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MOLECULAR GENETIC ASSESSMENT OF *JATROPHA CURCAS* L. GERMPLASM OF DIVERSE ORIGIN ALONG WITH ITS WILD RELATIVES FOR VARIOUS EARLY GROWTH AND ESTABLISHMENT RELATED TRAITS

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ABSTRACT

Jatropha curcas L. is promising crop with drought tolerance and potential crop for biodiesel. In present study, 33 accessions of *Jatropha curcas* L. were collected from different eco-climatic zones along with two wild relatives, *J. foetida* and *J. gossypifolia,* were screened for genetic diversity using RAPD and ISSR markers. Further the same has been evaluated for their ability for early field establishment on the basis of phenotypic parameter. Out of 25 RAPD and 14 ISSR primers, 16 RAPD and 6 ISSR primers gave reproducible amplification banding patterns with total 88 polymorphic bands across genotypes. The polymorphic information content was highest for the primer OPB 10 (0.43) followed by the primers OPH 7 (0.42). UPGMA cluster analysis of genetic similarity indices grouped all the accessions into four major groups. Jaccard's coefficient of similarity varied from 0.42 to 0.97, indicating high level of genetic variation across genotypes under study. Significant phenotypic variation in terms of total chlorophyll content, growth and establishment across genotypes was also observed. Present study highlights collection, conservation and characterization of potential *Jatropha* genetic resources and their utilization as breeding materials for their growth and early establishment in field conditions.

KEY WORDS: ***Jatropha*, Genetic Diversity, Phenotypic Diversity, RAPD, ISSR, Polymorphism**

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INTRODUCTION

Jatropha, also known as physic nut is Euphorbiaceae family member encompassing around 175 species. *Jatropha* has become a good choice as alternative crop with about 39% of seed oil. [1,2] Earlier reports have emphasized the necessity of a systematic study for understanding the degree of genetic variation in native populations' of *Jatropha,* for the selection of elite germplasm of high yielding genotypes.[3] Understanding the genetic variation using molecular markers across populations and their impact on phenotypic parameters of plant will form an ideal bench for identification and selection of varieties for breeding and cultivation. RAPD and ISSR are being popular as their application does not need any prior sequence information. In *Jatropha* there is immediate need to breed for superior genotypes aiming at high oil content, early maturity, high fruit bearing, pest, disease and drought resistance.[4] Earlier studies on characterization of *Jatropha* germplasm confined to locally available germplasm.[5] Ranade[6] employed single primer amplification reaction (SPAR) to evaluate genetic diversity among 21 genotypes of *J. curcas* which exhibited low level of variation highlighting the necessity to develop large number of markers which can be utilized for genotypic characterization. With the several reports on less genetic polymorphism and high phenotypic variation within *Jatropha* collections, there is uncertainty on extent of genetic contribution to *Jatropha* morphology and other yield related characters, some suggest for systematic comparative evaluation of distinct collections under same climatic conditions.[7] Thus, in present study phenotypic and molecular marker analysis have been carried out to investigate genetic variation among different genotypes of *J. curcas* collected from the distinct climatic zones of the globe along with two wild relatives. This study would act as initial step towards selection of elite breeding genotypes that are efficient in terms of growth and early field establishment.

MATERIALS AND METHODS

Experimental site is located in the Yogi Vemana University, Kadapa (AP., India, 14.47°`N, 78.71°E). The place has received a mean rainfall of 16.23 mm and mean air temperature of 32.64 ^{0}C during the experimental period. Irrigation was provided to the plants whenever it is needed until the sampling was done.

Plant materials collection

A total of thirty three genotypes of *Jatropha curcas,* are obtained from the fields of Naturole, Hyderabad which include the accessions collected from varied climatic conditions in Africa and India (Table:1), and other two wild relatives of *Jatropha, J. foetida and J. gossypifolia* were collected from Kadapa, Andhra Pradesh (India) which were used in present study.The plants were authenticated by by Botanist Dr. P. Chandra Obul Reddyand Plant taxonomist Dr. A. Madhusudhana Reddy and specimen herbariums were submitted and maintained in Yogi Vemana University Herbarium. All the genotypes were raised in the net house conditions and were transplanted to the fields at Yogi Vemana University, Kadapa, India located at 14.47°N 78.92°E latitude, altitude and at a sea level of 90m

Phenotypic characterization

Thirty five varieties were raised for three months under standard field conditions in the red loamy soil supplemented with form yard manure. Three months after field transfer, phenotypic parameters like total chlorophyll content, Internodal length, specific leaf area, petiole length, number of leaves per shoot, leaf angle and leaf dry weight were recorded. All the readings were recorded in triplicate.

Chlorophyll Estimation

Total Chlorophyll was estimated using SPAD Chlorophyll Meter Reading (SCMR) in triplicates using Minolta SPAD-502, Konica, Japan. SCMR was measured during 9.00-11.00 A.M using the 4th and 5thfully expanded leaf from the top of the stem as described by Rao and care was taken to ensure that the SPAD meter sensor fully covered the leaf lamina and that interference from veins and midribs were avoided.[8]

Measuring petiole length, leaf angle and Internodal length

Phenotypic parameter like leaf angle, Internodal length and petiole length were measured at fully expanded 4thand 5th leaves from the top using scale and by taking shoot at the centre.

Leaf number

The total numbers of leaves present on each for the three month old shoot were recorded manually.

