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Evaluation of seed traits in *Jatropha curcas* L. for enhancement of oil yield

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Jatropha curcas has received considerable attention from researchers as a potential source of non-edible vegetable oil which is eminently suitable for production of liquid bio fuel, meeting international standards. For any tree improvement and breeding programme, study of variation among the populations is prerequisite as it helps in the detection of relative performance of various traits of economic value. 28 candidate plus trees were selected from the states of Jharkhand, West Bengal and Bihar in Eastern India. Nine seed parameters were measured and their genetic values and correlation was calculated to serve as base information for further improvement and breeding of *J. curcas* in Eastern India. Significant genetic differences exist in all the seed characteristics among the different candidate plus trees of *J. curcas*. Positive direct effect of seed width and protein content was observed on seed oil content. Indirect effect of seed traits via seed width was positive. Thus, seed width can be taken as a criterion for selecting trees with good oil yield. The variations in most studied parameters were under genotypic control among seed sources. Highest coefficient of variation was found for seed oil content emphasizing the need of wide scale screening and selection of superior genotypes to capture the existing variability.

Key words: Candidate plus trees, correlation, genetic gain, heritability, seed oil.

INTRODUCTION

Jatropha curcas L. is a small multipurpose tree with valuable attributes and considerable economic potential that grows in the equatorial Americas and has spread over other tropical countries cultivated worldwide for production of non-edible seed oil. In recent years *J. curcas* has drawn attention as a source of seed oil that can provide an economically viable substitute for motor fuel (Openshaw, 2000; Adebawale and Adedire, 2006; Chen et al., 2006). *J. curcas* is among the most suitable tree species for production of biodiesel as it can be cultivated as a quick yielding plant even in problem soils and adverse environmental conditions. Potentially high yield of oil per unit land area in *J. curcas* is second only to oil

palm (Fairless, 2007). Furthermore, the quality of oil in its seeds is suitable for production of biodiesel as they contain more than 75% unsaturated fatty acids (Biello, 2009). Among the oil bearing trees, *J. curcas* is desired due to its drought hardiness, rapid growth, easy propagation, small gestation period, wide adaptability, production on good and degraded soils and optimum plant size that makes seed collection more convenient (Jones and Miller, 1991; Francis et al., 2005).

Worldwide introduction of *J. curcas* for varied purposes had met with limited success due to unreliable and low seed set as well as oil yields resulting in poor economic returns (Singh et al., 2010). *J. curcas* is a wild species and no varieties with desirable traits for specific growing conditions are available, which makes its cultivation a risky business (Jongschaap et al., 2007). The crop is also

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characterized by variable and unpredictable yield due to unidentified reasons (Ginwal et al., 2004). The positive attributes of this plant are not fully understood in terms of breeding and utilization (Fairless, 2007) which limits its large-scale cultivation and warrants the need for genetic improvement and breeding of superior genotypes of the species.

Selection is the most important activity in all tree breeding programmes (Zobel and Talbert, 1984). *J. curcas* being highly cross-pollinated species is anticipated to contain wide genetic variability offering significant scope for selecting superior genotypes which will help in the improvement of productivity. Knowledge of genetic relationship and variation in the species is a prerequisite in any breeding programme because it permits the organization of germplasm, including elite lines and provides for more efficient selection (Karp and Edwards, 1998). However, the major constraint in achieving higher quality oil yield of *J. curcas* is lack of information about its genetic variability, oil composition and absence of suitable ideotypes for different cropping systems.

