DISCUSSION
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Temperature and relative humidity has been indicated to be important environmental factors, which influences the seed longevity during processing and storage of seeds. Therefore, the present investigations on *Baliospermum* has been undertaken to understand the effect of different temperatures and relative humidity on the seed storage.

5.1 Changes during seed development

Seeds start to develop after fertilization, going through a number of morphological and physiological changes until they reach the stage of dispersal. From a seed conservation point of view, the first important physiological change that seed must take place during the development is that the seeds should have acquired the ability to germinate. Furthermore, our studies have indicated the orthodox (desiccation-tolerant) nature of *Baliospermum* seeds (Table 4), therefore storage of seeds is possible by drying the seeds to low moisture content (moisture content 5% fresh weight), in a dry room or in desiccators (with a drying agent such as silica gel) depending on the facilities available.

In *Baliospermum* maximum viability in seeds occurred when the fruit colour is greenish yellow and started splitting from its edges. These studies are in accordance with the earlier results on pigeon pea, neem and *Jatropha* (Eesewara *et al*., 1998, Kaushik, 2003 Harrington, 1972). The changes that occurred in the seeds beyond physiological maturity are mainly due to dehydration without accumulation of reserves (Ovcharov *et al*., 1966). Photosynthetically active vegetative parts of plants act as a source to the developing seed during reproductive phase. The change in colour of these parts indicate stage when such vegetative tissues are no longer photosynthetically functional and stop supplying nutrients to the reproductive parts thus marking the physiological maturity of the seeds. Morphological characters of capsule, pod and seed parts are utilized to assess physiological maturity in different crops. Turning of *Baliospermum* seeds brown or turning of capsule from green to yellow depict physiological maturity (Table 1). Therefore, in *Baliospermum* seed and capsule colour are good indicators of physiological maturity and can be used as an indicator of physiological maturity which does not require
a destructive sampling of the capsules. Ohland and Fehr *et al.*, used change of pod colour to yellow as an index for harvesting in soybean.

The present studies indicated that for *Baliospermum* seeds 0.1% TZ solution at 30°C for 17 hours after pre moistening of seeds are suitable conditions for quick viability test as the potential viability was at par with the laboratory germination test (ISTA, 1993). Among the different stages, the first category is recognized as fully viable with undifferentiated embryo (Plate 3). The evaluation of these categories as viable seeds is supported by staining of all essential structures of seeds which is recognized as necessary for development of a normal seedling. Results of seeds revealed that potential viability, as estimated by TZ test is significantly and positively correlated with actual germination percentage. In *Quercus falcate* and *Pinus elliottii* significant correlation of TZ test result with germination was found (Bonner, 1984, Alvarez *et al.*, 1992).

Apart from the visual indices, physiological maturity in various crops has been assessed on the biochemical changes during maturation. The presence and accumulation of the low to medium molecular weight proteins during the developmental stages is further confirmed by the protein profiles (SDS PAGE) of the seeds protein profile during the various developmental stages. During the 1st stage of development, no bands were observed indicating absence of protein at such an early stage. In the later stages some of the new proteins have been developed along with some of the proteins which were present have been broken down in to simpler forms (Plate 4).

### 5.2 Lab Germination Vs Field Emergence

Field emergence of the various conditional seeds did not correlated with the laboratory germination test. It may be due to the adverse environmental condition in addition to the seed deterioration caused by high moisture and high temperature storage of seeds. The experiment was terminated.

### 5.3 Seed Storage Behaviour

Under the present investigation *Baliospermum* seeds were found to be desiccation tolerant. The freshly harvested seeds having a moisture content of 18.09% with 80% viability on desiccation exhibited moisture content of 5% with 100% viability. Further
desiccation resulted in lowering of moisture content and hence worth decline in seed viability.

Desiccation tolerance of seeds may vary within genus, depending on habitat and on the characteristics of each species (Tompsett, 1984, Radhamani and Chandel, 1996). Seeds of other species are also sensitive to drying but to varying degrees. The degree to which water loss is tolerated varied with the species and the physiological maturity of the seed at harvest (Chandel et al., 1995). The results of the experiments on seed desiccation demonstrated that the *Baliospermum* seeds are not sensitive to desiccation (Table 4).

The combine factors i.e. high moisture content at shedding, shape, taxonomy and desiccation tolerant of seed confirms the seed storage behaviour of particular species. *Baliospermum* seeds are small globose, thrive well in sub tropical conditions and desiccation tolerant indicating the seeds to be orthodox in nature.

