The Bioscan 9(3): 1131-1134, 2014
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

BIOCHEMICAL CHARACTERIZATION OF JATROPHA (*JATROPHA CURCAS* L.) ACCESSION IN EASTERN HIMALAYA, INDIA

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KEYWORDS
Jatropha curcas
Seed yield
Oil content
Cellulose
Crude protein
Peroxidase

Received on :
02.02.2014

Accepted on :
22.07.2014

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ABSTRACT

Adaptive trials on *J. curcas* were undertaken at ICAR RC for NEH Region, Jharnapani during 2007-2012 with 11 accessions of *Jatropha* collected from different parts of India. The aim of the study was to determine the source variation in *J. curcas* accessions collected from 11 locations of Nagaland and to identify the best sources to be utilized for reforestation and future genetic improvement work. All the accessions of jatropha differed significantly in the respect of seed yield, oil content and oil yield, crude protein, cellulose, hemi-cellulose and enzyme activities. The provenance Molvum produced significantly higher seed yield (0.88 t ha^{-1}). Such variation among the different accession may be due to different intensities of natural selection acting upon the traits in their natural habitat. Among all accessions of *jatropha,* Molvum recorded significantly higher cellulose (23.1%) followed by Rangapahar. Similarly, the maximum hemicellulose was recorded with Molvum which was statistically at par with Piphema, Ruzaphema, Rangapahar but significantly superior over all the remaining accessions, while, the lowest hemicellulose (10.8%) was obtained with Seithekiema. Significantly maximum lignin of 23.3% was obtained under Molvum but it was statistically comparable to Rangapahar. The minimum lignin (13.1%) was found under Seithekiema. Crude protein content was recorded maximum with Molvum (15.7%) which was comparable with Jharnapani, Mediziphema and Ruzaphema and significantly higher to rest of the other accession.

INTRODUCTION

Biofuel is renewable and benign to environment and has showed a great potential in coping with worldwide energy crisis and the increasingly serious environmental problems. (Anand *et al.*, 2011). Biodiesel derived from vegetable oil is an important renewable source of energy which is biodegradable in nature. Increasing rate of depletion of fossil fuel leads people to think about alternative sources of energy (Navanita and Goswami, 2010). Use of vegetable oil as an alternative fuel for diesel engines is not a new concept. *Jatropha* has drawn the attention of researchers in recent years due to its emergence as a highly suitable feedstock plant for biodiesel production. Many non-edible tree-born oil-seed species such as Jatropha, Karanj, Pilu, Mahua, Sal and Cheura, widely found in India, are suitable for bio-diesel production. *Jatropha curcas* has been identified as a promising biofuel crop by NOVOD Board, Government of India and many other Research Institutes within and also outside the country. Its superiority over other bio-fuel crops and tree borne oil seeds is due to its oil quality, suitability to diesel engine, high oil content, short gestation, crop architecture, adaptability and familiarity with the farmers in India. One of the major problems for the poor yield of *Jatropha* is the poor quality of the planting material. Seeds were collected locally as bulk without any selection, grading, testing and were mass propagated and marketed. Lack of quality seedlings and potential hybrids are the causes for concern. Identification of a good location is important as it would help in continuous collection of seed for propagation and seedling production. Since the prices of edible vegetable oils are higher than that of diesel fuel, non-edible crude vegetable oils like *Jatropha* are preferred as potential low priced Biodiesel (Singh and Singh, 2010). The seeds of *jatropha* contain about 30% oil which is suitable for biodiesel. It can be grown under different land use situations and be easily propagated by seed or cuttings and starts bearing fruiting within 2-3 years. It can be commercially exploited in 4-5 years and lasts for about 50 years (Kumar *et al.*, 2005). The oil can be combusted as fuel without being refined. It burns with clear smoke-free flame, tested successfully as fuel for simple diesel engine. The use of this plant for large-scale bio-diesel production is of great interest with regard to solving energy shortage, reducing the carbon emission and increasing income of the farming community. The utilization of *Jatropha. curcas* oil as a fuel for diesel engine has tremendous scope and thus contributing to the growing needs of energy resources in the country (Raiger *et al.*, 2011).

