



Divergence of seed quality and storability traits in *Pongamia* genotypes

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Abstract

Pongamia is an important renewable resource for biodiesel production. Evaluation of this legume tree will provide an insight in the understanding ways to improve seed storability through breeding and enable us to meet the future seed demand for taking up of afforestation programme. Twenty four *Pongamia pinnata* accessions were collected from M/s Tree Oils India Limited, Zaheerabad and evaluated for sixteen seed quality parameters and grouped into 6 clusters. The genotypes in cluster II showed more number of accessions (17) followed by cluster I (3) and remaining clusters showed one accession each. There is a wide genetic diversity existed between cluster IV (TOIL13) and III (TOIL17), followed by cluster I (TOIL 2, 19 and 9) and IV (TOIL13) and crosses between genotypes of these clusters may result in substantial segregates. Cluster II exhibited high cluster means for 9 characters. Traits like seedling vigour index-II and seed germination after aging and initial speed of germination contributed more towards genetic diversity in *Pongamia* genotypes for seed storability.

Keywords: Clustering, Divergence, *Pongamia*, Seed Quality, Storability

Introduction

Pongamia pinnata (L.) Pierre (also known as *Millettia pinnata* or Indian Beech) locally known as Karanja is an indigenous tree to India is a perennial, fast-growing, leguminous tree, widely distributed on the Indian subcontinent, south-east Asia, Oceania, northern Australia, the East-African coast and southern China (Murphy *et al.*, 2012). In addition *Pongamia* has been introduced to other parts of the world, including the United States (Kazakoff *et al.*, 2011). It is a versatile leguminous tree (Savita *et al.*, 2010) and as a potential source of biodiesel (Naik *et al.*, 2008). As a nodulating and nitrogen-fixing legume the nitrogen (N) fertilizer requirements of *Pongamia* are potentially minimal. This is an advantage for a biofuel crop as N fertilizer inputs detract from the net energy gain, a fact often overlooked in choosing biofuel crops (Hill *et al.*, 2006). *Pongamia pinnata* is regarded as a sustainable biofuel feedstock of the future because of its abundant production of oil-rich seeds, tolerance to abiotic stress, and ability to undergo biological nitrogen fixation (minimizing nitrogen inputs). However, it needs extensive

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domestication through selection and genetic improvement. Genetic diversity in plant species is a gift to mankind as it forms the basis for selection and further improvement of species. Genetic diversity within a population refers to the number of different alleles (the alternate forms of genes) of all genes and the frequency with which they appear. Variation is high when there are many different alleles of all genes and many different combinations of those alleles. The information on the genetic structure and diversity relationship of TOILS provides a basis for planning and conducting future collections and efficient utilization of genetic resources to realize the potentiality for maximizing seed storability. Various statistical tools like Mahalanobis D^2 analysis, canonical and principal component analysis are helpful in deriving genetic information from quantitative data. The D^2 statistic is one of the powerful tools to assess the relative contribution of different component traits to the total diversity and to quantify the degree of divergence between populations and to choose genetically diverse parents for obtaining desirable recombination. Diversity in plant genetic resources (PGR) provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics,



which include both farmer-preferred traits (yield potential and large seed, etc.) and breeder preferred traits (pest and disease resistance and photosensitivity, etc.). From the very beginning of agriculture, natural genetic variability has been exploited within crop species to meet subsistence in food requirement and now it is being focused to surplus food for growing populations. Keeping in view of the importance of the crop and its propagation by seeds, the present experiment was designed with the objective to identify genotypes of *Pongamia pinnata* with good seed storability.

Materials and Method

The experiment was conducted at the Department of Seed Science and Technology, Seed Research and Technology Centre, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad during 2014 and 2015 to study the existence of genetic diversity among 24 genotypes of *Pongamia pinnata* and performance of seed quality parameters during seed storage. The extensive survey was under taken across forest areas of Adilabad and Ranga Reddy districts of Telangana and collected 8700 genotypes of pongamia. These genotypes were planted during August, 2003 in the nursery and fields of Tree Oils India limited, Zaheerabad. From these genotypes, 110 were screened out based on the characters like viz., early maturity, seed oil content, crop canopy with less branching, less height and girth and good yield potential. Finally, 24 genotypes were screened based on their efficiency and performance. The individual tree was identified based on their phenotypical characteristic. Seeds were collected from candidate plus trees identified at farm of Tree Oils India Limited, Zaheerabad, Telangana, India during the month of February, 2014. Seed physiological studies were carried out during the reported period under study. Standard germination test was conducted as per ISTA (2007) using sand method (Mariappan *et al.*, 2014) and germination per cent was expressed on the number of normal seedlings at 21 days. Seedling Vigour Index I and Seedling Vigour Index-II were calculated as suggested by Abdul-Baki and Anderson (1973). The seedling length was measured on linear scale from 10 normal seedlings, which were randomly selected from the standard germination test. The product of

the seedling length and germination percentage was tabulated for estimation of Seedling Vigour Index I. For seedling dry weight, ten normal seedlings from each replication of the germination test were selected at random and kept for oven drying overnight and were weighed in mg (ISTA, 1999) and the values were multiplied with germination percentage for calculation of the Seedling Vigour Index II. Data were analyzed statistically by adopting CRD (Completely Randomized Design) techniques, as described by Panse and Sukhatme (1985). The data recorded as percentage were transformed to the respective angular (arc sine) values before subjecting them to statistical analysis. The traits were analyzed using Analysis of variance (ANOVA) to understand the significant difference among the traits of TOILs under consideration (Gomez and Gomez, 1984). A measure of group distance based on multiple characters was given by Mahalanobis (1936) using D^2 statistic by which, genetic divergence between genotypes was estimated.

