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Pre-storage seed treatments for improved germinability and field performance of urdbean

N. LAYEK, S.K. MISHRA, B.K. DE and A.K. MANDAL

Institute of Agricultural Science, University of Calcutta, 35 Ballygunge Circular Road, Kolkata 700 019, India

ABSTRACT

Pre-storage dry seed invigoration treatments of freshly harvested urdbean seeds (*Vigna mungo* L. Hepper) cv. Sarada with aspirin, bleaching powder, iodinated calcium carbonate and red chilli powder showed significantly improved post-storage germinability, field performance and productivity over control. However, pre-storage wet treatments did not show any beneficial effect on storability and field performance over control. Physiological and biochemical studies indicated higher membrane integrity and greater enzyme activity of the dry treated seeds than untreated control. The lipid peroxide formation and volatile aldehyde production were also significantly lower in the treated seeds than in control. Thus, pre-storage dry treatments may be suggested for the improvement of storability and field performance of urdbean seeds.

Key words: Germination, Storability, pre-storage treatment, Membrane integrity, Electrical conductance

In India, poor to medium quality seeds are used for planting field and horticultural crops resulting in substantial yield loss (Banerjee 1984). Seed may undergo certain irreversible changes after attaining physiological maturity that may reduce seed quality leading to loss of vigour and viability and consequently reduction in yield potential of the standing crop (McDonald 1999). Storing seeds in gunny bags or earthenware vessels absorbs lot of moisture from atmosphere during monsoon months and hastens seed ageing. Mid-storage hydration-dehydration (H-D) treatment helps to maintain vigour, viability and productivity of several leguminous and non-leguminous crop seeds (Basu 1994). However, drying of seeds after hydration is problematic during monsoon months. In addition, hydration-dehydration treatment is ineffective in freshly harvested seeds of many crops due to soaking injury (Saha and Basu 1984).

Dry-dressing treatments of freshly harvested (high-vigour) or high-medium-vigour seeds with halogenated compounds like bleaching powder, iodinated calcium carbonate, *etc.*, retard seed deterioration after subsequent storage (Mandal *et al.* 2000). However, farmers may consume surplus edible seeds that have been treated with potentially harmful seed protective chemicals. Under such situation, treatment with natural crude plant materials or with very low concentrations of non-toxic chemicals or pharmaceutical products would be desirable for improved storability and field performance. Our objectives were to standardize a suitable dry treatment for improvement of germinability and field performance of high-vigour urdbean seed.

MATERIALS AND METHODS

Freshly harvested urdbean (cv. Sarada) seeds were cleaned and thoroughly sun dried for 4-5 days to a moisture content of about 8.9% for safe storage and then stored in 2.5 litre capacity rubber stoppered glass bottles under ambient conditions. Pre-storage seed invigoration treatments were given to one month-old (high-vigour) seeds. Seeds were dry-dressed with finely powdered chemicals, pharmaceutical formulations and crude plant materials such as aspirin (active ingredient, acetyl salicylic acid) @ 50 mg/kg of seeds, bleaching powder (active ingredient, calcium hypochlorite) @ 2 g/kg of seeds, calcium carbonate @ 3 g/kg of seeds, iodinated calcium carbonate (30 mg iodine impregnated with 3 g of calcium carbonate) @ 3 g/kg of seeds and red chilli powder @ 1 g/kg of seeds following the method of Mandal *et al.* (2000). After treatment, glass bottles (treated and untreated) were thoroughly shaken once in a day up to 7 days for thorough mixing of finely powdered chemicals pharmaceutical products and crude plant materials with the seeds and kept at room temperature.

