

Effect of different hydration-dehydration and dry-dressing treatments on viability of green gram (*Vigna radiata* wilczek L.) seeds

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ABSTRACT

Under West Bengal conditions, maintenance of vigour and viability of green gram seed (*Vigna radiata* Wilczek L) is a serious problem from harvest to next sowing. To over come this problem, experiments were conducted under ambient laboratory conditions to slow down the ageing process of the seeds during storage. High vigorous seeds of 2-3 months old were taken and subjected to Hydration dehydration (H-D), Moisture-equilibration & drying (ME – D) and dry – dressings with powdered plant materials of Ashwagandha root (200 mg kg⁻¹), Zinger rhizome (100 mg kg⁻¹), Turmeric rhizome (100 mg kg⁻¹), *Trigonella* seeds (200 mg kg⁻¹), jute leaf (100 mg kg⁻¹) and pharmaceutical preparations of vit C (celin tablet) and salicylic acid (Disprin tablet) were done. Among the treatments plain H – D with water for 3 hours and slow imbibition for 48 hours with ME – D showed significantly good effect over untreated control in respect of germination percentage and seedling growth. The treatment effect were magnified under accelerated (40°C + 98% R.H.) and natural ageing (27 ± 5°C and 73 ± 7% R.H.) conditions where as in dry dressing with powered plant materials had significant beneficial effect in maintaining seed vigour and viability over control. Among the treatments with powdered plant material such as Turmeric rhizome and jute leaf as well as pharmaceutical preparation vit C (celin tablet) had highly beneficial effect under accelerated and natural ageing conditions. Peroxidase enzyme assay study showed betterment effect in all the treatments indicating that free-radical formation due to ageing quenched by the treatments over control.

Key words : Hydration-dehydration (H-D.), moisture-equilibration & drying (ME – D), dry dressing, accelerated ageing, natural ageing.

Green gram (*Vigna radiata* Wilczek L.) seeds after harvest passes through the monsoon season and shows a significant fall in germinability by the time of next planting. According to Roberts (1972) the primary cause of loss of viability may be developed due to its extrinsic factors namely fungi, bacteria, nematodes, moisture, temperature and ionizing radiation and intrinsic or physiological, biochemical and molecular basis of ageing. To over come this problem, many seed treatments have been practiced mostly in two broad groups in most of the orthodox seed such as hydration-dehydration treatments with mild solution of inorganic salts and another with dry dressings with organic materials to slow down the ageing process at the time of storage or at mid-storage intervals showed very much effective results. The beneficial effect of hydration-dehydration treatments as well as with dry dressing helps to decrease/eliminate free-radical formation due to lipid peroxidation during ageing at storage, which damages the bio-membrane of a cell. Present study also emphasize that seed ageing is also related with aldehyde production and this seed treatment some how minimize the aldehyde production and check the damage of bio-membrane.

MATERIALS AND METHODS

Procurement of seeds

Seeds of green gram [*Vigna radiata* wilczek (L.)] have been collected from Instructional Farm, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal. Seeds after collection were cleaned and well dried thoroughly to the moisture level of 9% and then stored in bottles with properly sealed mouth after testing the germination percentage of seeds.

Seed variability test

Seed viability test were conducted as per ISTA rules in the laboratory condition by keeping the treated along with control seeds in perforated paper packets in desiccator (8" diameter) over water in such a way that, there would be no contact of paper packets containing seeds with water. Lid of the desiccator closed air tight and the whole setup kept in BOD at 40°C and 98% RH over a period 8 days for ageing. Then the seeds were set for germination test in glass-plate method.

Seedling growth

Seedling growth were recorded under laboratory conditions (in glass plate method) after 144 hours of germination set up were done in 3 replications and their mean germination percentage were recorded.

Statistical analysis

Statistical analysis was done adopting the method of Analysis of variance by Fisher 1948. Randomized Block design was adopted for analysis of data.

