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Natural variation in fatty acid composition of *Sapindus* spp. seed oils

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ABSTRACT

Sapindus mukorossi and *Sapindus delavayi* seeds were collected from 60 populations distributed over a wide geographic area (a range of 12.0° latitude, 19.0° longitude, and 1800 m elevation) across southern China. The variation in fatty acid composition of seed kernel oil between the two species and among individuals was determined, and relationships with geographical and environmental factors were analyzed. The oil characterization allowed the identification and the quantification of 12 fatty acids. The C16–C20 fatty acids accounted for more than 98% of the total content, of which oleic acid was the most abundant (more than 50%). The fatty acid composition was generally stable between the two species and among individuals despite the wide geographic range from which the samples were collected, and the oil showed good potential for biodiesel production. Oleic acid showed insensitivity to environmental factors. Correlation analysis showed that environmental factors had a strong influence on the composition of other fatty acids, such as C20:1 (20.95%), C18:2 (7.56%), C20:0 (6.01%), and C16:0 (4.55%), and explained 36.68% of the total variance. Among the environmental factors, elevation, maximum temperature, and precipitation significantly contributed to variation in seed kernel oil fatty acid composition among populations. Furthermore, the cooperativity of elevation and maximum explained 30.13% of the variance ($P < 0.01$). Thus, environmental factors, especially elevation, maximum temperature, and precipitation, of the original provenance should be considered during genotype selection.

1. Introduction

Plant seed oils are useful commodities, either as food for humans or animals, or as industrial materials. Plant seed oils are an increasingly important bioenergy resource because they are renewable and environmentally safe (Jia and Sun, 2012). Inedible plant seed oils with high contents of medium chain monounsaturated fatty acids (e.g., oleic acid) show considerable potential as resources for biodiesel production. Currently, seed oils of *Brassica napus* and *Jatropha curcas* are the main bio-oils used in industry, and the demand for raw materials is growing in developing countries such as China (Maghuly and Laimer, 2013; Mazumdar et al., 2012). However, the yields of bio-oils fluctuate widely because the cultivated plants are not always adaptable to the environmental conditions (Yang et al., 2013). Therefore, bio-oil plants that show greater environmental adaptability are useful to supplement biodiesel production.

Sapindus has a large distribution range worldwide. The 13 member species are distributed in tropical to subtropical areas. Four *Sapindus* species are indigenous to China, of which two, *Sapindus mukorossi* and *Sapindus delavayi*, are widely distributed across southern and western China. The species show high seed yield, and the seed oil contains a high concentration of medium-chain monounsaturated fatty acids, especially oleic acid (C18:1). In a previous study, seed oils from *Jatropha curcas* and *S. mukorossi* were mixed to produce biodiesel, and produced a very good blend (Chen et al., 2013). Analyses of the chemical composition of *Sapindus saponaria* seed oil resulted in improved processing of high-quality biodiesel (Lovato et al., 2014). The seed oil is also a potential raw material for epoxy esters production (Sun et al., 2011). Saponins extracted from the fruit pericarp of *S. mukorossi*, *S. saponaria* and *S. trifoliatus* were shown to have surfactant and anti-inflammatory properties (Flechas et al., 2009; Jia and Sun, 2012; Morikawa et al., 2009).

Currently, considerable efforts are focused on identification of suitable *Sapindus* germplasm for breeding programs and large-scale cultivation. Research on genetic diversity and phenotypic variance in *Sapindus* has included analyses of biochemical indices with the aim of identifying lines that produce seed oil with high fatty acid contents, and those that show wide adaptability in cultivation (Diao et al., 2014). Although these studies have made good progress in

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evaluating the germplasm and selecting suitable lines, additional research on the fatty acid composition and adaptability of *Sapindus* is required. Most previous studies have focused on the fatty acid composition of *Sapindus* trees in natural stands (Zhao et al., 2014). However, limited information is available on the effects of environmental factors on fatty acid composition.

In this study, we investigated two *Sapindus* species distributed widely across southern China (*S. mukorossi* and *S. delavayi*). The objectives were to analyze variation in seed oil composition, and to investigate the relationship between environmental factors and fatty acid composition of the seed oil of the two *Sapindus* species. The information generated will be useful for breeding programs and to aid selection of the most adaptable lines for large-scale cultivation.

2. Materials and methods

2.1. Plant material

Seeds of *Sapindus* species were collected from populations at 60 distinct localities covering most of the *Sapindus* distribution area in China in November 2013. The two most widely distributed wild *Sapindus* species in China were sampled (47 *S. mukorossi* populations and 13 *S. delavayi* populations). With the support of local research institutions, samples were collected from 14 provinces or direct-controlled municipalities (Henan, Sichuan, Yunnan, Guizhou, Jiangxi, Guangxi, Guangdong, Fujian, Zhejiang, Jiangsu, Anhui, Hunan, Hubei, and Chongqin), across a wide area of southern China (a range of 12.0° latitude, 19.0° longitude, and 1800 m elevation) (Fig. 1). Only mature seeds were collected, because mature seeds have a stable fatty acid composition (Diao et al., 2014). To ensure that mature seeds were collected, fruits in which the pericarp was shrunken and a yellow or red color were collected.

