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Effects of Seed Invigoration Treatments with Bio-priming on Germination, Vigour and Seedling Growth in Black Gram (*Vigna mungo* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A study had been conducted in black gram to observe the effect of different seed invigoration treatments with bio-priming for seed germination, seedling growing and vigour. The variety of black gram i.e., Uttara, was selected and seed had been treated with control (T_1); Seeds soaked in distilled water/ hydropriming (T_2); *Rhizobium leguminosarum* 10% (T_3); *Rhizobium leguminosarum* 15% (T_4); *Rhizobium leguminosarum* 20% (T_5); *Rhizobium leguminosarum* 25% (T_6); *Rhizobium leguminosarum* 30% (T_7) respectively. Treated seeds were grown in different glass plate and patri plate at Seed Testing Laboratory, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India. Seed invigoration with *Rhizobium leguminosarum* at 20% followed by T_4 was found

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very effective for seed germination with vigorous seedlings. Higher seed germination percentage (82.60), shoot length (17.89 cm), root length (12.99 cm), seedling fresh weight (1.87 g), seedling dry weight (0.19 g), seedling vigour index I (2393.62) and seedling vigour index II (15.96) results were observed for the seed bioprimed with *Rhizobium leguminosarum* at 20%. Seed treatment with *Rhizobium leguminosarum* at 20% recorded better performance than rest the treatments for all characters observed. The present investigation clearly depicted that the germination, vigour and seedling growth revalidated seed lots can be improved by pre-sowing and invigoration treatments.

Keywords: Bio priming; black gram; germination; Rhizobium leguminosarum; vigour.

1. INTRODUCTION

Blackgram, (*Vigna mungo* L. Hepper), is a popular legume crop that plays a significant role in the agricultural landscape. It is also called black lentil or urad dal, blackgram. It is cultivated primarily for its nutritious seeds, which are a staple food in many countries around the world. Blackgram is an annual plant belonging to the fabaceae family and is native to the Indian subcontinent. It is a warm-season crop that thrives in tropical and subtropical regions, making it an essential component of the agriculture sector in countries such as India, Pakistan, Bangladesh, and Myanmar. India is the world's largest producer as well as consumer of black gram [1]. In India, black gram is third most important pulse crop grown under rainfed, rice fallow, irrigated conditions and during kharif, rabi and summer seasons, which matures in 90-100 days and it enriches soil with nitrogen [2]. Cultivating blackgram requires well-drained soil and a warm climate, with temperatures ranging between 25 to 35 degrees Celsius. The primary reasons for pulses' low production, according to [3], include the use of inferior seed, poor crop management, and cultivation in dry, marginal soils. Farmers often rotate blackgram with other crops to enhance soil fertility and prevent the build-up of pests and diseases.

From a nutritional standpoint, blackgram seeds are a rich source of protein, dietary fibre, vitamins (particularly B vitamins), and minerals such as iron, potassium, and magnesium. Its consumption provides various health benefits, including improved digestion, increased energy levels, and support for muscle and tissue repair. In traditional medicine, blackgram is believed to possess antioxidant and anti-inflammatory properties, contributing to overall well-being. Besides its nutritional significance, blackgram holds cultural and culinary importance in many cultures. It serves as a primary ingredient for preparation of dahl and for different food preparations like idli, dosa and non-fermented foods [4], with rice flour.

Compared to existing field application systems, seed treatments with bioagents offer affordable and comparatively non-polluting delivery systems for protective chemicals. When applied to seeds, bioprotectants have the potential to boost plant growth as well as protect and colonise roots. However, compared to chemical seed treatments, biological agents have a tendency to be less consistent and effective. There is a need for seed treatment methods that will increase the effectiveness of biological agents, and “bioprimin”" is one such effort in this area. Many soil and seed-borne ailments may be successfully controlled by treating seeds with bio-control and priming substances [5].

Seed priming with distinct bio-priming like *Rhizobium leguminosarum* can be done to enhance the germination and vigour as these are vital stage of a plant life. Accordingly, this investigation was thus undertaken to assess the achievements of bio-priming treatments on black gram seed in relation to its germination and seedling growth.

