

Research Article

Effect of Various Seed Priming Methods on Germination Characteristics of *Black Gram*

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Abstract

Black gram despite being one of the major grain legumes in the mid-hills of Nepal, poor germination is a problem in its cultivation. Seed priming is an accepted technique to improve seed germination. An experiment was therefore, conducted to study effect of priming using various priming agents on germination characteristics of *Black gram*. The experiment was laid out in Completely Randomized Design replicated thrice using Hydropriming, Osmo-priming (PEG-600@1%, 2%, 5% and 10%), Halo-priming (NaCl@ 1% and 2%) and Hormonal priming (GA3 @5 and 10ppm) as treatments and compared with unprimed seeds as control treatment. Germination characteristics studied included features of overall germination efficiency, speed of germination and traits relating to vigor of germinating seedlings. Priming with PEG@2% showed a better overall germination parameters and seedling characteristics including a higher seedling length (8.979cm), dry weight (1.308gm) and germination percentage (96%) indicating higher seedling vigor. In addition, seeds primed with PEG 5% exhibited quicker germination and seedling development with higher germination index (103.3), coefficient of velocity of germination (0.355) and lower Mean germination time (2.696days). However, seed primed with PEG@1% and 10% exhibited only less mean germination time than control and there was no difference between the two treatments. Halo-priming of seeds with NaCl@1% showed better results in root length (2.12cm) and mean germination time (3.257days) than in control and was better than NaCl@2%. Hormonal priming didn't improve any germination characteristics except mean germination time where GA3@10ppm was better. Osmo-priming with PEG600 up to 5% concentration was found beneficial

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for better germination and crop establishment. So, the further trials should be conducted in field condition to verify this concept and work more for enhancing the productivity of *Black gram*.

Keywords: GA3; NaCl; PEG-600; Seed germination; Seed priming

Introduction

Black gram (*Vigna mungo* L. Hepper) is a leguminous crop belonging to the family Leguminosae and subfamily Papilionaceae. It is popular among the countries around Indian sub-continent and is one of the most consumed legumes in Nepal. It is grown in rainy season mainly in a cereal-pulse cropping system primarily to conserve soil nutrients, soil moisture and soil itself. It not only helps in enhancing soil fertility through biological nitrogen fixation but also increases the yield of the following crop [1]. It is normally grown in areas with an average temperature of 27-30°C and an annual rainfall of 600-1000mm. In higher rainfall areas it may be grown in the dry season on residual moisture. A dry harvest period is preferable as this forces the crop to mature and reduce the risk of weather damage. It prefers to grow on heavier, well-drained; cotton-black soil with pH 6-7 but it is also grown on lighter soils. *Black gram* has good tendency to resist drought but frost and prolonged cloudiness can create serious problems in its cultivation. Since it is cultivated on marginal lands under rainfed conditions, lack of nutrients and moisture has resulted in poor germination and establishment. Germination is one of the yields determining factors but it is a challenge to facilitate good germination in soil with poor fertility. Seed priming is accepted technique to improve seed germination.

Priming is a pre-sowing treatment in which seeds are partly hydrated such that there is initiation of germination process with no observable radicle emergence [2]. Increase in germination rate, greater germination uniformity, vigorous growth, early flowering and higher yield can be observed with the use of primed seeds [3,4]. Various priming methods are available to enhance seed germination. Hydro-priming is achieved by submerging seeds in water whereas in osmo-priming, osmotic solutions having low water potential are used to control water uptake. In haloprimering seeds are soaked in solutions of inorganic salts [5]. There are many studies reporting the effects of priming on seeds. According to [6] priming improves the performance of seeds by coordinated germination. In a study conducted by [7] in *Black gram*, the improvement of primed seeds to germinate in field conditions could be credited to activation of cells which resulted in enhancement of mitochondrial activity leading to the formation of more high energy compounds and vital biomolecules which were made available in early germination stage [8] concluded that seed priming is a suitable method to improve germination, reduce seedling emergence time and improve stand establishment and yield [9] conducted an experiment to evaluate effect of seed priming on seed germination and yield of corn and found that priming methods and duration increased germination percentage and germination rate [10] conducted an experiment to study the influence of haloprimering and

organic priming on germination and seed vigour in *Black gram* and concluded halopriming showed better result in comparison to organic priming. Similar conclusion was obtained from [11] in his experiment to observe the suitable method of priming for wheat seeds [12] concluded that priming with NaCl can prepare a suitable metabolic reaction and can improve seed germination under undesirable condition such as salinity stress [13] reported that maximum radicle length of cultivar Sardari (*bread wheat*) was obtained at 20% PEG6000 solution primed for 24h where germination rate and radicle length decreased with increasing concentrations.

