Effect of Carbohydrate source on Seed Germination in *Adansonia digitata* L.: A Unique Tree Species

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Abstract

*Adansonia digitata* L. (Bombacaceae) is a majestic tree known for its unique medicinal properties and charismatic attractive appearance. In India, the tree is not indigenous and the germination of seeds under natural conditions is a limiting factor for plant regeneration. **Objectives:** The present study aims to determine a suitable carbohydrate for seedling growth *in vitro* and to produce vigorous plantlets. **Experimental:** Glucose, sucrose, maltose, fructose and commercial sugar at varied concentrations (2, 4, 6 and 8%) were incorporated in WPM solid medium with 0.8% Difco bacto agar. Above media without any carbon source was used as control. 30 seeds were used for each treatment and experiment was repeated thrice. Seeds were incubated in 16 hour/8 hour photoperiod for 30 days. **Results:** Sucrose was found to give highest germination rate. 72.2% germination in 30 days was achieved with 4% sucrose. The growth performance of plantlets varied with the sugar composition. Medium with 6% sucrose gave the best performance with maximum average shoot and root length 6.73 and 5.16 cm respectively. **Conclusion:** Optimization of carbohydrate source will help to standardize a protocol and preserve this genetic resource of great economic value. The results from this study will be used for plant regeneration through micropropagation.

Citation:
1. Introduction

*Adansonia digitata* L. (Baobab) is one of the most widely used indigenous priority tree species in sub-Saharan Africa. It belongs to the family Bombacaceae which is represented by 30 genera, six tribes and about 250 species. It is a widely distributed species and is reported from most of the semi-arid and sub-humid regions of Africa as well as western Madagascar (Diop et al., 2005). The tree is multi-purpose and offers protection, provides food, clothing, medicine and raw materials for many useful items (Igboeli et al., 1997; Venter and Venter, 1996; Rahul et al., 2015).

The present status of distribution of *A. digitata* in India is not very encouraging. However in Madhya Pradesh about 100 cultivated plants are found in block Mandu of Dhar district. Adansonia is regarded as the “Queen of all the carbon storage trees” and possess high water holding capacity (Sugandha et al., 2014). Water storage capacities, range from 1000-9000 litres per tree (Gebauer et al., 2002; Tukur, 2010). Each part of this drought resistant tree is extremely useful.

1.1 Justification of research and scope:

Natural regeneration of *A. digitata* is very poor as seeds suffer from acute dormancy due to their hard, impermeable seed coat. There are no reports on seed germination in the field, but *in vitro* tests have reported low germination rate, between 0-10 % for untreated seeds. The seeds seem to have seed coat induced dormancy (Etjere et al., 1984), have very hard seed coats and germination is usually less than 20% due to the seed coat dormancy (Danthu et al., 1995). Uncontrolled grazing and bush fires further reduce the stock of seeds in the soil (Sidibe et al., 2002). It was therefore inferred that in vitro seed germination is the only alternative for producing high quality plantlets. These will provide elite explants for micropropagation and callus suspension studies. Also, there are no reports on the effect of sugars on germination responses in vitro, the present study is focussed to enhance *in vitro* seed germination using different sugars and will contribute in the conservation of this valuable tree species. Through clonal propagation and planting, there is potential for reducing hunger and rural poverty in some of the earth’s most difficult to feed locations.

2. Experimental

2.1 Objective of Research

Carbohydrates are the most important source of energy amongst all the macronutrients available in any culture medium. Sugars play an important role as an energy source as well as osmotic agent. The aim behind this study was to determine a suitable carbohydrate and standardize a protocol for maximum in vitro seed germination response and plantlet growth.

2.2 Collection of Plant material

The plant was identified with the help of voucher specimens deposited to Botanical survey of India, Western regional office, Pune and was given the accession number (SUSADDI).

Two locations of Madhya Pradesh were selected for collection of plant material, namely Mandu (Dhar) and Bhopal. Fully mature ripe fruits (Figure 1A, 1B.) were collected in the months of December-January respectively. The fruits were cracked open and the seeds were separated from the dried fruit pulp (Figure 2A, Figure 2B, Figure 2C). The seeds were nicked using mortar and pestle and surface sterilized 4% NaOCl for 10 minutes.

