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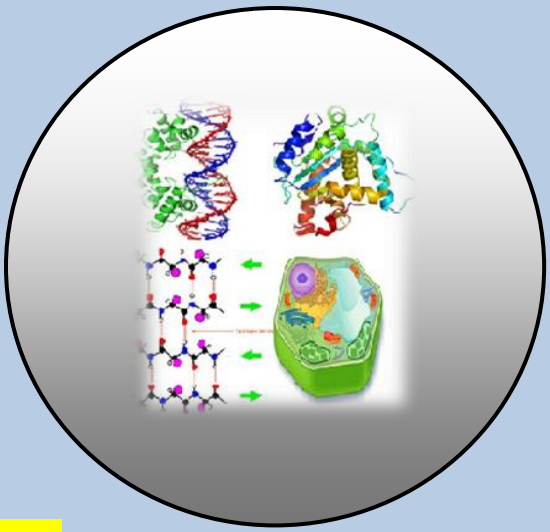

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Comparative Study on the Metabolic Constituents and Genetic Profile of *Jatropha glauca* and *Jatropha curcas*

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ABSTRACT

Jatropha has received much attention as a potential renewable feedstock for producing biodiesel and biofuel that may replace petroleum in many developed and developing countries. The aim of this study was to compare the biochemical constituents as well as the genetic profiles of the cultivated and domestic jatropha (J. curcas and J. glauca) plants. Seeds' extracts of Jatropha curcas and J. glauca were screened for their accumulated metabolic compounds. The genetic differences between the two species were assessed using RAPD markers. Results indicated that J. glauca has more sugar, amino acids and saponin contents than J. curcas while the later has more protein, flavonoids, steroids, terpenoid and resins contents. No reducing sugars, starch, phenolic compounds or tannins were detected in the two species and there was no variation in the amount of the detected alkaloids. Out of the 40 amplified RAPD primers, 30 produced 175 bands of which 141 were polymorphic. The number of bands generated per primer varied from 1 (OPC17, OPH4, OPK17, OPR6 and OPY15) to 10 (OPR15) and the size of the amplified products varied from 100 to 1700 bp. The genetic similarity between the two species was 19.5%. Results also indicate that 12 RAPD primers have shown amplification products with J. curcas and never with J. glauca. On the other hand, 9 primers amplified DNA of J. glauca only. These observations therefore support the use of both J. curcas and J. glauca in herbal cure remedies. Future studies could be conducted to identify the generated specific amplification products in order to develop specific primers for the identification of these species.

Keywords: Jatropha species, Metabolites, Diversity, Markers, Saponin Content and RAPD.

INTRODUCTION

The genus *Jatropha* belongs to the family Euphorbiaceae and comprises around 175 species which are distributed mainly in tropical and subtropical regions of America, Africa and Asia (Trivedi et al., 2009; Dias et al., 2012). The genus name *Jatropha* derives from the Greek word jatros (doctor) and trophe (food), which implies its medicinal uses (Dehgan and Webster, 1979; Schultze-Motel and Mansfields, 1986). The plant has a high potential for greening and rehabilitation of wastelands and the seeds have a high oil concentration with excellent quality for conversion into biodiesel (Francis et al., 2005). Several *Jatropha* species are cultivated for their ornamental leaves and flowers, while some are grown in the tropics for their economic uses. *J. curcas* is a semi-evergreen shrub or small tree reaching a height of 20 ft. It can survive arid conditions; therefore, can be grown on drylands and wastelands. The seeds of this plant are highly toxic but produce oil that can be used as biodiesel after transesterification, besides that, in soap and candle making (Jain et al., 2014). It was introduced to Sudan during the last century for commercial production of oilseeds. *J. glauca* occurs in Sudan, Eritrea, Ethiopia, Djibouti and Somalia, and extends to Yemen and Saudi Arabia. It is used in traditional medicine to treat constipation, earache and as an astringent (Schmelzer, 2007). The fact that *Jatropha* has adapted itself to a wide range of ecological conditions suggests that there exist considerable amount of genetic variability to be exploited for potential realization (Rao et al., 2008). Environmental factors in combination with genetic and physiological factors play an important role in determination of plant potential for seed quality. These characters appear to be under strong genetic control (Roy et al., 2004). The key for success of any breeding program lies in the availability of genetic variability for desired traits (Heller, 1996). Genetic resource through global exploration, introduction, characterization and evaluation will provide strong base for development of elite varieties by various improvement methods. The existing information regarding chemical constituents screening and the extent and pattern of genetic variation in *J. curcas* population is quite limited. Investigations on the phytochemical screening of *J.curcas* stem bark and leaf extracts revealed the presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids (Uche and Aprioku, 2008; Igbinosa et al., 2009; Namuli et al., 2011; Gupta et al., 2003). Common molecular markers have been used to assess the genetic diversity of *J. curcas* (Singh et al., 2010). However, there is no any documented report on the metabolites screening or the use of molecular markers to study the genetic variation in *J. glauca*. Therefore, the aim of this study was to compare the chemical constituents and the genetic profile of the cultivated *J. curcas* and the wild *J. glauca* in Sudan.

