**Nodulation of black gram as influenced by *rhizobium* inoculation using different types of adhesives.**

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**Abstract:** Beneficial roles of certain symbiotic nitrogen fixing bacteria such as *Rhizobium* on growth and yield of Black gram are well known.Three *Rhizobium* strains were isolated from different species of legumes. RLc107 from Lentil, RCa 220 from Chick pea and RVm 307 from Black Gram*.* To find out the effect of these strains on nodulation (Nodule number per plant, nodule fresh weight per plant and nodule dry weight per plant) of two Black gram varieties (BARI MASH-1 and BINA MASH-1), investigations were carried out in the field of Rajshahi University. For better seed inoculation of those varieties, four different types of adhesives like sucrose, peptone, molasses and glycerol were used. It was observed that *Rhizobium* inoculation improved nodulation in both the varieties than that of un inoculated control. The highest nodule number (58.45) per plant, nodule fresh weight (46.11mg) per plant and nodule dry weight (12.07 mg) per plant were observed in BINA MASH-1 when inoculated by RVm 307 with the adhesive peptone.

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**Introduction:**

The increasing demand for production of crops and food for such a vast population has led to an interest and necessity for the use of biofertilizers for the betterment of the crops and even for the health of soil. Biofertilizers can be a very good complimentary to the chemical pesticides as they not only kill the harmful insects but also the beneficial insects such as pollinators.

By the turn of this century, food requirement is increasing day by day. One of the key limiting factors in crop productivity is the availability of nitrogen. Because of the constraints on the production, availability and use of chemical nitrogenous fertilizers, biologically fixed nitrogen will play an important role in increasing the crop production. Amongst the leguminous crops, Black gram is one of the most important crop, like other pulse crops. it has unique characteristics of maintaining and restoring soil fertility through biological nitrogen fixation and its deep root system also maintains physical properties of soil. But there are many agro-ecological, biological and management related constraints that are responsible for the lower productivity of blackgram. Non-availability of proper biofertilizer and inadequate use of macro and micronutrients are some of the important factors that are responsible for lower yield of black gram. For higher yield of Black gram, an optimum rhizobial population in the rhizosphere, artificial seed inoculation with an efficient rhizobial strain is necessary.

But many leguminous seeds contain certain water soluble toxic compound such as α,ϒ –diaminobutyric acid which adversely affect the viability of *Rhizobium* (Jacobs and Daad,1959; Millington,1959; Thomson,1960).Therefore, it is essential to ensure the adhesion and survivality 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 nodulation of two Black Gram varieties 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 (Mutant variety); three *Rhizobium* strains such as RLc 107 (isolated from Lentil); RCa 220 (isolated from Chick Pea) ; RVm 307 (isolated from Black Gram) and four different types of adhesives namely sucrose, peptone, molasses and glycerol were used as experimental materials.

The seeds of Black Gram varieties were disinfected with 0.2% HgCl2 (3-4 mins.) followed by 6-7 washings with sterile water. The disinfected seeds were then suspended in 50 ml thick suspension (1012 cells/ml) of *Rhizobium* in presence of 1% sucrose, peptone, molasses and glycerol separately for 30 minutes. These seeds were air dried and sown in the field with three replications. Nodulation data were collected at flowering stage because at maturity most of the nodules were disintegrated and decomposed. For collection of this data, three plants were carefully uprooted from each experimental plot by digging 15 cm around the plant using a spade and washed with clean tap water to remove all attached soil from the roots and the nodules. The nodules were counted and carefully picked using a pair of tweezers, oven dried and dry matter weights determined. Data were analyzed by DMRT and ANOVA.

**Results:**

Data pertaining to the number of nodules per plant are presented in Table 1. It was evident that differences between the means of treatments were significant. Plant inoculated with different *Rhizobium* strains produced significantly higher number nodules as compared to that of un inoculated control. The highest number (58.45) of nodules were obtained with the strain RVm307 using the adhesive peptone in BINA MASH-1 and the second highest (56.23) number of nodules were obtained with the same strain but using the adhesive sucrose in the same variety (Fig.1) which was statistically similar to the effect of RCa 220 with the adhesive peptone. The lowest number (33.45) of nodules were obtained with RLc 107 in BARI MASH-1 using adhesive glycerol, which was statistically similar to the effect of same strain with adhesive molasses in the same variety (Table 1). Among the different types of adhesives, peptone showed the better result than other adhesives in both the varieties (Fig.2).

Analysis of variance (Table 4) showed that except replication, interaction between variety and adhesive & strain and adhesive , all the sources had a significant effect in nodule number per plant.