Wet treatments were given to urdbean seeds following the method of Saha and Basu (1984). It was given by three different ways.

i) **Soaking-drying (S-D)** : Urdbean seeds (100 g) were soaked in double volume of distilled water for 1 h at room temperature (29 ± 1°C) with occasional stirring. After decanting off the excess water, the seeds were first surface dried with blotting papers and then dried back to its original moisture content in a drying cabinet over a current of dehumified air at 35 ± 1°C for about 72 h (Mandal and Basu, 1983).

ii) **Moist sand conditioning-drying (MSC-D)** : Seeds were preconditioned by a slow and progressive rise in the moisture content which were achieved by keeping the seeds (100 g) in moist sand (sand : seed :: 3 : 1). For this purpose, sand was sterilized with concentrated sulphuric acid to kill the microbes and then washed thoroughly and finally dried. Air-dried sand was moistened with water @ 7% (300 g sand + 21 ml water) at room temperature. Seeds were then thoroughly mixed with

the moist sand and kept covered for 8 h at room temperature (29 ± 1°C). After stipulated period, seeds were sieved to remove the sand followed by drying in the cabinet over a current of dehumified air at 35 ± 1°C for about 72 h.

iii) Moist sand conditioning followed by soaking-drying (MSC-D): Seeds (100 g) were pre-conditioned with moist sand for 8 h following the above noted method and then soaked in water for 1 h at room temperature (29 ± 1°C) followed by drying to its original moisture content (Saha *et al.* 1990).

After 7 days of treatment, all the treated and untreated seeds were subjected to natural ageing under ambient conditions (average relative humidity 82 ± 3.2% and temperature 30 ± 1.6°C) for 190 days. For this purpose, treated and untreated seeds were stored in perforated paper packets (each packet with equal number of holes and containing equal amount of seeds) and then put into the cloth bag and kept in the laboratory shelf for natural ageing. Germination test of the treated and untreated seeds (minimum 400 seeds for each treatment as specified by ISTA 1976) were carried out immediately after treatment (before ageing) and after 190 days of natural ageing following the method of Punjabi and Basu (1982). Data on germination percentage and seedling length were recorded after germination for 5 days at 29 ± 1°C temperature.

Field experiments were carried out at Calcutta University Agricultural Experimental Farm at Baruipur, 24-Parganas (S), West Bengal during pre-*kharif* season of 2003-04 and 2004-05 using randomized block design (RBD) with 3 replications for each treatment. After final land preparation, plot was divided into 3 blocks each containing 9 subplots measuring 10 m^2 (4 m ' 2.5 m) in size. A fertilizer dose of N:P:K was given @ 20 : 40 : 20 kg/ha, respectively. During final land preparation, whole amount of nitrogen, phosphate and potassium was added as a basal dose. Seeds were sown @ 12 kg/ha giving a spacing of 30 cm between the rows and 10 cm between plants. The crop received a total of three irrigations; one at the same date of sowing (post-sowing); one at flowering stage and another at pod filling stage and necessary cultural practices were done throughout the cropping period. Data on field emergence was recorded after 15 days of sowing. Plant height, yield per unit area and other yield attributing data *viz.*, number of pods per plant, pod weight per plant, number of seeds per pod, and 1000-seed weight were recorded replication-wise for each treatment.

To study the membrane permeability, 35 seeds of each treatment were soaked in 30 ml of distilled water for 1 h and 30 minutes at 29±1°C temperature. Electrical conductivity of seed leachate was recorded with a conductivity bridge following the method of Anderson *et al.* (1964). Sugar from seed leachate was estimated using anthrone reagent as described by Mc Cready *et al.* (1950). The dehydrogenase activity of the treated and untreated seeds was determined after 24 h of imbibition by taking 8 embryonic axes in a glass vial containing 2 ml of 0.2% tetrazolium chloride solution and incubated for 3 h at 30°C in dark. The embryonic axes were then thoroughly washed with distilled water and 4 ml of methoxyethanol (methyl cellosolve) were added and kept for 8 h at 28°C for the extraction of the red coloured formazan and the absorbance of the solution was recorded at 470 nm (Kittock and Law 1968).

