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Isolation, Characterization and Selection of *Rhizobium* Strains for Cultivation of *Sesbania sp.*

Md. Zakaria Hasan[1*], Mohammad Shah Alam[2], Musharraf Hossain Mian[3] and Md. Zahurul Islam[4]

CSISA-CIMMYT, Faridpur Hub[1]
Dept. of Agriculture Extension, Ministry of Agriculture[2]
Dept. of Soil Science, Bangladesh Agricultural University, Mymensingh[3]
Dept. of Soil Science, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh[4]

Corresponding author[*]: *z.hasan@cgiar.org*

Abstract

The experiment was conducted to isolate Rhizobium form the root nodules of Deshi dhaincha (Sesbania aculeate) and African dhaincha (Sesbania rostrata), characterization of isolates for authentication, testing their performance as the inoculants and selection of better performed strains for future use as biofertilizer. Nineteen rhizobial strains were isolated from root nodule of dhaincha from 5 Agro Ecological Zones (AEZ 9, AEZ 11, AEZ 12, AEZ 13 and AEZ 28) of Bangladesh. The strains were characterized through morphological, biochemical, microscopic and growth observation. Experiment was conducted in bacteriologically controlled condition using sterile sand in plastic pot in glasshouse. Two varieties of Sesbania named Deshi dhaincha and African dhaincha were used as test crop varieties. Five plants were grown in each plastic pot. After 25 days of sowing plants were harvested and data on nitrogen content of shoot, shoot dry weight, root dry weight, nodule number and nodule dry weight of Sesbania cultivars in glass house were collected. Rhizobial inoculation had significant and positive effect on nitrogen content of shoot, shoot dry weight, root dry weight, nodule number and nodule dry weight for both varieties of Sesbania in sterile condition. The strain DhM3 recorded the highest nitrogen content of shoot, shoot dry weight, root dry weight, nodule number and nodule dry weight. The strain DhSk2 ranked second and DhSk4 ranked third in respect of growth, nodulation, nitrogen fixation and biomass production. Therefore, these three strains can be used for Rhizobium inocula production for cultivation of Sesbania but field level extended studies recommended

Key words: *Dhaincha, AEZ, Rhizobium, Sesbania sp., Nodule and Biofertilizer*

I. Introduction

Soils with adequate fertility status are essential for crop productivity and sustainability. Leguminous plants proved to be useful tool for improving soil fertility through regenerative means. Leguminous plants in association with *Rhizobium* can fix significant amount of atmospheric nitrogen from air which contributes to 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% (33 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 potential in nitrogen fixing bacteria is cultivated in Bangladesh. Its inoculation with superior rhizobial strains is essentially required to increase the yield of legumes through nitrogen fixation (*Athar, 1998*).

Rhizobium-legume symbiosis can increase yield with subsequent decrease in pollution (Freiberg *et al*., 1997). Rhizobial isolates vary in their nitrogen fixation potential and in improving the vegetative and reproductive growth of different crops under varying environmental conditions (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). This research was conducted to achieve two major objectives – firstly, isolation and characterization of rhizobia from Deshi and African dhaincha and secondly, the effect of isolated rhizobia inoculants on growth, nodulation and biomass production of Deshi dhaincha (*Sesbania aculeata*) and African dhaincha (*Sesbania rostrata*) plants.

II. Materials and Methods

Nodules of Sesbania plants were collected from five selected Agro Ecological Zones- AEZ 9: Old Brahmaputra Floodplain (BAU Farm, Mymensingh), AEZ 11: High Ganges River Floodplain (Ishurdi, Pabna), AEZ 12: Low Ganges River Floodplain (Faridpur Sadar), AEZ 13: Ganges Tidal Floodplain (BINA Substation, Satkhira) and AEZ 28; Madhupur Tract (Dhonbari, Tangail). Roots with intact nodules were carried out to BNF (Biological Nitrogen Fixation) Laboratory, Division of Soil Science, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh.

Larger, healthy and mature nodules were selected. After separation nodules were washed in running tap water and transferred to sterile vial. Selected nodules were then immersed into 70% alcohol solution for 30 seconds and in 3% hydrogen peroxide solution for 3 minutes. Hydrogen peroxide treated nodules were then rinsed with sterile mild hot water for several times. Nodules were then cut transversely with sterile blade and the milky suspension directly from the cut nodule was streaked out on congo red yeast extract mannitol agar (CRYEMA) plates using inoculation needle under laminar flow hood. Plates were incubated for 7 days at 28º C temperature.

