Moist sand conditioning to minimize loss of viability in cocoa (Theobroma cacao Linn.) seed

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Abstract
Conditioning of cocoa seeds using five per cent moist sand impregnated with Jalsakthi 10 per cent solution in polyvinyl bags minimized the rate of drying and loss of viability up to 40 days under ambient conditions. The leakage of solutes was more in untreated seeds. The lowest safe moisture contents to retain 50 per cent viability and above appears to be 22 per cent.

Keywords: Cocoa, Theobroma cacao, Jalsakthi, Seed viability, Moist sand.

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Introduction
Cocoa (Theobroma cacao Linn.) is raised as an inter crop in coconut, arecanut and palm gardens. It is estimated that 0.3 million hectare of suitable land is available for further expansion of cocoa as a mixed crop (Rethinam, 2000). Non-availability and short-lived nature of seeds are the limiting factors for the development of cocoa industry in India. Unlike traditional areas of moist warm tropical forest environment suitable for hilly plantation crops, the area identified in India for cocoa cultivation is completely different, favouring seed deterioration. Cocoa being recalcitrant (Evans, 1953), conventional methods of dry low temperature for seed storage is unfavourable, since they do not withstand desiccation.

Moisture retaining environment within pods coated with paraffin wax (Friend, 1964) or imbibed storage (Villiers, 1972) could retain viability for a very short period and a storage environment rich in oxygen, in the presence of a fungicide is essential (King & Roberts, 1982). Different methods suggested for recalcitrant seed storage (King & Roberts, 1982; Bhattacharyya & Basu, 1992) are elaborative and require skill. An easy and inexpensive method under controlled, subimbibed moisture content, with minimum desiccation under ambient conditions will prove beneficial. Keeping these factors into consideration, studies were initiated in cocoa to minimize loss of viability.

Materials and Methods
The seeds extracted from freshly harvested mature fruits of cocoa cv. ‘Foreestro’ obtained from the plantation maintained by Cadburies India Ltd. at Coimbatore, South India formed the basic material. The experiment comprised of following two components:

1. Assessment of critical moisture content: To assess the recalcitrant nature under Coimbatore conditions, evaluation of critical moisture content in relation to viability maintenance was done. Immediately after extraction seeds were spread over a plastic tray and kept under ambient conditions (mean temp. 27.2±4°C, RH 74 ± 4%) in shade with free ventilation. Seed samples were taken daily and estimated for moisture and germination up to 10 days.
2. Identification of an easy and inexpensive method for viability maintenance: The method described by Bhattacharyya and Basu (1992) for Jack seeds was followed with some modifications. Sterilized sand of 0.8 mm size was used for conditioning the seeds. Four treatments were imposed. Air dried sand was moistened to 5 per cent (w/w) respectively with water, Jalsakthi (an inert, hygroscopic starch polymer) (10%) and polyethylene glycol (PEG) 6000 (−4 bar). The treated and untreated seeds were covered with pre-moistened sand with the respective solutions at a ratio of 1:2 (seed and sand) and stored in loosely bound polythene bags (700 gauge) and cloth bag for 40 days under ambient conditions (mean temp. 27.7±4°C, RH 72 ± 3%). Samples were drawn at an interval of eight days and analyzed for moisture content, germination and electrical conductivity.

The moisture content was analyzed by low constant oven method using four replicates each of 5g coarsely ground seed material. Since the moisture content was above 30 per cent, seeds were pre-dried at a constant temperature of 130°C for five minutes and then at 105°C for 16 hour (ISTA, 1999). The germination test was conducted in sand medium using 100 seeds, in a germination room maintained at 25±2°C and 95±2% RH. The final count was taken on 22nd day and expressed as percentage (ISTA, 1999). The root length (cm) and shoot length (cm) were measured from all normal seedlings grown on seeds stored in all the treatments. The root length (6.32 cm), shoot length (22.40 cm), dry matter production (118 mg) and vigour index (Abdul) were measured from all normal seedlings irrespective of treatments. The rate of decline in moisture content for seeds stored in cloth bags was 6.30 and 16.01 per cent in untreated, 5.99 and 15.18 per cent in moist sand, 5.30 and 13.82 per cent in Jalsakthi and 5.83 and 14.99 per cent in PEG after 16 and 40 days of storage, respectively and it was less in seeds stored in polyvinyl bags (Fig. 1).