2.3 Effect of sugars on seed germination

Glucose, sucrose, maltose, fructose and commercial sugar at varied concentrations (2, 4, 6 and 8%) were incorporated in ½ WPM liquid medium and WPM solid medium with 0.8mg/l BAP and their effect on in vitro seed germination was studied. Above media
without any carbon source was used as control. All solid media contained 0.8% Difco bacto agar. 30 seeds were used for each treatment and experiment was repeated three times.

2.4 Effect of sucrose concentration on seed germination

Four sucrose concentrations (2-6%) were tested. The basal medium used was ½WPM (for solid medium, 0.8% Difco bacto agar was added). 30 seeds were used for each treatment and the experiment was repeated 3 times.

2.4 Cultural conditions

Seeds were incubated in 16-hour/8-hour photoperiod for 30 days in light with photosynthetic photon flux of 6μmolm⁻²s⁻¹ and then shifted to plain media. Germination percentage was noted after 25-30 days and length of shoot, root and no. of opened leaves was recorded after 60 days.

3. Results

Amongst all sugars tested, sucrose was found to promote highest germination rate followed by fructose, maltose, glucose and commercial sugar. Sucrose at 4% concentration gave 72.2% germination in 30 days. The maximum average shoot and root length 6.73 cm and 5.16cm respectively were recorded at 6% concentration after 2 months. Sucrose at 8% concentration gave 57.7% germination in 30 days and 74.4% germination in 60 days with average shoot length of 5.63cm and root length of 3.46cm. At 6% sucrose concentration even though the germination percentage was not very high (36.6% in 1 month) it was found that this concentration gave the highest number of opened leaves (Table 1, Figure 3.).

Fructose gave 51.1% germination at 4% concentration in 30 days which later increased to 61% in 60 days. The shoot and root length, 3.2cm and 2.63cm respectively at this concentration were very low. While fructose at 6% concentration even though gave only 42% germination in 30 days the shoot (5.3cm) and root lengths (3.2cm) were higher than those induced at 4% fructose.

6% maltose was found effective in inducing germination in 64.4% (almost same as control) of the seeds in 60 days. The root and shoot length recorded at this concentration after 2 months was 3.30cm and 4.33cm respectively. At 2% glucose level, no germination was observed in 30 days. At 6% concentration the germination percentage recorded was 41.1% (less than control) in 60 days. Among all the carbon sources sucrose at 4% concentration was found best for germination of seeds.

Increased concentration of maltose (8%) has an inhibitory effect on the growth of shoot and root. Limited growth and shoot length were observed on medium with glucose and maltose. An opposite response was observed at the highest sucrose concentration (8%) where the shoot and root length was 4.50cm and 3.33cm.

At lower sucrose concentration (2.0-2.5%), 37.7% germination was obtained where shoots and roots never elongated beyond 3.23cm and 3.06 cm respectively. 3.5% sucrose was better as compared to the other lower sucrose concentrations (Table 2, Figure 3.).

4. Discussion

Maximum seed germination was recorded with sucrose in the medium. Sucrose not only promoted seed germination but average root/shoot length as well as large number of opened including cotyledonary leaves were also seen. It is cheap, readily available, relatively stable to autoclaving and readily assimilated by plant cells. Monosaccharides like glucose and fructose are quickly caramalised by autoclaving and hence are not convenient to use. The presence of a carbon source in the culture medium during in vitro culture is fundamental to the propagation of woody species as these not only act as energy source and osmotic regulators but also influence growth and tissue differentiation (Dobranszki, 2010). Santana et al., 2008

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Figure 1A: *Adansonia digitata* at Berasia Bhopal

Figure 1B: *Adansonia digitata* bearing rat shape fruits at Mandu Dhar, Madhya Pradesh

Figure 2A: Fruits 20-25 cm in length

Figure 2B: Breaking of fruits shows the fruit pulp with seeds inside

Figure 2C: Mature seeds after nicking
Table 1: Effect of different sugars on seed germination

