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SHORT COMMUNICATION

SEED TREATMENTS FOR IMPROVED STORABILITY AND FIELD PERFORMANCE OF GRAM (*CICER ARIETINUM* L.)

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Pre-storage dry treatments of freshly harvested Bengal gram seed (high-vigour) with calcium hypochlorite (common bleaching powder, @ 2 g / kg of seed), iodinated calcium carbonate (30 mg iodine impregnated with 3 g of calcium carbonate, @ 3 g / kg of seed), alcohols such as methanol and isopropanol (1 ml each of methanol and isopropanol mixed with 3 g of calcium carbonate @ 3 g / kg of seed) and wet treatments such as moisture equilibration-drying (hydrated over saturated atmosphere for 24 h followed by drying back to its original moisture content) significantly improved post-storage germinability over untreated control. The treated seeds also showed significant increase in yield and its attributes per unit area over control. The refrigerated control (seeds kept in the refrigerator after harvest) which was taken as a reference treatment has shown better results in improving storability and field performance over control and other treatments. Physiological and biochemical studies on treated seeds showed greater membrane integrity as measured by reduced leakage of electrolytes, sugars and amino acids over untreated control. Dehydrogenase enzyme activity was significantly higher in the treated seeds than the control. The dry and wet treated seeds also showed lower lipid peroxide formation than the control. On the basis of present findings, dry treatments of seeds with calcium hypochlorite and iodinated calcium carbonate and wet treatments such as moisture equilibration-drying in freshly harvested Bengal gram seeds (high-vigour) are suggested for improved storability and field performance.

***Key words*: Germinability, gram, seed treatment, vigour, yield.**

In the eastern parts and the coastal belts of India, it is difficult to maintain seed vigour and viability of stored seed under ambient conditions because of high humidity and high temperature. During March-April, the crops are harvested and seeds are stored in moisture permeable containers under ambient conditions that would show a rapid fall in germinability and by sowing time in November-December, the viability may still go down to half or even less. Monsoon considerably raises the ambient relative humidity (average 58 % in March to 82 % in July) and as a result, the seed stored in gunny bags absorb a lot of moisture from the atmosphere, greatly hasten the ageing process of the seed.

Hydration-dehydration treatments of stored seed of agriculturally important crop plants have been reported to minimize deterioration during subsequent storage under ambient warm humid conditions and increase yield over their respective control (Basu and Dasgupta 1974, Basu 1976, Mandal and Basu 1982). However, legume seeds would be adversely affected because of soaking injury during rapid imbibition of water (Powell and Matthews

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1978, Saha and Basu 1984). Therefore, a suitable hydration-dehydration treatment to alleviate soaking injury would be desirable for the maintenance of vigour and viability of gram seed. But hydration-dehydration treatment of large seed stocks has one disadvantage that is the problem of drying back to the original weight which is essential for restorage of the seed till sowing in the field. The efficacy of dry-dressing treatments with halogenated compounds, non-toxic chemicals, pharmaceutical products and crude plant materials in high-vigour wheat and soybean for the retention of germinability and field performance have been reported by Mandal and Basu (1986) and De *et al*. (2003). Keeping these aims in view, the present study was undertaken for identification of suitable, inexpensive and easily practicable dry treatments with halogenated compounds and alcohols for better storability and field performance of gram seeds.

Seeds of gram (*Cicer arietinum* L. cv. Deshi BG 240) were collected from the State Seed Corporation Ltd., Government of West Bengal and dried in the sun to a moisture content of 9.5 % and then stored in rubber stoppered glass bottles (2.5 ltr. capacity). The experiments were conducted in three consecutive years (1995-96, 1996-97 and 1997-98). Dry-dressing treatments were given to freshly harvested gram seeds (1-month-old) with calcium hypochlorite (common bleaching powder @ 3 g / kg of seed), calcium carbonate (3 g / kg of seed), iodine (30 mg of iodine was thoroughly mixed with 3 g of calcium carbonate @ 3 g / kg of seed); methanol and isopropanol (1 ml each of methanol and isopropanol was mixed with 3 g of calcium carbonate, @ 3 g / kg of seed) and then stored in rubber-stoppered glass bottles (300g seeds in 500 ml capacity bottle) at room temperature (29 ± 1^0C), which were gently shaken once daily for seven days to thoroughly mix the chemicals with the seed.

