The Andhra Agric. J 71 (3): 323-327, 2024
doi :10.61657/aaj.2024.138

Efficacy of bio-priming in enhancing blackgram seed germination and vigour

A Bavana Keerthi, P Kishore Varma, S L Bhattiprolu, N Kamakshi and A Janaki prasad
Department of Plant Pathology, Acharya N G Ranga Agricultural University,
Agricultural College, Bapatla - 522101, Andhra Pradesh, India

ABSTRACT

A study was conducted to test *in vitro* efficacy of six bioagents in improving seed germination and vigour of blackgram seed. Among the bioagents tested in roll paper towel method and plastic cup method. Biopriming helps in improving germination percentage, shoot length, root length and seed vigour of the blackgram seed over the controls *i.e.,* Hydroprimed and Unprimed seed. Among the bioagents tested, *Pseudomonas fluorescens* is found effective than other bioagents by improving the seed vigour by 88.42% over unprimed seed in roll paper towel method and 86.33% over unprimed seed in plastic cup method.

Key words: *Biopriming, Plastic cup method, Roll paper towel method and Vigour.*

Blackgram is one of the most commonly grown pulse crop in India. However, the productivity was reducing year to year due to some abiotic and biotic stresses years (Nair *et al*., 2024). Healthy seeds are critical for the proper establishment of all crops because seed quality has a substantial impact on the crop's responsiveness to other inputs. It is predicted that high-quality seeds alone can enhance crop output by 18 to 20%, providing all other inputs are constant. Biopriming, a process in which seeds are treated with helpful microbes prior to sowing, has been shown to significantly improve seed germination and vigour. This strategy combines biological and physiological components to increase the overall health and performance of seeds. Biopriming promotes seed germination by making it faster and more consistent. Beneficial microbes promote water intake, boost germination enzyme activity, and break down seed dormancy, resulting in faster sprouting. Seeds Biopriming improves seedling vigour, which includes longer root and shoot lengths, higher biomass, and overall growth. This aggressive start allows seedlings to establish more efficiently in the field, resulting in stronger and more resilient plants. Biopriming helps to protect seeds and seedlings from a variety of diseases. Beneficial microbes utilized in biopriming can outcompete harmful pathogens, create antimicrobial compounds, and establish systemic resistance in plants, lowering disease incidence and severity. Bioprimed seeds exhibit greater resistance to abiotic conditions such as drought, salt, and severe temperatures. Beneficial microorganisms modulate stress-responsive pathways, enhancing the plant's ability to survive harsh environmental conditions. Biopriming is an environmentally friendly method that reduces the need for chemical treatments. By utilizing naturally occurring microbes. By utilizing naturally occurring microorganisms, it encourages sustainable farming practices while reducing the environmental impact of chemical seed treatments. Increased germination rate, seedling vigour, and overall plant health all contribute to higher agricultural yields and quality. Farmers may benefit significantly from this, as they will be able to earn higher returns on their seed and input investments.

MATERIAL AND METHODS

Healthy seeds of blackgram variety, LBG 623 were thoroughly rinsed under tap water and were surface sterilized with 1% sodium hypochlorite. Then, the seeds were washed repeatedly with sterilized distilled water to remove traces of sodium hypochlorite. After washing, the seeds were air dried and were separately soaked in a suspension of talc

based formulations (10g/l) of respective bioagents containing 10^8 CFU/g for 4 h. Soaked seeds were removed from the solution and spread over the blotter paper for drying up to original moisture content prior to sowing. Then the treated seed were tested for biopriming using two methods as follows:

Roll paper towel method

Hundred seeds from each treatment were tested for seed germination and vigour using roll paper towel method as described by Ladumor (2022). one sheet of germination paper was wetted by distilled water and hundred seeds each of respective treatment were placed on first sheet evenly. Second sheet of germination paper was placed on first sheet followed by wetting it carefully. Both sheets were rolled along with wax coated paper. The rolled papers were incubated in a seed germinator at 25 ˚C temperature for five days. At the end of incubation, rolled towel papers were carefully opened and recorded germination percentage, radicle and plumule length after five days and ten days. Unprimed and hydroprimed seeds serve as controls. At 5 DAS and 10 DAS, germination percentage, shoot length and root length were recorded. Further Seed vigour index was calculated.

