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Genetic diversity assessment in physic nut (*Jatropha curcas* L.)

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Abstract

Mahalanobis' D-square (D^2) statistics was applied to assess diversity in the 9 genotypes collected of semi-arid region of India (7 genotypes from Gujarat and Rajasthan for normal toxic and two from Orissa csmcri's plantation of non toxic nature. These genotypes were grouped into five. Cluster I and III had two genotypes, cluster II had three genotypes and cluster VI and V contributed as solitary germplasms. The genotypes in cluster II had the maximum divergence which was closely followed by cluster III. The maximum and minimum divergence was revealed between clusters I with cluster V and cluster I and cluster V with cluster IV, respectively. In general, cluster III and IV exhibited high and low mean values, respectively for most of the characters. It has been suggested that for varietal improvement, hybridization among the genotypes of divergent clusters should be done in order to obtain better results in terms of variability and diversity.

Keywords: Jatropha; *J. curcas;* Cluster pattern; D-Square analysis

Introduction

Jatropha curcas Linn. (Tropical physic nut) belonging to family Euphorbiaceae is a native species of tropical America, cultivated throughout the tropics and is sub-spontaneous in Mauritius and Seychelles (Baker, 1877). It has gained attention in tropical and subtropical countries as a potential bio-fuel crop. Major sources of bio-diesel include rapeseed (USA), sunflower (Italy and Southern France), soybean (USA and Brazil), Oil palm (Malaysia), linseed (Spain), cotton seed (Greece), beef tallow (Ireland) and Jatropha (Nicaragua and South America) (Jayasingh, 2004). Some Jatropha species are grown in the greenhouses for their ornamental leaves and flowers, while others are grown in the tropics for their economical use (anonymous 1959; Heller 1996), of late, the genus Jatropha has attracted the attention of several workers, particularly the species *J. curcas*, for the use of its seed oil as a commercially viable alternative source of fuel (Takeda 1982; Benerjee et al., 1985; Martin and Mayeux 1985; Openshaw 2000).

Physic nut *(Jatropha curcas)*, a drought perennial shrub has evoked interest all over the world as potential. The wide gap in potential and actual yield is due to the use of locally available 'wild' material. The limitations with the currently used germplasm in India is the lack of knowledge on the genetic base, poor yield, low genetic diversity and vulnerability to a wide array of insect pests and diseases under monoculture. No systematic breeding program for breeding superior high yielding genotypes has been initiated. Vast scope lies for genetic improvement of the Jatropha through genetic diversity study was done to understand the diversity in different germplasms for assessment and creation of diverse lines for future breeding.

Materials and methods

Climate and soil

This study was conducted at highly eroded forest land on hill slope (undulated) at Jatropha Research Station of Phytosalinity discipline, Central Salt and Marine Chemical Research Station (CSIR) Chorvadla, Bhavnagar (Gujarat, India; 21° 40' N, 071° 47' E) at an altitude of approximately 682 feet (207.9 meter). In this areas climate, mean annual rainfall averages 650 mm, the rainfall or South West Monsoon commences on middle June and extends till to September end/October beginning. More than 90 per cent of the total rainfall gets receives during the above 14-15 weeks period, while rest 38 weeks of the year remains dry. Even during these 14 weeks, the number of rainy days hardly exceeds 4 weeks. Average annual potential Evapo-transpiration (PET) is 1800 m, maximum and minimum temperature are 31.1 and 22.8°C, respectively. There is no salinity or alkalinity problem in this region. The soil is a gravelly sandy clay loam. The excessive drainage, rapid permeability, shallowness of the soil, lithic contact at a very shallow depth, presence of rock out crops in moderate amounts ultimately have resulted in and with poor irrigation and land capability

Survey and collection

Surveyed of Jatropha had been carried out extensively in the forest of Gujarat and seven normal/toxic genotypes selected on the phynotyppes, two non-toxic genotypes (PC-26, PC-27) were also collected from experimental site of csmcri, Gopalpura, Orissa.

