KFRI RESEARCH REPORT NO. 529
(Final Report of the KFRI RP 681.12 / 2014)

DNA BARCODING AS A VALUABLE MOLECULAR TOOL FOR CERTIFICATION OF BAMBOO PLANTING MATERIALS

Suma Arun Dev
Forest Genetics and Biotechnology Division

V.B. Sreekumar
Forest Ecology and Biodiversity Conservation Division

E.M. Muralidharan
Forest Genetics and Biotechnology Division

KERALA FOREST RESEARCH INSTITUTE
(An Institution of Kerala State Council for Science, Technology and Environment)
Peechi 680653, Kerala, India.

JULY 2017

ABSTRACT OF THE PROJECT PROPOSAL

1.	Project No.	KFRI RP 681.12/2014
2.	Title	**DNA barcoding as a valuable molecular tool for certification of bamboo planting materials**
3.	Principal investigator	Dr. Suma Arun Dev, Senior Scientist, KFRI
4.	Associate investigators	Dr. VB. Sreekumar, Scientist B, KFRI Dr. EM. Muralidharan, Principal Scientist, KFRI
5.	Research Fellow	Ms. Sijimol K.
6.	Objectives	Development of DNA barcodes in the priority bamboo species for the certification of planting materials (seedlings/culm cuttings/rooted culms)
7.	Duration	2 Years
8.	Funding Agency	National Bamboo Mission (NBM), Government of India

ACKNOWLEDGEMENTS

The authors are thankful to Dr. P.G. Latha, Dr. P.S. Easa, former Directors as well as Dr. Bransdon S. Corrie, Director, Kerala Forest Research Institute (KFRI) for providing the facilities to perform the project work and for their keen interests. The authors' record their gratitude for the financial support provided by National Bamboo Mission (NBM), Government of India, to carry out the research work at Kerala Forest Research Institute (KFRI). We are indebted to Kerala, Karnataka and Northeast Forest Departments for giving us permission to collect the required samples from the natural distribution zones of different commercial bamboo species. The meticulous laboratory and field works carried out by Ms. Sijimol K. and Ms. Prathibha P.S. during the tenure of the project are highly appreciated. We would also like to extend our thanks to the members of editorial committee for their helpful suggestions and comments on the project report.

CONTENTS

ABSTRACT

In bamboos, after the initial field level selection process for the superior mother clumps, the bulk planting materials generated viz. culm cuttings or rhizome transplants lack any distinguishing key morphological characteristics for traceability. These planting material generated at the nursery level are directly procured for the establishment of commercial plantations without any further verification. Very often misidentification and mixing up occur at the nursery level and the error is not discovered until several years have passed. The objective of the preset study is to develop suitable DNA barcodes in the commercially important bamboo species of India belonging to four genera. The study evaluated the potentiality of seven CBOL recommended standard DNA barcode regions to arrive at a species specific barcode. The *psbA-trnH* DNA barcode region generated unique species specific barcodes for the commercial bamboo species in the genera *Bambusa, Dendrocalamus, Melocanna* as well as *Ochlandra*. To achieve precise identity in bamboo species, any national certification agency set up for the purpose can utilize *psbA-trnH* DNA barcode region to tag the species identity and to establish the authenticity of the multiplied planting materials.

1. INTRODUCTION

Bamboos, woody perennials of the grass family Poaceae, provide livelihood for millions of people around the globe. India is well endowed with 136 species of bamboo resources in 23 genera, extending over 13.96 million ha and is the second largest bamboo reserve in the world (FSI, 2011). Bamboo is highly adaptable to a wide range of climatic and rainfall conditions and is the most suitable species for commercial forestry (Thokchom and Yadhava, 2015). Along with the traditional uses of bamboo poles for rural building construction, fencing, agricultural purposes and house hold articles, recently the 'green gold' of India has got immense demand as an industrial raw material with 1500 documented uses. The multiple harvests possible throughout the year together with the tremendous growth rate contributed towards the wide scale acceptability and commercial potential of bamboo species' among the farming community. In India, the last few decades have witnessed a considerable interest in bamboo cultivation owing to the huge demand of source material from the bamboo based industries (Mishra 2015).

Even though, India possesses 45 per cent of the global bamboo growth, the country contributes only 4 per cent towards the global trade with an annual productivity of only 1 MT/ ha (Tripathi *et al.,* 2006). India's share in the global bamboo market is estimated to be of US $1 billion and the global trade of bamboo products is estimated to be 8 billion USD with an expected growth of 17bn USD by 2017 (Omari, 2009). Improved productivity of bamboo plantations is thus imperative to meet the growing demand of 26.9 million tones as against the annual productivity of 13.47 million tons (Salam, 2013). Over exploitation of the available resources, poor performance of the unsuitable species, forest fires, grazing, gregarious flowering, among others contributed to the productivity decline albeit the existence of significant species diversity/ growing habitats of bamboos in India (NBM, 2013). Government of India through National Bamboo Mission (NBM) is trying to achieve a global share of 27

per cent through improved productivity of bamboo plantations and 13 commercially prioritized bamboo species are recommended for this purpose (Kumar *et al.,* 2005). In this regard, NBM has recommended priority bamboos species based on their annual productivity and suitability in different agro-climatic zones throughout the country (http://www.nbm.nic.in). Guidelines issued by the NBM aims at ensuring the use of superior planting stock for raising the bamboo plantations through a network of certified high-tech bamboo nurseries in the country. There are more than thousand NBM accredited nurseries in the country which ensure the quality of planting materials and suitability of clones/ species at a given site for the establishment of plantations.

The crucial step in any productivity improvement program is the proper identification, multiplication and supply of suitable species with guaranteed productivity. Unlike other plants, flowering in bamboos is an elusive physiological phenomenon which is unpredictable, long-periodic, gregarious, uncontrollable and usually plants die after flowering. Field identification of bamboo species is thus traditionally relied on phenetics /vegetative characteristics and is mainly by describing culm / culm-sheath characteristics (Clark *et al*., 2007; Filgueiras and Londono, 2006; Ohrnberger and Georrings, 1986; Triplett *et al*., 2006). The long and unpredictable flowering cycle also hinders the use of seeds as natural propagules in some species, and often, to meet the demand for propagules, other modes of propagation have to be resorted for plantation establishment. Species identification of the multiplied planting materials of bamboos at various age classes such as seedlings, rooted culm or branch cuttings, rhizome transplants and micropropagated plantlets is often not easy in the absence of any consistent vegetative characteristics and unintentional mixing of species/ clones is quite common at the nursery level. Therefore, to ensure the intended productivity and to avoid identification errors which can only be realized in later years, NBM envisages for a precise species certification program to certify the planting materials before the commencement of plantations. The standard criteria or tools for the certification of any forest reproductive material should be retrievable at any stage of the establishment of plantations thereby ensuring the genetic identity and productivity of

the established plantations. Nonetheless, morphology based identification keys are very useful for quick identification at the field; a supplementary molecular tool can bring in more precision to protect the genuine interests of bamboo growers and industries.

The tremendous advancement of molecular marker technologies holds promise to address this issue and yet only limited progress has been achieved with regard to the traceability systems in forest reproductive material employing molecular markers (Botta *et al*., 2001; Botta *et al*., 2004; Degen *et al*., 2010; Fostel, 2008; Konnert and Behm, 2006; Konnert and Hussendorfer, 2002; Ziegenhagen *et al*., 2003). The last decade witnessed the potentialities of DNA barcoding as a promising tool to supplement the species identification in plants as well as in animals. DNA barcoding is the process of identification of species based on short standard conserved region of the genome. DNA barcoding can thus transform conventional bamboo taxonomy by digitizing the identity of a given species and the short sequence DNA barcode can serve as an abbreviated label for the genome of the species (Hebert *et al*., 2004; CBOL, 2009).

Plastid gene sequences such as *rbcL, matK, rpS4, rpL16*, among others have contributed immensely to the current understanding of bamboo systematics and phylogeny (Kelchner and Clark, 1997). However, the feasibility of recommended conserved plastid barcode regions for the species discrimination in bamboos was tested only in a few instances. In temperate woody bamboos, four barcoding loci namely *matK*, *rbcL*, *psbA-trnH*, *ITS2* were analyzed and the combination of *rbcL+ITS2* was suggested as the potential barcode for species discrimination (Cai *et al*., 2012). Failure of *matK* to discriminate *Bambusa* species due to interspecific hybridization and polyploidy was recently reported (Das *et al*., 2013). Low discriminatory power of the core barcode (*rbcL+matK*) as well as greater discriminatory power of *trnG-trnT* spacer in bamboos was also suggested (Zhang *et al*., 2013). Sosa *et al*. (2013) recommended *matK+psbI-psbK* as the discriminant barcode loci in temperate bamboos.

