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Physicochemical properties of seeds and oil from an F_2 population of *Jatropha curcas* × *Jatropha integerrima*

Khin Thida One[a], Narathid Muakrong[b], Patcharin Tanya[c], Jérémy Valette[d], Philippe Girard[e], Peerasak Srinives[c,*]

[a] Tropical Agriculture Programme, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140 Thailand

[b] Plant Breeding Programme, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140 Thailand

[c] Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140 Thailand

[d] BioWooEB Unit, French Agricultural Research Centre for International Development (CIRAD), 34398 Montpellier Cedex 5, France

[e] CIRAD Representative in Thailand, Kasetsart University, Chatuchak, Bangkok 10900 Thailand

[*]Corresponding author, e-mail: agrpss@yahoo.com

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ABSTRACT: Jatropha or physic nut (*Jatropha curcas*) is being genetically improved in many countries for commercial plantation as a source of biodiesel. This study investigates the variation in physicochemical properties of seed and oil obtained from F_2 plants of a cross between *J. curcas* cv. "Chai Nat" and *J. integerrima* (a local dwarf ornamental type from Thailand). The results revealed a high diversity in properties of seed and oil showing a number of promising genotypes. Out of 296 F_2 plants derived from an F_1 plant, 42 of them showed small canopy size, high in seed yield, oil content, and oleic acid content, but low in linoleic acid content. Some plants showed a kernel to seed weight ratio of more than 0.8. Some plants had over 40% seed oil content. Phorbol ester content of their seeds varied from 0.7–4.6 mg/g, with an average of 1.9 mg/g. Seed yield of the F_2 plants showed a positive correlation with oil yield per plant ($r = 0.99$), while there were weak relationships between oil content with the other traits. A highly negative correlation was observed between oleic and linoleic acids ($r = -0.97$).

KEYWORDS: interspecific cross, phorbol esters, seed oil, fatty acid

INTRODUCTION

Jatropha curcas L., commonly known as jatropha, physic nut, or purging nut, belongs to the family Euphorbiaceae. It is a perennial oilseed shrub that originated in Tropical and Subtropical America. *J. curcas* is a drought-resistant non-edible tree that can thrive in a wide range of soils and climates. For these reasons, it is considered as the most promising biodiesel feedstock worldwide. *J. curcas* is a fast growing crop and can produce seeds for up to 50 years[1]. The full potential of *J. curcas* has however not been realized due to several technological and economic reasons[2]. Physic nut seed kernel contains 40–60% (w/w) oil[3], and the oil contains around 80% unsaturated fatty acid which makes it suitable for biodiesel production[4]. Physic nut oil can be converted to biodiesel using transesterification, the process depends on the free fatty acid content of the oil[5]. The resulting biodiesel can be used as a substitute for petroleum diesel fuel. The seed cake remaining after oil extraction is an excellent source of plant nutrients[6]. However, the presence of curcin and phorbol esters makes physic nut oil toxic[3], preventing its use as animal feed. Variations in physic nut seed edibility is due mainly to the level of phorbol esters in the seeds[7]. Despite the expectation on physic nut oil as a promising biodiesel feedstock, the production has not reached its full potential due to the lack of reliable cultivars. To make the production of physic nut profitable and sustainable, genetic improvement of oil yield and quality is required[8]. To commercialize physic nut, its seed yield should reach 4–5 t/ha per year (about 2.0 kg per plant per harvest)[9]. There are a few recognized varieties of *J. curcas* in the world and their differentiation is based upon the shape and size of canopy, fruit and seed, or contents of phorbol esters and curcin in the seed. However, this classification has an element of arbitrariness. For example, three