Plants, in general, might be expected to maintain high levels of genetic variation within their populations since their sessile nature often leads to the evolution of locally adapted ecotypes (Antonovics, 1971; Bradshaw, 1972; Jain and Bradshaw, 1966). This variation may be utilized in selection and improvement of the species. Gaining insights into the genetic variability of *J. curcas* provenances and elucidation of correlations between the genetic parameters and biochemical characteristics of seed provenances collected from different regions of the world would be a critical input for the selection of appropriate genotypes for cultivation and breeding purposes (Basha et al., 2009). Modern techniques have accelerated characterization of *J. curcas* germplasm at molecular level (Singh et al., 2010; Xu et al., 2012) and even whole genome sequencing (Sato et al., 2010). However, information regarding the extent and pattern of genetic variation in *J. curcas* population is limited barring a few recent studies (Kaushik et al., 2007; Rao et al., 2008; Gairola et al., 2011; Tripathi et al., 2013; Brasileiro et al., 2013). Therefore, genetic and correlation studies among the seed traits were carried out in candidate plus trees of *J. curcas* selected from a wide geographical range in three Eastern Indian states aiming at utilization in oil yield improvement.

MATERIALS AND METHODS

Twenty eight (28) Candidate Plus Trees (CPTs) of *J. curcas* were selected during 2007-2010 from naturalized areas of Eastern India (states of Bihar, West Bengal and Jharkhand) on the basis of seed bearing quality, crown size and tree health. The details of the selected plus trees are given in Table 1. Seeds of plus trees were collected to measure seed traits under laboratory conditions. These included seed length (cm), seed width (cm), hundred seed weight (g), 2D surface area (cm²), aspect ratio, germination percentage (%),

total soluble carbohydrate (mg/g), total soluble protein (mg/g) and seed oil percentage (%).

Total soluble carbohydrate and total soluble protein were as estimated according to standard methods of Dubois et al. (1956) and Lowry et al. (1951) on UV-Vis spectrophotometer (Pharos, Merck Germany). Seed oil content from seed kernel sample was studied using Soxhlet apparatus. 100 g of seed were oven dried at 40°C overnight before the seeds were broken to get the kernels. 10 g of these kernels were finely chopped, wrapped in Whatman's Filter paper Number 2 and kept in the middle chamber Soxhlet's apparatus for oil extraction. Empty weight of the boiling flask was taken. In the boiling flask, petroleum ether (boiling range 60 to 80°C) was maintained at about 70°C. The ether vapors after getting condensed in the condenser chamber got collected as liquid ether in the middle chamber. The kernels came in contact with ether and the oil in the kernels got dissolved with ether which led to the rise in ether level in the middle chamber. Oil mixed ether was then poured down into the lower boiling flask and the entire process was repeated once again which allowed ether to get evaporated and not the oil as the later had higher boiling range. The lower boiling flask was kept in boiling water bath for about 35 min to expel the ether and separate the oil. Final weight of the boiling flask with oil was taken and net weight of *Jatropha* oil obtained was calculated from the difference of the two weights of the boiling flask. Percentage of oil in seeds was measured as oil content percentage = weight of extracted oil (g)/sample weight x 100.

The data of seed traits was subjected to statistical analysis firstly to test the significance among the various CPTs by analysis of variance. The Statistical Package for Agriculture Research, version 2.0 (SPAR 2.0) was used for calculating various variability parameters (phenotypic coefficient of variability, genotypic coefficient of variability, heritability, expected genetic advance and genetic gain), correlation among different traits, path and Euclidean clusters analysis.

RESULTS AND DISCUSSION

Seed contain a lot of variation from one origin to another origin with regards to morphological variation and physiological differences which could be genetic in nature as a result of adaptation to diverse environmental condition prevailing throughout their distributional range (Mathur et al., 1984). Apart from age, vigour, crown exposure and genotype of mother tree, soil and climate of the place of seed origin are important factors affecting the seed traits (Salazar and Quesada, 1987). Due to its wide geographical distribution, there is considerable scope of genetic variation in *J. curcas* seeds, which are the principal means of propagation. Significant trait differences were observed in seed characters namely: seed morphology and oil content as well as in growth characters including seed yield in the progeny trial of *J. curcas* for evaluating genetic association, and variability in seed and growth characters (Rao et al., 2008). Variability in seed traits and oil content of 24 accessions of *J. curcas* collected from different agroclimatic zones of Haryana state, India were assessed to record significant differences in seed size, 100-seed weight and oil content between accessions (Kaushik et al., 2007).

