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Variability, Heritability and Association Studies in Seed Attributes of Jatropha Hybrid Genetic Resources

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Jatropha has emerged as one of the potential biofuel crop across the world. However, the yield instability coupled with the associated productivity issues are the major factors delimiting the promotion of Jatropha as an economically viable crop. Hence attempts were made to improve the productivity and vigour through interspecific hybridization programme which resulted in development of promising back cross clones. These were evaluated for variability and genetic attributes of seed physical characteristics. All the 27 Jatropha back cross clones exhibited significant variability in seed attributes. Six back cross clones viz., HC 23, HC 21, HC 17, HC 14, HC 8 and HC 6 exhibited superiority in terms of hundred seed weight and three back cross clones HC 23, HC 6 and HC 1 exhibited higher oil content. The genetic estimate study indicated that seed oil content expressed higher PCV and GCV and hundred seed weight exhibited higher heritability. The path analysis indicated that the seed length exerted maximum positive direct effect on oil content followed by hundred seed weight. In a holistic perspective, six back cross clones for hundred seed weight and three back cross clones for seed oil content expressed superiority which could be used in further improvement and promotional programme of Jatropha as biofuel crop.

Key words: Bio-fuel, jatropha, heritability

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India is targeting economic growth rate of 8-9% in coming years and there will be a substantial increase in demand for oil to manage transportation and to meet various other energy needs (Kureel, 2007). The growing consumption of energy has resulted in increasing dependency on fossil fuels. The consumption of crude oil was about 185 MT for the year 2007-08 but 80 per cent of this demand was met by imports (TERI, 2007).Thus the energy security has become a key issue for the nation as a whole. Even though hundreds of Tree borne oil seeds (TBO's) are available. Jatropha is an important crop to meet the energy demands of future.

Jatropha [*Jatropha curcas* (L)], an exotic non-edible oil seed bearing shrub and traditionally grown as a hedge plant in India (Heller, 1996). It is widely considered as a potential feed stock crop for bio-diesel production because of its shorter gestation period, smaller canopy suitable for high-density planting, convenient seed collection, higher seed oil content, easy propagation through seeds/ cuttings, drought hardiness, long productive period (40 years), rapid growth, non-grazing habit by animals, production on good and degraded soils and wider adaptability compared to most of the other non-edible oilseed yielding species (Martin and Mayeux, 1985; Francis *et al*.,2005 ; Sujatha, 2003).

In spite of these numerous favorable attributes, 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. The success of a breeding program depends largely on the knowledge of the genetic variability available and of estimates of genetic parameters of the main trait (Freitas *et al.,* 2011).Considering the economic importance of Jatropha,the present study was conducted in already established trial of 27 promising backcross derivatives at Forest College and Research Institute, Mettupalayam during the year 2010- 2011. The objective of this study is to investigate the variability, heritability and association characters among the seed attributes.

Materials and Methods

Interspecific hybridization programme has been attempted between *Jatropha curcas* and eight other *Jatropha* species to develop new hybrids with higher yield potential and resistance to root rot and frost tolerance. The cultivated species *J. curcas* was used as the female parent and the species, *viz*. *J. integerrima*, *J. podagrica*, *J. villosa*, *J. tanjorensis*, *J. gossypifolia*, *J. glandulifera*, *J. multifida* and *J. maheswari were* used as pollen donors. Hybridization between *J. curcas* and *J. integerrima* produced successful hybrids with more seed set, whereas the other crosses failed to produce seeds due to existence of crossability barriers either in pre-zygotic state or in post-zygotic state. Even in the successful hybrid though the F_1 progeny has exhibited vigorous growth, the fruit was smaller in size resembling *J. integerrima* characters. Hence, a backcross was attempted to get progeny with unique fruit, seed and oil yield characteristics (Parthiban *et al*., 2009). The BC_1F_1 exhibited wide variability in terms of fruit size and yield attributes. Among them 27 back cross derivatives were selected for further evaluation through clonal evaluation using rooted cuttings. These back cross derivatives were established in the RBD design in an espacement of 3m x 3m with 5 replications and in each replications, 5 ramets was used for observation. The seeds collected from this field were assessed for variability, heritability and association characters.

The data on seed length, seed breadth, seed length:breadth ratio, hundred seed weight and oil content were investigated in laboratory. These physical characters of seed were measured using vernier caliper. The seed parameter studies were carried out with a random sample of 20 seeds from each back cross clone with five replications. Oil was extracted by means of solvent extraction using petroleum ether (40 – 60_o C) as solvent (A.O.A.C. 1975). Data thus obtained were subjected to statistical analysis. Analysis of variance was carried out following the procedure given by Panse and Sukhatme (1978). The variability, heritability in broad sense (Lush, 1940), ge-netic advance as per cent of mean (Johnson *et al.,* 1955), phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) as per cent of mean (Burton, 1952) and path analysis (Dewey and Lu, 1959) were worked out for all the parameters.