### 5.4 Seeds response to different storage conditions

Physiological reactions, related to seed longevity, change with increase in seed moisture content. These effects are deleterious with increased moisture content and hence seed deterioration leading to loss of germination progressed very rapidly at higher humidity and higher temperature (Harrington, 1973, Bass et al., 1963, Barton 1941; Kang and Umoh, 1996).

The seeds equilibrated at 33% RH, the germination percentage was best recorded at -20°C, where the viability was extended up to a period of 15 months which are the preferable limit for long term conservation of germplasm (IPGRI, 1998). Whereas, at low RH (5%) which is an ultra day condition the viability of seeds was better. Increased rates of seed deterioration has been reported by Vertucci & Ross (1990) & Vertucci et al. (1994) in many orthodox species stored above or below the optimum moisture content at which maximum longevity of viability was observed. The results of the present studies suggested that seed storage at high RH of 33% and with the maintenance of high internal seed moisture content (7%) deterioration of *Baliospermum* seeds was faster.

The present study showed that germinability of *Baliospermum* seeds can be maintained for a prolonged duration if the seed moisture content is maintained at a lower level of 3 – 5%. Analysis of data further revealed that RH or seed moisture content has a
more pronounced effect on seed germinability as compared to temperature. At low RH of 5% & moisture content 3% the viability decreased to 70.0 whereas at high RH 33% & moisture content 7% the viability was reduced to 44.0 at the end of 9 months of storage at ambient temperature. Deteriorative reactions frequently proceed in the seed more readily if the moisture content is higher; and consequently the moisture condition would constitute a threat to longevity of seed survival.

**Vigour & related parameters**

Loss of viability always precedes loss of vigour. The changes in seedling vigour index also closely followed the changes in the viability since vigour index is directly dependent upon shoot length, root length and germination percent. Loss of vigour is associated with seed deterioration and is influenced by composition of seed, particularly the food reserves or the efficiency of mobilization of nutrients.

In the present study, the highest vigour index was observed in control seeds, which decreased with seed storage in different conditions of temperature and humidity. The present study showed a decrease in shoot length which was found to be directly proportional to seed viability and it also confirms with the earlier reports in a number of crops (Harrington, 1972; Dey and Basu, 1982; Yadav et al., 1987; Dharamlingam and Basu, 1990, Bhattacharya and Bhattacharya, 1988; Perry, 1977). Shanmugavel (1993) observed a close association between the loss in seed germination and reduction in seedling vigour in soybean during the course of ageing at 70% RH. In the present investigation, it has been observed that seedling length and vigour index declined steadily over the storage period and were more pronounced at higher humidities and higher temperatures.

Seed deterioration is generally initiated in the meristematic tissue. Meristematic areas and particularly the radicle meristem appear most prone to deterioration. At least two reasons are forwarded to explain this observation. Generally, the radicle of most seeds is located at the funicular end of the seed, an area permitting rapid entry and penetration of water during imbibition (McDonald, 1999) along with likely intake of oxygen as well. In addition, meristematic regions are energy intensive and possess high numbers of mitochondria compared to other cellular tissues. Lipid peroxidation
occurring with mitochondria of the radicle tip would explain the reduced seedling growth characteristics of deteriorated seeds.

**Electrical conductivity of seed leachates**

Membrane is the most important site of a seed which appears to be affected by seed deterioration/ageing (Ching and Schoolcraft, 1968). Degradative changes in cellular membranes are (is one of) the early events of seed ageing (Heydecker, 1972). Enhanced solute leakage from imbibed seed is associated with the loss in seed vigour and viability (Mathews and Bradnock, 1968; Dadlani and Agrawal, 1985). In the present study also, increase in electrolyte leakage was noted before the reduction in germination.

The decrease in membrane integrity and occurrence of membrane lesions might play a significant role in the deterioration of seeds. It has been supported by the work on solute leaching accompanying a fall in germinability and viability (Powell and Mathews, 1977; Halder *et al.*, 1982). The increase in the amount of electrolytes is found to be proportional to the seed deterioration, attaining maximum values when seeds lost viability completely during storage at high humidity (Table 8). At these levels the seeds showed very low germination. Therefore seed deterioration primarily results in loss of solutes following an increase in the electrical conductivity which could be interpreted as irreversible changes in membrane structure and loss of unique chemical structure of membranes essential for viability. In present study, *Baliospermum* seeds showed reduced level of leaching under high RH (33%) & low temperature (-20°C) in comparison to seed stored at high RH (33%) and ambient temperature. The leaching of total electrolyte was always less in seeds stored under dessication at 3% moisture content.

