

# Genetic associations, variability and diversity in seed characters, growth, reproductive phenology and yield in *Jatropha curcas* (L.) accessions

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**Abstract** A thorough and extensive wild germplasm exploration survey was undertaken and 32 high yielding candidate plus trees (CPTs) of *Jatropha curcas* from different locations from a latitudinal and longitudinal spread between 12°41' and 22°E longitude and 77° and 84°40'N latitude covering 11 locations in an area spread of 150,000 km<sup>2</sup> was collected for evaluating genetic association, and variability in seed and growth characters. Significant trait differences were observed in all the seed characters viz., seed morphology and oil content as were observed in growth characters viz., plant height, and female to male flower ratio and seed yield in the progeny trial. Broad sense heritability was high in general and exceeded 80% for all the seed traits studied. Female to male flower ratio showed near to 100% heritability followed by yield (83.61) and plant height (87.73). The path analysis revealed that female to male flower ratio had highest positive direct relationship with seed yield (0.789), followed by number of branches (0.612) and number of days from fruiting to

maturity (0.431). Negative indirect effects were seen in number of days from flowering to fruiting which indirectly and negatively influenced yield through plant height and number of branches. Hierarchical clustering by Ward's minimum variance cluster analysis showed phylogeographic patterns of genetic diversity. *K*-means clustering revealed that trees from different geographic regions were grouped together in a cluster and as were trees from the same geographical area placed in different clusters suggesting that geographical diversity did not go hand in hand with genetic diversity. In addition clustering identified promising accession with favourable traits for future establishment of elite seedling seed orchard and clonal seed orchard for varietal and hybridization programmes.

**Keywords** Biodiesel · Diversity · Germplasm · Phylogeography

## Introduction

The oil yielding plant *Jatropha curcas* (L.) (*Jatropha*) or physic nut is a multipurpose and drought resistant large shrub or small tree. It is a native of tropical Central America; it has now been domesticated in a widespread manner in Africa and Asia mainly due to its ability to grow in a number of climatic zones in tropical and subtropical regions of the world particularly in marginal lands. *Jatropha* is easy to establish, grows relatively quickly and is hardy. *Jatropha* has immense economic potential and ecological and environmental significance. The uses of this crop range from traditional medicine for common human and animal ailments; protection against land erosion, as a boundary fence or live hedge to newly found highly economic potential of fossil fuel replacement

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(Openshaw 2000). This latter use has significant implications for meeting the demand for rural energy and counter greenhouse gas accumulation in the atmosphere. The biodiesel property of this crop has fuelled intense research in this crop in the recent years (Agarwal 2007).

In spite of these numerous favourable attributes, even half the complete potential of the crop *Jatropha* is yet to be realised. One of the important reasons for this apart from agronomic, socio economic and institutional constraints is the fact that there is a distinct lack of planned rational conventional breeding programs. The very fact that *Jatropha* has adapted itself to a wide range of edaphic and ecological conditions suggests that there exists considerable amount of genetic variability to be exploited for potential realisation. Ginwal et al. (2005) reported variability of *J. curcas* in central India which was mainly limited to seed source variation in morphology, germination and seedling growth. Kaushik et al. (2007) have reported divergence in seed oil traits of *Jatropha* from a limited number of locally collected accessions. Work on heritability estimates in growth and yield attributes is distinctly lacking in *Jatropha*, since heritability estimates in perennial plants decrease with age because of compounded environmental effects masking genotypic differences, there is an urgent need for the knowledge on these estimates in progenies. Comprehensive work on collection of germplasm and evaluation of growth, morphology, seed characteristics and yield traits is still in its infancy.

One of the important limitations for selection for improvement is the extent of diversity present in the germplasm. Quantitative characters such as yield and its determinants exhibit substantial degree of interaction with the environment, thus it is imperative to analyse the variability present in the germplasm and partition it into genotypic, phenotypic nature. Release of high yielding cultivars is impossible without ascertaining the magnitude of variation present in the available germplasm, interdependence of growth pattern with yield, extent of environmental influence on these factors, heritability and genetic gain of the material. Selection for improved biomass is best estimated by growth attributes and should be made during the first 2 years especially in trees and shrubs of perennial nature. Subsequently environmental effects will have caused too much variation to distinguish effectively between genotypes (Dierig et al. 2001).

Acquiring and documenting such in-depth knowledge of variability in perennials crops is a mammoth task. Although initial selection based on seed characters would simplify the task a great deal, there still will exist several limitations and doubts in these selected plants because of season and time bound expression of genes of interest.

Considering the multifarious uses of *Jatropha*, evaluation of progenies is an essential aspect which plays an

important role in developing standard agro techniques for this crop which is particularly lacking when it comes to agroforestry and block plantations (Carlowitz Von 1986). Considerable genetic variation in growth, chemical composition of seed and seed traits at the level of provenance, variety or progeny has been reported in most out-crossing multipurpose tree species such as *Albizia*, *Acacia*, and *Prosopis* (Costa et al. 2005; Wanyancha et al. 1994; El Amin et al. 2006; Goel and Behl 2001), which are widely used in agroforestry systems. The knowledge on this type of variation would be useful for genetic selection especially in a crop where the desired ideal type is yet to be defined due to its wide adaptability and end use pattern. Knowledge of genetic variation in branching pattern, female to male flower ratio, pest resistance, drought hardiness and yield attributes in wild germplasm of *Jatropha* can be of great potential in tree improvement programmes, particularly selection of genotypes having more oil content and yield.

The necessity to obtain adequate information on these aspects and to assess the magnitude of genetic variability and their corresponding heritability is imperative for any breeding program to succeed. Hence a large scale collection of wild germplasm from selected plus trees, conservation and evaluation program of various *Jatropha* accession was taken up with the objectives of progeny trial, genetic analysis of variability, broad sense heritability and genetic advance in order to estimate the extent to which improvement is possible through selection. Further, the aim of such attempts is to establish seedling seed orchard and/or clonal seed orchard to meet the requirement of nursery stock from elite planting material to cater to the massive biodiesel plantation programmes.

