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ABSTRACT

Jatropha (*Jatropha curcas* Linn.) is an oil-bearing species with multiple uses and considerable potential as a bioenergy crop. The present investigation has been undertaken to assess the variability in seed traits, moisture percentage and oil content of 25 accessions of *Jatropha curcas* collected from different agroclimatic zones of North-East India. There were significant differences ($P < 0.05$) in seed size, 100 seed weight and oil content among the accessions. The maximum seed weight was recorded in J-22 collected from Baramura, Tripura and the minimum in J-13 collected from Roing, Arunachal Pradesh. Oil variability ranged from 25% (J-20, collected from Mokochung, Nagaland) to 36% (J-18, collected from Mamit, Mizoram). The moisture percentage in the sun-dried seeds varied from 6.06% (J-5, collected from Lambding, Assam) to 11.32% (J-6, collected from Lakhimpur, Assam). When the oil percentage of seeds is correlated with the sun-dried seed moisture percentage and 100 seed weight, it has been found that the oil yield is negatively correlated with the seed moisture. On the other hand, the seed weight has strong positive correlation with the oil yield. The toxic phorbol ester content of *Jatropha curcas* seeds cultivated in North East Institute of Science & Technology, Jorhat, Assam in non-defatted and defatted whole and kernel seed meals were also assessed. Detoxifications of phorbol esters from *Jatropha curcas* by chemical treatments were performed. The effects of the chemical treatments were successful in detoxification of phorbol esters content.

Key words: *Jatropha curcas*, Oil percentage, Moisture percentage, Phorbol esters, Detoxification

The world reserve of primary energy and raw materials are limited and according to an estimate, the reserves will last for 218 years for coal, 41 years for oil and 63 years for natural gas [5], [8], [15]. The present energy scenario has stimulated active research interest in non-petroleum renewable and non-polluting fuels. *Jatropha curcas* L. also known as Physic nut belongs to the family Euphorbiaceae has been identified as a potential biodiesel crop because of the presence of 40-50% oil [9]. It is a native of Mexico and Central American region [11]. In India it is introduced by the Portuguese in 16th Century [2]. *Jatropha curcas* is a multipurpose species with many attributes and considerable potential. The oil from the seed is the most valuable end product. Except being an oil yielding plant, it is a rich source of herbal drugs also. In nature wide range of variability exists for various qualitative and quantitative characters of *Jatropha curcas*. The availability of variability for

a given crop is the basic prerequisite in improvement programme and correlations between different characters provide a realistic basis for deciding upon a suitable selection criterion [20]. So far as *J. curcas* is concerned, little work has been done on germplasm collection and evaluation of oil content of seeds. The potential major constraint in the wide spread acceptance of *Jatropha* as a source of biodiesel could be the presence of phorbol esters, which, when consumed by man and animal, are toxic and are also co-carcinogens [7]. In man, hazard of conditional carcinogenesis may be associated with exposures to plants of the Euphorbiaceae containing diterpene ester type tumour promoters. This makes the oil unsuitable for food and feed applications. The phorbol ester and their different derivatives are also reported to induce a remarkable diversity of other biological effects at exceptionally low concentration. These are responsible for skin irritant effects and tumor promotion because they stimulate protein kinase C (PKC), which is involved in signal transduction and developmental processes of most of the cells and tissues, producing a variety of biological effects in a wide range of organisms [7]. The biological effects of these compounds in addition to tumour promotion, bring about a wide range of biochemical and cellular effects, alter cell morphology, serve as lymphocyte mitogens and induce platelet aggregation [3]. The whole seed as well as dehulled seed meal are reported to be highly toxic in animal studies [1]. In spite of the food-related uses of the *Jatropha*

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curcas, information on their toxic components (phorbol esters) in *Jatropha curcas* seeds are scanty or non-existent in the literature. In the present study 25 different accessions of *Jatropha curcas* collected from seven North-Eastern states of India were screened and evaluated. The objective of the study was to understand the pattern of variation existing in different population of *J. curcas* in respect of seed morphology and the effect of some chemical treatments on detoxification of phorbol esters of *Jatropha curcas* seeds. Such an investigation may help in early evaluation of criteria for selection of some prominent traits both in the laboratory and nursery conditions, which may be related to subsequent performance in the field. Also, it will lead to the development of economically viable techniques for detoxification of *Jatropha* meal to check the efficacy in animal model.

