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Efficacy of Hydropriming Employing Slow Hydration Followed by Soaking for Improved Germination and Early Seedling Growth of Black Gram (*Vigna mungo* L. Hepper)

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Abstract: ***Seed priming employing slow hydration viz. moist sand conditioning for 12 h and moist sand conditioning followed by soaking for various durations significantly improved vigour of the seedling as measured by root and shoot length over non - primed (control) black gram seeds (1 - month old) immediately after treatment. There was a marginal difference on germination percentage between the primed and non - primed seeds. Among the treatments, moist sand conditioning followed by soaking for 2 h and then lightly air - drying performed better results in improving germination and early seedling growth. Physiological and biochemical studies showed reduced leakage of electrolytes and sugar in the primed seeds than the non - primed ones. Similarly, primed seeds showed higher dehydrogenase enzyme activity than the non - primed (control) seeds. Among the treatment, moist sand conditioning (12h) followed by soaking for 2 h and then light air - drying have shown better results in maintaining membrane integrity (reduced leakage of electrolytes and sugar) and higher dehydrogenase enzyme activity. The results of the present experiment indicate that hydropriming employing slow hydration by moist sand conditioning and moist sand conditioning (12h) followed by soaking for 2 h and then light air - drying under ambient conditions may be practised for improved germination and early seedling growth of blackgram.***

Keywords: Seed priming, Germinability, Black gram, Membrane functions, Enzyme activity.

1. Introduction

Blackgram (*Vigna mungo* L. Hepper) is an important nutritious pulse crop occupying unique position in Indian Agriculture, belongs to family Leguminoseae with chromosome number 2n=2x=22. 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. Blackgram crop is one of the important pulse crops, grown throughout the century. It belongs to the 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 $^{-1}$ which has been estimated to be supplement of 59 thousand tones urea annually. The pulse, black gram plays an important role in Indian 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. It can be used as green manuring crop which can efficiently fix the atmospheric nitrogen and thus helps in restoring the soil fertility.

Seed dormancy has been defined as the inability of a viable seed to germinate under favourable conditions. Dormancy in legume seeds is of relatively common occurrence and is frequently associated with the covering structures (Jackson and Blundel, 1963). Black gram is one of the important pulse crops with hard seeds which have about 3 - 4 months of physical seed dormancy. So, the hard seeds, fail to germinate even in when the favourable conditions are provided. Seed priming is given as a pre - sowing treatment to enhance seed performance concerning rate and uniformity of germination through controlled hydration of seeds. The seed priming is a process that initiates the pre - germinative metabolic activity before the actual radical emergence (Vinothini *et al.*, 2020). 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. It 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). Priming treatment greatly enhances seed germination and other seedling parameters in blackgram (Poovarasan *et al.*, 2019).

Hydro - priming involves soaking the seeds in water before sowing and may or may not be followed by air - drying of the seeds. In many agricultural areas, a major cause of poor stand establishment and low crop yield is unfavourable environmental conditions for seed germination and seedling emergence. However, rapidly germinating seedlings could emerge and produce deep roots before the upper layers of

the soil are dried out and crusted, which may result in good crop establishment and higher crop yield. Seed priming reduces the effect of salinity on the morphological parameter of the plants. Any factor that facilitates rapid germination may contribute to establishment of a successful crop. A low cost approach, designated as on farm seed priming involve soaking of seed in water before sowing. This pre sowing seed treatment, known as hydro - priming, allows the seed to imbibe water and go through the first phase of germination in which pre - germination metabolic activities are started while the latter two phases of germination are inhibited (Pill and Necker, 2001). Hydro - priming involves soaking seeds in water before sowing (Pill and Necker, 2001) and may or may not be followed by air - drying of the seeds. Hydro - priming may enhance seed germination and seedling emergence under both saline and non - saline conditions.

Soaking is not suitable for some plant species, as rapid hydration may cause leakage of essential nutrients out of the seed, resulting in seed damage besides soaking injury in leguminous seeds. To overcome these potential problems, various methods have been devised to deliver appropriate hydration to the seed. One of them is moist sand conditioning and another is moist sand conditioning soaking followed by light air - drying to improve germination and early seedling growth of blackgram.

2. Materials and Methods

Freshly harvested (1 - month old) Blackgram seeds (cv. Sonali) were pre - moistened with air - dried moist sand (6% moisture content) in the container and then seeds were thoroughly mixed with the moist sand (seed: sand:: 1: 3) and kept covered for 12 hours under ambient conditions (85±1% R. H. and temp 30±1°C). After the stipulated period, seeds were sieved to let the sand pass. After that, the seeds were soaked in double volume of water for various soaking durations, viz., 0.25, 0.5, 1, 2, 3, 4 and 6 hours followed by light air - drying under the fan prior to germination. Afterwards, seeds were placed for germination following the method of Punjabi and Basu (1982) with minor modifications. Before placing the seeds on the glass plate, the seeds were thoroughly slurry - dressed or pre - treated with Mancozeb to control fungal growth during germination. The germination percentage, root and shoot length of the seedling were recorded after 5 days of germination. Root and shoot length of normal seedling was measured to the nearest millimetre. Over 400 seeds for each treatment were employed for germination test (ISTA, 1996).

