



GENETIC IMPROVEMENT OF WOOD TRAITS AND CALORIFIC VALUE IN AN INTERSPECIFIC BACKCROSS POPULATION OF *JATROPHA CURCAS* L.

Anoop Anand Malik¹, Isiaka Ibrahim Muhammad^{1,2}, Vivek Kumar Singh¹, Shashi Bhushan Tripathi^{1*}

¹TERI School of Advanced Studies, 10 Institutional Area, Vasant Kunj, New Delhi 110 070, India

² Present Address: Laboratory of Plantation Science and Technology, Institute of Plantation Studies, Universiti Putra Malaysia 43400 Serdang Darul Ehsan Selangor, Malaysia

*Corresponding author's email: shashi.tripathi@terisac.ac.in

ABSTRACT

In this study, an interspecific BC₁ population of *Jatropha* was evaluated for wood related anatomical and physiological traits. Significant variability was present in the BC₁ plants for all observed traits. Moisture content had strong positive correlation with one-minute sap flow, fibre diameter and cortex index and a strong negative correlation with vascular bundle index. Calorific value on fresh weight basis had strong negative correlation with moisture content, one-minute sap flow, fibre length and fibre diameter and a significant positive correlation with vascular bundle index. However, calorific value on dry weight basis did not show significant correlation with any other studied trait. Significant positive heterosis over the mid-parent values was observed for pith index (~67%) and fibre length (~14%). On the other hand, negative heterosis was observed for vascular bundle index (34%), fibre diameter (26%) and one-minute sap flow (36%). No significant heterosis for moisture content and calorific value on fresh weight basis was found. Transgression was observed in 25, 37.5 and 62.5% of the BC₁ plants for pith index, vascular bundle index and cortex index, respectively. Further, transgression was observed in 12.5% of the BC₁ plants for moisture content and 16.6% of the BC₁ plants for calorific value on fresh weight basis. No significant transgression was observed for fibre length, fibre diameter and one-minute sap flow. Overall, there seems a great potential for improvement in *Jatropha* as a source of biomass for energy production.

KEYWORDS: Sap flow, wood fiber, fiber dimensions, fiber length, fiber width, Calorific value.

INTRODUCTION

Jatropha curcas (also known as Ratanjot or Physic nut) belongs to the family-Euphorbiaceae. It is a native of tropical America which was brought to India by the Portuguese during sixteenth century (Ginwal *et al.*, 2005). The plant has now found recognition as a potential biodiesel crop worldwide due to its economic, environmental and socioeconomic benefits (Heller, 1996). It can be grown on degraded and marginal soils with low rainfall and its oil can be used as fuel without any modification in the existing automobile engines (Gopinathan and Sudhakaran, 2009). However, the current level of productivity in *Jatropha* is not attractive to growers considering the associated costs. Consequently, research towards its genetic improvement has been initiated by several groups in India and abroad.

In addition to providing oil, *Jatropha* also has the potential to provide substantial amount of biomass in the form of pruned branches. In north India, pruning is done during winter when the plant is under dormancy. Pruning can be done at least once every year. Regular pruning keeps the plant height short which helps in manual harvesting of fruits (Ghosh *et al.*, 2011). Additionally, pruning leads to growth of increased number of branches and a bushy habit which is likely to increase the yield per unit area. As a by-product, this biomass can be a potential source for energy production through gasification and pyrolysis. Unfortunately, the current varieties of *J. curcas* have a succulent stem with moisture content of about 75% on

fresh weight basis which renders the wood useless as a bioenergy source. High moisture content also makes the plant susceptible to wind stress especially during the fruiting season. It leads to higher plant weight which makes the plants prone to lodging during windy monsoons and under water logged conditions. High water content may also lead to increased susceptibility of the plant to fungal diseases such as collar rot and stem rot (Latha *et al.*, 2009; Machado *et al.*, 2012). Wood is the most sustainable renewable source of energy which can provide an environment-friendly and cost-effective alternative to fossil fuels (Plomion *et al.*, 2001). In addition, wood also represents one of the most important sinks for atmospheric CO₂. Wood is primarily composed of cellulose, hemicellulose and lignin. Water is present in the living wood cells in the cell walls, in the cells as protoplasm and as free water in the cell cavities and spaces. The mature heartwood cells are eventually dead and do not contain protoplasm. Thoroughly air-dried wood may contain a moisture content of 8–16%. Within certain limits, an increase in water content makes the wood softer. The calorific value of wood is dependent on its water content and specific gravity. Moisture content in the fuel influences robust interior temperature history during pyrolysis because of endothermic evaporation (Chan and Kelbon, 1985). Therefore, decreasing wood moisture content is expected to improve in the quality of energy feedstock.

