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Hydro-Priming in Dry Bean (*Phaseolus vulgaris* L.)

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Abstract: Hydro-priming is a very important seed treatment technique for rapid germination and uniform stand establishment in various grain crops. The objectives of the study were to evaluate the effect of hydro-priming and hydration media on the germination of dry bean cultivars. Seed performance was evaluated based on germination test, seed moisture content, electrical conductivity and water uptake. The study constituted two set of experiments. The result of the first experiment revealed that seed priming, cultivars, and their interaction were significant (at $p = 0.01$) for percent germination at the 2nd, 4th and 8th day and normal seedlings percentage at the 8th day. Seeds failed to produce normal seedlings for both 4 and 8 h seed priming treatments, while the control (no priming treatment) produced large percentage of normal seedlings. The second experiment was designed to examine the cause for the failure of germination in the primed seed in the first experiment. There was significant difference between cultivars for germination percent at the 2nd day, while only the media of hydration was significant at the 8th day count. None of the factors were significant at the 4th day. There was significant difference among hydration media and cultivars for normal seedlings at the 8th day, while the interaction was non-significant. Completely immersing the seed in water-filled flask did not cause the failure of germination in the first experiment; rather hydration followed by dehydration treatment was the possible cause. The better performance of the control in both experiments indicated that hydro-priming seems unnecessary in dry bean.

Key words: Hydro-priming, dry bean, germination test, desiccation sensitivity

INTRODUCTION

Hydration is a prerequisite for seed germination. The water imbibed by the seed activates enzymes, which facilitate mobilization and translocation of reserves. Thus, imbibition of water is an essential step for the metabolism of stored starch and protein in the seed (Kikuchi *et al.*, 2006). This in turn is important in ensuring nutrient supply to the germinating embryo and to generate energy for the commencement of active germination and seedling growth.

The duration and extent of imbibition for seed germination depends on the cultivar, species and relative availability of moisture (Copeland and McDonald, 1995). Hydration under high moisture conditions can affect proper germination, mainly because of nutrient leakage. Simon and Rajaharun (1972) stated that sugars, organic and amino acids, among

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others are the major substances leaked during hydration. Simon and Rajaharun (1972) reported that under field conditions, the leakage is positively associated with poor seedling emergence. The leaked substance can support growth of fungal pathogens (Simon and Rajaharun, 1972).

Modi (2005) defined seed priming as an activity of allowing the seed to be hydrated followed by dehydration and hydration will commence again during germination. The capacity of the seed to tolerate the phenomenon of hydration followed by dehydration is a measure of the quality of the seed (Modi, 2005) and helps to ensure uniform emergence (McDonald, 2000).

Pre-hydration of seed is an important approach to enhance germination and emergence in drought affected areas, where adequate moisture is not available for proper crop establishment. Afzal *et al.* (2002) reported significant grain yield improvement in double cross hybrid maize treated with hydropriming. Similarly, hydropriming showed significant improvement in percentage and mean time of emergence on sorghum (Moradi and Younesi, 2009). However, hydropriming may not always be a good option especially, for crops such as legumes. This is mainly because of sensitivity of some legume seeds to leakage and loss of desiccation tolerance through priming.

Quan *et al.* (2004) studied the effect of pre-imbibition on desiccation tolerance and leakage on mung bean and reported significant increase of leakage at the initial phase of imbibition, which declined at the latter stages (Simon and Rajaharun, 1972; Quan *et al.*, 2004). Dehydration of pre-imbibed seeds drastically reduced the survival of the seed, fresh weight of the seedling and length of radicle and hypocotyls of the surviving seeds (Quan *et al.*, 2004). The authors concluded that dehydration in mung bean seed causes loss of desiccation tolerance of pre-imbibed seeds.

Cracking of the cotyledon in bean seeds increased imbibition of water and subsequent failure of germination. Dickson *et al.* (1973) and McCollum (1953) described cotyledon cracking as a physiological disorder of legume crops during or after germination, having a characteristic feature of transverse fracture on the cotyledon. Mazibuko and Modi (2005) reported faster imbibition of water in the large seeded cultivars than the small seeded, which was also associated with cotyledonal cracking. This indicates that large seeded beans are more liable to cotyledonal cracking than small seeded beans.