Results and Discussion

Twenty-four accessions of *Pongamia* were placed under six clusters on the basis of Mahalanobis D^2 cluster analysis. The maximum number of accessions (seventeen) were grouped in cluster II, followed by cluster I with three accessions (Table 1 and Figure 1). Whereas, cluster III to VI had one accession each. In the present study, the clustering pattern of genotypes is based on seed storability behavior related to genetic diversity. This was proved by tendency of genotypes from cluster I showing nil germination after six months of seed storage.

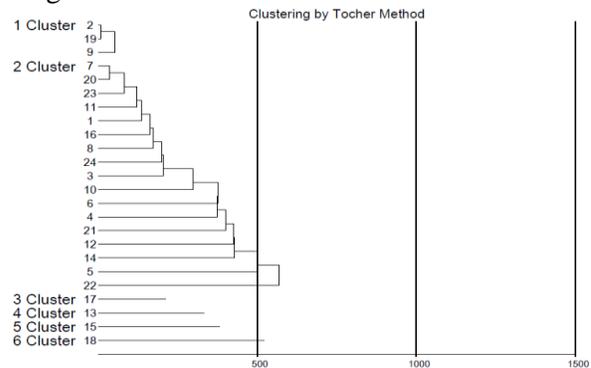


Figure 1. Clustering of 24 genotypes of *Pongamia pinnata*

Divergence of seed quality and storability

The clustering pattern in this study revealed that seed physiological diversity was related to genetic diversity. Intra- and inter-cluster distance ranged from 0.00 to 19.92 and 22.81 to 154.39, respectively. Intra-cluster distance was maximum (19.92) in cluster II with 17 accessions and minimum (0.00) in cluster III, IV, V and VI with one accession each (Table 2). Highest inter-cluster distance was observed between cluster IV and V (154.39) followed by cluster I and IV (152.20), suggesting that there is wide genetic diversity between these groups. The minimum

Table 1. Clustering pattern for seed physiological and storability traits among 24 genotypes of *Pongamia pinnata* by Tocher's method

Clusters	Number of genotypes	Genotypes
Cluster I	3	TOIL 2, TOIL 19 and TOIL 9
Cluster II	17	TOIL 7, TOIL 20, TOIL 23, TOIL 11, TOIL 1, TOIL 16, TOIL 8, TOIL 24, TOIL 3, TOIL 10, TOIL 6, TOIL 4, TOIL 21, TOIL 12, TOIL 14, TOIL 5 and TOIL 22
Cluster III	1	TOIL 17
Cluster IV	1	TOIL 13
Cluster V	1	TOIL 15
Cluster VI	1	TOIL 18

Table 2. Intra cluster (Diagonal) and inter cluster average distance (D^2) in *Pongamia* genotypes (Tocher Method)

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	7.44	145.84	134.68	152.20	22.88	137.73
Cluster II		19.92	30.09	27.43	149.70	34.20
Cluster III			0.00	32.08	136.01	22.81
Cluster IV				0.00	154.39	42.39
Cluster V					0.00	140.46
Cluster VI						0.00

inter-cluster distance was between cluster IV and III (22.81) followed by cluster I and V (22.88). Similarly, considerable genetic differences existed in pod and seed morphological characters and oil content among various seed sources of *Pongamia pinnata* (Naresh Kaushik *et al.*, 2007). Since, wide diversity exists between these clusters; the crosses between TOILs of these clusters may result in substantial segregates for sixteen quantitative traits and helps in further selection for overall improvement of species for seed storage. This kind of study can help to identify *Pongamia pinnata* genotypes with better seed storability. Cluster means expressed significant variation among clusters for all the traits, particularly for seed germination after aging (Table 3). Cluster II with seventeen genotypes recorded maximum cluster

means for nine traits viz., speed of germination-BA (11.79), seedling length (cm)-BA (52.67), seedling dry weight (g)-BA (3.33), seedling vigour index-I-BA (4374.64), seedling vigour index-II-BA (278.13), number of trifoliolate leaves-BA (1.72), seedling length (cm)-AA (33.93), seedling dry weight (g)-AA (2.74) and seedling vigour index-II-AA (156.56) followed by cluster IV (TOIL 13) for five traits viz., seed germination (%)-BA (85), speed of germination-AA (0.68), seedling vigour index-I-AA (2117), number of trifoliolate leaves-AA (1.50) and seed germination (%)-AA (64.33). Cluster VI (TOIL 18) recorded maximum cluster mean values for number of simple leaves-BA (3.01) and number of simple leaves-AA (2.03). In general, the cluster II and cluster V had highest and lowest mean values for most of the



