

Dual inoculation of salt tolerant *Bradyrhizobium* and *Glomus mosseae* for improvement of *Vigna radiata* L. cultivation in saline areas of West Bengal, India

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ABSTRACT

This study is aimed as to evaluate the interaction between salt tolerant *Bradyrhizobium* sp. and *Glomus mosseae* in the rhizosphere of legume crop *Vigna radiata* L. under pot culture and field conditions in different saline zones of West Bengal, India. *Bradyrhizobium* sp. when inoculated alone showed marked increase in number of nodules, root and shoot length, total plant biomass, arbuscular mycorrhizal fungal (AMF) colonization and population etc. when compared with plants inoculated only with AMF. However, when used in combination, the inoculants showed marked change in the above mentioned parameters over single inoculation of both salt tolerant AM fungi and *Bradyrhizobium*. These results suggest that AMF along with *Bradyrhizobium* can greatly help in establishment of *V. radiata* L. cultivation in the saline soils of West Bengal, India. The increased production of the legume crop could also lead to further benefit of the poor farmers by up lifting their socio-economic conditions with the net profit achieved by cultivating this crop in saline stress condition of West Bengal as a second crop during rabi season.

Keywords: *Vigna radiata* L.; Arbuscular Mycorrhizal Fungi; *Bradyrhizobium*; Salinity; *Glomus mosseae*

1. INTRODUCTION

Salinization and nutrient depletion are serious and growing problems of agricultural land in different parts of the world [1,2]. Soil salinity inhibits plant growth by reducing the ability of the plant water uptake and ion-excess, which affects the cellular metabolism [3,4]. Moreover, it induces nutritional imbalance in plants and

thereby reduces the yield of many crops. This ranges from a slight crop loss to complete crop failure depending on the type of crop and severity of the salinity problem. Though several treatments and management practices are available to reduce salt levels in the soil, there are situations where it is either impossible or too costly to attain desirably low soil salinity levels. Reclamation and management of such saline soils are therefore essential to meet the surplus need of food for ever increasing population of developing countries.

In India, coastal saline soils are spreaded over an area of approximately 3.1 million hectare including eight coastal states [5]. Among these, the state of West Bengal has the highest area (820×10^3 hectare) of coastal saline land encompassing five districts namely North 24-Parganas, South 24-Parganas, Haora, East Medinipur and West Medinipur.

The salinity response of legumes in general varies greatly depending on factors like climatic conditions, soil properties, salt tolerance and the stages of crop growth [6-8]. Successful cultivation of legumes can be achieved by the selection and/or development of a salt-tolerant legume/*Rhizobium* combination although high salinities are known to affect rhizobial activities. The legume-*Rhizobium* symbioses and nodule formation in legumes are more sensitive to salt or osmotic stress. Salinity is reported to affect the infection process by inhibiting root hair growth and decreasing the number of nodule per plant and the amount of N_2 fixed per unit weight of nodules. These cause a decrease in the yield of leguminous crops in saline soils due to the lack of the successful symbiosis.

In addition, mutualistic association of arbuscular mycorrhizal fungi (AMF) also improves plant salinity tolerance by virtue of the recognized role of mycorrhizae in plant growth performance [9]. On the contrary, mycorrhizal infection can be suppressed by high salinity depending on the species or the origin of the fungus [10]. However, a synergistic association of arbuscular my-

corrhizal fungi and rhizobia with leguminous crops has been found to cause an increase in nodulation, nitrogen fixation as well as growth and yield of legumes. Such an effective improvement of plant growth varies with the host genotype. Therefore, the development of host specific, salinity tolerant rhizobia—AMF symbiosis could be an effective approach for the successful cultivation of legume crops in the rabi season as a second crop in the saline tracts.

So far, the response of the pulse crop mung (*Vigna radiata* L.) to dual inoculation of *Rhizobium*—arbuscular mycorrhizal fungus and their cultivation under salt stressed conditions have not yet been evaluated under the agro climatic conditions of West Bengal, India. The present investigation is, therefore, an attempt to inoculate *Vigna radiata* L. with salinity-tolerant rhizobia and AM fungi and to evaluate growth and yield performance of the crop in saline belts of West Bengal which is characterized by mono-crop *aman* rice cultivation during the *kharif* season (June-November) only. Cultivation of *V. radiata* L. in these fallow lands as a second crop during the *rabi* season has also been attempted to improve the socio-economic status of the cultivators of saline zones of West Bengal, India.

2. MATERIALS AND METHODS

2.1. Source of Legume Cultivar and Microbial Culture

Vigna radiata L., cultivar B-1, the salt tolerant legume was selected during the course of screening of pulse crops for tolerance to salt stress. Seeds of this salt tolerant cultivar were obtained from the Oil and Pulse Seed Research Station, Department of Agriculture, Government of West Bengal, Behrampore, Murshidabad, India and used throughout the present study.

Salt tolerant, streptomycin resistant *Bradyrhizobium* CAN-11 was isolated from root nodules of salt tolerant cultivar B-1 of *V. radiata* L. following the method of [11]. The strain was maintained by regular sub culturing on slopes of yeast extract mannitol agar medium. *Glomus mosseae* (BAS-I) tolerating NaCl was isolated from saline tracts of West Bengal, India and multiplied in open pot culture of *Zea mays* L. following the method.

2.2. Inoculum Development

The streptomycin resistant, salt tolerant *Bradyrhizobium* sp. (CAN-11) was grown in yeast extract mannitol agar medium containing 400 µg/ml of streptomycin 48 - 96 h and harvested by centrifugation at 10,000× g for 10 min. Inoculum of salt tolerant mycorrhizal complex *Glomus mosseae* (BAS-I) was prepared in an open-pot

culture of *Zea mays* L. The soil of the pot was inoculated with spores, mycelia of *G. mosseae* (BAS-I) and pieces of infected *Zea mays* roots. After 50 days of growth of *Zea mays*, the soil of the pots was taken out, properly dried in an open air and stored in polypackets for future use.

2.3. Cultivation in Earthen Pots

The earthen ware pots (15 cm dia.) filled with 2 kg saline soils of respective saline zones of West Bengal and autoclaved twice at 121°C for 1 h. Seeds of *V. radiata* L. cultivar B-1 were surface sterilized in ethanol: H₂O₂ (1:1) for 3 min, washed with sterile distilled water and germinated. Five healthy germinated seeds per pot were sown during March/April and the pots were covered with cellophane topped paper cylinder. After two weeks, the tops were removed and the plants were transferred to glass house maintained with a day/night temperature of approximately 28°C/20°C, 75% - 85% relative humidity (RH) and a photoperiod of 12 - 13 h. After 7 days, each pot was inoculated with 1 mL of bacterial suspension (10⁸ cells mL⁻¹) [12] and/or with 10 g of mycorrhizal inoculum (50 propagules g⁻¹ of soil) [13].