varieties are frequently mentioned by researchers; the Cape Verde variety that has spread all over the world, the Nicaraguan variety with few but large fruits, and a non-toxic Mexican variety that has only a trace of seed phorbol esters[10–12]. Thus there is an urgent need to develop high seed- and/or oil-yielding varieties of physic nut by breeding technique, beginning from assessing genetic diversity among the local and introduced accessions. To gain maximum benefit from breeding efforts, identification of breeding lines with target traits in genetically polymorphic background is desirable. However, the success from conventional breeding technique is limited due to narrow genetic base of *J. curcas*. Using different molecular marker systems, many researchers have reported too low level of genetic diversity to improve the genotypes of *J. curcas*. The genetic diversity assessment on hybrid progenies at both intraspecific and interspecific levels should be carried out since the genus *Jatropha* comprises about 175 species. Agronomically important species of *Jatropha* are *J. curcas*, *J. integerrima*, *J. podagrica*, *J. multifida*, *J. gossypifolia*, *J. villosa*, *J. glendulifera*, and *J. maheshwarii*[13]. Sun et al[14] found that *J. integerrima* carries useful traits such as growth and seed traits. However, there has been no report utilizing dwarf character in *J. integerrima* to reduce canopy size and increase harvest index of the progenies. Interspecific hybridization was suggested as one of the most feasible approaches for physic nut improvement by transferring the desirable traits from one species to another. The most successful interspecific cross reported so far is between *J. curcas* and *J. integerrima*[15,16]. Many reports have been published on the physicochemical properties of different *J. curcas* accessions from different countries[6,17], with limited attention to chemical diversity as an indicator of genetic variation. Oil content and fatty acid composition in *J. curcas* samples collected from three regions of China and one region of India were determined[18]. Seed phorbol ester and curcin contents, together with genetic diversity in multiple provenances of *J. curcas* from Madagascar and Mexico were reported[7]. High oleic acid and total oil content are desirable traits for *J. curcas* breeding. Low phorbol ester content is appreciated as it would improve economic viability of *J. curcas* cultivation through the value of the seed cake. Variations in oil content can be generated by genetic as well as environmental factors[19,20] and the fatty acid composition could be altered to some extent through interspecific hybridization[21]. Up until now, only limited information is available on variation of these traits among the interspecific hybrids of *J. curcas*. The initial success in generating low phorbol ester interspecific BC_1 hybrids of *J. curcas* was achieved by Popluechai et al[22]. However, there has been no study on physicochemical diversity of interspecific F_2 hybrids of *J. curcas*. The current research therefore aims to investigate diversity of the F_2 progenies derived from *J. curcas* × *J. integerrima* based on yield and biochemical properties of seed and oil.

MATERIALS AND METHODS

Experimental sites

Field study was carried out at the experimental field of the Department of Agronomy, Kasetsart University, Kamphaeng Saen, Thailand; while biochemical analysis was conducted at Biomass for Energy Research Unit, CIRAD, Montpellier, France during November 2011 to August 2013. The experimental field is located at the latitude 14.01° N and longitude 99.58° E with the average temperature of 28.1 °C, relative humidity of 76%, and annual rainfall of 1328 mm. The soil type is sandy clay with pH ranging between 6.7 and 7.2 and EC of 0.6 mS/cm.

Plant materials

The plant materials were derived from the cross between a plant of *J. curcas* (Thai local cv "Chai Nat") as female parent and a plant of *J. integerrima* (Thai local dwarf ornamental type) as male parent. There were 9 F_1 plants obtained. One F_1 plant was self-pollinated to produce 296 F_2 plants used in this study. Three plants each of both parents, Chai Nat and dwarf *J. integerrima*, were also grown to collect seeds as checks in this experiment. All plants were sown from seeds in bags in September 2011 and transplanted to the field in November 2011. The spacing used was 1.5 m × 1 m, giving a crop density of 6667 plants per ha. Farm yard manure was incorporated at the rate of 100 g per plant prior to transplanting. A compound fertilizer (15-15-15: N-P_2O_5-K_2O) was given to each plant at 30 g per plant at the third month after transplanting. The experimental field was irrigated by a drip pipe system at the rate of 4 l/plant at monthly interval. Among 296 F_2 plants, 42 of them (∼14%) possessed desirable characters in canopy size, and seed size with sufficient seed yield for further analyses, and thus were marked for additional study.