MATERIAL AND METHODS

Plant materials

Seeds of cultivated *Jatropha curcas* were collected from ALrwakeep Research Station, National Center for Research, Sudan, while seeds of the indigenous *Jatropha glauca* were collected from Sinkat, Red Sea State, Sudan where it grows as a wild plant. The seeds for both species were identified at the Department of Biology and Biotechnology, Faculty of Science and Technology, AL Neelain University.

Screening for chemical composition

Preparation of seeds crude extracts

Dry seeds of jatropha (*J. curcas* and *J. glauca*) were thoroughly washed using tap water and rinsed with distilled water.

The seeds were dried at room temperature and then pulverized to a fine powder with the aid of a mortar and pestle. The extract was prepared by weighing out 20 g of the milled powdered seeds and soak in 100 ml of 60% conc. Methanol in a conical flask. The contents were stirred vigorously with a glass rod for proper extraction. The extract was then filtered using Whatman No.1 filter paper. The filtrate was air dried at 28°C., reconstituted in 20 % Dimethylsulphoxide (DMSO) solution and then kept at 4°C prior to determination of metabolites.

Qualitative estimation of biochemical compounds

Total proteins, amino acids, total sugars, reducing sugars and starch contents were determined according to the methods of Gornall et al. (1949), Moore and Stein (1957), DuBois et al. (1956), Simoni et al. (2002) and Wagner (1993), respectively. Secondary metabolites such as Alkaloids, phenolic compounds, Saponins, tannins, flavonoids, steroids and terpenoids were measured according to the methods described by Singh et al. (2012). Resins were detected following the Crecelius and Lefkovitz (1997) method.

Determination of genetic variation

DNA extraction

Genomic DNA of each cultivar was isolated by a sap-extraction method (CIMMYT, 2005) from 100 mg of fresh leaf tissues. Leaves of 2-week-old seedlings were placed in a blender and mixed with 1 ml of extraction buffer (50 mM Tris–HCl, 25 mM EDTA, 1 M NaCl, 1% CTAB, 1 mM 1, 10-phenathroline, and 0.15% 2-mercaptoethanol) was slowly added to the rollers, and immediately mixed with the sap before collection in 1.5 ml micro centrifuge tubes. The extract was incubated at 60°C for 1 h, and then mixed with equal volume of chloroform-isoamyl alcohol (24:1). After centrifuging at 12,000 rpm, the supernatant was transferred to a new tube and incubated with isopropanol for 30 minutes to precipitate the DNA in a pellet from. The pellet was dried and re-suspended in 200 ml of TE buffer (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0). The DNA solution was mixed with 20 ml of 8M ammonium acetate and 400 ml of cold absolute ethanol for 30 min, centrifuged for 10 min, and air-dried at room temperature. The DNA was then re-suspended in 300 ml of TE buffer and stored at -20°C till used.