Thus the present study envisages developing DNA barcodes for the commercially important bamboo species including both native and exotic species which are recommended by the NBM, Government of India and are in cultivation for a long

time at different agroclimatic zones of India. The developed barcode can be eventually utilized as a certification tool for the multiplied planting materials in the NBM accredited nurseries throughout India. The certification process can ensure the identity of the intended species suitable for the particular planting site and thus the productivity in the long run can be guaranteed. The bamboo growers may be sensitized to ensure that the plantations in future should only be established with a certified planting stock of guaranteed productivity.

2. MATERIALS AND METHODS

Material and sampling

Leaf samples were collected from reserved forests and protected areas throughout the distribution zones in India with prior permission from concerned State Forest Departments and the details are given in Table 1. For the preparation of voucher specimens, one or two leaves with culm sheath were collected without damaging the plants.

Thirteen commercially important bamboo species representing the genera such as *Bambusa balcooa* Roxb., *B. bambos* Voss, *B. nutans* Wall ex Munro, *B. pallida* Munro, *B. tulda* Roxb, *B. vulgaris* var. *vulgaris* Schrad ex Wendle, *Dendrocalamus asper* Baker ex Heyne, *D. giganteus* Munro, *D. hamiltonii* Ness and Arn ex Munro, *D. strictus* Nees, *Melocanna baccifera* Kurz., *Ochlandra travancorica* Benth and *Oxytenanthera parviflora* Brandis ex Gamble (Table 1) were selected for the development of DNA barcodes. Leaf samples from multiple accessions were collected and dried in silica gel. In addition to the NBM listed priority species several other Northeast bamboo species such as *Bambusa* (*B. jaintiana, B. manoharanii, B. teres, B. multiplex*), *Dendrocalamus* (*D. brandisii, D. hookeri, D. longispathus*) and *Melocanna baccifera,* which are generally in cultivation were also considered for the development of DNA barcodes. The samples were authenticated at Kerala Forest Research Institute (KFRI) andvoucher specimens were deposited in the Kerala Forest Research Institute (KFRI) herbarium.

Table 1. Priority bamboo species listed by National Bamboo Mission (NBM), Government of India

Name of Species	Distribution	Propagules used
Bambusa balcooa Roxb.	Northeastern India. Tripura, Nagaland, Meghalaya, Assam, West Bengal, Uttar Pradesh	Branch/ culm cuttings Tissue culture plantlets
B. bambos Voss	Wide distribution in India	Seedlings
B. nutans Wall ex Munro	Himachal and North eastern states, West Bengal, Orissa, Sikkim, UP	Culm/ branch cuttings, Offset plantings
B. pallida Munro	Northeast India, Bhutan, Myanmar	Seedlings Culm cuttings, rhizome/ Offset plantings
B. tulda Roxb	Assam, Bihar, Meghalaya, Meghalaya, Mizoram, Tripura	Seedlings Culm/ branch/ rhizome cuttings Tissue culture plantlets
B. vulgaris var. *vulgaris* Schrad ex Wendle	Northeast and central India	Culm/ branch cuttings
Dendrocalamus asper Baker ex Heyne	Exotic and cultivated in Northeast India	Culm/ branch cuttings Tissue culture plantlets
D. giganteus Munro	Exotic and cultivated in Northeastern India and West Bengal	Seedlings Culm/ branch cuttings, rhizome/ Offset plantings
D. hamiltonii Ness and Arn ex Munro	Central, Northeast India, Sikkim, West Bengal, Assam	Seedlings Culm/ branch cuttings, rhizome/ offset plantings
D. strictus Ness	Throughout India	Seedlings Culm/ branch cuttings, rhizome cuttings
Melocanna baccifera Kurz	Northeast India	Seedlings Rhizome cuttings
Ochlandra travancorica Benth	Kerala, Tamil Nadu	Seedlings Rhizome cuttings
Oxytenanthera parviflora Brandis ex Gamble	Assam, Mizoram	Rhizome cuttings

DNA extraction, PCR amplification and Sequencing

Total genomic DNA was extracted from either fresh or silica dried leaves using modified Cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990) as well as using DNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. Seven candidate barcode loci of the plastid genome (four coding regions such as *matK, rbcL, rpoB, rpoC* and three intergenic spacers namely *psbA-trnH, psbK-psbI* and *atpF-atpH*) were evaluated to identify the discriminant DNA barcodes for priority bamboo species. Primer details and reaction conditions standardized for the polymerase chain reaction (PCR) amplification are listed in Table 2.

Table 2. Primer sequences of four candidate DNA barcodes and their PCR conditions

Barcode region	**Primer**	**Primer sequence 5′-3′**	**Reaction condition**
rbcL	1F 724R	ATGTCACCACAAACAGAAAC TCGCATGTACCTGCAGTAGC	94°C 5 min. 94°C 1 min. 60°C 1min. 72°C 1min. 34 cycles 72 °C 10 min.
matK	472F 1248R	CCCRTYCATCTGGAAATCTTGGTT GCTRTRATAATGAGAAAGATTTC TGC	94°C 10 min. 94°C 1 min.57°C 1min. 72°C 1.5 min. 34 cycles 72 °C 12 min.
psbA-trnH	*psbA* *trnH*	GTWATGCAYGAACGTAATGCTC CGCGCATGGTGGATTCACAATCC	94°C 5 min. 94°C 1 min. 60.5°C 1min. 72°C 45 min. 34 cycles 72 °C 10 min.
rpoC	*rpoC* Forward *rpoC* Reverse	GGCAAAGAGGGAAGATTTCG CCATAAGCATATCTTGAGTTGG	94°C 5 min. 94°C 1 min. 57°C 1min. 72°C 1min. 34 cycles 72 °C 10 min.
rpoB	*rpoB* Forward *rpoB* Reverse	AAGTGCATTGTTGGAACTGG GATCCCAGCATCACAATTCC	94°C 5 min. 94°C 1 min. 59°C 1min. 72°C 1min. 34 cycles 72 °C 10 min.
atpF-atpH	*atpF* *atpH*	ACTCGCACACACTCCCTTTCC GCTTTTATGGAAGCTTTAACAAT	94°C 5 min. 94°C 1 min. 57°C 1min. 72°C 1min. 34 cycles 72 °C 10 min
psbK-psbI	*psbK* *psbI*	TTAGCCTTTGTTTGGCAAG AGAGTTTGAGAGTAAGCAT	94°C 5 min. 94°C 1 min. 61°C 1min. 72°C 1min. 34 cycles 72 °C 10 min

Amplification of genomic DNA was performed in a PTC-100 thermocycler (BIO-RAD, India) in a final volume of 20 µL reaction mixture containing 50-100 ng DNA, 10X Taq buffer with 1.5 mM $MgCl_2$, 200 µM dNTPs, 10 pm of each primer, and 2U Taq DNA polymerase (Invitrogen, Bangalore). The amplified products were resolved in 2 per cent agarose gel and documented using a gel documentation system (Syngene, UK). PCR products were further purified using a Nucleospin Gel and PCR Clean-up kit (Macherey Nagel, USA) as per the manufacturer's protocol and quantified using Nanodrop (Thermo Scientific, USA). Sequencing was performed using Sanger dideoxy chemistry in both the forward and reverse directions (Chromous, Bangalore).

Sequence analysis

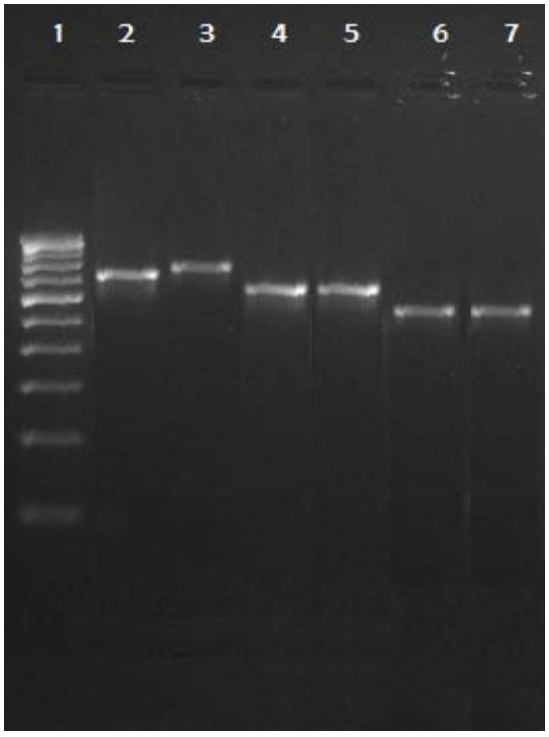
The chromatograms were edited and trimmed using *BioEdit* software (Hall, 1999). The edited sequences were aligned using *Clustal W* (Thompson *et al*., 1994) and submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank/) as well as BOLD http://www.barcodinglife.org**.** Homology searches were performed using *BLAST* (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the identity of the sequences.