Plastic cups method

After biopriming with different treatments, the treated seeds were sown in plastic cups (200 mL) filled with equal ratio of sterilized soil and sand, along with controls having hydroprimed and unprimed seed. At 5 DAS and 10 DAS, germination percentage, shoot length and root length were recorded. Further Seed vigour index was calculated.

Shoot length (cm)

List of treatments used for evaluating seed biopriming efficacy for enhancing seed germination and seed vigour

Treatment	NAME OF THE TREATMENT
T1	*Trichoderma asperellum*
T2	*T. harzianum*
T3	*Pseudomonas chlororaphis*
T4	*P. fluorescens*
T5	*Bacillus subtilis*
T6	*B. inaquosorum*
T7	Control (Hydro primed seed)
T8	Absolute control (Unprimed seed)

Germination percentage

Germination percentage was calculated as per the given formula:

$$\text{Per cent seed germination} = \frac{\text{No. of seeds germinated}}{\text{Total number of seeds examined}} X100$$

Shoot length is measured in centimeters using meter scale.

Root length (cm)

Root length is measured in centimeters using meter scale.

Seed vigour index (SVI)

Calculated seedling vigour index by formula:
SVI-I = Germination (%) X (Shoot length + Root length) in (cm).

RESULTS AND DISCUSSION

The biopriming efficacy of six bioagents was tested in blackgram by roll paper towel method and plastic cup method. In both the methods, the observations were recorded at 5DAI and 10DAI.

In roll paper towel method, observations regarding percentage of seeds with aerial growth of fungi, germination percentage, shoot length and root length were recorded. At 5DAI, the percentage of fungal growth on seeds ranged between 0-11.33% and significantly less than hydroprimed (20.67%) and unprimed seed (24.00%). Zero per cent growth of mycelium was observed when treated with. *Subtilis* and *B. inaquosorum*. The germination percentage of different treatments ranged between 91.33-100% and significantly higher than hydroprimed and unprimed seed. The 100% germination was recorded *in T. asperellum.* The seed vigour ranged between 2070-2950 in all treatments which is significantly higher than hydroprimed (1698.06) and unprimed seed (1189.29). However, higher seed vigour was observed with *P. fluorescens* (4064.96), followed by *T. asperellum* (3807.00) and *P. chlororaphis* (3696.66) (Table 1).

At 10DAI, the percentage of seeds with aerial growth of seed mycoflora was comparatively high (5.67-38.00%) than at 5DAI and significantly lower than in hydroprimed (41.00%) and unprimed seed (87.33%). The germination percentage ranges between 95.33-100% which is higher than

hydroprimed (94.67%) and unprimed seed (89.00%). Seed vigour of all treatments are on par with each other and significantly higher than the hydroprimed (2540.00) and unprimed seed (2157.36) (Table 2).

In plastic cup method, the germination percentage in bioagents ranged between 83.33-90.00% at 5DAI over hydroprimed (76.67%) and unprimed seed (66.67%). The shoot length and root length in different treatments ranged between 13.00-17.67cm and 4.50-5.33cm over hydroprimed (14.67cm, 4.50cm) and unprimed seed (8.00, 3.50cm). Highest seed vigour was observed in *fluorescens* (2040.30) followed by (1935.34) which is on par with other treatments over hydroprimed seed (1469.76) and Unprimed seed (766.71). The lowest seed vigour is observed in *harzianum* (1458.28). The per cent increase of seed vigour over unprimed seed ranged between 90.20 - 152.42% .