Healthy intermediate branches (with pale green color and slender whitish pots) of *Jatropha curcas* was selected and collected through the field surveys representing the states of Rajasthan and Gujarat and performance from nursery stage (3 month) to first yield (1.5 years old) plants. Each parental plant chosen randomly from each population, which were situated about 40m apart from each other. A uniform pretreatment was given to the branch cutting prior to sowing them in Liquid Sea Weed Fertilizer (18 amino acids, macro-elements such as nitrogen, phosphorus and potassium, more than 40 microelements, natural growth regulating substances, such as gibberellic acid,) for speedy root initiation- kept soaked for 24 hours at 5% concentration. Branch cuttings were sown directly in polythene bags (one cutting per bag) containing potting mixture of sand, soil and farm yard manure in the ratio of 1:1:1 (by volume) in three replication of 100 cutting each using completely

randomized block design as per ISTA rules (1996). Prior to sowing, the polythene bags containing potting mixture were treated with fungicide (0.2% Bavistin) and kept covered with plastic sheet for 24 hours. Daily sprouting observation was taken still sprouting in all treatment was completed (up to 20 days after sowing) branch cuttings were maintained in the nursery till their field planting. Four month old branch cuttings were planted in the field (pit) in July 2003 in and completely randomized block design with three replications and 3 m distance. All the necessary cultural practices were followed for raising good stand of Jatropha. The observations on five randomly selected plants in each population were recorded on 10 quantitative traits viz., plant height, plant canopy, coral diameter, number of primary branches, number of secondary branches, number of tertiary branches, average leaf area, petiole length, flower bud setting and basal height for 1st branch initiation.

Extraction of seed oil was carried out following the method of Anon (1965); in which the seed kernels were separated and powdered. The oil was extracted from the samples with known weight in chemical extraction using hexane (50-80 ^{o}C) for 6 hours without interruption by gentle heating it. Hexane was evaporated on a water bath until remained. The oil yield was expressed in term of percentage of powdered sample.

The genetic diversity was estimated using the Mahalanobis D^2 statistics (Mahalanobis, 1936). Tracing D^2 as a generalized distance, the criterion used by Tocher as described by Rao (1952) was applied for determining the clustration group. Average intra and inter cluster distances were determined using GENRES version 3.11, 1994 Pascal Intl. Software and suggested by Singh and Chaudhary (1977).

Results and discussion

The analysis of variance showed significant differences among the genotypes in respect of all characters and indicated high genetic variability. The differences among the genotypes were also found when aggregate effect of all characters was tested by Wilk's criterion. The D^2 values for all 64 comparisons between pairs of genotypes are given in table 1. On the basis of divergence, 9 genotypes under investigation have been grouped into five clusters (Table 2), indicating wide diversity in the experimental material for majority of the characters. Maximum number of genotypes (3) was included in cluster-II. The cluster I, and III contained two genotypes each. Cluster IV and V both had a solitary genotype. This pattern of clustering indicated that there was no association between eco-geographical distribution of genotypes and genetic diversity as genotypes selected under diverse locations, gets cluster together. This kind of genetic diversity might be due to differential adoption, selection criteria, selection pressure and environment (Vivekananda and Subramanian, 1993). This indicated that genetic drift produce greater diversity than the geographic diversity (Singh et al., 1996; Selvakumar et al., 1989). The divergence within the cluster indicates the divergence among the genotypes in the same cluster. On the other hand, inter-cluster divergence suggests the distance (divergence) between the genotypes of different clusters (table 3). The tendency of genotypes from diverse eco-geographic regions to group together in the same cluster or scattered distributions of genotypes of same geographic origin in different clusters have been observed in the present study.

Table 1. Value of D^2 for combinations of 9 genotypes in *Jatropha curcas* L.

	1	2	3	4	5	6	7	8
1	0.00	113.05	210.63	497.41	339.94	352.66	294.68	233.24
2		0.00	176.57	382.91	284.86	470.39	425.72	275.65
3			0.00	259.49	291.75	292.71	195.80	239.22
4				0.00	153.48	742.04	718.25	578.41
5					0.00	920.15	822.23	702.24
6						0.00	47.65	48.66
7							0.00	113.55
8								0.00

Table 2. Clustering of *Jatropha curcas* genotypes using Tocher's method.

Cluster	Genotypes	No of genotypes
I	Ranpur and Rintoda	2
II	Buret, Chikla and Kgr	3
III	Pc-26 and Pc-27	2
IV	Shamlaji	1
V	Znjmer	1

The clustering pattern did not indicate any relationship between genetic diversity and geographical distribution. The result is in accordance with the findings of kumar (1991) and Swain and (1997), cluster II contained three genotypes and showed maximum intra cluster distance (12.913, table 3) because the genotypes used for breeding program were from different locations. Thus these three genotypes in cluster II were most heterogeneous and this cluster was best for within group hybridization. Genotypes from this cluster could also be exploited in hybrid development program, due to their wide intra-cluster distances within the group genetic distance, closely followed by cluster-III.