However, there was no adequate information on the effect of hydropriming on dry bean and the response of different varieties to hydropriming. In addition, information was scant on the effect of hydration interval on the success of hydropriming treatment. Moreover, the effect of the mechanism of hydration technique, i.e., completely immersing the seed and partially soaking, on the germination of dry bean seed was not known. Hence, the objectives of the study were: (1) to understand the effect of hydration intervals followed by dehydration, (2) to detect cultivar difference in response to hydropriming treatment, (3) to detect the presence of interaction between cultivars and hydration interval followed by dehydration treatment and (4) to determine the effect of hydration (without dehydration), cultivar and the interaction of hydration (without dehydration)×cultivars.

MATERIALS AND METHODS

Three dry bean cultivars, namely mangeni, 52-1 and 45-1, which were obtained from Pro-seed Company, were used for the study. The seed size was large, intermediate and small for cultivars 52-1, 45-1 and mangeni, respectively with respective average 100 seed weight of 34.72, 28.95 and 20.01 g. A total of 20 seeds were used for each treatment. The experiment

was designed in a 3 varieties×3 hydration intervals factorial arrangement in a Completely Randomized Design (CRD) with three replications. The experiment was conducted during the period August-October, 2007 in the seed laboratory of the University of Kwazulu Natal, South Africa. The three hydration intervals were the control (no hydration), 4 and 8 h hydration followed by dehydration. Hydration was done by completely immersing the seeds in water filled flasks. The dehydration was done using saturated lithium chloride salt for 24 h, to reduce the moisture content of the hydrated seeds to a level nearly equivalent to the control treatment (no hydration). The amount of water imbibed by the seeds was measured by taking the weight difference of the hydrated seed and initial seed weight. Seed moisture content in the control treatment was measured by grinding the seed in to flour and splitting it in to two replications; and measuring the weight of the grinded seed before and after oven drying at 130°C for an hour. Electrical conductivity was measured using Metrohm, 644 conductometer for each of the treatments.

In the second experiment, two types of hydration treatments i.e., hydrating the seed by completely immersing in water-filled flask and partially soaking the seed in a petridish, were compared with the control treatment (seed with no hydration treatment). The experiment was laid out in three hydration media x three cultivars using CRD in a factorial arrangement with two replications. In this experiment, the hydration time was constant i.e., 4 h. In contrast to the first experiment, no dehydration treatment was applied. Counting of the number of seeds that germinated (radicle protruded) was done every 24 h until the 8th day of germination. On the 8th day, the final counting of normal, abnormal and dead seeds was done. Normal seedlings were those which produced healthy basic seedling parts as per described by ISTA (1985).

As all the recorded data were counts and showed lack of normality, square root transformation was used (Gomez and Gomez, 1984) and analysis of variance for the factorial CRD design was done using Genstat software (VSN International, 2008) for both experiments according to the procedures described by Gomez and Gomez (1984). The significance of treatment means was determined using LSD (at $p = 0.05$).

RESULTS

The statistical analysis showed that percent germination was significant (at $p = 0.05$ level of significance) for response of cultivars to seed priming and cultivar X seed priming treatment, while seed priming was significant (at $p = 0.01$ level of significance). The 4th and 8th day germination and normal seedlings percentages at the 8th day showed significant difference (at $p = 0.01$) for all the three factors i.e., the main effects: seed priming and cultivars and their interactions (Table 1). The interaction effects were significant for percent germination at the 2nd, 4th and 8th date of germination and normal seedlings percentage at the 8th date. The control treatment i.e., seed without priming showed consistent highest percentage of seedlings germinated (Table 1). Germination failure increased as the hydration interval increased from 4 to 8 h followed by 24 h dehydration treatment (Table 1). The control treatment x cultivars performance was the same at the 2nd and 4th day of germination, while slight difference was observed at the 8th day.

