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Seed invigoration treatment with sodium molybdate (Na_2MoO_4) Nutri-priming for improvement of quality performance of Bengal gram (*Cicer arietinum* L.)

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Abstract
The present investigation is dealing with the potentiality of micronutrient (Molybdenum) in terms of seed priming treatment. Since last two to three decades' various scientists are working with different seed invigoration techniques, one of them is seed priming. Seed priming is basically a controlled hydration process that involves exposing seeds to low water potentials that restrict germination, but permits pregerminative and physiological changes to occur. Therefore, upon rehydration, primed seeds may exhibit more uniform emergence, faster rate of germination, better tolerance to environmental stresses, and reduced dormancy in many species. Micronutrient priming has several beneficial effects in the morpho-physiological aspects of the plants. In most cases, micronutrient application through seed treatment has astonishingly performed better than other application methods. Being an easy and cost effective method of micronutrient application, seed priming offers an attractive option for resource-poor farmers. The present experiment therefore an attempt has been made to study the effects of seed priming with T_1= 250 ppm Na_2MoO_4 for 6 hrs, T_2 = 250 ppm Na_2MoO_4 for 8 hrs, T_3 = 250 ppm Na_2MoO_4 for 10 hrs, T_4 = 500 ppm Na_2MoO_4 for 6 hrs, T_5 = 500 ppm Na_2MoO_4 for 8 hrs, T_6 = 500 ppm Na_2MoO_4 for 10 hrs T_7 = 750 ppm Na_2MoO_4 for 6 hrs, T_8 = 750 ppm Na_2MoO_4 for 8 hrs, T_9 = 750 ppm Na_2MoO_4 for 10 hrs, T_{10}= 1000 ppm Na_2MoO_4 for 6 hrs, T_{11}= 1000 ppm Na_2MoO_4 for 8 hrs, T_{12}=1000 ppm Na_2MoO_4 for 10 hrs and dry seeds as control in laboratory condition. From the experiment, it can be concluded that 500 ppm sodium molybdate soaking for 8 hrs was the best performer than other priming materials as it was highest performer in germination percentage (95.60), seedling fresh weight (1.54 g), seedling dry weight (0.15 g), seedling vigour Index-I (2010.60), time to 50% germination (2.68 days) and germination index (28.30).

Keywords: Germination, invigoration, priming, sodium molybdate, vigour

Introduction
Bengal gram (*Cicer arietinum* L.) belong to sub family Papilionaceae of the family Leguminaceae, is an important pulse crop of the semi-arid tropics, particularly in the rainfed ecology of the Indian subcontinent. It is the world's third-most important food legume (pulse) and is consumed as a high-quality protein food with India being the world's largest producer and consumer of pulses. Bengal gram contains 17-22% protein and 60-64% carbohydrates (Joshi, 2001) [13]. Bengal gram helps in sustaining the productivity of the cropping systems through their ability to fix atmospheric nitrogen. India is the largest Bengal gram producing country accounting for 64% of the global Bengal gram production.

Seed priming is a pre-sowing strategy for influencing seedling development by modulating pregermination metabolic activity prior to emergence of the radicle and generally enhances rapid, uniform emergence and plant performance to achieve high vigour and better yields. Seed priming is one of the most important physiological methods which enhances the seed performance and provides faster and synchronized germination (Chakraborty and Bordolui, 2021) [5]. It is a commercially used technique for improving seed germination and vigour. It involves imbibition of seeds in water under controlled conditions up to the point of radical emergence followed by drying the seed back to the initial moisture content of the seeds. The primed seeds give earlier, more uniform and sometime greater germination and seedling establishment and growth (Bradford, 1986) [3].

Among all known micronutrients molybdenum requirements are lowest amount (Mandal and Bose, 2019) [19]. But, it plays a key role in plants regarding nitrate assimilation and fixation. Nitrate from the soil is takenup and assimilated with the help of the enzyme Nitrate reductase which contains Mo.