At 10DAI, the germination percentage ranges between 86.67-96.67 over hydroprimed (83.33) and unprimed seed (76.67). All the treatments are on par with each other and significantly higher seed vigour was observed compared to hydroprimed (2069.08) and unprimed seed (1661.44) ranging between 2739.63-3095.76. However, the highest seed vigour was observed with *P. fluorescens* (3095.76), followed by *P. chlororaphis* (2867.23), *T. asperellum* (2830.70).

Therefore, *P. fluorescens*, *P. chlororaphis* and *T. asperellum* were found effective in both the methods and on par with other bioagents. So, using bioagents for biopriming helps in improving seed germination and vigour. Naik (2015) also reported seed biopriming with *viride* for 4 hours was best with maximum seedling emergence (85.6%) and minimum incidence of root rot and seedling blight (9.0 % and 8.2 % respectively) under greenhouse condition and *P. fluorescens* being the next best and at par with *T. viride* and successfully managed seed borne pathogens and enhanced seed and crop quality parameters in garden pea. Similar results were also observed by Deshmukh *et al.* (2016), Kasherwani *et al.* (2018), Kumar *et al.* (2021) and Ladumor *et al.* (2022).

At 10DAI, the percentage of seeds with aerial growth of seed mycoflora was comparatively high (5.67-38.00%) than at 5DAI and significantly lower than in hydroprimed (41.00%) and unprimed seed (87.33%). The germination percentage ranges between 95.33-100% which is higher than hydroprimed (94.67%) and unprimed seed (89.00%). Seed vigour of all treatments are on par with each other and significantly higher than the hydroprimed (2540.00) and unprimed seed (2157.36) (Fig. 1).

In plastic cup method, the germination percentage in bioagents ranged between 83.33-90.00% at 5DAI over hydroprimed (76.67%) and unprimed seed (66.67%). The shoot length and root length in different treatments ranged between 13.00-17.67cm and 4.50-5.33cm over hydroprimed (14.67cm, 4.50cm) and unprimed seed (8.00, 3.50cm). Highest seed vigour was observed in *fluorescens* (2040.30) followed by *subtilis* (1935.34) which is on par with other treatments over hydroprimed seed (1469.76) and Unprimed seed (766.71). The lowest seed vigour is observed in *harzianum* (1458.28). The per cent increase of seed vigour over unprimed seed ranged between 90.20 - 152.42%.

At 10DAI, the germination percentage ranges between 86.67-96.67 over hydroprimed (83.33) and unprimed seed (76.67). All the treatments are on par with each other and significantly higher seed vigour was observed compared to hydroprimed (2069.08) and unprimed seed (1661.44) ranging between 2739.63-3095.76. However, the highest seed vigour was observed with *P. fluorescens* (3095.76), followed by *P. chlororaphis* (2867.23), *T. asperellum* (2830.70).

Therefore, *P. fluorescens*, *P. chlororaphis* and *T. asperellum* were found effective in both the methods and on par with other bioagents. So, using bioagents for biopriming helps in improving seed germination and vigour. Naik (2015) also reported seed biopriming with *viride* for 4 hours was best with maximum seedling emergence (85.6%) and minimum incidence of root rot and seedling blight (9.0 % and 8.2 % respectively) under greenhouse condition and *P. fluorescens* being the next best and at par with *T. viride* and successfully managed seed borne pathogens and enhanced seed and crop quality parameters in garden pea. Similar results were also observed by Deshmukh *et al.* (2016), Kasherwani *et al.* (2018), Kumar *et al.* (2021) and Ladumor *et al.* (2022).

