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Physico-Chemical Control of Seed Deterioration

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Seed science research in our country, has made tremendous progress over the past few decades and Seed Research journal has been instrumental in marking and documenting new milestones in our journey of discovering and seeking new knowledge. Dr. Malvika Dadlani suggested considering re-printing classical research papers published in Seed Research. There are several papers that have been published in this journal decades ago which could be considered as classical papers, since they still continue to be standard reference papers for basic seed research experiments. Considering their relevance for the current younger generation of scientists and stakeholders, the editorial team has decided to publish one such paper in each issue of the journal. The paper on 'Physico-Chemical Control of Seed Deterioration' was first published in this journal in 1976 and is being reprinted in this issue, as the second one in the series of classic papers.

ABSTRACT: The loss of viability of seeds of wheat, rice, jute, sunflower and several pulses and vegetables, under ambient and accelerated ageing conditions, could be significantly slowed down by soaking the seeds halfway during storage, with water or dilute solutions (10^{-5}—10^{-2}M) of a range of chemicals for 2-6 hours, followed by drying back to original weight. Water soaking and drying itself has been found to be effective in majority of the seeds investigated. Instead of soaking in water, a light spray with water or solutions of the chemicals or even moisture equilibration with a saturated atmosphere, followed by drying back, prolonged the viability of wheat seeds. Soaking in double the volume of water, however, gave better results in majority of the materials. Treatment of fresh seeds or very old seeds was less effective than treatment of stored seeds (after 4-6 months of normal storage) of good germinability. A second treatment after several months of storage would further extend the viability. The soaking and drying treatments improved the immediate germinability of seeds but the beneficial effects were greatly magnified upon storage. Evidences have been presented to suggest that the treatments which extend seed viablity possibly do so by reducing the free radical damage to the cellular components.

The rapid deterioration of stored seeds is a serious problem in India when the high temperature and high relative humidity greatly accelerate the seed ageing phenomenon. The control of temperature and humidity during storage has a profound influence on the vigour and viability of seeds but such facilities are presently not available to all the seed producers and small cultivators. Seeds of *Rabi* (winter) crops such as wheat, pass through the hot and humid monsoon season in eastern India, resulting in a significant loss of viability. This is also true for the seeds of the major pulses and vegetables. Investigations were, therefore, undertaken to develop methods which could be followed for minimizing the loss of seed viability. The loss of viability of rice, jute and wheat seeds could be significantly slowed down by soaking the stored seeds in water or dilute solutions of salts, phenols, organic acids etc. followed by drying back to the original weight before storage under different conditions (Basu *et al.*, 1974; Basu and Das Gupta, 1974; Das Gupta and Basu, 1975). The method has since been tested with seeds of a wide range of plant species, employing different chemicals and in the present paper the results so far obtained are reported and discussed giving emphasis on the possible mode of action of the

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hydration and chemical treatments.

METHODS OF SEED TREATMENT AND AGEING

Seeds were collected after harvest from different sources, dried and stored in 1-3-litre capacity rubber-stoppered glass bottles till they were used. The stored seeds were given a soaking treatment in double the volume of water or dilute solutions of chemicals, in concentrations from 10^{-5} to 10^{-2}M for 2 to 6 hours depending on the material and ambient temperature. Thereafter the seeds were surface-dried with blotting papers and finally dried in the sun or in a drying cabinet, in air current at 35°C, to the original weight. The control seeds were not soaked but dried along with the other treatments (Basu and Das Gupta, 1974). After drying, the seeds were kept in desiccators over fused calcium chloride for one week for moisture equilibration before subjecting them to different ageing conditions. Apart from studying the natural deterioration under ambient conditions, accelerated ageing treatment at 100 per cent RH and 45°C was given for 8 days.

In some of the experiments, instead of soaking in water or chemical solutions, moisture equilibration with a saturated atmosphere for different periods (12 to 24 hours) or a light spray with water or chemicals, followed by drying back were attempted. Similarly, dry seeds were placed on moist filter papers for germination for different periods before drying back.