MATERIALS AND METHODS

Twenty-five *Jatropha curcas* accessions are collected from different parts of North-East India (Table 1). The experiment is laid out in a randomized block design (RBD) with

three replications in the experimental fields of North East Institute of Science and Technology, Jorhat, Assam with a spacing of 2.5m × 2.5m. Fully matured capsules are collected from the 2-3 years old plants. The seeds are manually separated from the capsules and shade dried for 30 days till the seeds become completely dry. Three samples were drawn from each seed lot and 100 random undamaged seeds (total 300 seeds) were measured for their length, breadth and thickness. To determine the moisture content of shade dried seeds, 3 samples each again containing 100 seeds from each accession has been randomly selected and kept in Hot Air Oven at a constant temperature of 70°C for overnight until the seed weight becomes constant. The oil content of the seeds was estimated by Soxhlet method using three replicates for each accession. The chemicals and reagents used for the detoxification of phorbol esters of *Jatropha curcas* seeds were purchased from HiMedia Laboratories Pvt. Limited, Mumbai, India. The used water was distilled using water distillation apparatus. Standard Phorbol ester (phorbol-12-myristate-13-acetate) was also purchased from Hi Media Laboratories Pvt. Limited, Mumbai, India.

Table 1 Passport data of the accessions collected

S. No.	Code	Place of collection and state	Latitude	Longitude	Annual rainfall (mm)	Temperature (°C)	
						Minimum	Maximum
1	J1	Jorhat, Assam	26° 73" N	94° 01" E	2052	10.8	31.9
2	J2	Bongaigaon, Assam	26° 55" N	90° 58" E	1614	12.9	31.7
3	J3	Golaghat, Assam	26° 45" N	97° 30" E	2072	10.8	31.9
4	J4	Tezpur, Assam	26° 60" N	92° 78" E	1563	11.0	31.2
5	J5	Lambding, Assam	25° 86" N	92° 59" E	1854	9.8	31.0
6	J6	Lakhimpur, Assam	27° 65" N	96° 25" E	2635	8.0	31.5
7	J7	Nagaon, Assam	26° 21" N	92° 41" E	1745	10.0	35.0
8	J8	Sibsagar, Assam	26° 15" N	95° 25" E	2142	15.0	28.0
9	J9	Goalpara, Assam	25° 54" N	91° 05" E	2424	7.0	33.0
10	J10	Nalbari, Assam	26° 25" N	91° 26" E	1500	10.0	36.0
11	J11	Dergaon, Assam	26° 41" N	93° 58" E	2050	10.0	35.0
12	J12	Naharlagun, Arunachal Pradesh	24° 44" N	93° 65" E	2688	8.0	32.0
13	J13	Roing, Arunachal Pradesh	28° 05" N	95° 89" E	2800	5.0	29.0
14	J14	Lamphelpat, Manipur	24° 44" N	93° 65" E	2027	8.0	34.0
15	J15	Senapati, Manipur	24° 20" N	93° 42" E	2593	3.0	32.0
16	J16	Ccpur, Manipur	24° 00" N	93.15" E	2745	0.0	41.0
17	J17	Kolasib, Mizoram	23° 44" N	92° 47" E	2800	11.0	30.0
18	J18	Mamit, Mizoram	23° 44" N	92° 48" E	3000	12.0	30.0
19	J19	Mon, Nagaland	26° 78" N	94° 77" E	1644	13.3	24.8
20	J20	Mokokchung, Nagaland	26° 44" N	94° 65" E	2330	9.0	25.0
21	J21	Kamalasagar, Tripura	23° 82" N	91° 65" E	2800	12.5	33.0
22	J22	Baramura, Tripura	23° 63" N	91° 25" E	2700	10.1	34.3
23	J23	Watrigithim, Meghalaya	25° 98" N	90° 68" E	3350	10.0	37.0
24	J24	Tura, Meghalaya	25° 31" N	90° 13" E	2600	7.0	30.0
25	J25	Borapani, Meghalaya	25° 39" N	91° 54" E	2138	<0.0	28.0