## Materials and methods

A thorough and extensive wild germplasm exploration survey was undertaken to identify high yielding candidate plus trees (CPTs) of *Jatropha* from different locations in Andhra Pradesh, India. Targeted districts were Adilabad, Chittoor, Cuddapah, Anantapur, Kurnool, Nellore, Prakasam, Guntur, Visakhapatnam, Vizianagaram and Srikakulam covering a latitudinal and longitudinal range between 12°41' and 22°E longitude and 77° and 84°40'N latitude. Collection of seeds/cuttings from selected plus trees were done during the fruiting season of *Jatropha* in 2004–2005 and 2005–2006. Criteria advocated by Cornelius (1994) were followed for selection of plus trees. The selection were made on phenotypic assessment of characters of economic interest, i.e., yield potential, number of capsules per cluster, branching pattern, crown spread, girth, disease resistance etc. Plants yielding more than 2 kg of

seed plant<sup>-1</sup> (dry seeds) and of more than 5 years of age were selected as plus trees. Care was taken to avoid trees infested with pests and diseases and isolated trees or wolf trees. Seed characteristics were analysed after seeds were separated from the fruit, cleaned and stored in muslin cloth bags at ambient conditions (26°C and 55% RH). All seed lots were dried in hot air oven at 60°C till constant weight. Five samples were drawn from each seed lot and 100 random undamaged seeds (total 500 seeds) were measured for their length, width and thickness. The oil content of 20 seeds was estimated by Soxhlet method using three replicates for each seed lot.

After collection, the germplasm was brought to Central Research Institute for Dryland Agriculture (CRIDA) and the seed/cuttings of germplasm of *Jatropha* collected during 2004–2005 and 2005–2006 were raised in the nursery for laying out trials in nursery as well as in field conditions to identify elite germplasm. Progeny trial (field and cultural conditions in Appendix I) with 29 progenies selected from the collected germplasm on the basis of oil yield and growth parameters in the nursery was initiated with three replications. Nine plants of each accession were planted at a spacing of 3 m × 3 m.

#### Recording of data

Data were recorded on all the 9 plants (aged 34 months) in each germplasm line in each replication for the following morphological traits:

1. Plant height (cm): the average height from the ground level to the tip of the inflorescence on the main stem at the time of harvesting was measured.
2. Branches/plant: the total number of branches growing from the main stem at different node positions, including the basal branches.
3. Flowers/plants: all the flowers in all the inflorescence per plant was counted at flower induction stage, i.e., during 2nd fortnight of June.
4. Female to male flower ratio was recorded by selecting the nine inflorescences randomly on each plant from the opening of the flowers to completion of flowering in each selected inflorescence by counting male and female flowers daily.
5. Days taken from initiation of flowering to fruiting: days were counted and recorded after emergence of 80% flowering till of 80% fruiting.
6. Days taken from fruiting to maturity: days were counted and recorded after emergence of 80% fruiting till physiological maturity of 80% of the fruits [~50 days after anthesis (DAA)].
7. Yield was recorded as of the seeds per plant.

#### Statistical analysis

The raw data was compiled by taking the means of all the nine plants taken from each line and replication for different traits in the experiment. The means were subjected to further statistical and genetical analysis. All statistical parameters, viz., mean, standard error, variance and coefficient of variation were analysed statistically using the software package Systat<sup>®</sup> for windows (Wilkinson et al. 1996). Correlation analysis was performed to determine the relationships between oil content and seed characteristics in seeds and growth, reproductive phenology and yield in progeny trials and all the component traits, both at genotypic and phenotypic levels according to Johnson et al. (1955). The variability, genetic advance as per cent of mean, phenotypic and genotypic variance, phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were worked out for seed and oil content and growth traits as suggested by different workers (Baezniger et al. 2004; Pliura et al. 2007; Yoshida et al. 2007). Path coefficients were then computed according to De Rodriguez et al. (2001) to separate the direct and indirect effects of correlation coefficient. A significant positive correlation between a trait and oil content yield, high positive direct effect by that trait on oil content/yield and minimal negative indirect effect by that trait on oil content/yield via other traits, were the three factors that categorised the trait as effective for selection for yield improvement.

Broad sense heritability in all the accessions was estimated by partitioning the variance in measurements into between-accessions and within-accessions components and applying these in the following function (Falconer and MacKay 1996).

$$H = \frac{\sigma^2g}{\sigma^2p}$$

$\sigma^2g$  is genotypic variance = (variance between-accessions – variance within-accessions)/ $n$ .

$\sigma^2p$  is phenotypic variance = [(variance between-accessions – variance within-accessions)/ $n$ ] + variance within-accessions.

$n$  = number of replicates per treatment.

Genetic advance was calculated as the difference between the genotypic mean of selected lines and genotypic mean of population. Genetic advance as percentage of mean was calculated by the following formula (Bhargava et al. 2007):

$$\text{Genetic advance(\%)} = \frac{\text{genetic advance(trait)}}{\text{mean(trait)}} \times 100$$

Both hierarchical Euclidian cluster analysis and non-hierarchical ( $K$ -means) Euclidian cluster analysis was done

in both initially collected wild germplasm and progeny trial.

The broad genetic divergence was calculated by using non-hierarchical Euclidian cluster analysis (Sachan et al. 2004). The Euclidean distances were calculated by the Wards method and dendrogram were constructed to examine the relationships within and between populations (Karon' ski and Calin' ski 1973; Sokal and Rohlf 2003).

## Results

### Variability in seed characteristics

Analysis of variance in the seed characters revealed that there was significant variation among all the wild accession studied in all the characters at  $P > 0.05$ . Maximum seed width (Table 1) of 2.25 cm was observed in CRDJ31 followed by 2.08 in CRDJ1. Minimum seed width was 1.77 cm in CRDJ18. Seed length had a range of 1.55–1.87 in CRDJ21 and CRDJ30 respectively. Hundred seed weight and oil content varied from 56.98 to 79.09 and 29.85 to 37.05 respectively in the *Jatropha* accessions. CRDJ1, CRDJ6 and CRDJ20 showed highly significant values for 100 seed weight as compared to other accession. The low ranking accession showed significantly different values from the rest. With regard to oil content percentage it was seen that CRDJ1, CRDJ17, CRDJ20, CRDJ21, CRDJ23, CRDJ27, CRDJ28 and CRDJ31 had more than 35% oil in seeds. The low ranking ones with less than 32% oil were CRDJ7, CRDJ11, CRDJ14, CRDJ24, and CRDJ25. Highest variance was seen in 100 seed weight (34.10) among all the characteristics recorded followed by oil content.