Results and Discussion

Variability studies in seed physical attributes

The back cross clones exhibited significant variation for seed physical attributes. Seven back cross clones *viz*., HC 1 (1.85 cm), HC 6 (1.83 cm), HC 11 (1.81 cm), HC 14 (1.84), HC 20 (1.82 cm), HC 23 (1.89 cm) and HC 24 (1.88 cm) recorded significantly higher values for seed length compared to general mean(1.72cm). Three back cross clones *viz*., HC 17 (1.14 cm), HC 22 (1.06 cm) and HC 26 (1.07 cm) recorded significantly higher values for seed breadth compared to general mean (0.93cm). Two back cross clones *viz.,* HC 15 and HC 24 (2.08) recorded significantly higher values for seed length/ breadth ratio compared to general mean (1.87) (Table 1).

Hundred seed weight ranged from 33.35 g (HC 19) to 69.02 g (HC 23). The average hundred seed weight recorded was 54.31 g. Compared to the general mean (54.31g), six back cross clones *viz*.,

Table 1. Variability in seed attributes of Jatropha back cross clones

Hybrids	Seed length (cm)	Seed breadth (cm)	Length/ breadth ratio	100 seed Weight (g)	Kernal oil content (%)	Seed oil content (%)
HC 1	1.85**	0.90	2.05	55.30	44.95**	42.28**
HC 2	1.63	0.83	1.94	42.85	32.25	24.97
HC 3	1.73	0.92	1.90	50.78	34.09	32.45
HC 4	1.56	0.87	1.80	55.57	35.42	34.03
HC 5	1.74	0.97	1.80	49.53	41.30**	33.80
HC 6	1.83**	0.94	1.96	63.97**	44.13**	40.84**
HC 7	1.73	0.89	1.95	58.16	36.75	35.66
HC 8	1.77	0.88	2.02	64.08**	28.30	26.65
HC 9	1.64	0.95	1.74	48.59	38.94	34.43
HC 10	1.76	0.89	1.95	55.21	32.52	28.65
HC 11	1.81**	0.94	1.93	48.75	29.04	27.24
HC 12	1.60	0.81	1.95	54.57	32.64	31.69
HC 13	1.66	0.86	1.93	57.31	37.21	34.84
HC 14	1.84**	1.02	1.84	64.90**	32.71	28.47
HC 15	1.79	0.88	2.08**	56.36	38.76	35.47
HC 16	1.41	0.90	1.58	50.32	37.15	29.00
HC 17	1.80	1.14**	1.60	64.42**	30.31	24.23
HC 18	1.70	0.91	1.87	52.49	46.46**	37.22
HC 19	1.59	0.98	1.62	33.35	31.29	22.51
HC 20	1.82**	0.92	1.95	65.48**	33.40	30.66
HC 21	1.72	0.91	1.91	46.49	38.19	34.59
HC 22	1.67	1.06**	1.57	57.37	33.70	28.86
HC 23	1.89**	1.01	1.89	69.02**	40.81**	39.22**
HC 24	1.88**	0.93	2.08**	56.56	26.49	24.67
HC 25	1.60	0.82	1.93	47.05	34.49	32.58
HC 26	1.80	1.07**	1.68	54.41	26.75	24.31
HC 27	1.74	0.92	1.88	43.57	33.66	31.84
MEAN	1.72	0.93	1.87	54.31	35.25	31.52
Sed	0.04	0.05	0.08	2.01	1.49	2.29
CD (.01)	0.09	0.12	0.21	5.28	3.90	6.00

**Significant at 1% level

HC 6 (63.97g), HC 8 (64.08g), HC 14 (64.90 g), HC 17 (64.42g), HC 20 (65.48g) and HC 23 (69.02g) registered highly significant values for this trait. Five back cross clones *viz.,* HC 1 (44.95%), HC 5 (41.30 %), HC 6 (44.13 %), HC 18 (46.46 %) and HC 23 (40.81%) recorded significantly higher values for kernel oil content compared to general mean (35.25%). Seed oil content varied between 22.51 per cent (HC 19) and 42.28 per cent (HC 1). The average seed oil content recorded was 31.52 per cent. Compared to the general mean (31.52%), three back cross clones *viz*., HC 1(42.28%), HC 6 (40.84%) and HC 23 (39.22%) registered significantly higher values for seed oil content (Table 1).