Germination tests were carried out in petri dishes lined with moist blotting papers at 28 ± 1°C. The seeds were first soaked in water for 1 to 2 hours before placing them in the dishes for germination. Experiment was conducted on sunflower *(Helianthus annuus)*, gram *(Cicer arietinum)*, lentil *(Lens culinaris)*, mung *(Phaseolus aureus)* arhar *(Cajanus cajan)* wheat *(Triticum aestivum)* paddy *(Oryza sativa)* jute *(Corchorus* sp.) and few vegetable seeds, but in this paper detailed data on wheat and paddy only are presented. Germination counts were taken at intervals and after 3-10 days, depending on the material. Data on the growth of root and shoot were recorded.

SOME EFFECTIVE TREATMENTS FOR DIFFERENT SEEDS

In wheat *(Triticum aestivum* cv. Sonalika) and rice *(Oryza saliva* cv. Ratna) soaking of seeds in water or solutions of various chemicals minimized the loss in germinability (Tables 1 and 2). Several free radical quenching agents such as ascorbic acid, *p*-aminobenzoic acid, cysteine, potassium iodide etc., at 10^{-4} and 10^{-3}M, considerably slowed down the loss of viability of wheat seeds (Table 3). The beneficial effects of the treatments were noted under 100 per cent RH and 45°C as well as under 36 per cent RH and 45°C; an environment not suitable for the growth of storage fungi.

In jute *(Corchorus capsularis* cv. JRC 212 and *Corchorus olitorius* cv. JRO 632), in addition to water soaking (3 hr at 25°C) and drying, sodium chloride, sodium phosphate (mono- and dibasic) and sodiuin thio-sulphate, each at 10^{-4}M, extended the viability of seeds stored under high temperature and high humidity conditions.

For slowing down the deterioration of sunflower (cv. EC 68414) seeds, apart from water soaking (3 hr at 25°C) and drying, sodium phosphate (dibasic), sodium chloride and the phenol, ferulic acid, each at concentrations of 10** and 10^{-3}M, gave good results.

Soaking in sodium thiosulphate and sodium phosphate (monobasic) at 10^{-4}M each, and sodium chloride (10^{-3}M) for 2.5 hr at 25°C followed by drying back extended the viability of carrot *(Daucus carota* cv. Nantes) seeds subjected to the accelerated ageing treatment of 100 per cent RH and 40°C for 15 days. In onion *(Allium cepa* cv. Red Globe), water soaking for 6 hr at 25°C was useful but in this material also further extension of seed viability was obtained with mono- and dibasic sodium phosphate (10^{-4}, 10^{-3}M), oxalic acid (10^{-4}, 10^{-3}M), boric acid (10^{-3}M) and mercuric chloride (10^{-8}M). Thiourea at 10^{-8}M and sodium phosphate (dibasic, 10^{-4}M) gave good results in okra *(Abelmoschus esculentus* cv. Pusa Sawani). In tomato *(Lyco-persicon esculentum* cv. Pusa Early Dwarf), brinjal *(Solarium melongena* cv. Muktakeshi) and chilli *(Capsicum annuum* cv. Suryamukhi), water soaking (3 hr at 25°C) and drying showed very good effects. In these

Table 1. Effects of different treatments (a) on the germinability (b) of wheat (cv. Sonalika) seeds subjected to accelerated ageing (100% RH and 45°C) for 8 days and natural ageing for 5 months

Treatments	Accelerated ageing (c)			Natural ageing		
	Germination (%)	Mean root length (mm)	Mean shoot length(mm)	Germination (%)	Mean root length (mm)	Mean shoot length (mm)
Control	67	126	25	62	89	17
.Water	82	159	31	84	96	24
Sodium phosphate (dibasic)	88	235	44	86	163	38
Sodium phosphate (monobasic)	88	209	43	82	122	34
Sodium chloride	S3	211	41	86	162	33
Sodium thiosulphate	85	217	41	91	129	25
Oxalic acid	87	225	37	84	127	24
Tannic acid	82	196	40	92	128	32
Boric acid	89	221	42	90	114	21
Mercuric chloride	82	201	34	70	124	22
*Control (original)	95	—	—	95	—	—