Confirmation of true F_1 plants by simple-sequence repeat markers

Leaves of *J. curcas*, *J. integerrima*, and their F_1 plants were extracted to obtain DNA for PCR amplification following the method of Laosatit et al[23]. The PCR

products were separated by electrophoresis in 5% denaturing polyacrylamide gel to identify the simple-sequence repeat (SSR) marker loci detected in each plant.

Seed data collection

The fruits of the selected 42 F_2 plants were weekly harvested for 4 consecutive months from May to August 2012 beginning from 6 months after transplanting, or at the plant age of 8 months. The seeds were accumulated from 3 harvesting periods, i.e., May–June, June–July, and July–August. Each period was treated as a replicate in a completely randomized design (CRD). Seeds from each harvest were immediately oven-dried at 45 °C for 3 days before determining percentage of kernel weight (per seed weight) and the dry seed yield per plant using a laboratory digital balance. Seed samples were kept at an optimum temperature before further analysis.

Biochemical analysis

All biochemical analyses were all determined in duplicate to allow testing of the difference between F_2 plants. In each determination, the samples were prepared from ground seeds to estimate the experimental error used for testing the significance.

Oil extraction

Seed samples were uniformly dried in an oven at 105 °C for 12 h prior to biochemical analysis. This was done as a standard practice for partially-dried F_2 seeds to remove all the moisture inside the seeds. The seed samples were manually ground using mortar and pestle until the particle size was smaller than 1 mm. Then 2 g of ground seed sample was put in a 22 ml cell containing a cellulose filter and dispersed by mixing with sand (general purpose grade) in a sandwiching manner between the two layers of sand to prevent compaction in the extraction cell. Oil extraction was done by an accelerated solvent extractor (ASE 350, Dionex, USA). Each sample was extracted by petroleum ether solvent, using the ASE conditions of 1000 psi, 60 °C, 7 min heat up, 4 × 5 min static cycles, 100% flush, and 90 s purge. After extraction, the sample cells were left to cool down for 15 min before evaporating the solvent using a personal solvent evaporator (GeneVac, EZ-2 series, USA). The solvent-dried bottles were put in a desiccator to cool down for 30 min and weighed. The total oil content (TOC) was calculated as $(M_2 - M_1)/M_\mathrm{b}$, where M_1, M_2, and M_b are the weight of empty bottle, the weight of the bottle containing oil after drying the solvent, and the dry weight of the sample, respectively[24]. Oil yield per plant was also calculated based on their per unit seed weight. Oil yield per plant was the oil yield determined at the first season of harvesting.

Fatty acid composition analysis

Fatty acid analysis was performed using the method of Akbar et al[25] with some modifications. Four major fatty acids present in jatropha seed oil were determined using gas chromatography (GC, Agilent 6890 series, USA; equipped with flame ionization detector and CP-WAX 58-CB capillary column, 25 m length × 0.32 mm diameter × 0.2 µm film thickness). About 0.1 ml of sample oil was converted to methyl ester using 0.5 ml 2 N KOH in methyl alcohol, in 3 ml hexane. The sample mixture was vortexed for 30 s and allowed to settle down for 2 min before injection. Then 1 µl of the top layer containing methyl ester was injected into the GC. The detector and injector temperatures were 250 °C and 240 °C, respectively. Injection was performed in a split mode (20:1) at 240 °C. Column temperature was programmed from 180 °C for 5 min, and increased at 2 °C per minute until 220 °C. Helium was used as the carrier gas at a flow rate of 2.0 ml per minute. The run time was 25 min. The peaks of methyl esters of four major fatty acids (oleic, linoleic, palmitic, and stearic) were identified by comparing their retention times with those of oil standards analysed under the same conditions. Relative amount of fatty acid was calculated based on the peak area of an individual fatty acid to the total peak area of total oil profile. Ten random samples were reanalysed to evaluate the accuracy of the method.