### Variability in growth and yield characteristics

Significant variation was observed in plant height recorded during the growth of progeny of 29 seeds selected from the initial 32 wild accessions of *Jatropha* germplasm (Table 2). Maximum plant height of 134.0 cm was recorded in CRDJ24 followed by CRDJ26 which recorded a height of 128.6. Low ranking group of less than 110 cm height consisted of 13 accession which were CRDJ4, CRDJ5, CRDJ6, CRDJ9, CRDJ11, CRDJ12, CRDJ13, CRDJ16, CRDJ17, CRDJ18, CRDJ19, CRDJ20, and CRDJ30. There was significant variation among the accession with regard to the number of branches in the plant with a mean of 6.33 branches per plant observed. The highest number of branches (12.25) was seen in CRDJ23 and was followed by only three other accession having more than 10 branches CRDJ19, CRDJ20 and CRDJ2 which had 10.0, 10.2, and 10.8 branches respectively. The number of flowers had a high degree of variability as CRDJ8 had 299 flowers which

**Table 1** Mean values for various seed characteristics of different *J. curcas* accessions

Acc. no	Length (cm)	Width (cm)	100 Seed weight (g)	Oil content(%)
CRDJ1	1.75	2.08	77.35	35.7
CRDJ2	1.68	1.87	63.46	34.32
CRDJ3	1.75	1.90	70.81	33.12
CRDJ4	1.75	2.10	75.6	34.68
CRDJ5	1.76	1.89	59.26	32.51
CRDJ6	1.72	1.95	79.09	34.48
CRDJ7	1.76	1.90	66.87	31.67
CRDJ8	1.79	1.85	64.31	32.52
CRDJ9	1.86	1.97	69.95	34.84
CRDJ10	1.71	1.80	61.24	34.42
CRDJ11	1.60	1.86	63.12	31.77
CRDJ12	1.65	1.92	74.34	32.08
CRDJ13	1.78	1.89	75.53	32.65
CRDJ14	1.72	1.89	72.14	30.53
CRDJ15	1.79	1.97	56.98	32.25
CRDJ16	1.79	1.95	70.21	32.14
CRDJ17	1.79	1.93	68.82	35.12
CRDJ18	1.55	1.77	65.11	34.17
CRDJ19	1.84	1.97	67.8	34.5
CRDJ20	1.76	1.94	78.34	36.51
CRDJ21	1.56	1.93	69.34	37.05
CRDJ22	1.70	1.87	72.34	32.06
CRDJ23	1.63	1.82	61.44	35.97
CRDJ24	1.74	1.95	57.11	30.66
CRDJ25	1.76	1.94	65.48	29.85
CRDJ26	1.67	1.82	67.87	32.9
CRDJ27	1.79	1.93	65.69	36.99
CRDJ28	1.73	1.91	67.39	35.51
CRDJ29	1.65	1.84	67.82	32.72
CRDJ30	1.87	1.93	66.98	32.92
CRDJ31	1.74	2.25	74.81	35.44
CRDJ32	1.80	1.96	68.85	35.6
LSDat 0.05	0.312	0.29	4.93	2.86

were almost three times compared to the lowest ranker CRDJ12 which had only 106 flowers. Eleven accessions namely CRDJ7, CRDJ8, CRDJ10, CRDJ11, CRDJ16, CRDJ17, CRDJ21, CRDJ24, CRDJ29, CRDJ32, CRDJ1 recorded more than 200 flowers and the rest had less than 200 flowers. CRDJ12, CRDJ13 and CRDJ4 recorded the least number of flowers of 106, 120, and 123 respectively. The ratio of number of female flowers to male flowers was highest in CRDJ5 (0.14) followed by lower values of 0.09 in six other accessions. Highly significant differences were observed in low rankers which consisted of eight

**Table 2** Mean values for various plant characteristics of different *J. curcas* accessions

Accession	Plant height (cm)	Number of branches	Number of flowers	Female to male flower ratio	Days taken from flowering to fruiting	Days taken from fruiting to maturity	Seed yield (g plant <sup>-1</sup> )
CRDJ03	124.52	7.90	152	0.06	8.0	46.0	226.00
CRDJ04	103.63	6.00	123	0.09	7.0	49.0	150.44
CRDJ05	109.74	9.55	174	0.14	7.0	47.0	213.42
CRDJ06	107.55	9.00	198	0.06	7.0	46.0	187.69
CRDJ07	113.00	9.350	245	0.09	5.0	50.0	144.64
CRDJ08	115.66	9.20	299	0.09	5.0	48.0	263.97
CRDJ09	108.00	7.50	169	0.04	7.0	47.0	186.70
CRDJ10	111.40	7.00	240	0.07	7.0	48.0	207.08
CRDJ11	108.00	7.65	207	0.08	8.0	41.0	100.17
CRDJ12	103.86	4.00	106	0.04	9.0	35.0	71.33
CRDJ13	107.82	7.70	120	0.06	8.0	49.0	153.12
CRDJ16	98.02	4.00	260	0.09	5.0	46.0	58.12
CRDJ17	87.34	7.00	295	0.06	7.0	50.0	203.67
CRDJ18	105.61	8.45	196	0.05	8.0	50.0	137.40
CRDJ19	97.24	10.00	197	0.08	5.0	52.0	232.40
CRDJ20	101.11	10.25	147	0.08	6.0	54.0	236.43
CRDJ21	127.87	6.50	222	0.05	8.0	53.0	123.32
CRDJ23	120.32	12.25	191	0.06	8.0	45.0	214.40
CRDJ24	134.00	6.00	214	0.07	8.0	47.0	154.85
CRDJ26	128.64	6.00	120	0.04	7.0	54.0	134.37
CRDJ27	115.53	4.00	159	0.04	7.0	49.0	41.70
CRDJ28	113.21	9.90	150	0.03	7.0	50.0	72.80
CRDJ29	113.39	4.00	226	0.09	7.0	53.0	44.90
CRDJ30	105.25	5.00	159	0.07	5.0	53.0	93.55
CRDJ31	121.97	7.00	290	0.06	8.0	52.0	110.30
CRDJ32	119.54	7.00	277	0.09	8.0	49.0	130.52
CRDJ01	126.56	7.00	244	0.05	8.0	48.0	155.64
CRDJ02	112.18	10.85	179	0.07	8.0	49.0	220.67
CRDJ15	119.93	4.00	154	0.03	5.0	53.0	36.60
LSD at 0.05	18.15	3.09	34.87	0.086	1.43	4.78	32.86

