



Analysis of Diversity and Distribution of *Pongamia* [*Pongamia pinnata* (L.) Pierre] Germplasm Collections from Two Distinct Eco-Geographical Regions in India

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Abstract

Collection and study of extent of diversity available in *Pongamia*-native oil bearing tree species is the fundamental and significant requirement for its effective utilization. Two survey missions undertaken in distinct eco-geographical regions of India viz. Chhattisgarh and Rajasthan states of India formed the basis of the present study. The data on *in-situ* traits viz. pod length, pod width, pod thickness, seed length, seed width, seed thickness and the nursery traits viz. seedling girth, seedling height and number of leaves was recorded. There was good variation for these traits among the accessions collected from both the states. The extent of variation was in conformity with the earlier studies from several other regions of the country, which implied that pod and seed traits have a limited range. It was also inferred that cross pollination (pollination by bees) and open pollination did not significantly impact the extent of variation among the germplasm collections from Chhattisgarh and Rajasthan respectively; and using the DIVA-GIS software we were able to identify the diversity rich pockets in these two states. The D² statistic grouped the accessions from Chhattisgarh into seven diverse clusters and those from Rajasthan into four. It was concluded that use of phenotypic data along with the molecular markers data would effectively bring out the extent of diversity for utilization in *Pongamia* crop improvement.

Keywords: DIVA-GIS; Diversity index; Heritability; Pollination; *Pongamia*; Variability

Introduction

The demand for different sources of energy viz. coal, electricity, natural gas and oil has increased tremendously as a result of population growth along with social and economic development drivers. Oil, being portable and efficient, is the most convenient source of energy. Although oil supplies match the demand, in the current scenario, the high greenhouse effect/impact of the fossil fuel, has made us to look for alternative sources [1]. Bio-fuels from tree borne oil seed is considered to be viable option [2]. Among the

tree borne oils *Jatropha*, *Pongamia*, *Simarouba* etc are listed as the potential sources. Of these, *Pongamia*, native to India is nitrogen fixing tree species with a potential of bio-amelioration of degraded lands [3]. It scores over the non-native species in important factors viz. acceptability by farmers, adaptability to the local environment and in lacking possible invasive effects of the introduced tree species.

Pongamia pinnata (L.) Pierre known as *Karanj* belongs to family Fabaceae; sub-family-Papilionoideae and tribe Millettieae. It is hardy, non-edible seed oil producing tropical tree species. The economic part, the seed, is borne in a hard pod which varies in shape and size. The pods generally bear up to 1-3 seeds/pod, although four seeds/pod have also been reported [1]. The pods are borne either singly or in clusters. The natural distribution of *Karanj* (*Pongamia*) is along coasts and river banks in India and Myanmar [2]. In India, its occurrence and distribution of diversity is reported throughout the country in general and particularly in the Western Ghats. The distribution of variability along the altitudinal gradient in specific areas/ regions of diversity in Andhra Pradesh and Orissa states in India, using the DIVA-GIS was reported [4]. Good phenotypic diversity and variation is observed in traits, viz. flower colour, branching pattern, palmate leaf number, leaf colour, pod size and shape etc. in view of it being a cross pollinated species. Its attribute as a cool shade giving tree has made it an important species of urban forestry and also traditionally used to package and conserve the freshness of hot season fruits like Mango and tender nuts of Palmyra palm.

Harrington [5] demonstrated the suitability of fatty acid methyl esters (FAMES) from *Pongamia* seed oil as a good fuel for diesel engines and several other research findings also confirmed the potential of *Pongamia* oil to be used as a biodiesel [6,7,8,9,10]. FAMES as biodiesel are environmentally safe, non-toxic and biodegradable [11]. The composition of the *Pongamia* seed oil and the properties of the FAMES meet the North American and European industry standards and its values for the pour and cloud points are satisfactory for tropical and some temperate regions [3]. *Pongamia* being a native, non-food species with good oil yielding potential has become a popular bio fuel crop. However, the major bottleneck is identification of diverse and promising lines for exploitation, which is a major challenge. Keeping this in view well planned surveys were undertaken by NBPGR throughout India.