Wood development takes place by a succession of five major steps, including cell division, cell expansion (elongation and radial enlargement), cell wall thickening (involving cellulose, hemicellulose, cell wall proteins, and lignin biosynthesis and deposition), programmed cell death, and heartwood formation (Plomion *et al.*, 2001). Wood is a product of secondary growth, a process, which is responsible for increase in stem diameter. During secondary growth, the cells of vascular cambium divide periclinally to produce secondary xylem on the inner side and secondary phloem on the outer side. Further later during differentiation, the walls of xylem cells become lignified which imparts them hardness and strength.

The wood of *Jatropha* is diffuse-porous composed of oval to polygonal vessels with thick walls and simple perforations (Oladipo and Illoh, 2012). The vessels are in radial multiples of 2-11 with 31–84% solitary vessels. Bajas-Morales (1985) reported a vessel length ranging from 402.56–527.62 μm and a vessel diameter ranging from 60.99–89.18 μm in *J. curcas*. On the other hand, xylem fibers measure 14.6–22 μm across and have a mean length of 550 μm (Bahadur *et al.* 2013). The wood density in *J. curcas* varies from 0.33 to 0.37 (Hooda and Rawat 2006). The wood burns quickly with energy content of 15.5 MJ Kg⁻¹ (Sotolongo *et al.*, 2009). These attributes make the wood less suitable as a fuel.

Variations in wood traits can occur due to genotypic differences as well as due to environmental factors such as plantation density, tree height and diameter, site quality, growth rate and crown size (Kellog and Barber 1981). In Nigeria, xylem fiber length in *J. curcas* was found to be different at four sites in 7-year old trees (Akachuku and Burley 1979).

Interspecific crosses in *Jatropha* have been successfully used to develop novel breeding populations with wide genetic variability. *J. integerrima* has been frequently used for interspecific hybridization with *J. curcas* due to its high compatibility (Kumar *et al.*, 2009; Sinha *et al.*, 2015). The species has a number of desirable traits such as increased flowering, increased cold tolerance, hard stem and low sap flow. Muakrong *et al.* (2014) studied the wood traits in an interspecific F₁ hybrid of *J. curcas* x *J. integerrima* and reported improvement in wood traits for fuel use such as a reduced moisture content of 46.56% and

an increased wood density of 0.62 g/cm³. The F₁ hybrid had lesser ash content (2.60%) than *J. curcas* (6.93%), and also a higher calorific value of wood (18.73 MJ/kg) than *J. curcas* (17.77 MJ/kg). In the present study, we studied the relationships among different wood anatomical traits and calorific value using a genetically diverse backcross population derived from interspecific hybridization.

MATERIALS & METHODS

The plant material used in this study included a set of 47 BC₁ individuals. The founder parents and the F₁ hybrid were also included in the study. The BC₁ progeny was derived from an interspecific hybridization between *J. curcas* (hereafter referred as Jc) and *J. integerrima* (hereafter referred as Ji). The F₁ hybrid was backcrossed to Jc, to generate the BC₁ population (Sinha *et al.*, 2015). The plants were grown with a spacing 2m x 3m under field conditions at TERI's experimental field station situated in Eluru, Andhra Pradesh. All BC₁ plants used in this study were at fruit bearing age at the time of sample collection. The physiological and anatomical traits analyzed included wood moisture content (in %), radial lengths of pith, vascular bundles and cortex (all measured in mm), length and diameter of xylem fibres (in μm), average calorific value of wood (in MJ/Kg) and one-minute sap flow (in μl). As for BC₁, every individual is a distinct genotype; data collection was done on a single plant per genotype. However, at least three technical replicates were used for every trait. Sampling and analysis of all genotypes including the parents and F₁ were carried out at the same time to minimize errors.

Measurement of different tissue zones in stem sections

Stems of one-year old branches were sampled from each BC₁ plant. Thin transverse sections of stem were taken, stained with safranin and mounted on glass slides. The pictures of stem sections were taken under light with the help of high definition camera. The image was used to measure the radial dimensions of the three major zones namely, pith, vascular bundle, and cortex in millimeters. These dimensions were expressed as fractions of the total stem radius (hereafter referred as index). For example, the ratio between pith radius and total radius was termed as pith index. All measurements were carried out using ImageJ software (Schneider *et al.* 2012).