Four combinations of inoculation were used: 1) *Bradyrhizobium* (CAN-11), 2) *Glomus mosseae* (BAS-I), 3) *Bradyrhizobium* (CAN-11) + *Glomus mosseae* (BAS-I) and 4) uninoculated pots served as control. Five replications per inoculation treatment were prepared. The plants were watered at a regular interval of 2 days. Pot soils were also supplemented with a basal nutrient solution containing (in mol·m⁻³): CaCl₂, 0.25; KCl, 0.15; K₂HPO₄, 0.06; MgSO₄, 0.25; FeEDTA, 0.12; and (in mol·m⁻³): H₃BO₄, 11.5; MgSO₄, 0.9; ZnSO₄, 0.2; CuSO₄, 0.07; and H₂MoO₄, 0.3. During experiments with AM fungi, P was omitted. Plants were harvested during May/June and the growth parameters were evaluated.

2.4. Cultivation in Field Plots

Field experiments were conducted in random block design (RBD) with three replications. The plot size was 5 m × 4 m with spacing of 30 cm × 10 cm between rows and crop. Seeds were inoculated with salt tolerant *Bradyrhizobium* (CAN-11) and *Glomus mosseae* (BAS-I) inoculants by mixing thoroughly with the slurry of inoculants. Prior to sowing of seeds, field soil was provided with fresh culture of mixed inoculants on the surface layer (2 - 3 cm deep) and mixed thoroughly with ladder ploughing. The fertilizer was added at the rate of N:K:P at 20:60:20 kg/ha, but during AMF experiments P was omitted.

Seeds were sown in March/April by broadcasting at the rate of 15 kg/ha. Three irrigations were given: first at pre-sowing, second at flowering/ pod initiations and the

third one during grain setting stage. Weeding was done regularly as and when required and the crop was harvested during May/June.

2.5. Estimation of Plant Growth

Plants were harvested after 90 days of growth. The growth of *V. radiata* L. was estimated by measuring the length of the plant aerial part and the biomass produced by shoots and roots. Shoots and roots were dried separately at 80°C for 48h and their dry weights were recorded.

Relative growth rate (*RGR*) for total plant (*RGR_t*), shoot (*RGR_s*), and root (*RGR_r*) were calculated on the basis of days of growth and expressed as dry weight of plants using the following formula:

$$RGR_i = (\ln W_{t_i} - \ln W_{t_o}) / t_i - t_o$$

where *i* = variable used (total plant shoot or root dry wt) to measure RGR (day⁻¹); *t_i* = is the total period of growth (day) from germination; *t_o* = is the initial period of growth of 45 days from germination; *W_{t_i}* = is plant dry weight (total shoot or root) at the end of the experimental period (90 day); *W_{t_o}* = is plant dry weight at the beginning of the experimental period (45 day).

2.6. Root Colonization and Spore Count of AM Fungi

Root colonization by AM fungi was studied following the method as described by [14] Colonization of AM fungi was determined by evaluating percentage of root segments containing arbuscules and Vesicles using grid-line intercept method of [15].

AM fungal spores were recovered from rhizospheric soil by wet sieving followed by sucrose gradient centrifugation method of [16]. Spores were counted under ×35 magnification in a dissecting microscope and the density (SD) was expressed as the number of spore's g⁻¹ dry soil.

2.7. Socioeconomic Study

A door to door survey on social stratification, population size and annual income of families in some selected villages of Kakdwip areas of South 24-Parganas were conducted.

2.8. Statistical Analysis

All data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range list [17]. Percentage data were arcsine—transformed before a statistical analysis.

3. RESULTS

In pot culture experiments with saline soils from South and North 24-Parganas and East Medinipur districts of West Bengal growth of inoculated *V. radiata* L. cultivar B-1 was in general higher than the non-inoculated ones. When applied as single inoculum, performance of *Bradyrhizobium* CAN-11 was much better than *G. mosseae* BAS-I. At the same time, a significant improvement of growth was noticed for the dual inoculation of CAN-11 and BAS-I (**Table 1**). All the growth parameters studied *i.e.* plant height, shoot and root length, shoot and root dry/fresh weight and shoot/root ratio showed noticeable improvement due to dual inoculation. In general, growth of the crop was better in South 24-Parganas compared to North 24-Parganas and East Medinipur districts of West Bengal, India.

The relative growth rate (*RGR*) for total plant (*RGR_t*), shoot (*RGR_s*) and root (*RGR_r*) were determined within a period of 90 days and it was found maximum in North 24-Parganas during double inoculation of *Bradyrhizobium* CAN-11 and *G. mosseae* BAS-I (**Figure 1**).

The number of nodules per plant after three months of inoculation depended on the microsymbionts utilized. The no. of nodules per plant varied between 29 (treatment with CAN-11), 25 (treatment with BAS-I) and 34 (treatment with BAS-I + CAN-11) in North 24-Pargana soil. In South 24-pargana soil it was maximum with 33 nodules plant⁻¹ with treatment of BAS-I and CAN-11; E. Medinipur has the lowest *i.e.* 29 nodules plant⁻¹ with treatment of BAS-I and CAN-11 (**Figure 2**) Fresh and dry weights of nodules were in relevance with number of nodules.

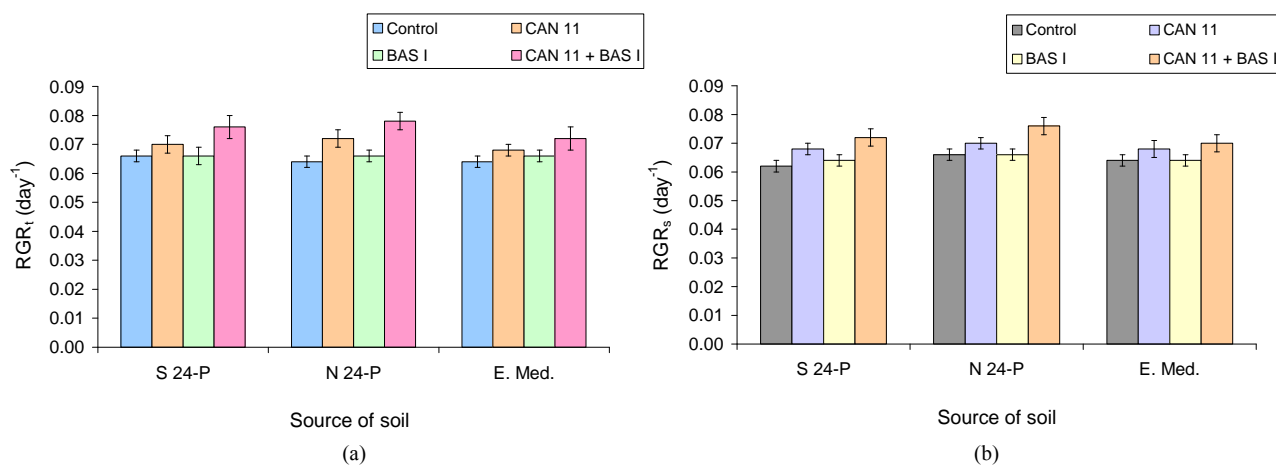
The level of mycorrhizal infection in roots of *V. radiata* L. cultivar B-I appeared to be less sensitive to NaCl and the mycorrhizal infection was maximum in all three districts of West Bengal with double inoculation (BAS-I + CAN-11) when compared to single inoculation by BAS-I or CAN-11 (**Figure 3(a)**). Similarly, the AM spore population in soil of all three districts was maximum during dual inoculation experiments. However, highest spore population was recorded in North 24-Parganas of West Bengal (**Figure 3(b)**).