accessions having values between 0.03 and 0.05. The least ratio was seen in CRDJ3. Apart from CRDJ5 other high rankers having a ratio of  $>0.09$  were CRDJ4, CRDJ7, CRDJ8, CRDJ16, CRDJ29 and CRDJ32. A maximum of 9 days and a minimum of 5 days were seen in all the accession to reach fruiting after initiation of flowering. The longest time taken was seen in only one accession (CRDJ12). Several accessions fell into the group of 7–8 days. The overall mean for the accessions to reach fruiting stage after initiation of flowering was 6.97 days. On the other hand the mean number of days taken from fruiting to maturity of the fruit in all the accession was 48.7 days. The least time taken was 35 days by CRDJ12 and the most time of 54 days was taken by CRDJ20 and CRDJ26. Significantly higher time of more than 50 days to come to maturity after fruiting was seen in as many as 11 accessions. The mean number of days taken for the fruits to

come to maturity was 48.72 days. Yield expressed as g plant<sup>-1</sup> was highest in CRDJ20 which recorded 263.97 and was closely followed by CRDJ20 and CRDJ19 with values of 236.4 and 232.4 respectively. Nine accessions namely CRDJ3, CRDJ5, CRDJ8, CRDJ10, CRDJ17, CRDJ19, CRDJ20, CRDJ23, and CRDJ2 were the high rankers with more than 200 g plant<sup>-1</sup> yield. Minimum yield of 36.6 g plant<sup>-1</sup> was seen in CRDJ15. The low yielders recording less than 100 g plant<sup>-1</sup> were CRDJ12, CRDJ16, CRDJ27, CRDJ28, CRDJ29, CRDJ20, and CRDJ15.

#### Genetic associations in seed characteristics

The amount of genetic variations and association was evident from the study of PVC, GCV and correlation analysis. Seed length exhibited a significant positive correlation (Table 3) at 0.05 probability level with seed width.

**Table 3** Genotypic (G) and phenotypic (P) correlation coefficients between seed physical attributes (length, width, and weight) and seed oil content in wild *J. curcas* accessions

Character	Genotypic/ phenotypic	Seed width (cm)	100 Seed weight (g)	Oil content (%)
Seed length (cm)	Genotypic	0.392*	0.024	-0.035
	Phenotypic	0.335*	0.021	-0.042
Seed width (g)	Genotypic		0.483**	0.269
	Phenotypic		0.436**	0.210
100 seed weight (%)	Genotypic			0.366*
	Phenotypic			0.344*

\* Significant at 5% level,

\*\* significant at 1% level

Whereas there was no evident correlation (genotypic and phenotypic) with 100 seed weight and oil content although, the  $r$  values were negative in the case of seed length and oil content. In contrast seed width exhibited a high ( $P < 0.01$ ) correlation with 100 seed weight at both genotypic and phenotypic levels and similarly showed a positive correlation with oil content although it was not significant. Hundred seed weight showed a positive correlation with seed oil content at 0.05 probably level.

The magnitude of PCV was higher than the corresponding GCV for all the characters studied though they were only small differences (Table 5). Phenotypic variance denoting total variance was maximum for 100 seed weight (34.84) among the seed the characters studied which was followed by oil content. On the other hand coefficient of variation values were highest in seed width as compared to other parameters observed. Broad sense heritability was high in general and exceeded 80% for all the seed traits studied. Oil content exhibited highest broad sense heritability of more than 99% followed by 100 seed weight (93.16%). Lowest heritability was seen in seed width which was 77.77%. In contrast seed width showed the second highest genetic advance of 4.98% following

the highest genetic advance of 7.21% exhibited by oil content.

#### Genetic associations in growth and yield characteristics

Plant height was significantly positively correlated with yield at 0.05 probability level (Table 4). There was a significant negative phenotypic correlation of plant height and female to male flower ratio. No evident correlation was observed between plant height and other characters although it exhibited positive values with days taken from initiation of flowering to fruiting and days taken from fruiting to maturity. Number of branches per plant showed high significant correlation ( $P < 0.01$ ) with yield at both genotypic and phenotypic levels with  $r = 0.605$  and  $r = 0.503$  respectively. Similarly it exhibited significant correlation with total number of flowers too although only at 0.05 probability level. Positive values of  $r$  were registered with other characters but without any significance. There was a positive genotypic correlation and phenotypic correlation of the total number of flowers with female to male ratio at 0.01 and 0.05 probability levels respectively. The next highest  $r$  values were seen for yield although not

**Table 4** Genotypic (G) and phenotypic (P) correlation coefficients between plant physical attributes and phenological attributes for selected *J. curcas* accessions

Character	Genotypic/ phenotypic	Branches	Number of flowers	F:M ratio	Initiation of flowering to fruiting	Fruiting to maturity	Yield (g plant <sup>-1</sup> )
Plant height (cm)	Genotypic	0.0956	0.0197	-0.234	0.287	0.178	0.357*
	Phenotypic	0.0765	0.0345	-0.321*	0.234	0.198	0.338*
Branches	Genotypic		0.387*	0.122	0.187	0.129	0.605**
	Phenotypic		0.367*	0.118	0.165	0.134	0.543**
Number of flowers	Genotypic			0.405**	-0.088	0.108	0.289
	Phenotypic			0.312*	-0.074	0.134	0.235
F:M ratio	Genotypic				-0.140	-0.014	0.476**
	Phenotypic				-0.134	0.017	0.421**
Days taken from initiation of flowering to fruiting	Genotypic					-0.441**	0.075
	Phenotypic					-0.389*	0.054
Days taken from fruiting to maturity	Genotypic						0.321*
	Phenotypic						0.319*

\* Significant at 5% level, \*\* significant at 1% level

**Table 5** Estimation of genetic variables for seed and oil traits in wild germplasm\* ( $n = 32 \times 3 \times 9$ ) and growth and reproductive phenological traits in progeny trial\*\* ( $n = 29 \times 3 \times 9$ ) of selected *Jatropha curcas* germplasm