Phorbol ester analysis

Phorbol ester (PE) content of seed oil was determined following the method of Makkar et al[3] with minor modification. Approximately 3 g of ground sample was subjected to ultrasonic wave (BioBlock Scientific 91631) for 5 min in the presence of about 40 ml of dichloromethane, then filtered to collect the filtrate. The extraction was done 4 times per sample, after which all four filtrates were pooled. The extracted solvent was dried under vacuum at 40 °C for about 30 min. The dried residue was dissolved in 5 ml of tetrahydrofuran, passed through a 0.45 µm nylon filter, and a sample of 20 µl was injected into the HPLC.

HPLC conditions to quantify phorbol esters

The high performance liquid chromatography (Kontron Instrument) consisted of a Kontron Instrument 525 pump, a Kontron Instrument 540 photo diode

array detector, and a Kontron Instrument 360 autosampler. The analytical column was a reverse phase C18 (ZORBAX Eclipse XDB-C18; Agilent, endcapped 5 µm) 250 mm × 4.6 mm i.d. protected by a guard column containing the same material as in the main column. Two solvents were used: (a) 350 µl of *o*-phosphoric acid (85%) in 1 l of acetonitrile and distilled water at 80:20 (v/v) ratio, and (b) acetonitrile. Both solvents were degassed by ultrasonication. Separation was performed at 25 °C, at the flow rate of 1 ml/min. Phorbol ester peaks were identified based on the retention time of standard phorbol ester, which appeared between 13 and 23 min. The peaks were integrated at 280 nm and the results were expressed as equivalent to phorbol-12-myristate 13-acetate (Sigma).

Statistical analysis

R program software (2.13.0 version)[26] was used for data analysis. Seed and oil characters of the selected F_2 plants were analysed for range, mean, standard deviation, coefficient of variation (CV), and correlation coefficient. To test the difference between F_2 plants for each trait, the data were analysed as a CRD with 3 replications for seed traits and with 2 replications for biochemical traits. Since the paternal parent *J. integerrima* did not set seeds in our study, only seeds of the maternal parent, *J. curcas* cv. Chai Nat was used as check in all evaluations.

RESULTS AND DISCUSSION

The hybridity of nine interspecific F_1 plants was clearly confirmed by SSR primers, i.e., the markers detected in all F_1 plants came from both parents (Fig. 1). Range, mean, standard deviation, and CV of seed and oil traits observed in the F_2 population are presented in Table 1. The F_2 plants were different in all characters indicating a wide variety of the sample.

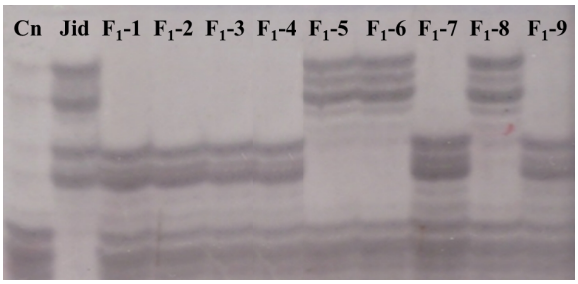

Fig. 1 SSR primers were used to confirm hybridity of 9 F_1 plants obtained from the cross *J. curcas* × *J. integerrima*. The figure shows the banding pattern of primer MPN 077.

Percentage of kernel weight and seed yield

Percentage of kernel weight to seed weight is a desirable trait for physic nut breeding programme. It reflects the oil yield as physic nut seed contains oil mainly in the kernel. Percentage of kernel weight among 42 F_2 plants varied from 52–81% with the average of 63% (Table 1). Distribution in percentage of kernel weight showed that about 69% of the population fell in the range from 61–70%. Other studies reported a range from 54–70% in wild and improved *J. curcas* accessions[3,6], while there was no such information on interspecific hybrids. Our F_2 population has two accessions possessing over 70% kernel weight. The highest percentage of kernel weight (81%) was observed in accession 198 followed by 73% in accession 291, indicating a higher variation in the F_2 population than the current *J. curcas* accessions.