Specific Leaf Area

Leaf surface area of the 5th or 6th leaf from top was measured using the leaf area meter (Li-COR Biosciences, USA). Leaf area was measured using a leaf area meter (LI-3000C LI-COR Biosciences.) and the leaf samples were oven dried at temperature 70°C for 36 hours before determine the leaf dry weight. Then SLA was calculated using the relationship as follows;

$$SLA = \text{Leaf area (cm}^2\text{) / Leaf dry weight (g)}$$

Isolation of genomic DNA and quantification

Total genomic DNA was isolated from 5 grams of young leaves using modified CTAB method.[9] Quantification of the DNA was done based on spectrophotometric measurement of UV absorbance at 260 nm. An aliquot of the DNA samples was diluted in TE buffer in a ratio of 1:1000 in a 1ml cuvette. Optical density was determined at 260 and 280 against TE buffer blank. The DNA concentration was calculated using the formula 1.0 OD corresponds to 50µg/ml of DNA. The ratio of OD 260 to OD280 was calculated to check the purity of DNA. DNA samples for analysis were diluted to 7.5 ng/µl for ISSR and SSR marker analysis.

RAPD's analysis

The genomic DNA is amplified with 25 RAPD (decamer) primers. The reaction mixture containing 1x PCR buffer containing (10mMmTris pH9.0, 50 mM KCL, 1.5 mM $Mgcl_2$), dNTP's each 100mM, 0.4mM of RAPD primer, 30 ng of total genomic DNA and 0.3 units of *taq* DNA polymerase (Genei, India). Polymerase reaction was performed in Thermocycler (Eppendorff, Germany) with initial denaturation of 94°C for 3 minutes followed by 40 cycles of 94°C of denaturation for 1 minute, annealing temperature of 37°C for one minute, extension time of one minute at 72°C. Final extension step is carried up to 10 minutes at 72°C. The amplicons were resolved on1.5% agarose gel in 0.5xTBE system with a voltage of 100V for two hours. The DNA is stained with Ethidium bromide and observed documented in Gel documentation unit (SynGene, UK).

ISSRs analysis

A total of 14 ISSR primers were employed for PCR amplification. The PCR reaction mixture containing 1x PCR buffer (10mM TRIS pH9.0,50mM KCL, 1.5mM $MgCl_2$), dNTP's each 100mM, 0.4mM of ISSR primer, 30 ng of total genomic DNA and 0.3 units of *taq DNA polymerase* (Genei, India). Polymerase reaction was performed in Eppendorf thermocycler with initial denaturation of 94°C followed by 40 cycles of 94°C of denaturation for 1 minute, annealing temperature of 45°C for one minute, extension time of one minute at 72°C. Final extension step is carried up to 10 minutes at 72°C. The amplicons were resolved in 1.5% agarose gel in 0.5xTBE system with a voltage of 100v for 2 hours. The DNA is stained with Ethidium bromide and observed and documented in Gel documentation unit (SynGene, U.K.).

Data analysis

Reproducible amplicons obtained after PCR amplification were scored as 1 and 0 for presence and absence respectively. The band profiles were scored only for distinct, reproducible bands for each RAPD and ISSR primers primer pair. Jaccard's similarity coefficient values were calculated and dendrograms based on similarity coefficient values were generated using unweighted pair-group method with arithmetic means (UPGMA) by the NTSYSpc 2.02j software.[10] The polymorphism information content (PIC) value of was also calculated using the formula 2f (1-f), where f is the frequency of bands present and (1- f), is the frequency of bands absent.[11]

Statistical Analysis

The mean of all the plants for each trait under each replication was subjected to analysis.[12] The estimate of genotypic variance and phenotypic variance were worked out according to the method proposed by Johnson[13] using mean square values from the ANOVA table. Phenotypic and genotypic coefficient of variance was calculated based on the method suggested by Burton.[14] Heritability percentage in broad sense was estimated as per the method described by Lush[15] and traits were classified as having high, moderate and low heritability as per the method of Robinson.[16] Genetic advance estimation and trait classification as high moderate and low genetic advancement in according to the method of Johnson.[13] STATISTICA Cluster analysis software was employed to develop Hierarchical clustering (joining) of Jatropha accessions based on morpho-physiological traits using Ward's minimum variance method with the Euclidean distances to measure the distances among the accessions.

RESULTS

In the present study, we have selected 35 *Jatropha* accessions, out of which 33 accessions belongs to the species *J.curcas* and other two are wild relatives of *Jatropha (J. gossypifolia* and *J. foetida)*. To access the phenotypic variability of the accessions seven morphological quantitative traits *i.e* chlorophyll content (SPAD reading); inter-nodal length; specific leaf area; petiole length; number of leaves per plant; leaf angle and specific dry weight of leaf were evaluated.The traits such as leaf angle, amount of chlorophyll present in leaves (SPAD) and number of leaves present in a particular shoot directly implies the photosynthesis capacity of the plants, which in turn effect the growth, establishment and overall physiology of plant. The three months old *Jatropha* accessions of VAR1 (Tandur), VAR4 (TNAU), VAR15 (ZH-3) and VAR24 (Samarlagondhi) recorded greater values for leaf angle, SPAD values and number of leaves compared to other accessions (Figure 2, 3, 4 and 5). Whereas the accessions VAR25-VAR32 (wild populations grown in Naturole farms Hyderabad) showed lesser No. of leaves per stem, low SPAD values and poor leaf angle (Figure 2, 3, 4 and 5). Specific leaf weight (the ratio of leaf weight to area) is reported as drought tolerant trait in several crops and has been suggested as a selection criterion for breeding programs targeting low rainfall areas. Genotypes with a high specific leaf weight (thick leaves), are thought to be an advantage for higher water use efficiency. The *Jatropha* accessions displayed a wide range of phenotypic variability for specific leaf area and specific leaf weight. The accessions of VAR1 (Tandur), VAR2 (ADI Biotech), VAR9 (DLR-2), VAR13 (ZH1), VAR18 (Veerareddy), and VAR19 (Mallisala) documented relatively high specific leaf area and specific leaf weight (Figure: 1, 7 & 8).A significant and large phenotypic variation was found between the accessions with a range of 17.7-42.1 for chlorophyll content (SPAD reading), 1.1-3.8 cm for inter-nodal length, 38.6-135.6 for specific leaf area, 5.65-33.3 cm for petiole length, 4.3-26.6 number of leaves per plant, 38.5°-60.7° of leaf angle and 0.16 -0.8 gm; for all the phenotypic quantitative traits (Table: 2). The phenotypic variation for all the quantitative traits is highly significant with respect to coefficient of variation. Six out of seven quantitative traits measured shown high coefficient of variation (CV%) of > 20 % among the accessions (Table: 2). All of the quantitative traits studied showed high heritability range from 61 – 100; whereas the mean of the percent genetic advancement range between 23.11 to 90.34. Our results highlight the wide range of genetic diversity and phenotypic variation present in the germplasm collection. The PCR amplicons with high reproducible visual bands were scored for both RAPD and ISSR markers, to evaluate the genetic relationships among the Jatropha accessions of *J.curcas.* Of all 25 RAPD primers used, 16 primers produced highly polymorphic reproducible banding patterns in, *J. curcas* genotypes, *J. foetida,* and

