

Original Research Article

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## Effect of Different Priming Methods on Nodulation in Blackgram (*Vigna mungo* L.) Seeds

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### ABSTRACT

#### Keywords

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Nodulation,  
PEG6000, CaCl<sub>2</sub>,  
NaCl, Tulsi and  
Curi leaf extract.

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The experiment was conducted in Field Experimentation Centre, Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad (U.P.) during *kharif* season 2016, in order to standardize the best method of priming specific to Blackgram (var. PU-31). Four methods of priming *viz.*, hydropriming, osmopriming, halopriming, and organic priming with control (Unprimed) were evaluated by screening 12 hour duration and different concentrations *viz.*, T<sub>0</sub>-Unprimed (Control), hydropriming - T<sub>1</sub>-Distilled water (hydration), osmopriming- T<sub>2</sub>-Polyethylene Glycol (PEG<sub>6000</sub>) 20%, halopriming- T<sub>3</sub>-Sodium Chloride (NaCl) 1%, T<sub>4</sub>-Calcium Chloride (CaCl<sub>2</sub>) 1%, organic priming- T<sub>5</sub>-Tulsi leaf extract (5%), T<sub>6</sub>-Curi leaf extract (5%). It was found that all the priming treatment showed significance difference with the control and the highest field emergence per cent and plant nodulation characters were observed for PEG<sub>6000</sub> priming. Highest nodulation was observed in PEG and CaCl<sub>2</sub>. Seed priming, its simplicity and no requirement for expensive equipment and chemical could be used as a simple method for overcoming related to a poor germination and seedling establishment and helps in sustaining agriculture and cost effective and economic, non-toxic and eco-friendly sources.

### Introduction

Pulses have been in focus in recent times due to the continuous upswing in their prices. Stagnant productivity coupled with declining availability has created substantial demand supply gap, forcing heavy import bill on the exchequer and affecting nutritional security of majority of the population for whom pulses are the one of the cheapest sources of protein. Besides serving as an important source of protein for a large portion of the global population, pulses contribute to healthy soils and climate change mitigation through their nitrogen-fixing properties (Mohanty and Satyasai, 2015). Pulses constitute an

important ingredient in predominantly vegetarian diet and are important source of protein that nutritionally balances the protein requirement of vegetarian population. They supply minerals and vitamins and provide an abundance of food energy. Pulses provide a cheaper source of nutrients/proteins as they generally contain nearly twice as much as protein as that of cereals and hence correctly called poor man's meat.

Pulses are also important for sustainable agriculture enriching the soil through biological nitrogen fixation, fixes about 40-50

kg of N/ha (Hariprasanna and Bhatt, 2002). Blackgram (*Vigna mungo* L.) is an important nutritious pulse crop occupying unique position in Indian Agriculture, belongs to family Leguminosae with chromosome number  $2n=2x=22$  (Malik, 1994).

Black gram is a third most important pulse crop of India representing 15% of the total pulse area and 9% total pulse production. Black gram gives low seed yield mainly due to poor management and low soil fertility. Nitrogen due to leaching and volatilization and phosphorous due to fixation may not be available adequately at flowering and pod formation stages of crop and result in shedding flowers and pods (Hamid, 1988). Blackgram crop is one of the important pulse crops, grown throughout the century. It belongs to the family Leguminosae and sub-family Papilionaceae. It is also cultivated in many other tropical and sub-tropical countries of the world, It is commonly known as urdbean. The crop is resistant to adverse climatic conditions and improves the soil fertility by fixing atmospheric nitrogen in the soils. It has been reported that the crop produces equivalent to 22.10 kg N ha<sup>-1</sup> which has been estimated to be supplement of 59 thousand tones urea annually. The pulse, black gram plays an important role in India diet. It contains vegetable protein and supplement to cereal based diet, it contains about 26% protein, which is almost three times that of cereals and other minerals and vitamins. Besides, it is also used as nutritive fodder, especially for mulch animals.

