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# *In vitro* Investigation of seed biopriming in black gram

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**Abstract**
The study aimed to evaluate the efficassy of seed biopriming on the seed germination and seedling vigour by controlling the seed mycoflora by Paper towel method in *in vitro* condition. Results revealed that average per cent seed germination, plumule length, radical length, seedling weight and seedling vigour index were significantly increased in seed biopriming with *T. harzianum*, *T. viride* or *P. flurescens* @10 gm talc base formulation/kg seeds followed by all the bioagents used over control.

**Keywords:** Black gram, biopriming, seedling vigour

## Introduction
Black gram (*Vigna mungo* (L.) Hepper) is commonly known as urd, udad, udid, mash and urad in India. Blackgram is originated from India (De candolle, 1986). Blackgram is an important pulse crop of Indian subcontinent, cultivated almost throughout the country in all the three seasons such as *Kharif, Rabi*, and summer. India is the largest producer of urdbean in the world. Urdbean (*Vigna mungo* L. Hepper) domesticated from *V. mungo* var. *silvestr* is belongs to the family *Fabaceae.* It has high nutritive value i.e. 25.21% protein, 1.64% lipid and 58.99% carbohydrates. They are excellent sources of B-complex vitamins. At 216 µg per 100 g, folates are plentiful in them. Folate, along with vitamin B-12, is one of the essential co-factors for DNA synthesis and cell division. Adequate folate in the diet around conception and during pregnancy may help prevent neural tube defects in the newborn baby. Urad beans also incredible sources of minerals. The beans are rich in iron at 7.57 mg per 100 g. Iron improves memory power, cognition and help prevent anemia. Other minerals in sample quantities in these beans are calcium 138 mg per 100 g, copper 0.981 mg per 100 g, magnesium 267 mg per 100 g, zinc 3.35 mg per 100 g and phosphorus 379 mg per 100 g (Anon., 2019) [1]. Blackgram suffers from several biotic and abiotic stresses, influencing production as well as quality of seeds. Seeds carries various mycoflora, externally or internally or both, which contribute a lot to quantitative and qualitative losses. The seed borne mycoflora cause pre and post- emergence mortality, seed rot, root rot, leaf blight, anthracnose, charcoal rot, wilt and many more symptoms, which impaire quality and quantity of black gram seeds and also render the seeds unfit for consumption. Seed borne pathogens are known to cause serious damage, right from sowing to harvest. The fungi *viz*., *Aspergillus flavus*, *Aspergillus niger*, *Alternaria alternata*, *Macrophomina phaseolina*, *Curvularia* spp., *Penicillium* spp., *Cladosporium cladosporioides*, *Fusarium* spp., *Drechslera* spp., *Rhizopus* spp. etc. were reported to be associated with black gram seeds (Rahiman *et al*., 1999; Shailbala and Tripathi, 2004, Rathod *et al*., 2012) [9, 10]. To increase the production of black gram qualitatively and quantitatively, farmer requires healthy quality seeds with high percentage of germination and purity. Hence, it necessitates the eradication of seed borne inoculum through various seed treatments and through the enforcement of proper domestic and international quarantine acts and procedures. Seed treatment is the oldest practice in plant protection and now, this is an attractive delivery system for either fungal or bacterial bioprotectants. The uses and expectations of seed treatments are greater today due to the impact of environmental regulations that have either banned or restricted the use of number of highly toxic fungicides such as organomercurials because of their residual toxicity. Seed treatments with bioagents provide economical and relatively nonpolluting delivery systems for protective materials compared to other field application systems. Bioprotectants applied to seeds may not only protect seeds but also may colonize and protect roots and increase the plant growth. However, biological agents have tended to be somewhat less effective and more variable than chemical seed treatments. Thus, seed treatment systems that will enhance efficacy of biological agents are needed and "biopriming" is one such attempt being made in this direction. Seed treatment with bio-control agents along with

priming agents may serve as an important means of managing many of the soil and seed-borne diseases, the process often known as bio-priming (Taylor and Harman, 1990) [13]. Thus the study was conducted to check the efficassy of various biopriming agents on the seed germination and seedling vigour by controlling the seed mycoflora by Paper towel method in lab condition.

