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RAPD analysis reveals the presence of mainland Indian and Indonesian forms of *Erianthus arundinaceus* (Retz.) Jeswiet in the Andaman–Nicobar Islands

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Three new accessions of *Erianthus arundinaceus* (Retz.) Jeswiet a wild relative of sugarcane, collected recently from the Andaman–Nicobar group of islands were characterized using RAPD markers, in relation to two distinct forms of the species from mainland India and Indonesia. Two of the clones from the Middle Andaman showed molecular profiles similar to the mainland Indian forms while the third clone from the Katchal Island of the Nicobar group showed profiles similar to the Indonesian forms. The study clearly establishes the presence of both mainland Indian and Indonesian forms of *Erianthus arundinaceus* in the Andaman–Nicobar group of islands. The distribution of the two forms was found to be in accordance with the geographical proximity of the collection sites with respect to the Indian subcontinent or the Indonesian Archipelago.

Keywords: Andaman–Nicobar, *Erianthus arundinaceus*, RAPD markers.

ERIANTHUS is a close relative of sugarcane (*Saccharum officinarum* L.) and an important member of the *Saccharum* complex[1]. There are several species of *Erianthus*, majority of which are found in the Indian subcontinent, including *E. arundinaceus*, *E. procerus*, *E. longisetosus*, *E. bengalense*, *E. ravennae*, *E. fulvus*, *E. elephantinus* and *E. hookeri*. This genus is believed to have originated in the Indo–Myanmar–China region, spreading subsequently to the adjoining areas[1]. *E. arundinaceus* is widely distributed in India, China, Myanmar, Thailand, Philippines, Indonesia and New Guinea.

The species that is considered most important among *Erianthus* for exploitation in sugarcane breeding is *E. arundinaceus* (Retz.) Jeswiet represented by cane-forming types, with tremendous ability for biomass production and a high level of tolerance to biotic and abiotic stresses. This species is extensively distributed in the Indian subcontinent as well as in most of the South East Asian countries and is considered to be an important source for breeding improved sugarcane varieties with high yielding ability, tolerance to environmental stress and resistance to diseases and pests. Serious efforts are underway to utilize *E. arundinaceus* in sugarcane breeding programmes in several countries including India. The major part of the *E. arundinaceus* germplasm available today is either from the Indian subcontinent or from the Indonesia–Papua New Guinea region, which form the major areas of diversity for the species. In view of its importance in sugarcane breeding, considerable attention had been given for the collection and characterization of the species[2].

Serious efforts to collect the wild germplasm of sugarcane in India began in 1933. Several *Erianthus* clones were collected from the distributional areas in the country during the explorations conducted over the years[3–6]. Consequently a large and well-characterized *E. arundinaceus* germplasm is available today for exploitation[2]. Though the existing collections represent most of the geographical diversity available in the country, representation from the Andaman–Nicobar group of islands was lacking till recently. The Andaman–Nicobar group of islands are positioned between the Indian subcontinent, the Malay Peninsula

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and the Indonesian Archipelago, which represent the major distributional areas of the species. The islands are rich in plant biodiversity and the flora is characterized by the presence of strictly endemic species as well as Indo-Myanmarese and Malaysian-Indonesian forms[7]. Collection of *Saccharum* germplasm from the islands was considered important in view of its strategic location and the presence of characteristically diverse flora. In this connection, some of the major islands of the Andaman–Nicobar group were surveyed during March 2003 for the collection of *Saccharum* germplasm, under the National Agricultural Technology Project on Plant Biodiversity. Though some of the major islands were surveyed, *Erianthus arundinaceus* could be located only at two locations in the Middle Andaman and one in the Katchal Island of the Nicobar group. Representative collections from these locations were brought to Coimbatore for conservation, characterization and utilization. The present study was an attempt to characterize the three *E. arundinaceus* collections from the Andaman–Nicobar Islands in relation to the mainland Indian and Indonesian forms using RAPD markers.

