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Study on Seed Morphology and Genetic Diversity of *Jatropha curcas* L. from Different Provenances

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Abstract: *Jatropha curcas* L., a multipurpose shrub has acquired significant economic importance for its seed oil which can be converted to biodiesel, is emerging as an alternative to petro-diesel. The present study aims at characterization of the seed morphology and genetic diversity of *Jatropha curcas* L. from eight different provenances for providing support for the breeding and allocation of seed. Five traits were investigated, including hundred seed weight, seed length, width, lateral diameter, seed length and width ratio. The genetic diversity of eight populations from different provenances was estimated using DALP method (5 primers). The results showed that seed morphology had significant variation among locations. Five DALP primers generated highly reproducible and stable DNA fragments. 219 of 244 loci were polymorphic, i.e., *PPB* was 89.75%. And POPGENE analysis indicated the total Nei's gene diversity (*H*) was 0.2878, the total Shannon's Information index (*I*) was 0.4331, and the coefficient of Gene differentiation (*Gst*) was 0.8200 among populations, namely 82.00% genetic variation occurring among populations and 20.00% remaining within population. It was suggested that a high level of genetic variation should be occur among the different populations of *J. curcas*. The high genetic differentiation among the populations could be caused by not only the limited gene flow (*Nm* = 0.1097) but also the genetic drift. The result indicates that the seed morphology among populations showed some certain differentiation. The eight populations had high level of genetic diversity and show apparent genetic differentiation. So that provenance selection has great potentiality.

Keywords: Different provenances, genetic diversity, *Jatropha curcas* L., seed morphology

INTRODUCTION

Jatropha curcas L., belonging to the family Euphorbeaceae, widely distributed tropical and subtropical areas (LI *et al*., 2009). *Jatropha curcas* is a multipurpose shrub with significant economic importance and having the capabilities to rehabilitate the degraded lands. At present, Jatropha's research is mostly concentrated in the areas of resource distribution, cultivation, grafting and nursery (Wenjun *et al*., 2008). *Jatropha curcas* widely used due to seed oil content as high as 40-50% and environmental protection so become a high quality woody biomass energy materials species, in recent years, caused extensive attention all over the world and set off a wave of development and utilization (Basha and Sujatha, 2009). Regarding Jatropha's research has made certain progress (Sudheer *et al*., 2009), but the basic biological research of *Jatropha curcas* seed is very limited, that makes big blindness in seed transfer aspect which seriously influence the scale cultivation and development.

As a result of *Jatropha curcas* distributed widely and distribution area discontinuous, in its distribution area due to the environmental selection pressure change, and caused geographical provenance of variation is inevitable. So to master the seed phenotypic variation regularity and provenance genetic relationship is the basis and precondition of identify the seeds, seeds regionalization, seeds inspection and sowing nursery work. Providing an important basis for the provenance test sampling and experimental design, and is also the basis for genetic breeding.

MATERIALS AND METHODS

Plant material: The experimental material for the present study consisted of eight *Jatropha curcas*

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Table 1: Origin of *Jatropha curcas* populations sampled

No. of pop	Locality	Altitude	East longitude	North latitude	Annual average temperature	Annual rainfall
JS	Jiangsu Suyang China	8~14 m	118°79′	34°12′	24°C	913 mm
GW	Guangxi Wuzhou China	1860 m	110°90′	23°60′	21°C	1503.6 mm
SC	Sichuan Panzhihua China	1980 m	101°56′	26°90′	19.2°C	1065.5 mm
YN	Yunnan Jinghong China	580 m	100°25′	21°90′	21.9°C	1500 mm
GL	Guangxi Liuzhou China	1241 m	109°40′	24°33′	20.1°C	1429.7 mm
ML	Oudomxay Province Laos	450 m	102°31′	19°33′	25°C	900 mm
LY	Shaye Wurie Laos	800 m	101°75′	19°25′	26°C	1250 mm
TG	Chiang Mai Thailand	305 m	98°58′	18°46′	22°C	1350 mm

Table 2: Sequences of the primer groups used in the present study

Primer	No.	Sequence (5'~3')
Selective primers	P_{234}	GTTTTCCCAGTCACGACCAG
Selective primers	P_{222}	GTTTTCCCAGTCACGACGT
Selective primers	P_{221}	GTTTTCCCAGTCACGACGC
Selective primers	P_{235}	GTTTTCCCAGTCACGACCAC
Selective primers	P_{243}	GTTTTCCCAGTCACGACTCGA
Reverse primer	R_2	AACAGCTATGACCATGA

populations accessions collected from different parts of China and Loas and Thailand. The list of population and their collection sites are furnished in Table 1.