Materials and Methods

The experiment was conducted in the Agronomy Lab of Institute of Agriculture and Animal Science, Sundarbazar, Lamjung. Locally available *Black gram* seeds were used in the experiment. The experiment was laid out in Completely Randomized Design (CRD) replicated thrice using Hydropriming, Osmo-priming (PEG-600@1%, 2%, 5% and 10%), Halo-priming (NaCl@ 1% and 2%) and Hormonal priming (GA3 @5 and 10ppm) as treatments and compared with unprimed seeds as control treatment. Presoaking was carried out for 24 hours at 25°C followed by shade drying for 48 hours except in case of hormonal priming in which presoaking was carried out for 2 hours at room temperature. Before the commencement of experiment, petri and solution dishes were thoroughly cleaned and seeds were rinsed with distilled water. Priming solutions were made with related concentrations using distilled water based on the formula:

- $X\% \text{ solution} = x \text{ gm solute} + (100-x) \text{ ml of solvent}$ (for PEG 600 and NaCl)
- $X \text{ ppm} = x \text{ mg of solute} + (1000-x) \text{ ml of solvent}$ (for GA3)

Petri dishes each having two layers of germination paper and 25 seeds were provided with control medium which were placed in a germinator at 25°C for 8 days and data were collected at every 24 hours. Seeds with ruptured seed coat and radical length of more than 2mm were considered to be germinated [14,15]. In table 1 each Petri dish ten seedlings were randomly selected for measurement of root length and shoot length. Seedlings were put in different envelopes and oven dried at 72°C for 48 hrs to measure the dry weight. Germination parameters like germination percentage, germination index, mean germination time and coefficient of velocity of germination along with seedling parameters like vigor index 1 & 2 were calculated.

Results

Seed germination

Seed priming had significant effect on germination percentage over unprimed one as shown in Table 2. Hydropriming and hormonal priming with GA3 couldn't improve germination percentage compared to control. As the concentration of PEG600 was increased above 1%, germination percentage also increased up to 5% and then it decreased with higher concentration. There was increase in germination percentage with increase in NaCl level.

Seedling length, dry weight and vigor

Table 2 revealed that Hydroprimed seeds induced longest root length while seeds primed with lower concentration of PEG600 and NaCl induced longer roots as compared to control. Priming with PEG 2% resulted in better shoot length. Better dry weight accumulation was seen with hydroprimed seeds. Osmopriming only improved dry weight accumulation at 2% concentration. Although halopriming induced better root length at lower concentration, it failed to improve dry weight. Hormonal priming wasn't able to induce better seedling length, dry weight accumulation and vigor. Overall priming with PEG 5% gave best results in germination and seedling characteristics. Hydropriming of seeds didn't improve SVI-1 but improved SVI-2. Seedling vigor increased above 1% up to 2% and decreased with increasing concentration. Halopriming and hormonal priming couldn't significantly influence seedling vigor.

Speed of germination

Speed of germination was analyzed with the help of germination index, coefficient of velocity of germination and mean germination time as shown in table 3. Though effect of priming on germination index and coefficient of velocity of germination was inconclusive, overall germination has been improved. Analysis of mean germination time gave satisfactory results to observe effect of seed priming. Unprimed seeds took maximum time to germinate. Hydropriming also reduced the germinating time. As the concentration of PEG increases above 1% MGT decreases up to 5% then MGT increases. Halopriming and hormonal priming gave better speed of germination at lower concentration and with increasing concentration germination slowed down.

Germination parameter	Unit	Formula calculation	Description of formula	Notes and Reference
Germination Percentage (GP)	%	$GP = (n/N) \times 100\%$	N = no of germinated seeds N= total no of seeds used	The higher the GP value, the greater the germination of seed population [16].
Mean Germination Time (MGT)	day	$MGT = \sum f_x / \sum f$	F = seeds germinated on day x	The lower the MGT, the faster a population of seeds has germinated [17].
Coefficient of Velocity of Germination (CVG)	day	$CVG = \frac{N_1 + N_2 + \dots + N_x}{100 \times (N_1 T_1 + \dots + N_x T_x)}$	N = No. of seeds Germinated each day, T= No. of days from seeding corresponding to N	The CVG gives an indication of the rapidity of germination. It increases when the number of germinated seeds increase and the time required for germination decreases. Theoretically, the highest CVG possible is 100. This would occur if all seeds germinated on the first day [18].
Germination Index (GI)	-	$GI = (8 \times n_1) + (7 \times n_2) + \dots + (1 \times n_8)$	n ₁ , n ₂ . . . n ₈ = no of germinated seeds on corresponding days; 8,7...1 are weights given to the number of germinated seeds on the first, second and subsequent days, respectively	In the GI, maximum weight is given to the seeds germinated on the first day and less to those germinated later on. The lowest weight would be for seeds germinated on the 10th day. Therefore, the GI emphasizes on both the percentage of germination and its speed. A higher GI value denotes a higher percentage and rate of germination [19]
Seedling Vigor Index-1 (SVI-1)	-	$SVI-1 = SL \times GP$	SL=seedling length(cm) GP=germination percentage	Abdul-Baki and Anderson [20]
Seedling Vigor Index-2 (SVI-2)	-	$SVI-2 = DW \times GP$	DW=dry weight(gm) GP=germination percentage	Abdul-Baki and Anderson

Table 1: Parameters calculated with details.

