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RESEARCH ARTICLE :

Effect of biopriming on seed quality parameters of blackgram (*Vigna mungo* L. *Hepper*.) seeds

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SUMMARY : The experiment was conducted in laboratory at Department of Seed Science and Technology, Madurai, Tamil Nadu. during 2015 in order to find out the effect of seed priming with biological agents on germination and seedling vigour were evaluated by given priming treatment with liquid formulation *viz.*, effective micro-organisms (EM), sulphur solubilising bacteria, pink pigmented facultative methylotrophs (PPFM), rhizobium + phosphor bacteria at 1 and 2 % concentration, coconut water at 1 and 2 % and cowpea sprout extract 3 % and seed quality parameters were recorded. It was found that all the priming methods showed significant differences with the control and the highest germination (93 %), root length (18.28 cm), epicotyl length (21.15 cm), hypocotyl length (9.76 cm), seedling dry matter production (0.279 g 10 seedlings^{-1}),vigour index (4573) and field emergence (92 %) were observed in the seed primed with PPFM 2%. The study helps to improve the quality of seeds with the help of seed priming treatments which are cost effective, economic, non-toxic and ecofriendly.

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KEY WORDS :
Blackgram, Biopriming, Seedling growth, Vigour index

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BACKGROUND AND OBJECTIVES

Pulse crops have a specific importance for the vegetarian population of our country because pulses are the major source of protein. However, due to population explosion and low productivity of pulse crops, per capita availability of pulses is consistently decreasing. Per capita availability of pulses per day is only 47g as against the minimum requirement of 104g as recommended by nutritional experts of World Health Organization/Food and Agriculture Organization.

Improvements in crop yields is a highly active area of research, and as a result, today's farms are much more productive than their counterparts from a century ago. However, as the world's population increases with a concomitant decrease in farming resources, more and more emphasis is being placed on enhancing crop yields. Farmers are presently seeking ways to expand their yields while limiting the use of dangerous fertilizers and pesticides. An avenue of research which has developed from the desire to avoid harmful chemical crop treatments is the treatment of seeds or the soil with non-toxic crop augmenter prior to sowing.

Seed priming is a controlled hydration

process that involves exposing seeds to low water potentials that restrict germination, but permits pre-germinative physiological and biochemical changes (Khan, 1992). Good seed germination and seedling quality characters are important for agriculture. Uneven or poor germination and subsequently inhomogeneous seedling growth can lead to great financial losses (Ghiyasi *et al.*, 2008). Karthika and Vanangamudi (2013) reported that, seed priming is an effective seed in vigouration method to increase the rate and uniformity of emergence and crop establishment.

Biofertilizers are biologically active products or microbial inoculants of bacteria, algae and fungi (Revathi *et al.*, 2013). Microbes are effective in inducing plant growth as they secrets plant growth promoters (auxins, abscisic acid, gibberellic acid, cytokines, and ethylene) and enhance seed germination and root growth (Santner *et al*., 2009). Many scientists and researchers are now recommending seed priming with liquid formulations of biofertilizers (Gomathy *et al*., 2007; Thamizh and Thangaraju, 2007 and Martin and Maria, 2009), since they spread well and mix uniformly without any sticking agent over the seed surface (Rice and Olsen, 1992). The main goal of this study was to look for the best biological treatments that could be applied to blackgram seeds to get good quality of seedlings.

RESOURCES AND METHODS

Studies were undertaken at the Department of Seed Science and Technology, Agricultural College and Research Institute, Madurai during 2015. Genetically pure and one month old seeds of blackgram [*Vigna mungo* (L) Hepper] cv. VBN (Bg) 4 and cv. MDU 1 were used for this study.

Good quality graded seeds were soaked in the ratio of 3:1 volume of freshly prepared solutions *viz.*, effective micro-organisms, sulphur solubilizing bacteria, Pink Pigmented Facultative Methylotrophs (PPFM), Rhizobium + Phosphobacteria Liquid Formulation (LF) and coconut water in two concentrations *viz.*, one and two per cent and three per cent cowpea sprout extract by adopting the priming method of slow moistening with wet gunny for 1 hour followed by soaking for 3 hours with priming solution replacement at 2 hours and dry back to original moisture content with two replications. During drying, seeds were spread evenly on a flat surface to ensure that no radicle emergence would occur as a result of overcrowding, retention of moisture and heat buildup. Water soaked seeds served as control. Both the seeds were evaluated for following seed and seedling quality characters.