2. MATERIALS AND METHODS

The laboratory experiment was carried out in seed testing laboratory, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India during 2022 following Complete Randomized Design with three replications. For this investigation, the seeds of black gram (Variety: Uttara) were collected from All India Coordinated Research Project (AICRP) on MULLaRP, ICAR, Kanpur. Seed priming was done with the solution of *Rhizobium leguminosarum* at different concentration. Dry seed was considered as control (T_1); Seeds soaked in distilled water/ hydropriming (T_2); *Rhizobium leguminosarum* 10% (T_3); *Rhizobium leguminosarum* 15% (T_4); *Rhizobium leguminosarum* 20% (T_5); *Rhizobium leguminosarum* 25% (T_6); *Rhizobium leguminosarum* 30% (T_7). Seeds were soaked eight hours for each treatment. The soaked seeds were then removed from the solution and dry for one hour. After that, seeds were placed for germination on standard germination papers using the Petri plate and glass-plate methods, and incubated for 7 days at 25 degrees Celsius with a relative humidity of 95% in a germinator. Different seed quality parameters like germination percentage, shoot length (cm), root length (cm), seedling fresh weight (g), seedling dry weight (g), seedling vigour index I [6], and seedling vigour index II (Abdul-Baki and Anderson, 1973) observations were recorded. The data were subjected to statistical analysis by using the online computer program 'OPSTAT' for proper interpretation.

2.1 Formulae of Different Seed Quality Parameters

2.1.1 Germination percentage:

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \text{X } 100$$

2.1.2 Root and shoot length (cm):

Root and shoot lengths were measured at 7 days after germination in the laboratory by glass plate method with the help of a meter scale and expressed in centimetre (cm).

2.1.3 Fresh weight (g):

Fresh weights of ten (10) seedlings were weighed in g at 7 days after germination in laboratory.

2.1.4 Dry weight (g):

The ten (10) seedlings were dried at 130^0C in the hot air oven for one hour and weighed in g to assess the dry weight.

2.1.5 Vigour index-I:

Vigour index-I (VI-I) was calculated by using the formula suggested by Abdul Baki and Anderson (1973): VI= G X L Where, 'G' indicates germination percentage and 'L' denotes average seedling length (cm)

2.1.6 Vigour index-II:

Vigour index-II (VI-II) was calculated by using the formula suggested by Abdul Baki and Anderson (1973): VI= G X D Where, 'G' indicates germination percentage and 'D' denotes average seedling dry weight (g).

3. RESULTS

3.1 Germination Percentage

Significant responses were noticed in the priming treatment of *Rhizobium leguminosarum* solution with different concentration of under laboratory condition. T_5 (82.60) recorded highest germination percentage followed by T_4 and T_2, while lowest germination percentage was recorded for T_1 (77.33) preceded by T_3 and T_7 respectively. But non-significant difference was observed in between T_1 and T_6, T_7; T_2 and T_3, T_4, T_6, T_7; T_3 and T_6, T_7; T_4 and T_5, T_6; T_6 and T_7 (Table-1). This result is in agreement with Choudhury and Bordolui [7]. Similar kind of experiment on chickpea was observed by Malathi and Doraisamy [8].

3.2 Vigour Index-I

Vigour Index-I showed a significant difference between priming treatments with varying *Rhizobium leguminosarum* concentration and duration. T_5 (2,393.62) was determined to have the highest value, followed by T_4 and T_6, respectively. But it was lowest for T_1 (1658.76), which was preceded by T_7 and T_2 (Table-1). Although the vigour index varied significantly, some non-significant differences between T_1 and T_7; T_2 and T_3; T_4 and T_5 were also seen. This result corroborates the findings of Ladumor and Singh [9] in black gram; Ray and Bordolui [10] in tomato.

3.3 Root Length (cm)

Significant difference was observed in root length for this bio priming. Maximum root length of seedling was observed for T_4 (12.99 cm) followed by $T_6$and T_5respectively, while it was minimum for T_7 (8.08 cm) preceded by T_1 and T_3 (Table 1). Although T_1 and T_3showed non-significant difference among themselves. The results are in agreement with the fact that root and shoot length increased in seeds due to priming as compared to non-primed seeds reported by Demir and Oztokat [11]; Choudhury and Bordolui [12].

3.4 Shoot Length (cm)

In case of shoot length, the longest shoot length of seedling was recorded for T_5 (17.89 cm) followed by T_4 and T_3 while shortest shoot length was observed in T_1 (12.16 cm) preceded byT_7 and T_2. Significant difference was noted for shoot length in overall though non-significant difference was observed in between T_2 and T_7; T_3 and T_4; T_6 and T_2, T_3, T_4, T_7 (Table-1). The result corroborates the findings of Mendez et al. [13].

3.5 Fresh Weight (g)

For this bio priming with *Rhizobium leguminosarum*, non-significant difference was observed in the fresh weight of the seedling. The seedling's fresh weight was discovered to be at its most for T_5 (1.87g), followed by T_4 and T_6, and at its lowest for T_1 (1.11g), which was preceded by T_7 and T_2 (Table-2). Ray and Bordolui [14] reported similar kind of experiment in marigold.