There is an extent of variation were noticed within the accessions of *Jatropha curcas*. Physiological and biochemical traits depending upon the environment-related conditions and may explain some of these associations. Furthermore, the different activities of enzymes in *Jatropha curcas* may indicate that there is a critical role-play in growth and development of the plant (Kumar *et al.*, 2007). It is therefore, exploration can be made to collect the superior plant materials on the basis of the studied morphological and biochemical traits. Polyphenol oxidase and peroxidase enzymatic activities showed large variation, which will not only help in estimating genetic

Table 1: Influence of different accessions on seed and oil yield and enzyme activities of *Jatropha*

Accessions	Seed yield (t ha^{-1})	Oil content (%)	Oil yield (t ha^{-1})	ADF (%)	NDF (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Crude protein (%)	Peroxidase (²%A420 by 0.001/mL /min)	Polyphenol oxidase (²% A420 by 0. 001/mL/min)
Jalukie	0.55	32.04	17.62	42.28	49.48	19.14	11.68	17.03	14.41	32.67	75.30
Jharnapani	0.70	30.11	21.08	43.01	50.08	17.55	11.39	15.10	15.14	34.89	80.30
Medziphema	0.68	31.36	21.17	43.11	48.96	16.49	11.95	16.35	15.02	33.87	78.02
Molvum	0.88	38.91	34.05	45.40	54.84	23.06	15.28	24.29	14.03	41.70	87.05
Piphema	0.85	35.54	30.21	43.85	50.82	20.80	14.38	20.90	15.74	37.97	82.91
Rûzaphema	0.70	34.35	24.05	42.90	49.88	19.54	14.97	19.34	15.03	34.69	81.01
Seithekiema	0.63	28.11	17.57	41.03	49.86	13.27	10.78	13.10	13.16	34.89	78.97
Khatkati	0.68	33.29	22.47	37.11	50.16	18.51	12.58	18.28	13.97	35.09	79.46
Rangapahar	0.73	37.43	27.14	36.03	48.96	22.69	14.68	22.42	13.97	30.97	81.95
Dhansiripar	0.80	32.31	25.85	42.08	50.24	19.24	11.68	17.30	14.21	34.68	85.11
Tolbibasti	0.70	32.92	23.04	41.90	46.75	18.17	12.53	17.91	14.03	32.87	79.10
SEm ±	0.02	1.00	0.78	1.19	1.45	0.58	0.39	0.58	0.40	1.05	2.33
CD (P = 0.05)	0.06	2.94	2.30	3.50	4.28	1.70	1.14	1.71	1.19	3.09	6.89

Table 2: Correlation between yield and quality traits of *Jatropha*

Traits	Seed yield (t ha^{-1})	Plant height (cm)	Collar diameter (cm)	No. of branches $plant^{-1}$	No. of fruiting branches $plant^{-1}$	No. of fruit $branches^{-1}$	1000-seed weight (g)	Oil content (%)
Seed yield (kg ha^{-1} $plant^{-1}$)	1.00							
Plant height (cm)	0.743**	1.00						
Collar diameter (cm)	0.284	0.381	1.00					
No. of branches $plant^{-1}$	0.827**	0.708*	0.151	1.00				
No. of fruiting branches $plant^{-1}$	0.859**	0.752**	0.066	0.948**	1.00			
No. of fruit $branches^{-1}$	0.756**	0.919**	0.159	0.813**	0.888**	1.00		
1000-seed weight (g)	0.188	0.123	0.603*	-0.051	-0.233	-0.194	1.00	
Oil content (%)	0.654*	0.556	0.143	0.473	0.478	0.537	-0.189	1.00

