the South Bay, since observations do not indicate any such signal either in terms of frequency of depressions or OLR anomalies over both north and south Bay of Bengal. The interaction between SST in north Indian Ocean and the convection or monsoon depression formation is complex[20]. Warm SST anomalies alone are not sufficient for initiation of intense convection over the Bay of Bengal or Arabian Sea area, leading to the genesis of meso-scale systems and hence their intensification to monsoon depressions or cyclonic storms. Rather, favourable atmospheric environmental conditions like low-level convergence and vertical wind shear are foremost conditions. The results of the present study indicate that although the number of LOPARS has been increasing during the last two decades, the dynamic conditions such as wind shears, MSLP, moisture, etc. are not favourable for their intensification to depressions and cyclones. Thus, there is a decrease in the number of depressions and cyclones during the last two decades. However, it is essential to examine why convection becomes weaker in spite of increased SST over the Bay of Bengal during the past years.

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Genetic diversity of black gram (*Vigna mungo*) landraces as evaluated by amplified fragment length polymorphism markers

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Twenty-six landraces of black gram collected from Orissa, India were analysed for genetic diversity using amplified fragment length polymorphism (AFLP) markers. Seven primer combinations were used for AFLP analysis. The percentage polymorphism across the samples varied from 74.5 to 93%. The level of rare and common alleles contributing to the diversity in the sample was analysed using the Shannon–Weiner (SW) diversity index. The SW index revealed that three samples of the entire twenty-six contributed significantly to the overall diversity of the sample set. A dendrogram was constructed based on the UPGMA clustering method, which revealed three major clusters. A principal component analysis of the same dataset revealed similar results to that of the dendrogram, with the first principle component accounting for 58% of the total variation. The analysed samples formed five significant groups. Three samples were distinct in their clustering and remained separate from the other samples. Influence of soil pattern and topography in the genetic make up of the landraces was visible and seemed to contribute to the genetic distinctness of the landraces. This genetic diversity could well be utilized for future breeding work.

BLACK gram (*Vigna mungo*) is a tropical leguminous plant, which belongs to the Asiatic *Vigna* species along with *V. radiata*, *V. trilobata*, *V. aconitifolia* and *V. glaberecence.* It is cultivated as fallow crop after rice cultivation in India. It is grown in various agro-ecological conditions and cropping systems with diverse agricultural practices[1]. In various parts of India, particularly in the hilly regions, a number of traditional landraces are still being cultivated as inter crops between rice, sugarcane, cotton, groundnut and sorghum cultivating seasons. These landraces possess unique traits (e.g. disease tolerance, abiotic stress tolerance, pest tolerance), which have been identified by the farmers. The farmers have been conserving these landraces for generations through *in situ* on-farm conservation practices where they collect the seeds of the germplasm and use the same seeds for raising the next season. This practice is commonly seen in a large part of Orissa.

In recent years, there has been significant decline in the pulse production in India. Lack of suitable varieties and

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genotypes with adaptation to local conditions is among the factors affecting the production. *V. mungo* forms one of the important constituents in the dietary practices of the local communities. Through national germplasm collection initiatives, a number of accessions representing *V. mungo* from diverse agro-climatic zones are being maintained in the germplasm collection at National Bureau of Plant Genetic Resources (NBPGR), Indian Institute of Pulses Research (IIPR), etc. Efforts to genetically improve the crop are still at a low ebb, barring few efforts to identify important morphological descriptors and develop advanced breeding lines for locale-specific cultivars of this crop[1].

These efforts are impeded due to lack of distinctive morphological attributes in the species, despite their wide distribution across diverse agro-climatic zones. This is exemplified by the fact that till date no subspecies and varietal delineation has been reported and recorded in the species. In the absence of distinct morphological attributes, genetic characterization of diverse genotypes and documentation of diversity among the genotypes are of utmost significance in genetic improvement of this important legume species. The present investigations were undertaken to analyse and assess the nature and extent of genetic diversity among the genotypes representing a narrow geographical region of western Orissa.

DNA-based marker systems have been used, both alone and in tandem with morphological markers in order to obtain more reliable information on the genetic diversity existing in a number of species groups[2]. A variety of DNA-based marker systems have been developed to study the genetic variation in various crop species and have been identified as reliable marker systems. Random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) are a few of the techniques, which have been developed and employed for this purpose. Among the various molecular marker systems available for studying genetic diversity, AFLP[3] has been the preferred method of choice. AFLP combines the features of easy handling of RAPD[4] and the robustness of RFLP[5]. It does not require prior sequence information and has been used in analysing the genetic variability in a variety of crop species[6–13].