PCR amplification

For genetic diversity studies 40 RAPD primers (Table 1) were used to amplify genomic DNA. The primers were purchased from Gene link, Inc. and Operon Tech., NY 10532. The code number and sequences of RAPD primers are shown in Table 1.

PCR amplification reactions were carried out in a total volume of 20 µl for RAPD primers. The PCR mixtures contained (Final concentration): 5X FIRE Pol PCR Master Mix (Ready to load), 5X reaction buffer (0.4M Tris-HCL, 0.1M $(NH_4)SO_4$, 0.1% w/v Tween 20), 12.5 mM dNTPs, 50ng of the primer under test, 1 U Taq polymerase and 20 ng template DNA.

Data scoring and analysis

Data generated from the chemical constituents screening were analyzed by independent two samples T-test using SPSS statistical software v. 16. Scores were recorded as numbers for three replicates. DNA fragments obtained by both marker types were scored as present (1) or absent (0) and the polymorphism percentage for each primer was detected. Similarity between the two species was calculated on the basis of the scores according to Jaccard's similarity coefficients (Jaccard, 1908). The PAST 3.01 software package was used for analysis.

RESULTS AND DISCUSSION

Table 2 shows the primary metabolites measured in the two species. Results indicated that *J. glauca* has more sugar and amino acids contents than *J. curcas* while the later has more protein contents. No reducing sugars or starch were detected in the two species. The variation in amino acids contents could be attributed to the different geographical regions of the two species. Sinkat area, from where *J. glauca* seed were collected has strong rainfall season. Nadja et al. (2013) reported that the plant amino acid compositions may be affected by the climatic conditions due to the proline methodism which is a typical mechanism of biochemical adaptation subjected to stress condition.

Secondary metabolites screening (Table 2) revealed that there is no variation in the amount of the detected alkaloids in the two species. *J. curcas* was found to accumulate higher flavonoids, steroids, terpenoid and resins contents. These compounds are known to be biologically active and therefore aid the antimicrobial activities of *J. curcas*. These secondary metabolites exert antimicrobial activity through different mechanisms (Najda et al., 2013). Flavonoids are well known for their ability to inhibit pain perception (Okwu and Josiah, 2006). Flavonoids as antioxidants also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation (Oweyele et al., 2005). On the other hand, higher saponins contents were determined for *J. glauca*. High saponification value indicated that oils are normal triglycerides and very useful in production of liquid soap and shampoo industries (Akbar et al., 2009). Results of this study also indicated that there was no phenolic compounds or tannins detected in both plants. The reduction or absence of phenols may be due to enzymatic activity of polyphenol oxidase. Tannin, Alkaloid, Saponin and Phytate are the major causes of toxicity in *Jatropha curcas* which has to be lowered to levels that do not elicit toxic response for *Jatropha curcas* meal to be used as an ingredient in livestock feed (Becker and Makkar, 1997). Absence of tannins in the two *Jatropha* species of Sudan could increase their potentiality to be used in animal nutrition.

Genetic variation between *Jatropha* species

Unlike the morphological and biochemical markers, whose variations can occur due to the environmental fluctuations, the molecular marker will be stable and reproducible (Nuel *et al*., 2001; Huang *et al*., 2003). Therefore, the characterized germplasm and the identified markers can be a good source of plant genetic resources and can further be exploited for genetic improvement of the species through marker assisted breeding. In this study, the two *Jatropha* species were analyzed using 40 random primers of which 30 produced reproducible polymorphic banding patterns (Table 3). A total of 175 bands were scored of which 141 (80.6%) were polymorphic. The number of bands generated per primer varied from 1 (OPC17, OPH4, OPK17, OPR6 and OPY15) to 10 (OPR15). Eighteen primers generated 100% polymorphic bands. The size of the amplified products varied from 100 to 1700 bp. RAPD profiles of five representative primers are shown in Fig 1. The similarity between the two species, based on their genetic backgrounds studied by RAPD primers, was 19.5% indicating a great genetic variation between them. Results in Table 4 also indicate that 12 RAPD primers have shown amplification products with *J. curcas* and never with *J. glauca*. On the other hand, 9 primers amplified DNA of *J. glauca* only. These primers could be furtherly studied to confirm their specificity in the identification of each species.

