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Intraspecific variation and interrelationships between morphology, nutritional content and enzymatic activity of *Jatropha curcas* L.

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The study was conducted to assess intra-specific variation and interrelations among morphological, nutritional and biochemical traits in 27 accessions of *Jatropha curcas* L. Principal component analysis (PCA) explained 58% of the total variation in the measured traits and revealed that there was negative correlation between four morphological traits and all nutritional compounds such as crude protein, neutral detergent fibre, acidic detergent fibre, lignin, hemicellulose and cellulose. Larger plants tended to contain lower concentration of protein and other nutritional compounds. Female flower/inflorescence showed maximum variation, while the difference for the number of male flowers/inflorescence was narrow. This might be explained by the difference in intracellular and extracellular enzymatic

activity at different development stages in *J. curcas* L. A strong correlation between plant height and branch length, number of branches and collar diameter was observed, which can help in the selection of superior genotypes of this species. The highest genetic variation was noticed for polyphenol oxidase analysis. PCA analysis showed that peroxidase activity was associated with cellulase, acid detergent fibre and protein content, whereas polyphenol activity was associated with neutral detergent fibre and lignin content.

Keywords: Enzymatic activity, intraspecific variation, *Jatropha curcas* L., nutrient content.

INDIA produces only 30% of its annual crude oil requirement of 111 mt and hence depends on imported crude oil to the tune of Rs 102,500 crores for meeting the requirements, resulting in a huge burden on the country's economy. This together with the rapid depletion of fossil-fuel reserves, high prices and associated environmental degradation with their use has prompted a search for alternative fuels in the world. In this scenario, the production and use of biofuels is one of the ways to reduce our dependence on fossil fuel and protect the environment. Biodiesel produced from edible and non-edible oils of plant origin and animal fat provide alternative sources of biofuel. As India is not self-sufficient in edible-oil requirement, focus is drawn on the production of biodiesel from non-edible oil-seeds. According to an estimate, even 5% replacement of fossil fuel by biodiesel will help save foreign exchange of over Rs 4000 crores annually¹. Many non-edible tree-borne oil-seed species such as *Jatropha*, Karanj, Pilu, Mahua, Sal and Cheura, widely found in India, are suitable for biodiesel production. Among these, *Jatropha curcas* is recognized as most potential species for biodiesel production, since the seeds contain high oil content (30–38%) and could be grown under different land-use situations². It can be easily propagated by seeds or cuttings and starts bearing within 2 to 3 years. Also, it can be commercially exploited in 4–5 years and lasts for about 50 years³. The species is not grazed by cattle and withstands extreme drought conditions. Out of 146 mha of wasteland existing in India⁴, plantation of *Jatropha* will help greening a large portion of it. As *Jatropha* is fast-growing and produces huge quantity of vegetation, it will add substantial quantity of organic matter to the soil through litter fall and fine root turnover. The increased organic matter will improve the physico-chemical properties of degraded lands. Considering its potential as a bio-energy crop, efforts are being made worldwide to enhance the genetic potential of *Jatropha* for economical biodiesel production. The availability of genetic variability and the knowledge of inter-relationships between different morphological and economic traits such as seed yield and seed biochemical traits are a prerequisite for systematic *Jatropha* genetic improvement. Therefore, better knowledge of physiology and biochemistry of *J. curcas* is

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Table 1. Jatropa accessions along with their locations

Accession	Location	Accession	Location
NRCJ-1	Dhodar, Ratlam (Madhya Pradesh)	NRCJ-16	Mangal Mahudi, Dahod (Gujarat)
NRCJ-2	Dhodar, Ratlam (Madhya Pradesh)	NRCJ-17	Limkhera, Dahod (Gujarat)
NRCJ-3	Dhodhar, Ratlam (Madhya Pradesh)	NRCJ-18	Piplod, Dahod (Gujarat)
NRCJ-4	Jaura, Ratlam (Madhya Pradesh)	NRCJ-19	Sant Road, Panchmahal (Gujarat)
NRCJ-5	Mundari, Ratlam (Madhya Pradesh)	NRCJ-20	Maharashtra
NRCJ-6	Palash, Ratlam (Madhya Pradesh)	NRCJ-21	Maharashtra
NRCJ-7	Karvar, Jhabua (Madhya Pradesh)	NRCJ-22	Maharashtra
NRCJ-8	Godaria, Jhabua (Madhya Pradesh)	NRCJ-23	Dantiwara (Gujarat)
NRCJ-9	Thandla, Jhabua (Madhya Pradesh)	NRCJ-24	Andhra Pradesh
NRCJ-10	Meghnagar, Jhabua (Madhya Pradesh)	NRCJ-25	Ambikapur (Chhattisgarh)
NRCJ-11	KVK, Jhabua (Madhya Pradesh)	NRCJ-26	Rahuri (Maharashtra)
NRCJ-12	Pitol, Jhabua (Madhya Pradesh)	NRCJ-27	Lalitpur (Uttar Pradesh)
NRCJ-13	Dahod Local (Gujarat)		
NRCJ-14	Banswara Local (Rajasthan)		
NRCJ-15	Jakot, Dahod (Gujarat)		