The small seeded cultivar Mangeni produced significantly higher percentage of germinated seed in the control treatment in all the 2nd and 8th date and percent normal seedlings at the last date of germination evaluation. However, cultivars Mangeni and 52-1 did not produce significantly different percent germination in all the 4 h hydration treatment at the 2nd, 4th and 8th date of germination and percent of normal seedlings at the

Table 1: The percent germination (radicle protruded) and number of normal seedlings at the final date of counting of three common bean cultivars treated for hydration interval (0, 4 and 8 h) followed by 24 h dehydration (ANOVA and mean separation is done using square root transformation)

Cultivars	Hydration intervals									Normal seedlings at the 8th day		
	2nd day of germination			4th day of germination			8th day of germination			Control	4 h	8 h
	Control	4 h	8 h	Control	4 h	8 h	Control	4 h	8 h			
45-1	76.7b	0.0b	0.0	96.7a	0.0b	0.0b	98.4b	0.0b	0.0b	88.4b	0.0	0.0
52-1	43.4c	16.7a	0.0	83.4b	26.7a	1.7a	90.0c	31.7a	4.7a	70.0c	0.0	0.0
Mangeni	90.0a	15.0a	0.0	100.0a	25.0a	1.7a	100.0a	30.0a	1.7a	100.0a	0.0	0.0
Mean	70.0	10.6	0.0	93.4	17.2	1.1	96.1	20.6	2.8	86.1	0.0	0.0
F test	*			**			**			**		
§SED	2.32			1.75			1.91			0.21		
†LSD 5%	4.9			3.71			4.05			0.44		
‡CV%	29.9			20.5			21.8			3.64		

*Significant at 5% level of significance, **Significant at 1% level of probability, figures followed by the same letter are not significantly different, §SED = Standard error of difference, †LSD = Least significant difference and ‡CV% = Percent coefficient of variation