The increase in the electrical conductivity was primarily related to seed deterioration whereas the maximum conductivity could be interpreted in terms of changes in membrane structure.

Basically membranes are composed of proteins and phospholipids arranged in a fluid bilayer. Koostra and Harrington (1969) have shown in their studies that a decline in the polar lipids, chiefly owing to their oxidation may be the immediate cause of leaky membranes in seeds. Thus, the breakdown of lipids results in the production of free radicals that deactivate enzymes, proteins and nucleic acids and ultimately lead to the
disruption of cell membranes, which results in the loss of their semi permeable character. In orthodox seeds the membranes generally loose their functional properties with dessication, which are normally reconstituted with the re-imbibitions (Simon, 1983).

**Total soluble proteins**

The total soluble proteins of aged seeds decreased progressively with the storage over the control seeds. This decline could be attributed to either decreased rate of synthesis of proteins or to increased activity of proteinases, or the combination of two. All treatments showed decline in the protein content to the different extent. The results indicated that the total soluble proteins ranged from 0.8 mg/ml/gm of seed weight to 2.8mg/ml/gm (Table 9). Cherry and Skadsen (1986) hypothesized that the irreversible loss of some essential proteins in the aged seeds leads to loss of seed viability. The decline in the total protein content due to impaired proteins biosynthetic activity with the gradual loss of seed viability have been reported in seeds of rye (Hallam et. al., 1973), pea (Bray and Chow, 1976), sal (Nautiyal et. al., 1985) and pigeon pea (Kalpana and Madhav Rao, 1997).

The decrease in the level of proteins with increasing level with storage time could be due to the denaturation of proteins during dessication of seeds. In addition to this, it is well demonstrated that free radicals produced during seed deterioration as a result of lipid auto-oxidation may be responsible for the denaturation of protein. Similar loss of seed viability with a decrease in protein and sugar have been also shown earlier by several workers (Nautiyal and Purohit, 1985, Chandel et. al., 1995).

**Dehydrogenase activity**

The data as periodic of Absorbance (A) of formazon revealed that high intensity of formazon was retained at low RH. In our studies, it was found that the dehydrogenase activity expressed in terms of absorbance at 534 nm was as high as 0.9312 at -20°C temperature with moisture content 5% (Table 10). Ray and Gupta (1980) also noted reduced dehydrogenase activity in terms of formazon formation in rice seeds undergoing deterioration.
Accelerated Ageing

Accelerated ageing tests are employed for predicting the seed storage potential and to study the process of seed deterioration. Artificial ageing treatments take advantage of the fact that seed ageing process is determined by the seed moisture level and the temperature. Manipulation of the factors therefore, hastens movement of seeds through the pattern of deterioration.

The mechanism causing deterioration is not fully understood. Changes which occur during storage are associated with deterioration such as delayed germination, reduced seedling vigour; decreased tolerances to adverse germination condition and loss of germinability have been reviewed by several workers (Bewley & Black, 1982; Abdul-Baki & Anderson, 1973). The changes associated with deterioration of seeds in general are categorized as constitutive and enzymatic changes during seed deterioration.

Effect of ageing on Physiological Parameters

Germination percentage

There was a significant declined in the germination percentage with the accelerated ageing. These results are in accordance with earlier results reported by Rao & Singh (1992) in sunflower. This is attributed to DNA degradation with ageing which leads to impaired transcription causing incomplete or faulty enzyme synthesis essential for earlier stages of germination.

Vigour Index

The data revealed that vigour index was 2748 in 100% viable control seeds which declined to 430 after four days of ageing (Table 11). With the declining germination percentage there is a decline in seedling length which resulted a simultaneous decline in vigour index during accelerated ageing.
Effect of Ageing on Biochemical Parameters

Electrical Conductivity

Increased leakage of electrolyte after the seeds were subjected to accelerated ageing was mainly due to membrane deterioration and metabolic changes in the seeds after accelerated ageing. The EC of the leachate was significantly high in the aged samples (1.7872 as compared to the control (1.2978) (Table 11). The increase in EC is a clear indication of deterioration of seeds by accelerated ageing. Wilson & McDonald (1986b) suggested that ageing of seeds leads to lipid peroxidation and lipid peroxides which leads to membrane perturbations leading to electrolyte leakage and exudation of simple sugars.

Dehydrogenase

The dehydrogenase activity was high in control seeds (0.9632), and it showed a gradual decline with accelerated ageing (0.1741) (Table 11). This reduction in activity is in coherence with seed germinability, which was also showed a reduction ranges from 100% to 0%.

