Effect of rhizobium inoculants on nodulation growth and yield of black gram varieties

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Abstract: An experiment was conducted at the Field laboratory of the Department of Soil Science, Bangladesh Agricultural University, Mymensinh during October 2012 to January 2013 to observe the response of two black gram varieties viz BARI Mash-2 and BARI Mash-3 to inoculation of four inoculants viz. BINA SP 10, BINA 441,BINA 301 and their mixture. The experiment was laid out split plot design with three replications having 30 plots. The size of each unit plot was 4m x 3m. All the growth characters including total nodule weight, shoot and root weights varied significantly due to the varieties and Rhizobium inoculants. Varietal effect on nodulation and weight of nodule were significant at 45 days of crop growth. The variety BARI Mash-3 gave the highest number of nodule and nodule weight and the BARI Mash-2 recorded the lowest. The effect of all inoculants was statistically similar but superior to uninoculated control. Two varieties were not found statistically different in terms of producing both seed and stover yields. There was significant effect of inoculants on both seed and stover yields. There was no interaction effect of the varieties and inoculants on seed yield but interaction was significant in case of stover yields. The highest seed yield of 741kg/ha and stover yields of 1074kg/ha were recorded with BARI Mash-3 variety. The highest seed yield 771 kg/ha stover yield 1108 kg/ha were recorded with the mixed inoculants and BINA SP 10 inoculants, respectively. The highest seed yield of 789 kg/ha was obtained due to the interaction of BARI Mash-3 x Mixed inoculants. So BARI Mash-3 x mixed inoculants appeared to be a better combination for successful balckgram production in this soil.

Key words: Rhizobium, inoculation, nodulation, yield and black gram.

Introduction

Blackgram (*Vigna mungo* L.) is one of the widely grown pulse crops in Bangladesh for both human consumption, animal fodder as well as for soil building purpose. But high cost and environmentally risky chemical fertilizers pose serious and continuous problem for increasing black gram production in developing countries including Bangladesh (Khan *et al*., 2014; Rahman,2013; Rani and Kodandarawaiah, 2011; Sharma and Khurana, 2012). These problems are likely to become more serious in future. Biological nitrogen fixation (BNF) resulting from a symbiosis between legume crops and root nodule bacterium *Rhizobium* can ameliorate these problem by reducing the N-fertilizer inputs required to ensure high productivity (Gupta and Namdea, 2008). The successful growing of black gram is dependent on the availability of its microsymbiont bacteria in soil. All effective *Rhizobium* strains are not present in all soils of Bangladesh (Chanda *et al.,* 2009; Bhuiyan and Obaidullah, 2008; Bhuiyan *et al*., 2008). In this situation inoculation can meet the challenge of providing superior strains in the soil, so that the most effective nodulation and nitrogen fixation are obtained. Thus, it was thought that there is a scope for utilizing the effective Rhizobial strains for obtaining more yield of black gram under field conditions which may also play a vital role in improving soil environment and agricultural sustainability. With this view in mind, a piece of research work was conducted to evaluate the performance of four *Rhizobium* inoculants on the growth, yield and nitrogen content of two varieties of black gram viz. BARI Mash-2 and BARI Mash-3; and to investigate the interaction effect of *Rhizobium* inoculants with black gram varieties.

Materials and Methods

The present investigation was conducted at the Field laboratory of the Department of Soil Science, Bangladesh Agricultural University, Mymensinh during October 2012 to January 2013 to observe the response of two black gram varieties viz BARI Mash-2 and BARI Mash-3 to inoculation of four inoculants viz. Control (I_0), BINA SP 10 (I_1), BINA 441(I_2), BINA 301(I_3) and BINA mixed (I_4). The experiment was laid out in split plot design with 3 replications. The total number of unit plots was 30 (10 treatment × 3 replications). The size of each unit plot was 4m × 3m and plot to plot distance was 1m. The seeds were collected from Pulse Research Institute Ishwrdi, Pabna. Strains were collected from Biofertilizer Laboratory of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. The mixed culture was prepared by mixing equal amount of fully grown individual culture broth in sterile conical flask. From the ready broth, 20 ml were taken out by sterile syringe and injected into polythene packed having sterile 50 g of peat in each packet. The inoculated packets were then incubated at 28°C for two weeks and were ready for seed inoculation. Seeds of black gram were taken in small polythene bag equal in each for 30. Out of 30 polythene packets 24 packets were made ready for mixed with 40% gum acacia per 1kg seed. Then the selected inoculum was mixed with the seeds (@ 50g inoculum/kg seed) for each treatment and mixed well with the seeds by shaking the bag thoroughly. For each inoculant separate polythene bag was used and care was taken to avoid contamination of the inoculants. Seeds were sown on the furrow during second week (13th) of October and the furrows were covered by soils soon after seeding. Uninoculated seeds and inoculated seeds were sown in the morning, respectively. The line to line (furrow to furrow) distance was maintained at 30 cm with continuous distribution of seeds in the line. Intercultural operations were done as and when necessary. Data were recorded on Plant height, Number of nodules, Dry weight of nodules, Shoot weight, Root weight; Pods/plant, Seeds/plant,100 seed weight, Stover yield, Grain yield. Data of all the above characters have been analyzed statistically following split-plot design using M-stat programme (Freed, 1992).