Field trial of *V. radiata* L. was made under random block design using dual inoculation of *Bradyrhizobium* CAN-11 and *G. mosseae* BAS-I. Cultivations were conducted in three different districts viz. North 24-Parganas (Deuli, Kharampur and Saimalpur), South 24-Parganas (Basanti, Canning and Kakdwip) and East Medinipur (Kanthi and Tamluk) during 2005 and 2006. Growth performance of the legume was in general superior in South 24-Parganas than in North 24-Parganas and E. Medinipur. In South 24-Parganas alone performance was best in Basanti was followed by Canning and Kakdwip

Table 1. Effect of salt tolerant *Bradyrhizobium* CAN-11 and *G. mosseae* BAS-1 on the growth of *Vigna radiata* cv. B-1 in pot culture.

Source of soil	Control (uninoculated soil)	Soil inoculants		
		CAN-11	BAS-I	CAN-11 + BAS-I
South 24-Parganas^A				
Plant height (cm)	55.90 ± 0.35 ^e	59.40 ± 0.34 ^e	58.90 ± 0.32 ^{ab}	62.04 ± 0.30 ^c
Plant biomass, dry (g)	3.20 ± 0.12 ^b	3.46 ± 0.10 ^{bc}	3.40 ± 0.12 ^d	3.54 ± 0.14 ^d
Shoot length (cm)	44.80 ± 0.24 ^d	46.80 ± 0.12 ^c	46.20 ± 0.36 ^e	49.80 ± 0.42 ^f
Shoot weight, dry (g)	2.44 ± 0.10 ^a	2.88 ± 0.10 ^d	2.81 ± 0.12 ^c	2.98 ± 0.16 ^b
Root length (cm)	11.10 ± 0.12 ^d	12.60 ± 0.20 ^e	12.42 ± 0.14 ^d	13.10 ± 0.20 ^d
Root weight, dry (g)	0.76 ± 0.06 ^f	0.86 ± 0.04 ^b	0.82 ± 0.04 ^a	0.94 ± 0.08 ^c
Shoot/Root ratio (length)	4.03 ± 0.14 ^c	3.71 ± 0.08 ^d	3.72 ± 0.12 ^b	3.80 ± 0.12 ^b
Yield of grains (g)	1.84 ± 0.14 ^e	2.89 ± 0.30 ^d	2.71 ± 0.22 ^a	3.70 ± 0.24 ^c
North 24-Parganas^B				
Plant height (cm)	58.00 ± 0.34 ^d	60.60 ± 0.36 ^a	59.04 ± 0.28 ^a	63.26 ± 0.40 ^b
Plant biomass, dry (g)	3.58 ± 0.08 ^c	3.77 ± 0.12 ^d	3.64 ± 0.12 ^d	4.14 ± 0.06 ^c
Shoot length (cm)	46.60 ± 0.42 ^{bc}	47.40 ± 0.40 ^b	47.20 ± 0.48 ^c	49.40 ± 0.42 ^c
Shoot weight, dry (g)	2.64 ± 0.08 ^d	2.67 ± 0.14 ^d	2.66 ± 0.12 ^e	2.86 ± 0.12 ^a
Root length (cm)	11.40 ± 0.12 ^{ef}	13.20 ± 0.24 ^{ef}	12.84 ± 0.18 ^d	13.86 ± 0.30 ^b
Root weight, dry (g)	0.94 ± 0.04 ^d	1.10 ± 0.04 ^c	0.98 ± 0.02 ^b	1.28 ± 0.08 ^{cd}
Shoot/Root ratio (length)	4.88 ± 0.14 ^c	3.59 ± 0.10 ^d	3.67 ± 0.06 ^c	3.56 ± 0.12 ^d
Yield of grains (g)	2.24 ± 0.06 ^d	3.89 ± 0.08 ^a	3.09 ± 0.06 ^d	4.50 ± 0.10 ^a
East Medinipur^C				
Plant height (cm)	68.30 ± 0.48 ^e	71.60 ± 0.52 ^a	70.50 ± 0.48 ^a	75.00 ± 0.54 ^c
Plant biomass, dry (g)	2.10 ± 0.12 ^b	2.54 ± 0.12 ^d	2.28 ± 0.14 ^c	2.86 ± 0.16 ^e
Shoot length (cm)	53.60 ± 0.34 ^d	55.10 ± 0.32 ^f	54.40 ± 0.36 ^e	57.20 ± 0.32 ^b
Shoot weight, dry (g)	1.78 ± 0.18 ^b	2.10 ± 0.16 ^a	1.82 ± 0.14 ^d	2.34 ± 0.16 ^a
Root length (cm)	14.70 ± 0.22 ^d	16.50 ± 0.28 ^b	16.10 ± 0.24 ^b	17.80 ± 0.28 ^d
Root weight, dry (g)	0.42 ± 0.06 ^f	0.49 ± 0.08 ^c	0.46 ± 0.06 ^c	0.52 ± 0.08 ^c
Shoot/Root ratio (length)	3.64 ± 0.12 ^c	3.64 ± 0.14 ^d	3.37 ± 0.10 ^a	3.21 ± 0.14 ^d
Yield of grains (g)	3.16 ± 0.32 ^d	4.44 ± 0.32 ^d	3.61 ± 0.36 ^c	5.21 ± 0.34 ^b

Soils of experimental sites were thoroughly mixed in equal proportion and used in pots. Soil salinity: ^A = 7.5 (dSm⁻¹), ^B = 6.5 (dSm⁻¹) and ^C = 7.2 (dSm⁻¹). ^{a,b,c,d,e,f}Mean data in each vertical row followed by different letters are significantly different ($P \leq 0.05$) as per Duncan's Multiple Range Test ($n = 5$). Values indicate mean of five replicates \pm S.E.