The harvested seeds were cleaned and the whole and kernel seeds were grounded using a mechanical grinder. The whole seed meal and kernel seed meal were divided into two portions. One portion of the whole and kernel seed meal were not defatted whereas the other portion were defatted in Soxhlet apparatus using petroleum ether (boiling point of 40–60°C) for 16 hours. The defatted whole and kernel seed meal was air dried at room temperature and stored in a separate plastic container at 4°C until required for further analysis. The powdered whole and kernel seed meal (non-defatted and defatted) was divided into five equal parts of 500 g each. The first part was treated as control, in which the samples were not treated with any chemicals. The second part was treated with distilled water in the ratio of 1:5 (w/v) and immediately autoclaved at 121°C for

25 min. The samples were then dried in hot air oven at 40°C. The third part was treated with 0.07% NaHCO₃ solution in the ratio of 1:5 (w/v) and immediately autoclaved at 121°C for 25 min. The samples were then dried in hot air oven at 40°C. Similarly, the fourth part was treated with 90% ethanol for 2 hours at room temperature (25 ± 2°C) with constant stirring; the sample to solvent ratio was 1:10 (w/v). The solvent was removed by filtration and the residue was dried in hot air oven at 40°C. In the fifth part, after treatment similar to the fourth, was air-dried, mixed with 0.07% NaHCO₃ solution in the ratio of 1:5 (w/v) and subjected to autoclaving at 121°C for 25 min and the residual was dried in hot air oven at 40°C. Statistical analysis was done according to the standard procedure [19].

RESULTS AND DISCUSSION

Significant differences ($P < 0.05$) occurred among the accessions for seed size (Table 2). Maximum seed length has been observed in J18 (19.10 mm) followed by J22 (18.75 mm) and lowest in J8 (16.15 mm). The low-ranking accessions varied significantly from the rest of the accessions. Seed breadth varies from 10.00 mm to 11.70 mm with maximum in J20 and minimum in J21. On the other hand, seed thickness varies from 8.00 mm in J13 to 8.90 mm in J3, J5, J14 and J15. Though the seeds were dried under similar condition they showed significant variation in their moisture content. Highest moisture percentage (Table 2) is found in J6 with 11.32% which is closely followed by J19 with 11.11% and lowest in J5 with 6.06%. The accessions also showed significant variability for 100 seed weight and oil content (Table 2). For 100 seed weight the top-ranking accession is J22 (69.00 gm) followed by J18 (68.50 gm). The top-ranking accessions differed significantly from the rest. There is also significant variability in oil content that varies from 25% in J20 to 36% in J18. The magnitude of

simple correlation coefficient among the six characters has been presented (Table 3). The seed length is positively correlated with the oil percentage and the sun-dried seed moisture shows negative correlation with the oil percentage. The 100 seed weight shows strong positive correlation with the oil content of the seeds. Variation is the phenomenon where individuals of a population differ from each other. The extent of variation in seed weight and oil content in the whole seed and kernel is large as compared to other traits. The consideration of seed weight in selecting and understanding the geographical variation has been advocated because of the least plasticity in this character [12]. The study of seed morphological characters of a natural population can be considered as a useful step in the study of the genetic variability. Various ecotypes/provinces/seed sources of *Jatropha curcas* exhibit variation in seed morphological traits [6], [14], [16]. Therefore, this kind of study can help to identify the better genotypes of *Jatropha curcas* having better yield and oil content and the best genotypes selected will improve energy plantations in the wastelands.