J. gossypifolia. The decamer primers were able to generate a minimum of two amplicons to maximum of ten amplicons with an average *PIC* of 0.07 to 0.5 across the accessions studied (Table: 3). A total of 6 ISSR primers, out of 14 primers used were selected based on their polymorphic banding pattern. These six 15-18 bp ISSR primers were able to amplify a minimum of two to five amplicons across the accessions with a total of 17 amplicons of varying size and *PIC* value of 0.06 to 0.26 among the accessions (Table: 4). A total of 85 polymorphic bands were scored from 16 RAPD and 6 ISSR primers. A dendrogram was constructed to reveal the genetic relationships among the *J. curcas* and wild *J. foetida, J. gossypifolia.* The UPGMA-based dendrogram showed that, all of the 35 clones could be classified into four groups at 80% similarity coefficient. The genetic distances among 35 genotypes were represented in (Table: 5). The similarity values in terms of genetic distance ranged from 0.5 to 0.97. A dendrogram generated by cluster analysis (UPGMA method) based on the 85 bands with 22primer (16 RAPDs & 6 ISSRs) revealed that 35 lines were separable into two major clusters, cluster 1 and cluster 2 (Fig: 10). Cluster 1 includes all thirty three genotypes of cultivated *Jatropha, Jatropha curcas* and cluster two contains other two wild varieties of *Jatropha (Jatropha gossypifolia, Jatropha foetida)*. Within the cluster 1, three genotypes VAR1 and VAR8 fall as a separate sub cluster, which were distinctly separated out from the others. The same has been reflected in the similarity table, where the similarity coefficient of the respective genotypes is in the range of 0.51 to 0.83 (VAR1), 0.53 to 0.75 (VAR16) and 0.49 to 0.78 (VAR18) in comparison with other genotypes under study. Similarity analysis of 33 *Jatropha curcas* varieties and two wild varieties *(J. gossypifolia J. foetida*) clearly showed that there is lot of variation in between genotypes ranging from 0.5 to 0.03. Interestingly the accessions collected from Karnataka- Mallisala, Delhi-adibiotech and Andhrapradesh- Chittoor, Maharashtra- Bombay genotypes very much close to the African–Congo and Uganda varieties. The DLR1-Himalayan variety showed greater genetic distance from all other genotypes. The Tandur variety of Andhra Ppradesh showed much variation with natural accession-7 of Andhra Pradesh even they are from closer areas. By the analysis of dendrogram as expected the two wild varieties of *Jatropha* fell in to separate group from that of the *Jatropha curcas* group. All the *Jatropha curcas* varieties are divided in to 2 major classes with Tandur-AP, Aleru-AP & DLR-1 Himalayas in one group and remaining 30 varieties as other group.

Table 1
Details of the Jatropha accessions collected and used in the present study

S. No / Variety	Name of the Source Place Where Procured	Code used	Species	Country of Origin
1	Tandur, Andhra Pradesh *	*VAR1*	*Jatropha curcas*	*India*
2	ADI Biotech, Delhi	*VAR2*	*Jatropha curcas*	*India*
3	SRIPHL, Rajastan	*VAR3*	*Jatropha curcas*	*India*
4	TNAU,Tamilanadu	*VAR4*	*Jatropha curcas*	*India*
5	Suresh Forestry, Chattisgarh	*VAR5*	*Jatropha curcas*	*India*
6	Sai Petro chemical, Maharastra	*VAR6*	*Jatropha curcas*	*India*
7	Maharastra, Maharastra	*VAR7*	*Jatropha curcas*	*India*
8	DLR-1, Himalayas	*VAR8*	*Jatropha curcas*	*India*
9	DLR-2, Himalaya	*VAR9*	*Jatropha curcas*	*India*
10	Bilaspur, Chatiisgarh	*VAR10*	*Jatropha curcas*	*India*
11	Karnataka, Karnataka	*VAR11*	*Jatropha curcas*	*India*
12	Surya peta, Andhra Pradesh *	*VAR12*	*Jatropha curcas*	*India*
13	ZH1, Zaheerabad, Andhra Pradesh *	*VAR13*	*Jatropha curcas*	*India*
14	ZH2, Zaheerabad, Andhra Pradesh *	*VAR14*	*Jatropha curcas*	*India*
15	ZH3, Zaherabad, Andhra Pradesh *	*VAR15*	*Jatropha curcas*	*India*
16	Aleru, Warangal, Andhra Pradesh *	*VAR16*	*Jatropha curcas*	*India*
17	Naturol office, Andhra Pradesh *	*VAR17*	*Jatropha curcas*	*India*
18	Vera Reddy, Nalgonda, Andhra Pradesh *	*VAR18*	*Jatropha curcas*	*India*
19	Mallisala, Karanataka	*VAR19*	*Jatropha curcas*	*India*
20	Congo1, Africa	*VAR20*	*Jatropha curcas*	*Africa*
21	Congo2, Africa	*VAR21*	*Jatropha curcas*	*Africa*
22	Uganda 1, Africa	*VAR22*	*Jatropha curcas*	*Africa*
23	Chittoor, Anahdra Pradesh	*VAR23*	*Jatropha curcas*	*India*
24	Samarlagondhi, Andhra Pradesh	*VAR24*	*Jatropha curcas*	*India*
25	Selection from wild populations grown in Farms of naturole hyderabad – 1	*VAR25*	*Jatropha curcas*	*India*
26	Selection from wild populations grown in Farms of naturole hyderabad – 2	*VAR26*	*Jatropha curcas*	*India*
27	Selection from wild populations grown in Farms of naturole hyderabad – 3	*VAR27*	*Jatropha curcas*	*India*
28	Selection from wild populations grown in Farms of naturole hyderabad – 4	*VAR28*	*Jatropha curcas*	*India*
29	Selection from wild populations grown in Farms of naturole hyderabad – 5	*VAR29*	*Jatropha curcas*	*India*
30	Selection from wild populations grown in Farms of naturole hyderabad – 6	*VAR30*	*Jatropha curcas*	*India*
31	Selection from wild populations grown in Farms of naturole hyderabad - 7	*VAR31*	*Jatropha curcas*	*India*
32	Selection from wild populations grown in Farms of naturole hyderabad – 8	*VAR32*	*Jatropha curcas*	*India*
33	Selection from wild populations grown in Farms of naturole hyderabad – 9	*VAR33*	*Jatropha curcas*	*India*
34	Yogi Vemana University, Kadapa	*VAR34*	*Jatropha ossipifolia*	*India*
35	Yogi Vemana University, Kadapa	*VAR35*	*Jatropha foitida*	*India*

