

## ***In vitro* Investigation of Seed biopriming in Green gram**

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

**Key Words:** Green gram, biopriming, seedling vigour.

### **Introduction:**

Greengram [*Vigna radiata* (L.) Wilczek] is an important pulse crop locally known as mung bean, golden gram, mung or moong (John, 1991) and considered to be native of India, Central Asia and grown widely in number of Asian countries including Africa, USA and Australia (Agrawal, 1989). The composition of greengram seed is approximately 25.0 to 28.0 per cent protein, 1.0 to 1.5 per cent oil, 3.5 to 4.5 per cent fiber, 4.5 to 5.5 per cent ash and 62.0 to 65.0 per cent carbohydrate on a dry weight basis (Singh *et al.*, 1970; Tsou *et al.*, 1979). Like other crops, greengram is attacked by many diseases during seed germination to seed production and maturity. Over 35 fungal pathogens, few viral, bacterial and nematode species are known to attack greengram resulting into substantial yield losses (Agrawal, 1989). Among these, seed borne fungal diseases are important in reducing the yield and seed quality of greengram (Sinnha and Prasad, 1981). Most of the fungal diseases such as anthracnose, leaf spots, leaf blights and root rot causing severe losses are seed borne in nature in greengram. Hence, seeds of moong have been reported to play an important role in the dissemination of various pathogens (Rangaswami and Prasad, 1960 and Rayen, 1961). The seed infecting fungi not only damages the quantity but also quality of seed. Similarly, nutritional status of greengram seeds is likely to be hampered by depleting the protein content and by the disturbance of the reducing sugar as well as starch content due to seed mycoflora (Bilgrami, *et al.* 1978). To increase the production of Greengram 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 biagents 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). Thus the study was conducted to check the efficacy 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 efficacy 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.

1. One kg greengram (GM-3) 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 greengram 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 efficacy *in vitro*. 6. Seeds with hydration and without any treatment served as control.

Treatment includes Talc base formulation of *T. viride* (TV-1), *T. harzianum* (TH-1), *T. fasciculatum* (TF-1), *P. fluorescens*-I (PF-1), *P. fluorescens*-II (PF-2), *P. aeruginosa* (PA-1), 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  $25^{\circ}$  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 (plumule, radical and remaining seed fresh weight) (gm), seedling dry weight (plumule, radical and remaining seed dry weight) (gm) and seedling vigour index were taken after seven days with standard scientific methods and formulae.

## Results and discussion:

Results revealed (Table -1) that average per cent seed germination, plumule length, radical length, seedling fresh, dry weight and seedling vigour index were significantly increased in all the treatments tested over control. Average seed germination was significantly higher in the seed biopriming with *T. harzianum* (92.58%) as compared to the rest but was statistically at par with *T. viride* (91.31 %) and *P. aeruginosa* (90.23 %) followed by all other treatments. Average plumule length of germinated seeds was larger in the seed biopriming with *T. harzianum* (11.61cm) and was significantly superior over the rest but was statistically at par with *T. viride* (11.50cm) and *P. aeruginosa* (10.51cm) followed by all other treatments. Average radical length of germinated seeds was more in seed biopriming with *T. harzianum* (10.60cm) as compared to the rest but it was statistically at par with *T. viride* (10.50cm). Next best in order to merit was *P. aeruginosa* (9.60cm) followed by *T. fasciculatum* (9.10cm). Average seedling fresh weight of germinated seeds was maximum in the seed biopriming with *T. harzianum* (726.92 mg) which was significantly superior over the rest of the treatments but was at par with *T. viride* (720.01 mg). Next best in order to merit was *P. aeruginosa* (658.32 mg) followed by *T. fasciculatum* (602.23 mg). Average seedling dry weight of germinated seeds was higher in seed biopriming with *T. harzianum* (161.51mg) as compared to the rest but was found at par with *T. viride* (160.00mg). Next best in order to merit was *P. aeruginosa* (146.31mg) followed by *T. fasciculatum* (133.83mg). Average seedling vigour index of germinated seeds was also found higher in seed biopriming with *T. harzianum* (2304.95) as compared to the rest but was statistically at par with *T. viride* (2258.42). Next best in order to merit was *P. aeruginosa* (1937.45) followed by *T. fasciculatum* (1778.59).