With regard to inter-cluster distance (Table 3), cluster V, showed maximum genetic distance from cluster I (50.02) followed by cluster V with cluster VI (46.47) suggesting wide diversity between these groups. Hybridization between parental lines selected from these clusters is likely to produce most variable progeny. Gohil and Pandya (2006) have also pointed out in *Salicornia brachiata* Roxb (a non traditional Oilseeds) that selection of parents for hybridization should be done from two clusters having wider inter-cluster distance to get maximum variability.

Table 3. Average intra and inter-cluster D^2 values and distance*.

	1	2	3	4	5
I	6.90	18.40	28.29	9.00	50.02
	(47.64)	(338.66)	(800.66)	(81.10)	(2502.07)
II		12.91	18.51	15.79	37.09
		(166.75)	(342.72)	(249.37)	(1376.36)
III			12.389	25.30	38.38
			(153.48)	(640.32)	(1473.54)
IV				0.00	46.47
				(0.00)	(2160.07)
V					0.00
					(0.000)

*= figures give in the parenthesis are distance D^2 values.

Table 4. Cluster wise mean values of fourteen characters in *Jatropha curcas* L.

Cluster	I	II	III	IV	V	Percent contribution
Plant height (cm)	128.91	124.61	153.53	119.43	137.66	5.6
Plant canopy (cm)	245.55	277.77	410	259.44	393.33	0
Collar diameter (cm)	13.57	13.16	16.75	12.08	15.21	2.8
No of primary branches	2.92	3.08	4.53	3.11	3.73	0
No of secondary branches	5.37	7.21	7.78	6.19	7.63	0
No of tertiary branches	7.45	9.04	7.36	5.95	11.11	0
No of Leaves	232.5	249.44	410	115	266.66	0
No of capsule	11.46	10.54	34.81	12.36	12.48	0
No of seeds	48.77	28.31	39.5	38.96	22.5	0
Basal height for 1st branch	10.45	6.87	4.15	9.25	7.5	0
Average seed wt.	0.505	0.482	0.577	0.49	0.83	0
Seed (g.)	23.77	15.82	63.2	18.54	12.65	0
Oil content (Kernel)	54.83	44.32	51.71	56.23	13.00	33.3
Oil content (Seed)	27.15	24.04	29.53	25.2	8.1	58.3

As far the cluster means are concerned, there was a wide range of variation observed for the entire characters (Table 4) cluster III had high mean value for plant height (153.53), plant canopy (410), collar diameter (16.75), number of primary branches (4.53), number of secondary branches (7.78), number of leaves (410), number of capsule (34.81), seed in gram (63.2) and seed oil content (29.53). Cluster V possessed good average seed weight (0.820) and (0.83), number of tertiary branches (11.11). Character contributing to highest oil kernel based content (56.23) was found in genotype Shamlaji (cluster-IV). Cluster I had high number of seeds (48.77) and basal height for 1st branch initiation (10.45). This indicated that cluster III contained genotypes with most desirable characters which could be directly selected and utilized for breeding program.

Cluster I showed the minimum intra genetic distance (6.90) between them revealing that these genotypes were somewhat similar in genetic constitution and hybridization amongst these groups not showing sufficient variability.

Contribution of characters to diversity

The contribution of individual characters to the diversity has been worked out in terms of number of times it appeared first (Table 4). The most important trait contributing maximum genetic diversity on the bases of appearance was seed oil content (58.33%, 21) followed by kernel based Oil content (33.3%, 12) as per cent contribution and rank total, respectively. The character contributing maximum diversity can be given more emphasis for the purpose of fixing priority of parents in hybridization program. It is suggested to effect crosses between genotypes selected from most distant clusters with high mean performance to get desirable transgressive segregants.

In general, the cluster III and IV exhibited high and low mean values, respectively for most of the characters (Table 4).

It is also suggested that for creating variability and developing the best selection a large number of divergent lines, instead of few should be used in the hybridization.

Conclusion

On the bases of observations recorded here on a divergent group of Jatropha genotypes, it is concluded that hybridization between the genotypes of variable clusters specially cluster – III, IV and V may help to produce wide spectrum of variation in the segregating progeny and thus may be helpful in hybridization program to cope up the fore coming limitations pertaining to improvement of bio-fuels species for bio-diesel industry.

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