(a) Treatment (3 hr at 25°C) given to 6-month-old seeds. Molar concentration of chemicals, sodium phosphate (di- and mono-basic), 10^{-4}; sodium chloride, 10^{-3}; sodium thiosulphate, 10^{-4}; oxalic acid and boric acid, 10^{-3}; mercuric chloride 10^{-5}.
(b) Percentage of germination recorded after 72 hr and root (total of all seminal roots) and shoot length after 96 hr.
(c) Data from Das Gupta and Basu (1975). *Germination percentage before ageing.

Table 2. Effects of different treatments (a) on the germiaability (b) of rice (cv. Ratna) seeds subjected to accelerated ageing (100% RH and 45°C) for 8 days and natural deterioration for 6 months (Basu and Pal, unpublished)

Treatments	Accelerated ageing(c)			Natural ageing		
	Germination (%)	Mean root length (mm)	Mean shoot length(mm)	Germination (%)	Mean root length (mm)	Mean shoot length (mm)
Control	50	88	30	25	3	3
Water	85	115	34	80	28	18
Sodium phosphate (dibasic)	92	145	40	90	30	19
Sobium phosphate (monobasic)	85	125	35	90	32	19
Sodium chloride	88	137	40	80	27	20
Sodium thiosulphate	85	115	33	90	36	25
Oxalic acid	86	116	37	85	30	19
Tannic acid	75	132	36	65	37	26
Boric acid	81	130	31	75	33	22
Mercuric chloride	73	126	34	95	36	25
Copper sulphate	65	135	36	85	35	35

(a) Treatment (6 hr at 25°C) given to 6-month-old seeds. Molar concentration of chemicals, sodium phosphate (dibasic), 10^{-4}; monobasic, 10^{-3}; sodium chloride, 10^{-4}; oxalic acid and boric acid, 10^{-3}; sodium thiosulphate and tannic acid, 10^{-4}; mercuric chloride and copper sulphate, 10^{-5}.
(b) Percentage germination recorded after 96 hr; root and shoot length after 120 hr.

Table 3. **Effect of aqueous spray(a) followed by drying back on the germinability(b) of wheat (cv. Sonalika) seeds subjected to accelerated ageing (100% RHand 45°C) for 8 days**

Treatments	Germination (%)	Mean root length (mm)	Mean shoot length (mm)
Control	67	137	24
Water spray	85	174	34
Sodium chloride	92	199	38
Sodium phosphate (dibasic)	95	197	39
Sodium thiosulphate	90	206	38
Tannic acid	80	185	34

(a) Eight-month-old seeds were spread in a single layer and sprayed (0.10 ml/g of seed) with a glass sprayer followed by drying back. Concentration of chemicals : sodium chloride and dibasic sodium phosphate 10^{-4}M, sodium thiosulphate and tannic acid, 10^{-5}M.

(b) Percentage of germination recorded after 72 hr and root and shoot length after 96 hr.

materials also, sodium phosphate (di- and mono-basic, 10^{-4}M) and tannic acid (10^{-6}, 10^{-4}M) further extended seed viability.

Treatment with tannic acid (10^{-5}M), thiourea (10^{-5}M), urea (10^{-4}M) and dibasic sodium phosphate (10^{-4}M) for 3 hr at 25°C was effective for gram (cv. S 4) seeds stored at 100 per cent RH and ambient temperatures for four weeks. Ascorbic acid (10^{-3}M), oxalic acid (10^{-5}M), boric acid (10^{-3}M) and sodium molybdate (10^{-5}M), proved effective in lentil (.cv. W. B. 81). Boric acid (10^{-3}M) gave good results in mung bean (cv. B 1) and arhar (cv. B 4) seeds as well (Basu *et al.*, unpublished).

