Industrial Crops and Products 50 (2013) 797–802

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

An evaluation of the factors influencing seed oil production in *Camellia reticulata* L. plants

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ARTICLE INFO

Article history:
Received 1 April 2013
Received in revised form 30 July 2013
Accepted 4 August 2013

Keywords:
Camellia seed oil
Fruit characteristics
Climatic factors
Soil type
Elevation

ABSTRACT

Camellia seed oil is well known for its industrial uses. However, despite the increasing use of this oil, few studies have investigated which factors impact seed oil production. Thus, the aims of this study are to identify which factors influence the percentage seed oil of *Camellia reticulata*, a by investigating both seed and environmental variables. Firstly, fruit traits were studied and correlated against percentage seed oil data, and then environmental variables linked to seed oil production were investigated. The first experiments analyzed how well fruit characteristics (size, length, weight, seed number, seed oil) of the Camellia plants predict percentage seed oil. The second experiment modelled the effect of environmental variables (soil, elevation, temperature and rainfall) on seed oil production. The results indicated that the kernel ratio per fruit, seed weight to fruit weight ratio and fruit weight positively influenced percentage seed oil while, when these previous effects are accounted for, the number of seeds reduced percentage seed oil. The environmental model showed that seed oil was influenced most strongly by elevation and soil type, with haplic Acrisols providing the highest seed oil. Thus it is clear that Camellia seed oil is affected by a variety of factors, and these should be taken into account when selecting species and cultivars for the planting of Camellia stands for oil production.

1. Introduction

Camellia is known worldwide for the production of tea, however, there is a growing industry utilizing the oil derived from Camellia seeds. Camellia oil, extracted from a number of different species, including *Camellia reticulata*, *Camellia sinensis*, *Camellia oleifera*, and *Camellia japonica*, has long been processed as an industrial oil, used in the production of cosmetics, soaps, hair oil, medicines and now it is generating interest as a biofuel source (Rajaei et al., 2008; Lin and Fan, 2011). With the very high production of tea worldwide, totalling 3.6 million tonnes annually, there is enormous potential for the Camellia oil industry (Wang et al., 2011; Chen et al., 2012). China alone harvests 650,000 tonnes of Camellia seeds each year, producing around 165,000 tonnes of seed oil, and this figure is predicted to rise to as much as 2.5 million tonnes by 2015 (McDowell and Owuor, 1992; Cao et al., 2005).

Camellia seeds are known to have a high oil content, on average ∼30% oil per seed, however, the seed oil content varies according to species, cultivar and environmental conditions, ranging between 24% and 50% (Ravichandran and Dhandapani, 1992; Xia et al., 1993; Yazicioglu et al., 1997; Sahari et al., 2004; Wang et al., 2011; Huang et al., 2010). The high seed oil variability is likely the result of a number of factors, but environmental variables such as soil, altitude, light, rainfall, humidity and temperature are likely to play a key role as they have been shown to influence the oil content of seeds for a variety of plants (Kang et al., 1993; Linder, 2000; Debin and Yangzhu, 2002; Junang et al., 2010). Furthermore, fruit traits such as seed size, fruit number, fruit size and dry weight have been linked to the oil production in seed oil plants (Li et al., 1992; Abdelgadir et al., 2010; Yanru and Zhangju, 2010). Thus, by understanding the impact of fruiting behaviour and fruit characteristics, and at a larger scale that of environmental conditions, seed oil production can, to a certain extent, be predicted and/or manipulated.

Seed oil from a variety of plants, including Camellia, can consequently be influenced by a variety of mechanisms. Given the growing demand for Camellia oil, it is important to understand exactly what is causing the variability in oil production, and what factors result in optimum seed oil production. The forecast increase in demand for Camellia oil will almost certainly entail an increase in Camellia cultivation and land use, and knowledge regarding the impact of environmental factors on oil production can help to enhanced productivity. Thus, we aim to determine which fruit

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http://dx.doi.org/10.1016/j.indcrop.2013.08.019

traits are associated with high seed oil production as well as which environmental factors most strongly influence oil production in *C. reticulata*, a species indigenous to Yunnan Province, China. Based on these findings, a map depicting the optimal growing areas for Camellia oil production in southwestern China will be produced.

2. Materials and methods

2.1. Sites, sampling, and environmental data

Plantation grown *C. reticulata*, cultivated from seed, with a minimum population size of 10 ha, were used for sampling of fruit and seed oil, during the period of March 2008 and August 2009. Sample populations were located at 8 different sites (Fig. 1), spread over in 3 districts: Tengchong, Changning, and Gaoligong Shan, all in western Yunnan Province, China. The elevation of the sites ranged between 1715 and 2290 m.a.s.l., and slope between 5° and 30°. For more detailed information regarding the locations and the environmental data of the locations refer to Table 1.