Seed yield of 42 F_2 plants ranged from 15.5–

Table 1 Percentage of kernel weight, seed yield, seed oil yield, fatty acid content, and phorbol ester content of 42 F_2 plants derived from *J. curcas* × *J. integerrima* compared to the parental *J. curcas* cv "Chai Nat".

Trait*	Range	F_2 Plants		Chai Nat
		mean ± SD	CV (%)	mean ± SD
Percentage of kernel weight	52–81	63 ± 5	8.0	64 ± 1
Seed yield per plant (g)	15.5–270.2	86 ± 61	71.1	66 ± 10
Total oil content (%)	21.7–41.5	33.2 ± 4.6	13.9	37.72 ± 0.23
Oil yield per plant (ml)	4.0–110.6	34 ± 26	75.8	29.13 ± 0.16
Oleic acid (%)	19.3–48.6	38.1 ± 9.0	23.6	43.34 ± 0.54
Linoleic acid (%)	25.6–60.1	40.1 ± 9.9	24.6	32.52 ± 0.86
Palmitic acid (%)	6.8–15.1	11.4 ± 1.8	15.6	14.90 ± 0.25
Stearic acid (%)	4.7–10.9	7.1 ± 1.4	19.3	7.41 ± 0.30
Phorbol ester content (mg/g)	0.70–4.62	1.9 ± 1.0	53.3	0.93 ± 0.03

* The F_2 plants are significantly different in the respective traits by an F-test at 5% probability.

270.18 g per plant with an average of 86.44 g, while their *J. curcas* parent "Chai Nat" gave 66.2 g (Table 1). Among the F_2 plants, plants no. 5, 116, 133, and 191 produced seed yield higher than 200 g per plant from which accession 116 gave the highest seed yield (270.18 g). Parthiban et al[15] reported seed yield of superior interspecific BC_1F_1 clones from Indian *J. curcas* // *J. curcas* / *J. integerrima* ranged from 250–357 g per plant. The seed yield in our study was lower mainly because it was the first harvest season of the plantation and partly due to a decrease in seed size as the effect of their male parent, *J. integerrima*. To confirm the potential of the F_2 plants, seed yield data should be further collected and recorded.

Oil content and oil yield

Seed oil contents among F_2 plants were significantly different ranging from 21.7–41.5% with an average of 33.2% (Table 1). Accession 4 showed the highest seed oil content (41.5%), whereas Chai Nat had 37.7%. Basha and Sujatha[13] reported seed oil content of Indian local *J. integerrima* at 28.7%. About two thirds of our 42 F_2 plants had oil content ranging from 31–40%. Based on the earlier reports, seed oil contents of *J. curcas* accessions varied from 17.4% in Malaysian accessions[27], and 17.5% in Indian accessions[28], to 64.3% in Chinese accessions[29], and 64.5% in 72 accessions from 13 countries[17]. Oil contents of non-toxic Mexican and toxic Indian *J. curcas* were from 35.4–40.7%[30], showing less variation than those from interspecific hybrids. Parthiban et al[15] reported seed oil contents of 27 superior backcross interspecific hybrid clones to fall between 37.0 and 55.3%. While Popluechai et al[22] observed that seed oil contents of ten interspecific BC_1 hybrid plants varied from 16.4–34.5%. The current F_2 population exhibited a higher range of oil contents than that of the BC_1 hybrids, and thus showed a high potential for further genetic improvement for seed oil content.

Oil yield of 42 F_2 plants ranged from 4.0–110.6 ml per plant (Table 1). The high range and standard deviation of these traits indicated that selection for high oil yield in this F_2 population will result in good response. The highest oil yield per plant was found in accession 116 followed by accession 5, 133, and 191 as a result of their high seed yield per plant. Generally, physic nut plants require about 4–5 years to reach its seed production potential[9]. Thus it is necessary to continue collecting seed and oil yields until they reach their full production capacity. There should be however no change in the seed physicochemical properties in relation to age of the physic nut plants.