For the pair-wise genetic distance (PWG) method, the genetic pair-wise distance (interspecific as well as intraspecific distances) was determined by *MEGA* v. 6.0 using the Kimura two-parameter distance model (K2P) adopting complete deletion option (Tamura *et al*., 2013). The interspecific divergence between the species was calculated using three parameters; (i) average interspecific distance, (ii) average theta prime (θ') and (iii) minimum interspecific distances. Intraspecific parameters; (iv) average intraspecific distance, (v) theta (θ) and (vi) coalescent depth were also calculated to characterize the intraspecific divergences (Meyer and Paulay, 2005). Barcoding gap was calculated by plotting intraspecific distances against interspecific divergences for each species (Meyer and Paulay, 2005; Meier *et al*., 2006). The significance of barcoding gap was assessed using the Wilcoxon matched pairs signed rank test in *SPSS* v 16.0 (SPSS Inc, 2007).

3. RESULTS AND DISCUSSION

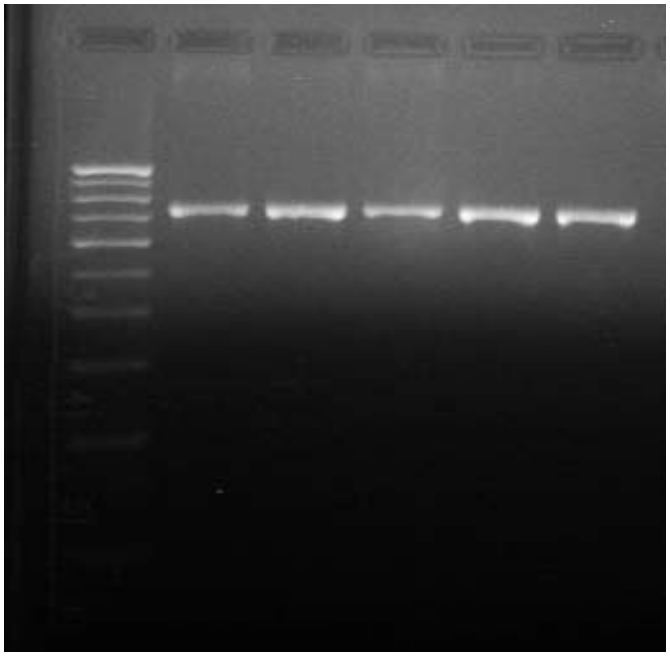
DNA barcode amplification, sequencing and alignment

An ideal DNA barcode is supposed to have adequate conserved regions, high PCR amplification efficiency, and enough variability for species identification (CBOL Plant Working Group 2009; Hou *et al.,* 2013; Pang *et al.,* 2010). All the studied DNA barcode regions (*matK*, *rbcL*, *rpoC, rpoB, psbK-psbI, atpF-atpH* and *psbA-trnH*) were successfully amplified with 100 per cent PCR efficiency using the primers recommended by CBOL (2009) (Figs 1-3).



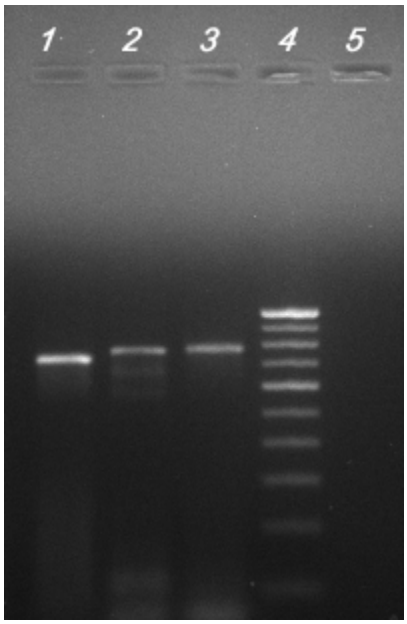
Lane1: 100bp DNA ladder
Lane 2& 3: *matK* (750bp)
Lane 4 & 5: *rbcL* (600 bp)
Lane 6 & 7: *psbA-trnH* (550bp)

Figure 1. PCR amplification of *matK, rbcL* and *psbA-trnH* in *Bambusa balcooa* and *B. tulda*



Lane1: 100bp DNA ladder
Lane 2 - 5: *atpF-atpH* (750bp)

Figure 2. PCR amplification of *atpF-atpH* spacer region in *Dendrocalamus asper, D. strictus, D. giganteus, D. hamiltonii* and *Ochlandra travancorica*



Lane1: *rpoB* (680 bp)
Lane 2: *rpoC* (700bp)
Lane 3: *rbcL* (750bp)
Lane 4: 100bp DNA ladder

Figure 3. PCR amplification of *rpoB, rpoC* and *rbcL* barcode regions in *Melocanna baccifera*

Six of the DNA barcode regions such as *rbcL, matK, rpoB, rpoC, psbK-psbI and atpF-atpH* displayed exactly identical sequences among all the analyzed species of *Bambusa* and *Dendrocalamus* (Figs. 4 - 7). Hence these six DNA barcode regions could not be recommended either for solving the taxonomic confusions or for the certification of planting materials in bamboos. The multiple sequence alignment (MSA) of *psbA-trnH* intergenic spacer barcode region showed species specific nucleotide differences in the studied bamboo genera such as *Bambusa, Dendrocalamus, Melocanna* and *Ochlandra.* The intergenic spacer *psbK-psbI* also showed species discrimination in the genus *Melocanna.*

The CBOL-Plant Working Group recognized *rbcL* as a potential barcode in plants. However, due to its low ability for species discrimination, most of the working groups suggested the use of *rbcL* in conjunction with other gene regions (Chase *et al.,* 2007; Hollingsworth *et al.,* 2009). Similarly, *matK* had proved its utility as a potential barcode in closely related groups, such as *Compsoneura* (Newmaster *et al.,* 2007), orchids (Lahaye *et al.,* 2008), sedges (Starr *et al.,* 2009) and *Acacia* (Newmaster and Ragupathy, 2009), but universality of this barcode remains uncertain in various taxa. In this study, *rbcL* and *matK* could not be used to differentiate bamboo species. Even though *rpoB* and *rpoC* has been reported as suitable barcodes (Lahaye *et al.,* 2008) due to the low interspecific divergence, this barcode region was reported as inappropriate supplementary barcode loci (Yan *et al.,* 2011). The present barcode analysis also revealed the low discriminatory power of *rpoB* and r*poC*. In addition to the candidate barcode regions described above, other plastid barcoding regions such as *atpF-atpH*, *psbK-psbI* and *trnL* were also recommended for species identifications (Taberlet *et al.,* 2007*).*

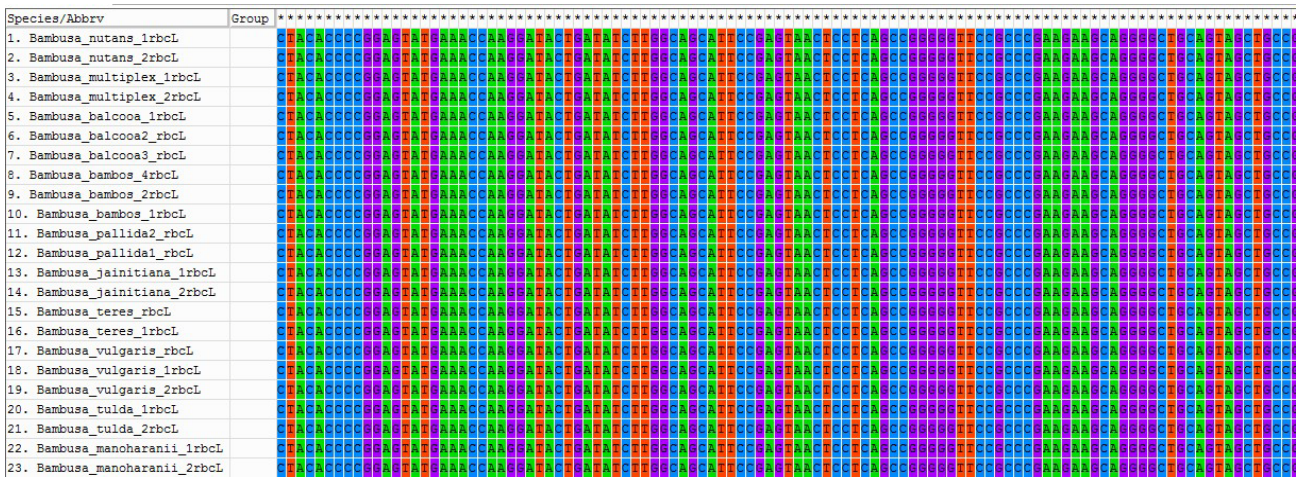


Figure 4. Multiple sequence alignment of *rbcL* barcode region in the genus *Bambusa*

