Deterioration of Lentil Seeds: Studies on Germination and Vigour in Relation to Membrane Leakiness and Lipid Peroxidation

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Accelerated ageing of seeds of lentil led to a significant decline in seed viability and seedling vigour. The same is true for controlled aged seeds (14% moisture content) stored at ambient and ±25°C temperatures. Under controlled ageing, no damage to normal germination and vigour was observed in seeds stored up to 90 days at ± 4°C, 60 days at –10°C and 30 days at –20°C temperatures. Increased electrical conductance, inorganic phosphate content, UV absorbancy of leachate, and lipid peroxidation accompanied seed deterioration which can be used as markers. These changes associated with ageing, are interpreted as results due to membrane deterioration.

Key Words: Accelerated Ageing, *Lens culinaris*, Lentil, Seed Sterage

Seeds lose vigour and ultimately their viability during ageing. Under low temperature and moisture conditions, orthodox seeds can be stored for a long period. Accelerated ageing of seeds over several days of exposure to high temperature and humidity has been recognized as a reliable method of predicting seed viability (Delouche and Baskin, 1973; Powell and Mathews, 1977). Such accelerated aged seeds are known to germinate and grow into seedlings comparable with naturally aged seeds (Bhattacharya *et al.*, 1985; Ganguli and Sen-Mandi, 1990). Although many hypotheses have been proposed for the mechanism of seed deterioration, such as lipid peroxidation and membrane damage, accumulation of toxic compounds, and degradation of nucleic acid and proteins (Roberts, 1972; Parrish and Leopold, 1978; Priestley, 1986), the exact mechanism of seed deterioration still remains obscure.

IBPGR (1976) has recommended the conservation of the orthodox seeds at –18°C with 3-7% moisture content (mc). Many genebanks operate following IBPGR's recommendations while sometimes seeds with higher mc need to be stored for short period before processing into genebanks when the amount of seeds received temporarily exceeds the seed drying capacity of a genebank.

The present investigation was undertaken to study some of the growth and biochemical characteristics of lentil seeds subjected to accelerated ageing as well as controlled ageing. Another objective of this study was to determine the optimum conditions for the short-term storage of lentil seeds with higher moisture contents.

Materials and Methods

Freshly harvested seeds of lentil (*Lens culinaris* Medic.; cv L-4147) was obtained from Division of Genetics, Indian Agricultural Research Institute, New Delhi. Initial mc of seeds was 7±0.5% as estimated by high-constant-temperature oven method (ISTA, 1985).

Accelerated Ageing

Seeds were spread uniformly on a wire mesh placed in an air-tight glass desiccator containing saturated solution of sodium chloride at the bottom to maintain 75-78% relative humidity. The desiccator was kept at 35±2°C (ambient temperature) in an incubator. Seeds were drawn for germination and biochemical tests after 7 days of ageing.

Controlled Ageing

A seed lot was conditioned to 14% mc by adding the required quantity of water using the formula:

$$W_F = W_I \frac{(100 - I_M)}{(100 - F_M)}$$ where

W_F = Final weight that will be attained by seeds after adding water

W_I = Initial weight of seeds

I_M = Initial mc of seeds

F_M = Final mc to which seeds were conditioned (*i.e.* 14% in present study)

Seeds added with required amount of water were sealed in Laminated Aluminium Foil (LAF) pouches and incubated at +10°C for 4 days to equilibrate the seeds to 14% mc which was further verified by high-constant-temperature oven method (ISTA, 1985). Seeds with 14% mc were divided into five lots and sealed hermetically in LAF packets (size: 10 cm x 10 cm) to maintain the same mc. These seed packets were stored constantly at ambient, +25°C, + 4°C, –10°C, and –20°C temperatures. Seeds were sampled at every 1 month interval for germination and biochemical estimations.

Germination and Seedling Vigour

To study seed germination and seedling vigour, three sets of 150 seeds each were placed between germination towel papers at 20±2°C in a seed germinator for 7 days. Each set consisting of three replications of 50 seeds each, was used to study seed germination, seedling vigour (shoot and root length) (ISTA, 1985), and to calculate mean germination time (MGT) by counting the germinating seeds everyday for 5 days (Ellis and Roberts, 1981).

Leachate Analyses

Twenty-five seeds were soaked in 25 ml deionised water for 24 h at 20±2°C. The leachate was collected and the conductance was measured using a digital conductivity meter (Control Dynamics). UV absorbancy of leachate was measured at 264 nm using a spectrophotometer (Backman DU 7400). The inorganic phosphate content (Pi) in the leachate was estimated by the method of Fiske and Subba Row (1925).

Lipid Peroxidation

It was estimated in whole seeds by following the method described by Heath and Packer (1968).