Moisture equilibration-drying (ME-D) treatment was given to the same seed lot following the method of Saha and Basu (1984). In this method, seeds were kept in an atmosphere of 100 % RH to raise seed moisture without direct contact with liquid water. Such a treatment would eliminate the possibility of leaching of substances from the seeds, besides a slow hydration would minimize soaking injury. To maintain the relative humidity, an enamel tray (38 cm × 30 cm × 6 cm), lined with moist blotting paper on all sides and containing 200 ml of distilled water were taken. A number of paper tray with seeds spread in a thin layer were arranged on the glass plate. The enamel tray with seeds was then covered with a heavy glass plate lined internally with thick layer of moist blotting papers to maintain 100 % relative humidity. The seeds were allowed to absorb moisture from saturated atmosphere (100 % RH) for 24 hours at room temperature (29 ± 1^0C). After moisture equilibration, seeds were dried back to its original moisture content at 35 ± 1^0C in a drying cabinet.

After 15 days of treatment, seeds were subjected to natural ageing under ambient conditions for 60 days and accelerated ageing at 93 % RH and 40^0C for 18 days. Germination test were carried out following the method of Punjabi and Basu (1982) to evaluate the treatment effects on germinability over untreated control.

Field experiments were conducted at Experimental Farm of Calcutta University at Baruipur, 24-Parganas (S), West Bengal, during winter seasons in three consecutive years (1995-96 to 1997-98) using completely randomized block design with 3 replications for each treatment. A fertilizer dose of N:P:K was given at the rate of 40:30:20 kg / ha respectively. The plot size was 10 sqm (4 m × 2.5 m) for each replication. During final land preparation, 50 % of the total nitrogen and full amount of phosphate and potassium were added to soil. Then the rest of the nitrogen was supplied in two equal split application; one after 21 days of sowing and another at flower initiation stage. Seeds were sown at the rate of 15 kg / ha in the 2nd week of November, and rows were kept apart at 40 cm. A post sowing irrigation was given on the same day and the crop received a total of 3 irrigations. After 25 days of sowing, plant population per plot was counted replication wise and then fixed to thirty per sqm. Data on grain yield and other yield attributes were recorded after harvest (105 days) replication-wise for each treatment.

To study the membrane permeability as evidenced by electrical conductance, leaching of sugar and amino acids were done following the method of Anderson *et al*. (1964); Mc Cready *et al*. (1950) and Moore and Stein

(1948) respectively. The dehydrogenase enzyme activity and lipid peroxide formation of treated and untreated seeds were estimated following the method of Kittock and Law (1968) and Bernheim *et al*. (1948).

Germination test conducted immediately after seed invigoration treatments did not show any beneficial effect on germination percentage over control (Table 1). But after 60 days of natural ageing under ambient conditions, all the treatments (dry and wet) showed significant improvement on germination percentage over control (Table 1). The vigour of the seedling as measured by root and shoot length were also significantly increased by iodine, methanol and isopropanol treatment over control (Table 1). Moisture equilibration-drying (ME-D) treatment also showed better results for the maintenance of vigour and viability of gram seeds over control. Among the treatment, iodine, methanol and isopropanol has shown much effectiveness in controlling seed deterioration of gram (Table 1). Vigour index (germination % × seedling length) was also high in these treated seeds than the control. Another control treatment (refrigerated seed) which was kept in the refrigerator (1 month) after harvest, taken as a reference treatment showed greater germinability and vigour index (Table 1).

Plant population per unit area was significantly increased by calcium hypochlorite, iodine, isopropanol (dry treatment) and moisture equilibration (wet treatment) over control (Table 2).Grain yield per unit area was significantly increased in all the dry and wet treatments over untreated control (Table 2). The refrigerated control (after harvest, seeds stored in refrigerator) which was taken as a reference treatment showed highest yield per unit area. But seeds kept in controlled condition i.e stored in refrigerator are not feasible to our small and marginal farmers due to the involvement of cost factor besides, non-availability of continuous power supply in the remote

Table 1. Effect of seed invigoration treatments on vigour and viability of gram seeds before and after natural ageing under ambient conditions for 2 months.