Genetic diversity studies in various species of the genus *Vigna* using DNA-based marker system are limited to a large extent. Few reports are, however, available in this regard dealing with diversity studies in *Vigna* species, namely *Vigna subterranea*[14], *Vigna unguiculata*[15] and *Vigna angularis*[2,16]. Among the various species of genus *Vigna*, black gram has been the least studied crop within the pulses and no international system under the CGIAR has this as a mandate crop[17]. However, there have been few efforts in this direction, but these diversity studies have been done using morphological data[1,18]. Till date no study on the genetic diversity of *V. mungo* using DNA-based markers has been reported. The present communication is a study undertaken to analyse the genetic diversity of *V. mungo* using AFLP marker system. In this communication we report the results of the AFLP study in 26 landraces of *V. mungo* collected from two regions, Thuamal Rampur and Lanjigarh in western Orissa, with different soil patterns. These are the regions where black gram is cultivated as a main crop all through the year and not as an inter crop as done in the other areas of the state. These regions also export black gram[19]. The objective of this study is to assess the genetic diversity in the black gram landraces from these two regions.

The present study included 26 landraces collected from the tribal farmers' field in Thaumal Ramapur and Lanjigarh regions, Kalahandi district, Orissa, India. Seed samples were collected from individual farmers. About 50 seeds from each landrace were collected and germinated under laboratory conditions. The young leaves collected from the samples were used for DNA extraction. The village and blocks from where the seed samples were collected and their maturation period are given in Table 1.

DNA was isolated using a modified CTAB (cetyl trimethyl ammonium bromide) method[20]. For each accession, about 5 g of bulked leaf tissue from 50 plants was ground to a fine powder using liquid nitrogen which was then suspended in 20 ml of extraction buffer (20 mM EDTA at pH 8.0, 100 mM Tris-HCl at pH 8.0, 1.5 M NaCl, 2% CTAB and 1% b-mercaptoethanol). The suspension was mixed well, incubated at 60°C for 45 min

Table 1. *Vigna mungo* landraces utilized in the study

Sample no.	Duration (months)	Region
1	4	Thuamal Ramapur
2	4	Thuamal Ramapur
3	3	Lanjigarh
4	4	Lanjigarh
5	4	Lanjigarh
6	4	Lanjigarh
7	4	Lanjigarh
8	4	Lanjigarh
9	4	Lanjigarh
10	4	Lanjigarh
11	4	Lanjigarh
12	4	Lanjigarh
13	4	Lanjigarh
14	4	Lanjigarh
15	4	Lanjigarh
16	4	Lanjigarh
17	4	Lanjigarh
18	4	Lanjigarh
19	4	Lanjigarh
20	4	Lanjigarh
21	4	Lanjigarh
22	3	Thuamal Rampur
23	3	Thuamal Rampur
24	3	Thuamal Rampur
25	3	Thuamal Rampur
26	3	Thuamal Rampur

followed by chloroform : isoamyl alcohol (24 : 1) extraction and precipitation with 0.6 volume of isopropanol at –20°C for 1 h. The DNA was pelleted down by centrifugation at 12,000 rpm for 10 min and was then suspended in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). DNA was purified from RNA and proteins by standard procedures[21], and its concentration was estimated by agarose gel electrophoresis and staining with ethidium bromide.

The genomic DNA (250 ng) was digested to completion with 5U of *Mse*I and *Eco*RI restriction enzymes (Boeringer-Manheim, Germany). The double-digested DNA fragments were ligated to 5 pmol *Eco*RI and 25 pmol *Mse*I adaptors. The adaptor-ligated DNA was diluted ten times with TE buffer and subjected to pre-selective amplification with *Mse*I adaptor + A and *Eco*RI adaptor + C primers. A 25 µl reaction mixture containing 2.5 µl of diluted DNA, 75 ng of each primer, 1X reaction buffer (10 mM Tris-HCl at pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 1.0 U of *Taq* DNA polymerase (Bangalore Genei, India) and 250 µM of dNTPs was prepared, and subjected to 20 cycles at 94°C for 30 s, 56°C for 60 s and 72°C for 60 s in a thermal cycler (Perkin–Elmer 9700). The amplified DNA was checked in 1.5% agarose gel, and diluted 25 times in TE buffer.