3.6 Dry Weight (g)

In terms of dry weight, the maximum dry weight of seedling was recorded for T_5 (0.19g) followed by T_6 and T_3 while minimum dry weight was observed in T_7 (0.12 g) preceded by T_1 and T_4 (Table-2). Non-significant difference was noted for dry weight in overall. This result corroborates the findings of Ray and Bordolui [15] in tomato.

3.7 Vigour Index-II

Due to priming with different concentrations of *Rhizobium leguminosarum*, the vigour index II showed non-significant difference between them. Lowest Vigour index-II was observed in T_7 (9.52) preceded by T_1 and T_4.While, T_5 (15.96) showed the highest germination index followed by $T_6$and T_3 (Table-2).Similar type of result was observed by Sujaya et al. [16].

4. DISCUSSION

Bio-priming play a great role in the growth promotion i.e. both vegetative and dry matter accumulation of plants. There are several reports regarding the use of microorganisms in the inoculation process for utilizing the maximum benefits in plant productivity as compared to the single inoculation [17], depicts the need to readdress the issue. Unpredictable of single inoculum might be due to higher resource competition with native microbes or survival problems in diverse ecological conditions [18]. Suitable microbes may produce a more synergistic effect on plant growth and development.

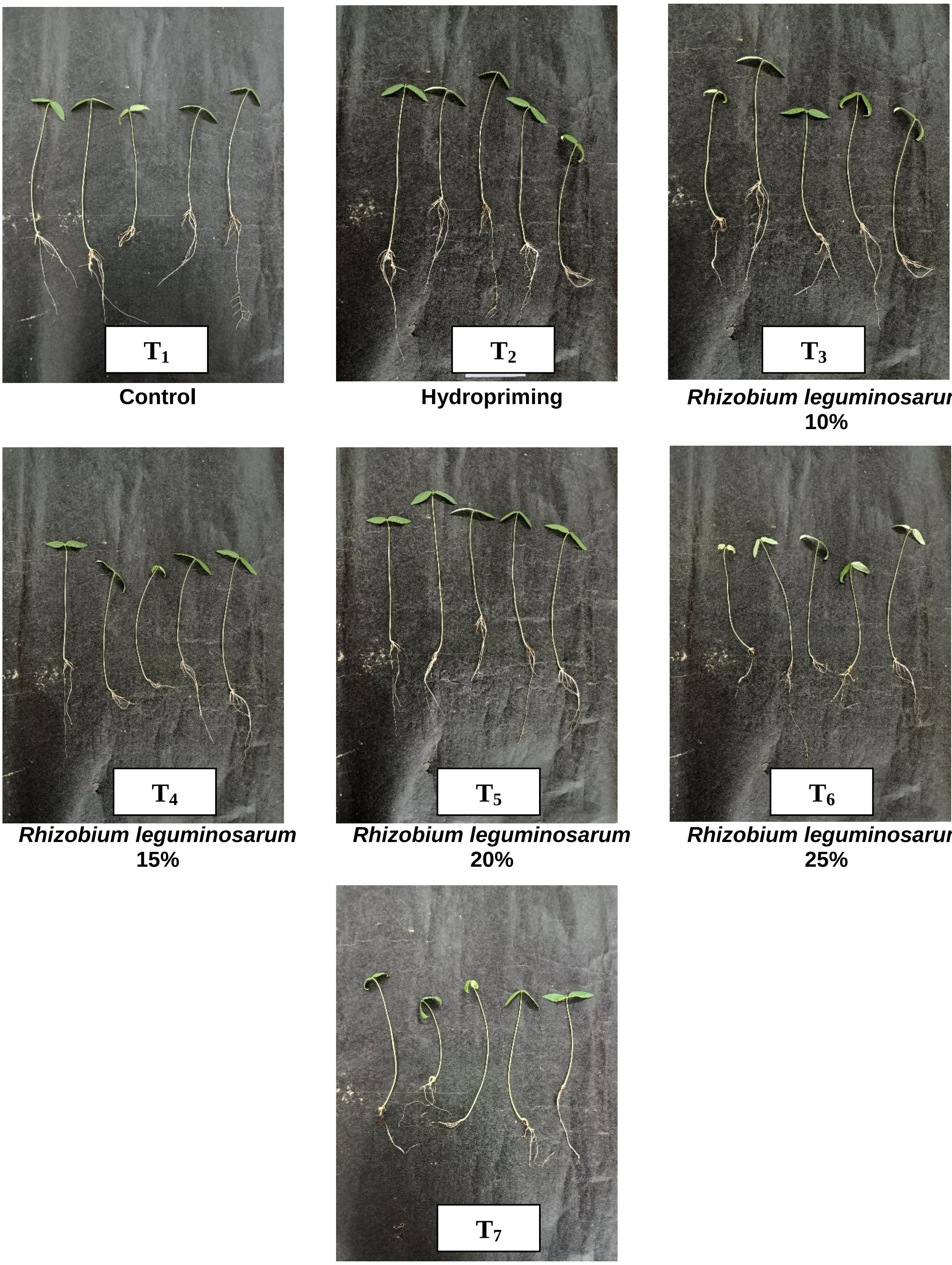
Like plants, *Rhizobium leguminosarum* are also able to produce certain phytohormones such as auxin, cytokinin, ethylene, gibberellin, abscisic acid. *Rhizobium leguminosarum* can also alter the production of phytohormones secreted by plants and thus play several roles in plant growth and development [19]. *Rhizobium leguminosarum* have a role in cell division stimulation, differentiation in meristematic tissues on the root, root hair proliferation, decrease on inhibiting lateral root formation, reducing root-shoot ratio and induce shoot growth [19].