Mo deficiency in plants leads to accumulation of nitrates in leaves, not being assimilated into proteins. In leguminous plants, Mo is associated with symbiotic nitrogen fixation in root nodules involving bacteria (*Rhizobium* sp.) to fix atmospheric nitrogen (Campo, Albino, and Hungria 2000) [4]. Therefore, the nitrogen-fixing ability of soil microorganisms is adversely affected by Mo deficiency in legumes, causing N-deficiency in them. Kumar Rao *et al*. (2004) [15] did an experiment with primed Bengal gram seeds at 500 ppm solution of sodium molybdate for 8 hours resulted in yield increase of 20% in field experiment as compared to soil application. Similar kind of observation was noticed by Kulkarni and Sharma (1998) [14]. In addition, seeds of common bean while primed with sodium molybdate were found to improve nodulation, dry matter accumulation, nitrogen fixation and yield (Mohandas 1985) [18]. Incorporation of Rhizobium culture to priming solution along with Mo can enhance the efficiency of productivity of seed. Priming of green gram (*Vigna radiata* L.) seed in sodium molybdate and rhizobia was found to improve nodulation, N fixation, nutrient uptake, plant growth and crop yield significantly as reported by Johansen *et al*. (2007) [12].

Materials and Methods

The experiment was carried out in seed testing laboratory, Department of Seed Science and Technology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India during 2021 following Complete Randomized Design with three replications. For this investigation, Anuradha variety of bengal gram was collected from AICRP on Chickpea, BCKV, Mohanpur, Nadia, West Bengal. Different seed primings were done with T_1= 250 ppm Na_2MoO_4 for 6 hrs, T_2 = 250 ppm Na_2MoO_4 for 8 hrs, T_3 = 250 ppm Na_2MoO_4 for 10 hrs, T_4 = 500 ppm Na_2MoO_4 for 6 hrs, T_5 = 500 ppm Na_2MoO_4 for 8 hrs, T_6 = 500 ppm Na_2MoO_4 for 10 hrs T_7 = 750 ppm Na_2MoO_4 for 6 hrs, T_8 = 750 ppm Na_2MoO_4 for 8 hrs, T_9 = 750 ppm $Na_2MoO_4$for 10 hrs, T_{10}= 1000 ppm Na_2MoO_4 for 6 hrs, T_{11}= 1000 ppm Na_2MoO_4 for 8 hrs, T_{12}=1000 ppm Na_2MoO_4 for 10 hrs.

Germination Parameters

Time to 50 % germination

According to Association of Official Seed Analysis (1983) [2] number of seeds germinated was recorded in daily basis. The time to obtain 50% germination (T_{50}) was calculated according to the following formulae given by Coolbear *et al*. (1984) [7] which was modified by Farooq *et al*. (2005) [9].

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{(n_j - n_i)}$$

Where, N stands for final number of germination and ni, nj are cumulative number of seeds germinated by adjacent counts at times ti and tj when ni< N/2 <nj.

Mean germination time (MGT)

Mean germination time (MGT) was calculated with the following equation suggested by Ellis and Roberts (1981) [18].

$$MGT = \frac{\sum D_n}{\sum n}$$

Where n indicates the number of seeds germinated on day D, and D is the number of days counted from the beginning of germination.

Germination percentage

Germination percentage (G) was calculated as:

$$\frac{\text{Number of normal seedlings produced}}{\text{Total number of seeds used}} \times 100$$

Where, X is the number of normal seedlings produced and Y denotes total number of seeds taken for germination (ISTA, 1996)] [11]. It is expressed in percentage.

Germination index (GI)

Germination index (GI) was calculated as described in the Association of Official Seed Analysts (AOSA, 1990) as the following formulae:

$$GI = \frac{\text{No. of germinated seeds}}{\text{Day of first count}} + - - - - + \frac{\text{No. of germinated seeds}}{\text{Day of last count}}$$

Germination Energy

According to Ruan *et al*. (2002) [24] energy of germination (GE) should be recorded at 4th day after planting. It is the percentage of germinating seeds 4 days after planting relative to the total number of seeds tested.