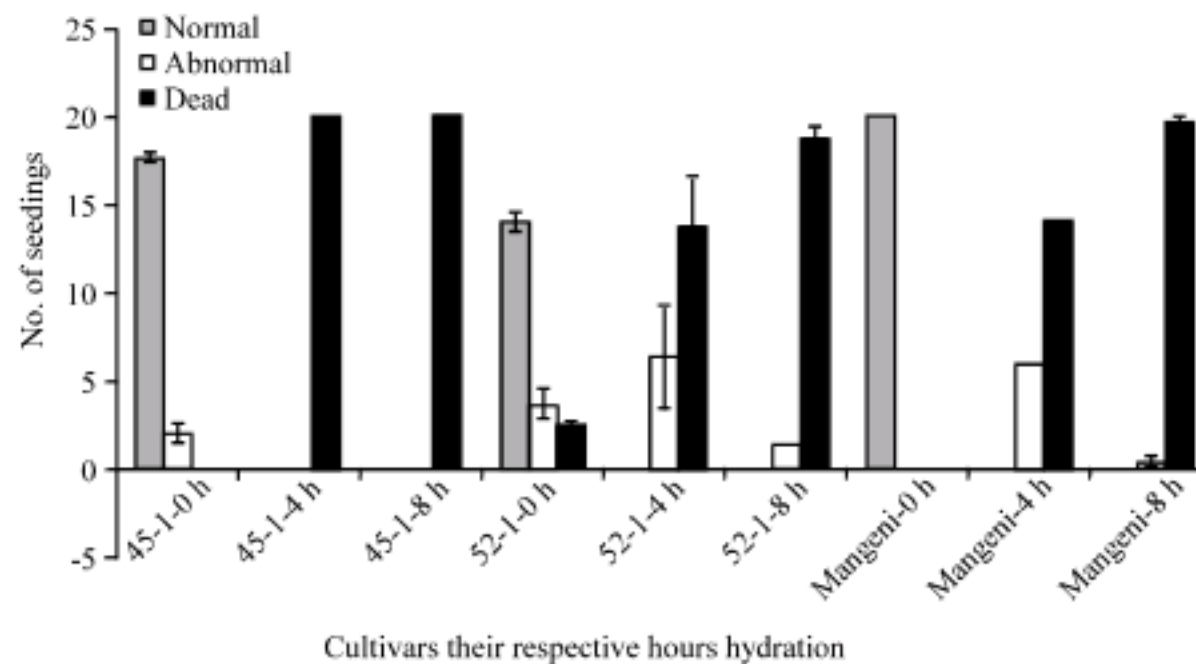


Fig. 1: The number of normally germinated, abnormally germinated and dead seeds obtained from seeds treated with 4 and 8 h hydration followed by 24 h dehydration along with the control (0 h hydration and no dehydration) evaluated at the 8th day of germination

last date (8th date) of evaluation (Table 1). Similarly, these two cultivars did not produce significantly different percent germination for the 8hour hydropriming treatment at 4 and 8th date of evaluation. Cultivars mangeni and 45-1 produced non-significantly different percent germination for the control treatment at the 4th date of evaluation. Cultivar 45-1 produced significantly lowest germination percentage in all the 4 h hydropriming treatment at the 2nd, 4 and 8th date of evaluation. All the cultivars treated with hydropriming failed to produce normal seedlings at the 8th date of evaluation. Similarly, there was no germinated seed at the 2nd date of evaluation for the 8 h hydropriming treatment (Fig. 1).

The complete failure of all the cultivars to produce normal seedlings at the final date of counting as a result of priming treatment led to the question, what caused the failure of germination? Is hydrating the seed by completely immersing in water-filled-flask or the dehydration of hydrated seed was the cause for the failure of germination? This was the reason for the initiation of the second experiment, which was designed to justify, whether the method of hydration treatment used in the first experiment caused the failure of germination or not, by comparing three treatments i.e., hydration by completely immersing the seed in

water filled flask, soaking the seed in petridish (without suffocating the seed; by avoiding completely immersing the seeds in water) and the control treatment, where no hydration treatment was applied. The result of this experiment indicated that significant difference ($p = 0.01$) was found only among cultivars at the second day of germination, while the type of hydration media was non-significant. Both of the main effects were non-significant on the fourth day germination, while only the type of germination media was significant ($p = 0.01$) at the 8th day of germination. The main effects i.e., cultivars and type of media of hydration were significant ($p = 0.01$) for the number of normal seedlings at the final day (8th day) of germination. There was no significant interaction between the cultivars and the type of hydration media for percent germination at the 2nd, 4 and 8th day and percent normal seedlings at the 8th day of germination.

The small seeded cultivar Mangeni showed significantly higher germination, followed by cultivar 45-1; while cultivar 52-1 gave the lowest germination percent at the second day of germination (Table 2), which is in line with the result of the first experiment. Mangeni and cultivar 45-1 gave the highest and non-significantly different percent germination, while cultivar 52-1 produced significantly lower percent germination of normal seedlings at the final date of germination (Table 2). On the other hand, the control treatment i.e., media of hydration (no hydration treatment) and seeds soaked in petridish did not show significant difference, while completely immersing the seed in flask produced significantly lowest germination percent for both percent germination at the 8th day and percent normal seedlings at the final date. However, none of the treatments showed complete failure of germination in this experiment. The correlation analysis showed that high and positive association was found between percent seeds failed to germinate (dead seeds and abnormal seedlings) with average conductivity and average moisture gain from imbibition (Table 3). Highly positive association was found between conductivity with seed weight and mean moisture gain from imbibition (Table 3).