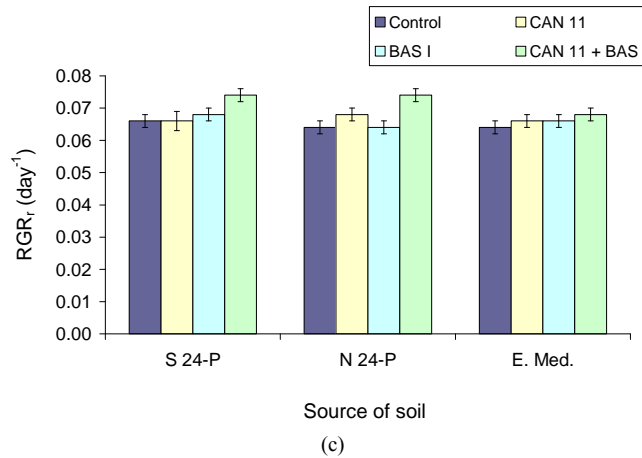


Figure 1. Relative growth rate of total plant RGR_t (a), total shoot RGR_s (b) and total root RGR_r (c) of *V. radiata* cv. B-1 inoculated with *Bradyrhizobium* CAN-11 and or *G. mosseae* BAS- I in pot culture. S 24-P = South 24-Parganas, N 24-P = North 24-Parganas and E. Med = East Medinipur.

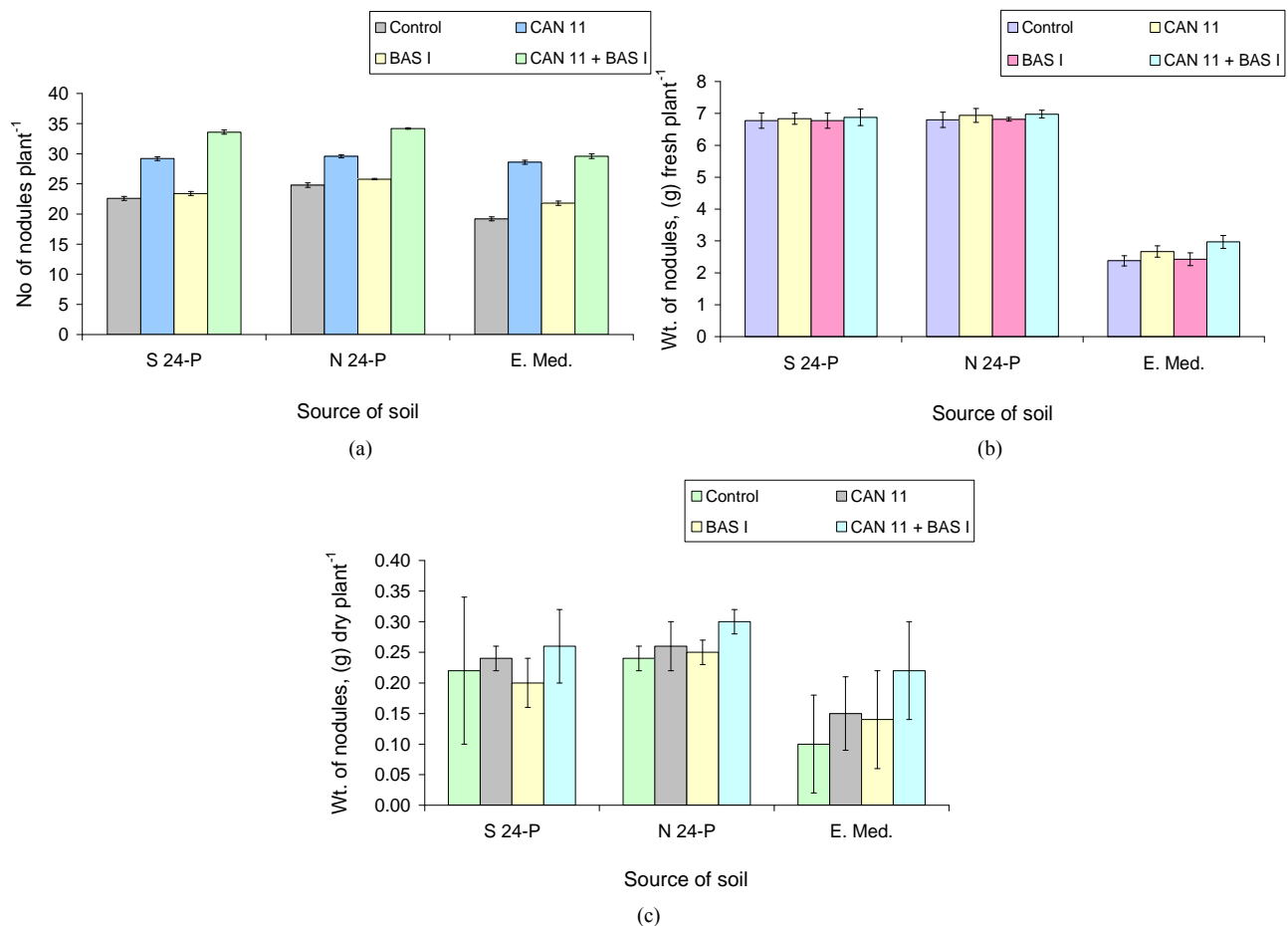


Figure 2. Effect of salt tolerant *Bradyrhizobium* CAN-11 and *G. mosseae* BAS-I inoculation on number of nodules plant⁻¹ (a), fresh wt. of nodules plant⁻¹ (b) and dry wt of nodules plant⁻¹ (c) of *V. radiata* cv. B-1 in pot culture. S 24-P = South 24-Parganas, N 24-P = North 24-Parganas and E. Med = East Medinipur.

(Table 2). In Table 3 we find that growth was maximum in Kharampur followed by Deuli and Saimalpur in North

24-Parganas. Similarly in Table 4 Tamluk was better than Kanthi of East Medinipur districts.