**** - Present in Telangana Stage***

Table 2
Genetic variability parameters for early field establishment traits in Jatropha accessions studied

S. No	Trait	Mean	Range	PCV (%)	GCV (%)	Heritability h² (%)	Genetic Advance	Genetic Advance as % of mean
1	Leaf Angle	50.4	38.5-0.75	10.4857	9.5819	83.5	11.6519	23.1157
2	Petiole length	10.8	6.2-15.05	21.3923	20.8621	95.1	5.78	53.711
3	Internodal length	2.4	1.1-3.8	29.61	27.13	83.9	1.58	65.61
4	Leaf surface area	73.5	37.9-33.1	30.34	23.71	61.07	35.97	48.92
5	Chlorophyll Content(SPAD)	28.3	18-50	23.9	22.31	86.64	15.51	54.82
6	Leaf dry wt	0.9	0.369-1.413	33.34	33.34	100	0.772	88.03
7	No.of leaves per stem	13.5	4.25-31	41.53	37.7	82.4	12.2	90.34

Table 3
Polymorphism Information Content (PIC) for Jatropha accessions studied using RAPD markers

S. No	Marker Type and Name	Sequence (5` - 3`)	Annealing Temperature	Number of Alleles	Polymorphic Alleles	PIC
1	OPA15	TTCCGAACCC	37	2	2	0.28
2	OPB 10	CTGCTGGGAC	37	2	0	0
3	OPC9	CTCACCGTCC	37	3	3	0.44
4	OPC10	TGTCTGGGTG	37	6	6	0.27
5	OPD02	GGACCCAACC	37	4	3	0.5
6	OPG11	TGCCCGTCGT	37	7	6	0.27
7	OPG13	CTCTCCGCCA	37	10	10	0.27
8	OPG14	GGATGAGACC	37	5	3	0.14
9	OPG16	AGCGTCCTCC	37	2	1	0.14
10	OPH17	CACTCTCCTC	37	5	5	0.42
11	OPH03	AGACGTCCAC	37	5	5	0.33
12	OPH07	CTGCATCGTG	37	5	5	0.42
13	OPK07	CTTGGGGGAC	37	4	4	0.39
14	OPK08	CCGAAGGGTG	37	3	1	0.07
15	OPO10	TCAGAGCGCC	37	5	5	0.33
16	OPV04	CCCCTCACGA	37	4	4	0.4

Table 4
Polymorphism Information Content (PIC) for Jatropha accessions studied using ISSR markers

S. No	Marker Type and Name	Sequence (5` - 3`)	Annealing Temperature	Number of Alleles	Polymorphic Alleles	PIC
1	816	CACACACACACACACAT	45	3	3	0.11
2	822	TCTCTCTCTCTCTCTCA	45	3	3	0.22
3	823	TCTCTCTCTCTCTCTCC	45	2	2	0.08
4	824	TCTCTCTCTCTCTCTCG	45	5	5	0.26
5	873	GACAGACAGACAGACA	45	3	2	0.16
6	880	GGAGAGGAGAGGAGA	45	1	1	0.06