Twelve each of mainland Indian and Indonesian forms of *E. arundinaceus* along with the three collections from the Andaman–Nicobar islands were used for the study (Table 1). The mainland Indian forms were selected to represent the different geographic regions of the country. DNA was extracted from the young leaves as per ref. 8, quantified by UV spectrophotometry and checked on 0.8% agarose gels. The DNA amplification conditions were as described by Williams *et al.*[9] with modifications. 12 ng of template DNA was taken in a 15 µl reaction. PCR reactions were carried out on a MJ Research PTC100 DNA Thermal Cycler, using 1 unit of *Taq* polymerase (Bangalore Genei, India). PCR consisted of 45 cycles, each of 94°C for 1 min, 37°C for 1 min and 72°C for 2 min and a final extension step of 72°C for 7 min. Twenty random primers (decamers) from Operon Technologies (Alameda, Calif, US) were used for the amplification. Ten of the primers, which were able to detect polymorphisms between the different genotypes, viz. OPH1, OPM14, OPR1, OPC16, OPV3, OPC4, OPU3, OPH14, OPU1 and OPC1 were selected for further analysis. Seven µl of the amplified products was separated on 1.5% agarose gels in 0.5X TBE buffer. 0.5 µg/ml ethidium bromide was added to both gel and buffer. Gels were visualized on a UV Trans illuminator and photographed. PCRs were repeated at least once for confirming the profiles and repeatable bands were scored for further analysis.

Table 1. Indian (Mainland, Andaman, Nicobar) and Indonesian forms of *E. arundinaceus* studied

Clone	Place of collection	Country
IND-99-871	Kerala	India
IND-99-884	Kerala	India
IND-99-907	Tamil Nadu	India
IND-99-957	Tamil Nadu	India
IND-02-1201	Andhra Pradesh	India
SES-149	Karnataka	India
IND-01-1105	Orissa	India
IND-01-1110	Orissa	India
SES-159	Orissa	India
SES-206	West Bengal	India
SES-288	Utter Pradesh	India
SES-347	Punjab	India
IND-03-1253	Betapur, Middle Andaman	India
IND-03-1255	Phoolthala, Middle Andaman	India
IND-03-1260	Katchal, Nicobar group	India
IK-76-101	Kalimantan	Indonesia
IK-76-22	Kalimantan	Indonesia
IK-76-80	Kalimantan	Indonesia
IS-76-178	Sulawesi	Indonesia
IS-76-169	Sulawesi	Indonesia
IS-76-142	Sulawesi	Indonesia
IJ-76-388	Irian Jaya	Indonesia
IJ-76-403	Irian Jaya	Indonesia
IJ-76-502	Irian Jaya	Indonesia
IJ-76-332	Irian Jaya	Indonesia
EA-Glongong	–	Indonesia
Timor wild	–	Indonesia

Clearly resolved bands were scored manually for their presence (1) or absence (0). Data on 190 clearly resolved bands generated by 10 primers were used for estimating the genetic similarity (s) among the clones based on Jaccard's similarity coefficient. The similarity matrix thus obtained was analysed using the UPGMA method to derive a dendrogram of the 27 clones. All these analyses were performed using NTSYS-PC software, Version 1.8 (ref. 10). The clustering was validated by bootstrap analysis using 1000 replicates using the software WINBOOT (IRRI). The genetic distance (d) between the clones was estimated as $d = 1 - s$, where s is the genetic similarity value. The average genetic distance within and between the groups was calculated from the pairwise distance values.

The 10 primers amplified 190 bands of which 141 were polymorphic. The high percentage of polymorphism may be due to the diverse nature of the material used in the study representing distinct geographical groups. When separately considered the mainland Indian forms were found to be more polymorphic (60.87%) compared to the Indonesian forms (33.6%). The number of fragments amplified by each primer varied from 14 (OPV3) to 23 (OPR11). The size of the fragments was in the range of 280 bp (OPR11) to 2617 bp (OPH11). The mainland Indian and Indonesian forms showed distinct molecular profiles with respect to all the primers. Four of the primers amplified six bands specific to the mainland Indian group while 7 primers amplified 8 bands specific to the Indonesian group. All the 10 primers were found to clearly differentiate the mainland Indian and Indonesian forms of *E. arundinaceus*. The RAPD profiles with respect to the primers OPH14 and OPM14 are presented in Figure 1. The number of bands amplified in the mainland Indian forms was higher (104–118) than that amplified in the Indonesian forms (98–112).