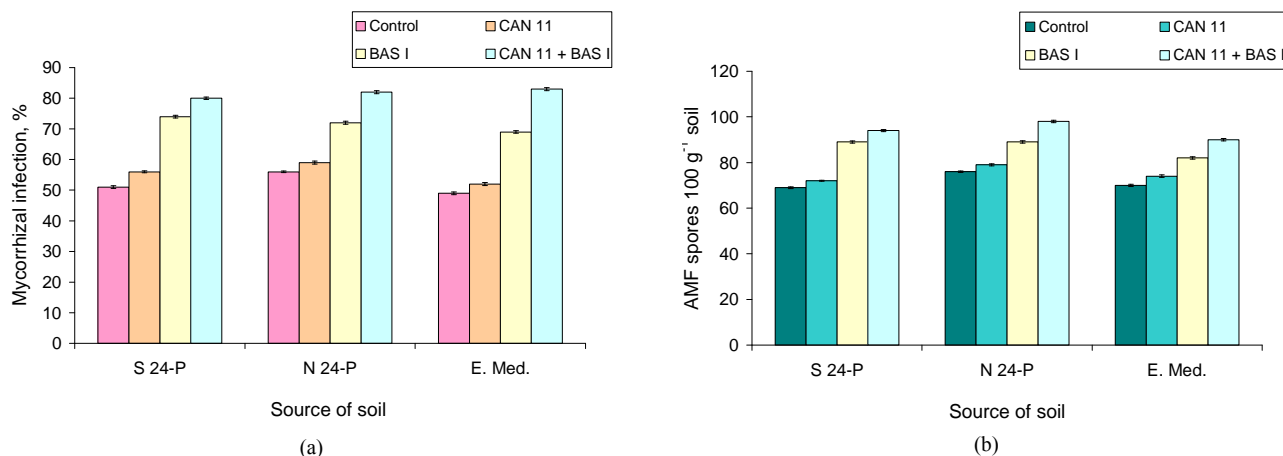


Figure 3. Incidence of mycorrhizal infection and population of AMF spores during growth of *V. radiata* cv. B-1 inoculated with *Bradyrhizobium* CAN-11 and or *G. mosseae* BAS-I in pot culture of S 24-P = South 24-Parganas, N 24-P = North 24-Parganas and E. Med = East Medinipur.

Table 2. Effect of inoculation of salt tolerant *Bradyrhizobium* CAN -11 and salt tolerant *G. mosseae* BAS-I on the growth of *V. radiata* cv. B-1 in different experimental fields of South 24-Parganas during 2005 and 2006.

Growth characteristics/plant ⁻¹	Experimental fields of South 24-Parganas					
	Basanti		Canning		Kakdwip	
	2005	2006	2005	2006	2005	2006
Plant height (cm)	69.00 ± 4.50 ^a	70.50 ± 4.52 ^c	68.60 ± 4.98 ^a	69.40 ± 4.46 ^a	64.00 ± 4.42 ^d	64.80 ± 4.44 ^a
Total plant biomass (g), fresh	20.80 ± 2.36 ^c	21.20 ± 2.34 ^b	19.80 ± 2.32 ^b	20.90 ± 2.36 ^c	20.10 ± 2.32 ^c	20.40 ± 2.28 ^b
Total plant biomass (g), dry	4.30 ± 0.24 ^d	4.20 ± 0.26 ^e	4.16 ± 0.18 ^c	4.32 ± 0.16 ^d	4.14 ± 0.20 ^f	4.18 ± 0.22 ^c
Shoot length (cm)	56.20 ± 3.48 ^e	56.80 ± 3.46 ^d	55.40 ± 3.50 ^d	56.30 ± 3.54 ^f	52.10 ± 3.44 ^b	52.40 ± 3.46 ^d
Shoot weight (g), fresh	16.12 ± 2.20 ^b	16.30 ± 2.22 ^a	15.90 ± 2.18 ^f	16.10 ± 2.16 ^b	15.60 ± 2.20 ^e	15.62 ± 2.28 ^e
Shoot weight (g), dry	3.28 ± 0.12 ^a	3.31 ± 0.10 ^b	3.10 ± 0.14 ^a	3.12 ± 0.12 ^c	3.06 ± 0.14 ^d	3.07 ± 0.16 ^b
Root length (cm)	12.80 ± 1.26 ^e	13.70 ± 1.28 ^e	13.20 ± 1.24 ^b	13.10 ± 1.24 ^d	11.90 ± 1.22 ^e	12.40 ± 1.20 ^d
Root weight (g), fresh	4.68 ± 0.10 ^d	4.90 ± 0.18 ^f	3.90 ± 0.16 ^d	4.80 ± 0.18 ^e	4.50 ± 0.20 ^b	4.78 ± 0.22 ^a
Root weight (g), dry	1.02 ± 0.08 ^a	0.89 ± 0.06 ^b	1.06 ± 0.08 ^e	1.20 ± 0.10 ^a	1.08 ± 0.08 ^{cd}	1.11 ± 0.14 ^c
Shoot/Root ratio (length)	4.39 ± 2.76 ^b	4.14 ± 2.70 ^c	4.19 ± 2.82 ^c	4.29 ± 2.85 ^c	4.37 ± 2.81 ^d	4.22 ± 2.88 ^b
No. of branches	4.92 ± 0.80 ^c	4.96 ± 1.22 ^d	4.84 ± 1.24 ^d	4.90 ± 1.24 ^b	4.70 ± 1.18 ^{de}	4.74 ± 1.22 ^d
No. of pods	17.60 ± 2.32 ^d	18.60 ± 2.32 ^e	16.80 ± 2.34 ^b	17.40 ± 2.36 ^d	16.60 ± 2.38 ^{ef}	17.00 ± 2.32 ^c
No. of seeds pod ⁻¹	8.40 ± 1.24 ^e	8.50 ± 1.26 ^a	6.80 ± 1.20 ^a	7.20 ± 1.18 ^e	6.60 ± 1.20 ^a	7.40 ± 1.18 ^b
Weight of seeds (100 nos.)	3.18 ± 0.12 ^a	3.20 ± 0.12 ^b	3.10 ± 0.14 ^{cd}	3.12 ± 0.16 ^a	3.12 ± 0.12 ^{bc}	3.12 ± 0.14 ^c
No. of nodule	34.20 ± 4.42 ^{bc}	35.10 ± 3.42 ^c	32.20 ± 3.44 ^d	33.40 ± 3.46 ^b	31.80 ± 3.38 ^d	32.60 ± 3.34 ^d
Weight of nodules (g), fresh	7.10 ± 1.24 ^c	7.12 ± 1.22 ^d	6.98 ± 1.32 ^{ef}	6.98 ± 1.34 ^e	6.60 ± 1.20 ^b	6.70 ± 1.28 ^e
Weight of nodules (g), dry	0.34 ± 0.06 ^{cd}	0.34 ± 0.06 ^f	0.30 ± 0.08 ^f	0.30 ± 0.08 ^d	0.28 ± 0.08 ^c	0.29 ± 0.06 ^a
Population of AMF (spores 100 g ⁻¹ soil)	106 ± 4.2 ^c	108 ± 2.12 ^a	98 ± 4.46 ^d	98 ± 1.22 ^c	96 ± 4.18 ^a	98 ± 4.14 ^b
Colonization of roots (%)	82 ± 3.8 ^b	82 ± 3.62 ^c	68 ± 2.86 ^{cd}	70 ± 4.54 ^f	66 ± 3.1 ^d	70 ± 4.26 ^c
Days of maturity	74 ± 5 ^d	73 ± 4 ^b	76 ± 4 ^d	73 ± 5 ^a	78 ± 4 ^b	77 ± 5 ^d
Yield of grain (kg·ha ⁻¹)	560 ± 21 ^c	568 ± 20 ^d	570 ± 25 ^a	575 ± 18 ^b	540 ± 14 ^c	548 ± 18 ^c
RGR _t (day ⁻¹)	0.076 ± 0.002 ^d	0.078 ± 0.002 ^e	0.066 ± 0.002 ^c	0.068 ± 0.002 ^c	0.066 ± 0.002 ^a	0.067 ± 0.003 ^c
RGR _s (day ⁻¹)	0.074 ± 0.002 ^b	0.076 ± 0.002 ^c	0.066 ± 0.002 ^c	0.068 ± 0.002 ^c	0.066 ± 0.002 ^d	0.068 ± 0.002 ^b
RGR _r (day ⁻¹)	0.074 ± 0.003 ^a	0.075 ± 0.002 ^d	0.066 ± 0.002 ^d	0.068 ± 0.002 ^d	0.064 ± 0.002 ^c	0.068 ± 0.002 ^a