Table 3. Cluster means for sixteen quantitative traits of *Pongamia* (Tocher's method)

Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cluster I	63.33	8.69	40.26	1.92	2565.63	125.26	2.07	1.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cluster II	82.75	11.79	52.67	3.33	4374.64	278.13	2.05	1.72	0.26	33.93	2.74	1934.40	156.56	1.74	1.39	56.84
Cluster III	45.00	3.38	40.36	1.49	1821.63	69.37	1.83	1.67	0.10	28.77	1.81	978.26	61.32	1.27	0.67	34.00
Cluster IV	85.00	8.03	43.80	2.52	3721.08	214.83	1.33	1.59	0.68	32.90	1.95	2116.75	125.34	1.31	1.50	64.33
Cluster V	30.00	3.11	48.52	0.61	1455.50	18.40	1.83	1.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cluster VI	80.00	7.79	39.75	1.75	3179.73	140.27	3.01	1.12	0.02	32.26	1.53	322.57	15.33	2.03	1.17	10.00

1=Seed Germination (%)-BA; 2=Speed of germination-BA; 3=Seedling Length (cm)-BA; 4=Seedling Dry Weight (g)-BA; 5=SVI-I-BA; 6=SVI-II-BA; 7=No. of simple leaves-BA; 8=No. of Trifoliate leaves-BA; 9=Speed of germination-AA; 10=Seedling Length (cm)-AA; 11=Seedling Dry Weight (g)-AA; 12=SVI-I-AA; 13=SVI-II-AA; 14=No. of simple leaves-AA; 15=No. of Trifoliate leaves-AA; 16=Seed Germination (%)-AA; SVI=Seedling Vigour Index; BA=Before Aging; AA=After Aging



Table 4. Relative contribution of different seed quality and storability characters towards genetic diversity in *Pongamiapinnata* genotypes

Character	Times ranked first	Contribution (%)
SVI-I-AA	98	35.51
Seed Germination (%) -AA	85	30.80
Speed of germination-BA	46	16.67
Speed of germination-AA	19	6.88
SVI-II-AA	10	3.62
Seedling Length (cm)-AA	8	2.90
No. of simple leaves-AA	4	1.45
SVI-II-BA	2	0.72
Seed Germination (%) -BA	1	0.36
Seedling Length (cm)-BA	1	0.36
No. of Trifoliolate leaves-BA	1	0.36
Seedling Dry Weight (g)-AA	1	0.36
Seedling Dry Weight (g)-BA	0	0.00
SVI-I-BA	0	0.00
No. of simple leaves-BA	0	0.00
No. of Trifoliolate leaves-AA	0	0.00

BA=Before Aging; AA=After Aging

traits, respectively. Cluster IV had maximum mean value for trait seed germination after aging. Similarly, Divakara and Rameshwar (2011) assessed 24 accessions and grouped them into 6 clusters on the basis of non-hierarchical euclidian cluster analysis and genotypes in cluster IV and cluster III were most heterogeneous and can be best used within group hybridization. The wide diversity exists between the cluster V and II, followed by cluster II and I and crosses between CPTs of these clusters may result in substantial segregates. It is revealed that the existence of substantial variation and diversity can be utilized for genetic resource conservation and further tree improvement programmes of the species. Mahendar Thudi *et al.*, (2010) assessed the molecular genetic diversity in 48 *Pongamia pinnata* accessions collected from six

different states of India by employing amplified fragment length polymorphism (AFLP) marker system and five AFLP primer combinations produced 520 discernible fragments, of which 502 (96.5%) were polymorphic. Quanyi Jiang *et al.*, (2012) also demonstrated an abundance of nucleotide core repeats in the *Pongamia* genome, large genetic and phenotypic diversity among randomly sampled *Pongamia* trees, restricted diversity in progeny derived from a single mature tree, stability of PISSR markers in *Pongamia* clones and genomic DNA sequences within PISSR markers. PISSRs provide a valuable biotechnology tool for assessment of genetic diversity, gene tagging and molecular breeding in *Pongamia pinnata*. Relative contribution of different seed quality and storability characters towards genetic diversity in *Pongamia pinnata* genotypes was



presented in Table 4. Among 16 traits, SVI-I-AA showed maximum (35.51 %) contribution towards diversity followed by seed germination (%)-AA (30.80%) and speed of germination-BA (16.67 %). From the above study, the traits like seed germination and seedling vigour index were highly correlated with seed storability traits of tree seed. Hence, identification of good TOILs may be advantageous based on initial seed germination and SVI. Cluster II and cluster I showed maximum intra-cluster distances. Maximum inter-cluster distance was between cluster IV and V, followed by cluster I and IV, indicating that there was wider genetic diversity between the trees in these groups. Since, wide diversity exists between these clusters; the crosses between TOILs of these clusters may result in substantial segregates for sixteen quantitative traits and helps in further selection for overall improvement of species for seed storage and the seeds of these TOILs may be important for massive afforestation programme. The present study can however serve as a pointer at later stages of study especially on seed quality enhancement of pongamia.

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