PHYSIOLOGICAL BASIS OF SEED TREATMENT

The beneficial effects of the water soaking and chemical treatments on the maintenance of seed viability are yet to be fully elucidated. Seed invigoration for imparting resistance to stress conditions have been strongly advocated by Henckel (1961). There are, however, conflicting reports on the question of seed invigoration. Salim and Todd (1968) pointed out that there could be no generalization and the chemicals and concentrations had all to be right for success. Woodstock (1969) reported the beneficial effects of preliminary imbibition of a 2 per cent solution of potassium nitrate and potassium dihydrogen phosphate followed by drying back on the germination of tomato and pepper seeds. Heydecker (1972) believes that seeds could be invigorated. He points out that during the first 24 hours from the beginning of imbibition 'seeds go through a series of irreversible steps towards visible germination; yet can be dried back without suffering. The results of the present investigation clearly demonstrated that deterioration of the treated seeds was greatly minimized, there was a significant increase in growth and vigour of plants from the treated seeds.

Leaching of inhibitors

In the soaking and drying treatments, the seeds are kept immersed in the aqueous solutions. Thus, the leaching of toxic metabolites from the seed may promote subsequent germinability. Das Gupta and Basu (1975) observed slightly greater leaching of growth inhibiting substances from wheat seeds in the presence of dibasic sodium phosphate. However, we have since noted that hydration unaccompanied by leaching, as in the case of hydration by moisture equilibration with a saturated atmosphere or by a light aqueous spray, would also considerably prolong the viability of wheat seeds (Das Gupta and Basu, unpublished).

Hydration versus germination

In the present mode of treatment, the seeds are first hydrated with or without chemicals before drying back. As germination also involves hydration, it may be argued that during soaking the seeds would become physiologically advanced by carrying out some of the initial steps of germination. The reactions of this phase, which is compatible with drying back, may be analogous to the complex and irreversible "Phase I" reactions of germination as discussed by

Heydecker (1974). The subsequent improvement in germinability of the stored seed could be due to the fact that such "advanced" seeds would retain the ability to carry on from where they left off upon reimbibition (cf. Heydecker, 1974). Because of possible impairment of the capacity to carry out the initial reactions of germination due to deteriorative senescence, the control seeds would lose viability. The failure of the Phase I reactions may thus be a critical factor in the loss of seed viability. For elucidating the role of hydration, it was therefore considered necessary to verify whether the beneficial effects on viability could be obtained if the seeds were soaked to imbibe water but not allowed to germinate. This led us to the question of separation of the hydration effect from germination and we made three approaches : (1) using dormant seeds in which the seeds would imbibe water without germinating, (ii) employing seeds in which germination could be regulated by a physical block such as light or chilling and, (iii) using a germination inhibitor such as abscisic acid. In the dormant seeds of rice cv. Sitabhog, the soaking and drying treatment not only broke dormancy but also extended the viability of seeds aged at 100 per cent RH and 45°C. The effect on viability was, however, not as pronounced as that obtained with stored non-dormant rice seeds. In lettuce seed cv. Sutton's A 1, requiring a pre-germination chilling, the seeds were treated with sodium phosphate (dibasic, 10^{-4}M) solution without chilling and that extended the viability of seeds under accelerated ageing (seeds were chilled before the germination test). In wheat cv. Sonalika the presence of abscisic acid (10^{-5}M) during the soaking treatment with sodium phosphate (dibasic) did not counteract the extension of viability of artificially aged seeds, thoroughly washed before the germination tests to remove as far as possible the residual abscisic acid. These preliminary observations indicate the possibility that the hydration effect may be independent of germination.

Role of cellular repair system

Whether the cellular repair system, which can restore much of the age induced damage (Berjak and Villiers, 1972), plays a major role during the short term hydration of seeds before drying back is yet to be critically elucidated. The time available for an extensive repair system to be operative in the present mode of treatment may be rather insufficient. For the initiation of protein synthesis in the seed, a greater time gap between imbibition and drying may be necessary (*vide* Bhat and Padayatty, 1975; Dobr-zanska *et al.*, 1975). It has further been shown that a simple aqueous spray, containing the requisite concentrations of the effective chemicals, followed by immediate drying back (within about 15 minutes the seeds would be dry), would also prolong the viability of wheat seeds (Table 3).