** Correlation significant at 0.01; *Correlation significant at 0.05

relationship between the germplasm, but also in evaluating capability of drought tolerance and enhancing the flowering and fruiting (Low and Merida, 1996).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 *Jatropha curcas* for high yielding potential. ADF, NDF, CP, lignin and hemicellulose content represent the nutritive value of *Jatropha curcas*. This information is very much useful to the oil cake industry based on *jatropha*. The study of peroxidase and polyphenol oxidase enzyme activities will help in improving the nutritive value as both the enzymes are found to be positively correlated with nutritive content. However, many specific questions about its production, commercialization and genetic improvement work are still in their infancy. Not much work on genetic improvement and environmental interaction aspects of this species has been taken up so far in India. Systematic provenance trails at different locations have not yet been carried out with *J. curcas* to the necessary extent in the India (Saikia *et al.,* 2009).

MATERIALS AND METHODS

A total 11 accessions of *Jatropha curcas* were selected for the present study (Fig. 1). Seeds of these accessions were sown in the nursery (8.0 m × 5.0 m) to raise the seedlings and were planted in the field during July 2008. Two, three, four and five year's old plants were evaluated for seed yield and biochemical constituent analysis in completely randomized block design (CRBD) with three replications. The observations were taken from second year after transplanting of plants. Data on seed yield was recorded and seeds were subjected for analysis of bio-chemical traits such as oil content (%), oil yield (t ha^{-1}), ADF (%), NDF (%), cellulose (%), hemicellulose (%), lignin (%), CP (%), peroxidase and polyphenol oxidase in seeds were recorded. The oil is extracted by solvent extraction by using the hydraulic press or a simple screw press (Patil *et al.*, 2003). Acid detergent fibre (ADF) and natural detergent fibre (NDF) were analysed with an Anokm Fiber Analyzer (ANKOM 220). The method suggested by Ven Soest (Robbins, 1993) was employed for estimation of lignin. Plant materials (seed) were homogenized in the ratio of 1:2 (w/v) with 0.1 M sodium 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 buffed pyrogallol in phosphate buffer (pH 6.0) along with 0.25mL enzyme sources. Absorbance at 240 nm at room temperature was measured up to 15 min and activities were presented (Anbgelini *et al.*, 1990) as changed in absorbance by 0.001mL^{-1} min^{-1}, with slight modification. Polyphenol oxidase was measured at A495 nm with chatecol as substrate (Luhova *et al.*, 2003). Total leaf crude protein (CP %) was determined by estimating nitrogen content and multiplying factor of 6.25 (A.O.A.C., 1970). The

Figure 1: Variation in seed colour of *Jatropha* provenances

pooled data fives were analyzed statistically by analysis of variance methods (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Molvum gave the highest seed yield of 0.88 t ha^{-1} which was statistically at par with Piphema and significantly superior to rest of the accessions. The lowest seed yield (0.55 t ha^{-1}) was recorded for Jalukie. The higher yield of Molvum might be due to its better growth and yield attributing traits (no. of branches $plant^{-1}$ no. of fruiting branches $plant^{-1}$ and 1000-seed weight) and finally affect the yield. Similar findings were also reported by Gurunathan and Srimathi (2012).

