African Journal of Agricultural Research Vol. 7(10), pp. 1487-1491, 12 March, 2012
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.732
ISSN 1991-637X

Full Length Research Paper

The traits, oil content and correlation studies of seed and kernel in *Jatropha curcas* L.

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Accepted 22 February, 2012

The single seed, kernel traits and oil content of 2000 seed samples of *Jatropha curcas* collected from China, Laos, Cambodia and Burma were analyzed based on no injury and contamination Nuclear Magnetic Resonance (NMR) technology. This study showed that there was a significant phenotypic variation for the seed weight and oil content in *J. curcas* (the coefficient of variation was 15.54 and 19.36%, respectively). The single seed weight was weak and negatively correlated with the seed oil content (r = -0.07). Based on the analysis of various seed traits, a new parameter WTRKS (width and thickness ratio of kernel to seed), was established. Analysis showed that the correlation between WTRKS and the oil content was highly positive (r = 0.92). Besides, WTRKS was also positively correlated with the single seed weight and the single kernel weight (r = 0.32 and r = 0.40). Therefore, WTRKS can be regarded as an important parameter for the improvement of both seed oil content and single seed weight of *J. curcas*.

Key words: *Jatropha curcas*, NMR, oil content, seed weight, biofuel.

INTRODUCTION

Jatropha curcas L. (Euphorbiaceae) is an ideal oil-bearing tree, which is adapted to aridity, high temperature and barren soil, and is also resistant to diseases and insect pests (Openshaw, 2000; Zahawi, 2005; Francis et al., 2005). The seed of *J. curcas* has oil content as high as 35% with abundant oleic and linoleic acid. As a result, it is especially suitable for use as biofuel (Ravindranath et al., 2003; Senthil et al., 2003; Forson et al., 2004; Mohibbe et al., 2005; King et al., 2009). *J. curcas* possesses several properties, including unisexual flowers, hermaphrodite flowers with superior ovary with three loculi and one ovule in each locule, and generally, three seeds in one fruit (Qiu et al., 1996; Wu et al., 2011); the seed nearly elliptical and the oil existing mainly in the white kernel (Figure 1).

As a biomass energy plant, *J. curcas* has drawn a lot of attention of researchers. Genetic improvement of *J. curcas* has been initiated (Koonin, 2006; Ranade et al., 2008). Flight tests had been completely powered by a blend of jatropha oil and conventional jet fuel (Evans, 2008; Garthwaite, 2009; Austin, 2009). As a raw material for biofuel production, the biofuel yield is directly influenced by the seed oil yield of *J. curcas* which is influenced by the seed yield and its oil content, and the seed weight is one of the factors affecting the seed yield. Therefore, studies of the seed weight and the oil content of *J. curcas* are of great significance in improving its seed oil production. Some studies show that the oil mobilization was initiated during germination, and that the oil was consumed during early seedling development which indicated that the oil content of matured seeds may affect the seedling quality. Studies in India showed that there were significant differences (P<0.05) in seed size, 100-seed weight and oil content between 24 accessions (Kaushika et al., 2007). However, few studies have been performed on the traits, oil content and correlation of a single seed and kernel in *J. curcas*. 2000 jatropha seed samples were assessed based on no injury and contamination NMR technology in this study.

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MATERIALS AND METHODS

2000 seed samples of *J. curcas* plants growing under natural field conditions were randomly selected from Sichuan, Yunnan, Hainan, Guangxi, and Guizhou provinces of China, and from Laos,

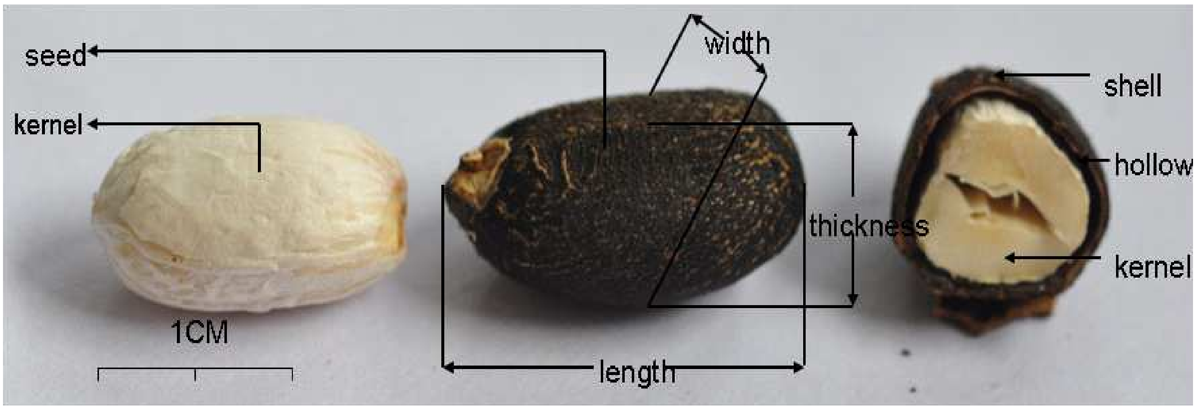

Figure 1. The structure of seed and the measurement of length, width and thickness of seed and kernel of *J. curcas.*

Table 1. Statistics of seed oil content and other characteristics of J. curcas, and their correlations with WTRKS.

