of lentil. Lens Newsletters, 1994. 21: 39-40.
- Shah, N.H., Hafeez, F.Y., Hussain, A. and Malik, K.A. Response of lentil to nitrogen and phosphorus fertilizer and inoculation with *Rhizobium leguminosarum* by vicia strains. Aust. J. Expert. Agri., 2000. 40: 93-98.
- Shishidu, M. and Pepper, I.L. Identification of dominant indigenous *Rhizobium* merlotic by plasmid profiles and intrinsic antibiotic resistance. Soil Biol. Biochem., 1990. 22: 11-16.