NRCJ, National Research Centre Jatropa.

needed to optimize its growth, fruiting cycle and different development stages, which will ultimately enhance seed yield, resulting in enhanced biodiesel production. The aim of this study was to assess the intraspecific variability and intercorrelations of morphological and nutritional traits as well as different enzymes produced in *J. curcas* L.

Twenty-seven accessions of *J. curcas* were used in the present study (Table 1). Seeds of these accessions were sown in the nursery to raise the seedlings and were planted in the field during August 2004. Two-year-old plants were evaluated for morphological traits and biochemical constituents analysis in randomized block design with three replications. The plantation was done at 2 × 2 m spacing. Every replication consisted of ten plants for each accession. Data on morphological traits such as plant height, collar diameter, number of branches, branch length, male flowers/inflorescence and female flowers/inflorescence were recorded.

Total leaf crude protein (CP) was determined⁵ by estimating nitrogen content and multiplying by a conversion factor of 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed with an Ankom Fiber Analyzer (ANKOM 220). NDF is the cell-wall fraction of the total fibre content and includes cellulose, hemicellulose and lignin. The method suggested by Ven Soest was employed for estimation of lignin. The ADF fraction contains cellulose and lignin, and is closely related to indigestibility of food items⁶.

Plant materials were homogenized in the ratio of 1 : 2 (w/v) with 0.1 M phosphate buffer (pH 6). Extract was centrifuged at 20,000 rpm for 20 min at 4°C. The enzyme sources were obtained as supernatant after centrifugation. Peroxidase was estimated spectrophotometrically in 0.5 ml 0.01 M buffered pyrogallol in phosphate buffer (pH 6) along with 0.25 ml enzyme sources. Absorbance at 420 nm at room temperature was measured up to 15 min and activities were presented⁷ as change in absor-

bance by 0.001 ml⁻¹ min⁻¹, with slight modification. Polyphenol oxidase (EC 1.10.3.1) was measured at A495 nm with catechol as substrate⁸.

Pearson correlation was used to assess the association between different traits. Data on measured variables of 27 germplasm of *J. curcas* were subjected to principal component analysis (PCA) to detect variation in patterns among morphological traits, major nutritional compounds (CP, ADF, NDF, cellulose, hemicellulose and lignin) and enzyme activities (peroxidase and polyphenol oxidase). Following PCA, Varimax orthogonal rotation was applied to construct a new, more easily interpretable pattern of component loadings. In the ordination diagram of the PCA analysis, distribution of each variable was plotted according to its PCA score coefficient. This plot was used to detect relationships among morphological traits, nutritional compounds and enzyme activities. Statistical analysis was carried out using SPSS⁹.

Analysis of variation revealed highly significant differences among the accessions for the characters branch length, ADF, NDF, cellulose, hemicellulose, lignin, crude protein, peroxidase and polyphenol oxidase (Table 2). Variation in morphological trait, nutritive content and enzymatic activity among individual germplasm is given in Table 3. Among the morphological traits, the number of female flowers/inflorescence had the highest variation (CV = 49.90%), whereas the number of male flowers/inflorescence had the lowest variation (CV = 5.81%). The main structural carbohydrates were NDF and ADF, comprising >50 and >38% of dry mass respectively. Variation for NDF and lignin was less (CV = 0.25 and 0.27% respectively), but was much higher for hemicellulose and ADF (CV = 3.67 and 1.03% respectively). Polyphenol oxidase had the highest variation (CV = 2.98%) among the analysed enzymes.