Fatty acid composition in oil

In physic nut, seed oil is composed of four major fatty acids, viz. palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2) (Table 2). Basha and Sujatha[13] were able to obtain a sufficient seed sample from their *J. integerrima* to determine the composition. While our *J. integerrima* set only empty fruits and seeds and thus we could not collect enough seed for oil and phorbol ester analyses. Likewise, the F_1 seeds were difficult to obtain and were not analysed in our experiment. Seed oil of the F_2 plants contained high level of unsaturated fatty acids. The most dominant unsaturated fatty acids were oleic and linoleic acids, amounted from 70.5% (accession 136) to 82.2% (accession 272) of the whole fatty acid profile (Table 2). Dominant saturated fatty acids in physic nut oil were palmitic and stearic acids. Four major fatty acids of seed oil varied among the F_2 plants. The contents of oleic acid ranged from 19.3–48.6%, linoleic acid from 25.6–60.1%, palmitic acid from 6.8–15.1%, and stearic acid from 4.7–10.9% (Table 1). Parental *J. curcas* (Chai Nat) oil contained 43.3% oleic acid, 32.5% linoleic acid, 14.9% palmitic

Table 2 Comparison of fatty acid composition of seed oil from *J. curcas* and *J. integerrima* from the literature versus F_2 plants derived from *J. curcas* × *J. integerrima* in this study.

Fatty acid	Composition (%)		
	Malaysian *J. curcas*[25]	Indian *J. integerrima*[13]	Current F_2 plants from *J. curcas* × *J. integerrima*[*]
oleic (18:1)	44.7	12.0	19.3–48.6
linoleic (18:2)	32.8	74.8	25.6–60.1
palmitic (16:0)	14.2	8.6	6.8–15.1
stearic (18:0)	7.0	4.4	4.7–10.9
others	1.3	0.2	0.8–1.7
saturated	21.2	13.0	14.7–25.0
unsaturated	77.5	86.7	70.5–82.2

[*] The F_2 plants are significantly different in the respective traits by an F-test at 5% probability.

Table 3 Fatty acid composition of four selected F_2 plants with desirable fatty acid content.

Plant no.	Oleic (%)	Linoleic (%)	Palmitic (%)	Stearic (%)	Unsaturated fatty acid (oleic + linoleic) (%)
5	48.3	28.9	12.9	7.4	77.2
198	46.9	25.6	14.6	10.4	72.5
201	48.6	31.1	11.8	6.7	79.7
272	34.8	47.3	6.8	8.8	82.2

Table 4 Correlations among physicochemical characters of seed and oil, and phorbol ester content of 42 F_2 plants generated from *J. curcas* × *J. integerrima*.

	PKW	SYP (g)	TOC (%)	OYP (g)	OL (%)	LNL (%)	PM (%)	ST (%)
SYP (g)	0.15							
TOC (%)	0.44**	0.24						
OYP (g)	0.19	0.99**	0.35*					
OL (%)	0.06	0.01	0.14	0.02				
LNL (%)	−0.13	−0.09	−0.18	−0.11	−0.97**			
PM (%)	0.26	0.16	0.17	0.17	0.19	−0.32*		
ST (%)	0.20	0.23	0.03	0.23	0.36*	−0.38*	−0.14	
PE (mg/g)	0.36*	0.28	0.37*	0.30*	−0.27	0.26	−0.13	0.05

PKW = percentage of kernel weight; SYP = seed yield per plant; TOC = total oil content; OYP = oil yield per plant; OL = oleic acid; LNL = linoleic acid; PM = palmitic acid; ST = stearic acid; PE = phorbol esters.
* significant at 5% probability ($df = 40$). ** significant at 1% probability ($df = 40$).