CONCLUSION

All the bioagents showed significant

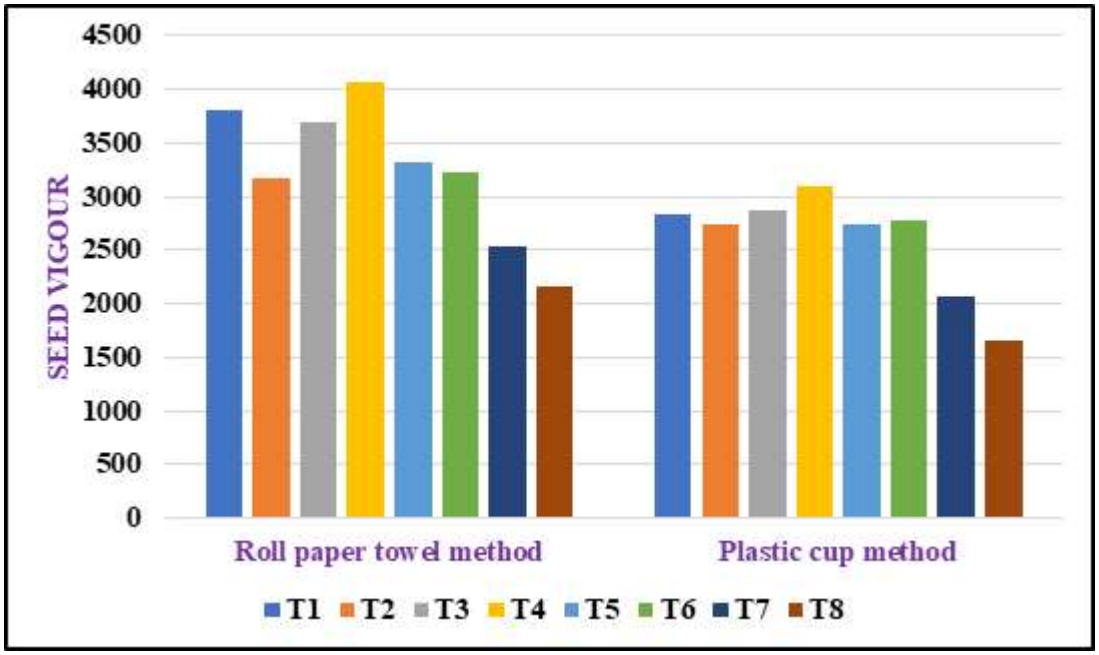

Fig. 1. Efficacy of seed biopriming with bioagents in blackgram assessed by roll paper towel and plastic cup method

T1 - *Trichoderma asperellum*, T2 - *T. harzianum*, T3 - *Pseudomonas chlororaphis*, T4 - *P. fluorescens*; T5 - *Bacillus subtilis*, T6 - *B. inaquosorum*, T7 – Hydroprimed seed, T8 – Unprimed seed.

Table 1. Effect of seed biopriming with selected bioagents on germination and vigour of blackgram seeds using roll paper towel method at 10DAI

Sl. No.	Treatment	10 DAI					
		Percentage of seeds with aerial growth of fungi	Germination percentage	Shoot length (cm)	Root length (cm)	Seed Vigour Index (SVI)	Per cent increase of seed vigour over unprimed seed
1	*Trichoderma asperellum*	5.67(2.38)^{f}	100.00^{a}	24.00^{b}	14.07^{a}	3807	76.47
2	*T. harzianum*	25.00(4.98)^{c}	95.33^{bc}	21.67^{c}	11.67^{c}	3178.3	47.32
3	*Pseudomonas chlororaphis*	10.00(3.15)^{e}	99.00^{a}	24.67^{b}	12.67^{b}	3696.66	71.35
4	*P. fluorescens*	15.00(3.87)^{d}	98.33^{ab}	27.67^{a}	13.67^{a}	4064.96	88.42
5	*Bacillus subtilis*	10.00(3.16)^{e}	98.00^{ab}	21.67^{c}	12.27^{bc}	3326.12	54.18
6	*B. inaquosorum*	38.00(6.16)^{b}	95.67^{bc}	22.00^{c}	11.67^{c}	3221.21	49.31
7	Hydroprimed seed	41.00(6.40)^{b}	94.67^{c}	17.00^{d}	9.83^{d}	2540	17.74
8	Unprimed seed	87.33(9.34)^{a}	89.00^{d}	15.67^{d}	8.57^{e}	2157.36	0
	P value	3.01e-15 ***	1.1e-05 ***	1.37e-09 ***	1.05e-09 ***		
	Sem (±)	0.143	0.943	0.565	0.261		
	C.D. ($P \leq 0.05$)	0.429	2.83	1.694	0.783		
	CV (%)	5.028	1.7	4.492	3.833		

Figures with similar alphabets doesn't differ significantly, DAI – Days after inoculation, Figures in parenthesis are square root transformed value '***' = 0.001 level of significance.