Treatment	Germination Percentage	Root Length	Seedling Length	Dry weight	Vigor Index-1	Vigor Index-2
Control	74d	1.663cd	6.537cde	0.818b	483.9c	60.6d
Hydropriming	77.33cd	2.263a	7.56b	1.337a	586.1bc	103.6ab
PEG 1%	82.67bcd	1.857bc	6.933bcd	0.878b	572.7bc	73cd
PEG 2%	89ab	2.103ab	8.797a	1.380a	784.9a	122.7a
PEG 5%	96a	1.583cd	6.573cde	0.954b	631b	91.6bc
PEG 10%	78.67bcd	1.443d	5.973e	0.929b	470.3c	73.6cd
NaCl 1%	75.33cd	2.12ab	7.34bc	0.984b	552.2 bc	74.6cd
NaCl 2%	85.33bc	1.337d	6.213de	0.849b	532bc	73.1cd
GA3 5ppm	75.33cd	1.693cd	6.617cde	0.894b	500.7c	67.4d
GA3 10 ppm	76cd	1.86bc	7.207bc	0.844b	547.1bc	64.5d
Grand Mean	81	1.792	6.975	0.987	566	80.5
LSD	9.89	0.3482	0.7907	0.1742	111.6	21.06
CV%	7.2	11.4	6.7	10.4	11.6	15.4

Table 2: Effect of different priming methods on germination percentage, root length, seedling length, dry weight, seedling vigor index-1 and seedling vigor index-2.

Treatment	Germination Index	Coefficient of Velocity of Germination	Mean Germination Time
Control	86.67ab	0.2608b	4.152e
Hydropriming	76ab	0.3155ab	3.213bc
PEG 1%	101.33ab	0.2847b	3.272bc
PEG 2%	99.33ab	0.3145ab	3.139b
PEG 5%	103.33a	0.3554a	2.696a
PEG 10%	88ab	0.3025ab	3.516cd
NaCl 1%	101ab	0.3104ab	3.257bc
NaCl 2%	98.67ab	0.296ab	3.710 d
GA3 5ppm	74.33b	0.289 b	3.201bc
GA3 10 ppm	83ab	0.2792b	3.706 d
Grand Mean	91.2	0.3008	3.386
LSD	23.84	0.05492	0.2978
CV%	15.4	10.7	5.2

Table 3: Effect of different priming methods on speed of germination.

Discussion

Seed priming seems to influence the germination characteristics and initial seedling growth of *Black gram*. Hydropriming had an adverse effect on germination percentage but a positive role towards speed of germination also found similar results. Earlier and synchronized germination in hydroprimed seeds is may be due to increased metabolic activities [21]. This can be associated with longer root length resulting in greater dry matter accumulation and seedling vigor [22] found that high seedling dry weight was seen with hydroprimed seeds.

Osmopriming with PEG gave the best results in germination and its speed which is due to reactive oxygen species which hastened amylase activities and starch breakdown [23,24] found that osmopriming can enhance DNA replication and DNA repair and promote mobilization of reserved materials which contribute to initiate seed germination. Overall parameters are positively affected by increasing concentrations of PEG but further increase can reduce the seedling parameters such as germination percentage and seedling length. This reduction in germination and length is due to increase in osmotic potential in primed seeds which can alter rate of water uptake.

Although haloprimed seeds performed better than seeds treated with GA3, its performance is inferior to osmopriming. Halopriming accelerated seed germination at lower concentration due to anti-oxidant increment as glutathione and ascorbate in seed which led to higher germination speed via reduction of lipid proxidation activity [25]. Halopriming slowed down germination at higher concentration which may be due to toxic effect of sodium and chloride ions or by creating osmotic pressure [26]. According to [27], there is increase in germination time and decrease in water uptake with increasing osmotic potential. This has led to poor root and seedling length resulting in fewer dry weight accumulation and poor vigor.

Conclusion

Hydropriming of seeds is a cheap and convenient way to improve crop establishment. Halopriming of seeds with low osmotic potential of NaCl of 1% gives better crop establishment than unprimed one. Hormonal priming with GA3 didn't improve germination and seedling characteristics but slightly reduced germination time at lower concentration. Maybe the effect of hormonal priming will be more significant if grown in different medium. Osmopriming with PEG600 up to 5% concentration was found beneficial for better germination and crop establishment.

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