The electrical conductivity was measured by soaking a single seed in 10ml of deionised water for four hours with a digital conductivity meter and expressed in d/Sm. Four replicates were used in each analysis on moisture content, germination, seedling measurements and electrical conductivity. The data were statistically analyzed after Snedecor and Cochran, 1967.

Results and Discussion

The initial moisture content observed on the first day (35.46%) got reduced to 12.00 per cent after nine days of storage with a concomitant reduction in viability (12%), root length (6.32 cm), shoot length (22.40 cm), dry matter production (118 mg) and vigour index (339). The viability potential reached zero after 10 days (Table 1). Recalcitrant seeds are killed if their moisture content is reduced below some relatively high critical value of 20-35 per cent. The rate of reduction in germination was gradual up to seven days of desiccation and had a steep fall thereafter. In the present study, the critical moisture content for loss of viability to zero level was 12 per cent and the lowest safe moisture content for retaining of viability to a minimum of 50 per cent appears to be 22 per cent (Table 1). Recalcitrant seeds are killed if their moisture content is reduced below some relatively high critical value of 20-35 per cent. The rate of reduction in germination was gradual up to seven days of desiccation and had a steep fall thereafter. In the present study, the critical moisture content for loss of viability to zero level was 12 per cent and the lowest safe moisture content for retaining of viability to a minimum of 50 per cent appears to be 22 per cent (Table 1) and is in conformity with Poulsen and Eriksen (1992) in Quercus robur Linn. and Gunasekaran (1997) in clove seeds.

Storage of cocoa seeds in five per cent moist sand incubation alone or impregnated with Jalsakthi (10%) or PEG (−4 bar), revealed that, the moisture content was not altered in seeds stored in polyethylene bag (35.46 to 35.71%) irrespective of treatments. The rate of decline in moisture content for seeds stored in cloth bags was 6.30 and 16.01 per cent in untreated, 5.99 and 15.18 per cent in moist sand, 5.30 and 13.82 per cent in Jalsakthi and 5.83 and 14.99 per cent in PEG after 16 and 40 days of storage, respectively and it was less in seeds stored in polyvinyl bags (Fig. 1). The electrical conductivity of seed leachate, showed an increasing trend and the rate of increase was higher in cloth bags compared to polyvinyl bags. When stored in cloth bags, the increase was 160 and 382 per cent in untreated, 146 and 361 per cent in moist sand, 105 and 312 per cent in Jalsakthi and 143 and 353 per cent in PEG, while in polyvinyl bags, it was 168 and 347 per cent in untreated, 121 and 295 per cent in moist sand, 91 and 250 per cent in Jalsakthi and 114 and 284 per cent in PEG. The rate of increase in general was less in polyvinyl bags (Fig. 1). The highest germination was observed in Jalsakthi treatment stored in polyvinyl bags (94, 80, 64, 50, 39.3 and 30%) at all periods compared to cloth bags (94, 70, 52, 38, 21 and 8%). This was closely followed by PEG. Moist sand conditioning alone had a marginal effect (Fig. 1). Visual observation on fungal load, indicated heavy incidence of Aspergillus spp. particularly in polyvinyl bags from eight days of storage, in all treatments. The incidence was less in seeds stored in cloth bags.

