



## Seed Source Variation and Biochemical Characterizations of *Pongamia pinnata* Genetic Resources

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**Studies on Seed source variation and biochemical characterizations of ten selected *Pongamia pinnata* genetic sources were carried out at Forest College and Research Institute, Mettupalayam in the Year 2009-10. The studies revealed that among the selected seed sources, Karamadai seed source shows superiority over others in terms of seed length (24.45cm), seed breadth (15.46cm), seed length to breadth ratio (1.90 cm), hundred seed weight (144.30g) and oil content in (24.56%). The values for saponification and Iodine number of most sources fell within the acceptable limits of Indian and international biodiesel standards. Only the free fatty acid percentage and acid value exceeded the permissible limits. But this does not subdue the utility of these seed sources which exhibited superiority in terms of growth, physico-chemical attributes and yield promoting their deployment for large scale biofuel plantation establishment.**

**Key words:** Pungam, seed source, variation, saponification value, Iodine number, free fatty acid, acid value.  
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Energy security has an important bearing on achieving national economic development goals and improving the quality of life. The level of per capita energy consumption has for long been considered as one of the key indicators of economic growth.

Biofuel is an eco-friendly, alternative diesel fuel prepared from domestic renewable resources i.e. vegetable oils (edible or non-edible oil) and animal fats. India has several hundred oilseed species that have enough potential for use in biodiesel production. Since India is deficient in edible oils, therefore, the non-edible oils could be the most desirable source for production of bio-diesel, especially tree borne oilseeds (TBO's) are the best and potential alternative to mitigate the current and future energy crisis and also to transform the vast stretch of wastelands to green oil fields. The potential species identified so far includes *Jatropha curcas*, *Pongamia pinnata*, *Madhuca latifolia*, *Garcinia indica*, *Azadirachta indica*, *Calophyllum inophyllum*, *Simarouba glauca* etc. Besides *Jatropha*, *Pongamia pinnata* is another versatile species India is focusing on promoting as biodiesel, as the country launches a nationwide biofuel mission (Pradhan, 2009).

*Pongamia pinnata* (Linn.) Pierre (Fabaceae) is the predominant species planted in homesteads as a shade or ornamental tree and along avenues. It is considered as one of the best alternative source for biodiesel (Srivastava and Verma, 2008) and is

one of the few nitrogen fixing trees (NFTs) to produce seeds bearing 30-40 per cent oil (Lakshmikanthan, 1978) which is thick yellow-orange to brown in colour. The oil has a bitter taste and a disagreeable aroma, thus it is not considered edible. In India, the oil is used as fuel for cooking, illuminant, lubricant, water-paint binder, pesticide, soap making, tanning, folk medicine for the treatment of rheumatism, as well as human and animal skin diseases. It is also effective in enhancing the pigmentation of leucoderma or scabies affected skin.

Pungam oil has the requisite potential of providing a promising and commercially viable alternative to diesel as it has the desirable physico-chemical and performance characteristics comparable to diesel. Hence the study of seed variation and biochemical characterizations of *Pongamia pinnata* sources is vital for production of biofuel. (Sharma and Singh, 2008).

#### **Materials and Methods**

The investigations were carried out in the laboratory of Forest College and Research Institute, Mettupalayam in the year 2008-09. Ten pungam genetic sources were identified from various parts of Tamil Nadu viz., Coimbatore, Annur, Karamadai, Thalavadi (1), Thalavadi (2), Thalavadi (3), Sathyamangalam (1), Sathyamangalam (2), Sathyamangalam (3) and Pollachi. The variability in physical characteristics of identified pungam genetic sources viz., seed length, seed breadth, seed length-breadth ratio (cm), were measured using Vernier calipers. Besides these physical

characteristics, hundred seed weight and oil content in seed were also analyzed. The seed parameter studies were carried out with a random sample of 20 seeds from each seed source with five replications. The oil was extracted by means of solvent extraction using hexane (40 – 60<sup>o</sup> C) (Soxhlet extraction) A.O.A.C. (1975) and expressed in percentage. The determination of hundred seed weight was computed as per ISTA (1993) using 8 x 100 seeds counted at random, weighed and expressed in gram (g).

The chemical characteristics of the oil viz., free fatty acid content, acid value, iodine number and saponification value were evaluated for the selected progenies and are presented in the Table 1. The acid value and free fatty acid of oil was determined by the procedures of A.O.A.C. (1975). The iodine number and saponification value was determined by Hanus iodine method (A.O.A.C., 1975).

### Results and Discussion

The effective tree improvement programme depends upon the nature and magnitude of existing genetic variability and also on the degree of transmission of the desired traits. Since most of the plant characters of economic importance are polygenic in nature and are highly influenced by environmental fluctuations. The largest, cheapest and fastest gains in most forestry tree improvement programmes will accrue if use of suitable species and seed sources within species is assured (Zobel and Talbert, 1984).