Table 2 showed that the highest fresh weight (46.11 mg) of effective nodules was obtained with the strain RVm307 using peptone as an adhesive in BINA MASH-1 which was statistically similar to the effect of RCa 220 with the same adhesive in the same variety. Plants inoculated with RLc 107 using adhesive sucrose produced the lowest fresh weight (24.22 mg) of nodule in BARI MASH-1 which was statistically similar to the effect of same strain with other three adhesives. Seed inoculated with the strain always showed the higher fresh weight of nodule in comparison to that of control. Among the strains and adhesives, RVm307 and peptone showed the better result (Fig. 3&4).

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

|  |  |
| --- | --- |
| BARI MASH-1 | BINA MASH-1 |
| StrainsAdhesives | RLc 107 | RCa 220 | RVm 307 | Control | RLc 107 | RCa 220 | RVm 307 | Control |
| Sucrose | 35.56 n | 37.56 m | 40.34 k | 32.75 q | 50.56 f | 54.19 cd | 56.23 b | 44.68 i |
| Peptone | 37.33 m | 39.45 kl | 41.96 j | 33.00 q | 53.33 cde | 55.78 b | 58.45 a | 44.67 i |
| Molasses | 34.34 op | 36.90 m | 39.23 l | 30.89 r | 49.33 g | 52,89 e | 54.34 c | 44.23 i |
| Glycerol | 33.45pq | 35.23 no | 37.89 m | 31.56 r | 48.11 h | 51.44 f | 53.23 de | 44.34i |

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

**Table 2:** Nodule fresh weight (mg) per plant of the two varieties treated with different *Rhizobium* strains using different types of adhesives

|  |  |
| --- | --- |
| BARI MASH-1 | BINA MASH-1 |
| StrainsAdhesives | RLc 107 | RCa 220 | RVm 307 | Control | RLc 107 | RCa 220 | RVm 307 | Control |
| Sucrose | 24.22 jk | 26.33 hij | 27.33 fghij | 19.78 l | 37.33 de | 40.22 cde | 42.oo bc | 30.11 fgh |
| Peptone | 28.11 fghij | 29.55 fghi | 30.66 f | 21.11 kl | 42.78 abc | 44.77 ab | 46.11 a | 31.22 f |
| Molasses | 26.55 ghij | 28.77 fghij | 31.00 f | 20.89 kl | 37.44 de | 39.77 cde | 40.55 cd | 30.44 fg |
| Glycerol | 26.00 ij | 28.11 fghij | 29.55 fghi | 20.22 l | 36.55 e | 38.89 cde | 40.89 cd | 30.00 fghi |

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

Varieties, strains, adhesives & interaction between varieties and adhesives showed highly significant and the remaining sources showed non significant effect on this character (Table 4).

There was a significant variation in dry weight of nodule per plant of Black Gram treated with different *Rhizobium* strains (Table 3). BINA MASH-1 produced highest nodule dry weight (12.07 mg) when inoculated with the strain RVm307 using peptone as an adhesive and the lowest nodule dry weight (5.033 mg) was observed in BARI MASH-1 when treated with RLc 107 using the adhesive molasses. In both the varieties, treatment with strains and adhesives showed better result than control and among the strains and adhesives, RVm307 (Fig.5) and peptone (Fig.6) gave the better result. In this case, except replication, interaction between variety and adhesives & variety, strains and adhesives, all the other sources showed significant effect on the above character (Table 4).

**Table 3:** Nodule dry weight (mg) per plant of the two varieties treated with different *Rhizobium* strains using different types of adhesives

|  |  |
| --- | --- |
| BARI MASH-1 | BINA MASH-1 |
| StrainsAdhesives | RLc 107 | RCa 220 | RVm 307 | Control | RLc 107 | RCa 220 | RVm 307 | Control |
| Sucrose | 5.090 jkl | 5.897 ij | 7.473 h | 4.190 lm | 9.481 def | 10.37 bcd | 10.77 bc | 7.240 h |
| Peptone | 6.730 hi | 6.837 hi | 8.437 g | 4.513 klm | 10.73 bc | 11.18 b | 12.07 a | 7.360 h |
| Molasses | 5.033 jkl | 5.887 ij | 6.527 hi | 4.250 lm | 9.207 efg | 10.08 cde | 10.34 bcd | 7.043 h |
| Glycerol | 5.523 j | 5.300 jk | 5.957 ij | 3.867 m | 8.926 fg | 9.870 cdef | 10.70 bc | 7.197 h |