Variable	Mean	Minimum	Maximum	Range	CV*	Pr>|t|	WRTKS (Sig.)	
Seed length (cm)	1.789	1.576	1.958	0.382	4.449	<0.0001	0.15	<0.0001
Seed width (cm)	1.096	0.957	1.461	0.504	4.905	<0.0001	-0.01	0.5737
Seed thickness (cm)	0.849	0.701	0.992	0.291	4.972	<0.0001	0.11	<0.0001
Kernel length (cm)	1.459	1.144	1.638	0.494	4.967	<0.0001	0.18	<0.0001
Kernel width (cm)	0.808	0.467	1.011	0.544	11.02	<0.0001	0.63	<0.0001
Kernel thickness (cm)	0.676	0.339	0.931	0.592	12.744	<0.0001	0.68	<0.0001
Single seed weight (g)	0.681	0.383	0.948	0.565	15.543	<0.0001	0.32	<0.0001
Single kernel weight (g)	0.438	0.203	0.686	0.483	18.669	<0.0001	0.40	<0.0001
Oil content of a seed (%)	32.105	11.754	48.423	36.669	19.367	<0.0001	0.92	<0.0001

*CV: Coefficient of variation.

Cambodia and Burma in 2009 and 2010. The length, width and thickness of the seed and the kernel were measured by vernier caliper, and the weight of single seed and kernel as well as, the oil content of a single seed were determined.

The seed weight and oil content were measured according to the following protocol: a single seed of *J. curcas* (kernel and seed shell) was placed in a weighing disk and dried at 30℃ for 24 h. Subsequently, it was transferred to a desiccator, and the seed weight was determined after cooling by electronic balance and the seed oil content determined using NMR technology (Wu et al., 2010). The kernel weight and oil content were immediately determined and data obtained were analyzed using Statiscal Analysis Software (SAS).

RESULTS

Properties of seed

The seed of *J. curcas* is the major organ for the storage of oil. The length, width and thickness measurements of the seed and the kernel are shown in Figure 1. Table 1 gives the data on range and mean value for the length, width, thickness and weight of seed and kernel, and the oil content of a single seed. There were very significant differences among single seeds of *J. curcas* in thickness, width, length and weight of seed as well as kernel, and the oil content (Pr>|t| < 0.0001), in which the variation in the seed oil content was the highest with a coefficient of variation of 19.36%, followed by the single kernel weight and the single seed weight with coefficients of variation of 18.66 and 15.54%, respectively. The variation in kernel thickness and width was lesser with a coefficient of variation of 12.74 and 11.02%, respectively. While the variations in the length, width and thickness of the seed and the kernel length were relatively low (with the coefficient of variation of 4.44, 4.90, 4.97 and 4.96%, respectively).

Correlation analysis for different seed properties

It can be seen from Table 2 that the correlation between the seed length and the kernel length was the highest (correlation coefficient r = 0.70) of the length, width and thickness correlations studied. The length and width of the seed were positively correlated with the seed's thickness significantly (r = 0.66 and 0.60). The length, width and thickness of the seed were positively correlated in a significant way with the width and thickness of kernel,

Table 2. Correlation among sizes of seed and kernel, weight and oil content of a single seed (the correlation and significance in each of the items).

