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Yield Response of Black Gram to Inoculation by Different *Rhizobium* Strains using Various Types of Adhesives

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ABSTRACT

Black Grams are capable of fixing atmospheric nitrogen through *Rhizobium* species living in its root nodules. To evaluate the effect of different *Rhizobium* strains on yield of Black Gram, a field experiment was conducted in the Rajshahi University field of Bangladesh. Three *Rhizobium* strains (RLc 107, RCa 220 and RVm 307), four different types of adhesives (Sucrose, Peptone, Molasses and Glycerol) and two Black Gram varieties (BARI MASH-1 and BINA MASH-1) were used as an experimental material. Data were recorded on number of pods per plant, number of seeds per plant, 100-seed weight and seed yield per plant. The yield of Black gram was significantly affected by *Rhizobium* inoculation and the better result for all the characters were obtained in BINA MASH-1 when seeds were inoculated with RVm 307 using the adhesive peptone.

Key words: Adhesives, black gram, inoculation, *Rhizobium*, yield

INTRODUCTION

Black gram (*Vigna mungo* L. Hepper) is a grain legume widely cultivated in both tropical and sub-tropical countries of the world. It is a part of diet for millions of people in many countries and a cheap source of protein with 17-34% of protein in seeds (Gour, 1993). An important feature of this plant is its ability to establish a symbiotic partnership with specific bacteria, setting up the biological N_2-fixation process in root nodules by *Rhizobia* that may supply the plant's needs for N (Mahmood and Athar, 2008; Mandal *et al*., 2009). Benificial role of *Rhizobium*-Legume symbiosis are well known. However, all the legume crops are not sustaining the same strains of the bacteria and the influences of these different strains of bacteria on growth and yield of pulse crops may not be equal. Examination of the differential roles of different strains of *Rhizobium* isolates on each and every crop is therefore significant to understand the best suitable strain for the optimum growth and productivity of the crop under a particular environmental condition. Survival of a large population of inoculated *Rhizobium* on the surface of legume seeds is necessary for improving the chance of root-hair infection and thereby nodulation.

Many leguminous seeds contain certain water soluble toxic compound such as α-diaminobutyric acid which adversely affect the viability of *Rhizobium* (Millington, 1995; Jacobs and Daad, 1959; Thomson, 1960). Therefore, it is essential to ensure the adhesion and survivability of large number of *Rhizobia* per seed to compete with indigenous microorganisms. To ensure maximum number of viable cells per seed, different types of adhesives like sucrose, peptone, molasses etc., may be used. The present study was carried out to investigate the effect of three *Rhizobium* strains on yield of Black Gram using four different types of adhesives.

MATERIALS AND METHODS

To evaluate the response of Black Gram to *Rhizobium* inoculation, a field experiment was conducted at the University of Rajshahi, Bangladesh. For this study, two Black Gram varieties i.e., BARI MASH 1 and BINA MASH 1; three *Rhizobium* strains RVm 307 (isolated from Black Gram), RCa 220 (isolated from Chick Pea) and Rlc107 (isolated from Lentil); four different types of adhesives namely sucrose, peptone, molasses and glycerol were used as an experimental materials.

The seeds of Black Gram varieties were disinfected with 0.2% $HgCl_2$ (3-4 min) followed by 6-7 washings with sterile water. The disinfected seeds were then suspended in 50 mL thick suspension (10^{12} cells mL^{-1}) of *Rhizobium* in presence of 1% sucrose, peptone, molasses and glycerol separately for 30 min. These seeds were air dried and sown in the field with three replications. Data on number of pods per plant, number of seeds per plant, 100-seed weight and seed yield per plant was taken and analyzed statistically.

RESULTS AND DISCUSSION

Pod No. per plant: The data regarding pod per plant of Black gram as affected by *Rhizobium* inoculation are presented in Table 1. It was obvious that different treatments significantly affected the pod number per plant. The highest number (20.34) of pod per plant was observed in BINA MASH-1 when the seeds were inoculated with RVm 307 using the adhesive peptone. The lowest number (12.67) of seed per plant was seen in BARI MASH-1 when inoculated with RCa 220 by the adhesive glycerol. Table 5 showed that all the sources had highly significant effect in pod No. per plant except replications.

Basu and Bandyopadhyay (1990) reported that seed inoculation and application of nitrogen increased number of pods per plant of *Vigna radiata*. Hoque and Haq (1994) reported that seed inoculation increased number of pods per plant in lentil. Rashid *et al.* (1999) reported that *Rhizobium* inoculation +20 kg N ha^{-1} increased pod yield significantly. Karadavut and Ozdemir (2001) and Fatima *et al.* (2008) reported that seed inoculation significantly increased number of pods per plant in chickpea. In our experiment, it was observed that seed inoculation with different *Rhizobium* strains gave the better result in both the varieties in comparison to that of control. These results are in confirmation with that of Bhuiyan *et al.* (2008) and Malik *et al.* (2006) who concluded that pod per plant of mung bean and soyabean is significantly increased by inoculating with *Bradyrhizobium*. Similar results were also obtained by Aslam *et al.* (2010) and Anjum *et al.* (2006) and among the adhesives, peptone showed better result as reported by Saha and Kapadnis (2001).