The laboratory germination test was carried out in using 4 × 100 seeds in paper medium. The test conditions of 25 ± 2°C temperature and 95 ± 3 % relative humidity were maintained in the germination room. At the end of seventh day, number of normal seedlings, abnormal seedlings and dead seeds was counted and the mean was expressed as per cent (ISTA, 1999) and also measuring the root, epicotyl and hypocotyl length of seedlings using measuring scale. The mean values were expressed in cm. Speed of germination was calculated using the formula of (Maguire, 1962) and the result was expressed in number.

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \text{------------} + \frac{X_n - X_{n-1}}{Y_n}$$

where,

X_1 - Number of seeds germinated at first count

X_2 - Number of seeds germinated at second count

X_n- Number of seeds germinated on n^{th} day

Y_1 - Number of days from sowing to first count

Y_2 - Number of days from sowing to second count

Y_n- Number of days from sowing to n^{th} count

The seedling dry matter production were determined using normal seedlings used for growth measurement were placed in paper cover and dried under shade for 24 hr and then in a hot air oven maintained at 85°C for 24 hr and the weight was recorded using an electronic balance. The mean weight was expressed as g 10 seedlings^{-1}. Vigour index values were computed using the following formulae and the mean values were expressed in whole number (Abdul-Baki and Anderson, 1973).

Vigour index 1 = Germination percentage x Total seedling length (cm).

Vigour index 2 = Germination percentage x Dry matter production (g).

For calculating field emergence per cent, hundred seeds replicated four times were sown in flat beds in field condition. The number of seedlings emerged were counted on seventh day and the mean was expressed in per cent. All the data were analysed according to the methods suggested by Panse and Sukhatme (1985).

OBSERVATIONS AND ANALYSIS

It is clear from data (Table 1) irrespective of biopriming treatments, VBN (Bg) 4 recorded the higher speed of germination of 22.96 which was 2.09 per cent higher than MDU 1(22.49) Irrespective of varieties, the maximum speed of germination occurred in T_6- PPFM 2 % (23.91) which was 11.46 per cent higher than T_{12} - water soak (21.45). Similar results were found by Ambika and Bala Krishnan (2015) and Aamir (2015) in cluster bean, Tiwari (2014) in pigeonpea, Hamidi (2013) in sunflower. The values of interaction for biopriming treatments and varieties were non-significant for speed of germination

Germination percentage was highest for bioprimed seeds of both varieties and all the biopriming treatments are significantly different and statistically superior from the control and reveals the benefit of different biopriming treatments over control. It is evident from the table that significantly maximum increase in mean seed germination per cent was registered by T_6- PPFM 2% (88.00%) which was 23 per cent higher than T_{12} control (65%).No significant results were obtained among varieties and interaction of priming treatments with varieties for germination per cent. Irrespective of varieties, the seeds primed with PPFM 2 per cent recorded the lower abnormal seedlings and dead seeds per cent (3 and 4 %) which was 6.00 and 7.00 per cent lower over water soak (Table 1).

Seedling length measured in terms of root, epicotyl and hypocotyl length also improved when subjected to biopriming treatment. Irrespective of varieties, seeds bioprimed with PPFM 2 per cent showed highest root, epicotyl and hypocotyl length *viz.*, 18.28, 21.15 and 9.76 cm, respectively and which was 12.70, 10.50 and 10.50

Table 1 : Effect of biopriming on speed of germination, germination (%), abnormal seedlings (%) and dead seeds (%) of black gram seeds