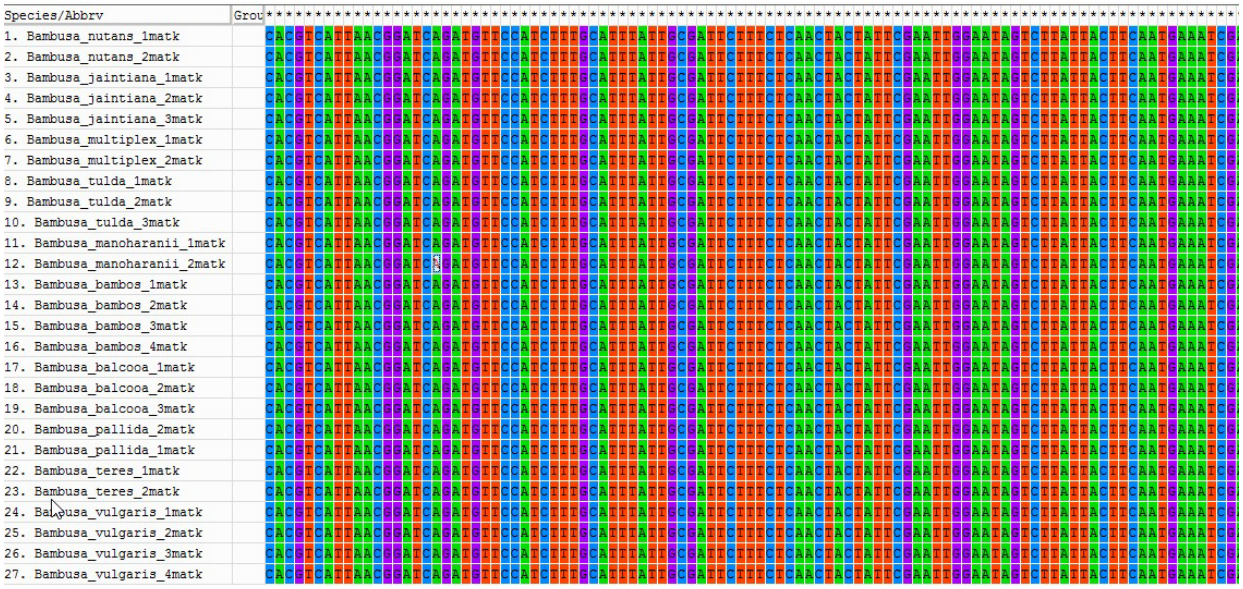


Figure 5. Multiple sequence alignment of *matK* barcode region in the genus *Bambusa*

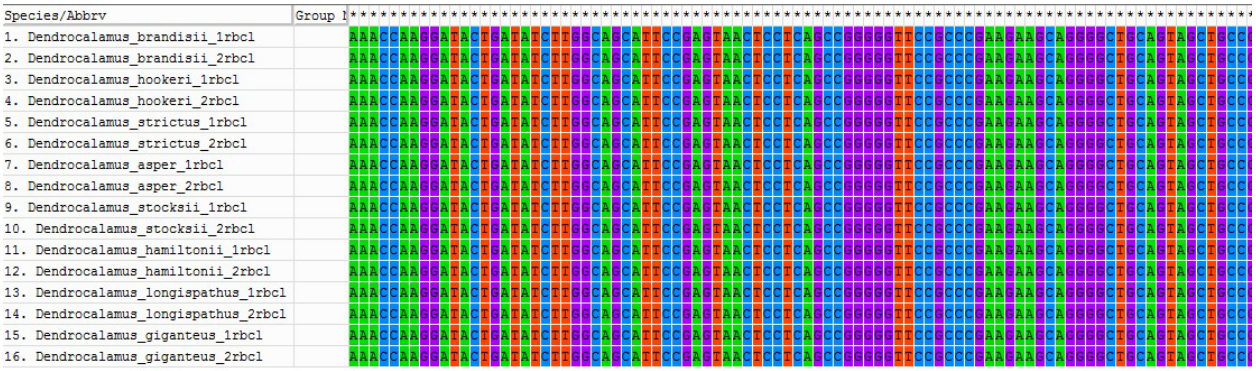


Figure 6. Multiple sequence alignment of *rbcL* barcode region in the genus *Dendrocalamus*

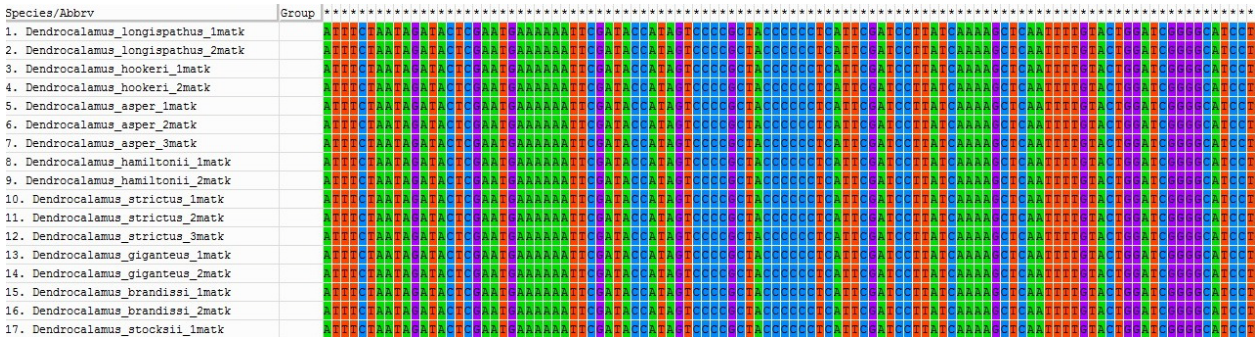


Figure 7. Multiple sequence alignment of *matK* barcode region in the genus *Dendrocalamus*

psbA-trnH has the potentiality as suitable marker for species discrimination between closely related taxa due to the high rate of sequence variation present generally in this intergenic spacer region (Bruni *et al*., 2012 Kress and Erickson, 2007; Newmaster *et al*., 2007). It has also been recommended as one of the best performing locus for various taxa in terms of PCR amplification success, sequencing and species resolution (Newmaster *et al*., 2007; Lahaye *et al*., 2008). Species specific differences were observed in the *psbA-trnH* intergenic spacer barcode region including the mononucleotide repeats of the genera *Bambusa* and *Dendrocalamus* whereas the genera *Ochlandra* and *Melocanna* also showed additional unique nucleotide differences. Thus *psbA-trnH* can serve as a potential DNA barcode for species identification of the various studied bamboo taxa (Fig. 8).

Sequence length and basic sequence statistics like conserved sites, variable sites, singletons and informative sites of *psbA-trnH* spacer region based on the *CLUSTAL X* alignment as well as with alignment explorer in *MEGA v.6.0* are provided in the Table 3.

Figure 8. *psbA-trnH* DNA barcode for the studied bamboo taxa

Table 3. Basic sequence information of *psbA-trnH* spacer region in the genera *Bambusa*, *Dendrocalamus* and *Melocanna*

Comparison	*Bambusa*	*Dendrocalamus*	*Melocanna*
Sequence length (bp)	636	708	665
Conserved sites	613	696	657
Variable sites	23	12	8
Informative sites	21	11	8
Singleton site	2	1	0

Sequence analysis of *Bambusa*

Multiple sequence alignment (MSA) of the *psbA-trnH* intergenic spacer region showed nucleotide changes unique to the species in most cases (Fig. 9). The major nucleotide changes are transitions/ transversions as well as insertions/deletions of nucleotides in the analyzed barcode region. In most of the species, deletions of mononucleotide thymine repeats in various numbers were obvious (Table 4).

