Stage-specific metabolism of triacylglycerols during seed germination of Sacha Inchi (*Plukenetia volubilis* L.)

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**Abstract**

**BACKGROUND:** A detailed study was carried out on Sacha Inchi (*Plukenetia volubilis* L.) to investigate the mobilization of storage lipids during seed germination.

**RESULTS:** Thin layer chromatography analysis of the total lipids showed a rapid decline in the triacylglycerol (TAG) and diacylglycerol (DAG) contents after the early stages (3–10 days after imbibition (DAI)) followed by a steady breakdown during the later stages (20 and 30 DAI) of germination. Trace amounts of monoacylglycerols (MAG) were identified during the final stage (30 DAI). Further, gas chromatography analysis showed an increase in the major unsaturated fatty acid (linoleic and linolenic) content from 3 to 10 DAI followed by a slow decline. In addition, the major saturated fatty acid (palmitic and oleic) content showed a decrease during the early stages (3–10 DAI) and an increase during the later stages (20 and 30 DAI).

**CONCLUSION:** The present study provides the first report on the metabolization of TAG along with fatty acid changes during the seed germination of Sacha Inchi.

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Keywords: Sacha Inchi; triacylglycerols; seed germination; gas chromatography; fatty acids

**INTRODUCTION**

Sacha Inchi or Inca peanut (*Plukenetia volubilis* L., Euphorbiaceae) is an oleaginous plant rich in oil (550 g kg\(^{-1}\)) and protein (290 g kg\(^{-1}\)). An important aspect of this plant is its abundance of unsaturated fatty acids (90% of total fatty acids), particularly linoleic acid (\(\omega-6, 37%\)) and linolenic acid (\(\omega-3, 45%\)).\(^1\) As a rich source of unsaturated fatty acids, the oleaginous seeds are suitable for human consumption, since these fatty acids are important in preventing coronary heart diseases, pathogenesis and hypertension.\(^2\) Although several studies have reported the oil metabolism changes during seed development, the metabolism during germination in this oil plant is unknown. Therefore it was of interest to analyse the metabolic changes in lipids during seed germination.

**RESULTS AND DISCUSSION**

Initially, mature seeds of Sacha Inchi (*P. volubilis* L.) (XTBG-PV003) were obtained from field-grown plants at Xishuangbanna Tropical Botanical Garden (21° 56′ N, 101° 15′ E, 600 m a.s.l.), Chinese Academy of Sciences, Yunnan, China. Batches of 20 seeds were germinated on sterile filter paper in petri dishes (150 mm × 25 mm) filled with sterile water. Pre-incubated seeds (4°C) were placed in a growth chamber at 18°C under dark and at 26°C under light conditions. Germination was defined by the appearance of the radicle at the axial end. The fresh weight of the cotyledons increased rapidly after 3 DAI, whereas the dry matter showed a prominent decline during the same period (data not shown). Previously, closely associated findings were reported during the seed germination of cotton,\(^3\) buckthorn\(^4\) and linseed.\(^5\) In addition, steady rates of cotyledon reserves were observed during the seed germination of Sacha Inchi. The cotyledons were pale green in colour during the initial stages of seed germination (3–10 DAI), which was followed by the appearance of thickened dark green tissues during the later stages (20 and 30 DAI). Supporting this finding, Chavan et al.\(^6\) stated that the oxidation and breakdown of stored macromolecules such as lipids and proteins during germination were responsible for the consistent decline in the dry matter.