The variability estimates *viz.,* Genotypic coefficient of variation(GCV), phenotypic coefficient of variation (PCV) and genetic advance as per cent of mean is presented in Table 2.The phenotypic and genotypic coefficient of variations for seed length was 7.11 and 6.34 per cent, respectively. Seed length recorded high heritability value of 0.79 and the genetic advance as per cent of mean was 11.64. Seed breadth exercised phenotypic and genotypic coefficients of variations of 10.92 and 7.53 per cent,

Table 2. Genetic estimates for seed attributed of Jatropha back cross clones

Traits	PCV	GCV	Heritability	GA(%) of mean
Seed length	7.11	6.34	0.79	11.64
Seed breadth	10.92	7.53	0.47	10.68
Length breadth ratio	9.89	7.11	0.51	10.53
100 seed weight	15.83	14.69	0.86	28.08
Seed oil content	19.33	15.95	0.68	27.12

respectively. It also recorded moderate heritability of 0.47 and the resultant genetic advance as percentage of mean was 10.68. The phenotypic and genotypic coefficient of variation for seed length/ breadth ratio was 9.89 and 7.11 per cent, respectively. It also recorded a moderate heritability of 0.51 and the genetic advance as per cent of mean was 10.53. Hundred seed weight exhibited phenotypic and genotypic coefficient of variation of 15.83 and 14.69 respectively. It recorded high heritability value of 0.86. The genetic advance as per cent of mean recorded by this trait was 28.08 which were highest among all the traits investigated. Seed oil content exhibited maximum phenotypic and genotypic coefficient of variation of 19.33 per cent and 15.95 per cent, respectively. This trait recorded high heritability value of 0.68 and the resultant genetic advance as percentage of mean was 27.12.

Genetic associations among seed physical attributes

Seed length expressed positive and feeble association with seed oil content both at phenotypic (0.137) and genotypic (0.146) levels. A positive and significant phenotypic and genotypic inter correlations were exhibited by seed length with seed breadth (0.355 and 0.409 respectively). Similarly, it also registered a positive and significant phenotypic and genotypic inter correlation with length: breadth ratio (0.349 and 0.470) and hundred seed weight (0.471 and 0.581) respectively. Seed breadth showed negative for phenotypic (-0.111) and significantly negative genotypic correlation (-0.373) with seed oil content. It recorded positive phenotypic and genotypic inter correlation with hundred seed weight (0.175 and 0.312). Seed breadth recorded negatively significant phenotypic and genotypic intercorrelation with seed length: breadth ratio (–0.745 and –0.613) respectively. Seed length: breadth ratio exhibited a positive phenotypic and significant genotypic association with seed oil content (0.202 and 0.464). The genotypic and phenotypic inter correlation with hundred seed weight was also found to be positive (0.161 and 0.210). Hundred seed weight expressed a positive and non-significant correlation with seed oil content at both phenotypic (0.211) and genotypic (0.269) levels (Table 3).

Table 3. Phenotypic and genotypic correlation coefficient among seed attributes on seed oil content

Traits		Seed length	Seed breadth	Length breadth ratio	100 Seed weight	Seed oil content
Seed length	P	1.000	0.355*	0.349*	0.471*	0.137
	G	1.000	0.409*	0.470*	0.581*	0.146
Seed breadth	P		1.000	-0.745*	0.175	-0.111
	G		1.000	-0.613*	0.312	-0.373*
Length breadth ratio	P			1.000	0.161	0.202
	G			1.000	0.210	0.464*
100 seed weight	P				1.000	0.211
	G				1.000	0.269
Seed oil content	P					1.000
	G					1.000

* Significant at 5% level

Path analysis

Among the four seed parameters studied, seed length (20.984) exerted maximum positive direct effect on oil content followed by hundred seed weight (0.686). Seed breadth (-24.045) and length: breadth ratio (-24.282) exhibited negative direct effect on seed oil content (Table 4).Seed length recorded a positive indirect effect through hundred seed weight (0.399) on seed oil content. The maximum negative indirect effect of this trait on seed oil content was exerted via seed breadth (-9.823) and length: breadth ratio (-11.413). Seed breadth exerted its maximum positive indirect effect through length: breadth ratio (14.885), seed length (8.572), and hundred seed weight (0.214) on seed oil content. Seed length: breadth ratio expressed positive indirect effect through seed length (9.863), seed breadth (14.739) and hundred seed weight (0.144) on seed oil content. Hundred seed weight exhibited positive indirect effect on seed oil content through seed length (12.186) .The negative indirect effect was expressed via seed breadth (-7.508) and seed length: breadth ratio (Table 4).