The *Eco*RI + 3 and *Eco*RI + 2 primers (30 ng) were end-labelled with g-^{32}P-dATP using T4 polynucleotide kinase (Life Technologies, USA) in a 50 µl reaction as recommended by the manufacturer. Selective amplification mixture (20 µl) was prepared with 0.25 µl labelled *Eco*RI primer, 0.3 µM *Mse*I + 3 primer, 5 µl diluted pre-selective reaction mixture, 1X buffer (20 mM Tris-HCl at pH 8.4, 1.5 mM $MgCl_2$, 50 mM KCl), 125 µM dNTPs and 1.0 U *Taq* DNA polymerase. Selective amplification was carried out for one cycle at 94°C for 30 s, 65°C for 30 s and 72°C for 60 s, the annealing temperature was lowered by 1°C for the next nine cycles, and followed by 23 cycles at 94°C for 15 s, 56°C for 30 s and 72°C for 30 s. Amplified products were mixed with equal volume of formamide-loading buffer, denatured at 90°C for 3 min, and resolved on 6% denaturing polyacrylamide gels (7.5 M urea) at 60 W constant power. The gels were dried and autoradiographed by exposing to X-ray film overnight. The seven primer combinations used for selective amplifications are reported in Table 2.

Each informative AFLP band was scored independently as 1 for 'presence' and 0 for 'absence'. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. The total diversity in the samples was calculated using SW diversity index[22], as $Hw = -\sum fi \times \ln(fi)$, where fi is the frequency of an AFLP band across all the samples. Genetic similarities among *V. mungo* landraces were calculated with the simple matching coefficient[23] by the NTsys-pc version computer software[24]. Then, the resulting similarity matrix was subjected to cluster analysis by the unweighted pair-group method with the arithmetic averages (UPGMA) cluster method and to principal component analysis.

Table 2. Total number of bands and percentage of polymorphism among the 26 *Vigna mungo* landraces

Primer combination	Total number of bands	Percentage polymorphism
E-ACGxM-CTA	47	85
E-ACGxM-CTG	49	93
E-AGCxM-CAT	65	90
E-ACGxM-CAG	51	74.5
E-ACGxM-CAA	67	86.5
E-AGCxM-CTT	59	81.3
E-AGCxM-CTC	59	91.5
		Average = 86

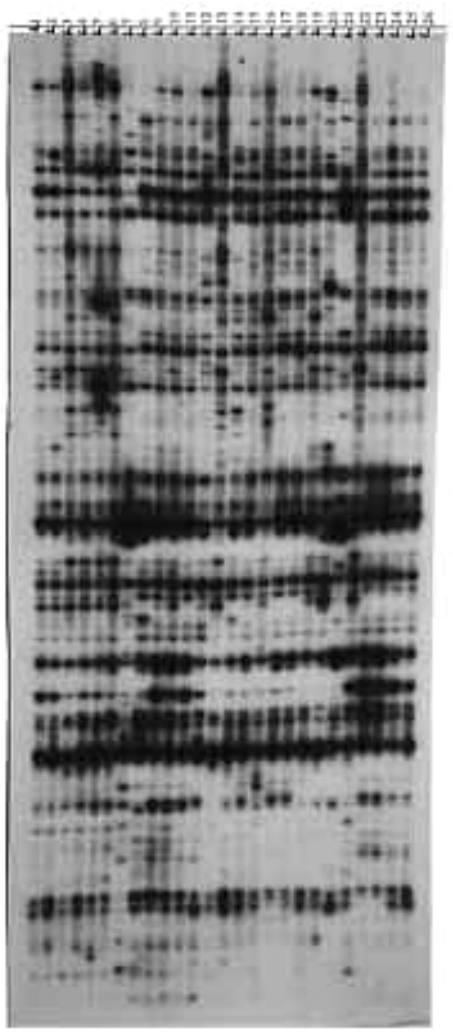

Figure 1. AFLP profile of 26 accessions of *Vigna mungo* landraces generated by selective amplification with *Eco*RI adaptor + ACG and *Mse*I adaptor + CTA primer combination and resolved in 6% denaturing polyacrylamide gel.