To study membrane permeability of treated and untreated seeds, the electrical conductance and leaching of sugar was measured following the method of Anderson *et al.* (1964) and Mc Cready *et al.* (1950) respectively. Twenty seeds from each treatment were soaked in 25 ml of distilled water for 30 minutes at 29 ± 1°C and then seed steeped water was decanted off and electrical conductance of seed leachate was recorded on a Conductivity Bridge (cell constant = 0.756). The amount of sugar leached out was determined by adding 4 ml of ice - cold freshly prepared Anthrone reagent (0.2% Anthrone in 98% sulphuric acid) to 2 ml of pre - cooled seed leachate in a hard glass test tube and kept in cold for 30 minutes for the development of bluish green colour. The intensity of the colour was measured on a Systronics Spectrophotometer at 580nm.

The dehydrogenase enzyme activity of treated and untreated seeds was estimated following the method of Kittock and Law (1968). Four uniformly sprouted embryos were dipped in 2 ml of 0.2% tetrazolium chloride solution and incubated for 3h in the dark at 29 ± 1°C. After incubation, the solution was decanted off and the embryos were thoroughly washed with distilled water and surface dried. Then four ml of 2 - methoxy ethanol were added and kept overnight for the extraction of red colour formazan. The absorbance of the solution was recorded on a Systronics Spectrophotometer at 470nm.

3. Results and Discussion

Hydro - priming employing slow hydration by moist sand conditioning and moist sand conditioning followed by soaking for various durations under ambient conditions significantly improved vigour of the seedling as measured by root and shoot length over untreated control (**Table 1**). Among the treatment, moist sand conditioning followed by soaking for 2 h has shown better results in improving germination and early seedling growth of black gram. There was a marginal improvement by the primed seeds on germination percentage over control, tested immediately after treatment. The vigour index was also higher in the primed pre - moistened seeds over untreated control.

Physiological and biochemical studies revealed that membrane functions as determined by electrical conductance and leaching of sugar along with dehydrogenase enzyme activity significantly improved by the priming treatment over non - primed (control) seeds. Most of the treated seeds showed reduced leakage of electrolytes and sugar than the untreated seeds (**Table 2**). Similarly, most of the primed seeds have shown higher dehydrogenase enzyme activity than the control (**Table 2**). Among the treatment, moist sand conditioning followed by soaking for 2 h and then lightly air - drying has shown reduced leakage of electrolytes and sugar along with higher dehydrogenase enzyme activity over control and other treatments.

Primed seeds usually have better and more synchronized germination (Farooq *et al.*, 2006), owing simply to less imbibition time (Brocklehurst and Dearman, 2008) and build - up of germination - enhancing metabolites (Farooq *et al.*, 2006), and improve seedling vigour and stand establishment (Diniz *et al.*2009). Seed priming in black gram has shown vigorous seedling growth at early stage and better germination. Pre - sowing seed priming with vermiculite and warm water (50ºC) soaking for 60 minutes improved the emergence responses of bitter gourd seeds under 25ºC and 20ºC (Lin and Sung, 2001). Pre - sowing wetting - drying treatments for enhancing germination and better seedling establishment have been proved very effective in seeds of a number of species (Hegarty, 1970). Mandal and Basu (1987) reported that pre - sowing hydration treatments improved field emergence and yield of wheat. Heydecker and Coolbear (1977) made an extensive survey of the work on pre - sowing wetting - drying treatments and concluded that

the seeds could be invigorated successfully for improving seedling vigour and better crop stand.

Potential implements for improved germination and transfer from dormancy by hydropriming are: initiation of water prompted metabolic cycles, improved repair of chemical action, formation of hormones and expanded accessibility of nutrients (Sebastian *et al*., 2014). Hydropriming conditioning advances the event of pre - germinative metabolic process empowering embryo growth, and subsequently speeding up germination and seedling vigour. A study found that by including enough oxygen, seed dormancy is broken and germination starts. Additionally, germination rate expanded when the seeds were hydroprimed with different duration of soaking (Afzal *et al*., 2012).

Biochemical evidences in favour of germination advancement by hydration - dehydration pre - treatments have been greatly emphasized by Sen and Osborne, (1974). According to Sen and Osborne (1974), the rate of RNA and protein synthesis in wetted - dried rye embryos were similar to those in embryos continuously germinated for the same period of total hydration. Heydecker (1974) was also of the opinion that hydration caused an advancement of the germination process which was compatible with the subsequent drying back.

