Short Communication

Received: 17 December 2013 Accepted article published: 13 August 2014 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6855

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

Umashankar Chandrasekaran[a,b] and Aizhong Liu[a*]

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.

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 (ω-6, 37%) and linolenic acid (ω-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 emerging radicle. At regular intervals (3, 6, 10, 20 and 30 days after imbibition (DAI)) the cotyledons were separated from the germinating seedlings, rinsed with distilled water and used for further analysis. Under the optimal conditions employed, a seed germination rate of *ca* 90% was achieved. After 3 DAI a softened seed coat was observed with the emergence 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

* *Correspondence Aizhong Liu, Key Laboratory of Tropical Plant Resource and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, 88 Xuefu Road, Kunming 650223, China. E-mail: liuaizhong@mail.kib.ac.cn*

a *Key Laboratory of Tropical Plant Resource and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, 88 Xuefu Road, Kunming 650223, China*

b *University of Chinese Academy of Sciences, Beijing 100049, China*

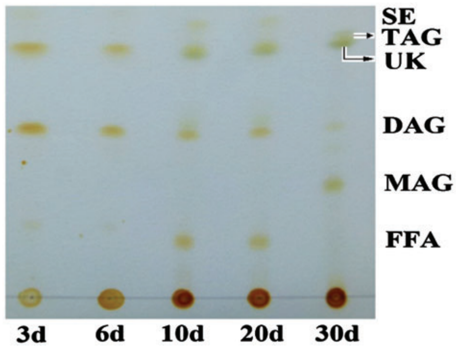

Figure 1. Mobilization pattern of neutral lipid components (3, 6, 10, 20 and 30 DAI). UK, unknown.

thus released undergo β-oxidation to produce adenosine triphosphate (ATP) as the major energy reserve within the seed.[7] Thin layer chromatography analysis of the total lipids (extracted from germinating cotyledons following Xu *et al.*[8]) 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 showed a 4% decrease in the total TAG content and an 8% increase in the FFA content at the end of seed germination.[5]

The increase in the FFA content suggests the possible biosynthesis of fatty acids during the later stages of germination as observed for soybean, flax and alfalfa.[9] In addition, increased SE levels were detected between 10 and 20 DAI, with trace amounts detected during all other stages, suggesting their active role in stabilizing the cellular membranes (by increased membrane synthesis) during the active germination process. Trace amounts of MAG were detected only during the late stage (30 DAI) of seed germination. The possible reason for such an action could be the effective activation of the enzyme esterases and hydrolases on MAG, which aid in the degradation of the lipid components, particularly in degrading the DAG and MAG.[10] The consumption of the TAG types at different rates during seed germination was a noted phenomenon in several oil seeds, with varying rates depending on the nature of the seeds.[4] In addition, the occurrence of an initial lag period in the process of TAG mobilization in germinating Sacha Inchi seeds was also observed in several other plant species.[11] At present, it is suggested that this phenomenon can be induced by several causes. On the one hand, they involve delays in the biosynthesis or activation of the enzymes concerned with this process, primarily lipase, in the transformation of these enzymes into oleosomes or in the formation of organelles such as glyoxysomes, mitochondria, etc., which are essential for TAG and fatty acid degradation. On the other hand, they involve a temporary inhibition of fatty acid breakdown under the action of excess soluble carbohydrates in the cotyledon tissues.[12] For gas chromatography analysis, fatty acid methyl esters (FAME) of total lipids (prepared and quantified as described elsewhere[13]) were analysed to assess the possible changes in the seedling TAG complex to study their fatty acid compositions.

Linoleic acid (18:2, ω-6) was the major fatty acid (60%) among the total lipids of Sacha Inchi seeds, followed by oleic acid, linolenic acid (18:3, ω-6), palmitic acid (16:0), stearic acid (16:1), myristic acid (14:0) and eicosanoic acid (20:0). Oleic acid (C18:1) was the predominant monounsaturated fatty acid (MUFA), whereas palmitic (C16:0) and stearic (C18:0) acids were the main saturated fatty acids (SFA) present. The fatty acids C14:0 and C20:0 began to appear in trace amounts only during the early stage (3 DAI) of germination (Table 1). 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.[14] 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,[15] 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

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

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

1 Hamaker B, Valles C and Gilman R, Amino acid and fatty acid profiles of the Inca peanut (*Plukenetia volubilis* L.). *Cereal Chem* **4**:461–463 (1992).
2 Fernandes G and Venkatraman JT, Role of omega-3 fatty acids in health and disease. *Nutr Res* **13**:19–45 (1993).
3 Joshi AC and Doctor VM, Distribution of fatty acids during germination of cotton seeds. *Lipids* **10**:191–193 (1975).
4 Pchelkin V, Tsydendambaev V and Vereshchagin A, Triacylglycerol metabolization during germination of sea buckthorn seeds. *Open Plant Sci J* **3**:1–6 (2009).
5 Wanasundara PKJPD, Wanasundara UN and Shahidi F, Changes in flax (*Linum usitatissimum* L.) seed lipids during germination. *J Am Oil Chem Soc* **76**:41–48 (1999).
6 Chavan JK, Kadam SS and Salunkhe DK, Changes in tannins, free amino acids, reducing sugars, and starch during seed germination of low and high tannin cultivars of sorghum. *J Food Sci* **46**:638–641 (1981).
7 Lin Y and Huang AHC, Lipase in lipid bodies of cotyledons of rape and mustard seedlings. *Arch Biochem Biophys* **225**:360–369 (1983).
8 Xu R, Wang R and Liu A, Expression profiles of genes involved in fatty acid and triacylglycerol synthesis in developing seeds of Jatropha (*Jatropha curcas* L.). *Biomass Bioenerg* **35**:1683–1692 (2011).
9 Huang AHC, Lin Y and Wang S, Characteristics and biosynthesis of seed lipases in maize and other plant species. *J Am Oil Chem Soc* **65**:897–899 (1988).
10 Galliard T, Degradation of acyl lipids: hydrolytic and oxidative enzymes, in *The Biochemistry of Plants: a Comprehensive Treatise*, Vol. **4**, ed. by Ian Morrison. Academic Press, New York, NY, pp. 85–116 (1980).
11 Yaniv Z, Shabelsky E, Schafferman D, Granot I and Kipnis T, Oil and fatty acid changes in *Sinapis* and *Crambe* seeds during germination and early development. *Ind Crops Prod* **9**:1–8 (1998).
12 Zimmerman DC and Vick BA, Hydroperoxide isomerase: a new enzyme of lipid metabolism. *Plant Physiol* **46**:445–453 (1970).
13 Bannon CD, Craske JD and Hai NT, Analysis of fatty acid methyl esters with high accuracy and reliability: II. *Methylation of fats and oils with boron trifluoride-methanol. J Chromatogr A* **247**:63–69 (1982).
14 Liu KS and Brown EA, Fatty acid compositions in newly differentiated tissues of soybean seedlings. *J Agric Food Chem* **44**:1395–1398 (1996).
15 Huang AHC, Oil bodies and oleosins in seeds. *Annu Rev Plant Biol* **43**:177–200 (1992).