Results and Discussion

Effect of variety and Rhizobium inoculants on number of nodules, nodule dry weight, plant height, dry weight of shoot, dry weight of root, N uptake of black gram at 45 days of sowing: There were significant variations

between the two varieties of black gram regarding formation of total number of nodules/plant and nodule dry weight recorded at 45 days of sowing (Table 1). The variety BARI Mash-3 recorded the highest number of nodules/plant (6.22) and nodule dry weight (18.28 mg/plant) which were statistically superior to that of BARI Mash-2 (5.04 and 12.93). The different *Rhizobium* inoculants produced significantly higher number of nodules/plant and nodule dry weight compared to that found in uninoculated control. The inoculant BINA 441 produced the highest number of nodules/plant (7.00) and nodule dry weight (20.17 mg/plant). The inoculants BINA SP-10 and BINA 301 produced identical number of nodules/plant (5.67) and nodule dry weight (6.95). Bhuiya *et al.* (2008) reported that inoculation significantly increased the formation of total nodules/plant and nodule dry weight.

Table 1. Effect of variety and Rhizobium inoculants on number of nodules, nodule dry weight, plant height,dry weight of shoot,dry weight of root, N uptake of blackgram at 45 days of sowing

Factor	Nodule no./plant at 45 days of sowing	Nodule dry wt. (mg/plant at 45 days of sowing)	Plant height (cm/plant) at 45 days of sowing	Shoot weight (mg/plant) at 45 days of sowing	Root weight at 45 days of sowing	N uptake by shoot (mg/plant) at 45 days of sowing)
Variety						
BARI mash-2	5.04 b	12.93b	29.57 a	1597.13	163.40 a	44.39
BARI mash-3	6.22 a	18.26a	28.66 b	1384.20	139.33 b	38.48
SE(m)+	0.39	1.73	0.334	NS	9.83	NS
Inoculant						
Control	3.53 c	7.83 c	26.40 d	1104.16 c	102.50 c	29.72 b
BINA SP-10	5.67 ab	15.17 ab	30.29 ab	1381.16 bc	158.50 b	41.32 b
BINA 441	7.00 a	20.17 ab	29.54 b	1511.66 b	161.33 b	39.43 b
BINA 301	5.05 b	13.33 b	27.99 c	1538.16 b	145.00 b	36.69 b
Mixed	6.95 a	21.50 a	31.34 a	1918.16 a	189.50 a	66.01 a
SE(m)+	0.626	2.74	0.52	101.22	15.55	6.25

In column figures having same letter(s) do not differ significantly by DMRT at 5% level of probability, NS=Non Significant

Table 2. Interaction effect of variety and Rhizobium inoculants on number of nodules , dry weight, plant height, dry weight of shoot, dry weight of root and N uptake by shoot of balckgram at 45 days of sowing .

Interaction	Nodule no./plant at 45 days of sowing	Nodule dry weight (mg/ plant) at 45 days of sowing	Plant height (cm/p at 45 days of sowing lant)	Shoot weight (mg/plant) at 45 days of sowing	Root weight (mg/plant) at 45 days of sowing	N uptake by shoot (mg/ plant) at 45 days of sowing
BARI mash-2 x control	3.06	566	26.69	1112.66	105.00	27.91
BARI mash-2 x BINA SP-10	5.43	8.00	30.28	1492.33	128.66	40.24
BARI mash-2 x BINA 441	6.83	14.33	30.37	1666.33	179.00	46.52
BARI mash-2 x BINA 301	3.86	19.33	28.82	1582.00	151.3	34.25
BARI mash-2x mixed	6.03	16.60	31.69	2132.33	203.00	73.05
BARI mash-3 x control	4.00	8.00	26.12	1095.66	100.00	31.54
BARI mash-3 x BINA SP-10	5.86	10.00	30.29	1270.00	138.33	42.40
BARI mash-3 xBINA 441	7.16	21.00	28.72	1357.00	143.66	32.35
BARI mash -3 x BINA 301	6.23	18.66	27.16	1494.33	138.66	39.13
BARI mash-3 x mixed	7.86	25.66	30.99	1704.00	176.00	46.97
SE(M)+	NS	NS	NS	NS	NS	NS

In column figures having same letter(s) do not differ significantly by DMRT at 5% level of probability, NS=Non Significant

Result in Table 1 showed that there were significant variation between the two varieties of black gram on plant height, dry weigh of shoot dry weight of root, and N uptake by shoot observed at 45 days of sowing. The tallest plant (29.57cm),highest amount of shoot weight(1597.13 mg/plant),root weight (163.40 mg/plant) and N uptake by shoot (44.39 mg/plant) were found in BARI Mash-2 which was significantly superior to BARI Mash-3 variety.