Hydration-dehydration treatment

To find out the efficacy of hydration-dehydration treatment on green gram seeds, soaking duration was standardized in laboratory by soaking the seeds in beaker taking 20 gm of seeds in 40 cc of water following the method of Dasgupta and Basu (1975) with little modifications. Seed soaking was done for 1 to 6 hours duration under ambient condition and the seeds were dried back to its original moisture content by spreading the seed over blotting paper under sun at least for 4 days or till it reaches its moisture content to original level. After thorough drying, the seeds were stored in paper packets and kept in desiccator over CaCl₂ for a week for stabilization of seed moisture.

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Moisture equilibration & drying treatment

Moisture-equilibration was done by keeping the seeds in moist sand (seed and sand ratio 1:3 i.e. 100 gm of seed with 300 gm of sand). Sterilized air dry sand were pre-moistured with water to 7% (100 gm dry sand + 7 ml water) and then seeds were thoroughly mixed with moist sand and kept covered for 24, 48, 72 hours respectively for slow absorption of moisture by the seed. After stipulated periods, seeds were taken out from the sand and was then dried in the sun till it reaches its original moisture content. Control seeds were neither soaked nor pre-moistened but were dried along with the treated seeds. After treatment, seeds were re-stored in paper packets in desiccator. Accelerated ageing were done to evaluate the effect of treatment by keeping the seeds in perforated paper packets in closed desiccator containing water of about 200 cc. and kept in B.O.D. at 40°C with 98% R.H. for different days for ageing. Germination test were done in glass-plates method and data of root and shoot growth and germination percentage were recorded after 6 days of germination according to ISTA rules.

Dry treatment

Harvest fresh seeds of green gram were dry dressed with finely powdered chemicals of pharmaceutical products and crude plant materials adopting the methodology of Pal and Basu (1988). Different plant materials and pharmaceutical products were thoroughly air dried and finely powdered before mixing with seeds. After dressing the seeds with powdered materials, seeds were kept in paper packets over CaCl_2 in dessicator or may be in glass bottles with air tight mouth. The chemicals of pharmaceutical origins and crude plant materials were used at following concentrations per kg of seed. Ashwagandha root powder @ 200 mg, turmeric rhizome powder @ 100 mg., *Trigonella* seed powder @ 200 mg, Jute leaf powder @ 100 mg, Zinger rhizome powder @ 100 mg, Celin tablet powder @ 100 mg, Disprin tablet powder @ 100 mg.

Peroxidase enzyme activity

The peroxidase enzyme activity were studied with treated and non-treated control seed under before, accelerated (40°C+98% R.H.) and natural ageing (27±5°C and 73±7% R.H.) conditions according to the method of Summer and Gijessing (1943) with slight modifications. Readings were taken in systronics 105 g spectrophotometer at 430 nm wave length.

RESULTS AND DISCUSSION

Standardization of soaking – drying durations for different hours starting from 1 hour to 6 hours were done in the laboratory. Germination percentage along with root-shoot growth were studied under three conditions such as before ageing, accelerated ageing and natural ageing conditions. Germination percentage and seedling growth were found more or less same as compared to control under before ageing conditions whereas under accelerated ageing and natural ageing conditions the effects were much more magnified over control (Table 1). It was observed that among the soaking duration, 3 hours was best in respect of germination percentage and root-shoot length over prolonged durations of soaking. However, all the

durations of soaking gave better result over control.

Standardization of slow hydration by moisture – equilibration & drying were done, where seeds were imbibed for 24, 48 and 72 hours in a moist sand media in a close container at 100% R.H. and $30 \pm 4^\circ\text{C}$ in ambient conditions. Experimental results showed that there was no significant difference in germination percentage and root-shoot growth in before ageing condition, but significant beneficial effect of treatment had been observed in accelerated and natural ageing conditions (Table 2). Among the different durations 48 hours showed maximum effect over other durations of slow imbibition and control treatments.

From the above experiments it may be concluded that the possible reasons for the betterment effect in maintaining seed vigour (vigour index generally measured by multiplying seedling growth with germination percentage) and viability by hydration – dehydration and moisture-equilibration-drying in green gram seed due to increase in DNA & RNA in the growing point, less active ribonuclease activity, active protein synthesis and better preservation of cellular membrane structure with allied sequential changes in the elasticity and viscosity of the protoplasm (Hanckel 72; Dell *et al.* 1994). According to Bokaria and Bandopadhyay (2002) this beneficial effect possibly due to reduction in lipid peroxidation reaction along with low formation of free-radicals by hydration-dehydration treatments.