We collated data for five candidate environmental variables that potentially affect (proximately or indirectly) the *C. reticulata* seed oil content: altitude, slope gradient, soil type, rainfall, and annual mean temperature. Altitude and slope were recorded in the field. For soil type we used the Soil and Terrain database (SOTER) for China version 1.0 compiled by the Institute of Soil Science, Chinese Academy of Science (ISSAS) and ISRIC-World Soil Information (Dijkshoorn et al., 2008). Rainfall and temperature data were provided by the Baoshan Meteorological Bureau and the Baoshan Statistic Year Book 2011 (CSA, 2012).

It should be noted that whilst individual elevation and slope measurements were present for each site, rainfall and temperature data were only available for the nearest weather station, which meant that nearby sites were assigned the same weather station value.

Sampling was carried out in a stratified random fashion within the populations, ensuring that there was a minimum of 40 m between sample plants to represent a maximum of the genetic diversity of the populations. In total 444 plants were sampled for the study. From each individual sample plant 30 mature fruits were randomly selected for further analysis.

2.2. Fruit and seed oil data

Fruit length and diameter were measured using LCD calipers (0.01 mm) within 24 h after fruit collection and an electronic balance was used to determine the fresh weight (g) of the fruit and the kernels. Subsequently, the fruit were dried for 10 days followed by the removal of the seeds for analysis. The following parameters were measured: number of seeds per fruit, number of kernels per seed, and seed and kernel weight (g). For the determination of seed oil content, healthy kernels were selected from the sampled fruit (30 per plant), totaling 12 g kernel material, and 3 replicates, of 12 g kernel material each, were used. The seed oil content was determined by initially grinding the kernel material into a powder form, from which 10 g was selected (using a 1/10,000 analytical balance) and the oil content obtained via the solvent extraction method of Shi et al. (2005) and percentage seed oil calculated.

2.3. Data analysis

We modelled the effects of fruit characteristics on percentage seed oil within a linear modelling framework. In order to establish significant covariates for the seed oil content, we first explored the data graphically, including checking for potential interaction effects between the candidate predictor variables (none were found). Then, in order to find the minimum adequate model, we fitted a multiple linear regression applying both a forward and backward stepwise predictor selection on the basis of the Akaike information criterion (AIC) and the partial F-statistic. While the forward selection included all candidate predictors, the backward dropping procedure only included the strongest non-collinear set (Pearson's $R < 0.7$) to avoid inflated standard errors and associated Type II errors (Zuur et al., 2007). To this end the relative explanatory power of all candidate predictors was established using hierarchical partitioning (Chevan, 1991). This procedure calculates the independent and conjoint contribution of all predictors to the total explained variance in a regression model over all possible candidate models (i.e. models with all combinations of predictors at each hierarchical level with 1 to N predictors). Model validation procedures followed Zuur et al. (2009) and indicated no problems associated with assumptions of normality and heterogeneity of variance; nor the presence of influential data points with a Cook's distance ≥ 1. Finally, we established the respective contribution of each variable towards explaining the variation in the seed oil content by decomposing the variance in a partial regression (Zuur et al., 2007). All analyses were carried out using the software environment R (R Development Core Team 2013).

The development of models to explore the environmental effects (topography, climate and soil) on C. reticulata seed oil content (using a total of 444 data points) followed the same analytical procedures presented above. In addition we tested whether a mixed modelling framework whereby the location of the plantation was fitted as a random factor was more adequate. Mixed models with random intercept and random intercept and slope were fitted using restricted Maximum Likelihood in the R package 'nlme' (Pinheiro et al., 2013). AIC statistics indicated that a simple linear model was adequate. The final model was spatially extrapolated to southwestern China whereby the topographic variables were based on the SRTM digital elevation model (Jarvis et al., 2008) with a resolution of 3 arc seconds (~90 m), and soil on SOTER China version 1.0 (Dijkshoorn et al., 2008).

3. Results

3.1. Fruit traits

The various fruit traits are shown in Table 2. Of the factors tested, percentage seed oil was positively influenced by the kernel ratio per fruit, the seed fresh weight to fruit weight ratio and, to a smaller extent, by fruit weight. Once these effects were accounted for the seed number had a negative effect on seed oil percentage. The final model took the form of seed oil percentage ~−20.59 + 0.75 kernel ratio per fruit + 0.41 seed weight percentage of total fruit weight + 0.02 fruit weight (g) – 0.28 numbers of seeds per fruit, and explained 68% of the variation in seed oil percentage ($F = 275.4$; d.f. = 525; $p < 0.001$; $R^2 = 0.68$). The kernel ratio per fruit explained 38% of the variation on its own whereas the other predictors explained less than 5% on their own. The fruit dimensions (length and diameter) were strongly positively correlated with fruit weight and not included in the model to avoid inflated standard errors.

3.2. Environmental variables

Simple variate–covariate plots showed that *C. reticulata* seed oil content decreased with elevation, slope and rainfall, and increased with temperature (Fig. 2a–d). It should however be noted that temperature was strongly correlated with elevation (Pearson's $R = -0.93$), and there was also a correlation between slope and rainfall (Pearson's $R = 0.71$). Significant predictors in the final