Table 5
Genetic distances between the Jatropha accessions studied

	Tandur-AP	ADIBiotech-Delhi	SKIPHL-Rajastan	TNAU-Tamilnadu	SureshForestry-Chattisgarh	SaiPetrochemical-Maharastra	Bombay-Maharastra	DLR1-Himalayas	DLR2-Himalayas	Bilaspur-Chattisgarh	Karnataka-Karnataka	Suryapet-AP	Zaheerabad1-AP	Zaheerabad2-AP	Zaheerabad3-AP	Aleru-AP	Natureloffice-AP
Tandur-AP	1.00																
ADIBiotech-Delhi	0.64	1.00															
SKIPHL-Rajastan	0.76	0.63	1.00														
TNAU-Tamilanadu	0.74	0.69	0.84	1.00													
SureshForestry-Chattisgarh	0.76	0.74	0.82	0.84	1.00												
SaiPetrochemical-Maharastra	0.58	0.92	0.68	0.75	0.75	1.00											
Bombay-Maharastra	0.60	0.97	0.66	0.73	0.77	0.95	1.00										
DLR1-Himalayas	0.77	0.50	0.72	0.67	0.63	0.56	0.53	1.00									
DLR2-Himalayas	0.65	0.74	0.77	0.75	0.70	0.77	0.75	0.69	1.00								
Bilaspur-Chattisgarh	0.58	0.90	0.68	0.77	0.77	0.95	0.93	0.53	0.80	1.00							
Karnataka-Karanataka	0.60	0.90	0.66	0.75	0.75	0.93	0.93	0.58	0.73	0.91	1.00						
Suryapeta-AP	0.73	0.68	0.78	0.85	0.83	0.67	0.67	0.64	0.69	0.69	0.69	1.00					
Zaheerabad1-AP	0.60	0.90	0.68	0.75	0.77	0.91	0.91	0.53	0.77	0.93	0.89	0.69	1.00				
Zaheerabad2-AP	0.70	0.75	0.72	0.76	0.78	0.72	0.74	0.64	0.74	0.72	0.69	0.80	0.74	1.00			
Zaheerabad3-AP	0.63	0.88	0.64	0.68	0.75	0.86	0.89	0.56	0.75	0.86	0.84	0.63	0.93	0.76	1.00		
Aleru-AP	0.83	0.58	0.75	0.73	0.75	0.59	0.59	0.78	0.68	0.59	0.59	0.74	0.59	0.67	0.61	1.00	
Natureloffice-AP	0.67	0.81	0.75	0.82	0.80	0.84	0.84	0.65	0.73	0.82	0.89	0.78	0.80	0.74	0.75	0.66	1.00
Verareddy-AP	0.60	0.90	0.70	0.75	0.77	0.93	0.93	0.51	0.77	0.93	0.89	0.67	0.89	0.72	0.86	0.64	0.84
Mallirala-Karnataka	0.59	0.91	0.65	0.74	0.74	0.94	0.94	0.59	0.76	0.92	0.90	0.64	0.90	0.70	0.90	0.58	0.83
Congo1-Africa	0.60	0.92	0.68	0.70	0.80	0.93	0.95	0.56	0.75	0.91	0.91	0.67	0.89	0.72	0.89	0.61	0.82
Congo2-Africa	0.58	0.90	0.66	0.70	0.75	0.91	0.93	0.56	0.75	0.89	0.91	0.65	0.86	0.69	0.89	0.61	0.82
Uganda-Africa	0.60	0.90	0.64	0.70	0.73	0.89	0.93	0.58	0.75	0.86	0.86	0.65	0.84	0.74	0.86	0.61	0.82
Chittoor-AP	0.61	0.86	0.63	0.74	0.69	0.85	0.90	0.59	0.74	0.83	0.88	0.66	0.88	0.70	0.90	0.60	0.81
Samarlagondhi-AP	0.80	0.69	0.81	0.86	0.82	0.70	0.70	0.64	0.69	0.73	0.73	0.83	0.70	0.78	0.68	0.75	0.75
Naturelfield1-AP	0.59	0.91	0.72	0.76	0.78	0.92	0.94	0.55	0.78	0.92	0.90	0.70	0.90	0.70	0.83	0.63	0.83
Naturelfield2-AP	0.64	0.70	0.67	0.72	0.69	0.72	0.72	0.64	0.69	0.72	0.72	0.73	0.69	0.75	0.69	0.69	0.81
Naturelfield3-AP	0.67	0.81	0.82	0.84	0.89	0.84	0.84	0.58	0.75	0.86	0.82	0.81	0.84	0.83	0.80	0.68	0.82
Naturelfield4-AP	0.70	0.77	0.83	0.85	0.88	0.81	0.81	0.64	0.81	0.83	0.78	0.77	0.83	0.80	0.81	0.72	0.81
Naturelfield5-AP	0.72	0.72	0.84	0.86	0.82	0.77	0.75	0.69	0.82	0.80	0.73	0.76	0.77	0.76	0.75	0.73	0.73
Naturelfield6-AP	0.66	0.80	0.78	0.81	0.88	0.81	0.83	0.59	0.76	0.85	0.81	0.77	0.81	0.82	0.81	0.67	0.81
Naturelfield7-AP	0.50	0.77	0.58	0.65	0.67	0.78	0.78	0.57	0.72	0.76	0.76	0.59	0.76	0.68	0.78	0.56	0.72
Naturelfield8-AP	0.72	0.79	0.82	0.84	0.85	0.83	0.83	0.61	0.77	0.83	0.80	0.76	0.83	0.80	0.80	0.69	0.80
Naturelfield9-AP	0.63	0.83	0.68	0.75	0.75	0.86	0.86	0.53	0.70	0.84	0.86	0.72	0.82	0.76	0.80	0.61	0.80
Jatropagossipifolia	0.57	0.55	0.42	0.42	0.49	0.51	0.51	0.55	0.49	0.51	0.51	0.48	0.56	0.50	0.60	0.53	0.42
Jatropafeitida	0.51	0.56	0.52	0.55	0.50	0.55	0.55	0.49	0.57	0.55	0.52	0.58	0.55	0.56	0.57	0.59	0.52