Twenty-six landraces were analysed using seven primer combinations. AFLP profile in the 26 landraces obtained through the use of *Eco*RI adaptor + ACG and *Mse*I adaptor + CTA combination is given Figure 1. The total number of AFLP loci recorded per primer combination varied from 47 in E-ACGxM-CTA to 67 in E-ACGxM-CAA (Table 2). The combined analysis of seven primer combinations showed 343 of 397 AFLP bands being polymorphic across the primer combinations. The percentage polymorphism across the landraces ranged from 74.5 (E-ACGxM-CAG) to 93 (E-ACGxM-CTG). The average percentage polymorphism across seven primers was 86%.

A dendrogram (Figure 2) was constructed based on simple matching coefficients, taking into account the presence or absence of the bands and ignoring their intensities. These showed three major clusters. The first cluster (cluster1) comprised of four landraces at a similarity of 78% (L2, L3, L4, L7). The second cluster (cluster 2) was a fairly larger one that included 16 (L8, L9, L10, L11, L12, L16, L17, L18, L19, L23, L24, L25, L26, L13, L15, L21) of the 26 landraces at 81% similarity. A separate cluster at 74% similarity comprised of two landraces (L5, L6), while the remaining four (L1, L22, L20, L14) formed operational taxonomic units. Principal component analysis (Figure 3) was performed using the same dataset in order to obtain a better understanding of the relationships of the landraces. The first principal component accounted for 58% of total variation. All the samples were tightly grouped and formed five distinct groups.

It has been reported that the ability to resolve genetic variation may be more directly related to the number of polymorphisms detected by the marker technique[2]. The percentage of polymorphic AFLPs, however, does not reflect on the influence of rare and common alleles on the genetic diversity, as a fragment of the lowest frequency is treated in the same weight as that of a fragment with the highest frequency across the genome. Hence SW diversity index was used to assess the genetic content of the samples (Table 4). SW diversity index value calculated for the samples collected from Thuamal Rampur and Lanjigarh was 44 and 74 respectively, thereby showing that landraces from Lanjigarh had a higher level of genetic diversity. Four samples, L1, L2, L5, L6, were of much interest from the results of the dendrogram. L1 formed an operational taxonomic unit; L2 clustered with the Lanjigarh samples, although it was collected from Thuamal Rampur. It was, however, interesting to note in Thuamal Rampur samples that the SW index values for samples (L23, L24, L25, L26) was 17.2, and L1, L2 together was 32 in comparison to the total SW value of TR landraces (44). This suggests that the genetic diversity in the Thuamal Rampur accessions was contributed significantly by two (L1, L2) of the total six landraces. This shows that L1 and L2 landraces could account for more number of rare alleles that are not shared with the other four samples in Thuamal Rampur landraces. In the Lanjigarh landraces, samples L5, L6 were found to be clustering separately. The SW index of the total sample set was found to be reduced (68.6) when L5, L6 were removed in comparison to the total SW index value (74.7) without removing them, thereby showing that these two samples are genetically unique and possess rare alleles.

The dendrogram and SW diversity index gives an idea about the nature of the individual samples in the whole sample set. L1, L5, L6 are found to be the most unique samples in the entire sample set. The SW values of the germplasm in the presence and absence of the three samples L1, L5 and L6, show that the number of rare alleles

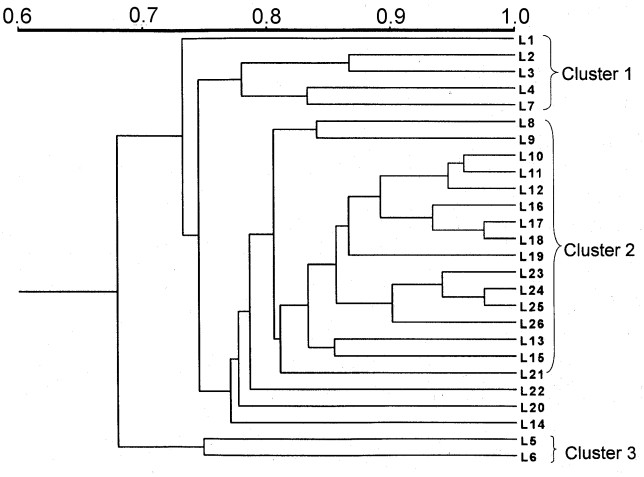

Figure 2. Dendrogram showing the relationships among the 26 accessions of *Vigna mungo* landraces that has been drawn based on simple matching coefficient.