Colonies were characterized through a systematic series of tests such as cultural tests (i.e. shape, size, opacity, elevation, surface, margin, consistency, emulsifiability, colour odour etc.), microscopic tests (i.e. simple staining, gram staining, motility observation etc.), biochemical tests (i.e. Congo red absorption test, BTB test, Hofer's alkaline broth test etc.) and nodulation tests.

Nodulation capacity of rhizobial isolates were determined by growing plants in plastic buckets containing 2 kg autoclaved sand (at 20 lbs pressure for 2 hours) in glass houses. Seeds of both Deshi dhaincha (*Sesbania aculeata*) and African dhaincha (*Sesbania rostrata*), were rinsed with concentrated Sulphuric Acid (H2SO4) for few seconds and immediately washed thoroughly by distilled water. Prior to sowing, seeds were treated with a thick Rhizobium suspension of each isolate. Ten seeds were planted in each bucket and thinned to keep five plants after germination. Sterilized Jensen's (1942) seedling solution was added to each bucket at one day intervals.

Plants samples were collected from the plastic buckets at 25 days of sowing. From each buckets 3 randomly selected plants were carefully taken out so that no nodule was left in the soil. Roots were washed and the Nodules from the root system of each plant were separately collected and counted. Oven dry weights of nodules were also recorded.

Data obtained from the pot experiment were analyzed statistically by F-test to examine whether treatment effects were significant or insignificant (Gomez and Gomez, 1984). The treatment means were evaluated by Duncan's New Multiple Range Test (DMRT).

III. Results and Discussion

Nineteen rhizobial isolates were obtained from root nodules of Deshi and African dhaincha plants. They were designated as-DhF1, DhF2, DhF3, DhF4, DhM1, DhM2, DhM3, DhM4, DhT1, DhT2, DhT3, DhT4, DhSk1, DhSk2, DhSk3, DhSk4, DhP1, DhP2 and DhP3, respectively. The colony characteristics of isolates did not vary widely. The isolates produced round, whitish, smooth surfaces with entire margin on (CRYEMA) plates.

All the isolates were found short rod in shape, motile in nature and gram negative in reaction. The bacterial isolates did not absorb congo red at young stage but absorbed slightly when cultures became old. All the bacterial isolates produced acid in variable amounts on BTB-YEMA plates. Among the nineteen isolates none had grown on Hofer's alkaline broth.

All the isolates showed good growth at temperature 28^0C and 32^0 C. Most of the isolates grew weakly at 14^0C. At 22^0C most isolates exhibited medium growth while seven isolates (DhT1, DhT2, DhSk2, DhSk3, DhP1, DhP2 and DhP3) recorded weak growth. All the isolates grew at 38^0C. Only nine isolates (DhF1, DhF2, DhT1, DhT2, DhSk1, DhSk2, DhSk3, DhSk4 and DhP1) showed good growth at 38^0C while rest ten showed medium growth. Most of the isolates do not grew at 45^0C.

Table 01. Effect of Rhizobium inoculation on nitrogen content of shoot, shoot dry weight, root dry weight, nodule number and nodule dry weight of *Sesbania sp*