Measuring method:

The 100-seed weight: The randomly selection 100 seeds each population and weigh it in the balance. Repeat it six times (Li *et al*., 2010).

Seed size: The randomly selection 30 seeds each population and measure seed length, width, lateral diameter for each seed by a vernier. Repeat it three times (LI *et al*., 2010).

DNA extraction: DNA isolation was performed on ten grams of leaf tissue ground in liquid nitrogen. Total genomic DNA was extracted individually from younger leaves of eight accessions of *Jatropha curcas* populations following the standard CTAB method with minor modifications (Doyle and Doyle, 1990). DNA concentrations were determined electrophoretically versus known amount of λ DNA as standards. For PCR, DNA samples were adjusted to a concentration of 2.5 ng/μL.

DALP analysis: Five primers (P1Pr1, P2P r1, P3P r1, P4P r1, P5P r1, Table 2) were used for amplification of DNA according to the method of Desmarais *et al*. (1998). PCR amplification was carried out in a 20 μL reaction mixture containing 2.5 ng of genomic DNA, 1 × PCR buffer containing 1.5 mM MgCl2, 0.5 μM of primer, 100 μM of each of the four dNTPs and 2 U of Taq DNA polymerase. PCR amplification was carried out in Perkin-Elmer 9700 thermaocycler (PE Corp., USA) with an initial denaturation at 95°C for 5 min followed by 36 cycles of denaturation at 94°C for 45 sec, annealing at 61°C for 30 sec and extension at 72°C for 2 min with a final extension at 72°C for 10 min.

The amplified PCR products were resolved by electrophoresis on 6% non-denaturing polyacrylamide gels (polyacrylamide: methylene bisacrylamide = 29:1) in 1 × TBE buffer by electrophoresis at 800 V for 150 min. Electrophoresed gel was detected by quickly silver staining and computer scanning. Every PCR reaction was repeated twice to check reproducibility of bands and a negative control (no DNA) was used in all reactions to avoid erroneous interpretations (Table 2):

Statistical analysis: The data of seed phenotypic traits were input into computer by a trained worker and analyzed by statistic software STATA 8.0, EXCEL and SPSS13.

DALP amplified fragments, with the same mobility according to the molecular weight, were scored by eyes for the presence (1), negative (0), or absence (.) of homologous bands. The resulting presence /negative /absence data matrices of the DALP phenotypes were analyzed using Popgene version 1.31 (Yeh *et al*., 1999) to estimate genetic diversity parameters: Percentage of Polymorphic Bands (PPB), Shannon's Index of phenotypic diversity (I), mean observed Number of alleles (Na), mean effective Number of alleles (Ne), Nei's gene diversity (H), total gene diversity (Ht), gene diversity within population (Hs), the coefficient of Gene differentiation (Gst) and the level of gene flow (Nm). To examine the genetic relationship at the species and population level, dendrograms were constructed by an Unweighted Pair Group Method of cluster Analysis using arithmetic averages (UPGMA) on the basis of the matrix of Nei (1978) unbiased genetic distance with Popgene. The tree was subsequently visualized with Treeview (Roderic, 2001).

RESULTS

Phenotypic traits: The same species or varieties seed size was considered to be relatively stable. Within populations, among populations or even individuals, seed size that there will be differences (Li *et al*., 2010).

Table 3: Results of variance analysis of seed phenotypic traits

Traits	Type III S.S.	df	M.S.	F	p
Seed length (mm)	50.510	7	7.210	9.46	0.000**
Seed width (mm)	14.343	7	2.040	7.24	0.000**
Lateral diameter (mm)	9.7170	7	1.380	3.87	0.001**
Seed length and width ratio	0.1600	7	0.023	3.15	0.004**
The 100-seed weight (g)	7344.30	7	1049.18	7.56	0.000**

**: Indicated variation analysis of significant at 1% levels respectively

Table 4: Multiple comparisons (LSD) of seed morphology of eight populations

Population code	Seed length (mm)	Seed width (mm)	Lateral diameter (mm)	Seed length and width ratio	The 100-seed weight (g)
GL	16.90a	10.13a	7.84a	1.67b	56.85ab
GW	17.05a	10.67bc	8.37b	1.60a	57.66ab
JS	17.15a	10.41ab	8.51b	1.65ab	59.20ab
LY	18.25bc	11.16d	8.62b	1.63ab	73.00c
ML	17.78b	10.92cd	8.49b	1.63ab	65.60b
SC	17.99bc	10.74bc	8.44b	1.68b	59.30a
TG	18.43c	10.93cd	8.73b	1.69b	73.14ab
YN	17.13a	10.75bc	8.44b	1.59a	55.85a

a, b, c, d: Indicated LSD analysis of significant at 1% levels respectively

Table 5: Correlation analysis of phenotypic traits of seeds in *Jatropha curcas*

Traits	Seed length	Seed width	Lateral diameter	Seed length and width ratio	The 100-seed weight
Seed length	1				
Seed width	0.54**	1			
Lateral diameter	0.55**	0.41**	1		
Seed length and width ratio	0.49**	-0.45**	0.17*	1	
The 100-seed weight	0.48**	0.37**	0.25**	0.126	1