There was strong correlation between plant height and (a) branch length ($r = 0.87$, $n = 27$, $P < 0.001$), (b) number

Table 2. Analysis of variance for different morphological, nutritional and biochemical traits in *Jatropha curcas*

Source of variation	df	Mean sum of squares													
		Plant height	Collar diameter	No. of branches	Branch length	Male flowers	Female flowers	ADF	NDF	Cellulose	Hemi-cellulose	Lignin	Crude protein	Peroxidase	Polyphenol oxidase
Treatment	26	235.12	0.70	1.80	172.85**	7.01	1.34	17.28**	5.96**	3.42**	13.74**	17.53**	4.41**	380.41**	567.48**
Replication	2	1091.10	10.34	4.25	931.12	194.04	102.37	0.24	0.02	0.009	0.13	0.003	0.13	1.26	5.79
Error	52	148.38	0.81	1.15	63.16	20.81	3.41	0.16	0.01	0.012	0.17	0.002	0.08	0.72	5.00

**Significant at 1% level.

Table 3. Mean, standard deviation (SD), critical difference (CD) and coefficient of variation (CV) for all accessions of *J. curcas*

Accession	Plant height (cm)	Collar diameter (cm)	No. of branches	Branch length (cm)	Male flowers/ inflorescence	Female flowers/ inflorescence	ADF (%)	NDF (%)	Cellulose (%)	Hemi-cellulose (%)	Lignin (%)	CP (%)	Peroxidase (▲ A 420 by 0.001 ml ⁻¹ min ⁻¹)	Polyphenol oxidase (▲ A 495 by 0.001ml ⁻¹ min ⁻¹)
NRCJ-1	175.30	6.20	7.93	123.33	77	3	41.49	51.15	20.64	9.66	19.40	15.4	56.07	75.12
NRCJ-2	172.48	6.04	7.09	132.96	76	4	42.35	50.75	21.51	8.40	19.35	15.8	52.08	102.69
NRCJ-3	158.60	4.97	5.80	114.65	76	3	37.28	50.68	21.07	13.40	15.26	16.5	48.88	90.84
NRCJ-4	148.27	4.73	5.27	109.46	77	3	41.47	50.10	20.55	8.63	19.95	14.4	45.28	62.87
NRCJ-5	169.23	6.21	7.57	124.83	80	3	40.54	51.65	20.81	11.11	19.07	15.1	40.75	61.93
NRCJ-6	160.87	5.36	6.20	118.70	79	3	36.72	49.25	20.55	12.53	15.06	14.6	34.09	71.66
NRCJ-7	170.53	6.06	7.23	124.03	77	4	40.16	49.33	21.41	9.17	18.08	14.5	31.96	76.85
NRCJ-8	166.20	6.27	6.30	124.81	78	4	40.74	50.11	21.66	9.37	18.15	14.4	29.43	67.53
NRCJ-9	150.91	4.70	5.64	108.02	77	4	40.93	51.45	20.57	10.52	19.19	15.2	31.16	61.80
NRCJ-10	156.07	5.81	5.30	119.17	80	4	36.08	48.35	22.06	12.27	19.08	15.3	35.16	59.14
NRCJ-11	159.71	5.68	5.80	117.86	79	4	41.45	49.56	20.80	8.11	16.66	14.6	28.50	68.19
NRCJ-12	152.28	5.92	4.75	105.91	79	4	39.28	49.66	20.61	10.38	17.99	13.9	28.63	82.18
NRCJ-13	158.83	5.47	6.47	111.34	78	4	37.63	49.18	20.13	11.55	16.38	14.2	32.50	78.05
NRCJ-14	156.78	5.03	5.70	116.58	79	4	40.84	51.49	20.92	10.65	17.77	15.3	28.63	71.12
NRCJ-15	140.77	5.11	5.10	103.07	79	3	34.34	48.29	19.07	13.95	14.42	14.0	28.37	65.80
NRCJ-16	157.47	5.66	5.22	112.12	79	3	39.74	54.05	20.49	14.31	18.37	12.4	28.50	63.13
NRCJ-17	164.26	5.92	5.76	113.50	80	4	38.50	49.35	19.56	10.85	15.64	13.8	30.90	68.33
NRCJ-18	161.33	5.25	6.90	114.77	79	5	40.18	49.73	19.71	9.55	17.68	14.1	20.77	88.04
NRCJ-19	151.80	5.08	6.10	110.28	78	5	38.81	48.34	19.32	9.53	17.63	14.3	27.43	85.51
NRCJ-20	153.79	5.07	6.03	115.18	77	4	40.13	50.25	20.48	10.12	17.99	13.7	25.70	80.71
NRCJ-21	156.27	5.00	6.40	106.10	81	3	38.98	51.91	20.23	12.93	16.64	12.7	22.11	62.60
NRCJ-22	140.95	5.27	5.30	102.06	80	3	37.60	48.32	21.43	10.72	17.13	11.8	29.83	88.57
NRCJ-23	163.23	5.48	6.60	123.72	75	3	37.67	51.29	18.86	13.62	21.02	12.0	28.37	81.91
NRCJ-24	167.83	5.99	5.90	124.80	80	3	34.72	48.86	21.27	14.14	12.39	12.0	27.13	77.38
NRCJ-25	171.93	5.13	5.60	120.51	80	5	38.40	50.91	20.35	12.51	16.49	15.6	38.62	77.52
NRCJ-26	155.67	4.94	6.43	117.16	79	4	37.10	51.70	18.19	14.60	25.10	13.1	27.83	76.32
NRCJ-27	157.06	5.34	6.24	112.96	80	4	33.50	48.54	17.47	15.04	15.13	13.6	29.17	78.72
Mean	159.20	5.47	6.10	115.85	78.48	3.70	38.76	50.16	20.36	11.39	17.67	14.16	32.88	74.98
S. Em ±	7.03	0.52	0.62	4.59	2.63	1.07	0.23	0.07	0.06	0.24	0.03	0.16	0.49	1.29
SD	12.18	0.90	1.07	7.94	4.56	1.85	0.40	0.12	0.11	0.42	0.05	0.28	0.85	2.24
CD	19.49	1.44	1.71	12.72	7.29	2.95	0.64	0.20	0.18	0.67	0.08	0.45	1.36	3.58
CV (%)	7.65	16.47	17.57	6.86	5.81	49.90	1.03	0.25	0.54	3.67	0.27	1.98	2.58	2.98