B. balcooa and *B. vulgaris* shared same type of deletion in thymine mononucleotides. Both these species are widely cultivated and morphological features are greatly influenced by selection process. Morphologically, these two species share several morphological characters like inflorescence large branched panicle with clusters of spikelets, rachis rounded, spikelets somewhat compressed, rachilla very short, glabrous, empty glumes 1-2, ovate-acute, faintly multinerved, margins ciliate towards apex. Stamens are 6 in number, acute and apiculate and ovary is narrowly oblong, hairy at apex, style dividing into three with plumose stigmas. The main differences between *B. balcooa* and *B. vulgaris* are the absence of transverse veinlets in lemma with ovate oblong lodicules in the former and presence of transverse veinlets in lemma with narrowly oblong lodicules in the latter.

Likewise, *B. balcooa, B. tulda* and *B. vulgaris* had a similar tranversion event (G > A). *B. balcooa* is morphologically allied to *B. bambos* in its lower leaflets, stout branching

appearance and thick clump formation. In *B. balcooa, B. tulda* and *B. vulgaris*, the inflorescence is clustered at nodes and glumes are persistent and shorter than spikelet.

B. nutans/ *B. teres* shared the type of deletion of thymine mononucleotides along with other specific nucleotide changes. Similarly, *B. nutans, B. tulda* and *B. vulgaris* had a similar transition event (C > T). Majumdar (1989) treated *B. teres* under synonym of *B. tulda* along with *B. nutans. B. teres* is distinct from these in having glabrous culm sheath proper, similar auricles erect at top of sheath proper, long ciliate ligule having white hair underneath blade, and dense hair at incurved leaf apex. *B. tulda* is a widely distributed species and morphologically highly variable in vegetative and reproductive characters. Munro (1868) while describing *B. nutans*, pointed that it might possibly be a mountain form of *B. tulda*. Gamble (1896) also pointed that both species are extremely difficult to separate as the leaves and culm sheath are almost similar. However the culm of *B. nutans* comes singly from a rootstock, whereas in *B. tulda* it is from the central tuft.

Morphologically distinct species like *B. multiplex, B. manoharanii, B. jaintiana* as well as *B. pallida* displayed unique barcodes with very little shared nucleotide changes. *B. pallida* is a quite distinct species with triangular culm sheath with sheath proper truncately cut at top, long imperfect blade as broad as top of the sheath proper and lanceolate spikelets with 3–8 fertile florets. *B. jaintiana* is a shrubby erect bamboo found in loose clumps. The culms are green becoming orange with age and young white powdery. *B. manoharanii* differs remarkably in other vegetative and floral aspects like culm-sheath short than internodes, auricles with short rounded shape, somewhat lobed with two small lobes on each sides and stamens are 6 in number with penicillate at apex with somewhat matching culm-sheath to B. *balcooa* (Kumari and Singh, 2009). *B. multiplex* is a morphologically variable widely cultivated perennial species with erect woody culms, nodal roots, synflorescence bractiferous inflorescence and caryopsis fruit. Among the ten species of the genus, *B. multiplex* had maximum level of unique nucleotide changes

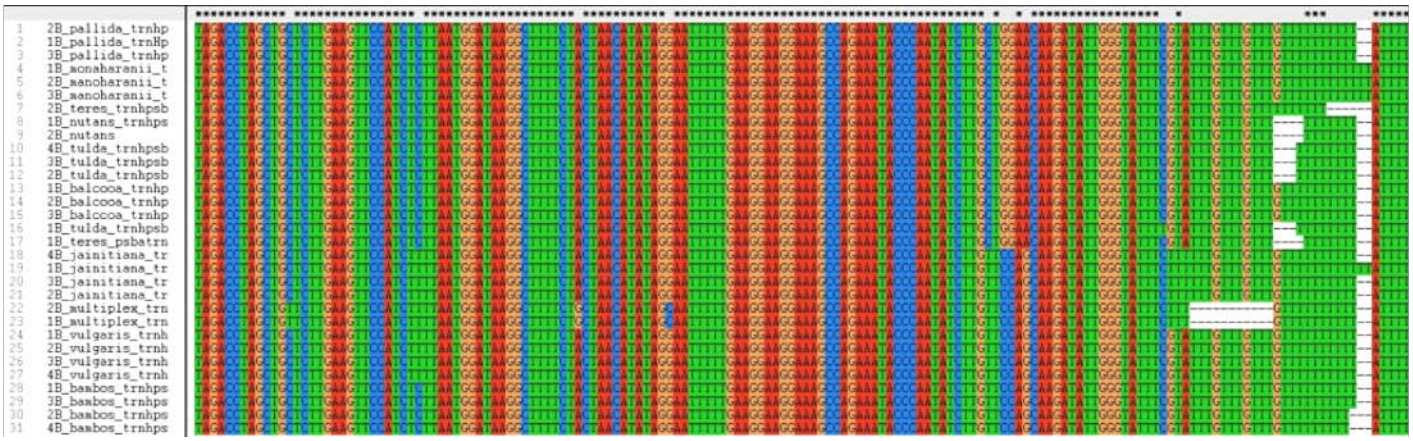


Figure 9. Multiple sequence alignment of *psbA-trnH* spacer region in the genus *Bambusa*

Table 4. Nucleotide differences in *psbA-trnH* spacer region in the genus *Bambusa*

Sl No	*Bambusa*	Nucleotide changes in *psbA-trnH* sequence
1	*B. nutans*	Deletion of TTTTTT mononucleotide repeats Transition – C > T Transversion – T > G at 2 places
2	*B. tulda*	Deletion of TTTTT mononucleotide repeats Transition – G >A at 2 places Transversion - T > G at 2 places
3	*B. balcooa*	Deletion of TT mononucleotide repeats Transition – G > A
4	*B. vulgaris*	Deletion of TT mononucleotide repeats Transition - G > A Transition - C > T
5	*B. bambos*	Deletion of TTT mononucleotide repeats
6	*B. pallida*	Deletion TT mononucleotide repeats Insertion of mononucleotide T at 2 places
7	*B. multiplex*	Deletion of TTTGTTTGTTT sequences Transition - C > T Transversion - G > C Transversion – C > A
8	*B. jaintiana*	Insertion of TTT in place of GTA Transition C > T Transversion G > C
9	*B. teres*	Deletion of TTTTTT mononucleotide repeats
10	*B. manoharanii*	Insertion of TT mononucleotide repeats Transversion T > G at 2 places

In the distance based analysis using *psbA-trnH* spacer region, three parameters (*viz.* average interspecific distance, theta prime and minimum inter-specific distance) were employed to characterize the interspecific divergence. The intra-specific variations were calculated by employing average intraspecific distance, mean theta, and coalescent depth (Table 5). Even though species specific nucleotide differences could be identified for each *Bambusa* species, most of the differences were located in the non-coding regions or in the mononucleotide repeats.

Table 5. Genetic divergence parameters in the genus *Bambusa* using MEGA

Parameters	***psbA-trnH***
Average intraspecific distance	0.0024±0.0010
Average theta	0.0008±0.0003
Average coalescent depth	0.0004±0.0001
Average interspecific divergence	0.0509±0.0012
Minimum interspecific distance	0.0000±0.0000
Average theta prime	0.0086±0.0033
Barcoding gap	0.0485±0.0002

Sequence analysis of *Dendrocalamus*

Out of the seven analyzed barcode regions, only *psbA-trnH* spacer region showed species specific nucleotide differences in the genus *Dendrocalamus* as in the genus *Bambusa* (Fig. 10). Unlike in the genus *Bambusa*, the species of the genus *Dendrocalamus* had lesser number of nucleotide changes and also shared some of the nucleotide changes. Both transitional and transversional nucleotide changes were present only in *D. hookeri* in addition to two major deletions. Transversion of G > C was observed in *D. hookeri* and *D. brandisii*. Both *D. brandisii* and *D. giganteus* had the same type of GTTTTT nucleotide deletions and they also have morphologically prominent auricles in culm sheath and their sheath is glabrous, ligule as compared to *D. strictus* and *D. hamiltonii.*

There are some unique nucleotide changes in *D. stocksii* such as an inversion of GTA to ATG and the insertion of AA nucleotides which are absent in all the other *Dendrocalamus* species. *Dendrocalamus stocksii* was initially known as *Oxytenanthera stocksii* which was shifted later on to the genus *Dendrocalamus* based on the similar morphological features such as basal nodes bearing aerial roots, erect culms and short internodes, large panicle of spikate heads, keeled palea, among others (Kumar *et al*., 2004). *D. brandissi* with its unique nucleotide changes is a morphologically distinct species with its mature culm smooth ashy-gray to greenish-gray coloured, loosely spaced and thornless. Both *D. asper* and *D. longispathus* showed only a deletion of T mononucleotide (Table 6).