*, **: Indicated significant correlation at 5% and 1% levels respectively

The results of variance analysis about the related characters of seed showed that each character of hundred seed weight, seed length, width, lateral diameter, seed length and width ratio was significantly different from eight different provenances. The result indicates that the seed morphology among populations showed some certain differentiation. Further summed up the data with LSD method, to reveal differences between different origin seed morphology (Table 3):

LSD multiple test results (Table 4) showed the 100-seeds weight in Chiangmai of Thailand population (TG) is the maximum (75.00 g), minimum is Panzhihua of Sichuang population (SC) and Jinghong of Yunnan population (YN). The Jatropha's seed size and shape in different producing area also have significant differences, from different origins of seed length, width, lateral diameter, side track performance view, the longest and larger seeds from Chiang mai, Thailand population (TG); followed by the Laos Shaye Wurie populations (LY); The average individual minimum is Guangxi Liuzhou population (GL) seeds, which the length, width, lateral diameter are significantly less than the other origins.

Seed length and width ratio as seed morphology research index reflected the shape of seed. Eight origins' seed length and width ratio changed between 1.59~1.69, LSD analysis shows that GL, SC, TG populations ratio of seed's seed length and width is the

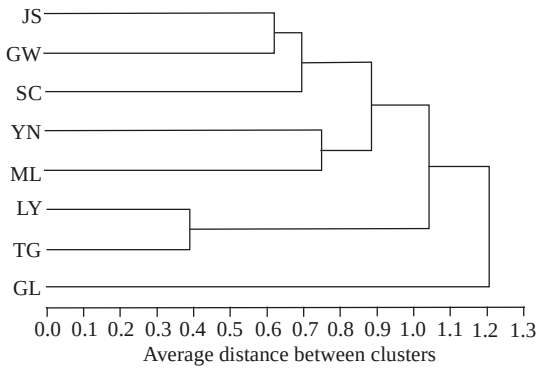

Fig. 1: The phylogenetic tree with AVE method of the seed populations sampled in *Jatropha curcas*

largest, those populations have extremely significant difference compare with others. The TG population seeds are the longest with lateral diameter is 8.73 mm, explain the seed shape approximately is long cylinder; and the seeds of GL population's seed length and width ratio (1.59) is the minimum, which was short cylindrical shape, other origin seeds ratio fall in between them. From different producing areas there were significant differences between seed traits, indicating that the geographical provenance of some genetic differentiation between the potential and the need for provenance selection.

Table 6: Genetic diversity of *Jatropha curcas* population

Population	Total number of loci	No. of polymorphic loci	PPB ① (%)	Na ②	Ne③	H④	I ⑤
JS	244	65	26.64	1.2664	1.0810	0.0577	0.0968
GW	244	60	24.59	1.2459	1.0706	0.0508	0.0859
SC	244	43	17.62	1.1762	1.0540	0.0366	0.0613
YN	244	49	20.08	1.2008	1.0792	0.0501	0.0800
ML	244	35	14.34	1.1434	1.0688	0.0433	0.0671
LY	244	24	9.840	1.0984	1.0428	0.0278	0.0439
TG	244	79	32.38	1.3238	1.1270	0.0824	0.1318
GL	244	71	29.10	1.2910	1.0943	0.0658	0.1091
Average	244	53.25	21.82	1.2182	1.0772	0.0518	0.0845
At species level	244	219	89.75	1.8975	1.4930	0.2878	0.4331

①: Percentage of Polymorphic Bands (PPB); ②: Observed number of alleles; ③: Effective number of alleles

Correlation analysis of phenotypic traits: Analysis of correlation showed there were significant correlation ship between hundred seed weight and seed length and width and lateral diameter, indicating that the size of the seed directly affect the weight of the seed (Table 5).

Clustering analysis of seed characters: The method of UPGMA were applied to analyze to the seed morphology of the eight populations in clustering (Fig. 1). The results of the cluster analysis showed that the seed from eight different populations divided into three categories: The first class is the small seed size and weight types, including JS, GW, SC, YN, ML populations; The second class is the larger seed types, including LY and TG populations. Guangxi Liuzhou populations (GL) alone for a class, the seed was significantly less than the seeds of other populations.

