Changes in Germination, Respiration Rate and Leachate Conductivity during Storage of Hevea Seeds

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ABSTRAK

ABSTRACT
Hevea seeds were stored imbibed at the temperatures of 10, 22 and 27°C. After each month of storage, changes in percentage germination, respiration rate and leachate conductivity were observed. There was a decrease in percentage germination, seedling height, seedling dry weight, respiration rate and an increase in leachate conductivity as duration of storage increased. Loss of membrane integrity was suggested as one of the causes of deterioration of seeds during storage.

INTRODUCTION
Rubber (Hevea brasiliensis Muell. - Arg.) seeds are considered to be recalcitrant in nature. Roberts (1973) classified recalcitrant seeds as those whose viability tends to decrease with a decrease in moisture content below some relatively high value, between 12 and 31%. Hevea seeds are killed by dehydration, high temperature or freezing (Chin et al. 1981). At present, no method is available for the storage of Hevea seeds beyond a year. Storage of Hevea seeds mixed with moist sawdust in perforated polythene bags at 7°C can maintain viability up to four months (Ang 1976). Treatment with polyethylene glycol 1500 was found to improve the storage life of seeds up to six months with 25% germination (Sakhibun 1981). Other than the studies carried out by Chin et al. (1981), Hor (1984) and Berjak et al. (1984), very little work has been done to study changes that occur in recalcitrant seeds during deterioration. Seed deterioration has been defined as irreversible changes that reduce survival capacity and lead to loss of vigour and germinability (Anderson 1973). It has been postulated that as the seed deteriorates, various functions of the seed are impaired in a definite sequence (Delouche 1969; Heydecker 1972). Thus, deterioration is not confined to any one cellular function, but is manifested in a variety of ways with anyone being sufficient to impede germination (Bewley and Black 1982).

According to Delouche (1969), delayed seed germination is one of the earliest physiological signs of deterioration. It occurs well ahead of decreased germination and has been used to index deterioration in a wide range of seeds including corn (Grabe 1965), sorghum and soybean (Teng 1977). Retardation of seedling growth was observed in a wide range of deteriorating seeds including broad beans, peas and barley (Abdalla and Roberts 1969), snap beans (Tooleeta L. 1957), and corn (Grabe 1965; Woodstock and Feeley 1965).

The decline in respiratory rate has also been reported for various seeds under different conditions of deterioration. These include natural age-

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ing (Woodstock and Grabe 1967; Kittock and Law 1986; Abdul-Baki 1969; Abdul-Baki and Anderson 1973), accelerated ageing (Abdul-Baki 1969; Parrish and Leopold 1978) and chilling injury (Woodstock and Feeley 1965; Leopold and Musgrave 1979). In most cases, respiration as measured by oxygen uptake is closely correlated with deterioration as measured by reduced germination.

Berjak and Villiers (1970) reported that membrane aberrations increased with increasing age of the maize embryos. More sugars and amino acids were leached from deteriorated than from vigorous seeds (Yaklich and Abdul-Baki 1975). An increase in leached solutes as viability declined was also reported by Maguire et al. (1973); Abdul-Baki and Anderson (1973); Parrish and Leopold (1978) and Abdul-Baki (1980). Seed viability has been highly correlated with leaching and conductivity of seed leachate and this phenomenon has been suggested as a good index for seed deterioration (Ching and Schoolcraft 1968).

The objective of this study is to observe and compare the changes that occur in Hevea seeds with those changes in orthodox seeds during storage at different temperatures. An imbibed storage method was used as this is the established method of storing Hevea seeds. It is hoped that through a better understanding of deterioration of the seeds during storage, a suitable method may be devised to improve the storability of Hevea seeds and also of other recalcitrant seeds.

MATERIALS AND METHODS

Seeds of clone RRIM 600 were obtained from Prang Besar Seeds Gardens located in Telok Intan, Perak, Malaysia. Sawdust was cleaned by soaking in water for three days, with daily changes of the water to remove toxic substances that might be present. It was then sterilised in an oven at 100°C for 48 hours and cooled before use. The sawdust was then mixed with distilled water up to 20% moisture content. Freshly harvested seeds were mixed in equal volume with moistened sawdust and were spread in shallow layers inside perforated black polythene bags (30 cm x 36 cm) with ten holes of approximately 0.5 cm in diameter in a split-plot design experiment. The seeds were stored at 10°C (cold room), 22°C (air-conditioned room) and 27°C (ambient room). Storage duration was for three months with the following changes being assessed after each month of storage.

Moisture Content

No rules are available for testing tree seeds in the 1985 International Rules for Seed Testing (International Seed Testing Association 1985). Therefore, in the present study, moisture tests were devised and conducted as follows: The seeds were first cracked open and the kernels cut into thin slices and weighed. The slices were then dried in an oven at 105°C for 16 hours and dry weight was recorded. The moisture content was determined as loss in weight and expressed as a percentage of the fresh weight of the kernels. Due to the difficulty of obtaining large amount of seeds, the moisture content was determined by sampling four replicates of 10 seeds each.