Traits	Range	Variance		Coefficient of variation		Heritability (broad sense)	Genetic advance as % of mean
		Genotypic	Phenotypic	Genotypic	Phenotypic		
Seed length*	1.55–1.87	0.23	0.28	5.44	5.76	82.14	3.87
Seed width*	1.77–2.25	0.07	0.09	11.34	12.03	77.77	4.98
100 seed weight*	56.98–79.09	32.46	34.84	7.34	7.89	93.16	4.22
Oil content*	29.85–37.05	10.23	10.27	11.67	12.12	99.61	7.21
Plant height**	87.30–134.00	99.05	112.90	26.71	28.43	87.73	68.75
Branches**	4.00–13.80	6.78	8.32	19.23	20.16	81.49	46.21
Number of flowers**	106–299	143.12	203.23	41.12	44.56	70.42	31.48
F:M ratio**	0.03–0.14	0.01	0.01	67.12	67.70	99.95	41.11
Days taken from Initiation of flowering to fruiting**	3.0–9.0	1.87	3.03	23.45	23.90	61.71	48.76
Days taken from fruiting to maturity**	35.0–54.0	15.98	25.31	15.39	15.76	63.13	16.79
Yield**	36.60–263.97	178.65	213.65	54.90	55.26	83.61	75.23

Seed length, seed width and plant height in cm; oil content in % and yield in  $\text{g plant}^{-1}$

significant. Female to male flower ratio did not exhibit any correlation with days taken from initiation of flowering to maturity of fruits. But there was a high significant correlation with yield at both genotypic and phenotypic levels. Values of  $r$  were negative for all other characters except for phenotypic correlation with days taken from fruiting to maturity. Days taken from initiation of flowering to fruiting was negatively correlated with days taken from fruiting to maturity in both genotypic and phenotypic levels although genotypic correlation was more significant than at phenotypic level. Phenotypic variance denoting total variance was maximum for yield followed by number of flowers among the growth characters studied (Table 5). Minimum variance was seen in female to male flower ratio. On the other hand female to male flower ratio exhibited high both high PCV and GCV followed by yield and plant height. Broad sense heritability was generally higher than 61.7% for all the growth characters. Female to male flower ratio showed near to 100% heritability followed by yield (83.61) and plant height (87.73). Days taken from flowering to maturity put together showed much less heritability near 60%. Similarly genetic gain for yield and plant were the highest (75.23 and 68.75 respectively) followed by other characters registering gains from 16 to 47% with the lowest being for days taken from fruiting to maturity (16.79). The path analysis revealed that female to male flower ratio had highest positive and direct relationship with seed yield (0.789) (Table 6), followed by number of branches (0.612) and number of days from fruiting to maturity (0.431). Plant height also positively and indirectly influenced yield through all traits except number of branches.

#### Genetic divergence in seed characteristics

Thirty-two accessions of *Jatropha* were placed in four clusters on the basis of  $K$ -means clustering, here it was seen that cluster I, II, III and IV had different number of accession grouped under them (Table 7). The maximum number of accessions were in cluster II (CRDJ3, CRDJ9, CRDJ16, CRDJ17, CRDJ19, CRDJ21, CRDJ 26, CRDJ27, CRDJ28, CRDJ 29, CRDJ32) and the minimum number were placed in cluster III (CRDJ5, CRDJ10, CRDJ15, CRDJ23, CRDJ24). Highest means for seed length and oil content was seen in cluster II and highest values for seed width and 100 seed weight were observed in cluster I. Lowest mean for 100 seed weight and oil content was seen in cluster III and IV respectively. The cluster I showed maximum intra-cluster distance (2.071) and the minimum (1.513) was seen in the cluster III (Fig. 1). Inter-cluster distance between cluster II and IV was the maximum at 3.102. Likewise the minimum inter-cluster distance of 1.987 was seen between cluster III and IV. Seed characters data of all the accessions were further hierarchically clustered according to ward with Euclidean distance. The dendrogram obtained by hierarchical clustering of seed characteristics (Fig. 2) showed that several clusters started at the lowest level and lead to the super cluster. Length of the horizontal branches between clusters indicated that there was a high degree of dissimilarity between clusters. The maximum number of members apart from the super cluster containing all the accession was populated by 25 accessions when cluster containing CRDJ24 and CRDJ22 were joined at distance of 23.826. This was followed by 16 members in a cluster containing CRDJ22 and CRDJ21 was

**Table 6** Path coefficient analysis results showing direct (in italic font) and indirect effects of 6 traits over seed yield in in progeny trial of selected *Jatropha curcas* germplasm

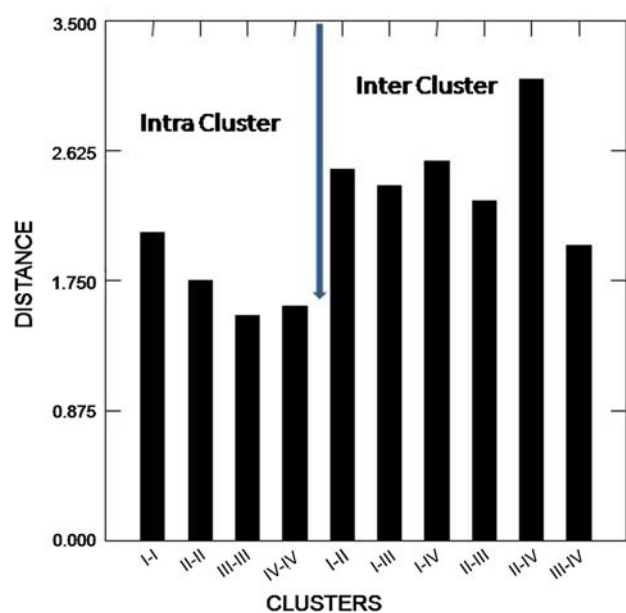
Trait	Plant height	Branches	Number of flowers	F:M ratio	Number of days from flowering to fruiting	Number of days from fruiting to maturity	Genotypic correlation coefficient
Plant Height	<i>0.143</i>	0.324	0.053	0.143	-0.178	-0.142	0.357*
Branches	-0.016	<i>0.612</i>	0.082	0.098	-0.112	-0.182	0.605**
Number of flowers	0.119	0.301	<i>0.213</i>	0.345	0.008	0.341	0.289
F:M ratio	0.043	0.087	0.112	<i>0.789</i>	0.132	0.110	0.476**
Number of days from flowering to fruiting	0.132	0.113	0.104	0.187	<i>0.264</i>	0.252	0.075
Number of days from fruiting to maturity	0.119	0.187	0.098	0.231	-0.213	<i>0.431</i>	0.321*