This article documents the variation in diversity observed, identification of diversity rich regions and diverse accessions for crop improvement

Materials and Methods

Two surveys were undertaken by National Bureau of Plant Genetic Resources, Regional Station, Hyderabad, India in the states of Chhattisgarh and Rajasthan, which are characterized by diverse agro-ecological traits for collection of *Pongamia pinnata* germplasm during April-May 2009. In the state of Chhattisgarh, the mission covered Jagdalpur, Kanker, Dantewada, Narayanpur, Dhamtari and Durg districts in the Bastar plateau. The surveyed area lies between 18.97.71-81.60.15 N to 21.10.87 - 81.26.73 E with an altitude ranging from 178 m to 889 m In Rajasthan, the mission was undertaken in the districts of Ajmer, Bilwara, Chhittorgah, Banswara, Udaipur,

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Dungarpur and Rajasmund. The surveyed area lies between 23.21.23-74.264 N to 25.58.14 - 75.0913 E. The general strategy adopted for germplasm collection was based on the theories suggested by Bennett, FAO and Huaman et al. [12,13,14]. Random sampling of matured pods from all sides of the trees was done. Latitude, longitude and altitude of the collection sites were recorded using Garmin 12, Global Positioning System (GPS). The Passport information consisting of the details with regards to the village, mandal and district of the site of collection were recorded. The collected germplasm was raised in nursery bags containing sand and clay in the ratio of 1:4 in polyhouse at ambient temperature with relative humidity ranging from 70 to 80 %. Data on seedling traits was recorded on two-month-old saplings. Average data for 10 nursery raised plants was recorded to study the diversity. The pod and seed data (recorded *in-situ*) along with the seedling (nursery) traits was subjected to statistical analysis using R 3.2.1 and GENRES softwares. DIVA-GIS software (developed by International Potato Centre, Lima, Peru) was also done to develop Grid maps on the diversity using point-to-grid analysis seeking square neighborhood method. Differentially colored grids were thus generated as Shanon diversity index to signify the extent of variation in the diversity. The diversity maps using GIS-DIVA software maps were generated for seed, pod and seedling traits.

Results and Discussion

Distribution of *Pongamia* is reported from India in diverse eco-geographical regions. There were reports of collection of germplasm from erstwhile Andhra Pradesh [1,15], wherein effort was made to collect phenotypically diverse germplasm lines to develop descriptor states and document the associated ITK and study the variability, heritability and group the accessions based on clustering analysis respectively. Mukta et al. [11] studied the variability in *Pongamia* for its potential for biodiesel traits. Scott et al. [3] published a timely review of the status of research on *Pongamia* and the way forward.

The two surveys undertaken in Chhattisgarh and Rajasthan resulted in the collection of 50 accessions from Chhattisgarh and 21 accessions from Rajasthan. The distribution of the *Pongamia* tree population in Chhattisgarh is denser and hence critical accession-wise evaluation based on plant traits was possible to sample the diverse accessions based on age of the tree, branching pattern, pod size, shape, bearing and also factors like assessment of local trees. Whereas, the distribution of the *Pongamia* in districts of Rajasthan was far and wide and the accessions were collected from single isolated trees. Solomon Raju and Purnachander Rao [16] reported reproductive behaviour of the crop leading to predominant cross fertilization, which is brought about by important pollinating insects such as *Apis dorsata*, *A. cerana indica*, *Amegilla* sp., *Megachile* sp., *Xylocopa latipes*, *X. Pubescens*, wasps viz., *Sphex* sp., *Vespa* sp.,

Ropalidia spatulata, *Delta pyriformes* and thrips (*Thrips hawaiiensis* and *Haplothrips tardus*). The distribution of *Pongamia* in Chhattisgarh models on cross pollination, as the dense population can easily be cross fertilized by bees and thrips, whereas the distribution of plant population in Rajasthan was not amenable for pollination by insects. Dhillon et al. [17] have reported cross-pollination up to 60-90% and open pollination 10-40% in *Pongamia*.