Effect of seed biopriming with selected bioagents on germination and vigour of blackgram seeds using plastic cup method at 10DAI

Sl. No.	Treatment	10 DAI				
		Germination percentage	Shoot length (cm)	Root length (cm)	Seed Vigour Index (SVI)	Per cent increase of seed vigour over unprimed seed
1	*Trichoderma asperellum*	93.33(9.66)^{ab}	23.33(4.83)^{a}	7.00(2.64)^{a}	2830.7	70.38
2	*T. harzianum*	86.67(9.31)^{abc}	24.67(4.96)^{a}	7.00(2.64)^{a}	2744.84	65.21
3	*Pseudomonas chlororaphis*	96.67(9.83)^{a}	22.33(4.71)^{ab}	7.33(2.71)^{a}	2867.23	72.57
4	*P. fluorescens*	93.33(9.66)^{ab}	25.67(5.06)^{a}	7.50(2.74)^{a}	3095.76	86.33
5	*Bacillus subtilis*	96.67(9.83)^{a}	21.67(4.65)^{ab}	6.67(2.58)^{a}	2739.63	64.89
6	*B. inaquosorum*	96.67(9.83)^{a}	21.67(4.65)^{ab}	7.00(2.64)^{a}	2771.53	66.81
7	Hydroprimed seed	83.33(9.13)^{bc}	18.33(4.27)^{bc}	6.50(2.55)^{a}	2069.08	24.54
8	Unprimed seed	76.67(8.75)^{c}	16.67(4.08)^{c}	5.00(2.24)^{b}	1661.44	
P value		0.00332 **	0.00449 **	0.000472 ***		
Sem(±)		0.176	0.152	0.057		
C.D. (P ≤ 0.05)		0.529	0.455	0.172		
CV (%)		3.216	5.652	3.841		

Figures with similar alphabets doesn't differ significantly, DAI – Days after inoculation, Figures in parenthesis are square root transformed value, '***' = 0.001 level of significance.

difference over hydroprimed and unprimed seed. However, Pseudomonas fluorescens is more effective in improving seed germination and vigour of blackgram.

LITERATURE CITED

Nair R M, Chaudhari S, Devi N, Shivanna A, Gowda A, Boddepalli V N, Pradhan H, Schafleitner R, Jegadeesan S and Somta P 2024. Genetics, genomics, and breeding of black gram [*Vigna mungo* (L.) Hepper]. *Frontiers in plant science*. 14: 1273363.

Ladumor S A, Singh P, Shekhada M R and Panara K N 2022. Field effect of seed bio priming on leaf blight (*Macrophomina phaseolina*) of blackgram. *The Pharma Innovation Journal*. 11(5): 1711-1714.

Deshmukh A J, Sabalpara A N, Prajapati V P and Shinde M S 2016. *In vitro* Investigation of seed biopriming in greengram. *International Journal for Innovative Research in Multidisciplinary Field*. 2(9): 262-265.

Kasherwani A, Lakpale N, Khare N and Tiwari P K 2018. Effect of seed borne mycoflora on seedling vigour of pea varieties and its management by seed priming. *Journal of Soils and Crops*. 28(2): 316-322.

Kumar D S S, Rao M S L and Manjunatha S E 2021. *In vitro* evaluation of seed dressing fungicides, bioagent and priming agents against pod blight complex of soybean (*Glycine max* (L.) Merrill) by rolled towel method. *Journal of Pharmacognosy and Phytochemistry*. 10(1): 1552-1555.

Received on 05.07.2024 and Accepted on 09.08.2024