Priming is the method used to improve stand establishment in several crops. Seed priming is a process in which seeds are imbibed either in water or in osmotic solution or combination of solid matrix carrier and water in specific proportion followed by drying before radical emergence. The theory of seed priming was proposed by Heydecker in 1973. Seed

priming is an effective technology to enhance rapid and uniform emergence and to achieve high vigour, leading to better stand establishment and yield (Harris *et al.*, 2007). Halopriming was conducted with salt NaCl 1% solution and CaCl<sub>2</sub> 1% solution concentration. Seeds were soaked 25<sup>0</sup>C for 14 hour in Petri plates. After dried and at room temperature and subjected to germination test, done at 25<sup>0</sup>C for 14 hrs. Evaluated the effects of NaCl priming with KNO<sub>3</sub> on the germination traits and seedling growth of four *Helianthus annuus* L. cultivars under salinity conditions and reported that germination percentage of primed seeds was greater than that of un-primed seeds Bajehbaj, (2010). Seed priming has presented promising, and even surprising results, for many seeds including the cereal seeds Bradford, (1986). In which organic priming plant extract is used after that drying, such as Neem leaf extract, Curi leaf extract, Tulsi leaf extract, neem seed kernel powder for maize. A higher concentration of this powder (10% w/w) was found effective over long period of time Sharma (1995).

### Materials and Methods

The experiment was conducted in Field Experimentation Centre, Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad (U.P.) during kharif season 2016, in order to standardize the best method of priming specific to Blackgram (var. PU-31). The treatments used at different concentrations for priming were T<sub>0</sub>-Unprimed (Control), T<sub>1</sub>-Distilled water, T<sub>2</sub>- Polyethylene Glycol (PEG<sub>6000</sub>) 20%, T<sub>3</sub>-Sodium Chloride (NaCl) 1%, T<sub>4</sub>-Calcium Chloride (CaCl<sub>2</sub>) 1%, T<sub>5</sub>-Tulsi Leaf Extract 5% and T<sub>6</sub>-Curi Leaf Extract 5%. After cleaning and grading, the seeds were soaked in respective priming solutions at different volume of seeds for

twelve hours. Then the seeds were air dried under the shade to bring back to their original moisture content and used for sowing on field.

### **Preparation of solutions**

For the preparation of solution one gram of each chemical was taken in a beaker. These chemicals were added separately in 1000 ml. of distilled water with constant stirring. The volume of solution will finally constituted to one liter, then it become 1000 ppm stock solution of each chemical.

The flasks containing chemicals was covered with muslin cloth to avoid any contamination. For the preparation of PEG<sub>6000</sub> (20%) solution 200 (g) PEG was a taken in a measuring flask made up to 1000 ml. distilled water, while for NaCl (1%) solution 10(g), CaCl<sub>2</sub> (1%) solution 10 (g), Tulsi leaf extract (5%) solution 50 (gm) and Curi leaf extract (5%) solution 50 (gm) was taken in a measuring flask and made up to 1000 ml with distilled water.

### **Preparation of plant leaf extract**

The fresh leaves of the tulsi and curi plants were collected separately and dried in shade. The shade dried leaves were powdered using mortar and pestle. Then exactly weigh fifty gram of leaf powder using weighing balance and dissolved in 100 ml. of distilled water which was measured already in the beaker to make 5% leaf extract. The leaf extract was filtered by using muslin cloth to remove unwanted material and leaf debris.

Randomized Block Design (RBD) (Panse and Sukhatme, 1967) with three replications was performed. Observations on field *viz.*, field emergence and nodulation characters were worked out and the data was statistically analyzed using ANOVA.

Number of seedling emerged on 15<sup>th</sup> days  
Field emergence (%) = -----×100  
Total no. of seeds sown

One hundred seeds from each treatment in four replications were used for field emergence studies. The seeds were sown in well prepared soil at 3 cm deep. The field emergence count was taken on the 15<sup>th</sup> day after sowing and the emergence percentage was calculated taking into account the number of seedlings emerged three centimeter above the s

### **Number of nodules per plant**

Ten plants from each treatment plot in three replication were uprooted 30 and 45 days after seeding (DAS), and the extent of nodulation was estimated by carefully washing the roots and detaching the nodules before counting (Khan *et al.*, 2006).