Estimation of variability using statistical measures

The descriptive statistic for pod, seed and seedling traits for both the states of Chhattisgarh and Rajasthan are provided in Table 1. In Chhattisgarh, maximum CV was recorded in the number of leaves per plant (33%) followed by seedling height (23%) and the lowest was recorded in pod length (5.4%). The maximum pod length recorded was 6.6 cm (VNKR-09-07) and there were five accessions which recorded a pod length of 6.0 cm and above; VNKR-09-49 recorded maximum pod width (3.0 cm). Pod thickness of 1.5 cm was recorded in VNKR-09-27, which was the highest. The seed length ranged from 1.8 (VNKR-09-38) to 2.7 (VNKR-09-18) and the maximum seed width was recorded in VNKR-09-44 (1.9 cm). Maximum seed thickness of 0.9 cm was recorded in VNKR-09-52. The seedling girth recorded was in the range of 2.8 cm (VNKR-09-03) - 5.4 cm (VNKR-09-42). The maximum seedling height of 34.1 cm was recorded in VNKR-09-47.

In Rajasthan, among the 21 accessions, maximum CV was recorded in number of leaves per plant (23%) followed by seed thickness (18.4%) and seedling height (18%). The maximum pod length and width was recorded in SNP-09-20 with 7.5 cm and 3.0 cm respectively and the same accession also recorded maximum seed length (1.9 cm), seed width (1.9 cm) and also number of leaves (20.0). Maximum seed thickness was observed in SNP-09-09 (0.8 cm) and also maximum seedling girth (6.1). The maximum seedling height was recorded in accession SNP-09-12 (41 cm). The variability assessment of the germplasm collected from Haryana [18], Maharashtra [19], Jharkhand [20], Andhra Pradesh [21] and Uttar Pradesh [22] was elucidated by different authors. The maximum pod length reported by Divakara et al. [20] was 65.5 mm and similar pod length was also recorded in the present study in Chhattisgarh however, in Rajasthan a pod length of 7.45 cm was recorded. Sunil et al. [1] reported maximum seed length, width and thickness of 2.6 cm, 1.8 cm and 1.0 cm respectively and similar results were reported from the observations in the present study. The studies depict the range of variation for pod and seed traits in the germplasm collected across the country and showed that what was observed in the present study is within the variation reported earlier for these traits. Analysis of variance revealed there was significant difference among the

Table 1: Summary statistics of the germplasm collected from Chhattisgarh and Rajasthan.

Trait	Pod length (cm)		Pod width (cm)		Pod thickness (cm)		Seed length (cm)		Seed width (cm)		Seed thickness (cm)		Seedling girth (mm)		Seedling height (cm)		No. of leaves		100-seed weight (g)	
	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R
Mean	4.9	5.4	2.1	2.3	1.1	1.0	2.3	1.9	1.5	1.5	0.8	0.7	4.2	4.7	22.6	28.0	12.8	14.5	150.7	164.5
Range	2.8	3.1	1.4	1.0	0.7	0.4	0.9	0.7	1.1	0.6	0.4	0.3	2.5	2.5	20.5	23.0	14.4	10.0	187.0	146.0
Minimum	3.8	4.3	1.6	2.0	0.8	0.9	1.8	1.2	0.9	1.3	0.5	0.5	2.8	3.6	13.6	18.0	5.3	10.0	75.0	124.6
Maximum	6.6	7.5	3.0	3.0	1.5	1.2	2.7	1.9	1.9	1.9	0.9	0.8	5.4	6.1	34.1	41.0	19.7	20.0	262.0	270.6
Standard Deviation	0.7		0.3	0.3	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.7	0.6	4.4	5.9	3.4	3.0	6.5	5.4
CV%	5.4	7.3	8.1	6.9	8.0	15.5	6.3	9.5	6.2	8.5	10.0	18.4	20.6	14.0	23.0	18.0	33.0	23.0	4.3	3.3

Note: C=Chhattisgarh; R= Rajasthan

accessions for all the traits recorded except for seed thickness among the accessions collected from both states (Table 2a and 2b).