The different inoculants showed beneficial effects on all the mentioned parameters at 45 days of sowing (Table 1). All the inoculants recorded statistically superior plant heights, highest amount of shoot dry weight of shoot, dry weight of root and N uptake by shoot compared to that found without any inoculants. Mixed inoculants produced the tallest plant (31.34 cm/plant), highest amount of shoot weight (1918.163 mg/plant), dry weight of root (189.50 mg/plant) and N uptake by shoot (66.01 mg/plant) which was statistically identical to that found in BINA SP 10 inoculant. The shortest values were found in uninoculated control on all the mentioned parameters.

Interaction effect of variety and Rhizobium inoculants on number of nodules , dry weight, plant height, dry weight of shoot, dry weight of root and N uptake by shoot of balckgram at 45 days of sowing: There were no significant interaction effect of blackgram varieties and *Rhizobium* inoculants in the formation of nodules/plant and nodule dry weight recorded at 45 days of sowing. The number of nodules and nodule dry weight ranged from 3.06/plant and 5.66 (mg/plant) in BARI Mash-2 without

any Rhizobial inoculant to 7.86/plant and 25.66 (mg/plant) in BARI Mash-3 variety due to inoculation with mixed inoculants.

The interaction effect of black gram varieties and *Rhizobium* inoculants were not statistically significant in receding plant height, highest amount of dry weight of shoot, dry weight root and N uptake by shoot at 45 days of sowing (Table 2). The tallest plant, highest amount of shoot weight, root weight and N uptake by shoot ranged from 26.12 cm, 1095.66 mg/plant, 100.00 mg/plant and 31.54 mg/plant recorded in BARI Mash-3 without any *Rhizobium* inoculant to 31.69 cm, 2132.35 mg/plant, 203.00 mg/plant and 73.05 mg/plant in BARI Mash-2 variety due to inoculation with mixed inoculant.

Effect of variety and Rhizobium inoculants on number of pods/plant, number of seeds/plant, 100 seed weight, Seed yield (kg/ha), Stover yield (kg/ha), N content in seed(kg/ha), N content in stover (kg/ha) and Total N uptake seed+stover (Kg/ha) of black gram: There were no significant variation in the number of pods/plant, number of seeds/plant, 100 seed weight, Seed yield (kg/ha), Stover yield (kg/ha), N content in seed(kg/ha), N content in stover (kg/ha) and Total N uptake seed+stover (Kg/ha)of two varieties of blackgram (Table 3). BARI Mash-3 produced 11.55 pods/plant, number of seeds/plant (59.62), 100 seed weight (4.05g), Seed yield (741kg/ha), Stover yield (1074kg/ha), N content in seed(30.94kg/ha), N content in stover(20.94kg/ha) and Total N uptake seed+stover (51.57Kg/ha) while BARI Mash-2 produced 11.02 pods/plant, number of seeds/plant (57.64), 100 seed weight (3.73g), Seed yield (729kg/ha), Stover yield (1058kg/ha), N content in seed (28.98kg/ha), N content in stover (20.50kg/ha) and Total N uptake seed+stover (49.48Kg/ha).

Table 3. Effect of variety and Rhizobium inoculants on pods/plant, number of seeds/plant and 100 seed weight, Seed yield (kg/ha), Stover yield (kg/ha), N content in seed(kg/ha), N content in stover (kg/ha) and Total N uptake seed + stover (Kg/ha) of black gram of black gram

Factor	Pods no./plant	Seeds no./ plant	100seed weight (g)	Seed yield (kg/ha)	Stover yield (kg/ha)	N content in seed (kg/ha)	N content in stover (kg/ha)	Total N uptake seed + stover Kg/ha)
Variety								
BARI mash-2	11.20	57.64	3.73	729	1058	28.98 b	20.50	49.48
BARI mash-3	11.55	59.62	4.05	741	1074	30.94 a	20.94	51.57
SE(m)+	NS	NS	NS	NS	NS	0.53	NS	NS
Inoculant								
Control	8.81d	48.30c	4.04	692 C	1001 c	28.82	22.39 a	51.21
BINA SP-10	12.60 ab	61.35b	3.91	766 a	1108 a	30.17	21.28 a	51.45
BINA 441	11.46 bc	57.65b	3.94	732 b	1055 b	30.83	20.91 ab	51.78
BINA 301	10.48 c	58.95b	3.89	718 bc	1059 ab	29.01	20.25 ab	49.44
Mixed	13.53 a	66.90a	3.69	771 a	1106 a	30.97	18.78 b	48.76
SE(m)+	0.53	1.68	NS	12	23	NS	0.97	NS