Cell membrane functions and free radical damage

The electrical conductivity of the leachates of treated seeds after accelerated ageing or natural deterioration was significantly lower than in the control. Das Gupta and Basu (1975) therefore suggested a beneficial effect of the chemicals on the cell membrane functions. Some sort of protective action of the treatments would, presum-ably extend seed viability. The question now rests on how the effective treatments would reduce the deterioration of cellular membranes.

The reaction of oxygen and the lipid constituents of the cellular membranes, to form free radical intermediates, is considered to be a basic reason of ageing and senescence of the cell (Tappel, 1973). The extension of seed viability by cathodic protection (Pammenter *et al.*, 1974) lends a strong support to the hypothesis of free radical damage as a major factor in seed deterioration. By providing a source of electrons the free radical damage could be significantly reduced (Pammenter *et al.*, 1974).

A more plausible explanation of the beneficial effects of hydration and chemical treatments could be based on the concept of free radical pathology as a vital factor in seed deterioration. We have noted that the deterioration of wheat seeds could be significantly reduced by water soaking as well as by soaking in dilute (10^{-4}—10^{-3}M) solutions of free radical controlling agents such as cysteine, *p*-aminobenzoic acid, ascorbic acid, potassium iodide etc. Those chemicals are commonly used as radioprotectors (Dertinger and

Jung, 1970) while treatment with radiosensitizing agents such as sodium azide, 2, 4-dinitrophenol and potassium cyanide, which accentuate free radical damage, accelerated the deterioration of wheat seeds (Table 4). The exact mechanism by which hydration followed by drying would control the free radical chain reactions at the molecular level is yet to be elucidated. It has, however, been shown that simple wetting and drying would wipe out 92 per cent of the radiation-induced signal in lettuce seeds (Haber and Randolph, 1967). Metal chelating agents such as EDTA, which prevent the formation of radical centres on unsaturated fatty acids (*vide* Demopoulos, 1973), significantly checked the deterioration of wheat seeds. Further, depending on cellular environment, sodium phosphate and sodium chloride may act as antioxidants or antioxidant synergists (Joslyn and Timmons, 1967; Nickerson, 1967).

Table 4. Effects of several free radical controlling agents (a) on the germirtability (b) of wheat (cv. Sonalika) seeds subjected to accelerated ageing treatments of 100% RH and 45°C for 8 days (Basu and Dasgupta, unpublished)

Treatments	Germination (%)	Mean root length (mm)	Mean shoot length (mm)
Control	48	127	24
Water	70	142	28
Cysteine	86	192	39
Ascorbic acid	85	181	37
p-Aminobenzoic acid	97	207	42
Potassium iodide	95	204	41
EDTA (Na-salt)	85	189	34
α-Tocopherol	90	185	36
Sodium azide(c)	70	139	29
Potassium cyanide(c)	60	108	22
2, 4-Dinitrophenol(c)	20	65	17

(a) Treatment (3 hr at 25°C) given to 9-month-old seeds. Molar concentrations of chemicals, a-tocopherol, 10^{-4}; others 10^{-3}.

(b) Percent germination recorded after 96 hr, root and 'shoot length after 120 hr.

(c) The last three chemicals viz. sodium azide, potassium cyanide and 2, 4-DNP accentuate free radical damage and act as radiosensitizers.

These observations, therefore could explain why hydration (by soaking unaccompanied by leaching and germination or even moisture equilibration with a saturated atmosphere) has such a great effect on seed viability. When the free radical quenching action of a chemical is added to that of water the effect would indeed be highly beneficial.

Further support to the concept of a protection from free radical damage to cellular components as a major reason of the beneficial effect of seed treatment, is obtained from our studies on X-ray-induced seed deterioration. Pre- and post-irradiation chemical treatments have shown significant promoting effect on the growth and vigour of X-ray irradiated wheat seeds, indicating a close parallelism between viability and radioprotection *vis-a-vis* free radical damage to the stored seed.

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