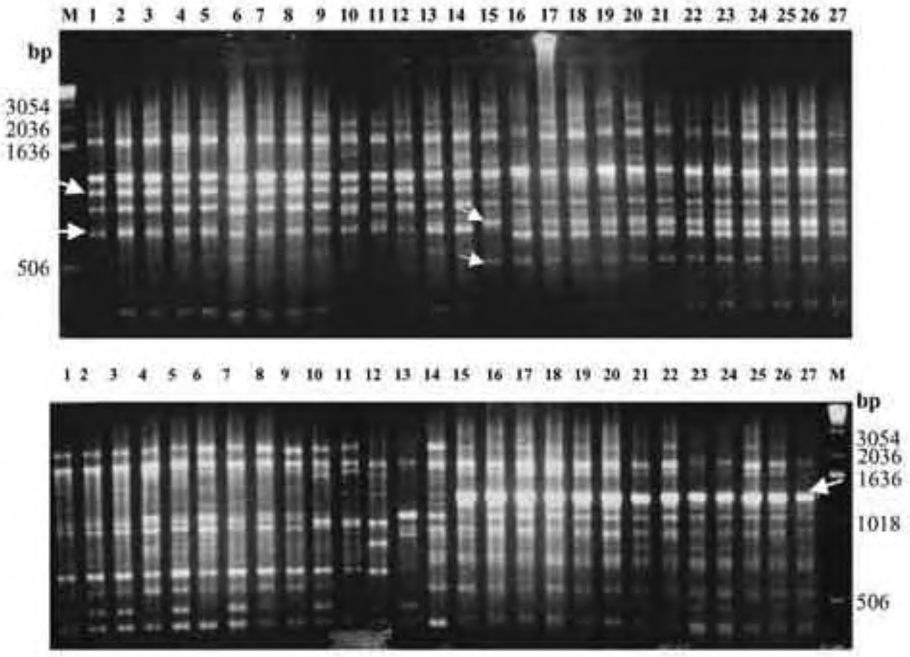

Figure 1. The amplification profile with respect to Indian (mainland, Andaman and Nicobar) and Indonesian *E. arundinaceus* clones for the primers OPH14 (top panel) and OPM14 (bottom panel). The bands specific to Indian and Indonesian forms are marked with arrow. Lanes 1–12, Indian clones (mainland); 13 and 14 Andaman clones; 15, Nicobar clone and 16–27, Indonesian clones. M-1 kb ladder.

Table 2. Genetic distance between Indian (Mainland, Andaman, Nicobar) and Indonesian clones of *E. arundinaceus*

	India			
Region	Mainland	Andaman	Katchal (Nicobar)	Indonesia
India – Mainland	**28.42**			
Andaman	37.15	**34.4**		
Nicobar	44.50	37.2	*	
Indonesia	49.20	44.99	24.2	**12.2**

*Only one clone available from Nicobar; Values in bold indicate within-group diversity.

The diversity within the two geographical groups is presented in Table 2. The diversity among the mainland Indian forms (di = 28.42) was higher compared to that of the Indonesian forms (di = 12.2). This is consistent with the higher geographic diversity present in the mainland Indian group compared to the Indonesian group. The mainland Indian clones drawn from diverse agro-climatic regions of the country, represented a much higher geographic diversity compared to the Indonesian forms. The Indonesian clones are from a relatively uniform environment, essentially tropical in nature. Berding and Roach[11] observed that *E. arundinaceus* from different provinces of Indonesia are uniform as far as morphological markers are concerned, which is consistent with the present results.

The genetic distance (Table 2) between the two *E. arundinaceus* groups was high (di = 49.2). The high molecular diversity between the two groups has been earlier reported by Besse *et al.*[12] based on RFLP markers on 42 Indonesian and three Indian clones. In fact the Indian forms of *E. arundinaceus* were found to be closer to the *Erianthus procerus* from India than to the Indonesian forms of *E. arundinaceus*. Based on AFLP studies on five Indonesian and two Indian *E. arundinaceus* clones, Besse and McIntyre[13] observed that *E. arundinaceus* has evolved differently in India and Indonesia. The cytological differentiation of the two groups of *E. arundinaceus* also needs to be emphasized in this context. The Indian forms of *E. arundinaceus* have a diploid chromosome number of $2n$ = 40, while the Indonesian group is represented by $2n$ = 60 types, which also contributes to their genetic differentiation.