Seedling parameters

Root lengths and shoot lengths of ten seedlings were measured at 8 days after germination by glass plate method in the laboratory with the help of a scale and graph paper and average was made out, expressed in centimetre (cm). Fresh weight of ten seedlings was measured with the help of a digital balance. Then seedlings were dried at 60-70 ℃ for two hours in hot air oven and weighed in a digital balance. Both seedling fresh weight and dry weight are expressed in gram (g).

Vigour index

Vigour index (VI) 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).

Results and Discussion

Germination Index

Germination Index significantly varied due to priming with different duration and concentration of sodium molybdate. Lowest Germination index was observed in control (21.10 days) preceded byT_4 and T_{12}. While, T_5 (28.30 days) showed the highest germination index followed by T_8 and T_7. But, non-significant difference was observed in between T_1 and T_9; T_4 and T_{12}; T_2 and T_6; T_2, T_3, T_7 and T_8. Similar type of result was observed by Mohandas (1985) [18] in bean (*Phaseolus vulgaris* L.); Choudhury, A. and Bordolui S.K. (2022) [6] in Bengal gram.

Root length

Root length significantly varied due to priming with different duration and concentration of. Lowest Germination index was observed in T_2 (3.42 cm) preceded by T_0, T_8 and T_7. While, T_{12} (6.34 cm) showed the highest Germination index followed

by T_9, T_5 and T_3. But, non-significant difference was observed in between T_0, T_7 and T_8; T_1, T_3, T_4, T_5, T_{10} and T_{11}; T_6 and T_7; T_9 and T_{12}. Root and shoot length increased in seeds due to priming as compared to non-primed seeds reported by Ray and Bordolui (2022b) [23]. Sharma and Parmar (2018) [25] conducted similar kind of experiment on rain-fed maize and pea.

Shoot length

Considering shoot length, the longest seedling shoot length was recorded for T_1 (15.70 cm) followed by T_5 and T_8 while shortest shoot length was observed in Control (7.56 cm) preceded by T_3 and T_4. Significant difference was noted for shoot length in overall through non- significant difference was observed in between T_9 and T_{10}; T_{11} and T_{12}; T_7 and T_{10}; T_7 and T_8; T_5 and T_8; T_1 and T_5. The result corroborates the findings of Hassan *et al*. (2018) [10].

Fresh weight (g)

Significant difference was observed in fresh weight for sodium molybdate. Highest seedling fresh weight was observed for T_8 (1.60 g) followed by T_5 and T_1while lowest was noted in T_0 (0.91 g) preceded by T_2 and T_7 respectively. But non- significant difference was noticed in between T_0 and T_{12}; T_1. T_3, T_5 and T_{10}; T_2, T_4, T_6, T_7, T_9 and T_{11}; T_5 and T_8. Singh *et al*. (2017) [26] noticed similar kind of experiment on seed quality parameters of field pea (*Pisum sativum* L.).

Dry weight (g)

In case of dry weight, it was significantly varied due to priming with different duration and concentration of sodium molybdate. Maximum seedling dry weight was noticed for T_5 (0.15 g) followed by T_{10} and T_3 while minimum (0.09 g) were noticed for T_0 and T_8 preceded by T_{11} and T_{12} respectively. Although non-significant difference was observed in between T_0, T_{11}, T_{12} and T_8; T_1, T_2, T_3, T_4, T_7, T_9, $T_{11}$and T_{12}; T_1, T_2, T_3, T_4, T_7, T_9, T_{10}, $T_{11}$and T_{12}; T_3, T_5 and T_{10}.

Table 1: Effect of priming on germination index, root length, shoot length, fresh weight and dry weight of Bengal gram

Treatment	Germination Index	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
T_0	21.10	4.00	7.56	0.91	0.09
T_1	25.38	5.03	15.70	1.44	0.12
T_2	27.06	3.42	11.19	1.20	0.12
T_3	27.46	5.46	10.53	1.40	0.13
T_4	23.06	5.28	10.58	1.28	0.12
T_5	28.30	5.68	15.35	1.54	0.15
T_6	26.96	4.90	10.50	1.25	0.09
T_7	27.45	4.39	14.79	1.22	0.12
T_8	27.46	4.15	15.22	1.60	0.09
T_9	25.52	5.94	14.25	1.30	0.12
T_{10}	24.82	5.30	14.49	1.43	0.14
T_{11}	26.10	5.31	12.07	1.33	0.11
T_{12}	23.34	6.34	12.16	0.90	0.11
S.Em (±)	0.16	0.235	0.146	0.048	0.008
LSD (0.05)	0.46	0.687	0.427	0.140	0.024