The effect of dry-dressing with plant materials and pharmaceutical preparations were taken on the basis of their anti-oxidant activity. Results showed that among the plant preparations under accelerated ageing & natural ageing conditions, turmeric and jute leaf treatment were significantly high than that of other treatment and among the pharmaceutical preparation celin was better over Disprin. Ageing of seeds magnified the treatment effect over control significantly. (Fig. 1). The beneficial effect of dry-dressing in maintaining vigour and viability of green gram seed due to anti-oxidant property of plant material as well as inhibition in aldehyde production during ageing process (Pal and Basu 1994 and De *et al.*, 1998).

Peroxidase enzyme activity was studied to ascertain the mechanism of action of treatment effect on free radical quenching. Result showed that peroxidase activity was significantly higher in seeds grown from the treated plants over control (Table 3) in both accelerated and natural ageing regime. These increase in peroxidase activity indicates that the treatments induce the resistance against the production of free radicals and protect the seeds from faster ageing process. It was observed from the results that O.D. values in treatments showed higher values compared to control, whereas non aged set, it showed more or less same values. From the above discussion it might conclude that the above treatments in maintaining vigour and viability of green gram seeds was to free-radical quenching effect proposed by Zhang *et al.* 1993, Pal and Basu 1994.

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Table 1 : Standardization of hydration-dehydration treatments of green gram (*Vigna radiata* wilczek L.) seeds under before ageing, accelerated ageing and natural ageing conditions.

Treatment	Before ageing (BA)				Accelerated ageing (AA)				Natural ageing (NA)			
	G%	Arc-Sin value	MRL (cm)	MSL (cm)	G%	Arc-Sin value	MRL (cm)	MSL (cm)	G%	Arc-Sin value	MRL (cm)	MSL (cm)
Control (Untreated)	98.66	83.45	9.65	18.23	50.66	45.40	5.53	11.21	54.6	47.64	5.23	9.12
1 hr.	98.66	83.45	9.93	18.93	75.66	60.47	5.63	12.46	77.0	61.34	6.12	10.40
2 hrs.	94.66	76.69	8.90	17.26	79.33	62.94	6.51	17.21	75.0	60.00	6.23	11.56
3 hrs.	94.66	76.69	10.46	20.72	93.33	75.00	9.59	20.10	87.2	69.04	9.46	19.98
4 hrs.	98.66	83.45	9.81	19.92	96.66	79.53	9.65	21.22	88.0	69.73	9.65	20.45
5 hrs.	90.66	72.24	9.22	15.53	90.66	72.24	7.51	14.63	78.4	62.31	6.98	12.19
6 hrs.	83.33	65.88	8.81	16.86	89.93	71.47	6.92	13.95	84.5	66.82	7.69	14.62
CD at 1%		2.47	6.74	8.72		1.10	3.19	6.67		2.00	2.84	3.40
CD at 5%		1.78	4.85	6.28		2.37	2.30	4.80		1.44	2.04	2.45

Accelerated ageing- 100% RH and 40°C for 7 days, Natural ageing – under ambient condition for 5 months (73 ± 7% RH and 27 ± 5°C), Germination data were recorded after 6 days, MRL – Mean root length (cm), MSL – Mean shoot length (cm), G % - Germination percentage

Table 2 : Standardization of moisture-equilibration-drying (ME-D) of green gram (*Vigna radiata* wilczek L.) seeds under before ageing, accelerated ageing and natural ageing conditions.

Treatment	Before ageing (BA)				Accelerated ageing (AA)				Natural ageing (NA)			
	G%	Arc-Sin value	MRL (cm)	MSL (cm)	G%	Arc-Sin value	MRL (cm)	MSL (cm)	G%	Arc-Sin value	MRL (cm)	MSL (cm)
Control (Untreated)	98.66	83.45	7.46	15.51	49.33	44.60	4.96	10.24	56.0	48.45	6.23	12.48
24 hrs.	98.66	83.45	7.39	14.99	90.67	72.24	5.26	11.60	73.2	62.87	7.64	17.20
48 hrs.	98.11	82.08	6.94	15.12	93.33	75.00	7.76	14.91	85.6	67.70	7.92	18.11
72 hrs.	94.66	76.69	5.84	13.65	90.66	72.24	5.12	10.98	70.5	57.10	6.99	14.64
CD at 1%		6.04	6.87	7.15		6.67	1.58	6.32		4.71	1.87	2.39
CD at 5%		4.15	1.00	4.92		4.58	1.08	4.34		3.24	1.28	1.64