Seed length, seed width and plant height in cm; oil content in % and yield in g plant<sup>-1</sup>

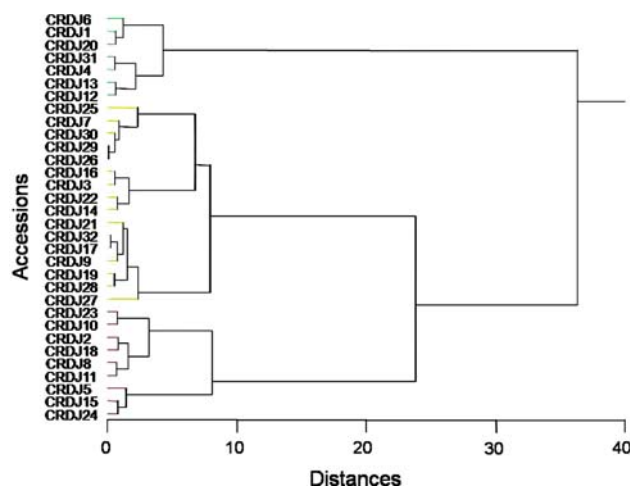
**Table 7** Composition of Euclidean clusters and cluster mean values obtained by *K*-means Non-hierarchical clustering for seed and oil traits in wild *Jatropha curcas* germplasm. Seed length, seed width in cm and oil content in %

Cluster	Number of accessions	Accession names	Seed length	Seed width	100 Seed weight	Oil content
I	9	CRDJ1, CRDJ4, CRDJ6, CRDJ12, CRDJ13, CRDJ14, CRDJ20, CRDJ22, CRDJ31	1.73	1.99	75.50	33.79
II	11	CRDJ3, CRDJ9, CRDJ16, CRDJ17, CRDJ19, CRDJ21, CRDJ26, CRDJ27, CRDJ28, CRDJ29, CRDJ32	1.75	1.92	68.60	34.59
III	5	CRDJ5, CRDJ10, CRDJ15, CRDJ23, CRDJ24	1.73	1.89	59.21	33.16
IV	7	CRDJ2, CRDJ7, CRDJ8, CRDJ11, CRDJ18, CRDJ25, CRDJ30	1.72	1.87	65.05	32.46

Seed length, seed width in cm and oil content in %

**Fig. 1** Estimates of intra-cluster (left of the arrow) and inter-cluster distance (right of the arrow) (four clusters) by *K*-means non-hierarchical clustering in seed and oil traits in *Jatropha curcas* wild accessions

joined at distance of 7.945. There were several clusters with two, three and four members as against only a few with more members than four.

**Fig. 2** Dendrogram constructed by Ward method of cluster analysis on the Euclidean distances by hierarchical clustering of seed characteristics of wild *Jatropha curcas* germplasm

### Genetic divergence in growth and yield characteristics

Six clusters were delineated by *K*-means clustering for the 29 accessions of *Jatropha* selected for progeny trial. Cluster I and II both had eight members in it and clusters IV and V had three members each. Cluster III had seven members in it (Table 8). Cluster IV consisted of high yielders (mean of



**Table 8** Composition of Euclidean clusters and cluster mean values obtained by *K*-means Non-hierarchical Clustering for growth and reproductive in progeny trial of selected *Jatropha curcas* germplasm

Cluster	Number of accessions	Accession names	Plant height	Branches	Number of flowers	FM ratio	Number of days to initial flowering	Number of days from fruiting to maturity	Yield
I	8	CRDJ2, CRDJ10, CRDJ11, CRDJ20, CRDJ21, CRDJ22, CRDJ24, CRDJ29	112.20	5.82	136.37	0.05	6.62	49.0	94.24
II	8	CRDJ1, CRDJ3, CRDJ4, CRDJ7, CRDJ15, CRDJ16, CRDJ18, CRDJ28	110.05	9.66	175.87	0.07	7.00	48.25	214.70
III	7	CRDJ5, CRDJ14, CRDJ17, CRDJ19, CRDJ25, CRDJ26, CRDJ27,	121.19	8.37	241.14	0.07	7.57	49.86	136.67
IV	3	CRDJ6, CRDJ8, CRDJ13	104.77	7.73	278.00	0.07	6.33	48.67	224.91
V	3	CRDJ9, CRDJ12, CRDJ23	106.43	5.22	231.00	0.05	6.67	46.67	67.73

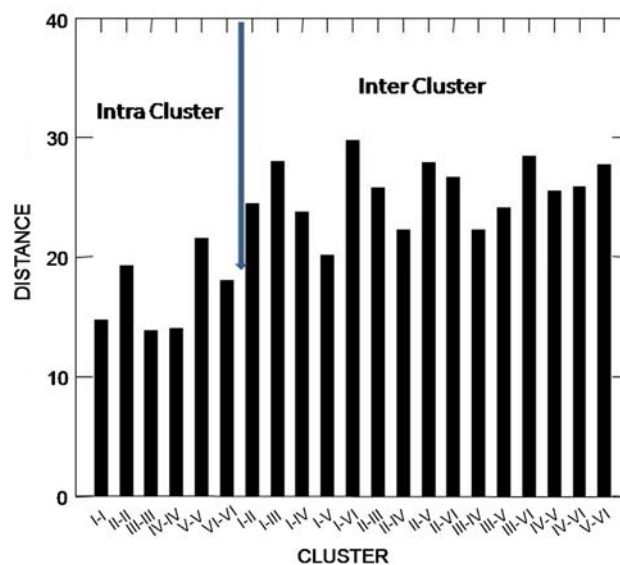
Plant height in cm and yield in g plant<sup>-1</sup>

224.91) which were CRDJ6, CRDJ8, and CRDJ13. Likewise Cluster V had low yielders (mean of 67.73) as its members which were CRDJ9, CRDJ12, and CRDJ23. Intra-cluster distances were generally less than the inter-cluster distance. Cluster III had the minimum intra-cluster distance of 13.87 followed by cluster IV (Fig. 3). Cluster I and VI were separated by the longest distance 29.76 between clusters followed by III and VI by a distance of 28.43. The minimum inter-cluster distance was seen between I and V followed by clusters both II and VI and III and IV (22.21). Hierarchically clustered data by wards method (Fig. 4) revealed that apart from the super cluster there were 3 clusters with more than 10 members wherein the cluster containing CRDJ11 and CRDJ19 joined at a distance of 205.8 had 18 members. This was followed by cluster containing CRDJ6 and CRDJ7 with 11 accessions and cluster containing CRDJ19 and CRDJ26 with 10 accessions as members. Most clusters contained two accessions as members under shorter distances.