We also observed significant variation among the candidate plus trees for all the seed parameters (Table 2).

Table 1. Location of selected candidate plus trees of *Jatropha curcas* in Eastern India.

CPT	Locality (District)	Latitude	Longitude	State
J ₁	Katangdri (Ranchi)	23° 34' 38.76"	85° 18' 34"	Jharkhand
J ₂	Hutar (Khunti)	23° 7' 54.2"	85° 16' 11"	Jharkhand
J ₃	Banai (Gumla)	22° 52' 15.88"	84° 49' 27.14"	Jharkhand
J ₄	Banai Bindratoli(Gumla)	22° 52' 15.88"	84° 49' 30"	Jharkhand
J ₅	Patura Dhauntatoli(Gumla)	22° 52' 12.43"	84° 49' 30"	Jharkhand
J ₆	Purnatoli (Simdega)	22° 41' 53.53"	84° 41' 56.24"	Jharkhand
J ₇	Fikpani(Simdega)	22° 41' 55.77"	84° 42' 1.15"	Jharkhand
J ₈	Jamtoli(Simdega)	22° 43' 40.9"	84° 41' 55.2"	Jharkhand
J ₉	Pandripani Karanjtoli(Simdega)	22° 30' 0.7"	84° 30' 40.33"	Jharkhand
J ₁₀	Silaphari Hundratoli (Gumla)	22° 2' 50.63"	84° 32' 31.45"	Jharkhand
J ₁₁	Hurudag (Hazaribagh)	24° 6' 32.53"	85° 12' 13.14"	Jharkhand
J ₁₂	Barhi (Hazaribagh)	24° 18' 16.37"	85° 25' 18.79"	Jharkhand
J ₁₃	Motileda Bermasia (Giridih)	24° 18' 10.68"	86° 21' 46.02"	Jharkhand
J ₁₄	Palunjia (Giridih)	24° 16' 10.31"	85° 55' 54.23"	Jharkhand
J ₁₅	Bardih (Bokaro)	23° 40' 7.15"	86° 3' 19.83"	Jharkhand
J ₁₆	Dantu (Bokaro)	23° 36' 16.94"	85° 56' 12.63"	Jharkhand
J ₁₇	Baghmundi (Purulia)	23° 11' 52.86"	86° 2' 38.82"	West Bengal
J ₁₈	Awagarh (Midnapore)	22° 25' 15.13"	87° 19' 33.91"	West Bengal
J ₁₉	Salboni (Midnapore)	22° 38' 32.12"	87° 19' 8.42"	West Bengal
J ₂₀	Pathrajuri (Midnapore)	22° 32' 27.08"	87° 18' 32.64"	West Bengal
J ₂₁	Bhadua (Midnapore)	22° 17' 25.28"	86° 56' 59.61"	West Bengal
J ₂₂	Satkui (Midnapore)	22° 22' 34.12"	87° 20' 33.36"	West Bengal
J ₂₃	Binpur (Midnapore)	22° 34' 38.17"	87° 0' 14.96"	West Bengal
J ₂₄	Rajgir Road (Nalanda)	25° 1' 54.43"	85° 25' 10.49"	Bihar
J ₂₅	Rajgir (Nalanda)	25° 1' 46.0"	85° 24' 59.83"	Bihar
J ₂₆	Kenar (Gaya)	24° 46' 2.87"	85° 16' 10.77"	Bihar
J ₂₇	Berhibigha (Gaya)	24° 48' 7.83"	85° 14' 37.62"	Bihar
J ₂₈	Wajirganj (Gaya)	24° 47' 53.14"	85° 2' 4.12"	Bihar