Accelerated ageing- 100% RH and 40°C for 7 days, Natural ageing – under ambient condition for 5 months (73 ± 7% RH and 27 ± 5°C), Germination data were recorded after 6 days, MRL – Mean root length (cm), MSL – Mean shoot length (cm), G % - Germination percentage

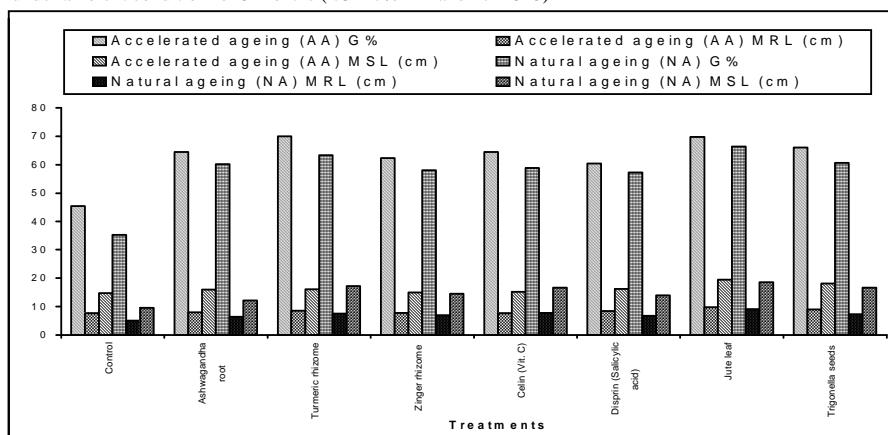
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Table 3 : Peroxidase activity of treated green gram (*Vigna radiata* wilczek L.) seeds under before ageing, accelerated ageing and natural ageing conditions.

Treatment	Before Ageing (BA)	Accelerated Ageing (AA)	Natural Ageing (NA)
	Change in Optical Density (O.D. value) gm ⁻¹ of tissue/5 min.	Change in Optical Density (O.D. value) gm ⁻¹ of tissue/5 min.	Change in Optical Density (O.D. value) gm ⁻¹ of tissue/5 min.
Control (Untreated)	0.32	0.25	0.22
Ashwagandha	0.32	0.30	0.28
Turmeric	0.35	0.27	0.26
Zinger	0.32	0.29	0.27
Celin	0.34	0.27	0.29
Disprin	0.33	0.27	0.24
Jute	0.33	0.29	0.25
Trigonella	0.36	0.26	0.24
3 hrs. WST	0.37	0.31	0.29
48 hrs. ME-D	0.36	0.29	0.25

O.D. values were measured in 430 nm in sytronics 105g spectrophotometer. Accelerated ageing- 100% RH and 40°C for 7 days
Natural ageing – under ambient condition for 5 months (73 ± 7% RH and 27 ± 5°C)

**Fig. 1 : Effect of dry dressing treatments of green gram (*Vigna radiata* wilczek L.) seeds under before ageing, accelerated ageing and natural ageing conditions.**

Plant powdered materials were taken for dressing in the ratio of (1) Ashwagandha root @ 200 mg kg⁻¹, (2) Turmeric rhizome @ 100 mg kg⁻¹ (3) Trigonella seed @ 200mg kg⁻¹ (4) Zinger rhizome @100mg kg⁻¹, (5) Jute leaf @100 mg kg⁻¹. Pharmaceutical preparations were taken in the ratio of (1) Celin @ 100mg kg⁻¹ (2) Disprin @ 100mg kg⁻¹. Accelerated ageing- 100% RH and 40°C for 7 days, Natural ageing – under ambient condition for 5 months (73 ± 7% RH and 27 ± 5°C), MRL – Mean root length (cm), MSL – Mean shoot length (cm), G % - Germination percentage

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