Table 2: The number of seeds germinated (radicle protruded) and number of normal seedlings at the final date of counting of three common bean cultivars tested for the type of hydration media (ANOVA and mean separation is done using square root transformation)

Cultivars treatments	Media of hydration		Treatments	Media of hydration	
	2nd day of germination	Normal seedlings at the 8th day		8th day of germination	Normal seedlings at the 8th day
45-1	81.7b	88.3b	Control	83.3c	70.0c
52-1	50.0c	63.3c	Completely immersed	98.3b	88.3b
Mangeni	100.0a	100.0a	Partially soaked	100.0a	93.3a
Mean	77.2	83.9	Mean	93.9	83.9
F-test	**	**	F-test	**	**
§SED	1.09	1.0	SED	0.74	1.0
†LSD 5%	2.52	2.23	LSD 5%	1.70	2.23
‡CV %	6.9	6.0	CV %	4.2	6.0

*Significant at 5% level of significance, **Significant at 1% level of probability, figures followed by the same letter are not significantly different, §SED = Standard error of difference, †LSD = Least significant difference and ‡CV% = Percent coefficient of variation

Table 3: Multiple correlations of the characters studied

Characters	(1)*	(2)*	(3)*	(4)*	(5)*
Conductivity	1.000				
Seed moisture content	0.610	1.000			
Number of dead and abnormal seedlings	0.661	-0.191	1.000		
Seed weight	0.963	0.800	0.436	1.000	
Mean moisture gain	0.860	0.121	0.951	0.692	1.000

*Number in bracket correspond to the type of traits listed in column 1 of the table in their respective order

There was no complete failure of germination in any of the factors in the second experiment (Table 2), as opposed to most of the 4 and 8 h hydropriming treatments (Table 1). There was higher number of dead seeds for cultivar 45-1 followed by cultivar 52-1 in completely immersed in flask treatment. On the other hand, abnormal number of seedlings was highest for cultivar 52-1 in both completely immersed and soaked in petridish treatments (Fig. 2). There were no dead and abnormally germinated seeds for both cultivars 45-1 and mangeni in both the control and soaking in petridish treatments. Exceptionally, some abnormally germinated seeds were recorded on the cultivar 52-1 in the control treatment. The percent (%) moisture content of the seeds before hydration treatment was the same for the two cultivars Mangeni and 45-1, while it was slightly higher for cultivar 52-1 (Fig. 3).

The conductivity of the large seeded cultivars 52-1 was higher for the control treatment followed by medium seeded cultivar 45-1, while the small seeded cultivar mangeni showed the lowest level of conductivity. Successive reduction in conductivity of the large seeded