The primary stage in lipid metabolism during seed germination is the release of fatty acids from the reserve triacylglycerols (TAG), accomplished via hydrolysis by the action of lipase. The fatty acids...
thus released undergo $\beta$-oxidation to produce adenosine triphosphate (ATP) as the major energy reserve within the seed. Thin layer chromatography analysis of the total lipids (extracted from germinating cotyledons following Xu et al.) showed neutral lipids (95%) as the major component. Neutral lipid fractions obtained were distributed in the form of TAG, diacylglycerols (DAG), monoacylglycerols (MAG), free fatty acids (FFA) and sterol esters (SE) (Fig. 1). The TAG and DAG contents in the cotyledon seedlings remained constant during the first 3 DAI (Fig. 1). A slow decline in the TAG and DAG contents was observed from 3 to 10 DAI, after which a rapid breakdown in the TAG content was observed during the final stages (20 and 30 DAI) of seed germination (Fig. 1). The steady mobilization of the TAG levels indicates that they are the major Sacha Inchi seed components involved in catabolism, which provide the substrate for oxidation during germination. Interestingly, a rapid increase in the FFA content was observed only between 10 and 20 DAI, which was also followed by a decline during the late stage of seed germination. In support, lipid changes of flax seed increased consistently during the early stages (3–10 DAI), after which a steady decrease was observed during the late stages (20 and 30 DAI) (Table 1). This increase in the percentage of linoleic and linolenic acids increased consistently during the early stages (3–10 DAI), after which a steady decrease was observed during the late stages (20 and 30 DAI) (Table 1). This increase in the percentage of linoleic and linolenic acids indicates a major reserve energy shift to the tissues that are rich in these fatty acids in the growing seedlings. Palmitic acid, which accounted for the major proportion of the SFA, and oleic acid, the major MUFA, showed a decrease during the early stages followed by an increase in their contents during the late stages of seed germination (Table 1). As TAG behave as the major energy suppliers for the germination process together with the parallel synthesis of TAG and oleosins in the region of the endoplasmic reticulum, the present study focused scrupulously on the TAG mobilization with the exemption of polar lipid investigation.

Table 1. Fatty acid composition changes (%) in total lipids of Sacha Inchi seeds during germination

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>3 DAI</th>
<th>6 DAI</th>
<th>10 DAI</th>
<th>20 DAI</th>
<th>30 DAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0 (myristic acid)</td>
<td>0.3 ± 0.1a</td>
<td>ND</td>
<td>0.8 ± 0.1b</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C16:0 (palmitic acid)</td>
<td>16 ± 0.4a</td>
<td>10 ± 0.3a</td>
<td>7.9 ± 0.4b</td>
<td>14 ± 0.5c</td>
<td>16 ± 0.3d</td>
</tr>
<tr>
<td>C18:0 (stearic acid)</td>
<td>0.3 ± 0.1a</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C18:1 (oleic acid)</td>
<td>17 ± 0.2a</td>
<td>10 ± 0.5b</td>
<td>12 ± 0.5c</td>
<td>13 ± 0.3d</td>
<td>41 ± 0.3e</td>
</tr>
<tr>
<td>C18:2 (linoleic acid)</td>
<td>49 ± 0.9b</td>
<td>58 ± 1.0a</td>
<td>57 ± 0.8a</td>
<td>53 ± 0.7a</td>
<td>42 ± 0.6a</td>
</tr>
<tr>
<td>C18:3 (linolenic acid)</td>
<td>10 ± 0.4b</td>
<td>21 ± 0.6a</td>
<td>20 ± 0.4a</td>
<td>19 ± 0.5a</td>
<td>ND</td>
</tr>
<tr>
<td>C20:0 (eicosanoic acid)</td>
<td>6.5 ± 0.3a</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Total saturated fatty acids</td>
<td>16</td>
<td>10</td>
<td>8.7</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Total monounsaturated fatty acids</td>
<td>17</td>
<td>10</td>
<td>12</td>
<td>13</td>
<td>41</td>
</tr>
<tr>
<td>Total polyunsaturated fatty acids</td>
<td>59</td>
<td>79</td>
<td>78</td>
<td>72</td>
<td>42</td>
</tr>
</tbody>
</table>

Values are mean ± standard error of triplicate measurements. Means with different letters in the same row are significantly different at $P < 0.05$ as determined by Fisher’s least significant difference procedure. ND, not detected.
However, a detailed study is warranted particularly on the polar lipid fractions to better understand their involvement in the seed germination process of Sacha Inchi. These results represent a first report on the lipid metabolism changes in germinating seeds of Sacha Inchi and provide an example of the selective utilization of fatty acids in the reserve oil during germination in a prospective approach that this study would provide useful information for the membrane turnover as well as for the growth and development of Sacha Inchi plants.

REFERENCES