Note: T_0 = Control, T_1= 250 ppm Na_2MoO_4 for 6 hrs, T_2 = 250 ppm Na_2MoO_4 for 8 hrs, T_3 = 250 ppm Na_2MoO_4 for 10 hrs, T_4 = 500 ppm Na_2MoO_4 for 6 hrs, T_5 = 500 ppm Na_2MoO_4 for 8 hrs, T_6 = 500 ppm Na_2MoO_4 for 10 hrs T_7 = 750 ppm Na_2MoO_4 for 6 hrs, T_8 = 750 ppm Na_2MoO_4 for 8 hrs, T_9 = 750 ppm $Na_2MoO_4$for 10 hrs, T_{10}= 1000 ppm $Na_2MoO_4$for 6 hrs, T_{11}=1000 ppm Na_2MoO_4 for 8 hrs, T_{12}=1000 ppm Na_2MoO_4 for 10 hrs.

Germination Percentage

Significant difference was observed in germination percentage. Among the priming treatments, with various duration and concentration of sodium molybdate, T_5 (95.60%) recorded highest germination percentage followed by T_8 and T_{10}. While lowest germination percentage was recorded for T_0 (85.83) preceded by T_1, T_{12} and T_2 respectively. But, non-significant difference was observed in between T_1, T_2, T_4 and T_{12}, T_2, T_7 and T_9; T_3, T_6, T_7, T_9, T_{10} and T_{11}; T_5 and T_8. This result is in agreement with the experiment conducted by Munawar *et al*. (2013) [20] on carrot (*Daucus carota* L.); Pradhan *et al*. (2013) [21] on Cowpea (*Vigna unguiculata* L. Walp).

Vigour Index

Considering vigour index, maximum value was calculated for T_5 (2010.60) followed by T_8 and T_{10} respectively; minimum vigour index was noted for T_0 (992.18) preceded by T_2 and T_6. Although, vigour index was significantly varied, but some non –significant difference was also noticed in between T_{11} and T_{12}; T_1, T_8, T_9 and T_{10}; T_6, T_3 and T_4. Masuthi *et al* (2009) [17] observed similar kind of experiment on wheat (*Triticum aestivum*) under manganese deficiency.

Time to 50 % germination (days)

Significant responses were noticed in the priming treatment with different duration and concentration of sodium molybdate under laboratory condition. Minimum time to 50% germination was recorded in T_5 (2.45 days) followed by T_6 and T_3. While, maximum time to 50% germination was observed for T_4 (3.61 days) preceded by T_{12} and T_0. Although, time to 50% germination was significantly varied, but some non –significant difference was also noticed in between T_0, T_4, T_{10} and T_{12}; T_1, T_9 and T_{11}; T_3, T_5 and T_6; T_6, T_7 and T_8; T_3, T_7 and T_8. Ray and Bordolui (2022a) reported similar type of result in tomato (*Lycopersicum esculentum*).

Mean germination time (days)

Considering Mean germination time T_4 (3.47 days) had the shortest mean germination time preceded by T_9 and T_6. While maximum Mean germination time was noticed in T_0 (4.40 days) followed by T_{12} and T_{10}. Though significant difference was observed in mean germination time but some non –significant difference was also noticed in between T_1, T_2, T_3, T_5, T_6, T_8, $T_9$and T_{11}; T_3, T_5, $T_{10}$and T_{12}; T_1, T_4, T_6 and T_7; T_0, $T_{10}$and T_4. This result is in agreement with the experiment conducted by Ullah *et al*. (2005) [27].