Table 2 Seed size, moisture and oil content variability table

S. No.	Accession Code	Seed length (mm)	Seed breadth (mm)	Seed thickness (mm)	Moisture %	100 seed weight (gm)	Oil %
		Mean±s.d.	Mean±s.d.	Mean±s.d.	Mean±s.d.	Mean±s.d.	Mean±s.d.
1.	J1	17.75±1.30	11.00±1.00	8.65±1.26	8.19±0.13	61.00±1.05	34.00±1.00
2.	J2	17.00±1.05	11.10±0.79	8.05±0.51	7.69±2.66	45.85±0.26	30.00±1.32
3.	J3	17.55±0.33	10.95±0.18	8.90±0.36	6.25±0.39	48.00±1.32	28.00±0.00
4.	J4	17.55±0.55	11.10±0.96	8.85±0.10	7.14±0.14	56.00±1.32	31.00±1.00
5.	J5	18.30±0.34	10.70±0.26	8.90±0.13	6.06±0.13	66.00±2.29	35.00±0.50
6.	J6	18.30±0.26	10.45±0.43	8.10±0.53	11.32±0.03	53.00±3.97	28.00±0.50
7.	J7	17.60±0.36	11.30±0.26	8.50±0.23	7.58±0.46	59.30±1.13	30.00±1.00
8.	J8	16.15±1.43	11.25±0.66	8.70±0.33	7.27±0.12	55.00±2.64	27.00±1.00
9.	J9	17.92±1.28	11.25±0.25	8.57±0.05	7.90±0.13	63.00±3.05	30.02±1.03
10.	J10	17.55±0.51	11.00±0.25	8.72±0.20	9.67±0.29	62.00±2.78	31.00±1.00
11.	J11	17.40±0.53	10.60±0.36	8.55±0.43	6.89±0.13	58.00±3.50	28.33±1.15
12.	J12	18.60±0.22	10.45±0.23	8.40±0.53	7.60±0.61	46.33±2.97	33.50±0.50
13.	J13	18.20±0.20	11.00±0.86	8.00±0.50	6.78±0.19	40.43±2.20	29.00±1.73
14.	J14	18.10±0.26	11.20±0.44	8.90±0.15	8.06±0.36	62.00±1.82	31.60±1.44
15.	J15	18.20±0.09	11.05±0.51	8.90±0.41	6.89±0.33	58.00±1.00	30.00±1.00
16.	J16	18.35±0.65	11.05±0.91	8.70±2.06	8.95±0.42	67.00±2.60	33.50±0.50
17.	J17	17.92±0.15	11.25±0.25	8.57±0.16	7.90±0.10	63.00±3.77	35.00±0.86
18.	J18	19.10±1.74	10.80±0.18	8.72±0.25	8.30±0.34	68.50±2.78	36.00±1.32
19.	J19	18.42±0.53	10.47±0.79	8.42±0.52	11.11±0.97	45.00±2.78	26.00±1.00
20.	J20	17.80±0.46	11.70±0.70	8.60±0.96	7.69±0.54	52.00±3.04	25.00±0.50
21.	J21	17.50±0.50	10.00±0.66	8.10±0.79	8.62±0.55	46.99±1.72	29.00±1.00
22.	J22	18.75±0.43	10.42±0.62	8.40±0.54	8.69±0.97	69.00±3.12	33.00±0.50
23.	J23	18.20±0.18	10.50±0.28	8.60±0.36	7.46±0.14	65.14±2.73	35.00±1.00
24.	J24	18.05±0.18	10.95±0.40	8.30±0.26	8.47±0.75	59.00±1.32	30.00±2.64
25.	J25	17.30±0.26	11.10±0.34	8.87±0.12	8.82±0.28	68.00±2.64	31.00±1.00
	SEM	0.57	0.46	0.52	0.56	2.04	0.90
	CV (%)	0.03	0.03	0.03	0.16	0.14	0.10
	CD (at 5%)	1.12	NS	NS	1.10	4.00	1.76

Table 3 Simple correlation matrix

	Seed length	Seed breadth	Seed thickness	Moisture %	100 seed weight	Oil %
Seed length	1.000					
Seed breadth	-0.357	1.000				
Seed thickness	-0.048	0.381	1.000			
Moisture %	0.231	-0.301	-0.328	1.000		
100 seed weight	0.225	0.142	0.566	0.017	1.000	
Oil %	0.465	-0.160	0.263	-0.165	0.633	1.000