Results

Accelerated Ageing

The results showed the significant decline in seed germination of lentil after 7 days of accelerated ageing at 75-78% RH (=13.5% mc) at 35°C. Also, data on MGT revealed the relevant changes indicating seed deterioration. In accelerated aged seeds, a significant loss in seedling vigour – both in root and shoot was observed (Table 1). The conductance of leachate measured almost twice as that of control seeds (Fig. 1a), whereas Pi content in the leachate was *ca.* four-fold (1830 µg/g fw against 470 µg/g fw in the control) (Fig. 1b). Also, UV absorbancy of leachate at 264 nm (Fig. 1c) and lipid peroxidation values (Fig. 1d) showed a significant increase in the accelerated aged seeds.

Controlled Ageing

Storage behaviour of controlled aged seeds was different under various temperature regimes. Seed germination decreased significantly at ambient temperature after 30 days of storage period. However, at other four temperature regimes tested, no marked decline in seed germination was observed during 30 days of storage. With the increase of storage period, seed germination and seedling vigour decreased significantly at ambient and +25°C temperatures in correspondence with significant increase in MGT (Table 1), conductance, Pi content, UV absorbancy of leachate, and lipid peroxidation (Fig. 1 a-d). Seeds stored at ±4°C up to 90 days and –10°C, up to 60 days did not deteriorate markedly and maintained full complement of viability and vigour as is evident by the trend in other biochemical parameters studied. However, there was a non-significant decrease in the root length and increase in the conductance value as well as UV absorbancy of the samples stored at –10°C after 90 days of storage. At –20°C temperature, no indication of seed deterioration was observed up to 30 days of storage (Table 1, Fig. 1 a-d).

Discussion

Accelerated ageing of lentil seeds resulted in a decline in germination and seedling vigour. These observations are in accordance with a number of reports on legume and other crops – soybean (Byrd and Delouche, 1971; Priestley and Leopold, 1979), pigeonpea (Kalpana and Madhava Rao, 1994; 1995) and *Dalbergia* (Thapliyal and Connor, 1997). Also, the other parameters on membrane leakiness studied are indicative of seed deterioration.

The increased leakage of solutes with both accelerated aged (at 35°C) and controlled aged seeds (at ambient and +25°C temperatures), suggests an increase in membrane permeability. In the present study, the increase in leakage of solutes with progressive ageing indicates that the membrane damage in the aged seeds were beyond repair and thereby increased permeability remained a feature associated with ageing of lentil seeds. Leakage of solutes has been reported to be a good index of seed vigour by several workers who also concluded that loss of integrity of plasma membrane in deteriorated seeds led to greater leaching of solutes into the imbibing medium (Parrish and Leopold, 1978; Kakefuda and Duke, 1982; Simon, 1984; Palanisamy and Karivartharaju, 1991; Chhetri *et al.*, 1993; Shen and Oden, 2000). In the present study, we demonstrate that lipid peroxidation is associated with deterioration of accelerated and controlled aged seeds of lentil. Investigations documented in literature do not rule out that lipid peroxidation is a cause of seed deterioration under various storage conditions. Lipids within the membrane are likely sites of ageing damage and lipoxigenase mediated peroxidase reactions are the probable chemical mechanisms for seed ageing (Koostra and Harrington, 1969; Wilson and McDonald, 1986). Gradual and progressive decline in

Table 1. Effect of storage conditions on germination, mean germination time (MGT) and seedling vigour of lentil

Storage condition	Days	Germination (%)	MGT (Days)	Vigour Shoot (cm)	Vigour Root (cm)
Control	0	100	1.18	11.48	8.31
Accelerated Ageing	7	72	1.54	7.83	4.68
Controlled Ageing					
Temperature ambient	30	92	1.33	8.45	5.19
	60	64	2.06	5.13	3.16
	90	26	2.42	1.97	1.11
+25°C	30	95	1.14	10.69	7.08
	60	81	1.34	7.72	6.28
	90	67	1.99	8.56	5.34
+4°C	30	96	1.08	11.66	8.54
	60	95	1.03	11.82	8.36
	90	98	1.14	11.09	8.07
-10°C	30	96	1.05	11.43	8.03
	60	96	1.16	10.90	7.46
	90	96	1.28	11.85	7.28
-20°C	30	99	1.06	11.61	7.65
	60	99	1.01	11.21	7.24
	90	92	1.29	11.29	7.02
LSD > 0.01P		7	0.26	1.36	0.85