Treatments	Nitrogen content (%) in shoot	Shoot dry weight (mg plant^{-1})	Root dry weight (mg plant^{-1})	Total nodule (no. plant^{-1})	Nodule dry weight (mg plant^{-1})
Variety					
Deshi dhaincha	5.84a	162.4a	45.12a	34.33b	17.85b
African dhaincha	4.76b	127.1b	40.63b	36.42a	21.07a
Significant level	1%	1%	1%	5%	1%
Rhizobial inoculation					
DhF1	4.63hi	117.70i	31.33gh	28.00i	11.16lm
DhF2	4.85gh	121.00i	27.33h	29.67hi	10.91m
DhF3	5.04fg	141.50g	34.67fg	33.17f-i	14.38j
DhF4	5.40def	144.70fg	31.33gh	33.50f-i	13.63j
DhM1	5.71bcd	159.00d	47.33e	44.50b	21.43f
DhM2	5.85bc	158.30de	47.33e	38.33c-f	20.53g
DhM3	6.06b	187.00a	62.33a	52.33a	31.62a
DhM4	4.93gh	157.70de	45.33e	40.67b-e	19.73gh
DhT1	4.94gh	127.30h	31.00gh	29.33hi	11.77l
DhT2	5.10fg	118.30i	29.33h	31.67ghi	12.62k
DhT3	5.10fg	142.00g	38.00f	36.33d-g	18.37i
DhT4	4.87gh	143.50fg	38.33f	35.17efh	19.32h
DhSk1	5.69cd	175.30b	56.67bc	39.17b-f	27.43c
DhSk2	5.82bc	157.00de	52.17cd	44.00bc	22.63e
DhSk3	6.08b	166.70c	60.00ab	37.67d-g	29.00b
DhSk4	5.24efg	153.50e	49.33de	38.67b-f	25.55d
DhP1	6.58a	147.30f	45.00e	35.67efg	26.33d
DhP2	4.37ij	169.30c	55.33bc	42.33bcd	25.60d
DhP3	5.55cde	161.30d	56.67bc	37.33d-g	27.23c
Control	4.27j	45.67j	18.67i	0.00j	0.00n
Significant level	*1%*	*1%*	*1%*	*1%*	*1%*
CV (%)	*5.52*	*2.73*	*8.33*	*12.39*	*3.60*

In a column, figures having similar letter (s) do not differ significantly as per Duncan's Multiple Range Test (DMRT).

Table 02. Interaction effect of Rhizobium inoculation on nitrogen content of shoot, shoot dry weight, root dry weight, nodule number and nodule dry weight of *Sesbania sp*

Treatment		Nitrogen content (%) in shoot	Shoot dry weight (mg plant$^{-1)}$	Root dry weight (mg plant$^{-1)}$	Total nodule (No. plant$^{-1)}$	Nodule dry weight (mg plant^{-1})
Variety × Inoculation						
Deshi dhaincha	DhF1	5.15i-l	141.30jkl	32.67i-l	26.67o	13.07no
	DhF2	5.38h-k	143.30ijk	26.67lm	28.67mno	12.07op
	DhF3	5.66d-j	162.70h	37.33hij	33.00h-o	13.73n
	DhF4	5.54e-k	164.70h	28.00kl	32.33i-o	13.63n
	DhM1	6.10de	172.00fg	49.33c-f	46.33abc	18.27kl
	DhM2	5.99d-g	176.70ef	50.67c-f	36.00e-n	18.07kl
	DhM3	6.66bc	208.00a	70.00a	52.00ab	26.43e
	DhM4	5.54e-k	176.70ef	41.33gh	40.00c-j	22.33h
	DhT1	5.50f-k	143.30ijk	30.00kl	27.33no	11.90op
	DhT2	5.78d-h	128.00no	28.00kl	30.00l-o	13.13no
	DhT3	5.72d-i	162.70h	31.33jkl	36.00e-n	15.93m
	DhT4	6.10de	161.30h	44.00fgh	34.67f-o	19.60ij
	DhSk1	6.72bc	191.30b	63.33b	37.67c-l	23.40gh
	DhSk2	7.50a	176.00ef	53.00c	42.67c-f	20.40i
	DhSk3	6.05def	184.00cd	68.00ab	36.00e-n	24.67f
	DhSk4	5.43g-k	171.70fg	54.67c	35.33e-o	18.53jk
	DhP1	6.94b	163.30h	51.33cde	36.33e-m	24.93f
	DhP2	4.65lm	190.00bc	62.00b	40.67c-i	24.27fg
	DhP3	5.99d-g	181.30de	64.00ab	35.00f-o	22.73h
	Control	4.48mn	48.67s	16.67n	0.00p	00.00r
African dhaincha	DhF1	4.11mno	94.00r	30.00kl	29.33l-o	9.25q
	DhF2	4.31mn	98.67r	28.00kl	30.67k-o	9.75q
	DhF3	4.42mn	120.30p	32.00i-l	33.33g-o	15.03m
	DhF4	5.26h-k	124.70nop	34.67ijk	34.67f-o	13.63n
	DhM1	5.32h-k	146.00ij	45.33d-g	42.67c-f	24.60fg
	DhM2	5.70d-i	140.00jkl	44.00fgh	40.67c-i	23.00h
	DhM3	5.46g-k	166.00gh	54.67c	52.67a	36.80a
	DhM4	4.31mn	138.70jkl	49.33c-f	41.33c-h	17.13l
	DhT1	4.37mn	111.30q	32.00i-l	31.33j-o	11.63p
	DhT2	4.42mn	108.70q	30.67jkl	33.33g-o	12.10op
	DhT3	4.48mn	121.30op	44.67efg	36.67d-m	20.80i
	DhT4	3.64o	125.70nop	32.67i-l	35.67e-n	19.03jk
	DhSk1	4.65lm	159.30h	50.00c-f	40.67c-i	31.47c
	DhSk2	4.14mno	138.00klm	51.33cde	45.33a-d	24.87f
	DhSk3	6.10de	149.30i	52.00cd	39.33c-k	33.33b
	DhSk4	5.04kl	135.30lm	44.00fgh	42.00c-g	32.57bc
	DhP1	6.22cd	131.30mn	38.67ghi	35.00f-o	27.73d
	DhP2	4.09mno	148.70i	48.67c-f	44.00b-e	26.93de
	DhP3	5.10jkl	141.30jkl	49.33c-f	39.67c-j	31.73c
	Control	4.05no	42.67s	20.67mn	0.00p	00.00r
Significant level		*1%*	*1%*	*1%*	*1%*	*1%*
CV (%)		*5.52*	*5.52*	*8.33*	*8.33*	*3.60*