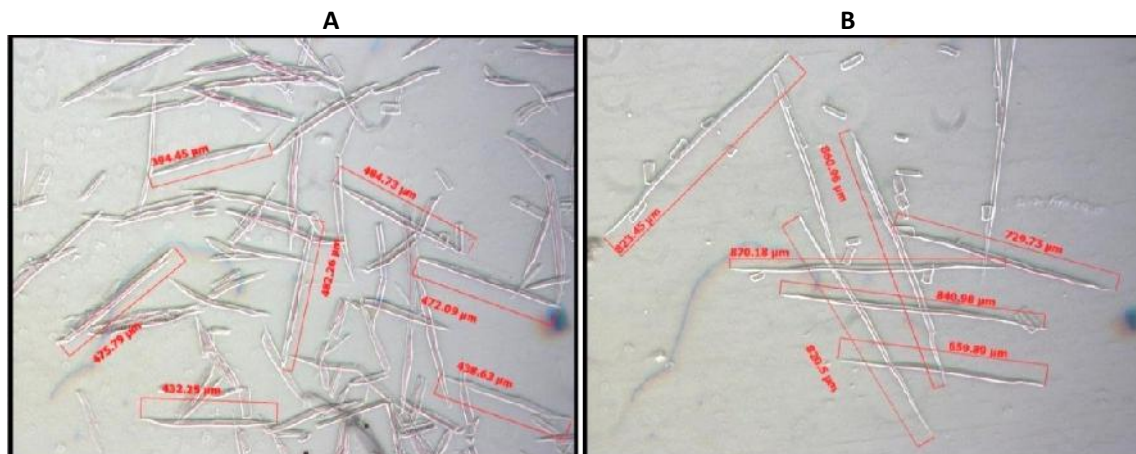


FIGURE 1: Photomicrograph of xylem fibres of Jc (Plate A) and Ji (Plate B) stained with safranin. Lengths of representative xylem fibres are shown in μm .

Measurement of fibre length and diameter

Wood fibers were extracted from one-year old hard stems. A small piece of wood (8-10 mm long) was incubated with 5-10 ml of 50% HNO₃ in a water bath at 80 ±2°C for 6-8 hours (Verweris *et al.*, 2004). The slivers were then cautiously rinsed, macerated and then stained with safranin. The fibres were then placed on glass slides and fibre dimensions (in µm) were measured under a compound microscope (ZEISS). For each sample, 25-30 measurements were taken (Fig. 1). Three replicates for each genotype were used (over 75 readings per genotype). Average fibre length and diameter was calculated for each genotype.

Wood moisture content

During winter (December-February), one-year old defoliated branches were collected from individual BC₁ plants. Five cuttings of 10cm length were bundled together as one unit and three such units were sampled from each BC₁ plant. The fresh weight of each bundle was recorded. The bundles were then oven dried at 60 ± 2°C for 15 days to reach a constant weight after which the dry weight was recorded. Wood moisture content on moist basis was calculated using the formula

$M = (W_w - W_o) / W_w$ where, W_w is fresh weight of wood and W_o is oven dry weight of wood.

One-minute sap flow

For every plant, newly developing branches which had grown ~1.5 feet in length were cut at about 20 centimeters from the top and the exuding sap from the attached end of the branch was collected for 60 seconds. The volume of this exudate was measured in microliters. To minimize variations due to weather and soil moisture, all plants were measured on the same day under same field conditions.

Gross calorific value of wood

The dried stems of different BC₁ genotypes obtained from the above-mentioned experiment were used for this analysis. Oxygen bomb calorimeter was used to estimate the calorific value of each BC₁ genotype (Gravalos *et al.*, 2016). The wood dust was converted into equal size tablets using a hydraulic press, weighed and was burnt in the presence of oxygen. The heat produced due to burning

of wood dust was recorded. Data was collected for three replications per BC₁ genotype.

Statistical analysis

Correlation analysis (P <0.05) was carried out for all parameters for which data was recorded. All data analysis was carried out using SPSS 17.0.