Table 1. Codes of RAPD primers used for characterization of the two jatropha species and their sequences.

No.	RAPD primer	Sequence (5' - 3')	No.	RAPD primer	Sequence (5' - 3')
1	OPA04	AATCGGGCTG	21	OPR05	GACCTAGTGG
2	OPA11	CAATCGCCGT	22	OPR06	GTCTACGGCA
3	OPA18	AGGTGACCGT	23	OPR10	CCATTCCCCA
4	OPB10	CTGCTGGGAC	24	OPR15	GGACAACGAG
5	OPD07	TTGGCACGGG	25	OPY01	GTGGCATCTC
6	OPG05	CTGAGACGGA	26	OPY02	CATCGCCGCA
7	OPH04	GGAAGTCGCC	27	OPY04	GGCTGCAATG
8	OPK08	GAACACTGGG	28	OPY07	AGAGCCGTCA
9	OPK09	CCCTACCGAC	29	OPY14	GGTCGATCTG
10	OPK10	GTGCAACGTG	30	OPY15	AGTCGCCCTT
11	OPK15	CTCCTGCCAA	31	OPY16	GGGCCAATGT
12	OPK16	GAGCGTCGAA	32	OPY17	GACGTGGTGA
13	OPK17	CCCAGCTGTG	33	OPY18	GTGGAGTCAG
14	OPL07	AGGCGGGAAC	34	UBC04	CCTGGGCTGG
15	OPL11	ACGATGAGCC	35	UBC09	CCTGCGCTTA
16	OPL16	AGGTTGCAGG	36	UBC17	CCTGGGCCTC
17	OPL17	AGCCTGAGCC	37	GLC15	GACGGATCAG
18	OPL18	ACCACCCACC	38	GLD07	TTGGCACGGG
19	OPL19	GAGTGGTGAC	39	GLI09	TGGAGAGCAG
20	OPL20	TGGTGGACCA	40	GLE10	CACCAGGTGA

Table 2. Primary and secondary metabolites analysis for the two *Jatropha* species*.

Test	*J. curcas*	*J. glauca*
Primary metabolites		
Total Sugars	+++	+++
Total Proteins	++	+
Amino acids	++	+++
Starch	_	_
Reducing Sugar	_	_
Secondary metabolites		
Alkaloids	+++	+++
Saponins	+	++
Tannins	_	_
Flavonoids	++	+
Phenolic compounds	_	_
Resin	++	+
Steroid	+++	++
Terpenoid	+++	++

* + compound is detected, - compound not detected.

Table 3. Number of generated bands, bands' sizes, percentage of polymorphism and specificity of each RAPD primer.

RAPD primer	Total No. bands	Bands size range	Polymorphism %	Specific for
OPA04	7	250-800	100	*curcas*
OPA11	6	150-600	16.70	-
OPA18	0	0	0	-
OPB10	8	250-1250	100	*glauca*
UPC04	0	0	0	-
OPC09	7	300-900	71.40	-
GLC15	5	175-800	80	-
UPC17	1	250	100	*glauca*
OPD07	2	300-350	100	*curcas*
GLD07	4	400-1700	75	-
GLE10	8	175-1400	100	*curcas*
OPG05	3	225-450	66.70	-
OPH04	1	300	100	*glauca*
OPK08	6	350-1100	100	*curcas*
OPK09	0	0	0	-
OPK10	0	0	0	-
OPK15	7	400-1250	57.10	-
OPK16	3	100-300	100	*curcas*
OPK17	1	150	100	*glauca*
OPL07	8	150-800	100	*glauca*
GLI09	7	225-800	100	*curcas*
OPL11	9	250-1200	44.40	-
OPL16	9	150-800	55.50	-
OPL17	0	0	0	-
OPL18	2	700-800	100	*curcas*
OPL19	7	300-1000	100	*curcas*
OPR05	8	250-1200	100	*curcas*
OPR06	1	500	0	-
OPL20	0	0	0	-
OPR10	7	250 - 1200	100	*curcas*
OPR15	10	300-900	70	-
OPY01	8	200-1500	12.50	-
OPY02	8	100-1200	100	*curcas*
OPY04	8	250-1200	87.50	-
OPY07	8	225-1000	100	*glauca*
OPY14	0	0	0	-
OPY15	1	500	100	*curcas*
OPY16	0	0	0	-
OPY17	5	225-1100	100	*glauca*
OPY18	0	0	0	-
TOTAL	**175**	**100-1700**	**80.6**	NA