Seed No. per plant: Seed inoculation with *Rhizobium* strains on number of seeds per plant were found significant (Table 2). The maximum number of seeds per plant (102.4) were recorded in

Table 1: Pod No. per plant of the two varieties treated with different *Rhizobium* strains using different types of adhesives

	BARI MASH-1				BINA MASH-1			
Strains adhesives	RLc 107	RCa 220	RVm 307	Control	RLc 107	RCa 220	RVm 307	Control
Sucrose	14.45^{gh}	14.56^{g}	13.22^{ij}	11.79^{l}	17.34^{c}	17.49^{c}	17.45^{c}	14.11^{h}
Peptone	12.95^{jk}	12.96^{jk}	15.22^{f}	11.99^{l}	18.45^{b}	18.50^{b}	20.34^{a}	14.23^{gh}
Molasses	12.96^{jk}	13.22^{ij}	13.45^{i}	11.66^{l}	17.22^{c}	16.23^{e}	18.45^{b}	14.22^{gh}
Glycerol	12.99^{jk}	12.67^{k}	13.34^{ij}	11.70^{l}	16.34^{de}	16.63^{d}	17.21^{c}	14.41^{gh}

Means followed by same letter(s) are statistically non significant at 5% level as tested by DMRT

Table 2: Seed No. per plant of the two varieties treated with different *Rhizobium* strains using different types of adhesives

	BARI MASH-1				BINA MASH-1			
Strains adhesives	RLc 107	RCa 220	RVm 307	Control	RLc 107	RCa 220	RVm 307	Control
Sucrose	52.08^{r}	51.90^{r}	52.33^{r}	48.56^{t}	68.69^{j}	70.63^{h}	85.45^{d}	56.35^{r}
Peptone	57.66^{l}	56.90^{r}	62.39^{k}	47.69^{u}	75.50^{e}	73.61^{f}	102.4^{a}	56.40^{r}
Molasses	53.92^{p}	53.25^{q}	54.61^{o}	47.90^{u}	72.54^{g}	69.58^{i}	89.57^{b}	55.66^{n}
Glycerol	52.17^{r}	50.67^{s}	56.33^{r}	47.52^{u}	70.44^{h}	70.11^{hi}	88.44^{c}	56.37^{r}

Means followed by same letter(s) are statistically non significant at 5% level as tested by DMRT

Table 3: A 100 seed weight (g) of the two varieties treated with different *Rhizobium* strains using different types of adhesives

	BARI MASH-1				BINA MASH-1			
Strains adhesives	RLc 107	RCa 220	RVm 307	Control	RLc 107	RCa 220	RVm 307	Control
Sucrose	4.463^{o-s}	4.523^{n-q}	4.580^{n}	4.437^{p-s}	6.323^{fgh}	6.403^{c-f}	6.507^{b}	6.147^{jk}
Peptone	4.493^{n-q}	4.523^{n-q}	4.713^{r}	4.487^{n-r}	6.387^{d-g}	6.463^{b-e}	6.863^{a}	6.200^{ij}
Molasses	4.423^{q-s}	4.487^{n-r}	4.547^{no}	4.380^{s}	6.293^{ghi}	6.367^{efg}	6.497^{bc}	6.023^{l}
Glycerol	4.387^{rs}	4.507^{n-q}	4.537^{nop}	4.257^{t}	6.230^{hij}	6.330^{fg}	6.480^{bcd}	6.077^{kl}

Means followed by same letter(s) are statistically non significant at 5% level as tested by DMRT

BINA MASH-1 when inoculated with RVm 307 using the adhesive peptone closely followed by same strain in the same variety (89.57) but using the adhesive glycerol. The minimum number of seeds per plant was recorded in BARI MASH-1 (50.67) when seeds were inoculated with RCa 220 by the adhesive glycerol. Table 5 showed that all the sources had highly significant effect on above character except replications. Anjum *et al.* (2006) reported that seed inoculation with *Rhizobium* and nitrogen fertilizer increased the number of seeds per plant in comparison to control. In the present investigation, it was observed that seed inoculation with different *Rhizobium* strains using different types of adhesives always gave better result than that of control in both the varieties. Similar results were observed by Malhur *et al.* (2003) and Yagmur and Kaydan (2011) and among the adhesives peptone showed the better result as observed by Saha and Kapadnis (2001).