We wanted to compare the extent of variability between the populations collected from Chhattisgarh and Rajasthan considering the typical distribution of diversity/sampling observed, as outlined above. The assessment of variability using simple measures of variability viz. Phenotypic and Genotypic coefficients of variation, heritability and genetic advance are provided in Table 3a and 3b. Phenotypic Coefficient of variation (PCV) was higher than Genotypic Coefficient of Variation (GCV) for all the traits signifying the influence of environment. Among the *in-situ* recorded plant traits highest PCV was recorded in 100-seed weight. 100-seed weight also recorded higher heritability (97% in both the regions). Similarly, high PCV and heritability values were recorded for pod length, seed length and pod breadth. Hence, these traits can be used for phenotypic selection in *Pongamia*. However, 100-seed weight is the only trait that has also recorded high Genetic Advance (GA) in addition to PCV and heritability. This indicates that heritability is due to additive gene effect and selection is effective in such cases. Similar finding was reported by Sunil et al. [1]. The comparison of variability of the accessions between two states revealed that there was not much difference in

the cross pollinated (Chhattisgarh) and open pollinated (Rajasthan) conditions, clearly showing the non influence in sampling strategy under cross and open pollinated conditions. The study by Sahoo et al. [23] on the inter and intra- population variability in *Pongamia* also pointed out that, within population variability was up to 32.34% and the molecular polymorphism up to 67.18% with ten ISSR hinting at modest levels of genetic variation. Jiang et al. [24] also reported that the seed oil content and composition varied between the trees and within the progeny of the single parent tree. Use of efficient molecular markers like AFLP for diversity analysis in *Pongamia* [25] along with important phenotypic traits would more distinctly bring out the variation. These findings were in line with the finding of Thudi et al. [26], who showed that diverse germplasm lines could be collected from Karnataka state only, as the accessions collected from these two states showed similar variability among the traits studied.

Diversity analysis using DIVA-GIS

We also aimed to identify the pockets of diversity within these two eco-geographic regions. DIVA-GIS geo-referenced software was utilized for this, using the input data of traits recorded and the geo-reference (latitude and longitude) values recorded at the time

Table 2 A and B: Analysis of variance for seed and seedling traits of the germplasm collected from Chhattisgarh and Rajasthan.

Chhattisgarh											
Mean sum of squares											
Source	Df	Pod length (cm)	Pod width (cm)	Pod thickness (cm)	Seed length (cm)	Seed width (cm)	Seed thickness (cm)	Seedling girth (mm)	Seedling height (cm)	No. of leaves	100-seed weight
Genotypes	49	1.2**	0.2**	0.3**	1.4**	1.0**	0.3**	1.0*	58.3**	35.0**	4330.3**
Replicates	2	0.9	0.6	0.2	1.2*	0.2	0.1	1.0	62.7	30.3	151.0*
Residuals	98	0.7	0.3	0.1	0.2	0.0	0.0	0.7	2590.7	16.4	42.0
Rajasthan											
Mean sum of squares											
Genotypes	20	1.4**	0.2**	0.1**	0.3**	0.1**	0.0	1.0*	104.8**	26.8**	3188.3**
Replicates	2	0.1	0.1	0.0	0.1	0.0	0.0	0.2	111.6*	5.4	241.7**
Residuals	40	0.2	0.0	0.0	0.0	0.0	0.0	0.4	25.1	10.7	29.0