2.2. Seed oil extraction

The kernels were shattered, dried, and then subjected to a four-step extraction process (heating for 30 min, condensation for 1 h and 20 min, predrying and organic solvent recycling for 10 min) using a FOSS Soxhlet extraction apparatus (Soxtec 2050, Höganäs, Sweden). The final 2-h extraction step ensured that all of the oil was extracted from the seed kernel mass. The organic solvent was petroleum ether and each sample weighed 10–15 g. After the four extraction steps, the extracted oil was dried at 80 °C for 10 min to remove the residual organic solvent. Compared with the traditional Soxhlet extraction technique, automated Soxhlet extraction has the advantage of not only a high recovery rate, but also a short extraction time and less solvent consumption, and use of the method is recommended by USA EPA 3541.

2.3. Fatty acids determination

To characterizae fatty acids, they must first be derivatized into fatty acid methyl esters. We used the GB/T 17376-2008 (ISO 5509:2000,IDT) transesterification method for derivatization (Ren et al., 2015). Each oil sample (0.06 g) was dissolved in 4 mL isooctane in a 10 mL test tube with a stopper. Then, 0.2 mL potassium hydroxide: methanol solution (2 mol/L) was added, and the mixture was mixed with a Vortex mixer for 30 s. Then, 1 g sodium bisulfate monohydrate was added to neutralize excess alkali. The mixture was mixed with a Vortex mixer for 15 s and then allowed to stand to clarify. The supernatant was analyzed by gas chromatography using an Agilent 7890A instrument (Agilent, Palo Alto, CA, USA) equipped with an HP-Innowax capillary column (30 m × 0.25 mm × 0.25 μm; Hewlett-Packard, Palo Alto, CA, USA) and a flame ionization detector. The conditions were as follows: initial column temperature, 140 °C for 1 min, heating at 4 °C/min to 250 °C, hold for 5 min; column flow, 2 mL/min; injection port temperature, 220 °C; split ratio, 1:20; sample size, 1 μL; detector temperature, 275 °C, tail gas, nitrogen; make-up flow rate, 25 mL/min; hydrogen flow rate, 30 mL/min; air flow rate, 400 mL/min. The components of the seed oil were quantified by the peak area normalization method and were identified by comparison of the retention time with fatty acid methyl ester standards.

2.4. Physicochemical properties of biodiesel

The physicochemical properties of biodiesel were calculated according to the equations proposed by Park et al. (2008), Ramírez-Verduzco et al. (2012), Ramos et al. (2009) and Wang et al. (2012). The physicochemical properties comprised cetane number (Ramírez-Verduzco et al., 2012), kinematic viscosity (Ramírez-Verduzco et al., 2012), oxidation stability (Park et al., 2008), iodine value (Wang et al., 2012), heating value (Ramírez-Verduzco et al., 2012) and cold filter plugging point (CFPP; Ramos et al., 2009). The total concentration of saturated fatty acids ($\sum$SFA, wt.%), the amount of monounsaturated fatty acids ($\sum$MUFA, wt.%), the amount of polyunsaturated fatty acids ($\sum$PUFA, wt.%), the ratio of unsaturated/saturated fatty acids ($\sum$UFA/$\sum$SFA) and the $\sum$PUFA/($\sum$SFA + $\sum$MUFA) ratio (Gornas and Rudzinska, 2014) were calculated. Each value was calculated based on the mean fatty acid methyl esters composition for each species.

2.5. Environmental factors

Location data were obtained at the sample collection sites with a GPS (JUNO® SCSD, Trimble). The location data comprised longitude, latitude, and elevation. Climate data were obtained from the National Meteorological Data of China (http://www.escience.gov.cn/metdata/page/index.html), which provides the closest approximated values for a site based on data collected across an extensive geographical range. Climatic variables consisted of annual average temperature, annual minimum and maximum temperatures, annual average relative humidity, annual minimum relative humidity, average annual precipitation, and mean annual sunshine duration.

2.6. Statistical analyses

Mean and standard deviation (S.D.) of each of the fatty acids from C16:0 to C24:1 were calculated. The analyses were conducted using the statistical packages STATISTICA 6.0 (StatSoft Inc., 2001). A canonical correspondence analysis (CCA) was conducted using fatty acid as the response variable, and environmental factors as the explanatory variable, to detect their effects on variation in fatty acid composition (Chen, 1990; Ghebretinsaea et al., 2008). An initial principal component analysis (PCA) was performed to examine the correlations between fatty acid composition and environmental factors with two principal components extracted for the response and explanatory variables separately from the CCA model. A second PCA was performed to examine the structure of the fatty acid data with two principal components extracted for only the response variable from the CCA model. Data for the 12 fatty acids detected at >0.1% were included in the CCA to explore variation among the *Sapindus* populations and the relationships with environmental factors. A potential analytical problem was that the environmental factors may have been correlated with each other or showed strong co-linearity. To avoid this problem, the CCA model was simplified by selection in the forward direction. To analyze the data from an objective perspective, geographical factors and