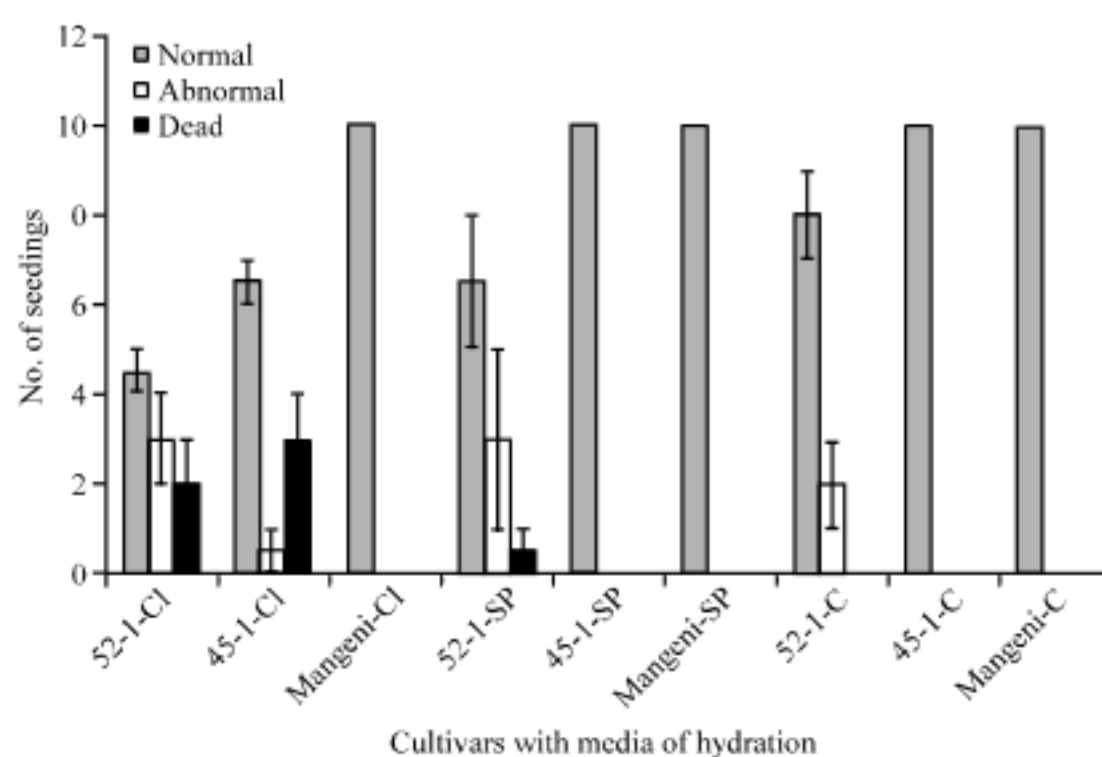


Fig. 2: Number of normally germinated, abnormally germinated and dead seeds obtained from three common bean seeds treated with three pre-hydration treatment. CI: Completely immersed, SP: Soaked in petridish, C: Control (no pre-hydration treatment) evaluated at the 8th day of germination