Phorbol esters content in whole and kernel of *Jatropha curcas* seeds are shown in (Table 4). Data indicate that whole and kernel seeds contained 26.12 and 46.01 mg/kg phorbol

esters, respectively in raw (non-defatted) seed. With meal (defatted seed) these contents were 23.25 and 42.33 mg/kg. Highly significant differences were ($P < 0.05$) observed in the

contents of phorbol esters in kernel seeds than those whole seeds. Similar results were also reported by Chivandi *et al.* [4], that phorbol esters (PEs) were present in high concentrations in raw *Jatropha curcas* kernels. Data in (Tables 5-6) indicate the effect of various chemical treatments on detoxification of phorbol esters of *Jatropha curcas* seeds. The results given in (Table 5) showed that, phorbol esters content of non-defatted whole and kernel *Jatropha* seeds were 25.77 and 45.13 mg/kg, respectively. It could be noticed that the phorbol esters levels is slightly affected by the treatment (Autoclave treatment at

121°C for 25 min) in whole and kernel of non-defatted seeds compared with untreated sample. Autoclave treatment at 121°C for 25 min showed that, phorbol esters content of defatted whole *Jatropha* and kernel seeds were 22.69 and 41.38 mg/kg respectively (Table 6). It could be noticed that the phorbol esters level is not affected significantly by the treatment in kernel seeds. These resulted are in agreement with those reported by [17-18] who reported that it is not possible to destroy phorbol esters by heat treatment because they are heat stable.

Table 4 Phorbol esters content in non-defatted and defatted *Jatropha curcas* seeds

Samples	Phorbol esters content (mg/kg dry weight basis)	
	Whole seeds	Kernel seeds
Non-defatted	26.12 ± 1.86	46.01 ± 2.16
Defatted	23.25 ± 0.01	42.33 ± 0.02

All values are means of triplicate determinations ± standard deviation

Table 5 Effect of chemical treatments of non-defatted *Jatropha curcas* on the detoxification of phorbol esters (on dry weight basis)

Treatments	Non-defatted whole seeds		Non-defatted kernel seeds	
	Content (mg/ kg)	Detoxification %	Content (mg/kg)	Detoxification %
Control (untreated)	26.12± 1.86	-	46.01±2.16	-
Autoclave at 121°C / 25 min.	25.77± 0.09	1.34	45.13±2.15	1.91
0.07 % NaHCO ₃ / 121°C / 25 min.	6.15±0.02	76.45	9.77±1.01	78.77
90 % ethanol /2 h	1.57±0.01	93.99	1.78±0.01	96.13
90 % ethanol /2 h followed by 0.07% NaHCO ₃ /121°C/25 min.	1.31±0.01	94.98	1.75±0.01	96.20
S.D. at 5%	1.04	-	1.09	-

All values are means of triplicate determinations ± standard deviation

Table 6 Effect of chemical treatments of defatted *Jatropha curcas* seeds on the detoxification of phorbol esters (on dry weight basis)

Treatments	Defatted whole seeds		Defatted kernel seeds	
	Content (mg/ kg)	Detoxification %	Content (mg/kg)	Detoxification %
Control (untreated)	23.25 ± 0.01	-	42.33 ± 0.02	-
Autoclave 121°C / 25 min.	22.69 ± 0.96	2.41	41.38 ± 0.93	2.24
0.07 % NaHCO ₃ / 121°C / 25 min.	4.92 ± 0.92	78.84	8.55 ± 0.95	79.80
90 % ethanol /2 h	1.34 ± 0.99	94.24	1.39 ± 0.99	96.72
90 % ethanol /2 h followed by 0.07% NaHCO ₃ /121°C/25 min.	1.21 ± 0.01	94.80	1.31 ± 0.91	96.91
S.D. at 5%	1.06	-	1.11	-

All values are means of triplicate determinations ± standard deviation (SD)