Table 4. Path coefficient analysis of seed parameters on seed oil content

Traits	Seed length	Seed breadth	Length breadth ratio	100 seed weight
Seed length	**20.984**	-9.823	-11.413	0.399
Seed breadth	8.572	**-24.045**	14.885	0.214
Length breadth ratio	9.863	14.739	**-24.282**	0.144
100 seed weight	12.186	-7.508	-5.095	**0.686**

Residual effect = 0.1740 (Diagonal values are direct effect)

The present study has conclusively established significant variations for seed physical characters. Seed size and weight are the two important characters for developing quality seedling production and reducing the nursery cost through selection of quality seeds, apart from selecting and delineating provenances (Bahar and Singh, 2007). In case of tree borne oil seed species genotypes with high oil content need to be selected so that a high seed yield really translates into high oil production (Kesari, *et al*., 2008). The study of pod and seed characters with oil content of the natural populations is often considered to be useful step in the study of genetic variability. Therefore, some of the basic material (seed) from the trees having more seed weight and oil content may be used for further improvement programme (Kaushik *et al*., 2007). Superiority in seed physical parameters *viz*., seed length, weight, length: breadth ratio and seed oil content was recorded by different back cross clones for all the characters. The back cross clone HC 23 recorded the highest and significant values for seed length (1.89 cm), HC 17 registered highest and significant values for seed breadth (1.14cm) and length: breadth ratio (2.08) in HC 15 and 24. Variability among hybrids for seed oil content and hundred seed weight had been observed in *Ricinus communis* (Saikia *et al.,* 2009 and Verma *et al*., 2007).

Similar variability in seed physical parameters of *Jatropha curcas* was earlier recorded (Kumar *et al*., 2003). A plethora of workers also reported significant variability for different seed physical attributes was observed among different *Jatropha curcas* sources (Ginwal *et al*., 2004; Das *et al*., 2010; Mohapatra and Panda, 2010; Rao *et al*., 2008).

In the present investigation, seed oil content registered highest GCV and PCV followed by hundred seed weight, seed breadth, seed length breadth ratio and seed length. The results are in association with the close findings of Das *et al*., (2010) and Rao et al., (2008) in *Jatropha curcas.*

The estimates of phenotypic and genotypic variances were close to each other. It indicates that the genotypic component was the major contributor to the total variance for these traits (seed length, breadth, 100-seed weight and oil content); i.e., most of the variability observed in the phenotype for these traits has more of a genetic than a non-genetic basis. This variability due to genotypic variance further indicates considerable scope for selection.

Hundred seed weight recorded the maximum heritability (0.86) and genetic advance estimate (28.08) per cent followed by seed oil content. The other seed parameters *viz*., seed length, seed breath and seed length breath ratio recorded the moderate heritability values. Such high heritability and genetic advance for seed parameters lie in close approximation with the findings of Das *et al*., (2010) and Rao *et al*.,(2008) in *Jatropha curcas*; Divakara *et al*., (2010) and Sahoo *et al.,* (2011) in *Pongamia pinnata*.

In the present study investigation, highly positive and significant phenotypic and genotypic correlation coefficient registered in hundred seed weight and exhibited positive association with seed oil content while seed breadth and length: breadth ratio exhibited positive and highly significant phenotypic and genotypic inter correlation with hundred seed weight. Similar positive and significant correlation of seed weight with oil content and other growth parameters were reported in *J. curcas* (Ginwal *et al*., 2004) and also in *Pongamia pinnata* (Divakara *et al*., 2010; Sahoo *et al.,*2011) which lend support to the present study. The correlation among seed breadth and hundred seed weight on seed oil content indicate that these physical parameters may be used to the advantage of the breeder for bringing simultaneous improvement of these traits easily. Heavier seeds have more oil and better seedling growth. The relation of seed weight and oil content existing in the current study indicate that seed weight as a factor could be exploited for screening genotypes to have an early indication on their oil yield and growth performance (Chauhan *et al*., 2005).

Path analysis gives an insight into complex relationship between different characters in a biological system (Table 4). The direct contribution of each component to the yield and the indirect effect which it has through its association with other components cannot be differentiated from mere correlation studies. Path coefficient analysis is further helpful in knowing the relative contribution of different traits to the trait of major interest (Divakara *et al*., 2010).

In the present investigation, seed length expressed maximum direct effect on seed oil content followed by hundred seed weight and length: breadth ratio. This suggests that the above mentioned traits be given utmost attention in the selection for seeds with high oil content. The seed characters *viz*., seed length, seed breadth and hundred seed weight exhibited their influence indirectly on seed oil content through seed breadth and hundred seed weight. However, variable results have been documented in *Madhuca latifolia* wherein hundred seed weight exerted maximum positive direct effect on seed oil content (Umesh Kanna, 2001) and the seed length to breadth ratio exhibited maximum direct effect on oil content in the same species (Jenner, 1995).

Conclusion

From the current study, it is known that back cross clones viz., HC 1, HC 6 and HC 23 performed exceedingly well in terms of seed oil content which is the most economic trait to meet future energy requirements. Hundred seed weight recorded maximum heritability combined with genetic advance and also the investigation exerted that seed length recorded maximum positive direct effect on oil content followed by hundred seed weight which shows that these parameters i.e. seed length and hundred seed weight could be selected for further Jatropha breeding/improvement programme.

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