## Materials and Methods

*In vitro* study to check the efficassy of seed biopriming on the seed germination and seedling vigour by controlling the seed mycoflora was carried out by Paper towel method. Method of seed biopriming was followed as per Taylor and Harman, 1990 [13].

1. One kg black gram (GU-1) seeds were taken from the previously collected seed samples.
2. Two liters suspension of talc based formulations (10gm/lit) of respective bioagents containing $10^8$ CFU/gm was prepared in sterilized distilled water.
3. One kg black gram seeds were then mixed in the above suspension and kept for 8 hrs.
4. Soaked seeds were then drawn out from the solution and spread over the blotter paper for drying.
5. Such seeds were used immediately for testing the efficassy *in vitro.*
6. Seeds with hydration and without any treatment served as control.

Treatment includes Talc base formulation of *Trichoderma viride, T. harzianum, T. virens, P. fluorescens, B. subtilis*, Seeds with hydration priming and absolute control. Talc based formulations of *Trichoderma* spp. and *Pseudomonas* spp. containing $10^8$ CFU/gm used in seed biopriming was the products of the Department of Plant Pathology, N.A.U., Navsari.

In case of Paper towel method, one sheet of germination paper was wetted by distilled water. Fifty 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 seed germinator at 25 $^0$C temperature for 7 days. At the end of incubation, rolled towel papers were carefully opened. The observations of the parameters like germination percentage (%), seedling lengths (plumule and radical length) (cm.), seedling fresh weight (gm) and seedling vigour index were taken after seven days with standard scientific methods and formulae.

## Results and Discussion

Data presented in the (Table 1) revealed significant effect of all bio-agents on Average seed germination, average plumule length, average radical length, average fresh weight of seedling and average seedling vigour index were significantly increased in all the treatments tested over control. Seed bioprimied with *T. harzianum* (92.67%) recorded maximum average seed germination which was at par with *T. viride* (89.33%) and next best treatment was *P. fluorescens* (86.67%) and *T. virens* (82.00%) which was followed by *B. subtilis* (79.33%). Lowest average seed germination was recorded in hydrated seed (75.33%) and control (72.00%). Maximum increase in average seed germination was recorded in *T. harzianum* (28.70%) followed by *T. viride* (24.07%), *P. fluorescens* (20.37%), *T. virens* (13.88%), *B. subtilis* (10.18%) and primed seeds with hydration (4.62%). Seed bioprimied with *T. harzianum* (12.03cm) recorded maximum average plumule length which was at par with *T. viride* (11.40cm) and next best treatment was *P. fluorescens* (9.80cm) and *T. virens* (9.43cm) which was followed by B. subtilis (8.80cm). Lowest average plumule length was recorded in hydrated seed (7.60cm) and control (6.37cm). Maximum increase in average plumule length was recorded in *T. harzianum* (89.00%) followed by *T. viride* (79.05%), *P. fluorescens* (53.92%), *T. virens* (48.16%), *B. subtilis* (38.21%) and primed seeds with hydration (19.37%).

