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Protein profiling of *Pongamia pinnata* L. accessions

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Abstract

Pongamia pinnata L. known as Karanja is gaining importance in Indian context as a renewable source of energy. The basic aim of this study was to establish the genetic relationship/ diversity in accessions of *Pongamia pinnata* based on protein profiling using SDS-PAGE. Dice similarity coefficient based dendogram generated two clusters. The cluster 1 embodied two accessions *viz.,* G10 and G3. The second cluster was further divided into two sub-clusters. G18 was placed alone in first sub-cluster while second sub-cluster was divided into two groups. In first group 4 accessions *i.e.,* G5, G11, G12 and G14 were placed. In second group 11 accessions *i.e.,* G1, G17, G2, G15, G8, G16, G9, G4, G13, G6 and G7 were present. The information generated can very well be utilized in future Karanja improvement programme to produce elite/hybrid varieties with characters of importance.

Key Words: Accessions, Biofuel, *Pongamia pinnata* L., Protein profiling, SDS-PAGE

Introduction

India ranks sixth in terms of consumption of energy, which is 3.5% of the total world's commercial energy. The current consumption of diesel in India is about 40mt (40% of the total petroleum product consumption) and the demand is growing at a rate 5.6% per annum. Biodiesel is a renewable energy resource generated from rehabilitation of waste and degraded lands. Bio-fuel is a fuel comprising of mono-alkyl esters of long chain fatty acids of vegetable oils or animal fats, which is derived either from plant or animal. Use of biofuel results in substantial reduction of un-burnt hydrocarbons by 30%, carbon monoxide by 20% and particulate matters by 25%. It has almost no sulphur.

Amongst the many species, which can yield oil as a source of energy in the form of bio-fuel, *Pongamia pinnata* L. has been found to be one of the most suitable species in India being widely grown. It belongs to family Leguminosae sp. (Papilionaceace). It is a medium sized evergreen tree with a spreading crown and a short bole. The natural distribution is along coasts and riverbanks in lands and native to the Asian subcontinent. It is a preferred species for controlling soil erosion and binding sand dunes because of its dense network of lateral roots. Its root, bark, leaves, sap and flower also have medicinal properties and are traditionally used as medicinal plants. It is N_2-fixing tree, not browsed by animals and oil is non-edible. It is tolerant to water logging, saline and alkaline soils, can withstand harsh climates (medium to high rainfall). It can be planted on degraded lands, farmer's field boundaries, wastelands / fallow lands and can be grown across the country. *Pongamia* seeds contain 30-35% oil. The present study was undertaken to establish the genetic relationship in accessions of *Pongamia pinnata* L. based on protein profiling using SDS-PAGE.

Materials and Methods

Eighteen accessions of *Pongamia pinnata* L. *viz.,* G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13, G14, G15, G16, G17, G18 collected from different parts of Uttar Pradesh, Madhya Pradesh, Rajasthan and Haryana were used in the present study. The seeds of these accessions were sown in nursery to raise the seedlings and were planted in field during August 2005. The leaves from three and half year old plants of these accessions were used to prepare protein samples. The present study was carried out in the tree improvement labortary of National Research Centre for Agroforestry, Jhansi during the months of January 2009 to June 2009. For protein profiling, fresh leaf samples were ground in buffer [(1ml Tris HCl (pH 6.8), 0.8 glycerol, 1.6ml SDS (10%) and 0.4 β mercaptoethanol)], centrifuged at 12,000 rpm at 4°C for 10 minutes and subjected to protein profiling using sodium dodecyl sulfate-polyacrylamide gel (10%) electrophoresis. Gel was stained using commassie brilliant blue solution.

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Table 1 : Accessions of *Pongamia pinnata* L. with their place of collection

S. No.	Accessions	Symbol used	Location
1.	NRCP-6	G1	Basi, Lalitpur (Uttar Pradesh)
2.	NRCP-7	G2	Basi, Lalitpur (Uttar Pradesh)
3.	NRCP-9	G3	Babina, Jhansi (Uttar Pradesh)
4.	NRCP-10	G4	Near Matatila Dam, Lalitpur (Uttar Pradesh)
5.	NRCP-11	G5	Matatila Dam, Lalitpur (Uttar Pradesh)
6.	NRCP-12	G6	Malthola, Lalitpur (Uttar Pradesh)
7.	NRCP-13	G7	Barodia, Lalitpur (Uttar Pradesh)
8.	NRCP-14	G8	Nandan Bara ki Nadi, Lalitpur (Uttar Pradesh)
9.	NRCP-16	G9	Bhagirathpur, Dholpur (Rajasthan)
10.	NRCP-17	G10	B.S.F. Campus, Tekanpur (Madhya Pradesh)
11.	NRCP-18	G11	B.S.F. School Campus, Tekanpur (Madhya Pradesh)
12.	NRCP-20	G12	Chhotikothi, Bassi, Jaipur (Rajasthan)
13.	NRCP-21	G13	Chhotowara Kala, Bharatpur (Rajasthan)
14.	NRCP-22	G14	Jansat, Panipat (Haryana)
15.	NRCP-23	G15	Solepur, Dholpur (Rajasthan)
16.	NRCP-24	G16	Near Ramnath City, Jhansi (Uttar Pradesh)
17.	NRCP-25	G17	Sariska, Alwar (Rajasthan)
18.	NRCP-26	G18	Dholpur (Rajasthan)