J₁₇ depicted the maximum seed length, seed width, 2D surface area, aspect ratio and germination percentage. Maximum 100-seed weight was found for J₁₉ (74.35) which were statistically at par with J₁₇, J₂₃, J₁₆, J₃, J₁₅, J₂₄, J₉ and J₁. J₁₆ (17.13) followed by J₁₈ exhibited highest value for total soluble carbohydrate. Maximum seed oil percentage was estimated in J₂₇ (45.38) and the minimum value in J₂₂ (27.77) (Table 2). Wide variation for seed traits are in conformity with earlier report in *J. curcas* (Anonymous, 2005; Ginwal et al., 2004; Kumar et al., 2003) and other tree species for example Japanese black pine (Miyata et al., 1991), and *Dalbergia sissoo* (Singh and Pokhriyal, 2001). Variations observed in seed traits are expected to be genetic in nature as a result of adaption to diverse environmental conditions where CPT selection was made.

The proportion of total variation, which is heritable, is termed as heritability in broad sense (Lush, 1937). Knowledge of its magnitude gives an idea about scope of effecting genetic improvement through selection. Heri-

tability in broad sense may give useful indication about the relative value of selection in the material at hand, to arrive at a more reliable conclusion. Variability and genetic parameters of seed and germination traits have been presented in Table 3. Highest coefficient of variation was exhibited for seed oil percentage (11.58) and the lowest for protein content (0.12). Total soluble carbohydrate depicted the maximum phenotypic (63.93) and genotypic coefficient of variability (63.92), heritability (0.999) and genetic gain (131.66). Seed width exhibited the lowest phenotypic (4.34) and genotypic coefficient of variability (4.20), genetic advance (0.09) and genetic gain (8.04). Lowest heritability was found for seed oil percentage (0.302).

Heritability and associated genetic gain should be considered jointly. Heritability estimates along with genetic gain is more useful than the heritability alone in predicting the resultant effect for selecting the best genotype for given trait (Johanson et al., 1955; Volker et al., 1990). Therefore, a heritability estimate alone does

Table 2. Variation in seed traits of candidate plus trees of *J. curcas*. Data in parentheses are transformed values.