RESULTS AND DISCUSSION

As the radial dimensions of pith, vascular bundles and cortex varied within the same plant due to differences in the overall thickness of branches, we converted these dimensions relative to the total radius to obtain normalized dimensions which were called pith index, vascular bundle index and cortex index, respectively. As compared to the radial dimensions, which were highly dependent on the total branch thickness, these values were more stable within a genotype (with standard deviation ranging from 5% to 20% of the mean value). The pith index in BC₁ plants varied from 0.19 to 0.49 with an average value of 0.34 whereas these values in Jc, Ji and F₁ were 0.22, 0.20 and 0.35, respectively (Table 1). The vascular bundle index in BC₁ plants ranged from 0.12 to 0.53 with an average value of 0.28 whereas in Jc, Ji and F₁, these values were 0.31, 0.52 and 0.42 respectively. The cortex index in BC₁ plants ranged from 0.24 to 0.50 with an average of 0.38 whereas the same in Jc, Ji and F₁ were 0.33, 0.28 and 0.35 respectively. The xylem fiber length in BC₁ plants varied from 437 to 827 µm with an average value of 588 µm whereas the same in Jc, Ji and F₁ were 775, 868 and 497 µm respectively. On the other hand, the xylem fiber diameter in BC₁ plants ranged from 15 to 22 µm with an average value of 18 µm. The xylem fiber diameter in the parents, Jc and Ji, and their F₁ hybrid was 14, 15 and 24 µm respectively. The moisture content in BC₁ plants varied from 61 to 82% with an average value of 70% whereas the same in Jc, Ji and F₁ were 74, 54 and 65% respectively. It was interesting to note that the wood calorific value on dry weight basis of Jc was about 15% higher than that of Ji. However, the wood calorific value on fresh weight basis of Jc was about 35% lesser than that of Ji which was mainly on account of high moisture content in Jc as compared to Ji (Table 1).

TABLE 1: Variations in anatomical and physiological traits in the parents, F₁ hybrid and BC₁ plants. Pith index = Pith radius/ total radius; Vascular bundle index =Vascular bundle radius/ total radius; Cortex index = Cortex radius/ total radius. Superscripts “h” and “l” for % transgression indicates transgression beyond the higher and lower value parents, respectively. NS= Not significant

Genotypes	Pith index	Vascular bundle index	Cortex index	Fibre length (µm)	Fibre diameter (µm)	Moisture content (%)	One-minute sap flow (µl)	Calorific Value Dry wt (MJ/Kg)	Calorific value Fresh wt (MJ/Kg)
Ji	0.20	0.52	0.28	868±92	15±2	54±1	10±5	18±0.9	8.2±0.4
Jc	0.35	0.31	0.33	497±69	24±5	74±2	550±17	20.7±0.4	5.4±0.1
F ₁	0.22	0.42	0.35	775±66	14±2	65±2	180±15	19.9±0.2	7±0.1
BC ₁ min	0.19	0.12	0.24	437±49	15±2	61±0	52±5	17±0.1	4.3±0
BC ₁ max	0.49	0.53	0.50	827±186	22±4	82±7	242±56	23.4±1	8±0.2
BC ₁ av	0.34	0.28	0.38	588±81	18±3	70±3	109±14	19.6±0.4	6.1±0.1
Mid parent value	0.20	0.50	0.30	682.22	19.6	64.0	280	19.32	6.80
% Heterosis	66.67	-34.04	4.76	13.55	-25.93	1.37	-35.71	2.80	2.28
% of transgression	48 ^h	37.5 ^l	62.5 ^h	NS	NS	12.5 ^h	NS	5.5 ^h	16.6 ^l

Significant correlation was observed between several of the studied traits (Table 2). Moisture content had strong positive correlation with one-minute sap flow, fibre

diameter and cortex index and strong negative correlation with vascular bundle index. As the parenchymatous cells of cortex are major sites for storage of water in plants, an

increase in the proportion of cortex is expected to increase water content. On the other hand, vascular bundles are largely composed of dead cells with thick cell walls and no cytoplasm, the water content of this tissue is expected to be very low. As expected, calorific value on fresh weight basis had strong negative correlation with moisture content, one-minute sap flow, fibre length and fibre

diameter and significant positive correlation with vascular bundle index. In a recent study, Primandari *et al.* (2018) also observed a direct correlation between colorific value and humidity in case of fruit hull of *Jatropha*. However, in our study, calorific value on dry weight basis did not show significant correlation with any other studied trait.

