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Research Paper

Tracking lipid profiles of *Jatropha curcas* L. seeds under different pruning types and water managements by low-field and HR-MAS NMR spectroscopy

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A R T I C L E I N F O

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A B S T R A C T

Jatropha curcas L. seeds have been used as a promising source of oil for biodiesel production. In the present work, the impacts of different pruning procedures and water management on the content and lipid profile of the *Jatropha* seed oils were evaluated via nuclear magnetic resonance (NMR). The experiment was conducted at Research Station of ESALQ/USP, located in Piracicaba (São Paulo), Brazil. *Jatropha* plants were cultivated over four years under two different water conditions (irrigation – I and rainfed – R) and three pruning types (without pruning – P0, with removal of twigs at a height of 1.5 m from the ground and 0.75 m from the central stem – P1, and with the pruning performed at a height of 2.0 m from the ground and 0.75 m from the central stem – P2). The evaluation of seed oil contents was performed via low-field NMR, while the fatty acid composition was assessed by HR-MAS NMR. Low-field NMR results showed no changes in the oil content of *Jatropha* seeds regardless the treatment employed. ^{1}H HR-MAS NMR spectral profile of seeds revealed the presence of well-known signals of triacylglycerols, with the predominance of signals coming from oleic acid (C18:1) and linoleic acid (C18:2). Quantitative analyses of *J. curcas* seeds showed that the combination of irrigation and pruning managements influenced statistically the contents of unsaturated fatty acids. Among the evaluated conditions, the lipid profile achieved with pruning at 2.0 m height and 1.5 m canopy diameter (named P2) under irrigation (I) conditions might be considered the most effective for biodiesel production.

1. Introduction

With the accelerated industrial development, depletion of world oil reserves and increase in product prices, stimulated new push for the search for alternative renewable fuel sources has started (Qu et al., 2012). Vegetable oils have been extensively studied for its use in different renewable energy programs. Mainly due to their availability, being readily provided by a large number of natural source, and to be considered a good alternative for the replacement of petroleum derivatives (Francis et al., 2005; Barros et al., 2015). Besides the environmental benefits, the use of these oils offers an alternative to overcome social and economic challenges faced by the population. For instance, the decentralized production can improve the quality of poor region's life (Martín et al., 2010). In addition, diesel derived from vegetable oils presents benefits when compared with fossil diesel, which includes the low toxicity, renewable property, better emission qualities, and the engine performance that is slightly affected when fossil diesel is fully or partially replaced by vegetable oil (Corrêa and Arbilla, 2006; Oliveira et al., 2006).

Jatropha is a Central America native plant, widely distributed around the world (Chitra et al., 2005). This plant has been considered an important feedstock for bioenergy production (Behera et al., 2010), presenting outstanding characteristics over other oilseeds normally used for commercial production, such as soybean, corn, and castor bean (Daniel, 2008). Among the attractive features of this crop, notably its drought tolerance, easy spreading under low fertility soils, fast growth, high yield seeds and oil content (with seeds ranging from 30 to 50%) (Annarao et al., 2008; Achten et al., 2010).

According to Kaushik and Bhardwaj (2013), the oil content of seeds, oil fatty acid composition, and seed yield indexes are the main parameters that should be considered in the renewable feedstock obtainment for biodiesel production. Since these parameters are directly

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related to agronomic conditions, studies that aim to identify the best crop managements to guarantee the success of the agronomic business should be performed. Despite the *Jatropha* importance, as a potential alternative feedstock source for biodiesel production, there are few studies reporting the influence of agronomical treatments on seed oil content and fatty acid composition (Abou Kheira and Atta, 2009; Deus et al., 2012; Tikkoo et al., 2013).

Fatty acids are the main components of vegetable oils. Due to their relevance in determining the biodiesel quality, lipid profiles of oils should be evaluated (Sinha et al., 2015). In this context, nuclear magnetic resonance spectroscopy has been successfully employed (Prestes et al., 2007; Niu et al., 2014). Low-field NMR (frequencies < 60 MHz) and high-resolution NMR have been widely used for determining the content and lipid profile of oils, respectively (Prestes et al., 2007; Annarao et al., 2008; Tikkoo et al., 2013; Niu et al., 2014; Parker et al., 2014; Santos et al., 2015).

Recently, Andrade et al. (2017) employed high resolution magic angle spinning NMR spectroscopy (HR-MAS NMR), a technique able to analyze samples in their native state, to track the changes in the fatty acid composition of *Jatropha* oil seeds under different conditions of irrigation and nitrogen fertilization. In order to deepen the knowledge about the agronomical treatments in this crop, this work aims to evaluate the effects of different pruning and irrigation managements on the oil content and lipid profile of *Jatropha* oil seeds by means of low-field NMR and ^{1}H HR-MAS NMR.