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Germination % after 30 days (mean±SD)</th>
<th>Germination % after 60 days (mean±SD)</th>
<th>Average shoot length (cm) (mean±SD)</th>
<th>Average root length (cm) (mean±SD)</th>
<th>Average number of opened leaves (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.7±1.96</td>
<td>64.4±11.69</td>
<td>2.23±0.40</td>
<td>1.93±0.35</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Sucrose 2.0%</td>
<td>27.7±1.96</td>
<td>43.3±10.0</td>
<td>3.43±0.30</td>
<td>2.66±0.20</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Sucrose 4.0%</td>
<td>72.2±1.90</td>
<td>74.4±1.90</td>
<td>5.83±0.20</td>
<td>3.83±0.35</td>
<td>4.0±0.0</td>
</tr>
<tr>
<td>Sucrose 6.0%</td>
<td><strong>36.6±3.35</strong></td>
<td>57.7±5.09</td>
<td>6.73±0.25</td>
<td>5.16±0.37</td>
<td><strong>6.0±0.0</strong></td>
</tr>
<tr>
<td>Sucrose 8.0%</td>
<td>57.7±5.09</td>
<td>74.4±3.81</td>
<td>5.63±0.23</td>
<td>3.46±0.49</td>
<td>2.0±0.0</td>
</tr>
<tr>
<td>Fructose 2.0%</td>
<td>45.5±3.86</td>
<td>62.2±6.96</td>
<td>3.40±0.40</td>
<td>3.16±0.35</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Fructose 4.0%</td>
<td>51.1±10.1</td>
<td>61.0±3.86</td>
<td>3.20±0.34</td>
<td>2.63±0.15</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Fructose 6.0%</td>
<td>42.2±1.90</td>
<td>46.6±6.65</td>
<td>5.30±0.62</td>
<td>3.20±0.30</td>
<td>2.0±0.0</td>
</tr>
<tr>
<td>Fructose 8.0%</td>
<td>8.86±3.35</td>
<td>49.9±6.65</td>
<td>3.40±0.36</td>
<td>4.13±0.32</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Maltose 2.0%</td>
<td>22.1±5.09</td>
<td>44.4±5.09</td>
<td>3.13±0.32</td>
<td>3.46±0.30</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Maltose 4.0%</td>
<td>33.3±3.30</td>
<td>29.9±3.35</td>
<td>3.13±0.41</td>
<td>3.86±0.35</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Maltose 6.0%</td>
<td>32.1±5.09</td>
<td>64.4±5.09</td>
<td>3.30±0.26</td>
<td>4.33±0.47</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Maltose 8.0%</td>
<td>0.0±0.0</td>
<td>23.3±13.3</td>
<td>2.66±0.15</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Glucose 2.0%</td>
<td>0.0±0.0</td>
<td>12.2±3.81</td>
<td>2.53±0.25</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Glucose 4.0%</td>
<td>26.6±3.35</td>
<td>21.1±10.18</td>
<td>1.46±0.41</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Glucose 6.0%</td>
<td>19.9±6.65</td>
<td>41.1±10.18</td>
<td>3.40±0.52</td>
<td>2.80±0.50</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Glucose 8.0%</td>
<td>39.9±3.35</td>
<td>38.8±1.96</td>
<td>1.13±0.20</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Commercial sugar 2.0%</td>
<td>19.9±3.35</td>
<td>28.83±5.09</td>
<td>1.36±0.41</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Commercial sugar 4.0%</td>
<td>31.1±1.90</td>
<td>28.86±1.96</td>
<td>1.66±0.32</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Commercial sugar 6.0%</td>
<td>0.0±0.0</td>
<td>12.2±3.81</td>
<td>2.5±0.3</td>
<td>2.4±0.1</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Commercial sugar 8.0%</td>
<td>0.0±0.0</td>
<td>22.2±10.7</td>
<td>3.26±0.40</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>
Table 2: Effect of sucrose concentrations (2-6%) on seed germination