Table 3. Soil pattern of Thuamal Rampur and Lanjigarh (in percentage)

Block	Alluvial soil	Black soil	Red soil	Red and yellow soil	Red and black soil
Thuamal Rampur	–	–	98	2	–
Lanjigarh	3.0	–	75	22	35

Table 4. Shannon–Weiner index for landraces collected form Lanjigarh and Thuamal Rampur

Primer combination	Thuamal Rampur landraces	Lanjigarh landraces
E-ACGxM-CTA	7.66	11.65
E-ACGxM-CTG	6.42	10.31
E-AGCxM-CAT	6.95	14.31
E-ACGxM-CAG	7.96	13.27
E-ACGxM-CAA	6.16	9.02
E-AGCxM-CTT	6.52	9.96
E-AGCxM-CTC	2.44	5.99
Total	44.13	74.53
Average	6.30 ± 1.82	10.64 ± 2.77

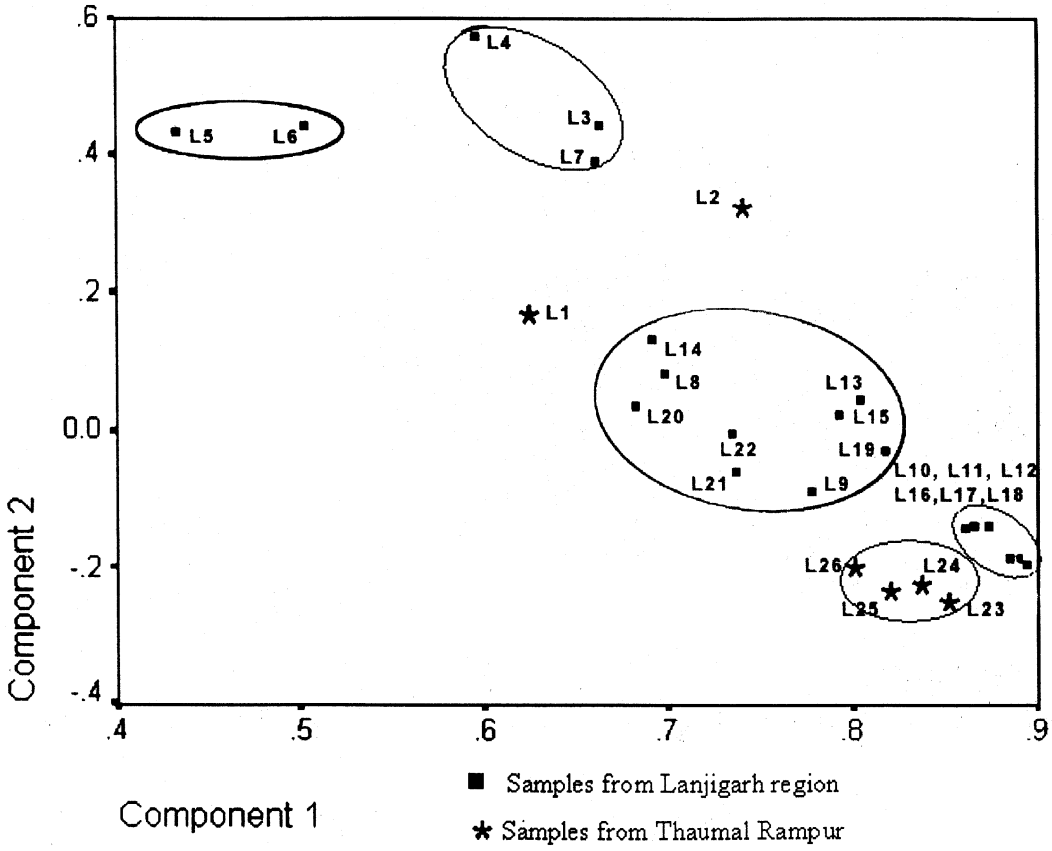

Figure 3. Cluster diagram showing the relationships among the 26 accessions of *Vigna mungo* landraces based on principal component analysis.

in these three samples is high and has contributed significantly to the overall genetic variation in the entire sample set.