Basic statistical parameters used to characterize interspecific (average interspecific distance, theta prime, and the minimum inter-specific distance) and intraspecific distances (average intraspecific distance, mean theta, and coalescent depth) are provided in Table 7.

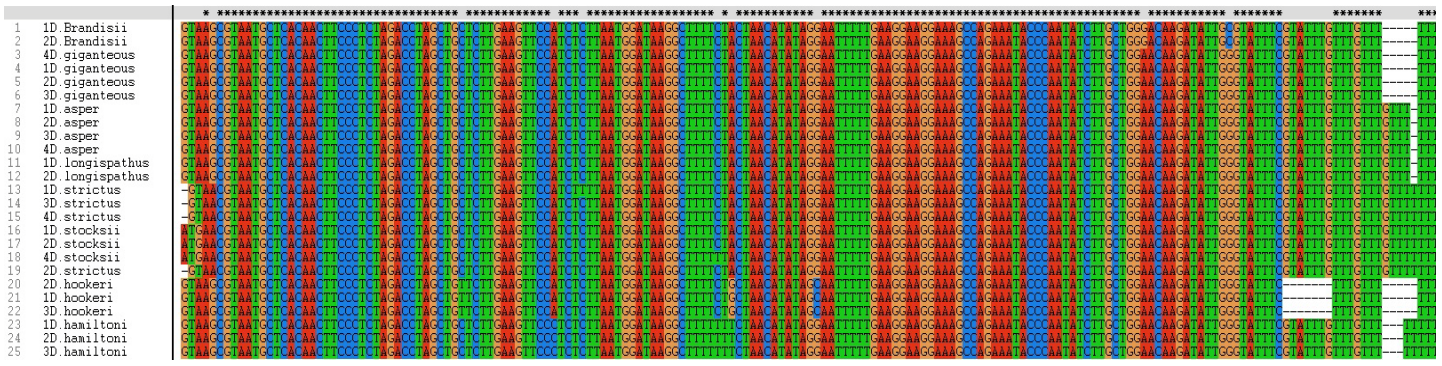


Figure 10. Multiple sequence alignment of *psbA-trnH* spacer region in the genus *Dendrocalamus*

Table 6. Nucleotide differences in *psbA-trnH* spacer region in the genus *Dendrocalamus*

Sl No	*Dendrocalamus*	Nucleotide differences
1	*D. strictus*	Deletion of G nucleotide
2	*D. stocksii*	Insertion of AA nucleotides Inversion of GTA to ATG
3	*D. hookeri*	Deletion of GTATTTG nucleotides Deletion of GTTTT nucleotides Insertion of T nucleotide Transition - A > G Transversion – G > C
4	*D. giganteus*	Deletion of GTTTTT mononucleotide repeats
5	*D. hamiltonii*	Deletion of TTT mononucleotide repeats
6 7	*D. asper* *D. longispathus*	Deletion of T in mononucleotide repeats
8	*D. brandisii*	Deletion of GTTTTT mononucleotide repeats Insertion of TG nucleotides Transversion G > C

Table 7. Genetic divergence parameters in the genus *Dendrocalamus* using MEGA

Parameters	*psbA-trnH*
Average intraspecific distance	0.0003±0.0002
Average theta	0.0015±0.0001
Average coalescent depth	0.0022±0.0026
Average interspecific divergence	0.0267±0.0006
Minimum interspecific distance	0.0000±0.0000
Average theta prime	0.0043±0.0021
Barcoding gap	0.0264±0.0004

Sequence analysis of *Melocanna* and *Ochlandra*

Out of the seven analyzed barcode regions, only *psbA-trnH* and *atpF-atpH* spacer regions showed nucleotide differences which were species specific (Table 8). Both *M. baccifera* and *M. clarkei* differed in terms of C > T and T > C transitions and G > C tranversion in their *psbA-trnH* sequences. Additionally, *M. baccifera* had two specific deletions of GTATTG and TTATTTT sequences (Fig. 11). Among the three genera in the subtribe Melocanninae, the *psbA-trnH* sequence of *Ochlandra travancorica* was more similar to *M. clarkei* than *M. baccifera*. Both *O. travancorica* and *M. clarkei* shared G nucleotide at two sites whereas it had undergone a transversional change (G > C) in *M. baccifera*. Similarly, a cytosine and an adenine nucleotide present in *O. travancorica* and *M. clarkei* respectively, had undergone transitional changes in *M. baccifera* (C > T and A > G). On the contrary, both *O. travancorica* and *M. baccifera* shared three major deletions such as GTATTTG, ATT and GTGGGTATTTTTTTTTT (Fig. 12). Even though, all these genera shared many nucleotide changes among them, each of them had unique species specific nucleotide changes as well. Kumari and Singh (2009) transferred *Arundinaria clarkei* to *Melocanna clarkei* based on the vegetative, flowering and fruiting characters which was additionally supported by SEM phylloderm analysis. *Arundinaria clarkei* Gamble ex Brandis is a less known bamboo as annotated by Gamble further described by Brandis (1906) with meager description. Camus (1913) treated it as a good species under the genus '*Arundinaria*' followed by Blatter (1929), Vermah and Bahadur (1980) and Shukla (1996).

The three parameters (*viz.* average interspecific distance, theta prime, and the minimum inter-specific distance) were employed to characterize interspecific divergence, and the average intraspecific distance, mean theta, and coalescent depth were employed to calculate intraspecific variation (Table 8).

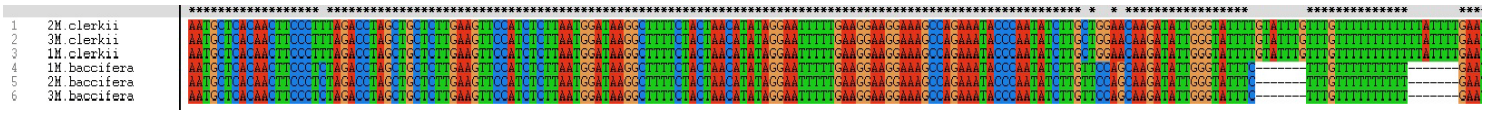


Figure 11. Multiple sequence alignment of *psbA-trnH* spacer region in the genus *Melocanna*

Table 8. Genetic divergence parameters in the genus *Melocanna*

Parameters	***psbA-trnH***	***atpF-atpH***
Average intraspecific distance	0.0062±0.0060	0.0012±0.0009
Average theta	0.0015±0.0015	0.0013±0.0011
Average coalescent depth	0.0034±0.0023	0.0016±0.0007
Average interspecific divergence	0.0125±0.0045	0.0022±0.0015
Minimum interspecific distance	0.0000±0.0000	0.0000±0.0000
Average theta prime	0.0073±0.0031	0.0020±0.0016
Barcoding gap	0.0063±0.0045	0.0010±0.0006

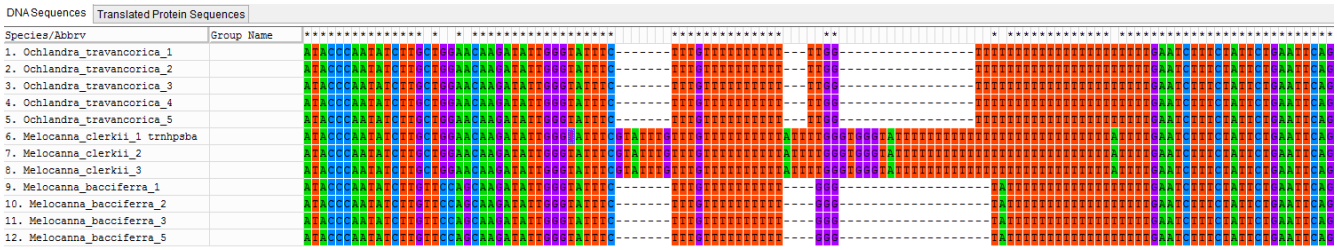


Fig. 12. Multiple sequence alignment of *psbA-trnH* spacer region in genus *Ochlandra* and *Melocanna*