Total Soluble Sugars

The amount of total soluble sugars present in accelerated aged samples and control showed an increasing trend with ageing (Table 12). Increase in the total soluble sugars in deteriorated seeds has also been reported earlier also. This increase is attributed to hydrolysis of polymeric storage compounds such as lipids, proteins and carbohydrates under unfavorable storage condition of high moistures and temperatures resulting in increase in their subunits viz. fatty acids, amino acids and soluble sugars.

Lipid peroxidation

An increase in the lipid peroxidation was reported in seed/embryonic axes of several recalcitrant seed species during dessication (Hendry et. al., 1992, Finch-Savage 1992a, Chandel et. al.,1995). Under the present studies, there is a progressive increase in the lipid peroxidation during storage in Baliospermum seeds. The values for Lipid
peroxidation are expressed in the form of Absorbance at 535 nm and it was 0.0906 for control seeds (100% viable) which increased to 0.2866 in five days of accelerated aged seed sample (Table 13).

These studies indicated a good correlation of increased electrolyte leakage with increased lipid peroxidation content, which indicates the destruction of membranes caused by peroxidation of lipids during storage. A decrease in the enzymatic protection was probably associated with loss of unsaturated bonds in lipids which can cause extensive disturbance to the ordered structure of membranes. Lipid peroxidation produces highly reactive free radical intermediates that can damage membranes, proteins and nucleic acids which were observed to precede the loss of viability in *Quercus ruber* axes (Finch-Savage, 1992a). The increase in the electrolyte leakage and the loss of seed viability probably resulted from the increase oxidative stress.

Francis and Coolbear (1984) reported that under ageing condition, concomitant with the fall of germination, there is a decline in phospholipids and phosphatidyl choline which are important membrane components. Changes in the membrane integrity (increase in EC values, lipid peroxidation and soluble sugars) and loss of enzyme activity and lowered protein values in seed storability of *Baliospermum* species might be the possible reason for the loss in seed viability during the storage at high humidities.

The increase in malondialdehyde throughout the levels of ageing in seeds has been reported by several workers (Harman and Mattick, 1976, Stewart and Bewley, 1980, Sung and Jeng, 1994). Changes in the membrane integrity results in the efflux of electrolytes in seed steep water (increase in EC values). Lipid peroxidation in stored seeds is the possible reason for the loss in seed viability during storage at high humidities. Hence it can be inferred that stress due to high temperature and humidity adds to the deleterious lipid peroxidation reactions. Perhaps the changes in seed MDA content during ageing support the hypothesis that loss of seed viability is associated with lipid peroxidation. Our findings of changes in the enzyme activity with seed deterioration were consistent with previous studies reporting that the peroxisomal catalase activity decreases during senescence (Kanazawa et. al., 2000).
Protein profile

The SDS-PAGE protein profile did not show significant changes in banding pattern during early deterioration but in the seeds with 0% germination no bands were observed. The intensity of sharp bands decreased as the viability decreased. The alteration in the banding pattern might be due to the breakdown of low molecular weight proteins under deterioration to low molecular weight proteins and free amino acids.

Enzymes

At biochemical levels, seed ageing is accompanied by a decline in metabolic activity upon germination, changes in enzyme activity (in most cases a decrease) and a decline in protein and nucleic acid biosynthesis.

A number of biological oxidations both enzymatic and spontaneous generate free superoxide radical (O_2\textsuperscript{-}) which is cytotoxic and in turn can react with H_2O_2 to produce singlet oxygen and the hydroxyl radical (OH). These can induce considerable destruction particularly to large polymers and to membrane lipids. In ageing tissues, the balance between free radical producing and scavenging reactions is disturbed in favour of the former. Hence free radical formation in stored seeds can result in progressive inactivation of enzymes, denaturation of proteins and membrane lipids. Several workers have reported a decline in the peroxide scavenging enzymes like catalase and peroxidase. Another group of enzymes which show a concomitant decrease with decline in viability are the dehydrogenase which controls the respiratory metabolism.

In present study, the membrane bound oxidative enzymes showed a positive correlation with seed viability. The healthy and viable seeds showed higher enzyme activity which reduced with seed deterioration. All enzymes studied viz., peroxidase, catalase, Acid phosphatase and lipoxygenase showed a decreasing trend till the germination is completely lost (Table 15, 16 and 17). Isozyme profile did not show any major difference in the banding pattern in all accelerated aged seed samples except in the seeds with 0% germination.