Research Journal of
Seed Science

ISSN 1819-3552

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Seed Science 8 (1): 22-29, 2015
ISSN 1819-3552 / DOI: 10.3923/rjss.2015.22.29

Enhancing Planting Value of Marginal Seed Lots of Blackgram through Hydration-Dehydration (HD) Treatment

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ABSTRACT
Seed invigoration is being utilized as a technique for enhancing quality of aged seeds. In pulses seed hardening is one technique involves Hydration Dehydration (HD). This study was conducted to investigate the response of seeds with marginal germination percentage of two varieties of blackgram (APK 1 and VBN 3), to hardening which consisted of soaking seeds in water/bio active solutions for 4 h and drying to initial moisture content. Seed quality assessment inferred that seed hardening generally improved seed germinability and vigor, seed response varying significantly with variety and initial vigor level. Excellent results were obtained in water soaked seeds rather than organics. Among organics, Prosopis leaf extract at 2% excelled in improving seed quality of aged seeds. Seed hardening is advantageous in two ways 1. Resource poor farmers could benefit from this cash-free technique and 2. Reduced seed rate of marginal seed lot.

Key words: Blackgram, two vigour level, response, hydration

INTRODUCTION
Productivity of pulses is low and static is due to the reason that pulses are grown almost entirely in marginal and rain fed areas combining with use of low quality seeds lead to poor population in the field and thus fail to fetches the interest of farmers to follow good management techniques, thus reduced yield. High seed quality is basic to good and healthy crops. However, seed quality can only be maintained up to certain level. In many parts of the world, especially those with hot and humid climates like India, maintenance of seed viability poses a serious problem. Seed, being a biological entity, undergoes deteriorative process, which might have enhanced by unfavorable conditions and deterioration has been believed to be an irreversible once started (Copeland and McDonald, 1995). Number of farmers are using marginal seed lots with poor germination in higher seed rate during unavailability of quality certified seeds. If the germination of marginal seed lot could be improved by certain practices the seed lots which are in the border of Minimum Seed Certification Standards (MSCS) levels could be well utilized for seed purpose.

Certain seed treatments are recently being utilized to improve viability and/or vigor of poor quality seeds and the results are positive. This is because seeds have an in-built system to counteract deteriorative events and under right conditions, have mechanisms for self-repair also (Coolbear, 1995). Seed enhancement, which aims to improve seed germination and seedling growth, encompasses many techniques performed on seeds after harvest and before sowing (Copeland and

McDonald, 1995; Taylor *et al*., 1998). One of such technique of seed enhancement is seed hardening where seed is allowed to imbibe moisture to a point where metabolic mechanisms are activated but not actual radicle emergence occurs (Bradford, 1985).

Seed hardening has been reported to induce drought resistance in plants and such seeds have the capacity to withstand dehydration and overheating. Other beneficial effects of hardening are inducing better root growth, higher rate of photosynthesis and dry matter accumulation (Henckel, 1964). Presowing seed hardening is the method that results in modifying the physiological and biochemical processes of seed to mitigate the adverse environment. Hardening of seeds resulted in the absorption of more water due to increase in the elasticity of cell wall and development of a stronger and efficient root system. Seed hardening can be done by using organic and inorganic products. For hardening instead water, organic solutions with bio active potentials being tried by number of researchers and could achieve significant results.

The dependency on use of inorganic fertilizer as a source of plant nutrients by farmers is further associated with land and soil degradation and environmental pollution. Plant extracts of some trees and crop residues have been reported to influence germination, crop growth and yield. Leaf extracts of plants reported to contain plant growth promoting substances, anti-oxidants and nutrients (Kamaraj and Padmavathy, 2012). The nutrient concentrations in components of *Prosopis juliflora* were quite high and said to contain 60, 57, 63, 31 and 63% of the total tree N, P, K, Ca and Mg, respectively (Maghembe *et al*., 1983).

In this background, the present investigation on seed hardening with different organics were tried to enhance the quality of seed lots with different germination level of blackgram.

MATERIALS AND METHODS

Seeds: For two varieties APK 1 and VBN 3, seed of naturally aged under ambient conditions of storage with 8% moisture and germination of 70 and 80%, respectively, were used.

Hydration-dehydration: The seeds with above standards were soaked in the ratio of 1:0.3 volume of freshly prepared leaf extracts viz., *Azadirachta indica*, *Prosopis juliflora*, *Moringa oleifera* and *Albiziaamara* in two concentrations viz., one and two per cent for 4 h along with water. The soaked seeds were dried back to original moisture content (8%). 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. Untreated seeds served as control. Both the seeds were evaluated for following seed and seedling quality characters.