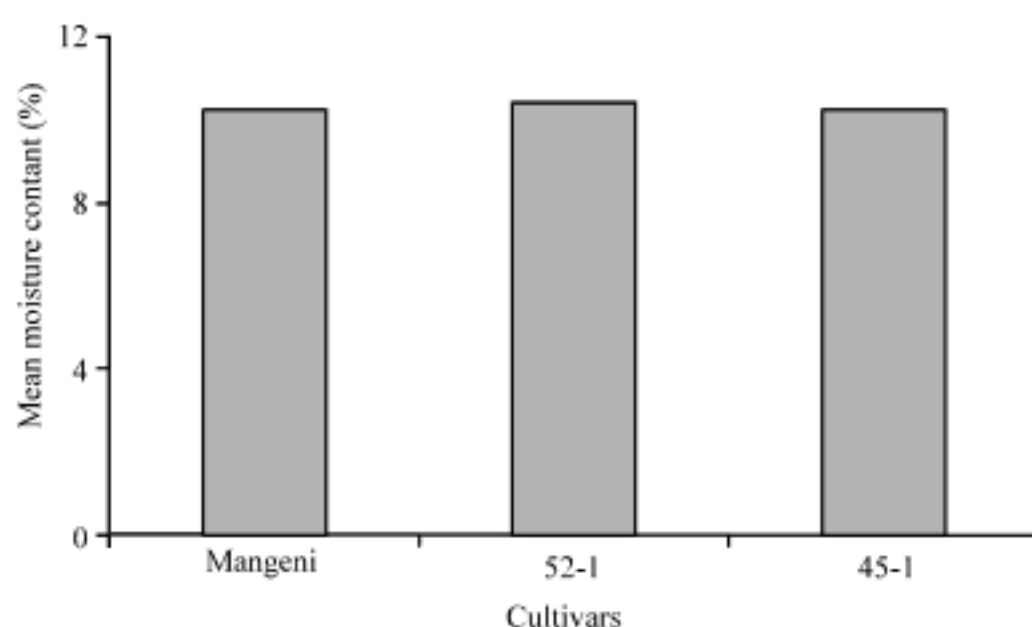


Fig. 3: Mean percent seed moisture content of three common bean cultivars

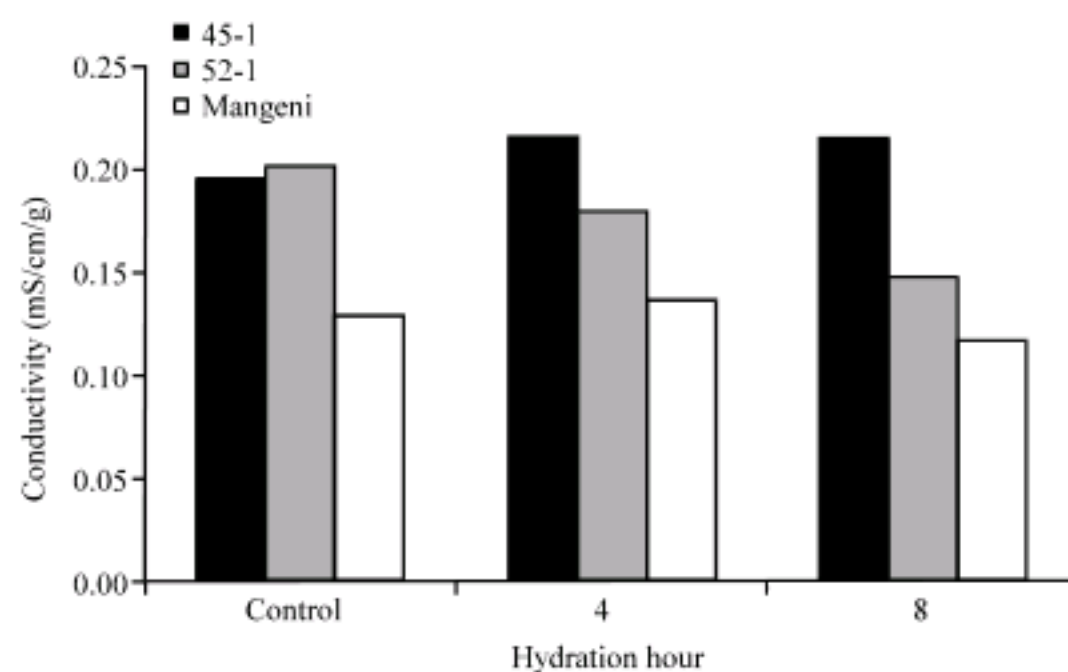


Fig. 4: Conductivity of three common bean cultivars hydrated at three time intervals (0, 4 and 8 h)

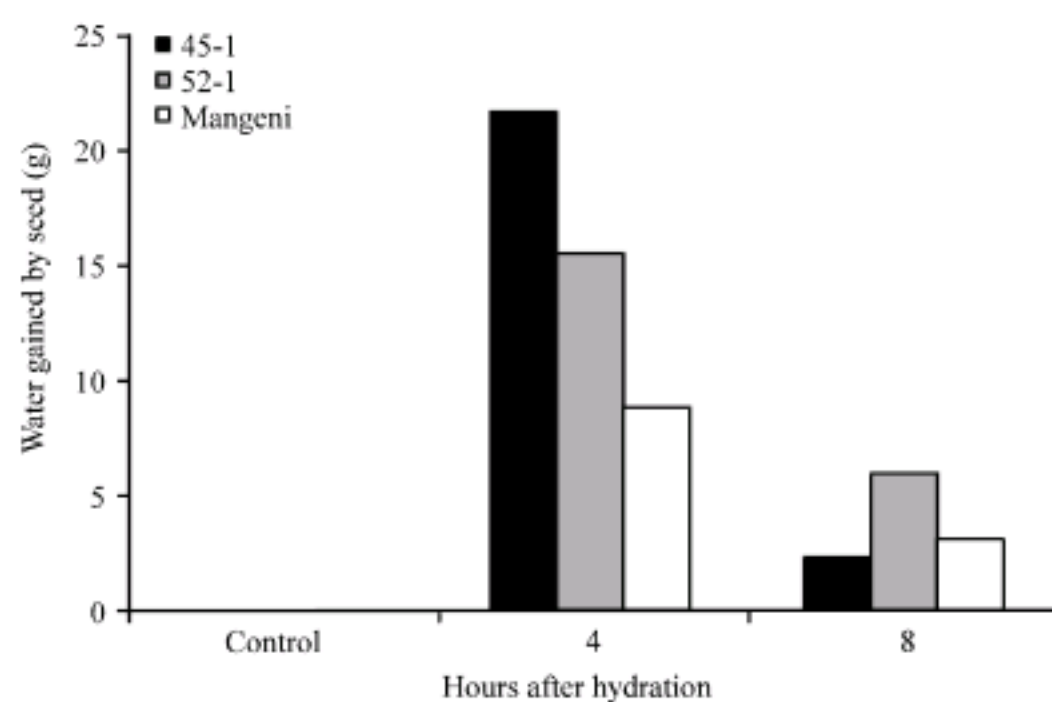


Fig. 5: Trend in water uptake in three common bean cultivars hydrated at three time intervals (0, 4 and 8 h)