## Discussion

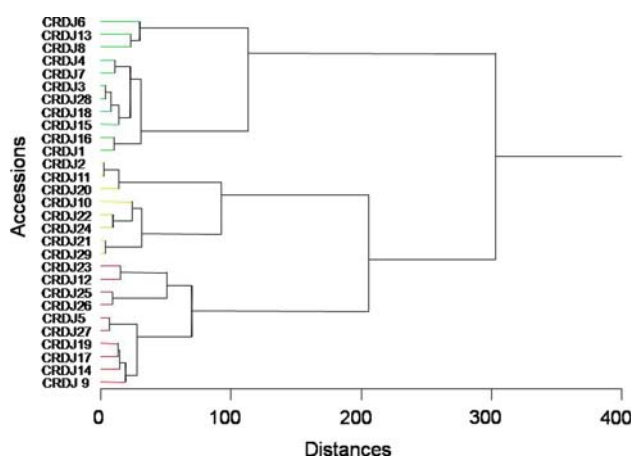
### Genetic associations

Seed morphological characters of the wild accessions are considered to be the first step in ascertaining genetic variability of the population. Multiple factors may induce and maintain variation in seed size some of which could be that large seeds may be favoured because they produce larger and more vigorous seedlings with better chances of survival than small seeds and conversely the small seeds may have a selection advantage due to wider and more effective dispersal. Therefore contradictory evolutionary actions mediated by biotic as well as abiotic agents potentially act



**Fig. 3** Estimates of intra-cluster (left of the arrow) and inter-cluster distance (right of the arrow) (six clusters) by *K*-means non-hierarchical clustering of growth and reproductive phenology in progeny trial of selected *Jatropha curcas* germplasm

upon plants to produce the seed size distributions observed in natural plant populations (Eriksson 1999). Seed oil content variation is more widely reported not only in annual crops but also in a wide variety of trees borne oil seed (Johansson et al. 1997; Kaura et al. 1998; O'Neill et al. 2003; Vollmann et al. 2007). The variation found in oil content in the present study along with other seed morphological attributes presents us with a viable selection alternative at a very early stage (collection of germplasm) from base seed material. This could be of use in improvement programmes especially considering the fact the *Jatropha* is a new crop where crop breeding is still in its infancy.



**Fig. 4** Dendrogram constructed by Ward method of cluster analysis on the Euclidean distances by hierarchical clustering of growth and reproductive phenology in progeny trial of selected *Jatropha curcas* germplasm

Variation in flowering and fruiting and number of flower and seed produced was clearly evident from the study. All the parameters studied exhibited considerable amount of variation in the progeny trial of the crop. Plant height and number of branches are important characters that can be looked upon as major selection indices when the objective is to incorporate *Jatropha* in an agroforestry system wherein balanced trade off can be made on yield considering the economic potential of the understory crop in the initial years of establishment. On the other hand the variation observed in reproductive characters can be useful in selecting plant types for block plantations with high yield as the primary objective.

Gains from tree breeding programmes depend on the type and extent of genetic variability. In the present study 100 seed weight, oil content, female to male flower ratio and yield showed distinctively higher heritability in the broad sense than other characters. Hence these characters can be viewed as best gain characteristics for *Jatropha* improvement more so because of its strong genetic control and a wide range of variability. Contrastingly the other characters like total number of flowers, days taken from flowering to maturity collectively may not be the target indices for *Jatropha* improvement as evident by low heritability and also due to the fact that environmental effects would have caused too much variation to distinguish effectively between genotypes. However, it should be noted that estimation of heritability is of little significance in coherent selection breeding programs unless accompanied by sufficient genetic gain (Tefera et al. 2003). Due to large differences in the phenotypic variation between different traits, genetic advance is not directly related to heritability values. In the present study, low to moderate genetic gain values for seed characters indicate that

improvement could be made in these characters. Genetic advance was considerably larger in the growth and yield traits as compared to the seed characteristics. This could be due to extreme variation in the material investigated, and smaller values for genetic advance are expected in further selection cycles in a more improved material. Within the growth traits yield and plant height showed maximum advance indicating that the progress in shifting the genotypic mean and gene frequencies of yield and plant height in the population could be achieved in the desired direction by selection.

Correlation coefficients revealed interesting relationship in the seed characters as well as growth parameters studied. Degree of correlation coefficient at genotypic level was higher than their corresponding phenotypic coefficient of correlations in all the parameters indicating the genetic association among the characters. The correlation matrix revealed that statistically significant correlation of seed weight existed with oil content, seed width, therefore, seed weight can be considered as important trait for early selection of seed sources. Similar results have been reported for other tree borne oils seeds (Kaura et al. 1998). Significant correlations between seed yield in progeny trial with female to male ratio, number of branches, plant height and days taken from fruiting to maturity also indicates that selection from early plantations of *Jatropha* can be made on the basis of these characters. This is further supported by the fact the number of flowers had a high positive association with female to male ratio forming an indirect positive association with yield. In addition the high degree of direct relationship between number of branches, female to male flower ratio and days taken from fruiting to maturity to yield indicates that selecting on the basis of these characters is all the more useful because of the difficulty in selecting plants solely based on yield as the full potential of yield is reached only after several years of growth in *Jatropha*. The correlations seen can be explained by the fact that during the phenological succession of appearance of physiological and morphological determinants of yield; the number of branches contributed to higher number of flowers which in turn contributed to higher number of female flowers which finally culminated in higher yield. It should be noted here that even though the female to male ratio is a unitary measure pertaining to the inflorescence per se the number of branches could have increased the total number of female flowers. Significant association among branches/plant, and number of flowers indicate that plants with good branching habit tend to develop more number of flowers. Similar relations have been reported in other crops as well (Bhargava et al. 2007; van Osteroma et al. 2006). Path analysis also revealed that there were indirect negative effects on yield. Negative indirect effects were seen in number of days from flowering to fruiting