<table>
<thead>
<tr>
<th>Sucrose (%)</th>
<th>% Germination (mean ± SE)</th>
<th>Shoot length (cm) after 2 months (mean ± SE)</th>
<th>Root length (cm) after 2 months (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>37.7±2.23</td>
<td>2.93±0.20</td>
<td>2.76±0.14</td>
</tr>
<tr>
<td>2.5</td>
<td>43.3±1.90</td>
<td>3.23±0.31</td>
<td>3.06±0.17</td>
</tr>
<tr>
<td>3.0</td>
<td>49.5±1.93</td>
<td>3.70±0.10</td>
<td>3.10±0.20</td>
</tr>
<tr>
<td>3.5</td>
<td>76.6±1.93</td>
<td>4.66±0.08</td>
<td>3.46±0.24</td>
</tr>
<tr>
<td>4.0</td>
<td>75.5±2.23</td>
<td>5.53±0.18</td>
<td>4.23±0.17</td>
</tr>
<tr>
<td>4.5</td>
<td>65.5±2.23</td>
<td>4.76±0.55</td>
<td>3.80±0.05</td>
</tr>
<tr>
<td>5.0</td>
<td>59.9±1.93</td>
<td>5.73±0.21</td>
<td>4.20±0.17</td>
</tr>
<tr>
<td>5.5</td>
<td>55.5±2.93</td>
<td>6.10±0.06</td>
<td>4.40±0.26</td>
</tr>
<tr>
<td>6.0</td>
<td>58.8±2.94</td>
<td>7.00±0.14</td>
<td>5.76±0.14</td>
</tr>
</tbody>
</table>

Figure 3: Effect of sucrose on seed germination. Error bars indicate standard error.
observed that, in addition to sucrose, aeration of the culture vessels can also stimulate autotrophy in in vitro cultivated plants of Annona glabra L. On the other hand, in the presence of glucose at concentration of 40gdmm⁻³, highest growth rate of shoots and roots was achieved in Dactylorhiza species (Wotavova-Novotnaet al., 2007).

In the present study, 3.5% concentration of sucrose (Table 2) favours maximum seed germination though better shoot and root length is at 6% sucrose concentration. Wainwright and Scarce, 1989 reported that low concentrations of sucrose in the culture medium can stimulate a beneficial change in the growth of plantlets in their transition from heterotrophy to autotrophy, thus facilitating their increase in their photosynthetic capacity. Leite and fortes, 2000 mentioned in their studies that high concentrations of sucrose in the culture medium can negatively affect the in vitro development of the aerial portion of plants as it can diminish their photoautotrophic capacity. Seed germination of Ochromapryramidale (Cav. ex. Lam. Urban.) seeds without GA₃ and 2, 2.5% sucrose was found to be 15 and 60% respectively after 60 days whereas the highest percentage of seed germination (75%) was obtained with the treatment 1.0 mg/l GA₃ and 2% sucrose (Alarcon et al., 2014). Maltose is hydrolyzed 20 times slower than that of sucrose, therefore the absorption and metabolism of maltose take a longer time than sucrose (Blanc et al. 2002).

**Conclusion**

Different carbohydrates, and their concentrations, significantly affected the success rate of germination and root shoot length but our results indicated that germination and seedling development of A. digitata was greatly influenced by sucrose.

**Research Highlights**

First report on the effect of Carbohydrates on in vitro seed germination of A. digitataseed samples collected from Dhar(Mandu) and Bhopal(Madhya Pradesh, India). Sucrose was found to promote highest germination rate followed by fructose, maltose, glucose and commercial sugar.

**Limitations**

A.digitata has limited occurrence and distribution. This restricted the availability of explants i.e. seeds. Moreover, Ex vitro germination of the seeds with or without seed coat was not successful. Therefore, in vitro studies especially of a woody tree species posed a big challenge. Many cultures were lost due to bacterial contamination.
Future Goals

Variables like use of growth hormones, gelling agents and light intensity etc. have to be optimized in order to generate a standardized protocol for in vitro seed germination and obtaining vigorous plantlet growth and explant material. The plantlets so generated ‘in vitro’ will be taken as explants for further regeneration studies.

Author’s contribution

The first author (SS) is responsible for conducting experiments and presentation of data on effect of carbohydrate source on seed germination in vitro studies and in the preparation of the initial draft of the paper. The second and third authors (RS and PV) helped in the final drafting of the manuscript.

Conflict of interest

The Authors have no conflict of interest to declare.

Acknowledgement

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