Variable	Seed length	Seed width	Seed thickness	Kernel length	Kernel width	Kernel thickness	Single seed weight	Single kernel weight	Oil content of a seed
Seed length	1.00	0.44 <0.0001	0.66 <0.0001	0.70 <0.0001	0.31 <0.0001	0.30 <0.0001	0.69 <0.0001	0.58 <0.0001	-0.13 <0.0001
Seed width	0.44 <0.0001	1.00	0.60 <.0001	0.28 <0.0001	0.23 <0.0001	0.34 <0.0001	0.54 <.0001	0.46 <0.0001	-0.24 <0.0001
Seed thickness	0.66 <0.0001	0.60 <0.0001	1.00	0.46 <0.0001	0.35 <0.0001	0.40 <0.0001	0.69 <.0001	0.61 <0.0001	-0.17 <0.0001
Kernel length	0.70 <0.0001	0.28 <0.0001	0.46 <0.0001	1.00	0.33 <0.0001	0.19 <0.0001	0.57 <0.0001	0.51 <0.0001	-0.04 0.0994
Kernel width	0.31 <0.0001	0.23 <0.0001	0.35 <0.0001	0.33 <0.0001	1.00	0.10 0.0002	0.45 <0.0001	0.46 <0.0001	0.47 <0.0001
Kernel thickness	0.30 <0.0001	0.34 <0.0001	0.40 <0.0001	0.19 <0.0001	0.10 0.0002	1.00	0.43 <0.0001	0.47 0.0001	0.54 <0.0001
Single seed weight	0.69 <0.0001	0.54 <0.0001	0.69 <0.0001	0.57 <0.0001	0.45 <0.0001	0.43 <0.0001	1.00	0.91 <0.0001	-0.07 0.0052
Single kernel weight	0.58 <0.0001	0.46 <0.0001	0.61 <0.0001	0.51 <0.0001	0.46 <0.0001	0.47 <0.0001	0.91 <0.0001	1.00	0.06 0.01
Oil content of a seed	-0.13 <0.0001	-0.24 <0.0001	-0.17 <0.0001	-0.04 0.0994	0.47 <0.0001	0.54 <0.0001	-0.07 0.0052	0.06 0.01	1.00

but the correlation coefficients were lower than 0.5; there were significant positive correlations also among the length, width and thickness of kernel with all correlation coefficients lower than 0.5. The correlation between the kernel width and kernel thickness was especially low with a correlation coefficient of 0.1. The length, width and thickness of seed and kernel were positively correlated with the weight of single seed and single kernel in a significant way such that the correlations between the length and width of seed and the weight of a single seed and a single kernel were high (r = 0.69, 0.69, 0.58 and 0.61). Besides, the single seed weight had a high positive correlation with the single kernel weight (r = 0.91).

The data in Table 2 also indicated that the seed oil content had a significant negative correlation with the length, width and thickness and the weight of the seed; the seed oil content had a weak correlation with the kernel length, and a slight positive correlation with the single kernel weight (r = 0.06), while it had a significant positive correlation with the width and thickness of kernel with low correlation coefficients (r = 0.447, 0.54, respectively). Therefore, a new parameter, WTRKS (width and thickness ratio of kernel to seed), expressed as equal to (kernel width/seed width) × (kernel thickness/seed thickness) was developed based on the correlation analysis that was previously mentioned to obtain a parameter of a relatively high correlation with the seed oil content (Table 2). The correlation analysis (Table 2) showed that the seed oil content had a positive correlation with WTRKS showing a high correlation coefficient (r = 0.92). Besides, the kernel width and length were also positively correlated with WTRKS in a significant way (r = 0.63, 0.68). The weights of a single seed and a single kernel were also positively correlated with WTRKS (r = 0.32, 0.40), while the seed length, seed thickness, and the kernel length were only slightly positively correlated with WTRKS. The correlation between the seed width and WTRKS was not significant.

Regression analysis on the seed oil content

Due to the high linear correlation between seed oil content and WTRKS, a linear regression analysis of seed oil content was performed with WTRKS. The regression analysis indicated that the relationship of the seed oil content and WTRKS could be described by a linear function, Y= A + b (WTRKS), where Y is the oil content of the seed, A being the intercept and b, the regression coefficient. The regression parameter estimation resulted in the following equation:

Single seed oil content (%) = -4.6582 + 61.6586 (WTRKS)

Where the lower limit of intercept A was -5.2881 and upper limit -4.0283 (confidence of 95%), and the lower limit of regression coefficient b was 60.6018 and its upper limit 62.7154 (confidence of 95%). Both intercept A and the regression coefficient b were very highly significant (Pr>|t|, <0.0001). The analysis variance of regression indicated that the regression effect as highly significant (Pr>F <0.0001) indicating that the regression equation was meaningful. Therefore, the parameter WTRKS is of practical significance for predicting the seed oil content.

DISCUSSION

The seed of *J. curcas* is the major organ for the storage of synthesized oil. The yield of oil was directly influenced by the seed weight and oil content. In this study, it was shown that there was significant phenotypic difference in the single seed weight and oil content; the former one was within the range from 0.383 to 0.948 g with an average of 0.681 g while the latter was within the range from 11.75 to 48.42% with an average of 32.10%, indicating that the variation was significant (the coefficient of variation was 15.54 and 19.36%, respectively). Some studies also showed that there were significant differences (P<0.05) in seed size, 100-seed weight and oil content, and also showed high heritability and genetic gain for oil content (99.00 and 18.90%) and seed weight (96.00 and 18.00%), respectively (Kaushik et al., 2007). Accordingly, the seed oil production of *J. curcas* can be greatly improved by the selection and breeding for high seed weight and high oil content.