Lipid peroxidation was studied by the thiobarbituric acid (TBA) colour reaction as outlined by Bernheim *et al.* (1948). Two hundred mg of dry urdbean seed powder were heated with 5 ml TBA reagent in a test tube for 15 minutes on a water bath and after cooling, five ml of methyl cellosolve were added to the tube and shaken. The mixture was then centrifuged for 10 minutes at 5000 rpm prior to the recording of per cent transmission at 520 nm.

Estimation of amino acid in the seed-steep water (35 seeds dipped in 30 ml distilled water for 5 h) was done following the method of Moore and Stein (1948). To 4 ml seed steeped water, 0.5 ml of 0.1 M acetic acid-sodium acetate buffer (pH 5.3) and 1 ml of 1% ninhydrin solution in dioxane were added and the reaction mixture was heated for 15 minutes in a water bath at 100°C for the colour development. The colour intensity was measured at 600 nm. The amount of amino acid was expressed as glycine equivalent per ml of leachate.

For the estimation of volatile aldehydes, emanating from treated and untreated seeds, the methods of Harman *et al.* (1982) were followed with minor modifications. Glass vial (15 ml) containing 10 ml of 3-methyl-2-benzo-thiazolinone hydrazone (MBTH) solution (0.2%) was kept at the bottom of air-tight plastic containers (8.5 cm diameter) and around the glass vial (containing MBTH solution) urdbean seeds were placed for germination. In one glass vial only, MBTH solution (0.2%) was taken and kept inside the empty containers as the control (blank). The whole set was kept at 23 ± 1.5°C for 48 h. The aldehyde emanated from the germinating seeds was trapped by MBTH solution. After 48 h of incubation, 3 ml of MBTH solution from each glass vial of different treatment sets including control was taken into test tube, in which 2.5 ml of 0.23% (w/v) ferric chloride solution was added and the mixture was incubated for 10 minutes and then 2.5 ml of acetone was added to each tube and tightly corked. After 30 minutes, the absorbance of the solution was measured on a Systronic Photoelectric Colorimeter at 635 nm.

The data obtained from laboratory germination test, field experiments and biochemical tests were analysed statistically following the method of analysis of variance (Fisher, 1948). Data on germination percentage were transformed to their respective arc-sin angle prior to statistical analysis and seedling length data were analysed as such.

RESULTS AND DISCUSSION

Germination tests (before ageing) did not show any significant difference on vigour and viability between the treated and untreated seeds (Table 1). Among the treatments, bleaching powder and red chilli powder showed marginal improvement on seedling length over control. But after natural ageing under ambient conditions for 190 days, all the dry treatments significantly improved germination percentage and seedling length over control (Table 1). Bleaching powder, red chilli powder and iodinated calcium carbonate has shown better results for the maintenance of vigour and viability of urdbean seeds over control. Seedling vigour as measured by root and shoot length and vigour index were also improved by dry treatments. Pre-storage wet treatments *viz.*, soaking-drying, moist sand conditioning-drying and moist sand conditioning-soaking-drying did not show any significant improvement on germinability over control (Table 1).

The field performance and productivity of the crop raised from the pre-storage dry treated seeds showed significant improvement in the field emergence per unit area, grain yield per unit area and other yield attributes such as number of pods, number of seeds and pod weight per plant including 1000-seed weight over control (Table 2). Among the dry treatments, bleaching powder, red chilli powder and

Table 1. Effect of pre-storage seed invigoration treatments on vigour and viability of urdbean seeds

Treatment	Germination before ageing				Germination under natural ageing (190 days)			
	(%)	Arc-sin value	Seedling length (mm)	Vigour index*	(%)	Arc-sin value	Seedling length (mm)	Vigour index*
Control	95	77.08	181	17195	48	43.85	114	5472
Aspirin	94	75.82	182	17108	58	49.60	124	7192
Bleaching powder	96	78.46	184	17664	67	54.94	129	8643
Calcium carbonate	94	75.82	183	17202	60	50.77	122	7320
Iodinated calcium carbonate	95	77.08	182	17290	62	51.94	125	7750
Red chilli powder	96	78.46	183	17568	65	53.73	127	8255
S-D	94	72.54	178	16732	47	43.28	110	5170
MSC-D	93	74.66	180	16740	48	43.85	110	5280
MSC-S-D	95	77.08	182	17290	50	45.00	113	5650
LSD at 0.05	-	NS	NS	-	-	3.1	7.9	-
LSD at 0.01	-	NS	NS	-	-	4.2	10.9	-