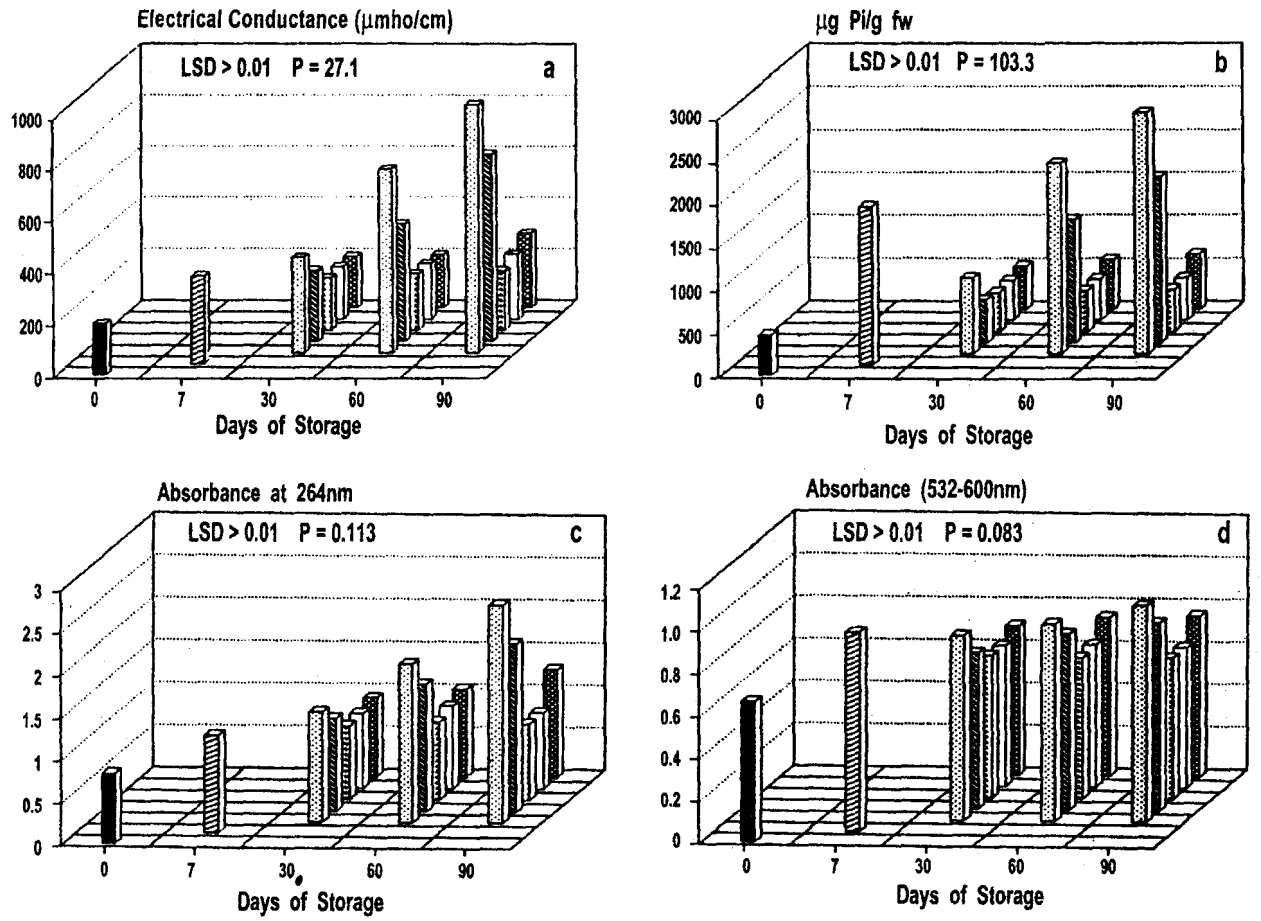

Fig. 1(a-d). Changes in electrical conductance (a), inorganic phosphate content (b), UV absorbance (C) and lipid peroxidation (d) at different storage regimes. Bars represent the storage treatments: control (■), accelerated ageing for 7 days (▨), accelerated ageing for 30, 60, 80, 90 days (▩) + 25°C (▤), + 4°C (▤), -10°C (□), –20°C (▩).

seed germination during longer exposure to sub-zero temperatures with high mc (14%) confirms the results obtained in lettuce (*Lactuca sativa*) stored at –20°C with high mc (12%) in which germination decreased with increased storage period (Kraak and Vos, 1987). It is presumed that damage to the seed viability and vigour at sub-zero temperature with 14% mc results from the freezable water in tissues which contributes to formation of ice crystals gradually, probably in intercellular space within the seeds (Levitt, 1980; Roberts and Ellis, 1989). It is suggested that seeds at around 14-15% mc and below it can be safely stored at –20°C (Roberts, 1972; Harrington, 1973). Our results showed that with higher mc (14%), lentil seeds can be stored safely but temporarily at +4°C at least up to 90 days and at sub-zero temperatures *i.e.* –10°C up to 60 days and –20°C up to 30 days before drying the seeds for long-term conservation in the genebank. Beyond this period, sub-zero temperatures proved to be detrimental to lentil seeds with 14% mc. Our present findings are consistent under accelerated and controlled ageing conditions, and clearly indicate that membrane deterioration including lipid peroxidation is closely linked with ageing of lentil seeds. The various parameters studied may be used as markers for seed viability and vigour.

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