of branches ($r = 0.70$, $n = 27$, $P < 0.001$), (c) collar diameter ($r = 0.64$, $n = 27$, $P < 0.001$). A positive correlation was detected between ADF and NDF ($r = 0.48$, $n = 27$, $P < 0.05$). The first three principal components accounted for 58% of the total variation. The first axis explained 31% of the variance and was positively correlated with four morphological variables that indicate plant size, but not with the number of male and female flowers (Figure 1). There was a pronounced negative trend between the four size parameters and all nutritional compounds. Thus, larger plants contained lower amounts of these compounds. The second axis explained 14% of the variance and was positively correlated with cellulose, ADF and CP and negatively correlated with hemicellulose (Figure 1). Thus plants which contained higher amounts of protein and ADF also contained lower amounts of hemicellulose. Whereas peroxidase activity seemed to be associated with cellulose, ADF and protein; the activity of polyphenol oxidase seemed to be associated with NDF and lignin (Figure 1). The third component explained 13% of the variation and was positively correlated with lignin and NDF.

Our study clearly indicated that there was a strong positive correlation between plant height and branch length, number of branches and collar diameter. Number of female flowers/inflorescence showed highest variation among the morphological traits. Only female flowers bear fruits and it is a most desirable trait in monoecious species. Selection of plants having more female flowers will significantly increase the seed yield, which is correlated with oil yield. Among the analysed enzymes, polyphenol oxidase showed highest variation (CV = 2.98%). The involvement of peroxidase in stress-related physiological process¹⁰, plant-pathogen interaction¹¹ as well as in plant growth was demonstrated¹². Polyphenol oxidase (EC 1.10.3.1), which belongs to the class of oxido reductase, also plays an important role in many physiological process. It is invol-

ved in disease resistance, flower shedding, seed development and other development processes^{12,13}. Researchers also correlated different enzymes with the percentage of oil in seeds of *Jatropha* and found that accessions which have greater laccase enzyme activity showed greater oil percentage¹⁴. Laccase and peroxidase polymorphism was also reported in germplasm of the National Agroforestry Repository^{14,15}. Peroxidase and polyphenol oxidase enzymes were found to be positively correlated with ADF, NDF and protein. Selection for peroxidase and polyphenol oxidase enzymes activities will improve the nutritive quality of *Jatropha*. Thus, these finding will be of use to *Jatropha* breeders for screening the breeding lines for further genetic improvement of this crop. Researchers have demonstrated the role of all these enzymes during the development process in several crops. Agrawal *et al.*¹⁶ reported that polyphenol oxidase, peroxidase and cellulase had a critical role in the physiological process during the development of soybean. Luhova *et al.*⁸ also reported the same in *Pisum sativum*. In several other crops and trees like tomato¹⁷, pine¹⁸ and oil palm tree¹⁹ different enzyme activities and their role in growth and enhancement of the development process have been reported.