Molecular markers were generally used for the species identification of the planting materials so far and this is the first report on the use of DNA barcoding for confirming the species identity of the planting material. Microsatellites have been widely used for

the identification and certification of cultivars and have been used for the certification of chestnut varieties to prevent its commercial exploitation (Botta *et al.,* 2001). Genomic variability assessed by DNA molecular markers was used as a discriminating tool for selection of genotypes for breeding programmes, to improve the *ex-situ* germplasm collection as well as for the certification of cultivars (Finkeldey *et al*., 2007). Antonova *et al.* (2006) developed marker system for the identification and certification of sunflower lines based on SSR. Microsatellites were employed for the registration and certification of planting material in *Eucalyptus* (Torres-Dini *et al.,* 2011), for the characterization of olive cultivars (Muzzalupo *et al.,* 2009) and for the certification of *Oryza sativa* varieties (Becerra *et al*., 2015). The identification and certification by means of ISSR has also been reported in Lupine Cultivars (Nam *et al.,* 2014). Forest certification schemes, state agencies such as customs offices, forest enterprises producing timber relied on molecular methods to improve the traceability of timber and offering opportunities to identify false declarations of the origin of timber (Finkeldey 2010). Even though the necessity of a viable molecular method for the certification of plant material through vegetative propagation has been discussed and suggested by various research groups (Alvarez *et al.,* 2001; Bekkaoui *et al.,* 2003; Fossati *et al.,* 2005; Rajora *et al.,* 2003; Zhou *et al.,* 2005), very limited reports are available on the actual implementation of the DNA barcode tool for the certification of vegetative propagation material.

4. SUMMARY AND CONCLUSIONS

Traditional species identification in bamboos is currently based on the vegetative characteristics of mature culms owing to the unique biological features. The long term unpredictable flowering hinders the conventional breeding for productivity improvement programs. Therefore commercial plantations are raised from the vegetatively multiplied planting stock produced in the various accredited nurseries across the country purely by the selection of genetically superior mother clumps based on field performance without any genetic basis. After the initial field level selection process for the superior mother clumps, the bulk planting materials generated from culm cuttings, or rhizome transplants lack any distinguishing key morphological characteristics for traceability. These planting materials generated at the nursery level are directly procured for the establishment of commercial plantations without any further verification. Very often misidentification and mixing up are happening at the nursery level and the assured productivity is not generally achieved at the time of harvesting.

This has necessitated the need for a certification agency at the national level and National Bamboo Mission (NBM), Government of India with the technical expertise from Bamboo Technical Support Group (BTSG) - Kerala Forest Research Institute (KFRI) proposed a certification frame work and guidelines in 2014 which recommends an integrated approach including DNA barcoding. In this regard, the present study was undertaken to test the feasibility of the standard DNA barcodes to discriminate the NBM listed priority species of bamboos. Among the seven candidate barcode loci of the plastid genome (four coding regions such as *matK, rbcL, rpoB, rpoC* and three intergenic spacers namely *psbA-trnH, psbK-psbI, atpF-atpH*) evaluated, the intergenic spacer region *psbA-trnH* barcode region only showed species specific differences for selected commercial species of bamboos which can be used as a potential DNA barcode for certification of planting materials in bamboos.

The study could generate species specific DNA barcodes for ten and eight analyzed species of the genera *Bambusa* and *Dendrocalamus* respectively, including the NBM listed priority species with unique species specific nucleotide differences in the *psbA-trnH* barcode region. The major nucleotide changes are transitions/ transversions as well as insertions/ deletions of nucleotides in the analyzed barcode region with maximum level of unique nucleotide changes in *B. multiplex*. Even though, most of the species in the genus *Bambusa* shared several nucleotide changes, the presence of unique species specific nucleotide differences can be utilized for the species identification of the planting materials of these bamboo species. In the genus *Dendrocalamus*, species specific *psbA-trnH* DNA barcodes were developed for six species i.e. *D. hookeri, D. brandisii*, *D. giganteus, D. hamiltonii* and *D. strictus.* Species such as *D. asper* and *D. longispathus* showed only a deletions inT mononucleotide repeats and the rest of the barcode region was exactly identical. Species specific *psbA-trnH* DNA barcodes were also obtained for two *Melocanna* species (*M. clarkei* and *M. bacciferra*) as well as *Ochlandra travancorica* belonging to the Melocanninae subtribe.

The current research to test the feasibility of DNA barcoding for the certification of bamboo planting material showed the efficiency of this technique as a reliable supplementary tool in the integrated approach for the proposed certification system in bamboos. To achieve the assured productivity in bamboo species, any national certification agency set up for the purpose can utilize *psbA-trnH* DNA barcode region to tag the species identity and to prove the authenticity of the multiplied planting materials in all the NBM recommended priority bamboo species.

5. REFERENCES

Alvarez A, Cervera MT, Agundez D, Alba N, Antonanzas GR, Zapater JM and Grau JM, Aplicacion de la tecnica AFLPs para la identificacion de clones del Género Populus, Simposio del Chopo, Zamora, 2001, pp. 381–389

Antonova TS, Guchetl SZ, Tchelustnikova TA and Ramasanova SA (2006) Development of marker system for identification and certification of sunflower lines and hybrids on the basis of SSR-analysis. Helia 29 (45): 63-72

Bekkaoui F, Mann B and Schroeder B (2003) Application of DNA markers for the identification and management of hybrid poplar accessions. Agroforestry system 59: 53–59

Blatter E (1929) Indian bamboos brought up-to-date. Indian Forester 55: 541-562

Botta R, Marinoni D, Beccaro G, Akkak A and Bounous G (2001) Development of a DNA typing technique for the genetic certification of the chestnut cultivars. Forest Snow and Landscape Research 76 (3): 425-428

Botta R, Marinoni DT and Bounous G (2004) Molecular Markers and Certification, Proceedings from the Workshop Biotecnolegia Forestal, Global Biotechnology Forum, Chile, 63-72.

Becerra V, Paredes M, Gutierrez E and Rojo C (2015) Genetic diversity, identification, and certification of Chilean rice varieties using molecular markers Chilean J. Agric. Res. 75(3), On-line ISSN 0718-5839.

Brandis D (1906) Indian Trees. Archibald Constable & Co. Ltd. London.

Bruni I, De Mattia F, Galimberti A, Galasso G, Banfi E, et al. (2010) Identification of poisonous plants by DNA barcoding approach. International Journal of Legal Medicine 124: 595–603.

Cai ZM, Zhang YX, Zhang LN, Gao LM and Li DH (2012) Testing four candidate barcoding markers in temperate woody bamboos (Poaceae: Bambusoideae). Journal of Systematics and Evolution 50 (6): 527-539.

Camus EG (1913) Les Bambusees: monographic. Biologic & Culture, Principaux usages. Paul Lechevalier. Paris.

CBOL PWG (2009) A DNA barcode for land plants. Proceedings of the National Academy of Sciences 106: 12794–12797.

Chase MW, Cowan RS, Hollingsworth PM, van den Berg C, Madrinan S, et al. (2007) A proposal for a standardised protocol to barcode all land plants. Taxon 56: 295–299.

Clark LG, Dransfield S, Triplett JK and Sanchez-Ken JG (2007) Phylogenetic relationships among the one-flowered, determinate genera of Bambuseae (Poaceae: Bambusoideae). Aliso 23: 315–332.

Das MM, Mahadani P, Singh R, Karmakar K and Ghosh SK (2013) MatK sequence based plant DNA Barcoding failed to identify Bambusa (Family: Poaceae) species from Northeast India. Journal of Environment and Sociobiology 10(1): 49-54.

Degen B, Holtken A and Rogge M (2010) Use of DNA-Fingerprints to Control the Origin of Forest Reproductive Material. Silvae Genetica 59(6): 268-273.

Doyle JJ and Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13-15.

Filgueiras TS and London OX (2006) A giant new Guadua (Poaceae:Bambusoideae) from central Brazil. In ''Semina´rio Nacional de Bambus'' (J. G. de Almeida and A. A. Teixeira, eds.), pp. 27–32.

Finkeledey R and Zeihe M (2004) Genetic implications of silvicultural regimes. Forest Ecology management 197: 231-244

Finkeldey R, Leinemann L and Gailing O (2010) Molecular genetic tools to infer the origin of forest plants and wood. Appl Microbiol Biotechnology 85: 1251–1258

FSI (2011) State Forest Report. Published by Forest Survey of India (Ministry of Environment & Forests) Kaulagarh Road , P.O -IPE Dehradun -248195. India.