	Verareddy-AP	Mallirala-Karnatak a	Congo1-Africa	Congo2-Africa	Uganda-Africa	Chittoor-AP	Samarlagondhi-AP	Naturelfield1-AP	Naturelfield2-AP	Naturelfield3-AP	Naturelfield4-AP	Naturelfield5-AP	Naturelfield6-AP	Naturelfield7-AP	Naturelfield8-AP	Naturelfield9-AP	Jatropagossipifolia	Jatropafeitida
Tandur-AP																		
ADIBiotech-Delhi																		
SKIPHL-Rajastan																		
TNAU-Tamilanadu																		
SureshForestry-Chattisgarh																		
SaiPetrochemical-Maharastra																		
Bombay-Maharastra																		
DLR1-Himalayas																		
DLR2-Himalayas																		
Bilaspur-Chattisgarh																		
Karnataka-Karanataka																		
Suryapeta-AP																		
Zaheerabad1-AP																		
Zaheerabad2-AP																		
Zaheerabad3-AP																		
Aleru-AP																		
Natureloffice-AP																		
Verareddy-AP	1.00																	
Mallirala-Karnataka	0.92	1.00																
Congo1-Africa	0.91	0.94	1.00															
Congo2-Africa	0.91	0.94	0.95	1.00														
Uganda-Africa	0.89	0.92	0.91	0.91	1.00													
Chittoor-AP	0.85	0.91	0.88	0.88	0.90	1.00												
Samarlagondhi-AP	0.70	0.67	0.70	0.68	0.68	0.69	1.00											
Naturelfield1-AP	0.92	0.91	0.92	0.92	0.90	0.86	0.74	1.00										
Naturelfield2-AP	0.78	0.73	0.72	0.72	0.72	0.75	0.77	0.73	1.00									
Naturelfield3-AP	0.84	0.81	0.84	0.82	0.80	0.76	0.86	0.88	0.76	1.00								
Naturelfield4-AP	0.83	0.80	0.81	0.81	0.78	0.77	0.81	0.84	0.73	0.94	1.00							
Naturelfield5-AP	0.73	0.74	0.75	0.75	0.73	0.72	0.88	0.78	0.69	0.89	0.88	1.00						
Naturelfield6-AP	0.83	0.80	0.83	0.83	0.78	0.75	0.83	0.84	0.75	0.97	0.93	0.88	1.00					
Naturelfield7-AP	0.78	0.84	0.81	0.85	0.81	0.77	0.58	0.77	0.70	0.72	0.70	0.67	0.73	1.00				
Naturelfield8-AP	0.85	0.82	0.80	0.83	0.80	0.78	0.80	0.82	0.76	0.91	0.92	0.86	0.87	0.71	1.00			
Naturelfield9-AP	0.86	0.83	0.84	0.84	0.80	0.81	0.77	0.83	0.76	0.86	0.83	0.75	0.83	0.74	0.86	1.00		
Jatropagossipifolia	0.47	0.55	0.53	0.56	0.49	0.55	0.48	0.48	0.50	0.44	0.43	0.51	0.45	0.57	0.49	0.51	1.00	
Jatropafeitida	0.55	0.56	0.55	0.59	0.57	0.60	0.55	0.56	0.58	0.52	0.53	0.57	0.51	0.60	0.56	0.55	0.65	1.00

Figure 1
Phenotypic variability among the Jatropha accessions in terms of leaf shape, color and total leaf area

Figure 2
Phenotypic variability among the Jatropha accessions for SPAD Chlorophyll reading

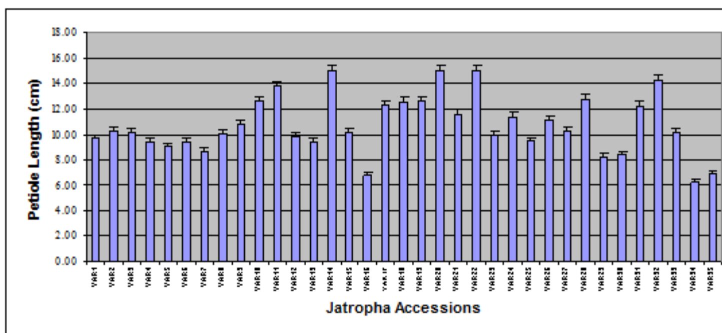

Figure 3
Phenotypic variability among the Jatropha accessions for Petiole Length

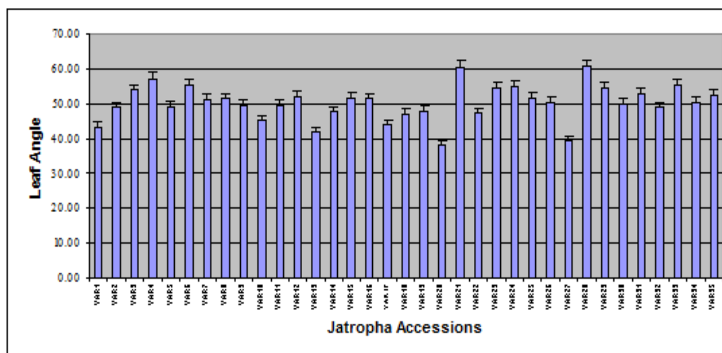

Figure 4
Phenotypic variability among the Jatropha accessions for Leaf Angle

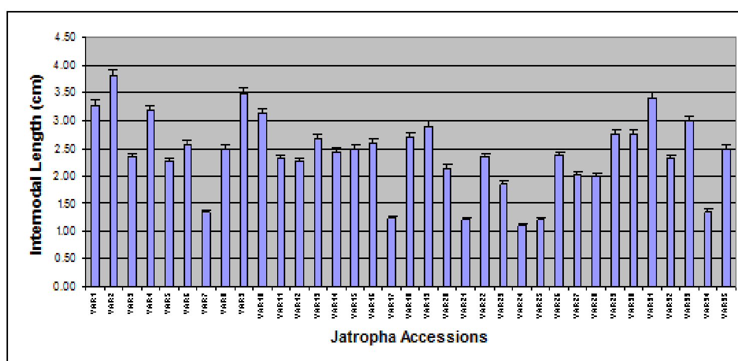

Figure 5
Phenotypic variability among the Jatropha accessions for Internodal Length

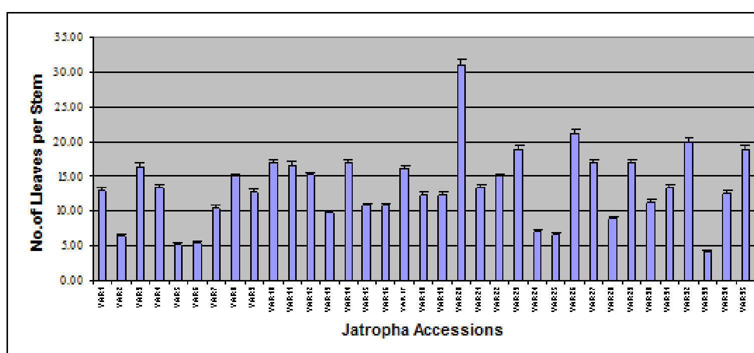

Figure 6
Phenotypic variability among the Jatropha accessions for Number of Leaves per stem