Treatments (T)	Speed of germination			Germination (%)			Abnormal seedlings (%)			Dead seeds (%)		
(Soaking at 3: 1 w/v ratio)	VBN (Bg) 4	MDU 1	Mean	VBN (Bg) 4	MDU 1	Mean	VBN (Bg) 4	MDU 1	Mean	VBN (Bg) 4	MDU 1	Mean
T_1 - Effective micro-organisms 1%	23.02	22.21	22.62	84 (66.42)	84 (66.42)	84 (66.42)	8 (16.43)	8 (16.43)	8 (16.43)	8 (16.43)	8 (16.43)	8 (16.43)
T_2 - Effective micro-organisms 2%	23.04	22.24	22.64	86 (68.03)	82 (64.90)	84 (66.42)	6 (14.18)	8 (16.43)	7 (15.34)	8 (16.43)	8 (16.43)	8 (16.43)
T_3 - Sulphur solubilizing Bacteria 1%	22.63	22.23	22.43	82 (64.90)	84 (66.42)	83 (65.65)	10 (18.43)	6 (14.18)	8 (16.43)	8 (16.43)	6 (14.18)	7 (15.34)
T_4 - Sulphur solubilizing Bacteria 2%	22.41	22.42	22.42	82 (64.90)	82 (64.90)	82 (64.90)	8 (16.43)	8 (16.43)	8 (16.43)	8 (16.43)	8 (16.43)	8 (16.43)
T_5 - Pink pigmented facultative Methylotrophs 1%	23.68	22.86	23.27	86 (68.03)	86 (68.03)	86 (68.03)	6 (14.18)	8 (16.43)	7 (15.34)	8 (16.43)	6 (14.18)	7 (15.34)
T_6 - Pink pigmented facultative Methylotrophs 2%	24.00	23.82	23.91	92 (73.57)	94 (75.82)	93 (74.66)	4 (11.54)	2 (8.13)	3 (9.97)	4 (11.54)	4 (11.54)	4 (11.54)
T_7 - Rhizobium+ Phosphobacteria LF 1%	22.97	22.49	22.73	86 (66.03)	84 (66.42)	85 (67.22)	6 (14.18)	6 (14.18)	6 (14.18)	6 (14.18)	6 (14.18)	6 (14.18)
T_8 - Rhizobium+ Phosphobacteria LF 2%	23.77	23.52	23.65	90 (71.57)	90 (71.57)	90 (71.57)	4 (11.54)	4 (11.54)	4 (11.54)	6 (14.18)	4 (11.54)	5 (12.92)
T_9 - Coconut water 1%	22.15	21.95	22.05	86 (66.03)	84 (66.42)	85 (67.22)	8 (16.43)	8 (16.43)	8 (16.43)	6 (14.18)	8 (16.43)	7 (15.34)
T_{10} - Coconut water 2%	23.36	23.16	23.26	88 (69.73)	88 (69.73)	88 (69.73)	6 (14.18)	4 (11.54)	5 (12.92)	6 (14.18)	8 (16.43)	7 (15.34)
T_{11} - Cowpea sprout extract 3%	22.74	21.78	22.26	84 (66.42)	84 (66.42)	84 (66.42)	6 (14.18)	8 (16.43)	7 (15.34)	10 (18.43)	8 (16.43)	9 (17.46)
T_{12} - Water soak	21.69	21.20	21.45	80 (63.44)	80 (63.44)	80 (63.44)	8 (16.43)	10 (18.43)	9 (17.46)	12 (20.27)	10 (18.43)	11 (19.37)
Mean	22.96	22.49	22.72	86 (68.03)	85 (67.22)	85 (67.22)	7 (15.34)	7 (15.34)	7 (15.34)	8 (16.43)	7 (15.34)	7 (15.34)
	T	V	T x V	T	V	T x V	T	V	T x V	T	V	T x V
S.E.±	0.35	0.14	0.50	0.28	0.114	0.39	0.51	0.22	0.72	0.49	0.21	0.70
C.D. (P = 0.05)	0.70**	0.29**	NS	0.56**	NS	NS	1.03**	NS	1.46	0.99**	NS	1.40*

(Values in parentheses are arc sine transformed values) * and indicate significance of value at P=0.01 and 0.05, respectively
T- Treatments; V –Variety. NS=Non-significant

Table 2 : Effect of biopriming on root length (cm), epicotyl length (cm) and hypocotyl length (cm) of black gram seeds