The results given in (Table 5) also showed the effect of NaHCO₃ treatment followed by autoclaving at 121°C for 25 min on detoxification of phorbol esters content on non-defatted *Jatropha* seed. The results indicate that, phorbol esters content were 6.15 and 9.77 mg/kg in non-defatted whole and kernel *Jatropha* seed, respectively. This treatment resulted in detoxification of the phorbol esters content by 76.45% and 78.77% of whole and kernel seeds, respectively. On the other hand, the effect of this treatment on the phorbol esters levels were 4.92 and 8.55 mg/kg in defatted whole and kernel seeds, respectively (Table 6). This treatment resulted in detoxification of the phorbol esters content by 78.84% and 79.80% of defatted whole and kernel *Jatropha* seed, respectively. These results are agreement with those reported by Herrera *et al.* [13], who reported that NaHCO₃ treatment decreased the phorbol esters content by 75.3%. The effects of (ethanol 90% for 2 hrs) treatment on detoxification the phorbol esters content in non-defatted whole and kernel seeds are presented in (Table 5). The results showed that the phorbol esters levels were 1.57 and 1.78 mg/kg of non-defatted whole and kernel seeds, respectively. The treatment (ethanol 90% for 2 hrs) was successful in detoxification the phorbol esters content by 93.99% and

96.13% of non-defatted whole and kernel seeds, respectively. On the other hand, the results given in (Table 6) showed that the phorbol esters content were 1.34 and 1.39 mg/kg of defatted whole and kernel seeds, respectively. The treatment (ethanol 90% for 2 hrs) was successful in detoxification the phorbol esters content by 94.24% and 96.72% of defatted whole and kernel seeds, respectively. Highly significant differences were observed with this treatment compared with the untreated samples. Phorbol esters are highly soluble in ethanol and that giving some possibility of detoxification of the meal [17]. The effect of the treatment (ethanol 90% + NaHCO₃/121°C/25 min) showed that, phorbol esters content of non-defatted whole and kernel *Jatropha* seeds were 1.31 and 1.75 mg/kg (Table 5), respectively compared with control. In general, from these results, it could be noticed that, the amount of detoxification of phorbol esters content were 94.98% and 96.20% of non-defatted whole and kernel *Jatropha* seed, respectively. This treatment was successful in detoxification of phorbol esters in the *Jatropha* kernel seeds to a maximum extend. Data showed highly significant differences between the treatment samples and the untreated samples (control). Regarding to, the effects of (ethanol 90% + NaHCO₃/121° C/25 min) treatments on

detoxification of phorbol esters in defatted seeds (Table 6), the results showed that, phorbol esters content were 1.21 and 1.31 mg/kg, defatted whole and kernel *Jatropha* seed, respectively compared with control. These results indicate that the detoxification of phorbol esters content were 94.80% and 96.91% of defatted whole and kernel *Jatropha* seeds respectively. This treatment was successful also in maximum detoxification of phorbol esters in the *Jatropha* kernel seeds. Statistical analysis indicated highly significant differences effects of this treatment on phorbol esters contents. Makkar and Becker [17-18] reported that heat alone cannot inactivate phorbol esters. However, an additional chemical treatment has been reported to decrease the phorbol esters level in *Jatropha* seeds to 75% [10].

CONCLUSION

The present study shows that there exists considerable amount of genetic variability in this species in North-East India with respect to seed traits and oil content. On the basis of our results, eventually, it may be concluded that the Mamit, Mizoram source is superior among all. Therefore, it is advisable that this seed source should be used for collection of bulk quantity of seeds to achieve better productivity. For handling of novel crops of this kind and their products, in terms of preventive toxicology, measures of protection and safety may be implemented. Due to the toxicity of the oil, special

precautions might need to be exercised during the processing of *Jatropha curcas* seeds and oils. The methods, described in this study, for detoxification of phorbol esters of *Jatropha curcas* seeds can play a very important role in this direction, so far as use in analytical chemical studies are concerned. The detoxification of phorbol ester using 90% ethanol/ 2 hrs followed by 0.07% NaHCO₃/ 121°C/ 25 min. has been found to be the best method to decrease the phorbol ester level in *Jatropha curcas* whole and kernel seed meal. The detoxified meal can be investigated using rat and fish as experimental models. Should it be found innocuous in the feeding studies, the seed meal would find application as livestock feed. In the long run, by selective breeding or by gene technological means, plant varieties may be generated from wild type species which are low in, or entirely free from, toxins. However, before a vision of this kind becomes reality, a considerable input of basic agricultural research guided by toxicological and analytical know-how will have to be invested.

Conflict of interest

There is no conflict of interest.

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