Bio-priming involves adjusting the assimilation potential of the priming solution using *Rhizobium leguminosarum*. This adjustment helps in regulating water uptake by the seeds, ensuring that they imbibe water at an optimal rate. Controlled hydration through bio-priming triggers various physiological processes required for germination, such as the mobilization of reserves, respiration, and cell expansion. It enhances the uniformity and speed of germination, resulting in more vigorous seedlings [20,21].

Table 1. Effect of priming on germination percentage, root length, shoot lengthand vigour index-I of black gram

Treatment	Germination Percentage (Tr value)	Shoot length (cm)	Root length (cm)	Vigour Index-I
T_1	77.33 (61.55)	12.16	9.29	1,658.76
T_2	79.76 (63.25)	14.05	10.38	1,948.85
T_3	77.95 (61.98)	15.79	10.00	2,008.89
T_4	81.50 (64.51)	15.94	12.99	2,358.17
T_5	82.60 (65.32)	17.89	11.09	2,393.62
T_6	79.43 (63.01)	15.16	12.23	2,175.31
T_7	79.12 (62.79)	13.74	8.08	1,727.24
SEm (±)	**0.53**	**0.47**	**0.26**	**50.60**
LSD (0.05)	**1.62**	**1.43**	**0.79**	**154.98**

Note: Control (T_1); Seeds soaked in distilled water/ hydropriming (T_2); Rhizobium leguminosarum 10% (T_3); Rhizobium leguminosarum 15% (T_4); Rhizobium leguminosarum 20% (T_5); Rhizobium leguminosarum 25% (T_6); Rhizobium leguminosarum 30% (T_7)



Control

Hydropriming

Rhizobium leguminosarum 10%

Rhizobium leguminosarum 15%

Rhizobium leguminosarum 20%

Rhizobium leguminosarum 25%

Rhizobium leguminosarum 30%

Fig. 1. Evaluation of seedling growth under different concentration of Rhizobium

Table 2. Effect of priming on fresh weight, dry weight, and vigour index-II of black gram

Treatment	Fresh weight (g)	Dry weight (g)	Vigour Index-II
T_1	1.11	0.13	10.30
T_2	1.47	0.15	12.25
T_3	1.56	0.16	12.47
T_4	1.77	0.14	11.42
T_5	1.87	0.19	15.96
T_6	1.73	0.17	13.75
T_7	1.43	0.12	9.52
SEm (±)	0.22	0.02	1.40
LSD (0.05)	N/A	N/A	N/A

Note: Control (T_1); Seeds soaked in distilled water/ hydropriming (T_2); Rhizobium leguminosarum 10% (T_3); Rhizobium leguminosarum 15% (T_4); Rhizobium leguminosarum 20% (T_5); Rhizobium leguminosarum 25% (T_6); Rhizobium leguminosarum 30% (T_7)

5. CONCLUSION

A higher level of hormones, such as gibberellins, may have been activated in seeds treated with *Rhizobium leguminosarum* @ 20%, which would have inspired the action of specific enzymes that aided in early germination, such as amylase. As a result, assimilations of starch would have been simpler to obtain. The best germination rate occurs when pre-germination metabolic processes are completed during seed priming, preparing the seed for germination. In order to improve blackgram performance, pre-sowing treatment with 20% *Rhizobium leguminosarum* for 8 hours is advised since it outperforms other treatments, including control, in terms of improving seed germination and seedling vigour.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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