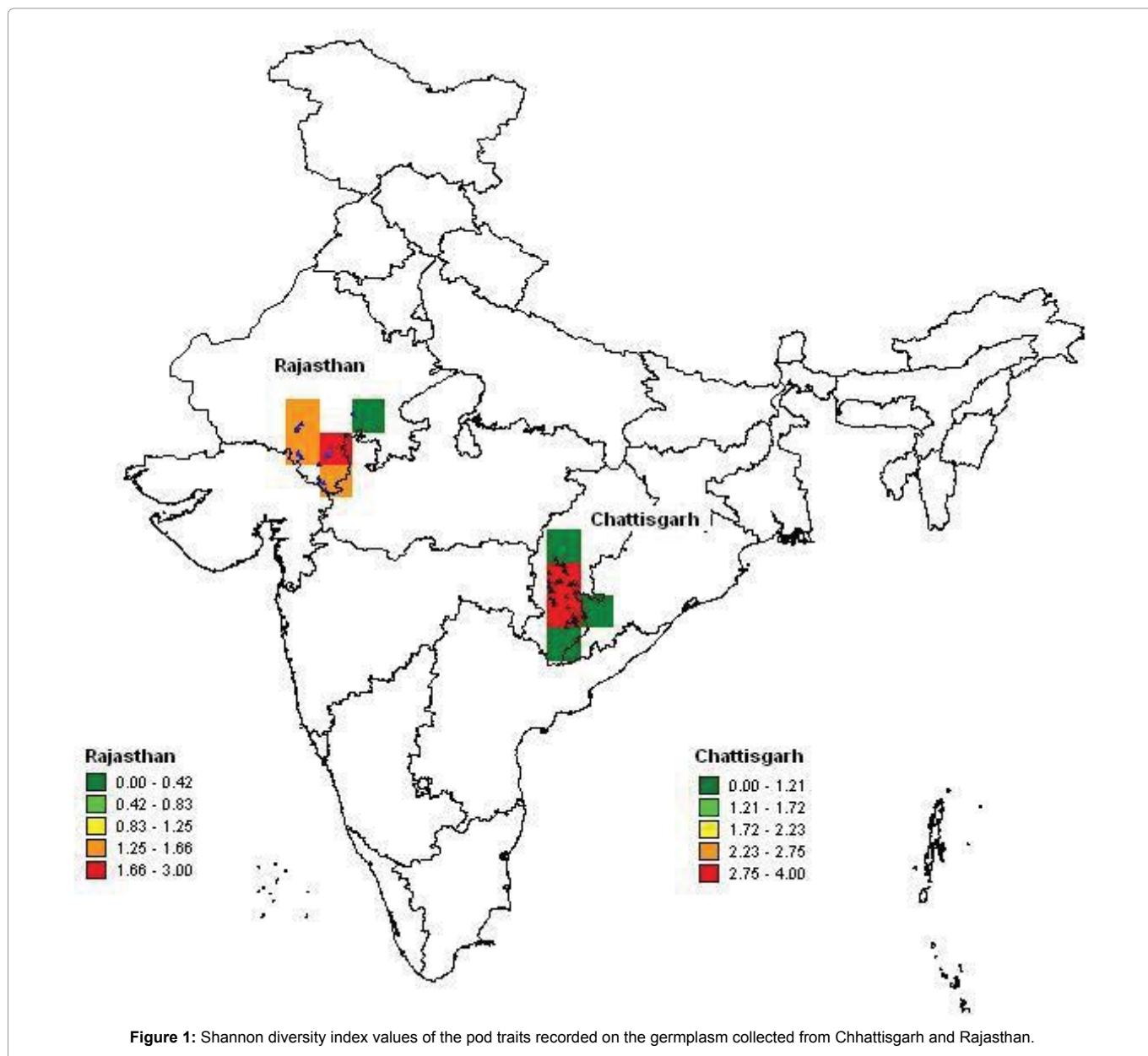
Note: **Significant at 1%; *Significant at 5%