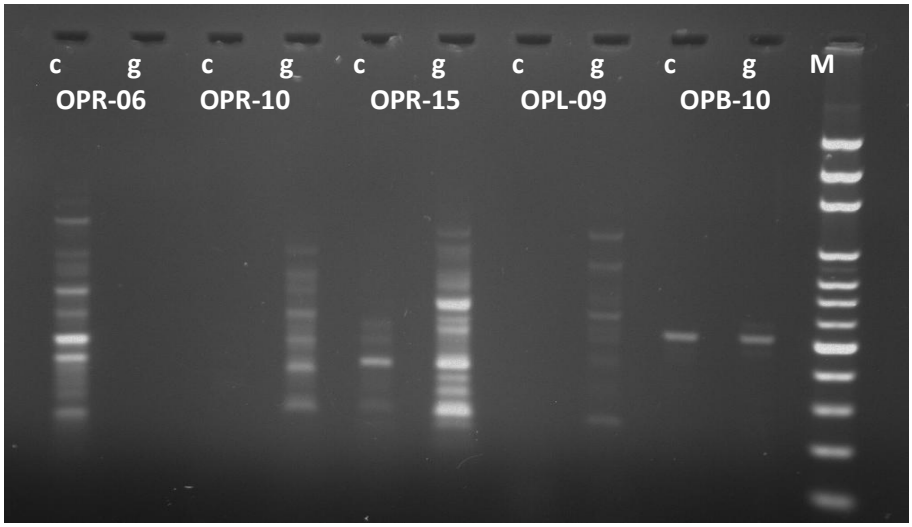

Fig. 1. RAPD-PCR amplification of the two Jatropha species.
Lanes g= *J. glauca*; c = *J. curcas*; M= 100bp DNA ladder.

To the best of our knowledge, there is no documented molecular markers study on *J. glauca*. There were very few studies carried to understand the genetic diversity using various marker systems in *J. curcas*. For example, Basha and Sujatha (2007) studied the extent of genetic diversity among toxic and non-toxic varieties using RAPD and the percentage of genetic similarity is found to be 96.3. In another study Sudheer et al. (2008) reported 84.91% similarity among toxic and non-toxic *J. curcas* by RAPD and identified the specific markers of RAPD for the varieties. Ram et al. (2008) assessed the genetic diversity of 12 *Jatropha* species based on RAPD markers and reported 80.2% polymorphism across the genotypes. Sudheer and Reddy (2014) studied the intraspecific genetic diversity of 63 *J. curcas* distributed in different parts of the globe using 52 out of 180 screened RAPD primers. Their results showed an overall polymorphism percentage of 76.96. However, till date no systematic studies were made on the analysis of genetic diversity among the selected germplasm; whose performance was evaluated in the field.

CONCLUSION

The two *Jatropha* species under study have comparable metabolic compounds. These observations therefore support the use of both *J. curcas* and *J. glauca* in herbal cure remedies. Results of the molecular screening indicate the great variation between the two species and the specificity of the tested DNA primers for each one. Future studies could be conducted to identify the generated specific amplification products in order to develop specific primers for the identification of these species.

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