Studies in India showed that phenotypic coefficient of variation of seed weight and oil content was higher than the genotypic coefficient of variation, indicating the predominant role of environment (Kaushik et al., 2007). To understand the influence of environment on the characteristics of the seeds of *J. curcas*, the average annual temperature and rainfall data in eight seed source areas were examined. The data of China came from the local meteorological stations, and the data of Laos, Cambodia and Burma were provided by the seed providers. The mean temperature and total rainfall values for Sichuan, Yunnan, Hainan, Guangxi and Guizhou provinces were respectively 21.2℃ and 310 mm, 20℃ and 630 mm, 23.8℃ and 1600 mm, 21.7℃ and 1304 mm, and 19.2℃ and 1200 mm. The respective data for Laos, Cambodia and Burma were 26℃ and 1300 mm, 28℃ and 1800 mm, and 27℃ and 2200 mm. However, the statistical analysis showed that there was no significant difference among the eight locations in the length, width, thickness, weight of the seeds and kernel and the seed oil content. In addition, there were no reports indicating that the size, weight and oil content of seeds of *J. curcas* in a certain region were significantly better than in the other regions. But, our research indicated that there was significant difference in oil content of seeds from different plants in the same area. In fact, there were different temperature and rainfall conditions during the different phases of the fruiting in *J. curcas* (there are normally 3 to 5 times that fruiting occurs in a year) in one area. Therefore, for better understanding of the environmental influence, it is necessary to under-take the study in the controlled environmental conditions.

Some studies showed that jatropha seed weight had positive correlation with oil content (Kaushik et al., 2007). This study showed that there was a slight negative correlation between the single seed weight and the oil content (r = -0.07), indicating that both characters could be improved at the same time. Some other studies also showed that the over-expression of diacylglycerol acyltransferase enhanced both seed oil content and seed weight in *Arabidopsis* (Jako et al., 2001). Therefore, it may be possible to improve seed oil content and seed weight of *J. curcas* by genetic engineering. The cor-relations between the seed length and thickness and the single seed weight were relatively high (r =0.69, 0.69), and could thus, be used for seed selection in breeding. For the oil production industry, the raw oil yield could be improved by selecting the seeds with high seed weight according to the seed length and thickness. However, the seed length and thickness had a slight negative correlation with the seed oil content (r = -0.13, -0.17). Thus, these two parameters were not suitable for selecting the seeds of high oil content.

Since the seed oil in *J. curcas* is mainly stored in the kernel, the parameters of kernel were expected to closely correlate with the oil content of the seed. The study showed that the width and thickness of kernel has a positive correlation with the oil content to a certain degree (r = 0.47, 0.54), while the correlation between the kernel length and the seed oil content was insignificant. Therefore, it is clear that accumulation of the oil per unit weight of seed could be reflected to a certain extent by the width and thickness of the kernel. The seed shell of *J. curcas* limits the space for the kernel to grow. The index WTRKS is the ratio between the width and length of kernel and the width and thickness of the seed, which reflects the variation in the width and thickness of the kernel when compared to that of the seed. The higher the WTRKS value of the seed, the higher the proportion of the seed space occupied by the kernel as reflected by its width and thickness (Figure 1). Therefore, WTRKS was positively correlated with the seed oil content (r = 0.92). The oil content of the seed could be predicted with reasonable levels of confidence by using the linear regression equation based on WTRKS index developed in this study without a direct measurement of the oil content. This is important for assessing the quality of produce from Jatropha plantations in remote areas where facilities for direct determination of oil might not be available. Besides, WTRKS also had a positive correlation with the single seed weight and the single kernel weight (r = 0.32 and 0.4). Therefore, WTRKS could also be used as a parameter for selecting the seeds of *J. curcas* with high oil content and high seed weight.

ACKNOWLEDGEMENTS

We are grateful to Lu-bin Tan, Hui-lan Wu and Yi He (Analytical and Testing Center of Sichuan University) for their advice, encouragement, and helpful suggestions. This research work was funded by the Cooperation Project Foundation of China and Holand (No. 2004DFB00300), the National Science Foundation (No. 30670204), “12th Five-Year Plan” to support science and technology project (No. No. 2011BAD22B00) and the Cooperation Project in industry, education and research of Guangdong province and Ministry of Education of China (No. 2009B090600069).

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