Seed quality characters

Germination (%) after (ISTA., 2003): Germination test was conducted using four replicates of hundred seeds each in sterilized sand medium in the germination room maintained at a temperature of 25±1°C and RH 96±2%. The germination was calculated based on the number of normal seedlings on seventh day and it was expressed in percentage.

Root length (cm): All normal seedlings at the end of the germination test were measured for root length. The length between the collar region and the tip of the primary root was measured and the mean value was recorded.

Shoot length (cm): All normal seedlings were measured for shoot length. The length between the collar portion and the tip of the shoot was measured and mean value was expressed.

Dry matter production (g seedlings^{-10}): All normal seedlings used for growth measurements were placed in a paper cover and dried under shade for 24 h and then in the hot air oven maintained at 85±1°C for 24 h. The dried seedlings were cooled in a desiccator for 30 min, weighed and expressed in g seedling^{-10}.

Vigour index: Vigour index value was computed using the formula suggested by Abdul-Baki and Anderson (1973) and expressed as whole number.

$$\text{Vigour index} = \text{Germination (\%)} \times \text{Dry matter production}$$

Electrical conductivity after (Presley, 1958): Four replications of 25 seeds from each treatment was drawn and prewashed well with distilled water to remove the adhering chemicals and then soaked in 25 mL of distilled water for 16 h at room temperature. After soaking, the seed steep water was decanted to obtain the seed leachate. The electrical conductivity of the seed leachate was measured in a digital conductivity meter with a cell constant of one and expressed as d Sm^{-1}.

Dehydrogenase activity (OD value) (Kittock and Law, 1968): Seeds from each treatment were taken and preconditioned by soaking in water for 6 h at room temperature. The seeds were prepared by peeling the seed coat to expose the embryonic axis. Then the peeled seeds were soaked in 0.25% solution of 2, 3, 5-triphenyl tetrazolium chloride and kept in dark for 2 h at 40°C for staining. The stained seeds were thoroughly washed with water and then soaked in 5 mL of 2-methoxy ethanol (methyl cellosolve) and kept in darkness for overnight for extracting the red coloured formazan. The intensity of red colour was measured in ELICO UV-VIS spectrophotometer (model SL-159) using blue filter No. 470 nm and the mean OD value was recorded.

Seed moisture content (%) (ISTA., 2003): The initial weight of the empty moisture bottle along with the lid was taken. The weight of the bottle along with the seed (coarsely ground) was recorded and kept in a hot air oven, maintained at 103±2°C for 16 h. After 16 h of drying, the samples were taken out and cooled in a desiccator for 30 min. Then, the final weight of the sample after drying was recorded. The moisture content was calculated and expressed in percent using the following equation:

$$\text{Moisture content (\%)} = \frac{M_2\text{-}M_3}{M_2\text{-}M_1} \times 100$$

Where:
M_1 = Weight of bottle alone
M_2 = Weight of bottle and ground seed material before drying
M_3 = Weight of bottle and ground seed material after drying

Experimental analysis: The trials were factorial in Completely Randomized Design (CRD) in three replicates. The data was analyzed using ANOVA after (Panse and Sukatme, 1985).

RESULTS

In general, the performance of hardened seeds are good and degree of response varied according to initial vigor level and variety. The treatments resulted in statistically significant results were produced and discussed.

Statistical analysis indicated the significant difference between treatments for all analyzed factors except root length.

Improvement in seed germination percentage was observed for both varieties but the percentage increase differed. Medium vigour VBN 3 seed responded extensively for HD, statistically significant improvement from 80-98% was noted in water soaked seeds. Though values are significantly differed, the response of low vigour seeds (APK 1) in terms of germination improvement was low for all treatments, the seed soaked in *Prosopis* leaf extract recorded highest improvement from 70-78% (Table 1).

Seedling length measured in terms of root and shoot length also improved when subjected to HD treatment. The improvement in seedling length is statistically significant for shoot length whereas, it is non-significant for root length. Water soaked seeds of seed lot with medium vigour showed an improvement from 14.5-18.2 and 14.3-15.8 cm for root and shoot length respectively. The low vigour seeds of APK 1 soaked in *Prosopis* solution recorded marginal increase from 11.8-13.0 and 20.4-23.0 for root and shoot, respectively. Accordingly, dry weight of seedlings also increased from 0.20-0.26 and 0.20-0.23 g seedling^{-10} for medium and low vigour seeds undergone HD treatment with water and *Prosopis*.

Vigour Index (VI), is a measure of the speed and uniformity in germination. In general, all seeds regardless of variety and vigor level responded favorably to HD treatment for vigour index. An increase in vigour index of about 54% for seeds of medium vigor (2160-3332), 20% for seeds of low vigour (2291-2752) were obtained, after these seeds were subjected to HD treatment (Table 1).