Treatments	Before ageing					Natural ageing				
	Germination (%)	Arc-sin value	Mean root length (mm)	Mean shoot length (mm)	Vigour index*	Germination (%)	Arc-sin value	Mean root length (mm)	Mean shoot length (mm)	Vigour index*
Control	97	80.0	168	40	20176	72	58.0	105	28	9576
Calcium hypochlorite	98	81.9	172	39	20678	88	69.7	113	31	12672
Calcium carbonate	98	81.9	170	37	20286	94	75.8	119	33	14288
Iodine	100	90.0	163	41	20400	100	90.0	130	37	16700
Methanol	100	90.0	171	40	21100	94	75.8	128	36	15416
Isopropanol	98	81.9	165	38	19894	85	67.2	127	34	13685
ME-D	100	90.0	174	42	21600	88	69.7	116	36	13376
Control (Refrigerated)	100	90.0	176	43	21900	100	90.0	123	46	16900
L.S.D. at 0.05P	-	NS	NS	NS	-	-	8.1	9	7	-

Treated and untreated seeds were placed for germination after 7 days of treatment (before ageing) and 2 months (natural ageing). Data on germination percentage, root and shoot length were recorded after germination for 7 days at 20 $\pm 1^0$C.

* Germination % × Seedling length

Abbreviations:

ME-D : Moisture equilibration followed by drying

Table 2. Effect of seed invigoration treatments on field performance and productivity of gram seed.

Treatments	Plant population/ m^2	Plant height (cm)	Number of pods/ Plant	Number of branches/ plant	No. of seeds/ plant	Grain yield (g/m^2)	1000-grain weight (g)
Control	58	52.8	13	6	20	120	193.4
Calcium hypochlorite	68	51.3	15	7	23	138	202.3
Calcium carbonate	55	53.9	14	6	23	135	200.6
Iodine	69	56.4	15	6	23	140	205.0
Methanol	64	46.2	12	7	22	133	199.9
Isopropanol	68	51.4	12	5	24	135	199.2
ME-D	69	57.0	17	6	23	142	205.6
Control (Refrigerated)	69	53.2	16	7	24	150	206.2
L.S.D. at 0.05P	10	NS	2	NS	NS	8.5	7.2

Plant populations were fixed to thirty per sqm.

Data on plant population and grain yield alongwith other yield attributes were recorded after 25 days and 105 days respectively.

areas. The stable character such as 1000-seed weight was also significantly increased in most of the dry and wet treatments over control (Table 2). But the number of seeds per plant were statistically non-significant between the treated and untreated seeds. Among the treatments, ME-D and iodine has shown better results in improving field performance and productivity over control.

There was no significant difference on membrane integrity as measured by electrical conductance, leaching of sugar, amino acid and dehydrogenase enzyme activity and lipid peroxide formation between the treated and untreated seeds when tested immediately after treatment (Table 3). But after accelerated ageing at 93 % RH and 40^0C temperature for 18 days, the membrane integrity such as leaching of electrolytes (electrical conductance), sugars and amino acids and lipid peroxide formations were significantly lowered in the treated seeds than the untreated control (Table 4). The dehydrogenase enzyme activity of treated seeds were also significantly higher than the untreated control (Table 4). Among the treatment, iodine has shown greater membrane integrity and enzyme activity with a reduced lipid peroxide formation than the control (Table 4).

Dry treatments of freshly harvested gram seeds with calcium hypochlorite (common bleaching powder) and wet treatments (ME-D) are much effective in slowing down seed deterioration and improvement of field performance and productivity. These result confirm the earlier observation of Rudrapal and Basu (1980) and Mandal and Basu (1986) on the efficacy of iodine and chlorine in controlling seed deterioration. Besides, dry treatments with alcohols viz. isopropanol and methanol at a very low concentration (1 ml of alcohol thoroughly mixed with 3 g calcium carbonate for 1 kg of seed) were also effective in maintaining vigour and viability of gram seed.

The role of iodine in the stabilization of double bonds of unsaturated fatty acid moieties of lipoprotein bio membranes as a possible reason for viability extension was suggested by Basu and Rudrapal (1980), besides the possibility of iodine acting as a free radical controlling agent (Pryor and Lasswell 1975). Chlorine (as released by bleaching powder, a source of chlorine) would also be more or less similar to iodine in seed protective action. In the present study, the chemicals viz. iodine, calcium hypochlorite and alcohols such as methanol and isopropanol were used at a very low doses for their

Table 3. Pre-storage dry and wet treatments on germinability, membrane permeability, lipid peroxidation and enzyme activity of gram seed just after treatment, i.e. before ageing conditions.