The Molvum provenance recorded significantly higher oil content of 38.9% and oil yield of 34.1 t ha^{-1} but was comparable to Rangapahar, however, the lowest oil content (28.1%) and oil yield 17.6 t ha^{-1} was recorded with Seithekiema. The maximum of Molvum oil content was due to its genetic potential and better utilization of nutrients. 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 percentage (Kumar *et al.*, 2006). The maximum ADF (45.4%) was recorded with Molvum which was statistically at par with Jalukie, Jharnapani, Mediziphema, Piphema, Ruzaphema, Dhansiripar and Tolbibasti but significantly superior over Seithekiema and Khatkati, whereas, Rangapahar which was lowest ADF (36.0%). Pooled data presented in Table 1 revealed that Molvum produced highest neutral detergent fibre (54.8 NDF %) which was comparable to Piphema (50.5%) and significantly superior to rest of the other accessions. However, Tolbibasti was noted the lowest values of NDF (46.7%). Among all accessions of *jatropha*, Molvum recorded significantly higher cellulose (23.1%) followed by Rangapahar. Tolbibasti was recorded lowest cellulose content (18.2%). Among the different accessions of jatropha, the maximum hemicellulose was recorded with Molvum which was statistically at par with Piphema, Ruzaphema, Rangapahar but significantly superior over all the remaining accessions, while, the lowest hemicellulose (10.8%) was obtained with Seithekiema. Significantly maximum lignin of 23.3% was obtained under Molvum but it was statistically comparable to Rangapahar. The minimum lignin (13.1%) was found under Seithekiema. Crude protein content was recorded maximum with Molvum (15.7%) which was comparable with Jharnapani, Mediziphema and Ruzaphema and significantly higher to rest of the others. Crude protein, minimum (13.2%) was recorded under Seithekiema. The peroxidase activity differed significantly due to different accessions. The highest peroxidase activity (41.7 ^{2}%A420 by 0.001 $ml^{-1}min^{-1}$) was found under Molvum which was significantly superior to remaining all the accessions. Whereas, the lowest (32.6^{2}%A420 by 0.001 mL^{-1} min^{-1}) was noted under Jalukie. Significant difference was recorded in polyphenol oxidase due to different accessions. The maximum polyphenol oxidase (87.1^{2}%A420 by 0.001 ml^{-1} min^{-1}) oxidase was recorded under Molvum which was similar to Jharnapani, Piphema, Ruzaphema, Rangapahar and Dhansiripar, while, minimum (75.3^{2}%A420 by 0.001 $mL^{-1}min^{-1}$) under Jalukie. 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. The involvement of peroxiadse in stress-related physiological process (Low *et al.*, 2014) as well as in plant growth was demonstrated (Kumar *et al.*, 2007). Polyphenol oxidase which belongs to the class of oxido reductase, also plays an important role in many physiological process. It is involved in disease resistance, flower shedding, seed development and other development processes (Huff and Dybing, 1980). Laccase and peroxidase polymorphism was also reported in germplasm of the National Agroforestry Repository (Kumar *et al.*, 2005). 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*. Agrawal *et al.* (2002) reported polyphenol oxidase, peroxidase and cellulose had critical role in physiological process during the development of soybean.

Correlations coefficient (Table 2) study revealed that seed yield was significantly and positively correlated with plant height (0.743), collar diameter (0.284), no. of branches plant^{-1} (0.827), and no. of fruiting branches plant^{-1} (0.859), no. of fruits branch^{-1} (0.756), 1000 - seed weight (0.188) and oil content (0.654). Such types of variation among different populations may be due to different intensities of natural selection acting upon the traits in their natural habitat. The significant (Genotypic × Environment) G × E interaction indicated that genotypes under different environment behaved differently for the expression of characters. It means a particular variety may not exhibit same phenotypic performance in different environments or varieties may respond differently to specific environment (Raiger *et al.*, 2011).

CONCLUSION

It can be concluded that the provenance 'Molvum' can be successfully cultivated in foot hill condition of Nagaland on account of its higher seed yield, oil content and better stability for favourable environment. However 'Molvum' which had higher seed yield with better stability across environments could be used in further multi-location trial of *J. curcas*.

ACKNOWLEDGEMENT

I thank the NOVOD Board, Gurgaon for financial assistance and ICAR RC for Eastern Complex, Patna for biochemical analysis. I am also grateful to Joint Director, ICAR Nagaland Centre Jharnapani for providing necessary facilities while conducting experiments and thank anonymous reviewers for their insightful suggestions and critical reviews.

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