*Vigour index = Germination % Seedling length

S-D: Soaking-drying; MSC-D: Moist sand conditioning-drying; MSC-S-D: Moist sand conditioning-soaking-drying; NS - Not significant

Table 2. Effect of pre-storage dry and wet physiological seed treatments on field performance and productivity of stored urdbean seed

Treatment	Field emergence (%)	Plant height (cm)	No. of pods/plant	Pod weight/ plant (g)	No. of seeds/plant	Seed yield/m^2 (g)	1000-seed weight (g)
Control	76	49.7	22	12.68	112	156.6	37.40
Aspirin	84	50.3	24	12.87	119	162.7	37.97
Bleaching powder	85	51.3	26	13.03	137	164.2	40.13
Calcium carbonate	83	52.4	24	12.86	118	160.6	37.12
Iodinated calcium carbonate	84	50.7	25	12.88	121	162.9	38.62
Red chilli powder	84	51.2	25	12.91	121	163.3	38.90
S-D	70	50.5	23	12.67	112	148.8	36.55
MSC-D	71	51.8	24	12.68	112	151.9	36.75
MSC-S-D	71	51.6	24	12.70	116	153.3	37.03
LSD at 0.05	5	N.S	1.4	0.18	5.9	4.8	0.57
LSD at 0.01	6.9	N.S	1.9	0.25	8.1	6.6	0.78

iodinated calcium carbonate has shown better results in improving yield and other yield attributes over control (Table 2). But pre-storage wet treatments (*viz.*, soaking-drying, moist sand conditioning-drying and moist sand conditioning-soaking-drying) did not show any improvement on field performance and productivity over control (Table 2). The ineffectiveness of wet treatment in freshly harvested seed (high-vigour) is probably due to soaking injury.

Germination test conducted immediately after treatment did not show any significant improvement on germination percentage and seedling vigour over control (Table 3). The membrane functions as evidenced by leaching of electrolytes, sugar and amino acid and enzyme activity *viz.*, dehydrogenase and lipid peroxidation activity and volatile aldehyde productions did not show any significant difference between treated and untreated seeds when tested immediately after treatment (Table 3). Only soaking-drying and moist sand conditioning followed by soaking and drying showed lower leakage of electrolytes in spite of similar germination percentage, probably because of the initial leakage at the time of soaking. But, after natural ageing under ambient condition for 167 days, all the dry treatments gave significantly better results in improving germination percentage and seedling vigour over control (Table 4). The electrical conductance of seed leachate, leakage of sugar and amino acids were significantly lower in the dry treated seeds than the untreated control (Table 4). The dehydrogenase enzyme activity was also significantly higher in the dry treated seeds than the untreated control (Table 4). Among the dry treatment, bleaching powder and red chilli powder showed better results in maintaining membrane integrity and enzyme activity. The pre-storage wet treatments did not show any beneficial improvement on membrane functions and enzyme activity over control.

Table 3. Effect of pre-storage seed invigoration treatments on physiological and biochemical parameters before ageing