2. Material and methods

2.1. Experimental area and plant material

Jatropha plants were cultivated over four years (Dec 2011–Aug 2015) in Piracicaba, southeastern Brazil (22°41′ S, 47°38′ W and 530 m altitude), using 4 months old seedlings at 3 m × 4 m spacing. The soil was a clay (sand, 220 g kg^{-1}; silt, 209 g kg^{-1}; clay, 571 g kg^{-1}) classified as a Nitisol (USDA) or Oxisol (FAO), with 1.4% organic matter content, and density 1.4 g cm^{-3}. The local climate is classified as Cfa according to the Köppen–Geiger world climatic classification (Peel et al., 2007). It has a subtropical humid climate with dry winter, presenting mean annual temperature of 21.6 °C and mean annual precipitation of 1328 mm (Marin et al., 2011). Jatropha plants were cultivated in two different areas, one under irrigation (I) by center pivot system and the other under rainfed condition (R). In early-September 2014, at the end of the stage of leaf senescence, all plants in both areas were pruned. The pruning treatments comprised of three pruning types: without pruning (P0), with removal of twigs at a height of 1.5 m from the ground and 0.75 m from the central stem (P1) and with the pruning performed at a height of 2.0 m from the ground and 0.75 m from the central stem (P2). In each area (I and R), the pruning treatments were arranged in a randomized complete block design, with 4 replicates of 16 plants per pruning type, resulting in 64 plants per pruning type (a total of 384 plants). A total of 3544 mm rainfall occurred over the four years of cultivation, while 1326 mm irrigation depth was applied in the irrigated treatments, totaling 4870 mm through the years. The respective amounts of rainfall and irrigation were 1117 and 287 mm water in the first year, 1195 and 230 mm in the second year, 588 and 731 mm water during the third year, and 644 and 78 mm water in the fourth year. The maximum evapotranspiration deficit recorded at the cultivation site was 36.8%.

From January to May 2015, fruits at the maturation period (presenting yellow, yellow–brown, and brown maturation color – Pessoa et al., 2012) were manually collected and dried out at the shadow under ambient temperature. Seeds were manually separated from fruits and again dried in a greenhouse at 60 °C until constant mass.

Table 1
Relation between the oil masses of *J. curcas* oil and their respective signal amplitudes acquired by the pulse sequences spin-echo and CPMG. Based on these results, the analytical curves were built.

Reference Sample	Oil Mass (g)	Signal Amplitude (a.u.)	
		Spin-echo	CPMG
1	0.25	5.93	8.07
2	0.50	11.83	15.73
3	0.76	18.02	23.59
4	1.00	23.80	31.50
5	1.25	29.49	38.70
6	1.50	35.72	48.10

2.2. NMR spectroscopy: oil content and lipid profile analysis

2.2.1. Low-field NMR experiments

Low-field NMR experiments were performed on a Bruker mq-20 spectrometer, equipped with a permanent magnet of 0.5 T and probe of 18 mm, observing ^{1}H at 20 MHz. The samples were directly transferred to NMR tubes and kept for 30 min in thermic blocks at 40 ± 2 °C. The oil analyses were performed using spin-echo and CPMG (Carr-Purcell-Meiboom-Gil) pulse sequences. In both sequences, it was employed 90° and 180° pulse length of 14.96 and 29.8 μs, respectively; echo time (τ) of 3.5 ms, 600 echoes, and 16 scans with 2 s of recycle delay (RD). The echo curves were registered with 600 data points. The analytical curves generated by both pulse sequences were used to determine the oil content in *Jatropha* seeds. For building of the analytical curves, six reference samples of *J. curcas* seed oils extracted by Soxhlet method were analyzed. The oil mass values (which varied from 0.25 to 1.5 g by an interval of 0.25 g) and their respective signal amplitudes are depicted in Table 1. Since these parameters have a proportional relation between them, it is possible to determine the oil content of a sample through its measured amplitude.

2.2.2. ^{1}H HR-MAS NMR experiments

For fatty acid composition analysis, ^{1}H HR-MAS NMR spectra were obtained from an NMR Bruker AVANCE 400 spectrometer at 294 K, operating at 9.4 T, observing ^{1}H at 400.13 MHz, equipped with a 4 mm four channel (^{1}H, ^{13}C, ^{15}N and D) HR-MAS probe. *Jatropha* seeds without shells were powdered in a mortar and 3 mg of the sample was inserted into a semi-spherical zirconium rotor (55 mm^{3}), followed by adding 40 μL of $CDCl_3$ containing 0.05% TMS (tetramethylsilane) as the internal reference. NMR spectra were acquired in triplicate with the following acquisition parameters: pulse sequence (zg), 20 s of relaxation delay (D1), 16 transients (NS), 64 K numbers of data (TD), acquisition time (AQ) of 7.97 s and the spectral window of 4111.842 Hz. During acquisition, the samples were spun at 5 kHz on the magic angle (54.74°), which was adjusted daily. The spectra were apodized via an exponential multiplication corresponding to a 0.3 Hz line broadening in the transformed spectrum and zero filled by a factor of 2. ^{1}H chemical shifts are given in ppm referenced to TMS signal at 0.00 ppm. Each treatment was represented by 15 seeds ($n = 90$). The determination of contents of saturated and unsaturated fatty acids was performed based in the quantification methodology proposed by Barison et al. (2010).

2.3. Statistical analysis

Analysis of variance (ANOVA) and F test were performed on the data through SAS 9.2 software (SAS Institute Inc., Cary, NC, USA). The comparisons between averages were performed by Tukey test at 5% level of probability, used as decision level for acceptance or rejection from the statistical significance in all analyses.