Dehydrogenase enzyme activity estimated in terms of OD value was increased (Fig. 1a) from 0.215-0.235 in water soaked seeds of marginal vigour seeds, where each and every treatment is statistically significant from each other, in case of low vigour seeds the increase is from 0.183-0.218. With regard to electrical conductivity of seed leachate, it is interesting to note the values were decreased in HD subjected seeds irrespective of treatments (Fig. 1c) in both the vigour lots and the values were statistically significant with each other. However, percentage of response to hydration varied according to initial vigour level.

Table 1: Seed quality characters of marginal seed lots of blackgram varieties APK 1 and VBN 3 after H-DH treatment

	Parameters observed									
	Germination (%)		Root length (cm)		Shoot length (cm)		DMP (g seedling^{-10})		VI	
Treatments/variety	APK 1	VBN3	APK 1	VBN3	APK 1	VBN3	APK 1	VBN3	APK 1	VBN 3
T0	70	80	11.8	14.5	20.4	14.3	0.20	0.20	2291	2160
T1	71	98	12.1	18.2	21.3	15.8	0.20	0.26	2397	3332
T2	78	88	13.0	17.2	23.0	15.6	0.23	0.26	2752	2673
T3	76	84	12.6	17.2	21.7	15.3	0.22	0.24	2542	2738
T4	75	82	12.7	17.3	22.4	15.2	0.22	0.26	2637	2878
T5	73	84	12.4	17.4	22.1	15.1	0.21	0.25	2526	2869
Mean	73	86	12.4	17.0	21.8	15.2	0.21	0.25	2524	2775
SE. d	0.235		NS		0.02369		0.00024		0.033	
CD (p = 0.05)	0.464**		NS		0.04664		0.00047**		0.066**	

T0: Control, T1: Water soaking, T2: *Prosopis juliflora,* 2%, T3: *Azadirachta indica* 2%, T4: *Moringa oleifera* 2%, T5: *Albizia amara* 2%, **Significant at p<0.05, NS: Not significant

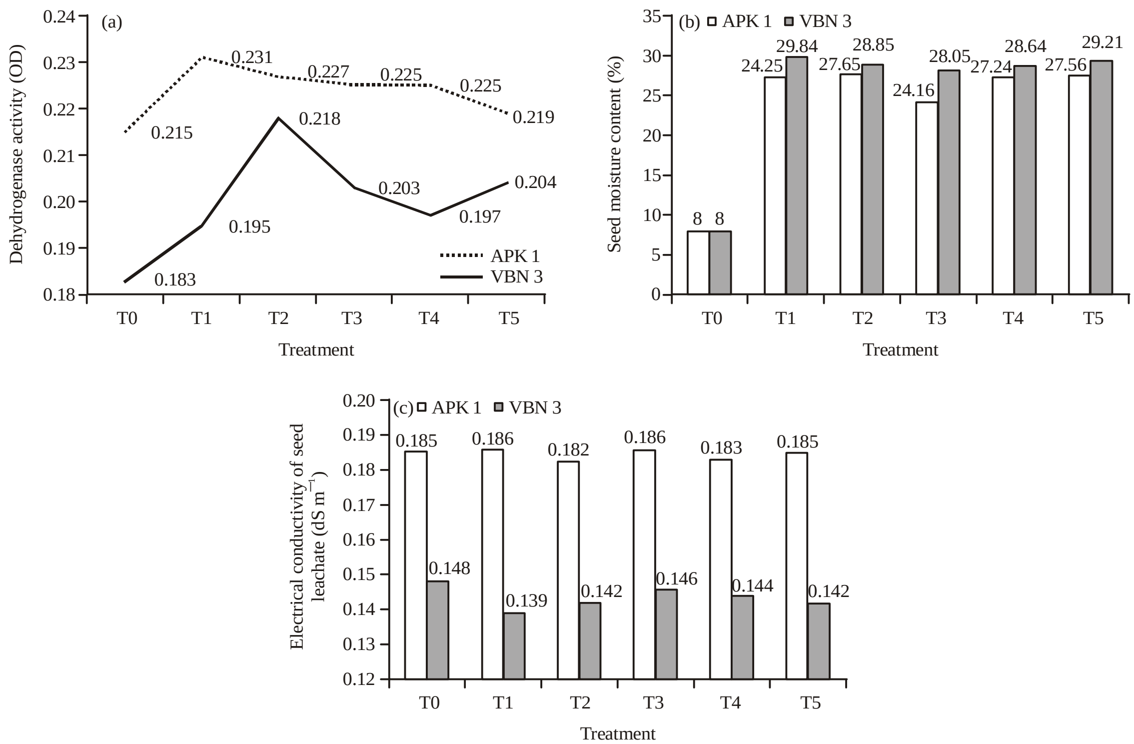

Fig. 1(a-c): Seed quality characters of marginal seed lots of blackgram varieties APK 1 and VBN 3 after H-DH treatment, (a) Dehydrogenase activity, (CD = 0.002**), (b) Seed Moisture content (%) and (c) Electrical conductivity of seed leachate (d Sm^{-1})

DISCUSSION

Hydration Dehydration (HD) treatment involves soaking of seeds in water/bio active solutions for specified period of time in order to allow the seeds to imbibe moisture from the environment to about 30% to initiate the pre germinative catabolic events. In case of blackgram, being proteinaceous seed, soaking of seeds in water/bio active organic solutions for 4 h, allowed seeds to absorb moisture up to 30%, irrespective of varieties and their initial vigour level (Fig. 1b).