In a column, figures having similar letter (s) do not differ significantly as per DMRT.

Performance of rhizobial strains on growth, nodulation and nitrogen fixation of *Sesbania sp.*

Nitrogen content in shoot

Data on nitrogen content in shoot have been presented in Table 01 and 02. The result showed highly significant effect of rhizobial inoculant, variety and their interaction on nitrogen content in shoot of *Sesbania*.

Effect of inoculation: Results on the effect of inoculation strains on nitrogen content in *Sesbania* shoot were highly significant compared to control. The strain DhP1 contained the highest nitrogen (6.58%) in shoot. Second highest nitrogen in shoot was found in treatments DhSk3 and DhM3. Treatments DhM2 and DhSk2 contained significantly lower nitrogen than DhP1, DhSk3 and DhM3 but higher than the others.

Effect of variety: The effect of varieties on nitrogen content in *Sesbania* shoot was significant in glass house. Deshi dhaincha contained higher nitrogen (5.84%) than African dhaincha (4.76%).

Interaction effect of inoculation × variety: Result revealed that the interaction effect of inoculants × variety on nitrogen content was significant. However highest content was recorded in variety DhSk2 in Deshi Dhaincha (6.98%) followed by DhP1 in Deshi Dhaincha (6.94%).

Shoot dry weight

The result showed highly significant effect of rhizobial inoculation, variety and their interaction on shoot dry weight of *Sesbania* have been presented in Table 01 and 02.

Effect of inoculation: The effect of rhizobial inoculation on shoot dry weight of *Sesbania* was found significant (Table 01). DhM3 the highest shoot dry weight (187mg plant^{-1}) whereas control produced the lowest. DhSk1 produced lower amount (175.30mg plant^{-1}) than DhM3 and significantly higher than other strains. Hoque (1986) reported significantly higher shoot dry weight due to inoculation with different inoculant strains.

Effect of variety: Shoot dry weight of Deshi dhaincha (162.40mg plant^{-1}) was significantly higher than African dhaincha (127.10mg plant^{-1}). Similar result was reported by Anonymous (1998).

Interaction effect of inoculation × variety: Highest shoot dry weight plant^{-1} was recorded in strain DhM3 in Deshi dhaincha (208mg plant^{-1}) followed by DhSk1 (191.30mg plant^{-1}) Deshi dhaincha. Inoculant strains gave significantly higher shoot dry weight over control. The above results were supported with the works of Islam (1999).

Root dry weight

The results showed significant effect of rhizobial inoculation, variety and their interaction on root dry weight of *Sesbania* have been presented in Table 01 and 02.

Effect of inoculation: The effect of inoculation on root dry weight (Table 01) showed that DhM3 produced the highest root dry weight (62.33mg plant^{-1}) whereas control recorded the lowest. The strain DhSk3 produced higher amount of root dry weight (60mg plant^{-1}) than other strains but lower than DhM3. Hoque (1986) reported significantly higher root dry weight due to inoculation with different inoculant strains.