In the present study, it was observed that all the moist sand incubation treatments were very effective in conserving the seed moisture content. Untreated seeds lost moisture rapidly, which paralleled the loss of germination particularly in cloth bags. After 40 days of storage seeds impregnated with Jalsakthi and PEG and stored in polyvinyl...
Fig. 1: Moist conditioning of cocoa cv. ‘Foresteró’ seeds on viability and membrane integrity
Table 1: Assessment of critical moisture content for vigour and viability in cocoa cv. ‘Forestero’

<table>
<thead>
<tr>
<th>Days after seed storage</th>
<th>Moisture content (%)</th>
<th>Germination (%)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Dry matter production (mg/10 seedling)</th>
<th>Vigour index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35.46 (36.54)</td>
<td>91 (72.61)</td>
<td>8.17</td>
<td>23.45</td>
<td>232</td>
<td>2877</td>
</tr>
<tr>
<td>2</td>
<td>32.02 (34.46)</td>
<td>88 (69.77)</td>
<td>7.91</td>
<td>23.26</td>
<td>224</td>
<td>2743</td>
</tr>
<tr>
<td>3</td>
<td>31.67 (34.24)</td>
<td>72 (60.67)</td>
<td>7.43</td>
<td>23.05</td>
<td>196</td>
<td>2316</td>
</tr>
<tr>
<td>4</td>
<td>30.10 (33.27)</td>
<td>67 (54.94)</td>
<td>7.21</td>
<td>22.97</td>
<td>188</td>
<td>2022</td>
</tr>
<tr>
<td>5</td>
<td>28.05 (31.97)</td>
<td>64 (53.13)</td>
<td>6.94</td>
<td>22.87</td>
<td>175</td>
<td>1938</td>
</tr>
<tr>
<td>6</td>
<td>24.23 (29.48)</td>
<td>59 (50.18)</td>
<td>6.78</td>
<td>22.76</td>
<td>169</td>
<td>1728</td>
</tr>
<tr>
<td>7</td>
<td>22.03 (27.99)</td>
<td>55 (47.87)</td>
<td>6.55</td>
<td>22.65</td>
<td>164</td>
<td>1602</td>
</tr>
<tr>
<td>8</td>
<td>17.64 (24.83)</td>
<td>21 (27.25)</td>
<td>6.40</td>
<td>22.50</td>
<td>142</td>
<td>603</td>
</tr>
<tr>
<td>9</td>
<td>13.03 (21.15)</td>
<td>12 (20.22)</td>
<td>6.32</td>
<td>22.40</td>
<td>118</td>
<td>339</td>
</tr>
<tr>
<td>10</td>
<td>12.00 (20.26)</td>
<td>0 (0.52)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

SEd (0.005) (0.828) 0.014 0.009 0.001 0.551
CD (P=0.05) (0.010**) (1.740**) 0.029** 0.020** 0.003** 1.157**

Figures in parentheses are arc sine values; ** Significant at 1% level

bags recorded higher germination (by 14 and 13 per cent, respectively), compared to seeds stored in cloth bags. The beneficial effects of Jalsakthi could be ascribed to the absorption, retention and slow release of moisture, resulting in reduced rate of moisture loss during the storage period. The loss of moisture in PEG treated seeds was similar to Jalsakthi only during the earlier period and not at the later period of storage. This may be due to the chemical composition of Jalsakthi, a hygroscopic polymer, responsible for the retention of moisture content. Loss of membrane integrity as a consequence of aqueous based process leading to considerable leakage of electrolytes and uneven distribution of tissue water observed in Ekbergia sp. (Pammentar et al., 1998) and Jack (Shyllamerlin, 1999) was evident in cocoa also. The electrical conductivity of seed leachate was more in untreated and the least in Jalsakthi and PEG treated seeds. Maintenance of viability up to eight months in cocoa seeds treated with PEG 6000, when stored at an RH of 98 per cent and 20°C as reported by King and Roberts (1982) could not be established in the present study, where PEG was less effective, probably due to higher ambient temperature and lower RH conditions (mean temp. 27.7±4°C, RH 72±3 %), wherein the loss of moisture was inevitable. The loss of moisture in seeds treated with Jalsakthi though minimum.
in polyvinyl bags at all periods of storage, it was not reflected in minimizing the decline in germination. This may be due to the fungal load, which could have altered the microclimate of seeds causing an increase in temperature, respiratory rate and heating, particularly under ambient conditions. However, this needs verification through further studies.

References