**Table 1:** *In vitro* efficacy of seed biopriming on black gram (Paper towel method)

| Sr.no. | Treatment | Av. seed germination | | Av. plumule length | | Av. radical length | | Av. fresh weight of seedling | | Av. seedling vigour index |
|---|---|---|---|---|---|---|---|---|---|---|
| | | % | Increase (%) | cm | Increase (%) | cm | Increase (%) | mg | Increase (%) | |
| 1 | *T. viride* | 71.01* (89.33) | 24.07 | 11.40 | 79.05 | 10.83 | 79.55 | 26.93** (721.67) | 68.35 | 2058.53 |
| 2 | *T. harzianum* | 74.53 (92.67) | 28.70 | 12.03 | 89.00 | 11.07 | 83.42 | 26.87 (725.00) | 69.35 | 2061.33 |
| 3 | *T. virens* | 64.91 (82.00) | 13.88 | 9.43 | 48.16 | 8.60 | 42.54 | 25.96 (614.33) | 43.31 | 1477.07 |
| 4 | *P. fluorescens* | 68.67 (86.67) | 20.37 | 9.80 | 53.92 | 9.53 | 58.01 | 24.79 (673.67) | 57.15 | 1675.93 |
| 5 | *B. subtilis* | 62.96 (79.33) | 10.18 | 8.80 | 38.21 | 8.13 | 34.80 | 24.56 (603.00) | 40.66 | 1342.87 |
| 6 | Hydrated seeds | 60.24 (75.33) | 4.62 | 7.60 | 19.37 | 7.10 | 17.67 | 21.97 (482.67) | 12.59 | 1105.93 |
| 7 | Absolute control | 58.06 (72.00) | - | 6.37 | - | 6.03 | - | 20.71 (428.67) | - | 892.67 |
| S.Em± | | 1.25 | - | 0.20 | - | 0.13 | - | 0.23 | - | 44 |
| CD at 5% | | 3.80 | - | 0.62 | - | 0.39 | - | 0.69 | - | 133.7 |
| CV% | | 3.30 | - | 3.77 | - | 2.52 | - | 1.61 | - | 5.03 |

Seed bioprimied with *T. harzianum* (725.00mg) recorded maximum average fresh weight of seedling which was at par with *T. viride* (721.67mg) and next best treatment was *P. fluorescens* (673.67mg) which was followed by *T. virens*

(614.33mg) and *B. subtilis* (603.00mg). Lowest average fresh weight of seedling was recorded in hydrated seed (482.67mg) and control (428.67mg). Maximum increase in average fresh weight of seedling was recorded in *T. harzianum* (69.12%) followed by *T. viride* (68.35%), *P. fluorescens* (57.15%), *T. virens* (43.31%), *B. subtilis* (40.66%) and primed seeds with hydration (12.59%).

Average seedling vigour index was recorded maximum in seed bioprimed with *T. harzianum* (2061.33) which was at par with *T. viride* (2058.53). Next best treatments were *P. fluorescens* (1675.93) which was followed by *T. virens* (1477.07) and *B. subtilis* (1342.87). Comparatively lower seedling vigour index was noticed in primed seeds with hydration (1105.93). It was the lowest in the control (892.67).

There was no any report on seed biopriming in black gram. They obtained more or less similar results as observed in the present study. Seed treatments with *T. viride* and *P. fluorecens* were reported as very effective to get maximum seed germination in black gram (Suradkar, 2010) [12]. Biopriming proved very useful for better seed germination, seedling vigour and disease management in groundnut (Malathi and Doraisamy, 2004) [6], pea (Mohamedy and Baky, 2008) [7], maize (Nayaka *et al*., 2008) [8], chilli, tomato and brinjal (Someshwar and Sitansu, 2010). Seed priming changes the physiology of the seeds that enhances the seed germination, seedling vigour (Khan, 1992) [4], along with solubilization of Molybdenum which gives rise to maximization of nodulation index in legume crops (Johanson, 2004 and Kumar Rao *et al*., 2004) [3, 5]. Thus, seed priming ultimately gives better crop stand with more productive plants (Rashid *et al*.; 2004). Along with these additions of bioagents during seed priming gives an additional dimension to seed priming for proper colonization of the bioagents to the seeds (Khan, 1992) [4].

## Conclusion

Seed biopriming with *T. harzianum*, *T. viride* or *P. fluorecens* @ 10 gm talc base formulation/kg seed is a suitable method not only to get high seed germination but also to get high vigourous seedling by enhancing plumule length, radical length, seedling fresh.

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