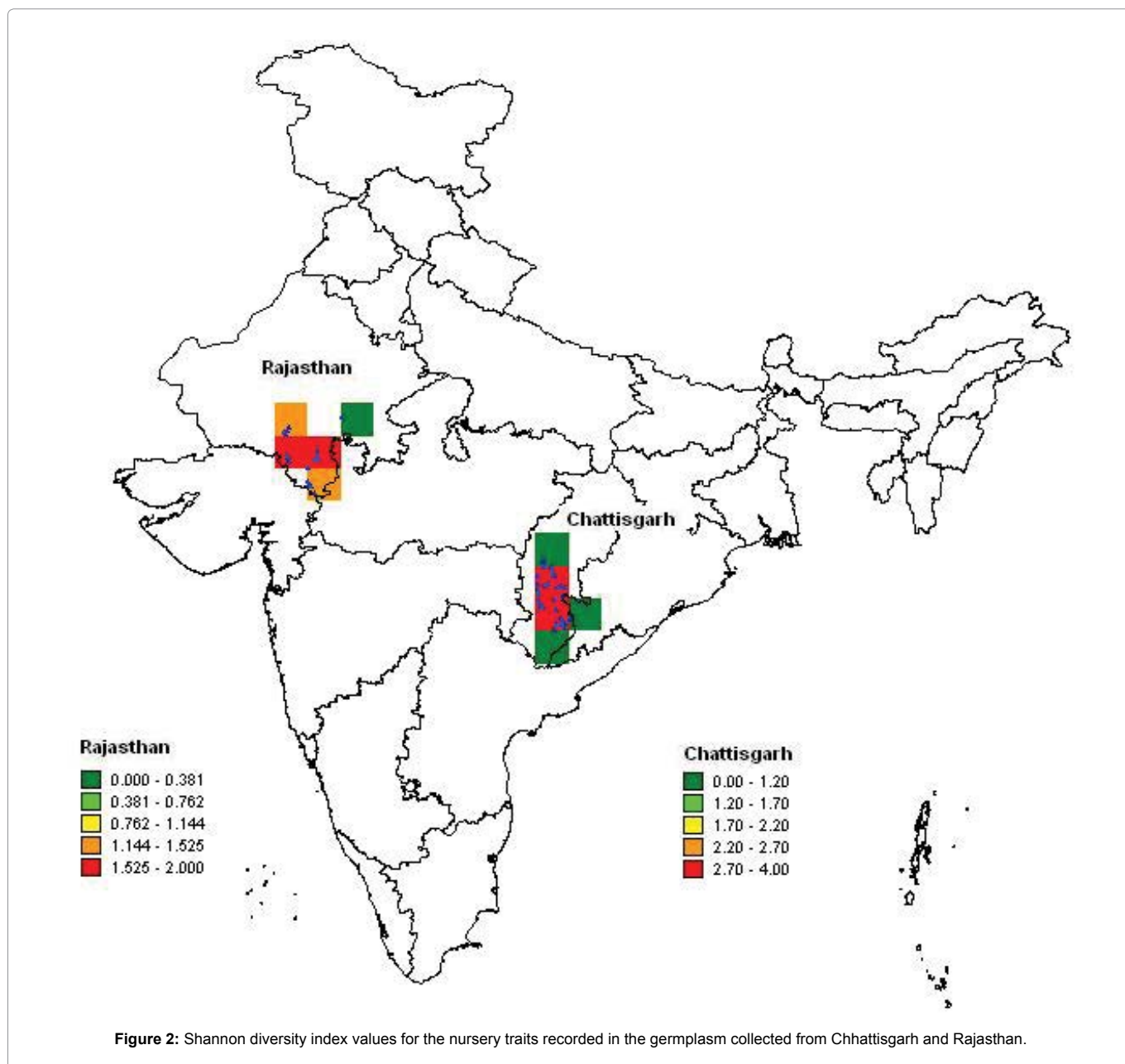
Table 3 A and B: Estimation of genetic variable for pod, seed and seedling traits in Chhattisgarh and Rajasthan.