In column figures having same letter(s) do not differ significantly by DMRT at 5% level of probability, NS=Non Significant

Table 4. Interaction Effect of variety and Rhizobium inoculants on pods/plant, number of seeds/plant and 100 seed weight of black gram

Interaction	Pods no./plant	Seeds no./plant	100seed weight (g)	Seed yield (kg/ha)	Stover yield (kg/ha)	N content in seed (kg/ha)	N content in stover (Kg/ha)	Total N uptake (Kg/ha)
BARI mash-2 x control	9.03	49.06	3.22	683	980 c	25.29 e	23.43 a	56.27 a
BARI mash-2 x BINA SP-10	12.36	60.06	3.92	766	1135 a	31.21 bc	23.17 a	54.38 a
BARI mash-2 x BINA 441	11.13	55.63	3.94	728	1095 a	28.70 cd	18.59 b	47.30 bc
BARI mash-2 x BINA 301	10.63	58.86	4.10	698	1005 c	28.77 cd	19.05 b	47.83 bc
BARI mash-2x mixed	12.86	64.56	4.16	752	1078 ab	30.93 bc	18.27 b	43.56 c
BARI mash-3 x control	8.60	47.53	3.93	701	998 c	26.12 de	21.35 ab	48.06 bc
BARI mash-3 x BINA SP-10	12.83	62.63	3.90	766	1082 ab	29.12 cd	19.39 b	48.52 bc
BARI mash-3 xBINA 441	11.80	59.66	3.94	736	1015 bc	32.97 b	23.23 a	54.36 a
BARI mash -3 x BINA 301	10.33	59.03	3.68	737	1139 a	29.25 cd	21.45 ab	51.05 ab
BARI mash-3 x mixed	14.20	69.23	4.17	789	1135 a	36.65 a	19.30 b	53.96 a
SE(M)+	NS	NS	NS	NS	32	1.20	1.37	2.27

In column figures having same letter(s) do not differ significantly by DMRT at 5% level of probability, NS=Non Significant

Application of different *Rhizobium* inoculants showed significant variation in all the mentioned parameters (Table 3). The highest number of pods/plant(13.53), number of seeds/plant (66.90)100 seed weight (3.99g), Seed yield (971kg/ha), Stover yield (1108kg/ha) and N content in seed (36.97kg/ha) were produced by mixed inoculant which was statistically identical with BINA SP 10 and superior to other inoculants. The highest N content in stover(22.39kg/ha) and Total N uptake seed + stover (51.21Kg/ha) were found in uninoculants control plants. The lowest number of pods/plant were produced by uninoculated control plants on all the mentioned parameters except N content in Stover and Total N uptake seed+stover. The lowest N content in Stover and Total N

uptake seed+stover were obtained in mixed inoculated plants. Podder *et al.,* (1999) reported that the effect of inoculation on soybean caused higher number of pods/plant over control.

Interaction effect of variety and Rhizobium inoculants on pods/plant, number of seeds/plant and 100 seed weight, Seed yield (kg/ha), Stover yield (kg/ha), N content in seed (kg/ha), N content in stover (kg/ha) and Total N uptake seed+stover (Kg/ha) of black gra: The interaction effect of black gram varieties and *Rhizobium* inoculants were not statistically significant in recording pods/plant, number of seeds/plant and 100 seed weight, Seed yield (kg/ha) except Stover yield (kg/ha), N content in seed (kg/ha), N content in stover(kg/ha) and Total N uptake seed+stover (Kg/ha) (Table 4). The number of pods/plant, number of seeds/plant and 100 seed weight, Seed yield, Stover yield and N content in seed ranged from (9.03), (49.06) (3.22 g), (683kg/ha) (980kg/ha) (25.29kg/ha) (Kg/ha) respectively recorded BARI Mash-2 variety without any Rhizobial inoculant to 14.20, 69.23, 4.17g,789 kg/ha,1136 kg/ha and 36.65 kg/ha in BARI Mash-3 variety due to inoculation with mixed inoculants respectively. The highest N content in stover and Total N uptake seed+stover were 23.43 kg/h and 56.36 kg/h and the lowest values were found in BARI mash -2 with mixed inoculants on all the mentioned parameters, respectively

The *Rhizobium* inoculation was beneficial on yield contributing characters and yield of blackgram. Between the two varieties tested BARI Mash-3 appears to be better than BARI Mash-2 variety. Mixed inoculant was comparatively more effective for BARI Mash-3 variety.

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