There was no any report on seed biopriming in greengram but seed priming (hydration) in green gram was studied earlier by earlier by Rashid *et al.*, (2004), Arif *et al.*, (2004), Khan *et al.*, (2008) and Umair *et al* (2010). They obtained more or less similar results as observed in the present study. Seed treatments with *T. harzianum* (Rajeswari, *et al.*, 1999 and Pooja *et al.*, 2003.), *P. fluorescens* or *T. harzianum* (Sarkar and Bhattacharya, 2008) and seed bacterization with *P. fluorescens* (Minaxi and Saxena, 2010a) or *P. aeruginosa* (Minaxi and Saxena, 2010b) were reported as very effective to get maximum seed germination, seedling vigour

index and management of *M. phaseolina* with an increase in yield in greengram. Biopriming proved very useful for better seed germination, seedling vigour and disease management in groundnut (Malathi and Doraisamy, 2004), pea (Mohamedy and Baky, 2008), maize (Nayaka *et al.*, 2008), soybean (Begum *et al.*, 2009), 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), along with solubilization of Molybdenum which gives rise to maximization of nodulation index in legume crops (Johanson, 2004 and Kumar Rao *et al.*, 2004). 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). However the production of metabolites such as siderophore (a source providing iron) and chitinase (a source providing protection against pathogenic fungi) by *P. fluorescens* BAM-4 (Minaxi and Saxena, 2010a) and the production of siderophore and hydrogen cyanide (HCN) on chrome azurol S, extracellular chitinase enzyme and an important antibiotic, phenazine-I in in vitro by *P. aeruginosa* RM-3 was also observed by Minaxi and Saxena (2010b) and are responsible for antibiosis and in inducing the systemic resistance in plants and overcoming the pathogen attack in the management of seed borne as well as soil borne diseases in green gram. The work done on seed biopriming in greengram here is the first time report and was more or less similar with the results obtained in other crops by these workers.

### Conclusion:

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

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**Table-1: *In vitro* effect of seed bioprimering on green gram (Paper towel method.)**

Sr.no	Treatment	Av. seed germination		Av. plumule length		Av. radical length		Av. fresh weight of seedling		Av.dry weight of seedling		Av. seedling vigour index
		%	Increase (%)	cm	Increase (%)	cm	Increase (%)	mg	Increase (%)	mg	Increase (%)	
1	<i>T.viride</i>	72.86* (91.31)	20.73	11.50	73.19	10.50	72.98	26.83* * (720.01)	73.12	12.65* * (160.01)	75.63	2258.42
2	<i>T.harzianum</i>	74.22 (92.58)	22.41	11.61	74.85	10.60	74.63	26.96 (726.92)	74.78	12.70 (161.51)	76.73	2304.95
3	<i>T.fasiculatum</i>	70.99 (89.11)	17.82	9.97	50.15	9.10	49.92	24.53 (602.23)	44.79	11.56 (133.82)	46.87	1778.59
4	<i>P.fluorescens</i> -I	67.80 (85.73)	13.35	8.81	32.68	8.04	32.45	23.48 (551.41)	32.58	11.06 (122.52)	34.47	1436.64
5	<i>P.fluorescens</i> -II	68.03 (85.99)	13.70	9.59	44.43	8.76	44.32	24.50 (600.73)	44.43	11.55 (133.53)	46.54	1627.41
6	<i>P.aeruginosa</i>	71.80 (90.23)	19.30	10.51	58.28	9.60	58.15	25.65 (658.32)	58.28	12.09 (146.31)	60.59	1937.45
7	Seeds with hydration priming	63.84 (80.56)	6.52	7.90	18.98	7.21	18.78	22.23 (494.50)	18.90	10.46 (109.61)	20.32	1147.86
8	Absolute control (without treatment)	60.42 (75.63)	-	6.64	-	6.07	-	20.39 (415.91)	-	9.54 (91.1)	-	822.21
S.Em.±		0.95	-	0.23	-	0.19	-	0.33	-	0.22	-	31.53
C.D. at 5%		2.88	-	0.68	-	0.55	-	1.00	-	0.66	-	95.64
C.V. %		2.39	-	4.08	-	3.60	-	2.33	-	2.31	-	3.43

\*Data outside the paranthesis are ARCSIN transformed data \*\* Data outside the paranthesis are SQRT transformed data, Data inside the paranthesis are original