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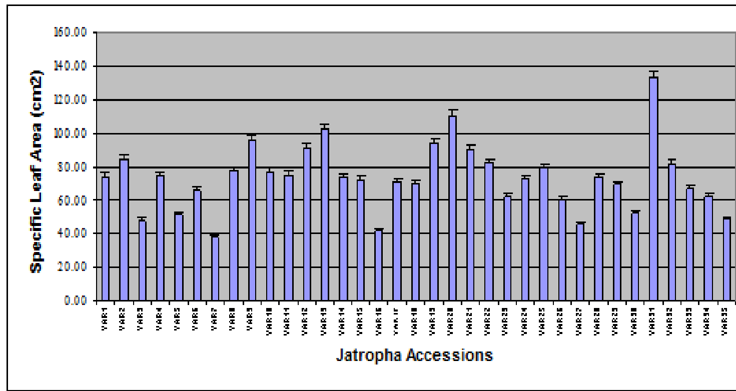

Figure 7
Phenotypic variability among the Jatropha accessions for Specific Leaf Area

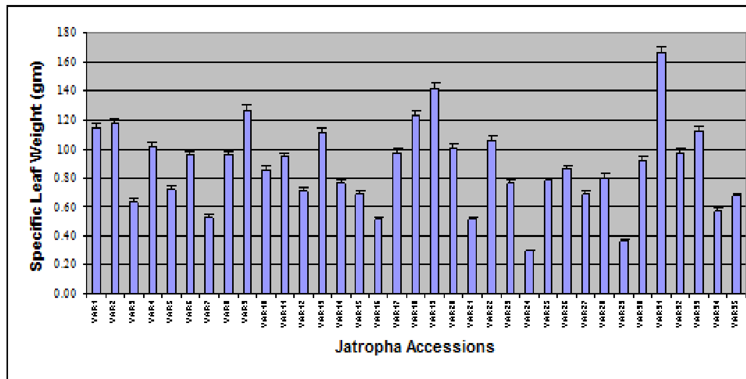

Figure 8
Phenotypic variability among the Jatropha accessions for Specific Leaf Weight

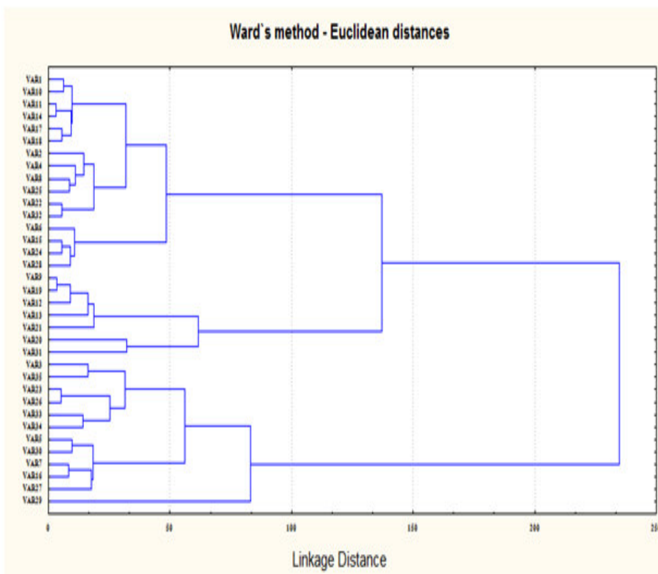

Figure 9
Ward's Minimum Variance Dendrogram for the Jatropha accessions studied (var – Variety)

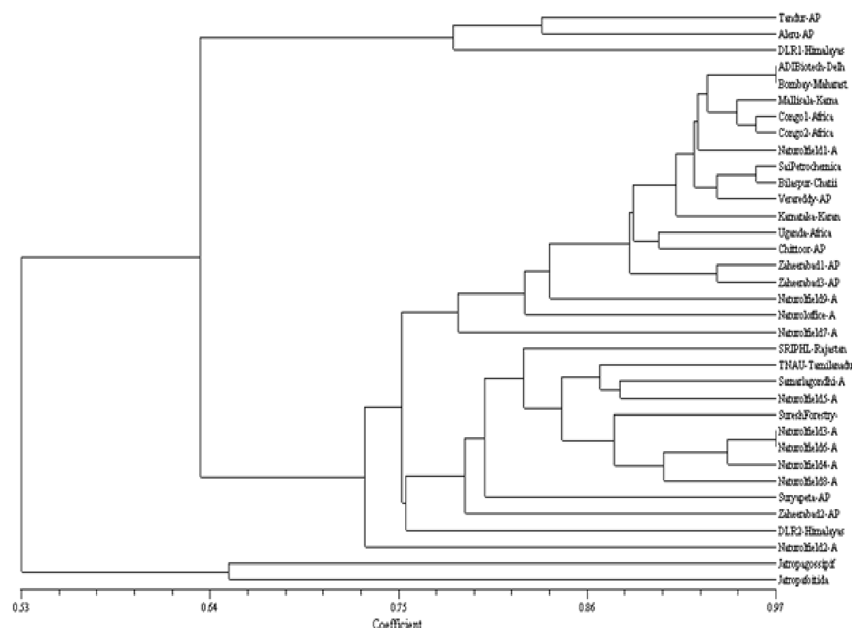

Figure 10
Phylogenetic relationships between 35 jatropha accesions created by using NTSYS-pc program based on DICE similarity coefficients computed from data matrix with 88 informative polymorphic DNA bands generated from 16 RAPD and 6 ISSR markers