CPT	Seed length (cm)	Seed width (cm)	100-Seed weight (g)	2D Surface area (cm ²)	Aspect ratio	Germination percentage (%)	Total carbohydrate (mg/g)	Total soluble protein (mg/g)	Seed oil content (%)
J ₁	1.52	1.06	46.48	10.11	1.43	54 (46.30)	2.52	14.8	42.17(37.40)
J ₂	1.61	1.07	53.50	10.89	1.50	64(52.14)	10.02	19.28	40.80(36.60)
J ₃	1.83	1.17	69.20	13.45	1.56	78(61.05)	1.88	11.63	40.60(36.48)
J ₄	1.45	1.06	40.10	9.61	1.37	51(44.58)	2.86	13.67	44.30(36.63)
J ₅	1.69	1.10	59.28	11.66	1.53	70(55.80)	2.54	14.83	38.08(35.00)
J ₆	1.77	1.13	57.05	12.53	1.56	70(55.80)	3.88	13.70	38.32(35.15)
J ₇	1.78	1.09	59.70	12.15	1.64	68(54.56)	7.35	15.81	37.35(34.56)
J ₈	1.58	1.11	65.00	11.03	1.43	74(58.38)	8.88	17.15	40.88(36.64)
J ₉	1.77	1.18	67.65	13.14	1.50	72(57.06)	1.21	11.32	40.36(36.34)
J ₁₀	1.57	1.04	44.23	10.22	1.50	52(45.15)	6.33	17.11	39.88(36.06)
J ₁₁	1.68	1.11	63.40	11.69	1.51	72(57.06)	2.15	12.06	41.25(36.86)
J ₁₂	1.78	1.13	51.80	12.66	1.57	62(50.45)	8.72	18.03	43.92(38.41)
J ₁₃	1.77	1.11	66.63	12.32	1.59	75(59.01)	3.76	15.59	35.01(33.18)
J ₁₄	1.67	1.08	58.38	11.26	1.55	69(55.18)	8.94	19.01	44.11(38.52)
J ₁₅	1.79	1.14	68.98	12.78	1.58	70(55.78)	10.4	19.35	42.59(37.64)
J ₁₆	1.70	1.21	70.10	12.92	1.40	82(63.90)	17.13	19.24	35.74(33.61)
J ₁₇	1.85	1.22	73.50	14.18	1.51	84(65.44)	4.02	13.72	42.59(37.64)
J ₁₈	1.73	1.11	60.33	12.08	1.56	68(54.56)	16.27	18.09	35.74(32.27)
J ₁₉	1.77	1.22	74.35	13.55	1.44	82(63.93)	6.09	13.93	42.31(37.48)
J ₂₀	1.79	1.13	62.28	12.65	1.59	71(56.42)	5.96	13.01	43.26(38.03)
J ₂₁	1.80	1.12	62.15	12.60	1.61	72(57.06)	2.25	11.06	37.10(34.42)
J ₂₂	1.44	1.08	49.58	9.80	1.34	58(48.61)	13.04	16.45	27.77(28.70)
J ₂₃	1.79	1.14	71.15	12.78	1.58	79(61.74)	9.32	19.16	38.97(35.53)
J ₂₄	1.78	1.14	68.28	12.72	1.56	69(55.18)	5.95	14.48	39.34(35.74)
J ₂₅	1.61	1.07	60.63	10.73	1.51	58(48.61)	6.93	17.21	38.76(35.40)
J ₂₆	1.77	1.12	63.98	12.41	1.58	55(46.87)	6.22	17.89	36.11(33.83)
J ₂₇	1.80	1.11	63.75	12.56	1.62	54(46.30)	7.78	18.66	45.38(39.25)
J ₂₈	1.49	1.13	65.65	10.55	1.32	56(47.45)	1.99	10.24	45.12(39.10)
LSD _{0.05}	0.018	0.0172	8.14	0.211	0.031	2.242	0.106	0.027	6.538

not necessarily mean an increased genetic advance. High heritability (broad sense) may be due to non-additive gene action so it shall be reliable only if accompanied by high genetic gain (Rawat and Nautiyal, 2007). In the present study, higher heritability values of the seed traits were generally accompanied by high genetic gain as earlier reported for seed weight in *Graewia optiva* (Uniyal, 1998) and *Celtis australis* (Jain, 1982). This indicates that high heritability leads to increased genetic gain if sufficient genetic variability exists in the germplasm.

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 from base seed material. Oil content is greatly influenced by environmental factors such as soil conditions (Srivastava, 1999). Phosphorus has been found to be the main requirement for increase in oil yield in the case of castor beans growing in East Africa (Geus and Jan, 1973). A drier climate is supposed to improve the oil yield in *J. curcas* seeds (Jones and Miller, 1991). These findings support the present research that oil content is mainly affected by environment conditions as compared to genetic makeup. We also recorded highest coefficient of variation for seed oil content, however it possessed lowest heritability. Heritability was high for total soluble carbohydrates, protein content, 100-seed weight, seed length and seed width, which shows that through clonal

Table 3. Mean coefficient of variation (CV (%)) and genetic estimates of seed traits of *J. curcas*