TABLE 2: Correlation among various anatomical and physiological traits. **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level

Pith index	-								
Vascular bundle index	-0.101	-							
Cortex index	-0.044	-0.406**	-						
Fibre length	-0.108	-0.049	0.015	-					
Fibre diameter	0.268	-0.150	0.057	0.451**	-				
Moisture content	-0.035	-0.389**	0.301*	0.151	0.377**	-			
One-minute sap flow	0.080	-0.091	-0.114	0.247	0.448**	0.394**	-		
Calorific value (dry weight)	-0.041	0.066	-0.054	0.034	0.074	0.187	0.326	-	
Calorific value (fresh weight)	-0.078	0.389*	-0.265	-0.351*	-0.522**	-0.878**	-0.429*	0.301	

There was significant positive heterosis over the mid-parent values for pith index (~67%) and fibre length (~14%) but negative heterosis for vascular bundle index (34%), fibre diameter (26%) and one-minute sap flow (36%) (Table 1). In contrast with the earlier findings by Muakrong *et al.*, (2014), there was no significant heterosis observed for moisture content. The calorific value on fresh weight basis of the F₁ was almost same as the calculated mid-parent value indicating little or no heterosis for this trait. However, the maximum calorific value on fresh weight basis in BC₁ was almost as high as that in the Ji parent which is desirable from the point of fuelwood quality.

An important observation in case of most of the studied traits was the presence of transgression which is defined as the appearance of individuals in segregating populations that fall beyond their parental phenotypes (Vega and Frey, 1980; de Vicente and Tanksley, 1993). Interspecific transgression is important with respect to crop improvement since it represents a potential source of novel genetic variation resulting in novel characters of agronomic importance (Lewontin and Birch, 1966). For anatomical traits, transgression was observed in 25, 37.5 and 62.5% of the BC₁ plants for pith index, vascular bundle index and cortex index, respectively. In the present study, transgression was observed in 12.5% of the BC₁ plants for moisture content and 16.6% of the BC₁ plants for calorific value on fresh weight basis. However, both these transgressions were towards the undesirable side and, therefore, will not be useful for fuelwood quality improvement. However, 5.5% of the BC₁ plants were found to have significantly higher wood calorific value than Jc parent on dry weight basis. No significant transgression was observed for fibre length, fibre diameter and one-minute sap flow.

All parts of *J. curcas* secrete large amounts of latex or sap on wounding. The latex of *J. curcas* contains haemostatic agents which can be used as anti-coagulants in biochemical and haematological analyses (Oduola *et al.* 2005). Further, the sap is highly reactive to metallic agricultural implements such as secateurs and spoils their sharpness. In the current study, average one-minute sap flow in BC₁ plants was one-fifth of that in the Jc parent.

Further, the minimum one-minute sap flow was about 11-times lesser than that in the Jc parent. Thus, our findings demonstrate that there is high potential to develop improved varieties of *Jatropha* with reduced sap flow which would be desirable under both manual as well as mechanical harvesting systems.

CONCLUSION

This study shows that there is great possibility of developing *Jatropha* varieties with improved quality wood biomass for fuel purpose. High correlation of wood calorific value on fresh weight basis with moisture content and one-minute sap flow shows that selection for these traits is likely to identify *Jatropha* genotypes with improved biomass having increased calorific value. Presence of transgression for wood calorific value on dry weight basis also shows the potential to improve the biomass quality through selection. Further, genotypes with reduced one-minute sap flow will be helpful in both manual as well as mechanical harvesting and pruning operations. Dual-purpose *Jatropha* varieties with improved oil as well as wood yields would increase the competitiveness of *Jatropha* as a bioenergy crop.

ACKNOWLEDGEMENT

The authors are also thankful to Dr. Leena Srivastava, Vice Chancellor, TERI School of Advanced Studies for her kind support.

Declaration of interest statement

Declaration of interest: None.

Data archiving statement

The above research work does not involve any molecular parameters and hence no data or sequences have been submitted to public databases.

REFERENCES

- Akachuku, A. and Burley, J. (1979) Variation of wood anatomy of *Gmelina arborea* Roxb in Nigerian plantations. IAWA Bulletin 4, 94-99.
- Bahadur, B., Chamundeswari, E. and Krishnamurthy, K. V., eds. Wood anatomy of Indian *Jatrophas*, *Jatropha*, Challenges for a New Energy Crop, Volume 2: Genetic