The HD generally improved the germination percentage of blackgram, which was more pronounced in moderate vigour than low vigour seed lot. This improvement could be attributed to modification of physiological and biochemical nature of seed embryo and its associated structures, i.e. pre-enlargement of the embryo (Austin *et al*., 1969), some preventive and restorative mechanisms active during the hydration phase of HD treatment (Basu, 1994) and biochemical changes like enzyme activation and release of enzymes (Lee and Kim, 2000; Basra *et al*., 2005; Ramamoorthy *et al*., 2009) that causes the early availability of high energy compounds and vital biomolecules to the germinating seedling. The beneficial effect of *Prosopis* leaf extract in low vigour seeds might be due to migration of mineral nutrients into the seed during hydration phase. Presence of Ca, an essential enzyme Co-factor in germination process and Mg, key factor of many metabolic process (Cakmak and Yazici, 2010) in the *Prosopis* leaf extract might have positively influences the germination.

The pronounced increase in seedling length, dry matter production and vigour index of *Prosopis* leaf extract soaked low vigour seeds may be due to activation of the growth promoting substances and translocations of secondary metabolites to the growing seedling. Physiologically active substances might have activated the embryo and other associated structures which resulted in the absorption of more water due to cell wall elasticity and development of stronger and efficient root system and that would have ultimately resulted in higher vigour index (Rangasamy *et al*., 1993). Many researchers also reported the benefits of seed hardening with *Prosopis* and pungam leaf extract to overcome the adverse condition (Rathinavel and Dharmalingam, 2000) in uppam cotton; (Kamaraj and Padmavathy, 2012) in green gram.

Biochemical analyses and hypotheses about seed deterioration suggest, impaired membrane activity during seed ageing is a factor leading to leakage of cellular solutions during seed soaking causes loss of metabolic energy for membrane transport system (Copeland and McDonald, 1995). In the present study hardened seed registered low electrical conductivity over non-hardened seeds. The probable reason for low electrical conductivity in the HD seeds presumed to be the quenching of free radicals which consequentially restore the membrane integrity. One possible mechanism put forth by Villiers and Edgcumbe (1975) and Burgass and Powell (1984), is that seeds undergo repair of biochemical lesions on vital cell organelles during hydration.

Greater response of seeds of moderate vigour further support to the idea of participation of a repair mechanism for correction of damages to organelles suffered by ageing (Basu, 1994), where the same is absent or non functional in low vigour seeds, so that low vigour could not improved beyond certain extend. The positive effects of hydration could be explained based on cellular corrective functions reflected over lower conductivity (Fig. 1c). A hydration level of greater than 18% (fresh weight basis) is essential for maintenance of lipid bilayer structure of cell membrane and for DNA repair mechanisms to occur (Basu, 1994; Bewley and Black, 1994). In the present study, blackgram seeds subjected to HD were able to achieve this hydration level. The results are in conformity with Sathish *et al.* (2011) in maize and Kausar *et al.* (2009) in sunflower.

Dehydrogenases contributed to the catalyses of stored products (Oaikhena *et al*., 2013) and function as components in the electron transport chain facilitating the transport of electrons from substrates to oxygen (Murray *et al*., 2009). In the present study, seed hardening resulted in higher activity of dehydrogenase indicated the high vigour. Similar results on high dehydrogenase activity of potassium and phosphorus soaked rice seeds was observed (Bam *et al*., 2006).

Among different solutions, water soaking could able to improve quality characters to a greater extent in case of moderate vigour seeds, where as in low vigour seeds failure of same indicates the need of some bio active substances to trigger the foresaid enzymatic activities and DNA repair mechanism, hence the *Prosopis* soaked seeds responded than other solutions. *Prosopis* leaves found to contain highest concentration of plant metabolite (Singh, 2012).

CONCLUSION

Hydration of moderate vigour blackgram seeds in water would improve the seed quality to Minimum Seed certification Standards (MSCS) but in case of low vigour, soaking in bio active solutions would improve the seed lot quality to the MSCS level. On the practical side, resource-poor farmers will gain from this cash-free technique. The technique may be recommended as a pre-sowing treatment for seeds of low to moderate vigor level.

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