bean cultivar 52-1 was found as the hydration increased from 0, 4 and 8 h (Fig. 4). All the cultivars showed increased water uptake at the initial stage of hydration, with subsequent decline, as the time of hydration increased. The water uptake rate of cultivar 45-1 was rapid and highest with subsequent sharp decline, as compared to the other two cultivars. Mangeni showed the lowest water uptake rate (Fig. 5).

DISCUSSION

The fact that dry bean seed did not respond to hydropriming in contrast to what is reported on sorghum and double cross maize hybrid (Afzal *et al.*, 2002) and Chick pea (Kaur *et al.*, 2002) might be due to several factors, of which the nature of the crop, which includes the nature of the embryo, cotyledon and nutritional composition of the seed, the nature of the seed coat, sensitivity of the crop to desiccation, leakage of substances and sensitivity of the seed to suffocation during hydration are the most important ones.

Different cultivars with varying seed sizes responded differently to seed priming treatment. The larger the seed size the lower the germination capacity of the seed and larger seeded varieties were more sensitive to desiccation that arose from dehydration of the hydrated seed. This is mainly because of the larger surface area of large seeded bean cultivars, which might have exposed them for more physical injuries and cotyledonal cracking. Leakage of substances will also be high from such large seeded cultivars due to the same larger surface area of the seed. The consistently lower number of normally germinated seedlings in the control (no priming) treatment on the large seeded cultivar is in agreement with the report of Mazibuko and Modi (2005), where the large seeded bean cultivars are more liable to cotyledonal cracking and subsequent failure to germinate.

The failure of germination has occurred in all the varieties at both 4 and 8 h seed priming treatments, however, the severe failure of germination in both the 4 and 8 h seed priming treatment in the cultivar 45-1 indicates the sensitivity of this cultivar to seed priming. The complete failure of germination that received seed priming treatment was more serious, when the hydration interval was extended. This might be due to more hydration time causes more leakage of substances and suffocation.

The lower percentage of normal seedlings in the hydrating seed by completely immersing in water indicates that completely immersing the seed when hydrating reduces the germination of the seed, which might be associated with leakage of substances and lack of aeration. This was evidenced by the positive association of number of seeds failed to germinate with average conductivity and average moisture content. This result is in agreement with the explanation given by McDonald (2000) that increasing the level of soil moisture content reduces germination significantly; probably due to suffocation. The positive association of conductivity with seed weight and moisture gained from hydration might also indicate that leakage of nutrients is higher in the large seeded bean cultivars. However, this need to be further studied to verify the result with large number of large seeded and small seeded cultivars.

The germination of significant number of normal seedlings in the fully immersed in water filled flask treatment indicated that the cause for the failure of germination in the first experiment was not the type of hydration method used, which was completely immersing the seed in the water filled flask. The possible cause might be the dehydration treatment, which was applied using concentrated lithium chloride solution following hydration. This result is in agreement with the result of Quan *et al.* (2004), where dehydration treatment of pre-imbibed seeds caused drastic reduction in the survival of mung bean seeds. Factors associated with large seed size, such as cotyledonal cracking and higher leakage of nutrients might be the cause for such abnormal germination of seeds.

The consecutive reduction in the conductivity of the large seeded bean cultivar indicates that this cultivar is more liable to leakage as the hydration time increases, in contrast to the small seeded cultivar mangeni. This is also one of the reasons for the lowest number of normally germinated seedlings of the large seeded cultivar 52-1. In general, the large seeded bean cultivar was inferior in performance, while more sensitivity to desiccation due to dehydration was observed in the medium seeded cultivar 45-1. The small seeded cultivar mangeni showed overall superior performance.

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