- Improvement and Biotechnology, DOI 10.1007/978-1-4614-4915-7_2, Springer Science+Business Media New York 2013,2, 2013.
- Bajaras-Morales, J. (1985) Wood structural differences between trees of two tropical Forests in Mexico. IAWA Bulletin 6, 355-64.
- Chan, W.C.R. and Kelbon, M. (1985) Modelling and experimental verification of physical and chemical processes during pyrolysis of a large biomass particle. Fuel 64, 1505-13.
- de Vicente, M.C. and Tanksley, S.D. (1993) QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134, 585-96.
- Ghosh, A., Chikara, J. and Chaudhary, D.R. (2011) Diminution of economic yield as affected by pruning and chemical manipulation of *Jatropha curcas* L. Biomass Bioenergy 35, 1021-29.
- Ginwal, H.S., Partyal, S.S., Rawat, P.S. and Srivastava, R.L. (2005) Seed source variation in morphology, germination and seedling growth of *Jatropha curcas* Linn. In central India. Silvae Genet. 54, 76-80.
- Gopinathan, M. and Sudhakaran, R. (2009) Biofuels: opportunities and challenges in India. In Vitro Cell. Dev. Biol. - Plant 45, 350-71.
- Gravalos, I., Xyradakis, P., Kateris, D., Gialamas, T., Bartzialis, D. and Giannoulis, K. (2016) An Experimental Determination of Gross Calorific Value of Different Agroforestry Species and Bio-Based Industry Residues. Natural Resources 7, 57-68.
- Heller, J. Physic nut. *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops: Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute, Rome, 1996.
- Hooda, N. and Rawat, V.R.S. (2006) Role of bioenergy plantations for carbon dioxide mitigation with special reference to India. Mitigation and Adoption Strategies for Global Change 11, 445-67.
- Kellog, R.M. and Barber, F.J. (1981) Stem eccentricity in coastal western hemlock. Canadian Journal of Forestry Research 11, 714-18.
- Kumar, R.S., Parthiban, K.T., Hemalatha, P., Kalaiselvi, T. and Rao, M.G. (2009) Investigation on cross-compatibility barriers in the biofuel crop *Jatropha curcas* L. with wild *Jatropha* species. Crop Sci. 49, 1667-74.
- Latha, P., Prakasam, V., Kamalakannan, A., Gopalakrishnan, C., Raguchander, T., Paramathma, M. and Samiyappan, R. (2009) First report of *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl causing root rot and collar rot disease of physic nut (*Jatropha curcas* L.) in India. Australasian Plant Disease Notes 4, 19-20.
- Lewontin, R. C. and Birch, L.C. (1966) Hybridization as a source of variation for adaptation to new environments. Evolution 20, 3 15-33.
- Machado, A.R., Pinho, D.B., Dutra, D.C. and Pereira, O. L. (2012) First report of collar and root rot of physic nut (*Jatropha curcas*) caused by *Neoscytalidium dimidiatum* in Brazil. Plant Disease 96, 1697-97.
- Muakrong, N., One, K.T., Tanya, P. and Srinives, P. (2014) Interspecific *Jatropha* hybrid as a new promising source of woody biomass. Plant Genetic Resources 12, S17-S20.
- Oduola, T., Avwioro, O.G. and Ayanniyi, T.B. (2005) Suitability of the leaf extract of *Jatropha gossypifolia* as an anticoagulant for biochemical and haematological analyses. Afr. J. Biotechnol. 4, 679-81.
- Oladipo, O.T. and Illoh, H.C. (2012) Comparative wood anatomy of some members of the genus *Jatropha* (Euphorbiaceae) found in Nigeria. Phytologia Balcanica 18, 141-47.
- Plomion, C., Leprovost, G. and Stokes, A. (2001) Wood Formation in Trees. Plant Physiology 127, 1513-23.
- Primandari, S.R., Islam, A.K.M.A., Yaakob, Z. and Chakrabarty, S. *Jatropha Curcas* L. Biomass Waste and Its Utilization, 2018.
- Schneider, C.A., Rasband, W.S. and Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9, 671-75.
- Sinha, P., Md Islam, A., Negi, M.S. and Tripathi, S.B. (2015) Analysis of genetic diversity and fatty acid composition in a prebreeding material of *Jatropha*. J. Plant Biochem. Biotech. DOI: 10.1007/s13562-015-0301-2.
- Sotolongo, A.J.A., Beaton, P., Diao, A. and De, O. (2009) *Jatropha curcas* L as source for the production of biodiesel: A Cuban experience. <http://hem.fryistrog.com/zamsi/papaer/w225/pdf,2009>.
- Vega, U. and Frey, K.J. (1980) Transgressive segregation in inter and intraspecific crosses of barley. Euphytica 29, 585-94.
- Ververis, C., Georghiou, K., Christodoulakis, N., Santas, P. and Santas, R. (2004) Fiber dimensions, lignin and cellulose content of various plant materials and their suitability for paper production. Ind. Crops Prod. 19, 245-54.