Studies in the present laboratory (Mandal and Basu, 1982 and 1987) suggested that the beneficial effects of pre - sowing soaking might also be obtained with shorter soaking duration. Many studies have indicated that a relatively short priming treatment is advantageous for extending the longevity and improving the vigour of stored seeds (Dearman *et al*.1986). However, others have reported that the storage life of seeds is shortened following priming (Sun *et al*.1997). Nonetheless, it is necessary to mention that different rates of water absorption and the duration of absorption may alter the redistribution of absorbed seed water among different binding sites (weak water - binding sites, strong water - binding sites, and multimolecular - binding sites) which is unclear and needs to be studied in relation to seed longevity (Vertucci and Leopold 1987). A significantly higher activity of dehydrogenase enzyme in primed seeds compared to non - primed seeds indicates that there was an early induction of metabolic activities for supporting rapid and synchronized germination in primed seeds. The increased activity of dehydrogenase enzyme may also protect the cell against membrane damage which occurs naturally due to lipid peroxidation during storage (Bailly *et al*.2000). A lower EC value in primed compared to nonprimed/ control supports this contention because EC has been found to be associated with the membrane permeability properties of cells (Simon 1974).

Whatever may be the exact mechanism operative in the seed priming treatment, moist sand conditioning for 12 h followed by soaking for 2 h and then lightly air - drying may be practiced for improved germination and early seedling growth of black gram seeds.

References

[1] Afzal, I., Hussain, B., Basra, S. M. A. and Rehman, H. (2012). Priming with moringa leaf extract reduces imbibitional chilling injury in spring maize. *Seed Sci. Technol.*, **40**: 271 - 276.
[2] Anderson, A. M., Hart, J. R. and French, R. C. (1964). Composition of germination technique and conductivity tests of cotton seeds. *Proceedings of the International Seed Testing Association*, **29**: 81 - 86.
[3] Bailly, C, Benamar, A, Corbineau, F and Côme, D. (2000). Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming. *Seed Sci. Res.*1**0**: 35 - 42.
[4] Brocklehurst, P. A. and Dearman, J. (2008). Interaction between seed priming treatments and nine seed lots of carrot, celery and onion. II. Seedling emergence and plant growth. *Annals of Applied Biol*ogy, **102 (3):** 585 - 593.
[5] Dearman, J, Brocklehurst, PA and Drew RLK. (1986). Effects of osmotic priming and ageing on onion seed germination. *Ann. Appl. Biol.1***08**: 639 - 648.
[6] Diniz, K. A., Silva, P. A., Oliveira, J. A. and Evangelista, J. R. E. (2009). Sweet pepper seed responses to inoculation with microorganisms and coating with micronutrients, amino acids and plant growth regulators. *Scientia Agricola*, **66 (3):** 293 - 297.
[7] Farooq, M, Basra, SMA and Wahid, A. **(**2006). Priming of field - sown rice seed enhances germination, seedling establishment, allometry and yield. Plant Growth Regulation **49,** 285 - 294.
[8] Harris, D., Rashid, A., Miraj, G., Arif, M and Shah, H. (2007). 'On - farm' seed priming with zinc sulphate solution - A cost - effective way to increase the maize yields of resource poor farmers. *Field Crops Res*.102 119 - 127.
[9] Hegarty, T. W. (1970). The possibility of increasing field establishment by seed hardening. *Horticulture Research*, **10**: 59 - 64.
[10] Heydecker, W. (1974). Germination of an idea: The priming of seeds. University of Nottingham, School of Agriculture. Report, Part III, 1968/1969, p.96 - 100.
[11] Heydecker, W. and Coolbear, P. (1977). Seed treatment for improved performance survey and attempted progress. *Seed Science and Technology*, **5**: 354 - 425.
[12] ISTA. (1996). International Rules for Seed Testing: Rules 1996. *Seed Sci. and Technol.*, **24**: Supplement 2, 1996.
[13] Jackson, G. A. D. and Blundell, J. B. (1963). Germination in Rosa. *Int. J. Hortic. Sci.*, **38:** 310 - 320.
[14] Kittock, D. L. and Law, A. G. (1968). Relationship of seedling vigour to respiration and tetrazolium chloride reduction of germinating wheat seeds. *Journal of Agronomy*, **60**: 286 - 288.
[15] Lin, S. S. and Sung, J. M. (2001). Pre - sowing treatments for improving emergence of bitter gourd seedling under optimal and suboptimal temperatures. *Seed Science and Technology*, **29**: 39 - 50.
[16] Mandal, A. K. and Basu, R. N. (1982). Alleviation of salt stress during germination and early seedling growth of wheat by hydration - dehydration pre - treatment. *Indian Agriculturist*, **26**: 121 - 128.