Values indicate mean of five replicates ± SE. ^{a,b,c,d,e,f} Mean data in each vertical row followed by different letters are significantly different ($P \leq 0.05$) as per Duncan's Multiple Range Test ($n = 5$). RGR_t, RGR_s and RGR_r are Relative growth rate of total plant, shoot and root respectively determined within a period of growth of 45 days.

Table 3. Effect of inoculation of salt tolerant *Bradyrhizobium* CAN-11 and *G. mosseae* BAS-I on the growth of *V. radiata* cv. B-1 in different experimental fields of North 24-Parganas during 2005 and 2006.

Growth characteristics/plant ⁻¹	Experimental fields of North 24-Parganas					
	Deuli		Kharampur		Saimalpur	
	2005	2006	2005	2006	2005	2006
Plant height (cm)	66.80 ± 4.62 ^a	67.20 ± 4.58 ^c	68.10 ± 4.48 ^c	68.70 ± 4.52 ^c	64.40 ± 4.60 ^a	66.10 ± 4.64 ^a
Total plant biomass (g), fresh	23.20 ± 2.28 ^b	23.60 ± 2.26 ^d	24.50 ± 2.30 ^d	24.90 ± 2.32 ^d	22.60 ± 0.30 ^b	23.00 ± 2.34 ^b
Total plant biomass (g), dry	4.42 ± 0.18 ^c	4.43 ± 0.16 ^a	4.50 ± 0.26 ^f	4.52 ± 0.22 ^a	3.92 ± 0.18 ^d	4.24 ± 0.24 ^d
Shoot length (cm)	52.80 ± 3.60 ^d	53.10 ± 3.58 ^b	53.70 ± 3.62 ^e	54.10 ± 3.56 ^b	51.10 ± 3.60 ^c	52.40 ± 3.62 ^e
Shoot weight (g), fresh	16.70 ± 2.28 ^c	16.90 ± 2.36 ^c	17.00 ± 2.34 ^a	17.50 ± 2.28 ^c	15.60 ± 2.30 ^e	15.80 ± 2.32 ^a
Shoot weight (g), dry	3.10 ± 0.16 ^{bc}	3.12 ± 0.18 ^d	3.14 ± 0.18 ^b	3.28 ± 0.16 ^d	2.90 ± 0.18 ^a	2.92 ± 0.20 ^b
Root length (cm)	14.00 ± 1.38 ^c	14.10 ± 1.32 ^f	14.40 ± 2.34 ^c	14.60 ± 1.36 ^b	13.30 ± 1.32 ^b	13.70 ± 1.38 ^c
Root weight (g), fresh	6.80 ± 0.68 ^d	6.70 ± 0.64 ^e	7.50 ± 0.60 ^d	7.40 ± 0.64 ^a	7.00 ± 0.66 ^d	7.20 ± 0.68 ^d
Root weight (g), dry	1.32 ± 0.28 ^{ef}	1.31 ± 0.30 ^d	1.36 ± 0.32 ^e	1.24 ± 0.34 ^e	1.02 ± 0.18 ^b	1.32 ± 0.18 ^e
Shoot/Root ratio (length)	3.77 ± 2.60 ^{bf}	3.76 ± 2.71 ^b	3.72 ± 1.54 ^a	3.70 ± 2.61 ^b	3.84 ± 2.72 ^c	3.82 ± 2.62 ^b
No. of branches	5.40 ± 1.18 ^a	5.60 ± 0.96 ^c	5.70 ± 0.88 ^b	5.80 ± 1.40 ^c	4.90 ± 1.34 ^b	5.10 ± 1.42 ^c
No. of pods	16.00 ± 2.10 ^c	18.50 ± 2.24 ^a	19.00 ± 2.78 ^c	19.80 ± 3.10 ^d	15.60 ± 2.36 ^d	15.90 ± 2.40 ^d
No. of seeds pod ⁻¹	8.10 ± 1.42 ^d	8.30 ± 1.46 ^e	8.50 ± 1.46 ^d	8.70 ± 1.38 ^{ac}	6.80 ± 1.32 ^a	6.90 ± 1.40 ^a
Weight of seeds (100 nos.)	4.10 ± 0.24 ^e	4.20 ± 0.26 ^d	4.40 ± 0.28 ^e	4.70 ± 0.26 ^e	3.70 ± 0.20 ^b	3.90 ± 0.32 ^b
No. of nodule	37.20 ± 3.62 ^b	38.40 ± 4.12 ^c	39.80 ± 3.24 ^a	40.40 ± 4.50 ^{bc}	35.40 ± 3.62 ^c	35.90 ± 3.72 ^d
Weight of nodules (g), fresh	7.10 ± 1.50 ^d	7.12 ± 1.62 ^b	7.22 ± 1.58 ^b	7.24 ± 1.38 ^c	6.84 ± 1.48 ^d	6.87 ± 1.54 ^e
Weight of nodules (g), dry	0.36 ± 0.02 ^a	0.38 ± 0.04 ^{ac}	0.39 ± 0.03 ^c	0.40 ± 0.04 ^{cd}	0.30 ± 0.02 ^c	0.32 ± 0.04 ^f
Population of AMF (spores 100 g ⁻¹ soil)	92 ± 5.4 ^c	94 ± 5.2 ^c	95 ± 4.8 ^d	95 ± 4.2 ^{ef}	86 ± 5.4 ^b	88 ± 5.2 ^c
Colonization of roots (%)	68 ± 4.4 ^b	70 ± 4.5 ^d	75 ± 4.4 ^c	77 ± 4.6 ^f	66 ± 3.8 ^c	68 ± 4 ^d
Days of maturity	69 ± 4.4 ^c	72 ± 4.5 ^e	73 ± 4.8 ^a	75 ± 4.2 ^a	68 ± 3.4 ^d	68 ± 4 ^a
Yield of grain (kg·ha ⁻¹)	580 ± 18 ^d	588 ± 20 ^f	594 ± 14 ^b	598 ± 20 ^b	612 ± 22 ^e	622 ± 24 ^b
RGR _t (day ⁻¹)	0.076 ± 0.002 ^c	0.078 ± 0.002 ^c	0.072 ± 0.002 ^c	0.074 ± 0.002 ^c	0.066 ± 0.002 ^d	0.068 ± 0.002 ^c
RGR _s (day ⁻¹)	0.076 ± 0.002 ^b	0.078 ± 0.002 ^d	0.072 ± 0.003 ^c	0.074 ± 0.002 ^c	0.064 ± 0.002 ^a	0.068 ± 0.002 ^d
RGR _r (day ⁻¹)	0.074 ± 0.002 ^a	0.076 ± 0.002 ^a	0.072 ± 0.002 ^d	0.074 ± 0.002 ^d	0.066 ± 0.002 ^c	0.068 ± 0.002 ^a