Germination

Seeds were germinated in accordance with the International Rules for Seed Testing (International Seed Testing Association 1985) using the in-sand method. Seeds were sown in boxes filled with sterilised moist sand. Initial and final counts were carried out after 14 and 21 days, respectively. Owing to the difficulty of obtaining large amount of seeds, four replicates of 25 seeds each were used in all germination tests. Seedlings were assessed as germinated when all essential structures were seen to have developed normally.

Seedling Height and Dry Weight

The height and dry weight of all normal seedlings from the first and final count of the germination test were measured. Seedling height was measured from the base of the hypocotyl to the tip of the shoot. The seedlings were then bisected at the base of the hypocotyl into shoot and root tissues. The seedling dry weight was obtained after drying in a 75°C oven for one week.

Conductivity of Seed Leachate

Seeds were thoroughly cleaned under the tap and rinsed six times in deionised water. Three replicates of ten seeds in each replica were completely submerged for one hour in 50 ml of deionised water at 22°C. After soaking, the water was decanted and its conductivity measured using a WTW conductivity meter (model LF42) with automatic temperature compensation to 22°C. The platinum electrodes were thoroughly rinsed with deionised water before each measurement.

Respiration Rate

Respiration rate was measured using a Gilson

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respirometer. Three replicates often embryonic axes in each replicate of each treatment were used. The embryonic axes were placed in the main body of the flask containing 2 ml of fresh sterile incubation medium. The sterile incubation medium consisted of 10 ml of streptomycin sulphate (500 mg/l), 10 ml of penicillin G (500 mg/l), 0.2 ml of Na₂HP₂O₄, 12H₂O, of 20% and 0.41 g KH₂P₀₄, made up to 100 ml with distilled water and sterilised. A small piece of fluted paper containing 0.2 ml freshly prepared potassium hydroxide was left in the centre well of each flask to absorb carbon dioxide. The flasks were allowed to equilibrate for approximately 20 minutes before respiration rate was measured. The respiration rate was measured after 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 hours of incubation at 30°C. All flasks were constantly shaken throughout the experiment. At the end of the incubation period, the axes were removed and their dry weight obtained after drying in a 75°C oven for one week.

RESULTS

The results of moisture content, percentage of germination, seedling height, seedling dry weight and leachate conductivity are given in Tables 1, 2, 3, 4 and 5, respectively.

The moisture content of seeds increased during the first month of storage at all temperatures. Subsequently, seeds stored at 10°C (cold room) and at 27°C (ambient room) maintained quite a constant moisture content. However, seeds stored at 22°C (air-conditioned room) continued to lose moisture during the second and third months of storage (Table 1).

There was no significant difference in percentage germination after one month of storage in all storage temperatures (Table 2). Percentage germination of those seeds stored at 10°C (cold room) and at 22°C (air-conditioned room) decreased significantly with increasing months of storage. For seeds stored at 27°C (ambient room), the decrease in percentage germination was less rapid.

Seedling height and dry weight increased after one month of storage at all temperatures and decreased with further storage (Tables 3 and 4). There was a significant temperature effect with months of storage. At the end of the storage period, seeds stored at 27°C had the greatest seedling height and dry weight, followed by those stored at 22°C and at 10°C, respectively.

There was an increase in leachate conductivity with increasing months of storage. However, the increase in conductivity of seeds stored at 27°C was significantly less than those of the seeds stored at 22°C and at 10°C, respectively (Table 5).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Moisture content (%) of Hevea seeds stored imbibed at 10, 22 and 27°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Months of storage</td>
<td>Storage temperature</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>10°C (cold room)</td>
<td>26.38</td>
</tr>
<tr>
<td>22°C (air-conditioned)</td>
<td>26.38</td>
</tr>
<tr>
<td>27°C (ambient)</td>
<td>26.34</td>
</tr>
<tr>
<td>Mean</td>
<td>26.37</td>
</tr>
</tbody>
</table>

L.S.D. (p = 0.05) for between months of storage within same temperature = 4.82
L.S.D. (p * 0.05) for between temperatures within same or different months of storage = 4.95
L.S.D. (p * 0.05) for between temperatures = 4.11 (Arcsin value)
L.S.D. (p = 0.05) for between months of storage = 4.52 (Arcsin value)

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Percentage germination of Hevea seeds stored imbibed at 10, 22 and 27°C (Values are arcsin transformed).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Months of storage</td>
<td>Storage temperature</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>10°C (cold room)</td>
<td>51.89</td>
</tr>
<tr>
<td>22°C (air-conditioned)</td>
<td>50.38</td>
</tr>
<tr>
<td>27°C (ambient)</td>
<td>51.89</td>
</tr>
<tr>
<td>Mean</td>
<td>51.39</td>
</tr>
</tbody>
</table>