Character	Range	Mean	CV (%)	Coefficient of variability		Heritability	Genetic advance	Genetic gain (%)
				Phenotypic	Genotypic			
Seed Length	1.43- 1.86	1.698	0.751	7.052	7.012	0.989	0.24	14.134
Seed Width	1.00- 1.23	1.12	1.084	4.338	4.2	0.937	0.09	8.036
100-seed weight	40.0- 74.6	61.323	9.389	16.425	13.478	0.673	13.97	22.603
2D Surface Area	9.56 - 14.37	11.964	1.246	10.118	10.041	0.985	2.46	20.56
Aspect Ratio	1.31- 1.65	1.517	1.442	5.783	5.601	0.938	0.17	11.206
Germination (%)	50.0- 85.0	67.464	2.349	14.46	14.269	0.974	19.57	29.008
Total soluble carbohydrate	1.18- 17.5	6.585	1.142	63.932	63.922	0.999	8.67	131.663
Total S. Protein	10.22- 19.38	15.589	0.122	18.258	18.258	0.999	5.86	37.591
Seed Oil Content	27.64- 45.42	39.922	11.581	13.863	7.62	0.302	3.44	8.617

Table 4. Simple Correlation between seed traits and germination percentage in *J. curcas*.

Character	Correlation with germination percentage
Seed Length	0.6080*
Seed Width	0.7277*
100-seed weight	0.6468*
2D Surface Area	0.7306*
Aspect Ratio	0.211
Total soluble carbohydrate	0.0786
Total soluble protein	-0.1187
Seed Oil Content	-0.0493

*Significant at 1%.

propagation, we can trap these qualities of mother plant. Manga and Sen (1996) in *Prosopis cineraria* and Mahadevan et al. (1999) in *Casuarina equisetifolia* have also recorded similar heritability of seed traits. Correlation is one of the important biometrical tools, which measure the degree and magnitude of association among different traits. In tree improvement programme, a clear understanding of association among different traits is of great importance as it illustrates whether the choice of one character confirm the appearance or disappearance of other. Significantly and positively correlation of 2D surface area (0.7306), seed width (0.7277), 100-seed weight (0.6468) and seed length (0.6080) were recorded with germination percentage at 1% level of significance.

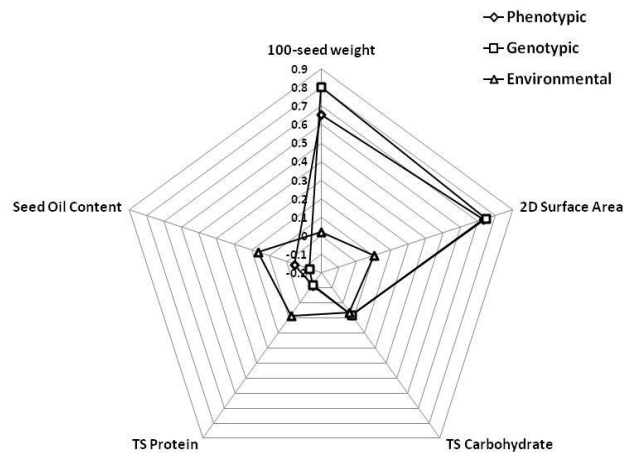
A negative but non-significant correlation was found of seed protein content and seed oil percentage with germination percentage (Table 4). Seed length, seed width and 100- seed weight has significant simple, phenotypic and genotypic correlation with germination percentage in the present investigation as previously reported in Douglas fir (Clair and Adams, 1991) and *P. cineraria* (Manga and Sen, 1995).

Proper utilization of observed variation in a species depends upon the knowledge the extent of variation and its cause, whether it is due to genetic (heritable) or the environmental and phenotypic (non heritable) factors. The related magnitude of these components determines the genetic properties of any particular species (Jain, 1982). Release of high yielding clones/cultivars cannot be done 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. 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). We also recorded highly significant and positive phenotypic as well as genotypic correlation of seed length, seed width, 100-seed weight and 2D surface area with germination percentage (Table 5). 2D surface area (0.732) had the maximum phenotypic correlation followed by seed width (0.730), 100-seed weight (0.653) and seed length (0.608). 100-seed weight (0.803) depicted the maximum genotypic correlation followed by seed width (0.761), 2D surface area (0.745) and seed length (0.619). Protein content and seed oil content showed non-significant and negative phenotypic and genotypic correlation. No combination had significant environmental correlation. Relative proportion of phenotypic, genotypic and environmental correlations for seed morphological and biochemical characteristics presents a contrasting scenario (Figure 1a and b) where in the case of the former genotypic correlations were stronger and significant while environmental correlations were strong though non-significant for seed oil and total protein. Thus, genotypic-/phenotypic variations scored over environmental variation. When more variables are included in the correlation studies, the inherent association becomes more complex. Under such circumstances, to understand the specific