Treatments (T) (Soaking at 3: 1 w/v ratio)	Root length (cm)			Epicotyl length (cm)			Hypocotyl length (cm)		
	VBN (Bg) 4	MDU 1	Mean	VBN (Bg) 4	MDU 1	Mean	VBN (Bg) 4	MDU 1	Mean
T_1 - Effective micro-organisms 1%	17.57	16.36	16.97	19.78	19.81	19.80	9.26	9.42	9.34
T_2 - Effective micro-organisms 2%	17.59	16.52	17.06	19.84	20.11	19.98	9.35	9.45	9.40
T_3 - Sulphur solubilizing bacteria 1%	17.21	16.14	16.68	20.06	19.62	19.84	9.40	9.20	9.30
T_4 - Sulphur solubilizing bacteria 2%	17.60	16.07	16.84	19.80	19.47	19.64	9.31	9.00	9.16
T_5 - Pink pigmented facultative Methylotrophs 1%	17.78	16.81	17.30	20.23	20.14	20.19	9.54	9.46	9.50
T_6 - Pink pigmented facultative Methylotrophs 2%	18.61	17.95	18.28	21.27	21.02	21.15	9.71	9.80	9.76
T_7 - Rhizobium+ Phosphobacteria LF 1%	17.63	17.02	17.33	20.21	20.19	20.20	9.49	9.42	9.46
T_8 - Rhizobium+ Phosphobacteria LF 2%	18.34	17.46	17.90	20.96	20.45	20.71	9.69	9.71	9.70
T_9 - Coconut water 1%	16.75	17.00	16.88	19.67	19.97	19.82	9.22	9.37	9.30
T_{10} - Coconut water 2%	18.11	17.21	17.66	20.72	20.30	20.51	9.58	9.55	9.57
T_{11} - Cowpea sprout extract 3%	17.3	16.25	16.78	20.10	19.74	19.92	9.43	9.26	9.35
T_{12} - Water soak	16.56	15.87	16.22	19.40	18.87	19.14	9.08	8.95	9.02
Mean	17.59	16.72	17.16	20.17	19.97	20.08	9.42	9.38	9.41
	T	V	T x V	T	V	T x V	T	V	T x V
S.E.±	0.27	0.11	0.37	0.28	0.11	0.39	0.17	0.07	0.23
C.D. (P = 0.05)	0.53**	0.22**	NS	0.56**	NS	NS	0.33**	NS	NS

* and ** indicate significance of values at P=0.01 and 0.05, respectively   T- Treatments; V – Variety.   NS=Non-signficant

Table 3 : Effect of biopriming on seedling dry matter production (g 10 seedlings^{-1}), vigour index and field emergence (%) of black gram seeds

Treatments (T) (Soaking at 3: 1 w/v ratio)	Seedling dry matter production (g 10 seedlings^{-1})			Vigour index 1			Vigour index 2			Field emergence (%)		
	VBN (Bg) 4	MDU 1	Mean	VBN (Bg) 4	MDU 1	Mean	VBN (Bg) 4	MDU 1	Mean	VBN (Bg) 4	MDU 1	Mean
T_1 – Effective micro-organisms 1%	0.256	0.254	0.255	3915	3830	3873	22	21	21	82 (64.90)	84 (66.42)	83 (65.65)
T_2 – Effective micro-organisms 2%	0.259	0.252	0.256	4023	3779	3901	22	21	21	84 (66.42)	85 (67.22)	85 (67.22)
T_3 – Sulphur solubilizing bacteria 1%	0.262	0.247	0.255	4033	3777	3905	21	21	21	82 (64.90)	82 (64.90)	82 (64.90)
T_4 – Sulphur solubilizing bacteria 2%	0.247	0.241	0.244	3827	3652	3740	20	20	20	80 (63.44)	80 (63.44)	80 (63.44)
T_5 – Pink pigmented facultative Methylotrophs 1%	0.267	0.265	0.266	4089	3991	4040	23	23	23	84 (66.42)	84 (66.42)	84 (66.42)
T_6 – Pink pigmented facultative Methylotrophs 2%	0.279	0.278	0.279	4584	4562	4573	26	26	26	92 (73.57)	92 (73.57)	92 (73.57)
T_7 – Rhizobium+ Phosphobacteria LF 1%	0.262	0.262	0.262	4070	3917	3994	23	22	22	84 (66.42)	84 (66.42)	84 (66.42)
T_8 - Rhizobium+ Phosphobacteria LF 2%	0.273	0.273	0.273	4409	4286	4348	25	25	25	87 (68.87)	88 (69.73)	88 (69.73)
T_9 - Coconut water 1%	0.251	0.258	0.255	3925	3893	3909	22	22	22	82 (64.90)	83 (65.65)	83 (65.65)
T_{10} - Coconut water 2%	0.270	0.269	0.270	4234	4141	4188	24	24	24	85 (67.22)	87 (68.87)	86 (68.03)
T_{11} –Cowpea sprout extract 3%	0.260	0.252	0.256	3934	3801	3868	22	21	22	76 (60.67)	80 (63.44)	78 (62.03)
T_{12}- Water soak	0.240	0.237	0.239	3603	3595	3599	19	19	19	74 (59.34)	76 (60.67)	75 (60.00)
Mean	0.261	0.257	0.259	4054	3935	3995	22	22	22	83 (65.65)	84 (66.42)	83 (65.65)
	T	V	T x V	T	V	T x V	T	V	T x V	T	V	T x V
S.E.±	0.004	0.002	0.006	57.25	23.37	80.97	0.35	0.14	0.49	0.95	0.39	1.35
C.D. (P = 0.05)	0.008**	NS	NS	115.12**	47.00**	NS	0.70**	0.28*	NS	1.91**	0.78*	NS