acid, and 7.4% stearic acid. Other published reports on fatty acid profile of *J. curcas* accessions and back-cross interspecific hybrids of *J. curcas // J. curcas / J. integerrima* indicated a range from 24.9–53.0% in oleic acid, 25.7–53.3% in linoleic acid, 4.2–18.9% in palmitic acid, and 2.3–9.2% in stearic acid[22,31]. Results of the current study were in accordance with their results. The corresponding fatty acid composition of *J. integerrima* was reported by Basha and Sujatha[13] at 12.0%, 74.8%, 8.6%, and 4.4% for oleic, linoleic, palmitic and stearic acids, respectively. Thus our F_2 progenies showed a wider variation in fatty acid compositions with desirable combination of the two unsaturated fatty acids. Higher poly-unsaturated fatty acid (linoleic acid) can negatively impact the oxidative stability of the derived fuel and cause high rate of nitrogen emission. For biodiesel production, the physic nut accessions possessing high mono-unsaturated fatty acid (oleic acid) and low poly-unsaturated fatty acid (linoleic acid) contents are desirable. Based on our results, plant no. 198 and 201 are desirable for their low linoleic and high oleic acid contents, respectively (Table 3). Plant no. 198 also possesses the highest percentage of kernel weight (81%).

Phorbol ester concentration

PE concentration in seeds of 42 F_2 plants varied ranging from 0.7–4.62 mg/g, with an average of 1.88 mg/g (Table 1). The F_2 population showed a wide range of PEs in the category of low to high toxic genotypes. The lowest PEs (0.7 mg/g) was observed in accession 231, whereas their maternal parent (Chai Nat) had 0.93 mg/g. Popluechai et al[22] reported that the PE contents in interspecific BC_1 hybrid seeds (*J. curcas* × *J. integerrima*) ranged from 0.04–9.04 mg/g, and that in *J. integerrima* kernels was as high as 7.92 mg/g. As compared to their results, the highest PE concentration was lower in our F_2 population than in their BC_1 progenies.

Correlation analysis

Seed yield of the F_2 plants had a positive correlation with oil yield per plant ($r = 0.99$) (Table 4). There were no strong relationships between oil content with other traits, except a low correlation ($r = 0.44$) with percentage of kernel weight. For oil composition, a highly negative correlation was observed between the two major unsaturated fatty acids; oleic and linoleic acids ($r = -0.97$). Our finding agrees with the report of Liu et al[21] implying that there could be common genetic factors affecting these two compositions. Although a high level of oleic acid suppresses the level of linoleic acid, developing high oleic genotypes is desirable for physic nut oil as a raw material for biodiesel. There was no high correlation between PE content and any other characters of seed and oil. A weak positive correlation was observed between PE content and the

percentage of kernel weight ($r = 0.36$), and total oil content ($r = 0.37$). It can be concluded that within the range of oil content under the present study, seed yield per plant is the most important contributing trait in physic nut to achieve high oil yield per plant, which can subsequently increase oil yield per ha. If the other yield components such as number of inflorescences per plant, number of female flowers per inflorescence, number of fruits per bunch, fruit size, and number of seeds per fruit are favourable, percentage of kernel weight is assumed to be the second most important character to ensure high seed and oil yield in physic nut.

Since there was no plant carrying all desirable characters from this pre-breeding work, the F_2 population still needs further improvement through hybridization and selection. Superior plants from the current study will provide a new gene source useful for physic nut improvement programs with particular objective to improve physicochemical characteristics of seed and oil feedstock.

Conclusions

F_2 progenies developed from *J. curcas* × *J. integerrima* showed a high diversity in seed characters, seed yield, oil yield, oil quality, and PE content. The progenies are smaller in plant size which should ease harvesting. Plants no. 5, 116, 133, and 191 are good genotypes for seed yield per plant, plant no. 4 and 262 for total oil content, and plant no. 198 and 201 for high oleic and low linoleic acid. Plant no. 198 can also be considered the best in seed filling giving the highest kernel percentage. Plant no. 5 possesses short plant height (135 cm), small canopy width (150 cm), high seed yield (237.14 g/plant), high oil content (37.9%), and high amount of unsaturated fatty acids (77.2%) being high in oleic (48.3%) and low in linoleic acid (28.9%). These traits are all important for biodiesel production from physic nut oil feedstock.

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