DALP data analysis:

Genetic diversity: Using Popgene version 1.31 (Yeh *et al*., 1999) to estimate genetic diversity parameters. In the eight populations of *Jatropha curcas*, a total of 244 DNA fragments ranging from 100 to 3000 bp were amplified with five primer groups, averaging 48.8 DNA fragments per primer groups. Altogether 219 were polymorphic among 244 DNA fragments, the Percentage of Polymorphic Bands (PPB) was 89.75% at the species level. The average number of DNA polymorphic band amplified by each primer groups was 43.8 in 8 populations, the average PPB was 21.82% at the population level. At the species level, the mean observed Number of alleles (*Na*) was 1.8975, the mean effective Number of alleles (*Ne*) was 1.4930, the mean Nei's gene diversity (*H*) was 0.2878, the Shannon's Index of phenotypic diversity (*I*) 0.4331. At the population level, *Na* was 1.2182, *Ne* was 1.0772, H was 0.0518, and *I* was 0.0845 (Table 6).

Genetic differentiation: Total gene diversity (*Ht*), gene diversity within the population (*Hs*), the coefficient of gene differentiation (*Gst*) and the level of gene flow (*Nm*) were computed with Popgene Version 1.31 (Yeh *et al*., 1999) and shown in Table 7. The *Ht* was 0.2878 and *Hs* was 0.0518. Moreover, the *Gst* was 0.8200, which could be interpreted as 82% of the total genetic divergence was attributed to among populations and the rest 18% was within populations. Meanwhile, it indicated that genetic divergence was extremely high among populations of *Jatropha curcas*. According to the value of *Gst*, it was estimated that the gene flow of populations (McDermott and McDonald, 1993) was 1.097 in *Jatropha curcas* (Table 7):

Table 7: Genetic differentiation among populations of *Jatropha curcas*

Species	Ht*	Hs*	Gst*	Nm*
Jatropha curcas	0.2878	0.0518	0.8200	0.1097
S.D.	0.0321	0.0022	-	-

*Ht: Total gene diversity; *Hs: Gene diversity within population; *Gst: The coefficient of gene differentiation; *Nm: Estimate of gene flow from Gst or Gcs. e.g., Nm = 0.5 (1 - Gst) /Gst

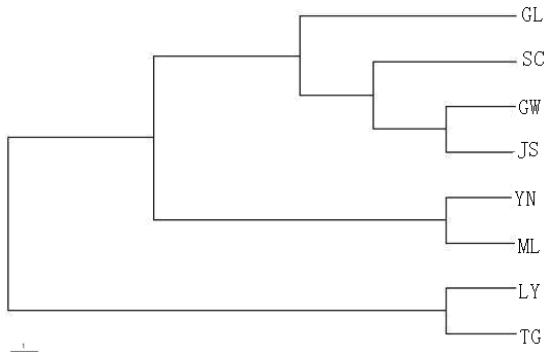

Fig. 2: UPGMA dendrogram of *Jatropha curcas* populations based on Nei (1978) genetic distance

Genetic relationship: The relationship of genetic identity among the eight populations of *Jatropha curcas* was shown in the UPGMA dendrogram (Fig. 2). The eight populations of *Jatropha curcas* were separated into three major groups, the first including pop LY and TG, and the second including pop YN and

ML and the third including pop GL, SC, GW and JS. It was suggested that there is obvious correlation between geographical distances and genetic relationships of the eight populations (Fig. 2)

DISCUSSION

The different provenances seed traits were significantly different. Multiple comparisons (LSD) of seed morphology showed seed size and shape were different among provenances. Correlation analysis of phenotypic traits indicating that the size of the seed directly affect the weight of the seed. It has been verified that the variance among provenances are obvious, and therefore provenance selection and breeding of fine varieties has great potentiality and necessity.

The *Ht* was 0.2878 and *Hs* was 0.0518. Moreover, the *Gst* was 0.8200, which could be interpreted as 82% of the total genetic divergence was attributed to among populations and the rest 18% was within populations. Meanwhile, it indicated that genetic divergence was extremely high among populations of *Jatropha curcas*. There are a great deal of variation between different provenances of *Jatropha curcas* and rich genetic diversity, and can provide germplasm for further breeding program and related studies.

The relationship of genetic identity among the eight populations of *Jatropha curcas* similar with morphological grouping results, and there is obvious correlation between geographical distances and genetic relationships of the eight populations. Therefore, seed traits can be used as an important indicator to distinguish provenances, and moreover, which can provide important reference value for provenance pre selection to breed and allocation of seed, etc.

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