The choice of provenance and seed source is important since it decides the genetic quality and the physiological potential. Seeds are influenced by their place of origin (Heydecker, 1972), especially due to environmental variations in latitude, altitude, rainfall, temperature, moisture and the external factors (Holzer, 1965; Mathur *et al.*, 1984 and Padmini and Bannerjee, 1986). Seed source variations were reported in many tree species (Bagchi and Dobriyal, 1990; Mishra and Bannerjee, 1995; Thapliyal and Dhiman, 1997; Pathak, 1998 and Gera *et al.*, 1999) dictated by environmental and edaphic factors and due to altitudinal variation (Bonner, 1984).

The present study revealed that significant amount of variability exists among different seed sources of *Pongamia pinnata* with respect to seed physical parameters investigated viz., seed length, seed breadth, seed length-breadth ratio, 100 seed weight and seed oil content (Table 1). The seed source Annur (24.45 cm) and Karamadai (23.61 cm) recorded the highest and significant values of seed length with Annur registering the highest and significant values for seed breadth (15.46 cm) also. Similarly the seed sources Annur and Sathya mangalam (1) recorded the highest and significant values for seed length breadth ratio of 1.90 cm and

1.60 cm respectively. The six selected genetic sources viz., Coimbatore (129.94 g), Annur (144.30 g), Karamadai (126.83g), Thalavadi (2) (122.47 g), Sathyamangalam (1) (120.43 g), Sathyamangalam (3) (140.70 g) and Pollachi (141.91 g) recorded the highest and significant values for 100 seed weight. While the seed source Annur from (24.56%), Karamadai (23.96%), Sathyamangalam (2) (22.95%), Sathya mangalam (3) (22.75%) and Pollachi (23.02%) recorded the highest and significant values for seed oil content. However, the genetic sources of Annur recorded highest and significant values for seed length, seed breadth, seed length-breadth ratio, 100 seed weight and seed oil content followed by Karamadai. All other seed sources exhibited significantly lower values compared to general mean (Table 1). Similarly variability in seed physical characters of *Pongamia pinnata* was documented earlier (Kumaran, 1991). Variation in seed physical parameters were documented in other tree species viz., *Azadirachta indica* (Kumaran, 1991; Sindhu Veerendra, 1995; Philomina, 2000; Bisht *et al.*, 2002; Vinod and Vijayakumar, 2002; Khajuria *et al.*, 2004; Chauhan *et al.*, 2005); *Madhuca latifolia* (George Jenner, 1995; Umesh Kanna, 2001; George Jenner *et al.*, 2003) and in *Simarouba glauca* (Sekar, 2003). 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 (collection of germplasm) to form base seed material.

With respect to biochemical characterizations, significant differences were recorded for all the chemical properties of the oil of pungam seed sources. Several chemical properties of the biodiesel allow it to burn cleanly and actually improve the combustion of petroleum diesel in blends. Some of the most important chemical properties which determine the suitability of oils for the utilization of biodiesel are free fatty acid (%), acid value, iodine value and saponification value. Standards for these properties have been established worldwide and in India, Bureau of Indian Standards has specified standards (IS: 15607, 2006). Among 10 seed sources, eight seed sources recorded significantly lower values for free fatty acid and acid value. While free fatty acid differed significantly among different seed sources. It ranged from 2.78 per cent Coimbatore to 4.27 per cent Thalavadi (1). The average value of free fatty acids was 3.47. Two seed sources viz., Thalavadi (1) (4.27%) and Pollachi (4.24%) possessed significantly higher free fatty acid content compared to general mean. Other seed sources expressed parity with general mean.

Among different seed sources significant variations was observed for acid value averaging at 6.62 and ranging between 4.87(Thalavadi - 2) and 8.47(Thalavadi -1). Thalavadi – 1 (8.47) and Pollachi (8.26) recorded significantly higher values over

**Table 1. Pongamia Seed source variations in seed physical attributes and Chemical Properties of selected pongam genetic resources**