Values indicate mean of five replicates ± SE. ^{a,b,c,d,e,f} Mean data in each vertical row followed by different letters are significantly different ($P \leq 0.05$) as per Duncan's Multiple Range Test ($n = 5$). RGR_t, RGR_s and RGR_r are Relative growth rate of total plant, shoot and root respectively determined within a period of growth of 45 days.

Parallel to this grain yield was maximum in Basanti followed by Canning and Kakdwip of South 24-Parganas (Table 2). Further it also revealed that maximum amount of grain (Table 3) was recovered in Saimalpur on North 24-Parganas whereas Kanthi had more yield (Table 4) than in Tamluk of East Medinipur districts.

Nodulation characteristics of *V. radiata* L. including the no. of nodules dry and fresh weight per plant after dual inoculation increases in all three districts i.e. South and North 24-Parganas and East Medinipur. Moreover, such improved features were better expressed in 2006 than in 2005 (Tables 2-4).

Table 4. Effect of inoculation of salt tolerant *Bradyrhizobium* CAN-11 and *G. mosseae* BAS-I on the growth of *V. radiata* cv. B-1 in different experimental fields of East Medinipur during 2005 and 2006.

Growth characteristics/plant ⁻¹	Experimental fields of East Medinipur			
	Kanthi		Tamluk	
	2005	2006	2005	2006
Plant height (cm)	64.2 ± 5.2 ^a	65.6 ± 5.18 ^b	66.4 ± 5.30 ^c	67.4 ± 5.42 ^a
Total plant biomass (g), fresh	19.8 ± 2.16 ^c	20.2 ± 2.42 ^c	20.8 ± 2.14 ^d	21.4 ± 2.32 ^d
Total plant biomass (g) Dry	3.60 ± 0.62 ^d	3.61 ± 0.50 ^d	3.94 ± 0.56 ^a	3.92 ± 0.58 ^b
Shoot length (cm)	49.8 ± 3.60 ^a	51.0 ± 4.24 ^c	52.1 ± 3.84 ^b	52.8 ± 4.10 ^c
Shoot weight (g), fresh	16.6 ± 1.82 ^b	16.9 ± 1.28 ^a	17.1 ± 1.64 ^c	17.6 ± 1.84 ^d
Shoot weight (g), dry	2.99 ± 0.42 ^c	3.1 ± 0.48 ^b	3.3 ± 0.52 ^d	3.2 ± 0.59 ^e
Root length (cm)	14.4 ± 1.64 ^d	14.6 ± 1.62 ^d	14.7 ± 1.84 ^e	14.9 ± 1.82 ^f
Root weight (g), fresh	3.20 ± 0.48 ^a	3.3 ± 0.54 ^c	3.7 ± 0.56 ^c	3.8 ± 0.48 ^b
Root weight (g), dry	0.61 ± 0.06 ^b	0.63 ± 0.04 ^a	0.64 ± 0.04 ^d	0.72 ± 0.06 ^c
Shoot/Root ratio (length)	3.45 ± 2.19 ^c	3.49 ± 2.61 ^b	3.64 ± 2.08 ^a	3.61 ± 2.25 ^d
No. of branches	4.84 ± 0.68 ^d	4.86 ± 0.64 ^d	4.92 ± 0.48 ^b	4.98 ± 0.42 ^c
No. of pods	21.2 ± 2.18 ^c	22.4 ± 2.42 ^c	22.8 ± 2.42 ^c	23.1 ± 2.46 ^f
No. of seeds pod ⁻¹	7.00 ± 0.86 ^a	7.1 ± 0.82 ^d	6.8 ± 0.90 ^d	6.9 ± 0.86 ^d
Weight of seeds (100 nos.)	4.22 ± 0.58 ^b	4.23 ± 0.46 ^b	4.24 ± 0.38 ^a	4.24 ± 0.44 ^c
No. of nodule	34.4 ± 3.20 ^c	35.2 ± 3.84 ^c	33.8 ± 3.68 ^b	34.1 ± 3.54 ^c
Weight of nodules (g), fresh	6.88 ± 0.86 ^d	6.91 ± 0.82 ^d	6.94 ± 0.92 ^c	6.98 ± 0.94 ^d
Weight of nodules (g), dry	0.28 ± 0.02 ^c	0.29 ± 0.04 ^{de}	0.30 ± 0.06 ^d	0.31 ± 0.06 ^a
Population of AMF (spores 100g ⁻¹ soil)	88 ± 6.12 ^b	92 ± 6.28 ^{ef}	90 ± 6.40 ^c	94 ± 6.48 ^b
Colonization of roots (%)	72 ± 4.80 ^a	76 ± 5.26 ^b	74 ± 5.24 ^d	78 ± 5.84 ^c
Days of maturity	78 ± 4 ^d	79 ± 3.9 ^c	69 ± 5 ^c	70 ± 5.0 ^d
Yield of grain (kg·ha ⁻¹)	590 ± 20 ^d	598 ± 12 ^d	586 ± 22 ^d	594 ± 24 ^a
<i>RGR_t</i> (day ⁻¹)	0.076 ± 0.002 ^c	0.078 ± 0.002 ^c	0.076 ± 0.002 ^a	0.078 ± 0.004 ^c
<i>RGR_s</i> (day ⁻¹)	0.076 ± 0.002 ^c	0.076 ± 0.002 ^c	0.074 ± 0.004 ^b	0.078 ± 0.002 ^f
<i>RGR_r</i> (day ⁻¹)	0.074 ± 0.002 ^b	0.076 ± 0.002 ^a	0.076 ± 0.003 ^c	0.078 ± 0.003 ^c

Values indicate mean of five replicates ± SE. ^{a,b,c,d,e,f}Mean data in each vertical row followed by different letters are significantly different ($P \leq 0.05$) as per Duncan's Multiple Range Test ($n = 5$). *RGR_t*, *RGR_s* and *RGR_r* are Relative growth rate of total plant, shoot and root respectively determined within a period of growth of 45 days.