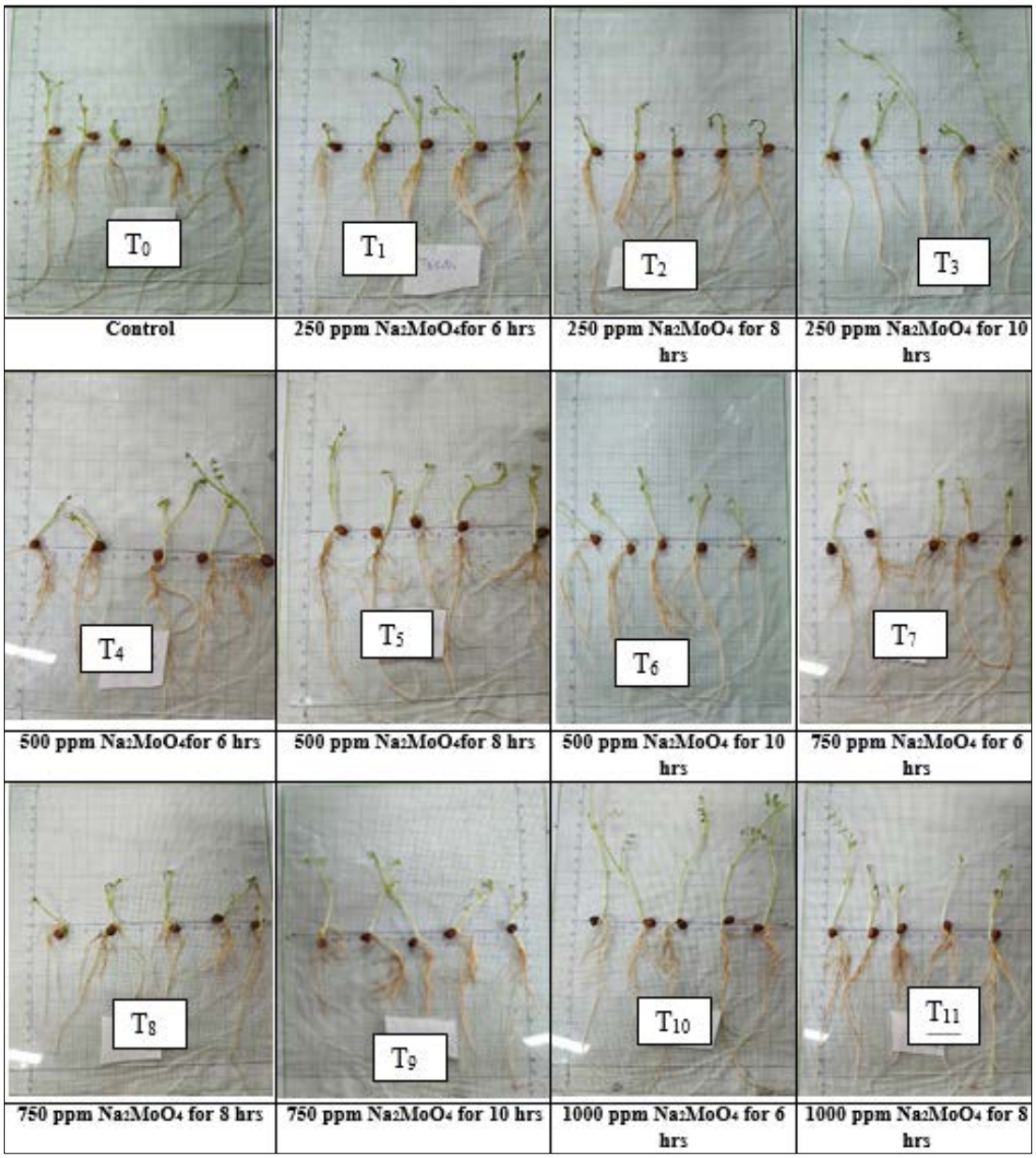
Germination energy (%)

Significant difference was observed in germination energy for sodium molybdate. The maximum energy of germination was recorded in T_7 (69.50) followed by T_2 and T_6 while it was minimum for T_0 (51.57) preceded by T_4 and T_{10}. But non-significant difference was observed in between T_o and T_4; T_3, T_5, T_9 and T_{11}; T_8 and T_{12}; T_3 and T_6. Mahajan *et al*. (2011) [16] observed in rice that seed priming treatments enhanced the energy of germination over that of untreated seeds. Johnson *et al*. (2005) came to a conclusion that low vigour seeds of Bengal gram, lentil and rice showed significant decrease in mean germination time and increase in germination index as well as germination energy over non-primed low vigour seeds after priming with micronutrient.