Seed sources	Seed length (cm)	Seed breadth (cm)	Seed length breadth ratio (cm)	Hundred seed weight (g)	Oil content in seed (%)	FFA %	Acid Value	Iodine Number	Saponification Value
Coimbatore	21.45	12.57	1.35	129.94*	18.12	2.78	5.29	80.21	178.67
Annur	24.45*	15.46*	1.90 *	144.30*	24.56*	3.83	6.55	97.81*	181.54
Karamadai	23.61*	13.60	1.34	126.83*	23.96*	3.49	6.80	88.40	184.74*
Thalavadi (1)	20.92	13.47	1.52	97.350	15.41	4.27*	8.47*	85.01	196.36 *
Thalavadi (2)	20.02	13.18	1.50	122.47*	19.98	2.84	4.87	95.16 *	190.74*
Thalavadi (3)	20.50	14.15	1.18	100.61	21.25	3.03	6.05	84.72	187.91*
Sathyamangalam (1)	21.53	13.33	1.60*	120.43*	14.52	3.48	7.18	88.18	187.93*
Sathyamangalam (2)	18.18	13.45	1.49	62.790	22.95*	3.40	6.31	94.83 *	176.72
Sathyamangalam (3)	21.18	13.27	1.52	140.70*	22.75*	3.42	6.43	84.62	182.33
Pollachi	21.36	12.98	1.61	141.91*	23.02*	4.24*	8.26*	88.84	185.12*
Mean	21.32	13.57	1.56	118.733	20.69	3.47	6.62	88.78	184.87
CD (5%)	0.9379	1.646	0.250	0.0170	0.9379	0.75	0.97	2.00	1.656

\*Significant at 5% level

general mean (6.62), while Coimbatore (5.29) and Thalavadi (2) (4.87) recorded lower acid values. All other seed sources exhibited parity with general mean. These values increased during storage and were attributed to ensure proper ageing properties of the biodiesel. High acid value can cause damage to engine parts and were reported in *Jatropha curcas* (Bhasabutra and Sutiponpeibun, 1982), *Pongamia glabra* (Raheman and Phadatare, 2004) and in *Madhuca indica* (Ghadge and Raheman, 2005).

In the present investigation, seed sources registered significant variations for saponification value with an average of 184.87 and ranging between 176.72 (Sathyamangalam (2)) and 196.36 (Thalavadi (1)). Six seed sources viz., Thalavadi (1) (196.36), Thalavadi (2) (190.74), Sathya mangalam (1) (187.93), Thalavadi (3) (187.91) and Pollachi (185.12) and Karamadai (184.74) recorded significantly higher saponification values compared to general mean. Four seed sources viz., Sathyamangalam (3) (182.33), Annur (181.54), Coimbatore (178.67) and Sathyamangalam (2) (176.72) recorded significantly lower values for saponification compared to general mean. Saponification value is an index of the average size of fatty acid present, which depends upon the molecular weight and percentage concentration of fatty acids components in the oil (Mohibbe Azam *et al.*, 2005).

The Iodine value differed significantly among different seed sources ranging from 80.21 (Coimbatore source) to 97.81 (Annur source). The average iodine value registered was 88.78 which measures the degree of unsaturation in Pongamia methyl esters as it restricts the fatty acid methyl esters from solidification, Pongamia methyl esters are not suitable for biodiesel (Mohibbe Azam *et al.*, 2005). In the current study, three seed sources viz., Annur (97.81), Thalavadi (2) (95.16) and Sathya mangalam (2) (94.83) proved to possess significantly higher iodine value while remaining

seed sources exhibited parity with general mean (Table).

The saponification value and Iodine number of most of the seed sources fell within the acceptable limits of the Indian and international biodiesel standards. Only the free fatty acid percentage and acid value exceeded the permissible limits. But this does not subdue the utility of these seed sources which exhibited superiority in terms of yield, growth and physico-chemical attributes.

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## References

- A.O.A.C. 1975. Official methods of analysis. Association of Official Agricultural Chemists, Washington, 12<sup>th</sup> edition. 488-492.
- Bagchi, S.K. and Dobriyal, N.D. 1990. Provenance variation in seed parameters of *Acacia nilotica*. *Indian Forester*, **116**: 958-961.
- Bhasabutra, R. and Sutiponpeibun, S. 1982. *Jatropha curcas* oil as a substitute for diesel engine oil. *Renewable Energy Review Journal*. **4**: 56-67.
- Bisht, S., Jain, A., Sharma N., Pandey, S. and Banerjee, S.K. 2002. Provenance variation in seed characteristics and germination behaviour of *Azadirachta indica* in Orissa. *J. of Non-Timber Forest Products*. **9**: 26-31.
- Bonner, F.T. 1984. Glossary of seed germination terms for tree seed workers, USDA Forest Service. Gen. Tech.Rep., Southern Forest Experiment Station, Starkville, Mississippi, USA. Gen. Tech. Rep, pp 30-49.
- Chauhan, K.C., Gautam, S. and Thakur, I.K. 2005. Genetic variability and correlation studies in seed and seedling traits of neem (*A. indica*). *J. Non-Timber Forest Products*, **12**: 69-75.
- George Jenner, V. 1995. Studies on genetic analysis of seed and juvenile seedling attributes in mahua (*Madhuca latifolia* Roxb.). M.Sc. Thesis. Tamil Nadu Agricultural University, Coimbatore - 3.