### **Nodules fresh weight**

After washing the root nodules from the field the nodules were detached from the plant roots and weighed in an electronic weigh balance for fresh weight of root nodules expressed in milligrams (mg).

### **Nodules dry weight**

After taking the fresh weight the root nodules they are kept in butter paper and kept in oven for drying at 80°C for 24 hrs and then weighed for dry weight of nodules expressed in milligrams (mg) (Khan *et al.*, 2006).

### **Results and Discussion**

According to the results, all studied traits were affected by the treatments and there was completely significant difference between control (non-primed seeds) and primed seeds (Table 1). Field emergence and nodulation

characters viz., Field emergence per cent, Number of nodules per plant, Nodules fresh weight (mg), Nodules dry weight (mg) were affected by PEG<sub>6000</sub> 20% concentration significantly recorded maximum where found in lowest control (Table 2).

Osmopriming with PEG (T<sub>2</sub>) recorded maximum field emergence per cent (82.67%) followed by halopriming with CaCl<sub>2</sub> (T<sub>4</sub>) (81.67%) where found in lowest control (T<sub>0</sub>) (75.33%). The interaction effect on emergence percentage is statistically significant Nagar *et al.*, (1998). Hussian *et al.*, (2014) states that Priming is one of the physiological ways which enhances performance of seed and seeds show rapid and synchronized germination and also show the importance of seed vigor and role of seed enhancement treatments in abiotic stress tolerance.

Osmopriming with PEG (T<sub>2</sub>) was observed numerically in maximum numbers of nodules (24.00) and (33.00) followed by halopriming with CaCl<sub>2</sub> (T<sub>4</sub>) (21.67) and (32.00) 30 and 45 DAS respectively. Unprimed plants produced lowest nodules (16.33) and (27.00) with 30 and 45 DAS respectively. Treatments T<sub>2</sub>, T<sub>4</sub> are statistically on par with each other for number of nodules per plant at 30 and 45

DAS. The interaction effect of priming methods on number of nodules per plant at 30 and 45 DAS was found to be significant. Premartne and Oertli (1994) reported that dry matter yield, nodule number, fresh weight of nodule per plant, average weight of nodule and the total nitrogen accumulation increased in soya bean when the seeds were primed with potassium at 5.0 mM concentration but the nitrogenase activity remains unaltered.

Data on nodule fresh weight per plant was found significant, osmopriming with PEG (T<sub>2</sub>) was observed with maximum fresh weight (135.00 mg) and (224.33 mg) followed by halopriming with CaCl<sub>2</sub> (T<sub>4</sub>) (132.67) and (220.00) 30 and 45 DAS respectively. Lowest fresh weight observed with unprimed (T<sub>0</sub>) (123.67 mg) and (191.33 mg) 30 and 45 DAS respectively. The interaction effect of priming methods on nodule fresh weight per plant at 30 and 45 DAS was also significant. Singh and Kataria (2012) studied the role of potassium on nitrogen fixation in alleviating water stress in chick pea and concluded that there was a corresponding increase in nitrogen fixation and its attributes with the increase in the potassium level.

**Table.1** Analysis of variance for 7 plant nodule characters in blackgram

S.No.	Characters	Mean sum of squares		
		Replications (df=2)	Treatments (df=6)	Error (df=12)
1.	Field Emergence Per cent	10.43 <sup>**</sup>	22.19 <sup>**</sup>	2.43
2.	No. of Nodules per plant at 30 DAS	11.62 <sup>**</sup>	20.08 <sup>**</sup>	1.84
3.	No. of Nodules per plant at 45 DAS	15.76 <sup>**</sup>	12.83 <sup>**</sup>	1.87
4.	Nodules Fresh Weight at 30 DAS	176.19 <sup>**</sup>	50.56 <sup>**</sup>	12.91
5.	Nodules Fresh Weight at 45 DAS	163.62 <sup>**</sup>	438.98 <sup>**</sup>	38.17
6.	Nodules Dry Weight at 30 DAS	22.05 <sup>**</sup>	29.19 <sup>**</sup>	3.38
7.	Nodules Dry Weight at 45 DAS	37.90 <sup>**</sup>	61.27 <sup>**</sup>	5.29