Table 5. Distribution of household according to annual income from present monocrop farming systems and introduced legume cultivation as second crop.

Village	Monocrop farming systems			Legume cultivation as second crop		
	No. of household	Annual income	% per household	No. of household	Annual income	% per household
Gabbani	168	≤15,000	80.00	-	≥15,000	Nil
	31	15,000 to 50,000	14.76	190	15,000 to 50,000	90.48
	11	50,000 to 100,000	5.23	20	50,000 to 100,000	9.52
Barbari	131	≤15,000	77.05	-	≥15,000	Nil
	33	15,000 to 50,000	19.41	160	15,000 to 50,000	94.12
	6	50,000 to 100,000	3.52	10	50,000 to 100,000	5.82

Villages: Gabbani and Barbari are from Canning district of South 24-Parganas, West Bengal.

Table 6. The cost benefit ratio of cultivation of mung (*V. radiata* cv.B-1) in the farmers field.

Particulars	<i>V. radiata</i> cv.B-1	
	Non-inoculated (Rs.)	Inoculated (Rs.)
Land preparation	2100	2100
Sowing of seeds	700	700
Seed treatment	750	625
Cost of AM + Rhizobia inoculum	Nil	250
Intercultural practices	1400	1400
Fertilizer (Basal dose N:P:K)	1400	1400
Plant protection chemicals	980	980
Harvesting	700	700
Threshing & Cleaning	980	980
Total cost	9010	9135
Yield/hectare	750 Kg	825 Kg
Total sell @ 25/Kg (grain)	18750	20625
Net profit	9740	11490

The level of mycorrhizal infection and density of AM spores in soil were better represented in the 2nd year irrespective of the localities. However, it was maximum in East Medinipur district when compared to South and North 24-Parganas of West Bengal, India.

During the course of socio-economic study, a door to door survey was conducted over 210 and 170 families of Gabbani and Barbari villages of South 24-Parganas with a total population of 1695 and 1500 respectively. As the area is mono crop rice area, 77% - 80% of household showed an average earning of Rs. 15,000 per annum, while only 3% - 5% could have earned Rs. 50,000 - 100,000 per annum (Table 5).

In an attempt to introduce mung cultivation the cost benefit ratio was calculated considering the inoculation of AM fungus and rhizobial strains. Total cost of the inoculated crop was more than the non-inoculated one, but as the yield of inoculated crop was much more, it showed a net profit of >10% over that of the uninoculated one (Table 6).

When this cropping system of pulse cultivation was introduced with salt tolerant inoculants, (rhizobium CAN-11 and AMF BAS-1) the socio-economic condition of the household farmers changed drastically. In both Gabbani and Barbari villages 90% - 94% household earned up to Rs. 50,000 per annum while 5.8% - 9.5% household could have earned up to Rs. 100,000 per annum (Table 5).

4. DISCUSSION

Salt salinity is known to affect drastically the growth and yield of various crop plants including the legumes [4]. However, improvements of legume crops by inoculation of rhizobia or AMF alone or in combination have been reported by several authors [18-23] under salt stress

conditions. In this study, the best salt tolerant streptomycin resistant strain of *Bradyrhizobium* CAN-11 (specific for *V. radiata* L.) and *Glomus mosseae* BAS-1 were utilized for cultivation of *V. radiata* in pot culture and under field conditions.

The experimental data (Table 1 and Figures 1-3) revealed that combined inoculation of *Bradyrhizobium* CAN-11 and *G. mosseae* BAS-I increased the yield of *V. radiata* cv. B-I followed by single inoculation of *Bradyrhizobium* CAN-11 and *G. mosseae* BAS-I than the control uninoculated set. All the growth characteristics of *V. radiata* cv B-I were also improved simultaneously. Moreover, in *V. radiata* cv B-I the relative growth rate of total plant, shoot and root was also best in combined inoculation than the single inoculation. [24] reported that *Rhizobium* and *Glomus* sp. significantly increased the shoot and root fresh and dry weight, number of nodules in faba bean under saline condition. [25] reported that alleviation of salt stress in *Lactuca sativa* could be achieved by inoculation with *Glomus* sp. Similar beneficial effects of dual inoculation of *Rhizobium/Bradyrhizobium* and AMF were reported on the growth characteristics and yield of *Medicago sativa* [26], tomato [27], soybean [28], maize [29], cotton [22] and *Lotus glaber* [20].

Field experiments conducted in the saline soils of Basanti, Canning and Kakdwip of South 24-Parganas; Deuli, Kharampur and saimalpur of North 24-Parganas and Kanthi and Tamluk of East Medinipur districts using single and dual inoculation of *Bradyrhizobium* CAN-11 and *G. mosseae* BAS-I to *V. radiata* cv. B-I (Tables 2-4). It was revealed that the crop grew well and produced yields appreciably. The growth parameters like height of the plants shoot and root-fresh and dry weights, number of seeds per pods were increased under normal condi-

tions. Similar observations have been reported in soybean [30], *Sesbania aegyptiaca* and *S. grandiflora* [21], *Vigna radiata* [31] and on *Pisum sativum* [32].

The beneficial effects of dual inoculation on the growth of *V. radiata* L. might be due to reduction in Na uptake and increased uptake of P, N and Mg and high content of chlorophyll in the inoculated plants which have played important roles in salinity alleviating mechanism of plants [21].

Further it was revealed that when the pulse cultivation was introduced in villages, namely Gabbani and Barbari of South 24-Parganas as a second crop utilizing salt tolerant *Bradyrhizobium* CAN-11 and *G. mosseae* BAS-I as the dual inoculation practices, the socio-economic conditions of the household farmers changed dramatically (Table 5). Similar beneficial report was reported by [33] have corroborated our findings with *V. radiata* and could find application in saline areas of West Bengal, India.

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