(Values in parentheses are arc sine transformed values) * and ** indicate significance of values at P=0.01 and 0.05, respectively
T - Treatment; V - Variety.   NS=Non-significant

per cent higher than water soaked seeds (Table 2). Irrespective of biopriming treatment, seeds of VBN (Bg) 4 (17.59 cm) recorded 5.20 per cent higher root length than MDU 1 (16.72 cm). Where the values of interaction for biopriming treatments and varieties were non-significant for root length. Similarly, seedling drymatter production also highest inthe seeds primed with PPFM 2 % (0.279 g 10 seedling^{-1})which was 16.74 per cent higher than the water soaked (0.239 g 10 seedling^{-1}) seeds. The values for varieties and interaction between biopriming treatments with varieties were non-significant for epicotyl length, hypocotyl length and seedling dry matter production (Table 3).

Vigour Index is a measure of the speed and uniformity in germination. In general, all seeds regardless of variety responded favorably to biopriming treatment for vigour index. For vigour index 1, results were significant for biopriming treatments and varieties while non-significant for biopriming treatments x varieties interaction. Irrespective of priming treatments, V_1 recorded the highest vigour index of 4054 which was 3.02 per cent higher than V_2 (3935). Irrespective of varieties, T_6 - recorded higher vigour index of 4573 followed by T_8 - (4348) which was 27.06 and 20.81 per cent than higher than T_{12} - (3599). For vigour index 2 [Seedling dry matter production (g 10 seedling^{-1}) x Germination (%)], Irrespective of priming treatments, both varieties were on par and recorded the vigour index value of 22. Irrespective of varieties, T_6 - (PPFM 2 %) recorded the maximum vigour index (26) which was 36.84 per cent higher than water soaked seeds T_{12} - (19). The interaction between varieties and priming treatments were non-significant (Table 3).

Field emergence percentage is an important index denoting optimum plant stand of a crop. Treatments with higher values of final emergence percentage are considered more beneficial compared with those having lower values of final germination count. In the present study, Irrespective of soaking method, V_2 - (84 %) recorded 1.00 per cent higher vigour index than V_1 - (83 %). Irrespective of varieties, T_6 - (PPFM 2 %) was significantly different from rest of the treatments by recording highest field emergence of 92 which is 17 per cent higher than T_{12} - (water soak) (Table 3). Non-significant differences were also recorded for interaction of priming treatments with varieties.

The possible reasons for this beneficial effect of PPFM during germination could be the presence of Indole-3-Acetic Acid (IAA) in supernatants of Pink – Pigmented Facultative Methylotrophic (PPFMs) bacterial cultures, production of urease enzyme (Holland and Polacco, 1992) and vitamin B_{12} (Basile *et al*., 1985) in stimulation of seed germination and seedling growth (Holland, 1997).

Conclusion :

It is concluded that seeds bioprimed with liquid biofertilizers such as PPFM at 2 % found to have improved the germination and seedling vigour of blackgram seeds under laboratory and field condition.

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