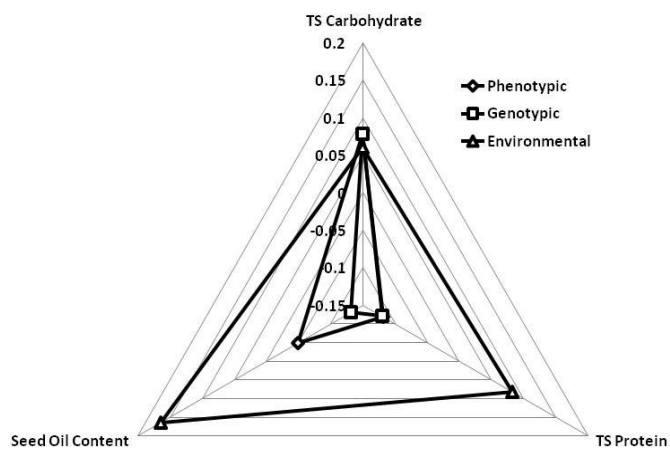
Table 5. Phenotypic, genotypic and environmental correlation between seed traits and germination percentage in *J. curcas*.

Character	Correlation with germination percentage		
	Phenotypic	Genotypic	Environmental
Seed Length	0.608*	0.619*	0.057
Seed Width	0.730*	0.761*	0.074
100-seed weight	0.653*	0.803*	0.022
2D Surface area	0.732*	0.745*	0.104
Aspect ratio	0.211	0.222	-0.044
Total soluble carbohydrate	0.079	0.079	0.060
Total soluble protein	-0.119	-0.120	0.082
Seed oil content	-0.049	-0.131	0.164

forces in building up of total correlation and to resort direct and indirect effects of different contributing traits on important desirable traits, path coefficient analysis is imperative. Perusal of Table 6 reveals that aspect ratio (4.9638) resulted in maximum direct effect on seed oil content, while seed length (-5.9372) depicted the maximum negative indirect effect. Seed length depicted the negative indirect effect on seed oil content by 100-seed weight, 2D surface area and germination percentage and positive indirect effect by seed width, aspect ratio, total soluble carbohydrate and protein content. Seed width showed positive direct effect (4.4862) on seed oil content and positive indirect effect via aspect ratio; however negative indirect effect via other seed traits. 100-seed weight depicted negative direct effect (-0.1241) on seed oil content. It revealed positive indirect effect via seed width and aspect ratio and via others negative indirect effects. 2D surface area revealed the negative direct effect (-0.4944) on seed oil content. It had positive indirect effect via seed width, aspect ratio and total soluble carbohydrate and negative indirect effect via other remaining traits. Aspect ratio resulted in positive indirect effects by seed width, aspect ratio, protein content and total soluble carbohydrate and negative indirect effect via other remaining traits. Germination percentage depicted the negative direct effect (-0.3556) on seed oil content and positive indirect effect via seed width and aspect ratio and negative indirect effect via other remaining traits. Total soluble carbohydrate resulted in negative direct effect (-1.0007) on seed oil content. It showed positive direct effect via seed width, 2D surface area and protein content. Via other traits, there were negative indirect effects. Protein content revealed positive direct effect (0.6484) on seed oil content. It had positive indirect effects via 100-seed weight, 2D surface area, aspect ratio and germination percentage; however negative indirect effects via seed length, seed width and total soluble carbohydrate.