- Gera, M., N. Gera and Ginwal, H.S. 1999. Seed source variation in germination and early growth among ten indigenous populations of *Dalbergia sissoo*. *Indian Forester*, **625**: 1190-1197.
- Ghadge, S.V. and Raheman, H. 2005. Biodiesel production from Mahua (*Madhuca indica*) oil having high free fatty acids. *Biomass and Bioenergy*, **28**: 601-605.
- Heydecker, W. 1972. Vigour. In: "Viability of seeds". (Ed. E.H. Roberts). Chapman Hall, London. pp 209-252.
- Holzer, K. 1965. Standardization of methods for provenance research and testing. In: Proc. of *IUFRO Congress*, Munchen, Germany, **111**: 672-718.
- ISTA. 1993. International rules for seed testing. *Seed Sci. & Technol.*, **21**: 1-288.
- George Jenner, V., Dasthagir, M.G., Parthiban, K.T. and Jude Sudhagar R. 2003. Variability studies on seed and seedling attributes in mahua (*Madhuca latifolia*). *Indian Forester*, **129**: 509-516.
- Khajuria, H.N., Chauhan, S.K. and Gera, M.K. 2004. Variation in seed characters and multiple seedlings in neem. *J. Non-Timber Forest Products*, **11**: 103-107.
- Kumaran, K. 1991. Genetic analysis of seed and juvenile seedling attributes in neem (*Azadirachta indica* A. Juss.), pungam (*Pongamia pinnata* Linn. Pierre). M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore -3.
- Lakshmikanthan, V. 1978. Tree Borne Oilseeds. Directorate 'of Nonedible Oils & Soap Industry, Khadi & Village Industries Commission. Mumbai, India, pp. 38-39.
- Mathur, R.S., Sharma, K.K. and Rawat, M.M.S. 1984. Germination behaviour of various provenances of *Acacia nilotica* spp. *Indica*. *Indian Forester*, **111**: 435-449.
- Mishra, C.M. and Banerjee, A.C. 1995. Provenance variation in *Casuarina* species with reference to germination and growth. *J. Trop. For.*, **11**: 209-211.
- Mohibbe Azam, M., Amtul Waris and Nahar, N.M. 2005. Prospects and potential of fatty acid methyl esters of some non-traditional seed oils for use as biodiesel in India. *Biomass and Bioenergy*, **29**: 293-302.
- Padmini, S. and Banerjee, A.C. 1986. Provenance trails of *Acacia nilotica*. *J. Tree Sci.*, **5**: 53-56.
- Pathak, N.N. 1998. Field evaluation and selection of *Acacia nilotica* provenances. *J. Trop. For.*, **14**: 197.
- Philomina, D. 2000. Genetic analysis of one-parent families for variability, diversity, stability and propagation techniques in neem (*Azadirachta indica* A. Juss). Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore - 3.
- Pradhan, R. C., Naik, S. N., Bhatnagar, N., Vijay, V. K. 2009. Physical Properties of Tree Borne Oil Seed: Potential Biodiesel Feedstock in India. *Assam University J. Sci., & Technol*, Vol 4, No 2. 46-53.
- Raheman, H. and Phadatare, A.G. 2004. Diesel engine emissions and performance from blends of Karanja methyl ester and diesel. *Biomass and Bioenergy*, **27**: 393-397.
- Sekar, I. 2003. Seed storage, seed source variations, molecular characterization and *in vitro* propagation in *Simarouba glauca* DC. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore - 3.
- Sharma, Y.C. and Singh, B. 2008. Development of biodiesel from karanja, a tree found in rural India. *Fuel*, **67**: 1740-2.
- Sindhu Veerendra, H.C. 1995. Variation studies in provenances of *Azadirachta indica* (the neem tree). *Indian Forester*, **121**: 1053-1056.
- Srivastava, P.K. and Verma, M. 2008. Methyl ester of karanja oil as an alternative renewable source energy. *Fuel*, **87**: 1673-7.
- Thapliyal, R.C. and Dhiman, R.C. 1997. Geographic variation in seed and seedling characteristics in *Pinus roxburghii* from Himachal Pradesh. *Ann. For.*, **5**: 140-145.
- Umesh Kanna, S. 2001. Genetic analysis, biochemical and molecular characterization of *Madhuca latifolia* Macb. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.
- Vinod, S. and Vijayakumar, N.K. 2002. Provenance evaluation in the seed and seedling characters of neem (*A. indica*). *Journal of Non-Timber Forest Products*, **9**: 54-57.
- Zobel, B.J. and Talbert, J. 1984. Applied tree improvement. John Wiley & Co., New York. 503 pp.