Table 2: Effect of priming on germination percentage, vigour index, Time to 50% germination, Mean germination time and Germination energy of Bengal gram

Treatment	Germination Percentage	Vigour Index	Time to 50% germination (days)	Mean germination time (days)	Germination energy (%)
T_0	85.83 (67.86)	992.18	3.55	4.40	51.57 (45.88)
T_1	88.23 (69.62)	1829.24	3.04	3.75	61.47 (51.61)
T_2	88.70 (70.35)	1295.62	3.27	3.86	67.57 (55.27)
T_3	91.80 (73.38)	1467.70	2.80	3.94	64.40 (53.35)
T_4	89.47 (71.04)	1418.64	3.61	3.47	51.58 (45.89)
T_5	95.60 (77.89)	2010.60	2.68	3.97	63.56 (52.85)
T_6	91.40 (72.93)	1407.64	2.78	3.74	65.47 (53.99)
T_7	90.47 (72.00)	1734.34	2.93	3.64	69.50 (56.46)
T_8	95.40 (77.62)	1847.89	2.85	3.89	57.33 (49.20)
T_9	90.47 (72.00)	1826.44	3.05	3.71	64.02 (53.12)
T_{10}	92.87 (74.55)	1838.04	3.53	4.14	53.66 (47.08)
T_{11}	92.20 (73.79)	1601.84	3.18	3.78	64.09 (53.16)
T_{12}	88.47 (70.14)	1636.94	3.58	4.22	56.87 (48.93)
S.Em(±)	0.669	27.157	0.055	0.096	0.379
LSD (0.05)	1.96	79.381	0.160	0.280	1.107

Note: T_0 = Control, T_1= 250 ppm Na_2MoO_4 for 6 hrs, T_2 = 250 ppm Na_2MoO_4 for 8 hrs, T_3 = 250 ppm Na_2MoO_4 for 10 hrs, T_4 = 500 ppm Na_2MoO_4 for 6 hrs, T_5 = 500 ppm Na_2MoO_4 for 8 hrs, T_6 = 500 ppm Na_2MoO_4 for 10 hrs T_7 = 750 ppm Na_2MoO_4 for 6 hrs, T_8 = 750 ppm Na_2MoO_4 for 8 hrs, T_9 = 750 ppm Na_2MoO_4 for 10 hrs, T_{10}= 1000 ppm $Na_2MoO_4$for 6 hrs, T_{11}=1000 ppm Na_2MoO_4 for 8 hrs, T_{12}=1000 ppm Na_2MoO_4 for 10 hrs.



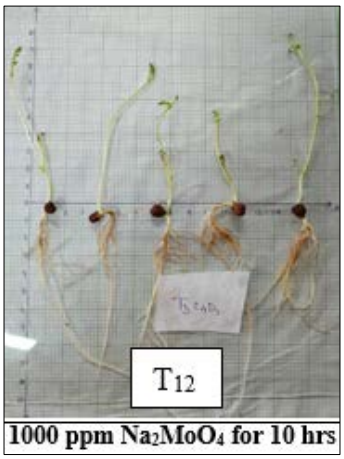


Fig 1: Evaluation of seedling vigour under laboratory condition

Conclusion

Various concentration and duration of sodium molybdate treated seeds of Bengal gram recorded higher seed quality parameters compared to control. Seeds treated with 500 ppm Na_2MoO_4 showed the significant results. It can be concluded from the results of the experiment that among all the treatments, 500 ppm Na_2MoO_4 for 8 hrs showed significant performance for seed quality parameter like germination (%), seedling fresh weight (g), seedling dry weight (g), seedling vigour Index-I, mean germination time (days), time to 50% germination(days) and germination index. Therefore, pre-sowing treatment with 500 ppm Na_2MoO_4 for 8 hrs is recommended for better performance of Bengal gram.

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