L.S.D. (p = 0.05) for between months of storage within same temperature = 7.87 (Arcsin value)
L.S.D. (p = 0.05) for between temperatures within same or different months of storage = 9.07 (Arcsin value)
L.S.D. (p = 0.05) for between temperatures = 4.11 (Arcsin value)
L.S.D. (p * 0.05) for between months of storage = 4.52 (Arcsin value)
TABLE 3

Seedling height (cm) from \( \sqrt{y} \) seeds stored imbibed at 10, 22 and 27°C

<table>
<thead>
<tr>
<th>Storage temperature</th>
<th>Months of storage</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>10°C (cold room)</td>
<td>0 1 2 3</td>
<td></td>
</tr>
<tr>
<td>22°C (air-conditioned)</td>
<td>27.19 30.02 34.12 14.13 27.86</td>
<td></td>
</tr>
<tr>
<td>27°C (ambient)</td>
<td>27.33 41.70 38.53 34.98 35.63</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>27.28 38.62 30.06 16.48</td>
<td></td>
</tr>
</tbody>
</table>

L.S.D. \((p = 0.05)\) for between months of storage within same temperature = 4.58
L.S.D. \((p = 0.05)\) for between temperatures within same or different months of storage = 4.21
L.S.D. \((p = 0.05)\) for between temperatures = 3.61
L.S.D. \((p = 0.05)\) for between months of storage \(= 2.65\)

TABLE 4

Seedlings dry weight (g) from \( \sqrt{y} \) seeds stored imbibed at 10, 22 and 27°C

<table>
<thead>
<tr>
<th>Storage temperature</th>
<th>Months of storage</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>10°C (cold room)</td>
<td>0 1 2 3</td>
<td></td>
</tr>
<tr>
<td>22°C (air-conditioned)</td>
<td>0.342 0.469 0.190 0.003 0.251</td>
<td></td>
</tr>
<tr>
<td>27°C (ambient)</td>
<td>0.339 0.553 0.503 0.127 0.381</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.342 0.672 0.555 0.518 0.522</td>
<td></td>
</tr>
</tbody>
</table>

L.S.D. \((p = 0.05)\) for between months of storage within same temperature = 0.08
L.S.D. \((p = 0.05)\) for between temperatures within same or different months of storage = 0.122
L.S.D. \((p = 0.05)\) for between temperatures = 0.091
L.S.D. \((p = 0.05)\) for between months of storage = 0.065

After one month of storage, there was no change in the respiration rate. However, the respiration rate increased significantly after two months of storage. Nevertheless, there was a significant decrease of more than 50 percent in the respiration rate at all storage temperatures after three months of storage (Table 6). There was no significant storage temperature effect on the respiration rate.

DISCUSSION

*Hevea* seeds stored imbibed deteriorated fastest when stored at 10°C, followed by 22°C and 27°C, respectively. Sakhibun (1981) also demonstrated that the best temperature of storing *Hevea* seeds imbibed was ambient temperature. Ang (1976) reported that the best storage temperature of imbibed *Hevea* seeds was 7-10°C with percentage germination of 25 percent after three months of storage. In the present study, when seeds were stored at 10°C, percentage germination was only 5 percent after the same period of storage.

The increase in leachate conductivity was correlated with percentage germination. Seeds stored
at 10°C leached highest followed by those seeds that were stored at 22°C and 27°C, respectively. Leachate conductivity was quite low at all temperatures because it was measured without removing the testa, hence, the thickness of the testa might have prevented much of the leachate from leaching out. However, a significant difference was observed between storage temperatures and months of storage. When whole soybean seeds were used to measure leachate conductivity (Tilden and West 1985), the result was equivalent to the result obtained in this study. It has been reported that there is often a high correlation between leaching and reduction in vigour (Abdul-Baki 1980). Increase in leachate conductivity is considered to result from degradation of cellular membranes and subsequent loss of control of permeability (Ching and Schoolcraft 1968). This is supported by Normah (1987) who observed tonoplast dissolution in Hevea seeds during storage.

The decrease in respiration rate with months of storage at all storage temperatures correlates with the reduction in the integrity of mitochondria. With months of storage, mitochondria had less cristae and some developed electron dense deposits and loss of membrane integrity was common (Normah, 1987).

Some of the changes revealed in this study on Hevea seeds seem to be similar to those observed in previous studies on orthodox seeds. Decreased respiration associated with deterioration was reported in maize (Woodstock and Grabe 1967), wheat (Kittcockand Law 1986), barley (Abdul-Baki 1969) and soybean (Leopold and Musgrave 1979) under natural ageing, accelerated ageing and chilling injury.

Changes that occur in Hevea seeds as shown in the present study may not be the only cause of deterioration of the seeds. Nevertheless, it was observed that imbibed storage of Hevea seeds was best at 27°C. More studies need to be carried out to find an improved method of storage of Hevea seeds as well as other recalcitrant seeds.

ACKNOWLEDGEMENTS

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