The 100 seed weight: Table 3 showed that the effect of *Rhizobium* strains on 100 seed weight was significant in both the varieties. In this case, the highest 100 seed weight (6.863 g) was observed in BINA MASH-1 when treated with RVm 307 by the adhesive peptone and the lowest seed weight (4.387 g) was observed in BARI MASH-1 when inoculated with the strain RLc107 using the adhesive glycerol. Analysis of variance (Table 5) showed that there was highly significant effect of variety, strains, adhesives and their interaction on 100 seed weight. The treated seed with different strains and adhesives always showed the higher result in comparison to that of control in both the varieties. Chetti *et al.* (1995) studied the effect of nitrogen and *Rhizobium* inoculation on the productivity of ground nut genotype and reported that both nitrogen application and inoculation had significant positive effects on 100-seed weight. Elsheikh and Elzidany (1997) reported that *Rhizobium* inoculation significantly increased 100 seed weight of faba bean. Aslam *et al.* (2010) stated that *Rhizobium* inoculation significantly increased 100 seed weight. Similar results were obtained by Alam *et al.* (1999), El-Hadi and El-Sheikh, 1999), Meena *et al.* (2001), Kyei-Boahen *et al.* (2002) and Anjum *et al.* (2006).

Table 4: Grain yield (g) per plant of the two varieties treated with different *Rhizobium* strains using different types of adhesives

	BARI MASH-1				BINA MASH-1			
Strains adhesives	RLc 107	RCa 220	RVm 307	Control	RLc 107	RCa 220	RVm 307	Control
Sucrose	3.533^{r}	3.913^{i}	3.715^{kl}	3.135^{r}	4.906^{d}	4.905^{d}	4.583^{f}	3.849^{j}
Peptone	3.552^{r}	4.079^{h}	4.050^{h}	3.739^{k}	4.905^{d}	5.084^{b}	5.845^{a}	3.875^{ij}
Molasses	3.674^{l}	3.850^{j}	3.533^{r}	3.292^{o}	4.682^{e}	4.976^{c}	4.337^{g}	3.847^{j}
Glycerol	3.463^{n}	3.575^{r}	4.033^{h}	3.333^{o}	4.376^{g}	4.582^{f}	4.337^{g}	3.837^{j}

Means followed by same letter(s) are statistically non significant at 5% level as tested by DMRT

Table 5: Analysis of variance of pod No. per plant, seed No. per plant,100 seed weight and grain yield per plant of two Black Gram varieties treated with different *Rhizobium* strains using different types of adhesives

		Mean square			
Sources	df	Pod No. per plant	Seed No. per plant	100 seed weight	Grain yield per plant
Replication	2	0.041 ns	0.268 ns	0.002 ns	0.004 ns
Variety (V)	1	331.638**	9354.194**	83.515**	19.598**
Strains (S)	3	42.391**	1920.658**	0.485**	2.822**
V×S	3	4.777**	746.093**	0.0074**	0.534**
Adhesives (A)	3	11.832**	164.129**	0.127**	0.932**
V×A	3	0.187*	3.464**	0.010*	0.171**
S×A	9	1.511**	38.364**	0.015**	0.250**
V×S×A	9	0.446**	6.712**	0.006*	0.196**
Error	62	0.054	0.114	0.003	0.001

*,**Indicates significant at 5 and 1%, respectively. ns indicate non significant

Grain yield per plant: Grain yield per plant was significantly affected by *Rhizobium* inoculation in both the varieties and increased the seed yield as compared to control (Table 4). Maximum seed yield (5.845 g) per plant was the variety BINA MASH-1 with the seed inoculation by RVm 307 with the adhesives peptone. The lowest seed yield (3.463 g) per plant was produced in BARI MASH-1 with the strain RLc 107 by the adhesive glycerol. Table 5 showed that without replication all the sources showed highly significant effect on the above characters. Kumaga and Ofori (2004), Malik *et al.* (2006), Fatima *et al.* (2007) and Shahid *et al.* (2009) reported on soyabean where seed inoculation and phosphorus application significantly increased seed yield. Ashraf *et al.* (2003) showed that seed inoculation with *Bradyrhizobium* strain significantly increased mungbean seed yield. Karadavut and Ozdemir (2001) reported that seed inoculation with *Rhizobium* significantly increased grain yield. In the present investigation, seed inoculation with different strains always increased grain yield in both the varieties in comparison to control. Our findings are in conformity with that of Fatima *et al.* (2008), who reported that application of *Rhizobium* inoculums generally increased yield components. Similar result were also obtained by Aslam *et al.* (2010), Anjum *et al.* (2006) and Sharma *et al.* (2001) and among the different types of adhesives, peptone gave the better result as reported by Saha and Kapadnis (2001).

CONCLUSION

In the present experiment, among the three different *Rhizobium* strains, RVm 307 (isolated from Black Gram) was found to be the most effective strain to enhance the yield of Black Gram. This result suggest that, the strain isolated from Black Gram is more effective in promoting yield than other strains, those were isolated from others leguminous crops.

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