A



B

Figure 1. A. Relative proportion of phenotypic, genotypic and environmental correlations for seed morphological characteristics. **B.** Relative proportion of phenotypic, genotypic and environmental correlations for seed oil and other biochemical traits.

Reliable classification of accessions along with identification of core subsets among the accessions with future utility for specific breeding purposes can be expedited through analysis of genetic diversity in germplasm collections. 28 CPTs of *J. curcas* were grouped in four clusters using non-hierarchical Euclidean Cluster Analysis (Table 7). However, *K*-means clusters clustering pattern in the present study revealed trees from different geo-graphic regions grouped together in a cluster and trees from the same geographical areas placed in different clusters indicating separation of geographical diversity from inherent genetic diversity of the germplasm. *K*-means clustering is done to understand the trend of evolution and

Table 6. Path coefficient analysis results showing direct (in italics) and indirect effects of seed traits on seed oil content in *J. curcas*.

Character	Seed length	Seed width	100-seed weight	2D Surface area	Aspect ratio	Germination (%)	TS Carbohydrate	TS Protein	Total effect on seed oil content
Seed length	-5.9372	2.7287	-0.0882	-0.4651	4.0357	-0.2201	0.0390	0.0001	0.0929
Seed width	-3.6112	<i>4.4862</i>	-0.1054	-0.4156	0.1637	-0.2704	-0.0151	-0.154	0.0787
100-seed weight	-4.2189	3.8082	<i>-0.1241</i>	-0.4150	1.3803	-0.2857	-0.0216	-0.074	0.0496
2D surface area	-5.5856	3.7715	-0.1042	<i>-0.4944</i>	2.8184	-0.2649	0.0257	-0.0665	0.1001
Aspect ratio	-04.8271	0.1479	-0.0345	-0.2807	<i>4.9638</i>	-0.0798	0.0500	0.1187	0.0583
Germination (%)	-3.6758	3.4121	-0.0997	-0.3683	1.1135	<i>-0.3556</i>	-0.0795	-0.078	-0.1313
TS carbohydrate	0.2314	0.0676	-0.0027	0.0127	-0.2479	-0.0283	<i>-1.0007</i>	0.5071	-0.1810
TS protein	-0.0012	-1.0617	0.0141	0.0507	0.9086	0.0428	-0.7826	<i>0.6484</i>	-0.1810

Table 7. Composition of Euclidean clusters and cluster mean values obtained by K-means Non-hierarchical clustering for seed and oil traits in *J. curcas*.

Cluster	Number of CPTs	CPT Codes	Seed oil (%)
I	10	J4, J6, J8, J12, J20, J21, J22, J25, J27	40.01
II	6	J1, J2, J3, J5, J7, J14	37.58
III	8	J9, J11, J16, J18, J19, J23, J24, J28	41.34
IV	4	J10, J13, J15, J26	40.36

choose genetically diverse parents for obtaining desirable recombination (Tams et al. 2006). Intra-cluster distances were lower (maximum 1.59 in III and minimum 0.853 in I) than that of the inter-cluster distances indicating the genetic similarity among the members within clusters. Maximum inter-cluster distance (6.323) between cluster I and IV suggests 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 distances for example II and III (2.162) should be avoided.

Present study suggests positive direct effect of seed width and protein content on seed oil content. Positive indirect effect of seed traits via seed width suggests that seed width can be taken as a criterion for selecting trees with good oil yield. It is clear that considerable genetic differences exist in all the seed characteristics among the different CPTs of *J. curcas*.

J₁₇ was found as a superior seed source on the basis of seed morphological characters. However, for current trait of interest in *J. curcas* that is seed oil, J₂₇ exhibited superiority. The variations in most studied parameters are under genotypic control among seed sources. Highest coefficient of variation was found for seed oil content emphasizing the need of wide scale screening and selection of superior genotypes to capture the existing variability. However, low heritability of seed oil makes the task difficult for breeders.

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