To conclude, there is an extent of variation within the accessions of *J. curcas*. Physiological and biochemical traits depending upon environment-related conditions may explain some of these associations. Furthermore, the different activities of enzymes in *J. curcas* may indicate that there is a critical role-play in growth and development of the plant. Our studies have revealed that all morphological traits, viz. plant height, collar diameter, number of branches and branch length are positively correlated. Therefore, exploration can be made to collect the superior plant materials on the basis of the studied morphological traits. Polyphenol oxidase and peroxidase enzymatic activities showed large variation, which will not only help in estimating the genetic relationship between the germplasm, but also in evaluating the capability of drought tolerance and enhancing flowering and fruiting¹⁰. Thus, quantification of peroxidase and polyphenol oxidase enzymes will be used not only as markers for estimation of genetic variation, but also for screening the breeding lines of *J. curcas* for high yielding potential. ADF, NDF, CP, lignin and hemicellulose content represent the nutritive value of *J. curcas*. This information is useful to the seed cake industry. The study has also revealed that estimation of peroxidase and polyphenol oxidase enzyme activities will help in improving the nutritive value, as both enzymes are found to be positively correlated with nutritive content. It can also be used as selection criterion for nutritive content, i.e. plants which show greater enzymatic activity will have more nutritive content.

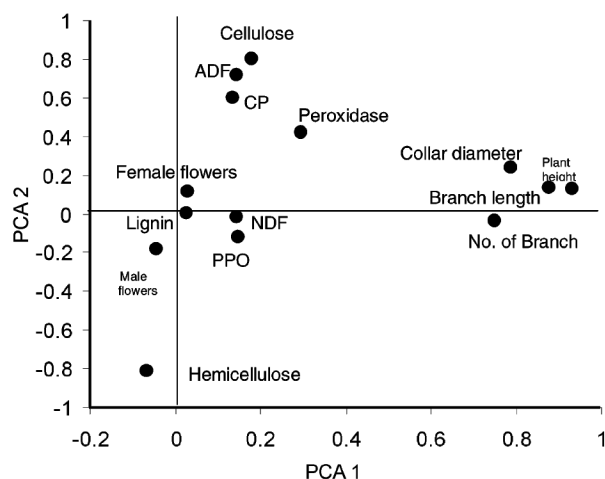


Figure 1. Principal component analysis ordination diagram of morphological and chemical traits of *Jatropha curcas* L. at NRCAF, Jhansi (India).

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Sand extraction from agricultural fields around Bangalore: Ecological disaster or economic boon?

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Sand supply from the riverbeds to Bangalore is not able to meet the demand of the booming construction sector. Enterprising farmers have taken up extraction of sand by washing surface soils of agricultural fields. Nearly 25% of the sand supplied is from this source. A field investigation and laboratory analysis was undertaken to understand the ecological and economic consequences of sand extraction. The study revealed that significant employment and economic gains are realized at an ecological cost. Loss of surface soils, nutrient losses, crop yield losses, siltation of tanks, excessive groundwater exploitation and soil erosion are taking place due to sand extraction. There are no quality differences between riverbed sand and soil extracted sand. A comprehensive policy is needed to make the enterprise ecologically tolerable and safe.

Keywords: Agricultural fields, eco-degradation, groundwater exploitation, sand extraction.

LAND is a limited resource having competing demands. The need to augment food production also from marginal lands has a serious impact on land use, resulting in accelerated land degradation. Progress in science and technology has eased out pressure on natural resources to some extent, but urban-centric development has resulted in imbalanced growth and exploitation of natural resources. Urban-centric economic growth has increased employment opportunities in these areas, whereas mechanization, stagnant prices and production, increased input costs in agriculture have made it unattractive leading to increased rural unemployment and migration. All these factors have put extraordinary pressure on urban infrastructure.

Riverbeds are major sources of sand and its accumulation as layers of deposits is a dynamic phenomenon. Major user of sand is the construction sector which is growing exponentially. Due to its increasing demand, sand is being over-extracted from different riverbeds. This is resulting in negative externalities on riparian habitats, like the riverbeds losing their ability to hold water. As sand is extracted rapidly, groundwater evaporates fast, reducing groundwater recharge, increasing initial and premature failure of irrigation wells and associated predicament in farming¹.

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