DISCUSSION

Jatropha, is a native to South Central America, which was introduced or spread to other tropical countries via Cape Verde islands and Guinea Bissau.[17] Though it is an introduced plant, its ability to thrive in harsh environments, easy propagation to a variety of edaphic conditions make itself to establish in wider agro-ecological zones across world.[18] There is virtually no information with regard to the number of introductions and the genetic diversity of *J. curcas* populations grown in India. Therefore, it is important to tap the existing genetic variation in the natural populations, which is a key for the *J. curcas* breeding for its ability as potent biodiesel crop species.[19] A wide variety of morphological, biochemical and molecular markers have been widely used for diversity studies in *Jatropha.*[20,21] Very limited phenotypic diversity studies based on plant height, number of primary branches, collar length, number of fruits per cluster and oil content provided reliable classification of *Jatropha* accessions for specific breeding purposes.[22-24] In the present study significant phenotypic variations was observed between the Jatropha accessions of India and South Africa based on the molecular genetic and phenotypic characters studied. Heller[17] tested 13 provenances in multilocation field trials in 1987 and 1988 in two countries of the Sahel region: Senegal and Cape Verde. Significant differences in the vegetative development except leaf shape were detected among the various provenances at all locations. Kaushik[13] studied divergence among 24 accessions using non-hierarchical Euclidian cluster analysis for seed traits in jatropha and suggested that the crossing between accessions of clusters IV and VI will result in wide spectrum of variability in subsequent generations. Gohil and Pandya[22] analyzed diversity based on phenotypic traits of nine *Jatropha* genotypes and suggested that for varietal improvement, hybridization among the genotypes of divergent clusters (clusters – III, IV and V) may be done in order to obtain better results in terms of variability and diversity. Rao[19] observed four clusters with phylogeographic patterns of genetic diversity among 32 high yielding candidate plus trees of *J. curcas* for seed traits. Though variety of markers has been adapted for the molecular genetic evaluation of the regional *J. curcas* lines, scanty studies has been made towards the integration of global germplasm for their ability for the field establishment and early growth. In the present study, we have collected 33 accessions of *J. curcas*, representing fairly good diversity across India, few accessions from Africa along with its wild relatives and assessed molecular diversity using RAPD and ISSR markers. The results clearly showed that RAPD and ISSR primers were more efficient in revealing DNA polymorphism among the genotypes. Analysis of genetic diversity in 33 *Jatropha* accessions revealed low genetic variability within the Indian accessions and with the African accessions. Most of the Indian accessions showed low genetic diversity with the Africal-Congo and Uganda varieties. However, high genetic diversity was observed between J.curcus and with the two wild varieties *(J. gossypifolia J. foetida*) ranging from 0.5 to 0.03. The use of RAPD & ISSR primers was also seen to be highly appreciable toward discrimination of *Jatropha* samples. The RAPD technique, shown to be more helpful over morphologic and chemical markers, provides an unlimited number of rapid inheritable genetic markers independent of environmental effects, which can be used for genetic diversity analysis and breeding purposes The technique is widely used for the estimation of genetic variability as well as cultivar identification /differentiation in various plant species Likewise, the greater usefulness of ISSR markers has already been established by plant breeders around the world. In the present analysis,

RAPD and ISSR data were used as support parameters for qualitative floral characters so that it could be possible to determine the extent of diversity and relation within and between the species. The results highlight the extent of phenotypic variability and genetic diversity present with in the collection from the India and their relationship with the collections from Africa. The collections of the Himalayan region (DLR1), Tandur region, and Aleru region shows not only distinct at molecular genetic level but also falls separate at phenotypic level for their ability for growth and early establishment in the field. The plant growth rate in terms of the total height is the shortest distance between the upper boundaries of the main photosynthetic axis of the plant and the ground level. The plants with good and better inter-nodal length will have additional advantage over others in terms of their establishment. It is associated with competitive vigor of a plant at whole plant level over a given period of time or between disturbances. Overall plant growth rate is determined by many factors such as canopy configuration, leaf shape, leaf angle and internode length.[25-27] The leaf angle determines the intensity of the light received and the rate of the photosynthesis. This in turn determines the whole plant growth rate. The varieties with ideal leaf angle may have greater advantage compared to others for early establishment. Earlier studies in other tree species shown reduction in leaf angle decreased net photosynthetic productivity[28]. In earlier studies, increase in net photosynthesis per unit land area and increased biomass partitioning of biomass showed to have impact on the net crop productivity[25,29] As in other cases of crop selection through breeding, it is clearly shown that the varieties with higher surface area are tend to have higher photosynthetic rate and will have faster growth capability with more vigor to early establishment[25]. In the present study, it is clear that the varieties with high specific leaf area, and SPAD meter (chlorophyll) content have the same vigor in the early field establishment. Studies on effect of plant architectural traits on light capturing, net photosynthesis and dry mass production in tomato clearly shown that overall growth rate depend on leaf angle, leaf curvature, internodal length etc., [3]. The *Jatropha* collections from the areas Karnataka, Zaherabad, Africa, had with good petiole length while, the lines from the TNAU, Maharastra and Africa has good leaf angle that influence the light perception and net photosynthesis. For Internodal length, lines from Tandur, Delhi, Himalayas has beet performance while for the number of leaves per stem has more in the varieties from Africa. Further apart from the molecular genetic analysis and phenotypic characterization, systematic studies on the selective lines was performed to evaluate their potential antidote properties of various solvent extracts and presented elsewere.[30] In the present study, it is clear that the overall phenotypic variation across the varieties also reflect at the genotypic level as shown in marker analysis. Our results highlights the finding of a genetic source of *J. curcas* of high genetic diversity with potential source as breeding materials for early field establishment and growth.

AUTHOR CONTRIBUTION STATEMENT

KVNRR, PR, KN conducted the experiments. LSP collected the germplasm. CVCMR performed the statistical analysis. AK, PCOR, ACS designed the experiment and wrote the manuscript. All authors scrutinized and corrected the manuscript.

CONFLICT OF INTEREST

Conflict of interest declared None.

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