Examination of morphological characters of the seed and plant material of these three landraces (L1, L5 and L6) showed no discrete differences from that of the other samples used in this study. A similar observation was made by Yee *et al.*[2], where morphological evaluation of seed size, colour, habit and, maturity of seeds provided no explanation as to why nine samples of their study showed distinct variation from the other samples. These three samples need to be studied further and analysed so that they can be used for further exploitation in the breeding programmes.

The objective of the present study was to assess the extent of genetic diversity among the 26 landraces being conserved and cultivated by the local communities. The black gram landraces have been collected from two geographical regions, Lanjigarh and Thuamal Rampur, possessing entirely different soil patterns (Table 3). Thuamal Rampur predominantly consists of red soil, with a limited area of mixture of red and yellow soil. Lanjigarh has a soil pattern which is a mixture of red, yellow, black and alluvial soils[25]. Tribal families have cultivated these landraces traditionally. The traditional agricultural practices in these tribal locations primarily involve cultivation of the seeds obtained from the same field in subsequent generations.

The homozygous genetic make-up of these landraces is well maintained, as the farmers collect and sow the same seeds for cultivation, supported by the fact that *V. mungo* is a self-pollinated crop. It is therefore envisaged that the genetic variation observed in these landraces might have resulted during the long cultivation history of the species, as an adaptation to the local agroclimatic conditions. Once these adaptive variations have been fixed in the genotypes, subsequently they could have passed on to the next generation. In the long run, these could have resulted in locally adapted genotypes. This agricultural practice is maintaining and also probably contributing to the genetic uniqueness by strengthening the specific adaptations obtained by the landraces. This is particularly evident from the fact that although the Thuamal Rampur landraces (L23, L24, L25, L26) have clustered with Lanjigarh, they remain distinct by subclustering among themselves.

The limited sample size utilized in this study revealed the presence of a unique genetic reservoir in the black gram landraces in Orissa. A similar kind of genetic distinctness has been reported in rice landraces collected from Orissa[26]. A study representing a large geographical area and also many number of samples from a limited geographical region will be of significance to identify

genetically superior genotypes and their correlation with the environmental attributes.

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Explosive pollen release, wind-pollination and mixed mating in the tropical tree *Shorea robusta* Gaertn. f. (Dipterocarpaceae)

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***Shorea robusta* in tropical India is wind-pollinated. Its gregarious occurrence; flowering during the dry season before the leaves unfold; conspicuous drooping inflorescence with pendulous flowers; massivity of blooming; copious pollen production (*c*. 60,000 per flower); explosive pollen release triggered by moderately gusty winds; pollen entrapment and transport in moderately turbulent wind; long period of pollen viability (*c*. 50 h); pollen grain size falling within the aerodynamic size range of 20–60 μm; pollen getting dispersed in single units; high pollen concentration in the air; strong protogyny with prolonged stigma receptivity (*c*. 50 h) – all constitute the adaptations for effective wind-pollination. The flowers are nearly similar in compatibility to auto, geitono and xeno pollen. The style emerges out a day before the unfolding of the corolla, an adaptive device for pollen reception from xeno and geitono sources; presumed cryptic self-incompatibility facilitates outcrossing at this stage of the flower. The mixed mating system and the stigma receptivity extending into day-2 of flower life when the corolla unfolds allow back-up auto-pollination should outcrossing fail.**

SHOREA Roxb. (Dipterocarpaceae) is a tropical genus widely distributed in India, Sri Lanka, Burma and other South-East Asian countries. It is highly valued for its strong and durable wood, resins, oils, camphor and turpentine. Indian forests harbour four species: *S. assamica* Dyer, *S. robusta* Gaertn. f., *S. roxburghii* (=*S. talura*) G. Don and *S. tumbuggaia* Roxb. Of these, *S. robusta* commonly called the sal tree, is the most important. It is the main source of construction and domestic timber and also dammars of India. It occurs extensively in parts of north, east and central India. The sal forests extend from the Himalayan foothills through central India, Madhya Pradesh, West Bengal, Orissa up to Visakhapatnam in Andhra Pradesh, often forming pure stands over extensive areas, and yielding large fruit crop adapted for wind dissemination. In the aseasonal rainforest of Malaysia, *Shorea* is dominant and its species are pollinated by thrips[1], and in the Sri Lankan forests, honey bees, *Apis dorsata* and *A. cerana indica*, are the pollen vectors[2]. In India, *S. robusta* is presumed to be either entomophilous or anemophilous[3], and the plant is a high pollen produ-

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