Effect of variety: The effect of varieties on root dry weight of *Sesbania* was significant (Table 01). Root dry weight of Deshi dhaincha was significantly higher than African dhaincha in both glass house and open soil condition. Similar result was reported by Anonymous (1998).

Interaction effect of inoculation × variety: Interaction effect of inoculants × variety on root dry weight was significant (Table 02). However highest root dry weight plant^{-1} was recorded in strain DhM3 in Deshi dhaincha (70mg plant^{-1}) followed by DhSk3 (68mg plant^{-1}) and DhP3 (64mg plant^{-1}).

Nodule number

Table 01 and 02 shows inoculation with *Rhizobium* inoculation increased the total nodule numbers which become statistically significant. *Rhizobium* inoculation × variety interaction effects on nodule number were also significant at both the conditions.

Effect of inoculation: Inoculant DhM3 (62.33) recorded the highest nodule number (Table 01). Next to the highest nodule number was showed by DhM1 (44.50). Treatment DhSK2 produced higher nodule than the other strains but significantly lower nodule than DhM3 and DhM1. Sattar *et al*. (1994) reported that inoculation increased effective nodule and nodule biomass. Vest *et al.* (1973) reported that *Rhizobium* strains differ in their ability to induce nodulation.

Effect of variety: Results indicated that the effects of varieties on nodule number were significant (Table 01). African dhaincha recorded higher number of nodule (36.42 plant^{-1}) than Deshi dhaincha (34.33 plant^{-1}).

Interaction effect of inoculation × variety: Result revealed that the interaction effect of inoculants × variety on nodule number of *Sesbania* was significant. However, highest nodule number plant^{-1} were recorded in DhM3 in both the varieties, African dhaincha (52.67 plant^{-1}) and Deshi dhaincha (52 plant^{-1}) followed by DhM1 in Deshi dhaincha (46.33 plant^{-1}) and DhSk2 in African Dhaincha (45.33 plant^{-1}). No nodule was found in control in sterile condition. Interaction effect of inoculation × variety in nodule number was supported by Hoque (1986).

Nodule dry weight

The results showed highly significant effect of rhizobial inoculation, variety and their interaction on nodule dry weight (Table 01 and 02).

Effect of inoculation: The effect of inoculation on nodule dry weight was presented in Table 01. Data showed that DhM3 recorded the highest nodule dry weight (31.62 mg plant^{-1}) where control produced no nodule. The strain DhSk3 recorded statistically higher amount of nodule (29 mg plant^{-1}) than other strains but lower than DhM3. Sattar et al. (1994) reported that inoculation increased nodule dry weight. Hoque (1986) reported significantly higher nodule dry weight due to inoculation with different inoculant strains.

Effect of variety: Result showed that the effects of variety on nodule dry weight of *Sesbania* were significant (Table 01). Nodule dry weight of African dhaincha (21.07mg plant^{-1}) was significantly higher than Deshi dhaincha (18.85mg plant^{-1}).

Interaction effect of inoculation × variety: Results revealed that the interaction effect of inoculants × variety on nodule dry weight of *Sesbania*were significant (Table 02). The highest nodule dry weight plant^{-1} was recorded in strain DhM3 in African dhaincha (36.80mg plant^{-1}) followed by DhSk3 (33.33mg plant^{-1}) and DhSk4 (32.57mg plant^{-1}) in African dhaincha. No nodule was found in control.

IV. Conclusion

All the strains increased nitrogen content of shoot, shoot dry weight, root dry weight, nodule number and nodule dry weight of Sesbania cultivars in glass of Deshi dhaincha (*Sesbania aculeata*) and African dhaincha (*Sesbania rostrata*) plants. The strain DhM3 produced the highest nitrogen content of shoot, shoot dry weight, root dry weight, nodule number and nodule dry weight. The strain DhSk2 ranked second and DhSk4 ranked third. So, the strains DhM3, DhSk2 and DhSk4 may be selected for producing inoculant for cultivation of Dhaincha in examined AES's. But conclusions and perspectives are valid under the present experimental conditions. Further experiments are needed to be conducted in soils of different AEZ's having wide variations in soil pH, organic matter and nutrient status for confirmation of the findings.

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