The two clones from the Middle Andaman showed higher diversity (d = 34.4) than either the mainland Indian (d = 28.42) or the Indonesian (d = 12.2) groups. These two clones were collected from areas separated by nearly 100 km and from distinct environments. IND03-1253 was collected from the Betapur area near Rangat in the Middle Andaman from among dense vegetation close to a brackish water stream while IND03-1255 was collected from the course of a fresh water stream deep inside the forest near Phoolthala in the Middle Andaman. Even conceding that they have a common origin and reached Andaman through the Indo-Myanmar route, their geographical separation by over 100 km might have contributed to their independent development and differentiation. The clone IND03-1260 from the Katchal Island showed higher diversity with the

mainland Indian group (d = 44.50) and the two Andaman clones (d = 37.2). This clone however, recorded a significantly lower genetic distance with the Indonesian group (d = 24.2), indicating that it probably belongs to the Indonesian group. The two clones from Middle Andaman were closer to the mainland Indian group (d = 37.15) than to the Indonesian group (d = 44.99).

The three clones of *E. arundinaceus* from the Andaman–Nicobar islands showed profiles of either mainland Indian or the Indonesian groups, with respect to all the primers. The two Andaman clones (IND03-1253 and IND03-1255 from the Middle Andaman) showed profiles similar to the mainland Indian forms, while the profile of the clone IND03-1260 from Katchal (Nicobar group) was identical to that of the Indonesian group (Figure 1). In the dendrogram based on 190 bands, the Indonesian and the mainland Indian clones formed discrete groups (Figure 2). The Andaman and the Katchal clones were unambiguously positioned along with the mainland Indian and the Indonesian clones respectively. This evidently shows that both mainland Indian and Indonesian forms of *E. arundinaceus* are present in the Andaman–Nicobar islands, consistent with the general floristic distribution in the islands. It is interesting that the two ecotypes of *E. arundinaceus* are present in this narrow stretch of islands strewn between Myanmar and the Sumatra coast of Indonesia. These islands, one of the richest landmasses in terms of plant biodiversity manifest plant elements of both Indo–Myanmarese–Thailand and Malay–Indonesian origin, apart from distinct endemic forms. The flora of the Andaman group of islands is reported to have closer affinity with the Indo–Myanmarese-Thailand flora, while the flora of Nicobar group of Islands are closer to that of the Malaysian–Indonesian order[7]. *Erianthus* is supposed to have originated in the North East India and spread through Myanmar and China to other parts of southeast Asia[1,14]. The distribution of the species extends from India through Myanmar to southeast Asia. The geographical proximity of the North Andaman island to Myanmar accounts for the presence of many of the plant forms of Indo-Myanmarese origin found in the island. Similarly the presence of many of the Indonesian plant forms, found in the Nicobar district is attributable to its proximity to the Sumatra province of Indonesia. The presence of the mainland Indian and Indonesian forms of *E. arundinaceus* in the Andaman–Nicobar Islands is thus found to be consistent with its geographical position and the general floristic distribution in the islands.

This study clearly establishes the presence of both mainland Indian and Indonesian forms of *E. arundinaceus* in the Andaman Nicobar group of Islands. This is a unique situation, characteristic of the Andaman–Nicobar Islands in view of its strategic location between the two biodiversity majors.