Forstel H (2008) Die natürliche Variation und die Messung der stabilen Isotope als Kontrollmethode. In: Gebhardt K (Ed.): Herkunftskontrolle an forstlichem Vermehrungsgut mit Stabilisotopen und genetischen Methoden. Nordwestdeutsche Forstliche Versuchsanstalt, Hann. Münden, pp. 16-36

Fossati T, Zapelli I, Bisoffi S, Micheletti A, Vietto L, Sala F and Castiglione S (2005) Genetic relationships and clonal identity in a collection of commercially relevant poplar cultivars assessed by AFLP and SSR. Tree Genetics and Genomes 11–19.

Gamble JS (1896) The bambuseae of British India. Ann. Roy. Bot. Gard. Calcutta 7(1): 1-133.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis: Department of Microbiology, North Carolina State University.

Hebert PDN, Penton EH, Burns JM, Janzen DH and Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator.* Proceedings of the National Academy of Sciences of the United States of America 101: 14812–14817.

Hollingsworth PM, Andra Clark A, Forrest LL, Richardson J, Pennington RT, et al. (2009) Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. Molecular Ecology Resources 9: 439–457.

Hou DY, Song JY, Shi LC, Ma XC, Xin TY, Han JP, et al. (2013). Stability and accuracy assessment of identification of traditional Chinese materia medica using DNA barcoding: a case study on Flos Lonicerae Japonicae. Biomed Research International: 549037 PMCID: PMC3687729

Kelchner SA and Clark LG (1997) Molecular evolution and phylogenetic utility of the rpl16 intron in Chusquea and the Bambusoideae (Poaceae). Molecular Phylogenetics and Evolution 8: 385–397.

Konnert M and Hussendorfer E (2002) Herkunftssicherung bei forstlichem Vermehrungsgut durch Referenzproben. Allg. Forst Jagdztg 173 (6): 97-104.

Konnert M and Behm A (2006) Proof of identity reproductive material based on reference samples. Mitteilungen der Bundesforschungsanstalt für Forst- und Holzwirtschaft Hamburg 221: 61 – 71

Kress WJ and Erickson DL (2007) A two-locus global DNA barcode for land plants: The coding rbcL gene complements the non-coding trnH-psbA spacer region. PLoS ONE 2: e508.

Kumar M, Remesh and Unnikrishnan N (2004) A new combination in Dendrocalamus (Poaceae: Bambusoideae**).** *SIDA, Contributions to Botany* -Vol. 21, No. 1, pp. 93-96

Kumar BM, Rajesh G and Sudheesh KG (2005) Above ground biomass production and nutrient uptake of thorny bamboo [*Bambusa bambos* (L.) Voss] in the home gardens of Thrissur, Kerala. Journal of Tropical Agriculture 43: 51–56

Kumar P and Singh P (2009) On the identity of *Arundinaria clarkei* Gamble ex Brandis (Poaceae: Bambusoideae) – its recollection and taxonomic position. Nelumbo 51:233-240

Kumari P and Singh P (2009) Two new species of *Bambusa* (Poaceae) from India. Kew Bulletin 64: 565-569.

Lahaye R, van der Bank M, Bogarin D, Warner J, Pupulin F, et al. (2008) DNA barcoding the floras of biodiversity hotspots. Proceedings of the National Academy of Sciences 105: 2923–2928.

Majumdar RB (1989) Bambusoideae. In: *Florae Indicae* Enumeratio: Monocotyledoneae. Karthikeyan S, Jain SK, Nayar MP and Sanjappa M (Eds.), Botanical Survey of India, Calcutta, pp. 254-274.

Meier R, Shiyang K, Vaidya G and Ng PK (2006) DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. Systematic Biology 55: 715–728.

Meyer CP and Paulay G (2005) DNA barcoding: error rates based on comprehensive sampling. PLoS Biology 3: 2229–2238

Mishra P (2015) Bamboo and its connectivity to the different fields of Economics: A potential resource of modern India International Journal Of Innovative Research and Development 4(2): 140-145.

Muzzalupo I, Stefanizzi F and Perri E (2009) Evaluation of olives cultivated in southern Italy by Simple Sequence Repeat Markers. Hort Science 44(3): 582-588

Nam IY, Zayakin VV, Artjuchova AV, Lukashevich MI and Kupzov NS (2014) Identification and Certification of Lupine Cultivars Using Molecular Markers. World Applied Sciences Journal 30 (7): 796-801

NBM (National Bamboo Mission, India) (2013). retrieved 17 December 2013 from http:nbm.nic.in/

Newmaster SG, Fazekas AJ, Steeves RAD and Janovec J (2007) Testing candidate plant barcode regions in the Myristicaceae. Molecular Ecology Notes 8: 480-490.

Newmaster SG and Ragupathy S (2009) Testing plant barcoding in a sister species complex of pantropical Acacia (Mimosoideae, Fabaceae) Molecular ecology resources 9: 172–180.

Omari 2009 Pang X, Song J, Zhu Y, Xie C and Chen S (2010) Using DNA barcoding to identify species within Euphorbiaceae. Planta Medica 76: 1784–1786.

Orhnberger D and Georrings J (1986) The Bamboos of the World. International Book Publishers, Dehra Dun, India.

Pang X, Song J, Zhu Y, Xie C and Chen S (2010) Using DNA barcoding to identify species within Euphorbiaceae. Planta Medica 76: 1784–1786.

Rajora OP and Rahman MH (2003) Microsatellite DNA and RAPD fingerprinting, identification and genetic relationships of hybrid poplar (Populus × canadensis) cultivars. Theoretical and Applied Genetics 106: 470–477.

Salam K (2013) Connecting the poor: Bamboo, Problems and prospect. South Asia Bamboo Foundation (SABF) retrieved 17 December 2013 from jeevika.org/bamboo/2g-article-for-nbda.docx

Shukla U (1996) Grasses of North-Eastern India. Scientific Publishers. Jodhpur.

Sosa V, Mejia-Saules T, Cullar MA, et al. (2013) DNA barcoding in endangered Mesoamerican groups of plants. Botanical Review 79: 469.

SPSS Inc. (2007) Released. SPSS for Windows, Version 16.0. Chicago, SPSS Inc.

Starr JR, Naczi RF and Chouinard BN (2009) Plant DNA barcodes and species resolution in sedges (Carex, Cyperaceae). Molecular Ecology Resources Suppl s1:151-63.

Taberlet P, Coissac E, Pompanon F, Gielly L, Miquel C, et al. (2007) Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. Nucleic Acids Research 35: e14.

Tamura K, Stecher G, Peterson D, Filipski A and Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729.

Torres-Dini D, Bennadji Z, Cabrera M, Centurion C, Resquin F and Balmelli G (2011) Use of SSR-Tools for clone certification in Uruguayan Eucalyptus grandis and Eucalyptus dunnii breeding programs. BMC Proceedings 5 (Suppl 7) : P58.

Thokchom A and Yadhava PS (2015) Bamboo and its role in climate change. Current Science. 108: 5

Thompson JD, Higgins DG and Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680.

Tripathi SK, Sumida A, Shibata H, Ono K, Uemura S, Kodama Y and Hara T. 2006. Leaf litter fall and decomposition of different above and below ground parts of birch (*Betula ermanii*) trees and dwarf bamboo (*Sasa kurilensis*) shrubs in a young secondary forest in Northern Japan. Biology and Fertility of Soils 43 (2): 237-436.

Triplett JK, Weakley AS and Clark LG (2006) Hill cane (*Arundinaria appalachiana*) a new species of bamboo from the southern Appalachian Mountains. Sida 22: 79–85.

Vermah JC and Bahadur KN (1980) Country report and status of research on bamboos in India. Indian Forest Records (new ser.) Bot. 6 (1): i-vii, 1-28.

Yan HF, Hao G, Hu CM and Ge XJ (2011) DNA barcoding in closely related species: A case study of *Primula* L. sect. Proliferae (Primulaceae) in China. Journal of Systematics and Evolution 49: 225–236.

Zhang YX, Xu YX, Ma PF, Zhang LN and Li DZ (2013) Selection of potential plastid DNA barcodes for Bambusoideae (Poaceae). Plant Diversity and Resources 35(6): 743-750.

Zhou CJ, Song HZ, Li JH, Sun JW, Jin DM, Zhang QW and Wang B (2005) Evaluation of genetic diversity and germplasm identification of 44 species, clones, and cultivars from 5 sections of the genus Populus based on amplified fragment length polymorphism analysis. Plant Molecular Biology Reports 23: 39–51.

Ziegenhagen B, Liepelt S, Kuhlenkamp V and Fladung M (2003) Molecular identification of individual oak and fir trees from maternal tissues of their fruits or seeds. Trees 17: 345– 350.