Treatments	Germination (%)	Arc-sin value	Seedling length (mm)	Electrical conductance (dSm^{-1})	Leakage of sugar (µg glucose equiv./ ml)	Leakage of amino acid (µg glycine equiv./ml)	Dehydro-genase activity nmol/h	Lipid peroxidation nmol/g tissue
Control	96	78.5	211	0.190	60	140	18.0	63.1
Calcium hypochlorite	98	81.9	208	0.189	58	142	18.2	61.8
Calcium carbonate	98	81.9	212	0.185	56	139	18.8	58.6
Iodine	99	84.3	202	0.180	59	138	20.6	61.8
Methanol	98	81.9	210	0.182	58	139	18.6	58.6
Isopropanol	98	81.9	214	0.187	58	140	18.3	58.6
ME-D	98	81.9	216	0.183	56	138	19.9	58.6
L.S.D. at 0.05P	-	NS	NS	NS	NS	NS	NS	NS

Treated and untreated seeds were placed for germination after 7 days of treatment.

Data on germination percentage and seedling length were recorded after germination for 7 days at 20±1^{0}C.

Table 4. Pre-storage dry and wet treatments on germinability, membrane permeability, lipid peroxidation and enzyme activity of gram seed after accelerated ageing at 93% RH and 40^{0}C temperature for18 days.

Treatments	Germination (%)	Arc-sin value	Seedling length (mm)	Electrical conductance (dSm^{-1})	Leakage of sugar (µg glucose equiv./ ml)	Leakage of amino acid (µg glycine equiv./ml)	Dehydro-genase activity nmol/h	Lipid peroxidation nmol/g tissue
Control	40	39.2	122	0.350	105	410	6.47	167.6
Calcium hypochlorite	65	53.7	137	0.310	99	361	12.84	108.0
Calcium carbonate	68	54.9	153	0.297	82	230	13.04	83.0
Iodine	80	63.4	164	0.275	70	235	16.33	79.8
Methanol	70	56.8	149	0.282	78	250	13.34	83.3
Isopropanol	63	52.5	133	0.206	88	261	13.34	86.5
ME-D	75	60.0	158	0.266	81	272	13.64	87.5
L.S.D. at 0.05P	-	8.6	12	0.048	9	19	4.6	6.4

Data on germination percentage and seedling length were recorded after germination for 7 days at 20±1^{0}C.

possible effectiveness in controlling free radical reactions as antioxidants, antioxidant – synergist and radioprotective agents (Demopoulos 1973b, Milvy 1973). Rudrapal and Basu (1980) suggested that the stabilization of unsaturated fatty acid components of lipoprotein membranes by treatments with halogens (iodine, chlorine and bromine) possibly reduced lipid peroxidation and free radical reactions. 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 (*Zea mays* L.) thereby confirming similar findings of this laboratory.

Regarding the mode of action of hydration-dehydration treatments, there are two main possibilities, viz. the involvement of cellular repair system in correcting age-induced biochemical lesions during seed hydration (Villiers and Edgcumbe 1975, Burgass and Powell 1984) and free radical and lipid peroxidation reaction in the stored seed (Berjak 1978, Buchvarov and Gantcheff 1984, Wilson and McDonald 1986b, McDonald 1999) that could be reduced by hydration-dehydration treatments (Saha and Basu, 1984). The mode of action of alcohols such as methanol and isopropanol in the viability maintenance are yet to be clearly understood.

Whatever may be the exact mechanism operative in the viability maintenance of gram seed, pre-storage dry treatments (high-vigour) with iodinated calcium carbonate and calcium hypochlorite or alcohols at a very low doses (1 ml of alcohol thoroughly mixed with 3 g calcium carbonate for 1 kg of seed) may be suggested for improved storability and field performance .

REFERENCES

Anderson, A.M., Hart, J.R. and French, R.C. (1964). Comparison of germination technique and conductivity test of cotton seed. *Proceed. International Seed Testing Asso*. **29**: 81-86.

Basu, R.N. (1976). Physico-chemical control of seed deterioration. *Seed Res.* **4**: 15-23.