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

**Table 4:** Analysis of variance of nodule number, nodule fresh weight and nodule dry weight of two Black Gram varieties treated with different *Rhizobium* strains using different types of adhesives

|  |  |  |
| --- | --- | --- |
| Source | df | Mean square |
| Nodule number | Nodule fresh weight | Nodule dry weight |
| ReplicationVariety (V)Strains (S)V×SAdhesives (A)V×AS×AV×S×AError | 2133339962 | 0.126 ns5325.707 \*\*394.979 \*\*17.544 \*\*58.380 \*\*0.152 ns3.415 \*\*0.640 ns0.388 | 6.942 ns3415.893 \*\*522.715 \*\*10.777 ns55.252 \*\*18.875 \*\*2.931 ns1.907 ns4.389 | 0.266 ns349.527\*\*47.763 \*\*2.053 \*\*8.470 \*\*0.110 ns0.698 \*0.286 ns0.294 |

\*,\*\* significant at 5% and 1% respectively; ns : non significant

|  |  |
| --- | --- |
| **Fig.1.** Effect of *Rhizobium* strains on Nodule no. of two Black gram varieties | **Fig.2.** Effect of different types of adhesives on Nodule no. of two Black gram varieties |
| **Fig.3.** Effect of *Rhizobium* strains on Nodule fresh weight of two Black gram varieties | **Fig.4.** Effect of different types of adhesives on two Nodule fresh weight of two Black gram varieties |
| **Fig.5.** Effect of *Rhizobium* strains on Nodule dry weight of two Black gram varieties | **Fig.6.** Effect of different types of adhesives on Nodule dry weight of two Black gram varieties |

**Discussions:**

An essential desired characteristic for the inoculum strains of Rhizobia is the highly effective nitrogen fixing ability with the intended host species and in some instances there is a requirement to effectively nodulate a wide range of host legumes. Other beneficial characteristics include stress tolerance, competitive ability against indigenous strain, genetic stability and satisfactory growth and survival during the production of commercial inoculums (Howieson *et al*. 2000b) .This study was undertaken to find out the relative ability of different *Rhizobium* strains for nodulation in Black Gram using different types of adhesives.

Nodule number per plant is very important character as there are evidences that the bacterium thrives in root exceretions, although no single component in the root exudates has any special root in stimulating its growth. Jagdale *et al.* (1980) stated that in field conditions nodulations were increased with the inoculation of *Rhizobium* in Bengal gram. However, in the present study, it was observed that nodulation was increased due to seed inoculation with different adhesives in comparison to that of control. Prasad *et al.* (1984) reported that number of nodules were found to increase with *Rhizobium* inoculation in comparison to control. Tippannavar and Desai (1992), Shah *et al*. (1994) and Biswas *et al*. (2003) reported that seed inoculation increased the number of nodules per plant. These results are also in conformity with the findings of El-Hadi and Sheikh (1999) as they reported that *Rhizobium* inoculation significantly increased total number of nodules per plant. Yadav *et al.* (1994) observed an increase in the number of nodules and grain yield due to grain inoculation. Roy *et al*. (1995) also reported that grams inoculation with *Rhizobium* increased nodule number per plant. Ahmed *et al*. (2006) reported that inoculation of soil and seed with *Rhizobium* in combination with nitrogen fertilizer significantly affected the growth and nodules formation in green gram. Similar results were also obtained by Alam *et al.* (1999), Solaiman and Rabbani (2006), Javaid (2009), Muhammad Aslam *et al.* (2010) and Javaid and Bajwa (2011). Among the different types of adhesives, peptone always showed the better result than other adhesives as observed by Saha and Kapadnis (2001).

Nodule fresh weight is an important character related to yield. In the present investigation, nodule fresh weight was always higher than that of control. In case of adhesives, peptone showed better performance. Javaid (2009) reported that nodule fresh weight was always increased with the inoculation of *Rhizobium* and soil amendments. Similar results were also obtain by Pawar and Ghulgule (1977), Vaishya *et al*. (1982) and Javaid and Bajwa (2011).

Nodule dry weight may be considered as a useful character for selecting efficient strains of *Rhizobium*. Dry weight of nodules per plant compared to number of nodules was more closely related to seed yield as reported by Khurana *et al.* (1984). This may also be used as a selection criterion for improvement in seed yield of blackgram. In the present investigation, highest nodule dry weight was observed in case of adhesive peptone as reported by Saha and Kapadnis (2001). From the table 3, it had been seen that nodule dry weight was always higher in case of treatment with *Rhizobium* using different adhesives in comparison to control. Eusuf Zai *et al.* (1999) conducted a pot experiment on chickpea and found that *Rhizobium* inoculation increased dry weight of nodules. This result was in agreement with Solaiman and Rabbani (2005) who reported that *Rhizobium* inoculant significantly increased dry weight of nodules per plant in edible-podded pea. Similar results were also obtained by Pawar and Ghulgule (1977), Vaishya *et al.* (1982), Hafeez *et al*. (2007), Talukder *et al*. (2008), Solaiman *et al.* (2010) and Javaid and Bajwa (2011).

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