	GCV (%)	PCV (%)	Heritability (%)	Genetic Advance (%)
Chhattisgarh				
Pod length	12.5	13.6	84.3	11.6
Pod breadth	12.1	14.6	69	4.4
Pod width	7.7	11.2	48	1.2
Seed length	9	11	67	3.4
Seed breadth	11.4	13	77.5	3.1
Seed width	10.8	14.8	53.7	1.2
Seedling girth	8.7	22.3	15.3	0.3
Seedling height	14.6	27.3	28.7	3.6
Number of leaves	20	38	27.3	2.7
100-seed weight	25	25.4	97	77
Rajasthan				
Pod length	12	14	73	1.1
Pod breadth	9.68	11.8	66.2	0.4
Pod width	10.6	18.7	32.1	0.1
Seed length	15.4	18.1	72.8	0.5
Seed breadth	9	12.3	53	0.2
Seed width	6.4	19.4	11	0
Seedling girth	9.4	17	31	0.5
Seedling height	18.3	25.6	51.3	7.6
Number of leaves	16	27.7	33.2	2.7
100-seed weight	19.7	20	97.3	65.9

of germplasm collection. The Figure 1 shows the diversity index values of the germplasm lines recorded for pod traits viz. pod length, breadth and width in both the states. The red grid standing for the more diverse lines followed by the rest as detailed in the legend. The Shannon diversity index values for the germplasm collected from Chhattisgarh ranged from 0-4.0 and those from Rajasthan recorded up to 3.0 only. The South Central region of Chhattisgarh emerged as the region with highest diversity within Chhattisgarh state and whereas South Eastern region of Rajasthan recorded highest diversity index values between 1.66 and 3.0. The seed traits viz. seed length, seed width and thickness recorded similar diversity index values as the pod traits. The maximum Shannon diversity index for other nursery traits including the number of leaves, seedling height and girth ranged from 2.7 to 4.0 in Chhattisgarh and the maximum value recorded in Rajasthan was in the range of 1.5 to 2.0 and the same is depicted in the Figure 2. The broad findings viz. high diversity regions

in Chhattisgarh are on the expected lines as the general density of the population was more in Chhattisgarh and the reproductive biology of *Pongamia*, as an outcrosser, giving rise to more diversity. Sunil et al. [4] had identified Rayagad district of Odissa state as the region for the collection of diverse germplasm lines for seed traits and oil content. Bastar region of Chhattisgarh, which forms a contiguous region of Odissa also can be tapped for diverse lines use of DIVA-GIS tool in identification of diversity rich regions was reported earlier in *Jatropha* [27], brinjal [28]. However, interestingly high mean values for all the traits were recorded in germplasm collected from Rajasthan except in case of pod thickness and seed length. The arid ecology and stressed and open pollination conditions prevailing, the isolated trees could not have been pollinated by bees as outlines by Solomon Raju and Purnachander Rao, [16]. Thus environmental conditions found in Rajasthan may be more suitable for the development of reproductive traits i.e. conditions suitable for quality over quantity of seed produced





and also the seedlings grown under optimal conditions expressing high potential value traits.

D² Analysis for identification of diverse groups

Further to identify the diverse group/accessions within these accessions. The pod, seed and seedling traits data was subjected to D² statistics. The accessions from Chhattisgarh were grouped into seven clusters and those from Rajasthan into four groups (Table 4a and 4b). Cluster II was comprised of maximum number of 45 accessions collected from Chhattisgarh and the rest of the six clusters comprised of two accessions each, whereas for Rajasthan, Cluster IV had 15 accessions and rest of three clusters comprised of two accessions each. The inter and intra cluster distances for the accessions from Chhattisgarh and Rajasthan have been provided in Table 5a and 5b. The maximum inter cluster distances in Chhattisgarh was recorded in

between cluster VI (57) and VII (432) followed by Cluster I (3.7) and VII (319), whereas the intra cluster distance was maximum in cluster VII. The maximum inter cluster distance for Rajasthan was recorded between I and IV and the maximum intra cluster distance was recorded in cluster IV. Selection of genetically divergent parents such as the accessions from clusters having maximum genetic distance would facilitate their exploitation by hybridization. The cluster means for each of the traits (Table 6a and 6b) revealed that cluster VII in Chhattisgarh and cluster IV from Rajasthan were conspicuous by recording higher 100-seed weight. Further, among the traits studied 100-seed weight emerged as the key character contributing to divergence both in Chhattisgarh (77%) and Rajasthan (75%).