Basu, R.N. and Dasgupta, M. (1974). Control of seed deterioration in wheat (*Triticum aestivum* L.). *Indian Agric.* **18** : 285-288.

Basu, R.N. and Rudrapal, A.B. (1980). Iodination of mustard seed for the maintenance of vigour and viability. *Indian J. Expt. Biol*. **18** : 492-494.

Berjak, P. (1978). Viability extension and improvement of stored seeds. *South African J. Sci.* **74** : 365-368.

Bernheim, F., Bernheim, M.L.C. and Wilbur, K.M. (1948). The reaction between thiobarbituric acid and the oxidation products of certain lipids. *J. Biol. Chem.* **174**: 257-264.

Buchvarov, P. and Gantcheff, T.S. (1984). Physiological influence of accelerated and natural ageing on free radical levels in soybean seeds. *Physiol. Plant.* **60** : 53-56.

Burgass, R.W. and Powell, A.A. (1984). Evidence for repair processes in invigoration of seeds by hydration. *Ann. Bot*. **53** : 753-757.

De, B.K., Mandal, A.K. and Basu, R.N. (2003). Seed invigoration treatments on different seed sizes of wheat (*Triticum aestivum* L.) for improved storability and field performance. *Seed Sci. and Technol*. **31**: 379-388.

Demopolus, H.B. (1973b). Control of free radicals in biological system. *Federation Proceed.* **32** : 1903-1908.

Kittock, D.L. and Law, A.G. (1968). Relationship of seedling vigour, respiration and tetrazolium chloride reduction by germinating wheat seeds. *Agron. J.* **60** : 286-288.

Mandal, A.K. and Basu, R.N. (1982). Dip-dry treatment for the maintenance of vigour and viability of stored wheat seed. Indian Agric. **26:** 271-278.

Mandal, A.K. and Basu, R.N. (1986). Vigour and viability of wheat seed treated with bleaching powder. *Seeds and Farms*. **12**: 46-48.

Mc Cready, R.M., Guggols, J., Silviers, V. and Owen, H.S. (1950). Determination of starch and amylase in vegetables. *Ann. Chem*. **22** : 1156-1158.

Mc Donald, M.B. (1999). Seed deterioration: physiology, repair and assessment. *Seed Sci. and Technol.* **27** :177-237.

Milvy, P. (1973). Control of free radical mechanism in nucleic acid systems: Studies in radio-protection and radiosensitization. *Federation Proceed*. **32** : 1895-1902.

Moore, S. and Stein, W.H. (1948). Photochromic ninhydrin method for use in the chromatography of amino acids. *J. Biol. Chem*.**176** : 367-388.

Powell, A.A. and Matthews, S. (1978). The damaging effect of water on dry pea embryos during imbibition. *J. Expt. Bot.* **29** : 1215-1229.

Pryor, W.A. and Lasswell, L.D. (1975). Diels-alder and 1,4-diradical intermediates in the spontaneous polymerisation of vinyl monomers. In: *Adv. Free Radical Chem*. (ed. Williams,G.H.). **5**: 27. Flek Sci., London.

Punjabi, B. and Basu, R. N. (1982). Testing germination and seedling growth by an inclined glass plate blotter method. *Indian J. Plant Physiol.* **25**: 289-295.

Rudrapal, A.B. and Basu, R.N. (1980). Iodine treatment of mungbean seeds for the maintenance of vigour and viability. *Current Sci*. **49** : 319-320.

Saha, R. and Basu, R.N. (1984). Invigoration of soybean seed for the alleviation of soaking injury and ageing damage on germinability. *Seed Sci. and Technol*. **12** : 613-622.

Sung, J.M. and Chiu, K.Y. (2001). Solid matrix priming can partially reverse the deterioration of sweet corn seeds induced by 2,2´-azobis (2-amidino propane) hydrochloride generated free radicals. *Seed Sci. and Technol*.**29** : 287-298.

Villiers, T.A. and Edgcumbe, D.J. (1975). On the cause of seed deterioration in dry storage. *Seed Sci. and Technol.* **3** : 761-774.

Wilson, D.O. and Mc Donald, M.B. (1986b). The lipid peroxidation model of seed deterioration. *Seed Sci. and Technol*. **14** : 269-300.