Treatment	Germination		Electrical conductance (dsm^{-1})	Sugar µg glucose equiv./ml	Amino acid glycine equiv./ml	Dehydro-genase enzyme activity (OD)	Lipid peroxide-tion (OD)	Volatile aldehydes (OD)
	(%)	Arc-sin value						
Control	94	75.82	0.0680	14.08	7.24	0.3979	0.0835	0.0856
Aspirin	95	77.08	0.0680	13.83	6.75	0.4001	0.8510	0.0851
Bleaching powder	95	77.08	0.0604	14.41	6.99	0.4023	0.0845	0.0835
Calcium carbonate	93	74.66	0.0529	14.66	7.50	0.3979	0.0798	0.0867
Iodinated $CaCO_3$	94	75.82	0.0604	13.75	6.99	0.4100	0.0767	0.0835
Red chilli powder	96	78.46	0.0529	13.58	7.36	0.3957	0.0788	0.0862
S-D	94	75.82	0.0226	13.25	7.12	0.4012	0.0814	0.0840
MSC-D	95	77.08	0.0302	13.33	7.24	0.3979	0.0829	0.0872
MSC-S-D	96	78.46	0.0151	13.00	6.87	0.3968	0.0783	0.0845
LSD at 0.05	-	NS	0.0164	NS	NS	NS	NS	NS
LSD at 0.01	-	NS	0.0226	NS	NS	NS	NS	NS

Table 4. Effect of pre-storage seed invigoration treatments on physiological and biochemical parameters after natural ageing for 167 days

Treatment	Germination		Electrical conductance (dsm^{-1})	Sugar µg glucose equiv./ml	Amino acid glycine equiv./ml	Dehydro-genase enzyme activity (OD)	Lipid peroxide-tion (OD)	Volatile aldehydes (OD)
	(%)	Arc-sin value						
Control	50	45.00	0.1410	32.91	18.88	0.1668	0.1972	0.209
Aspirin	60	50.77	0.0931	25.25	15.01	0.2472	0.1403	0.149
Bleaching powder	68	55.55	0.0881	22.25	13.89	0.2882	0.1221	0.128
Calcium carbonate	63	52.53	0.1007	25.33	15.76	0.2518	0.1355	0.151
Iodinated $CaCO_3$	65	53.73	0.0957	24.58	15.51	0.2588	0.1296	0.143
Red chilli powder	68	55.55	0.0906	22.58	14.14	0.2831	0.1237	0.137
S-D	48	43.85	0.0352	29.00	20.22	0.1561	0.2225	0.227
MSC-D	50	45.00	0.0428	29.58	20.01	0.1785	0.2000	0.206
MSC-S-D	52	46.15	0.0327	26.58	19.51	0.1904	0.1979	0.217
LSD at 0.05	-	3.6	0.0109	5.0	2.6	0.0238	0.0134	0.007
LSD at 0.01	-	5.0	0.0151	6.9	3.1	0.0325	0.0185	0.010

The lipid peroxidation activity and volatile aldehyde productions were significantly lower in dry treated seeds than the untreated control (Table 4). Among various dry treatments, bleaching powder and red chilli powder showed better results in reducing the lipid peroxide formation and aldehyde productions. The role of iodine in the stabilization of double bonds of unsaturated fatty acid moieties of lipoprotein biomembranes and controlling free radicals as a possible reason for viability extension has been suggested by Pryor and Lasswell (1975) and Basu and Rudrapal (1980). Chlorine would also be more or less similar to iodine in seed protective action (Mandal and Basu 1986). The role of certain chemicals, pharmaceutical products and crude plant materials has been reported as antioxidant, antioxidant-synergist and radio protective agents (Milvy 1973). Capsaicin, the most important constituent of chilli (*Capsicum frutescens* L.) is acknowledged as inhibitor of lipid peroxidation (Brand *et al.* 1990). The beneficial role of natural plant preparation could also be due to reduce lipid peroxidation. Sung and Chiu (2001) have given strong support to the concept of free radical induced lipid peroxidation as a causative factor of seed deterioration in sweet corn. The protein protective role of acetyl salicylic acid (aspirin) may be operative in viability maintenance. Takaki and Rosim (2000) have reported that aspirin application to *Raphanus sativus* seed would increase the tolerance to high temperature and synchronized seed germination.

The present study proved that bleaching powder @ 2 g, red chilli powder @ 1 g and iodinated calcium carbonate @ 3 g per kg of seed could be helpful for the maintenance of germinability and field performance of high-vigour urdbean seeds.

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