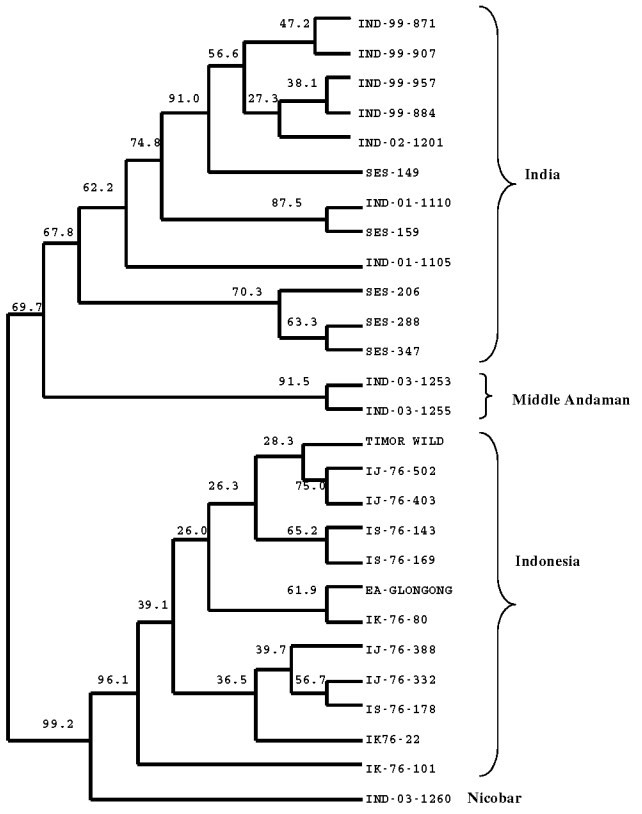

Figure 2. The dendrogram showing the clustering of *Erianthus arundinaceus* clones based on 190 markers. The tree is based on bootstrap analysis with 1000 replicates.

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A 1584-year ring width chronology of juniper from Lahul, Himachal Pradesh: Prospects of developing millennia-long climate records

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We report here a 1584-year (AD 420–2003) long ring width chronology of Himalayan pencil cedar from cold arid region in Lahul, Himachal Pradesh. Ring width variations in trees at this site are found to be associated with variations in precipitation from previous growth years' September to concurrent May. Potential of this chronology in developing millennia-long hydrological records is recognized. Strong relationship between the present Himalayan pencil cedar chronology and two Himalayan cedar ring width chronologies prepared earlier from stands adjacent to the present site, demonstrates the utility of tree ring data network of these species in developing robust reconstructions needed for better insight into climate variability in longer perspective in the precipitation-deficient trans Himalayan region.

Keywords: Hydrological records, *Juniperus macropoda*, Lahul, precipitation variability, ring width chronology.

THE Himalayan mountain system, the highest one on earth, has strong influence on regional and extra-regional circulation system. High-elevation mountain heating, snow/glacier albedo and glacier melt freshwater flux into the ocean provide a strong land–ocean–atmosphere interactive system. In view of this, long-term high-resolution climate records for this data-scarce region are important to develop better insight into the climate change behaviour on regional and global scale.

The trans Himalayan region holds a potential treasure of high-resolution proxy records of climate like tree rings, lake deposits, ice cores, etc. Among these, tree rings are unique in terms of the existence of precise dating control to the level of calendar years. Various workers have earlier developed tree ring chronologies from India to reconstruct past climatic variability patterns prior to instrumental records for distinguishing natural and anthropogenic climate change[1–7]. However, the tree ring chronologies reported so far span few centuries, except for the lone record of millennium-long chronology of Himalayan cedar from western Himalaya[8].

Here we report the tree ring chronology of Himalayan pencil cedar (*Juniperus macropoda* Boiss.), which is the longest so far for the Indian region. Potential of this chronology prepared from moisture-stressed site in developing millennia-long hydrolological records is recognized. Such long-term records are essential to understand natural climate variability and for the validation of climate prediction models.

Himalayan pencil cedar is found[9] in the inner arid regions of northwest Himalaya to as far as Kumaon at altitudes from 1500 to 4300 m. It is a cold and drought-hardy tree growing in areas where rainfall is as low as around 30 cm. For the present study, tree ring samples of Himalayan pencil cedar were collected from dry moisture-stressed site near Keylong, Lahul and Spiti district, Himachal Pradesh (Figure 1). This area lying on the northern side of Pir Panjal range in Lahul and Spiti district, falls in the monsoon arid transition zone and is considered to be a potential indicator of the northern limits of the intensity of monsoon. The area is influenced by monsoon in summer and westerlies in winter. As the area falls in the rain-shadow zone of Himalaya, precipitation occurring in winter and spring is higher than in summer and autumn. Westerly depressions during winter and spring bring cloudy weather and light rains and often cause heavy snowfall at high elevations[10]. The above ecological settings in the region show that the tree-ring records from this region should provide valuable information on long-term precipitation record.

We collected tree-ring samples from old as well as relatively younger trees of Himalayan pencil cedar during May–June 2004. Trees were growing on moisture-stressed south and southeast slopes at altitudes ranging from 2600 to 3300 m asl near Keylong (Figure 1). Old trees were characterized by crown dieback and prominent strip barks (Figure 2). For sampling healthy, undisturbed, distantly growing trees were selected. Increment borers were used

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