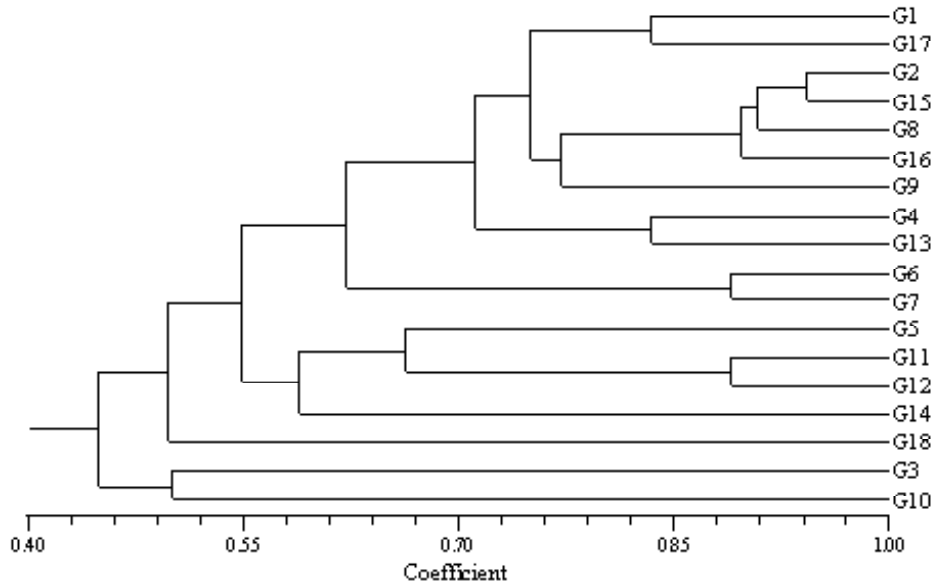

Fig. 1 : Cluster analysis based on protein profiling in different accessions of *Pongamia pinnata* L.

Results and Discussion

Analysis of soluble leaf protein profile through SDS-PAGE technique was used as a tool to establish the relationship and differences between all the eighteen accessions. In total 9 bands were scored having different intensities. Leaf protein profile of *Pongamia pinnata* L. revealed distinct protein bands with molecular weight ranging from 200 kDa - 40 kDa. Bands were scored in form of presence (1) and absence (0) in binary matrix and analyzed using computer software SPSS. The Dice similarity coefficient generated a dendogram for estimation of genetic variability that largely clustered these eighteen accessions of *Pongamia pinnata* L. in two main clusters (Fig.1). The first cluster had two accessions G10 and G3. The second cluster was further divided into two sub-clusters. G18 was placed alone in first sub-cluster while second sub-cluster was divided into two groups. In first group, 4 accessions *i.e.,* G5, G11, G12 and G14 were placed. In second group, 11 accessions *i.e.,* G1, G17, G2, G15, G8, G16, G9, G4, G13, G6 and G7 were present. The genetic identity was calculated and it is measured that genetic identity was lowest between G10 *vs* G11 and

Table 2 : Similarity coefficient in different accessions of *Pongamia pinnata* L.

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18
G1	0.00																	
G2	0.71	0.00																
G3	0.40	0.67	0.00															
G4	0.77	0.80	0.55	0.00														
G5	0.50	0.71	0.40	0.62	0.00													
G6	0.73	0.62	0.22	0.83	0.55	0.00												
G7	0.60	0.50	0.25	0.73	0.40	0.89	0.00											
G8	0.71	0.88	0.67	0.80	0.71	0.62	0.67	0.00										
G9	0.67	0.71	0.40	0.77	0.67	0.55	0.60	0.86	0.00									
G10	0.60	0.50	0.50	0.36	0.60	0.44	0.50	0.67	0.40	0.00								
G11	0.40	0.67	0.50	0.73	0.60	0.44	0.25	0.50	0.60	0.00	0.00							
G12	0.36	0.77	0.67	0.67	0.73	0.40	0.22	0.62	0.55	0.22	0.89	0.00						
G13	0.73	0.77	0.67	0.83	0.36	0.60	0.44	0.62	0.55	0.22	0.67	0.60	0.00					
G14	0.20	0.67	0.50	0.55	0.60	0.44	0.50	0.67	0.60	0.25	0.50	0.67	0.44	0.00				
G15	0.80	0.94	0.62	0.88	0.80	0.71	0.62	0.94	0.80	0.62	0.62	0.71	0.71	0.62	0.00			
G16	0.86	0.88	0.50	0.80	0.71	0.77	0.67	0.88	0.71	0.67	0.50	0.62	0.62	0.50	0.94	0.00		
G17	0.83	0.71	0.40	0.62	0.67	0.55	0.40	0.71	0.67	0.60	0.40	0.55	0.55	0.40	0.80	0.86	0.00	
G18	0.67	0.55	0.29	0.60	0.22	0.50	0.29	0.36	0.44	0.00	0.57	0.50	0.75	0.29	0.50	0.55	0.67	0.00

G10 *vs* G18. The genetic identity was highest between G15 *vs* G2, G15 *vs* G8 and G15 *vs* G16.

These similarities in polypeptide bands indicate the degree of homology among accessions. However, higher degree of dissimilarities in polypeptide banding pattern and low similarity index value among the accessions as revealed in the present study could be an effective measure in demarcation of species diversity as well as characterization and identification of accessions. Significant application of SDS-PAGE technique in elucidating species diversity, characterization and identification has also been demonstrated in many other species (Odeigah and Osanyinpeju, 1998; Odeigah *et al.* 1999). Similarly this technique has helped in studying genetic relationship among related species as well as in different accessions (Ghaffor *et al.*, 2002; Ladizinsky and Hymowitz, 1979). SDS- PAGE protein profiling had helped in identification and characterization of elite genotypes in different species (Srivastava and Gupta, 2002; Lone and Tewari, 2006). Therefore characteristic electrophoresis leaf protein profile recorded in all accessions could also be utilized in future Karanja improvement program to produce hybrid varieties with good oil content and other desirable characters.

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