\*\* Significant at 5% level of significance

**Table.2** Mean performance of blackgram for 7 plant nodule characters

S. No.	Treatments	Field Emergence %	No. of Nodules per plant at 30 DAS	No. of Nodules per plant at 45 DAS	Nodules Fresh Weight (mg) at 30 DAS	Nodules Fresh Weight (mg) at 45 DAS	Nodules Dry Weight (mg) at 30 DAS	Nodules Dry Weight (mg) at 45 DAS
1	T <sub>0</sub>	75.33	16.33	27.00	123.67	191.33	17.33	31.33
2	T <sub>1</sub>	76.67	17.67	28.33	125.00	196.67	19.67	34.67
3	T <sub>2</sub>	82.67	24.00	33.00	135.00	224.33	27.00	45.00
4	T <sub>3</sub>	80.33	20.33	30.67	128.33	207.33	22.00	38.67
5	T <sub>4</sub>	81.67	21.67	32.00	132.67	220.00	24.00	41.33
6	T <sub>5</sub>	78.33	18.33	29.93	126.33	201.00	20.67	35.67
7	T <sub>6</sub>	81.00	20.00	30.00	129.67	211.67	22.67	39.00
<b>Grand Mean</b>		<b>79.43</b>	<b>19.76</b>	<b>30.05</b>	<b>128.67</b>	<b>207.48</b>	<b>21.90</b>	<b>37.95</b>
C.D. (5%)		2.77	2.41	2.43	6.39	10.99	3.27	4.09
SE(m)		0.90	0.78	0.79	2.07	3.57	1.06	1.33
C.V.		1.96	6.87	4.55	2.79	2.98	8.39	6.06

Osmopriming with PEG (T<sub>2</sub>) was observed maximum nodule dry weight per plant (27.00 mg) and (45.00 mg) followed by halopriming with CaCl<sub>2</sub> (T<sub>4</sub>) (24.00 mg) and (41.33 mg) 30 and 45 DAS respectively. Lowest dry weight observed with control (T<sub>0</sub>) (17.33 mg) and (31.33 mg) 30 and 45 DAS respectively.

The interaction effect of priming methods and duration on nodule dry weight per plant at 30 DAS was found to be significant. George *et al.*, (2014) revealed the vital roles played by rhizobial inoculants supplemented with phosphorus and potassium has constructive effect in improving photosynthesis, nutrient uptake, nodulation, growth, yield and economic benefits in legumes.

Showed that interaction between seed priming media x cultivar x temperature and seed priming media x cultivar x priming duration have the beneficial effects on germination index, speed of germination, dry weight and seedling height Yari *et al.*, (2010)

The effect of priming method found to be significant in all the nodule parameters. Significant differences in all the nodule observations were observed due to different priming methods. Among different priming methods, seeds osmoprimed with PEG<sub>6000</sub> recorded maximum per cent of field emergence (82.67 %) followed by haloprimed (CaCl<sub>2</sub>) seeds (81.67 %) and found to be lowest in unprimed (control) seeds (75.33 %).

Number of nodules per plant (24 and 33) were highest in osmoprimed (PEG<sub>6000</sub>) seeds and lowest in unprimed (control) seeds (16.33 and 27.00) 30 and 45 DAS respectively. Nodule fresh weight was highest observed in plots having seeds primed with PEG<sub>6000</sub> (osmoprimed) (135 mg and 224.33 mg) and lowest in unprimed (control) seeds (123.67 mg and 191.33 mg) 30 and 45 DAS respectively. Nodules dry weight was observed to be higher in PEG<sub>6000</sub> primed seeds (27 mg and 45 mg) and found to be lowest in unprimed (control) seeds (17.33 and 31.33) 30 and 45 DAS respectively.

In conclusion, Priming increases the nodulation of blackgram seeds, significantly in field condition. osmopriming (PEG), Halopriming (CaCl<sub>2</sub>) significantly increased the nodulation of blackgram. Osmopriming with PEG showed maximum increase in nodulation. Priming of the blackgram seeds for 12 hrs, in which PEG best result to enhanced nodulation. These conclusions are based on the results of six months investigation and therefore further investigation is needed to arrive at valid recommendations.

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