Conclusions

The findings of the present study by phenotypic traits were in line

Table 4 A and B: Grouping of accessions collected from Chhattisgarh and Rajasthan into D² clusters.

Cluster No.	Accessions
I	VNKR-09-24, 30
II	VNKR-09-1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,25,26,27,28,29,31,32,34,35,36,37,38,39
III	VNKR-09-42, 44
IV	VNKR-09-47,48
V	VNKR-09-45,50
VI	VNKR-09-41, 49
VII	VNKR-09-40, 43

Rajasthan

I	SNP-09-11,12
II	SNP-09-8,9
III	SNP-09-6,14
IV	SNP-09-1,2,3,4,5,7,10,13,15,16,17,18,19,20,21

Table 5 A and B: Estimates of inter and intra cluster D² values for pod, seed and seedling traits for accessions from Chhattisgarh and Rajasthan.

	I	II	III	IV	V	VI	VII
I	3.7	90.0	101.0	32.0	122.0	38.0	319.0
II		94.0	60.0	70.0	88.0	155.0	195.0
III			8.9	53.0	26.0	174.0	100.0
IV				16.0	68.0	90.0	216.0
V					31.0	185.0	119.0
VI						57.0	432.0
VII							151.0

Rajasthan

	I	II	III	IV
I	2.8	29.0	38.0	114.0
II		3.7	17.0	111.0
III			13.0	94.0
IV				166.0

Table 6 A and B: Cluster means for the pod, seed and seedling traits for accessions from Chhattisgarh and Rajasthan.

Trait Cluster No.	1	2	3	4	5	6	7	8	9	10
I	4.5	1.9	1.0	2.3	1.3	0.7	3.7	15.7	9.1	110.0
II	4.9	2.0	1.1	2.2	1.4	0.7	4.0	22.0	11.6	150.0
III	5.1	2.3	1.1	2.3	1.5	0.7	4.4	25.5	15.5	168.5
IV	4.3	2.0	1.2	2.3	1.3	0.8	5.0	19.0	15.8	138.0
V	4.7	2.6	1.1	2.1	1.6	0.7	4.4	29.5	17.5	165.5
VI	4.6	2.1	1.0	2.0	1.3	0.6	4.5	21.0	14.6	91.0
VII	5.3	2.4	1.1	2.1	1.7	0.7	4.3	27.0	15.0	211.0

Rajasthan

Trait Cluster No.	1	2	3	4	5	6	7	8	9	10
I	5.0	2.0	1.0	1.4	1.3	0.7	5.3	25.3	15.0	155.0
II	5.2	2.4	1.0	1.7	1.6	0.7	4.3	23.0	15.0	148.0
III	5.7	2.5	0.9	1.9	1.4	0.6	4.7	25.8	11.5	154.0
IV	5.4	2.3	1.0	2.0	1.5	0.7	4.7	29.0	15.0	169.0

with those by Thudi et al. [26], who using AFLP molecular markers grouped 48 accessions of *Pongamia*, sourced from six different states into four clusters, they also reported an average genetic similarity of 0.61 among the accessions although the range recorded was from 0.28 to 0.90. This would imply that the variation was less than 40%

among these accessions. Although a high level of polymorphism is expected in an out-crossing species like *Pongamia*. However, the diversity reported using the above phenotypic traits may not be comprehensive. In this regard, the findings of Sharma et al. [29], who used microsatellite markers successfully in the diversity analysis

of 24 genotypes of *Pongamia* and Biswas et al. [30] who opined that use genetic and genomic tools to unravel the diversity and use of phenotype associated link markers may be the way forward for understanding the extent of diversity in the native population and tapping the diversity crop improvement.

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