which indirectly and negatively influenced yield through plant height and number of branches. Similar pattern was observed in number of days from fruiting to maturity and its negative influence on yield. This suggests that the number of days taken from fruiting to maturity although positively correlated may not be good selection index for selection of high yielders in *Jatropha*. This is explained by the fact that in a plant like *Jatropha* there could be two types of relationship of yield with flowering and fruiting phenology: (1) a positive influence which is genetically and physiologically manifested because the longer the fruit takes to mature there is more time for sink filling of the seed; (2) an indirect negative influence which is physiologically manifested because the longer the time taken to mature there is more instances of flower and fruit drop in addition to attack by external abiotic and biotic factors.

### Genetic diversity

Analysis of genetic diversity in germplasm collections can facilitate reliable classification of accessions and identification of core subsets of the accessions with future utility for specific breeding purposes. Hierarchical clustering by Ward's minimum variance cluster analysis method (Ward 1963) for both seed characters and growth data for all the accessions revealed the genetic relatedness between-accessions (Fig. 4). More truncations were seen in both seed characters and growth with two members in a cluster indicating high degree of genetic diversity among all the parameters studied. The dendrogram also were indicative of the relative involvement of different constituent traits to the total diversity and quantity the degree of divergence between populations. However *K*-means clusters clustering pattern in this study revealed that trees from different geographic regions were grouped together in a cluster and as were trees from the same geographical area placed in different clusters. This suggested that geographical diversity did not go hand in hand with genetic diversity.

*K*-means clustering is done to understand the trend of evolution and choose genetically diverse parents for obtaining desirable recombination (Tams et al. 2006). Generally the intra-cluster distances were lower than that of the inter-cluster distances indicating of the genetic similarity of the members of the clusters formed. Ten pairs of accessions (20 in all) out of the total 32 accession studied were placed in the same cluster when similarity in cluster membership was determined considering both seed characteristics and growth and yield characters. High intra-cluster distance in seed characters and growth attributes shown by cluster I and IV respectively indicated that of selection of parents for hybridization should be within these clusters in event of future hybridization programs with expectations for vigour in 100 seed weight and seed

yield respectively as the means for these values were high in these clusters. Accession CRDJ6 and CRDJ13 were placed in these clusters for both these characters. The maximum inter-cluster distance between cluster II and IV (seed characters) and cluster I and VI (growth and yield) indicates extensive genetic diversity between the trees in these groups and selecting of parents from these clusters would prove useful in developing novel hybrids. Selection of trees as parents from clusters with low inter-cluster distance should be avoided. *Jatropha curcas* originates from Central America and was distributed by Portuguese seafarers via the Cape Verde Islands to countries in Africa and Asia. Until now, before the new found bio fuel use of *Jatropha*, human intervention was minimal as it was a weed or sporadically grown as a live fence with minimal management. Our study here shows that after introduction of the plant in India there has been considerable activity of natural evolutionary forces that has created diversity among and variability within the species.

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### Appendix

Seedlings were raised in small 200 gauge plastic bag (size 8" × 5") by using selected seeds. The bags were filled with

**Table 9** Soil characteristics of the field and cultural practices followed in *Jatropha* progeny trial

Characteristics	Values
Soil order	Alfisol
Soil series	Hayathnagar
Coarse sand (%)	65.7
Fine sand (%)	11.0
Silt (%)	10.0
Clay (%)	13.3
Moisture (v/v) at field capacity ( $\text{m}^3 \text{m}^{-3}$ )	0.125
Moisture (v/v) at 50% field capacity ( $\text{m}^3 \text{m}^{-3}$ )	0.084
$\text{pH}$ (soil–water 1:2)	5.3
EC ( $\text{dsm}^{-1}$ )	0.087
Organic carbon (%)	0.37
CEC ( $\text{C mol (P}^+ \text{ kg}^{-1})$ )	14.5
Total P ( $\text{mg kg}^{-1}$ )	210
0.01 M $\text{CaCl}_2$ extractable sulphur ( $\text{mg kg}^{-1}$ )	7.0
Available N ( $\text{KMnO}_4$ method) ( $\text{mg kg}^{-1}$ )	65.0
Available P (0.5 M $\text{NaHCO}_3$ extractable) ( $\text{mg kg}^{-1}$ )	5.6
Available K ( $\text{mg kg}^{-1}$ )	80
Microbial biomass carbon ( $\text{mg kg}^{-1}$ )	108 (variable)

soil and organic manure and bio fertilizers viz., *Azotobacter* and *Phosphobacteria* at 1 g each and VAM 5 g. Bio control agents viz., *Trichoderma Viridi* and *Pseudomonas* at 1 g each was added in the pot mixture. One seed was planted in each bag. After 3 months, at the beginning of the rainy season and when appropriate soil moisture levels were reached in the field the plants were transplanted (Table 9).

The NPK requirement of this crop was taken as 46:48:24 kg/ha<sup>-1</sup> besides application of organic manure. Twenty grams urea, 120 g single super phosphate and 16 g murate of potash was applied in the planting pits after the plants established in the pits, optimum soil moisture was considered as a criteria for fertilizer application as it was a basically a dryland crop. Besides chemical fertilizer, bio fertilizers like *Azotobacter* 5 g, *Phosphobacteria* 5 g and VAM 20 g and bio control agents like *Trichoderma viridi* and *Pseudomonas* at 5 g each was applied per pit at the time of planting. The balanced dose of urea was applied in two splits at 10 g per plant at 1 month intervals after a month of basal dressing; this was done by closely monitoring the rainfall pattern and soil moisture levels. No irrigation was given.

Pruning was done between 90 and 120 days after planting on top of all plants at 25 cm. The top was cut off cleanly so as the cut top can produce 8–12 side branches. Initial pruning was done when leaves shed at 6–7 months age and successive pruning was done to maintain minimum of 30 branches with flowers or fruits at every 9 month interval.

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