[17] Mandal, A. K. and Basu, R. N. (1987). Midterm and pre - sowing hydration - dehydration treatments for improved field performance of wheat. *Field Crops Research*, **15**: 259 - 265.
[18] McCready, R. M., Guggolz, J., Silviera, V. and Owens, H. S. (1950). Determination of starch and amylase in vegetables. *Analytical Chemistry*, **22**: 1156 - 1158.
[19] Pill, W. G. and Necker, A. D.2001. The effects of seed treatments on germination and establishment of Kentucky bluegrass (*Poa pratense* L.). *Seed Science and Technology*, **29**: 65 - 72.
[20] Poovarasan, T., Lakshmi, S., Renugadevi, J and Senthil, A. (2019). Seed quality improvement with fruit extracts in blackgram. *J. Phytol.*, **14:** 16 - 20.
[21] Punjabi, B. and Basu, R. N. (1982). Testing germination and seedling growth by an inclined glass plate blotter method. *Indian Journal of Plant Physiology*, **25:** 289 - 295.
[22] Sebastian, J., Wong, M. K., Tang, E. and Dinneny, J. R. (2014). Methods to promote germination of dormant *Setaria viridis* seeds. *Plosone.*, **9:** 1 - 7.
[23] Sen, S. and Osborne, D. J. (1974). Germination of rye embryos following hydration - dehydration treatments. Enhancement of protein and RNA synthesis and earlier induction of DNA replication. *Journal of Experimental Botany*, **25**: 1010 - 1019.
[24] Simon, EW.1974. Phospholipids and plant membrane permeability. *New Phytol.*7**3**: 377 - 420.
[25] Sun, WQ, Koh, DCY and Ong, CM.1997. Correlation of modified water sorption properties with the decline of storage stability of osmotically - primed seeds of *Vigna radiata* (L.) Wilczek. *Seed Sci. Res.*7: 391 - 397.
[26] Vertucci, CW and Leopold, AC. (1987). The relationship between water binding and desiccation tolerance in tissues. *Plant Physiol.8***5**: 232 - 238.
[27] Vinothini, N., Manonmani, V. and Karthikeyan, S. (2020). Seed priming to mitigate the impact of elevated carbon dioxide associated temperature stress on germination in rice (*Oryza sativa* L.), *Arch. Agron. Soil Sci.*, **66:** 83 - 95.

Table 1: Efficacy of priming treatments employing slow hydration for improved germination and early seedling growth of black gram seeds (cv. Sonali)

Treatments/Soaking Duration	Germination		Mean root length (mm)	Mean shoot length (mm)	Vigour Index
	%	Arc - sin value			
Control (0 hr)	93	78.61	80	71	14043
Moist Sand Conditioning (12 hr)	88	69.43	94	93	16456
Moist Sand Conditioning (12 hr) +Soaking duration (h)					
0.25	95	80.79	106	100	19570
0.5	93	74.33	107	82	17577
1	93	69.39	99	89	17484
2	98	83.54	105	98	19894
3	95	80.79	112	76	17860
4	95	80.79	110	69	17005
6	93	78.61	103	66	15717
L. S. D at 0.05P	**-**	**NS**	**8.87**	**16.11**	**-**
L. S. D at 0.01P	**-**	**NS**	**12.89**	**23.43**	**-**

Abbreviation: - NS: - Non - Significant
Treatments were given to one - month old black gram seeds. After treatment, seeds were lightly air - dried under the fan and then put for germination. Data were recorded after 5 days of germination.
Vigour Index: - Germination percentage × Seedling Length
Germination percentage data were transformed to their respective arc - sin values prior to computation.

Table 2: Effect of priming treatments employing slow hydration on membrane permeability and enzyme activity of black gram seeds (cv. Sonali)

Treatments	Germination		Electrical conductivity (μscm^{-1})	Leaching of sugar (O. D. value recorded at 580 nm)	Dehydrogenase enzyme activity (O. D. at 470 nm)
	%	Arc - sin value			
Control (0 hr)	85	67.21	36.22	0.225	0.355
Moist Sand Conditioning (12 hr)	90	71.57	48.92	0.075	0.655
Moist Sand Conditioning (12 hr) +Soaking duration (h)					
0.25	95	77.08	22.44	0.032	0.629
0.5	90	71.57	23.67	0.035	0.509
1	85	67.21	31.38	0.030	0.577
2	95	77.08	11.84	0.045	0.707
3	90	71.66	16.78	0.086	0.345
4	90	71.57	43.83	0.095	0.655
6	85	67.86	43.20	0.105	0.595
L. S. D at 0.05P	**-**	**NS**	**14.56**	**0.07**	**0.018**
L. S. D. at 0.01P	**-**	**NS**	**21.18**	**0.12**	**0.023**

Other details are same as Table 1.