3 Biotech (2020) 10:59
https://doi.org/10.1007/s13205-019-2018-8

ORIGINAL ARTICLE

DNA barcoding as a valuable molecular tool for the certification of planting materials in bamboo

Suma Arun Dev[1] · K. Sijimol[1] · P. S. Prathibha[1] · V. B. Sreekumar[2] · E. M. Muralidharan[1]

Received: 25 June 2019 / Accepted: 16 December 2019 / Published online: 22 January 2020
© King Abdulaziz City for Science and Technology 2020

Abstract
DNA barcodes developed for selected commercially important bamboo species can be utilized for the certification of planting stock in bamboo nurseries in absence of discriminatory features at the juvenile stage. Planting materials such as micropropagated plantlets, rhizome transplants and culm cuttings, generated at nursery level are directly procured for establishment of commercial plantations without any further verification. Very often misidentification and mixing up occur at nursery level and the error is not discovered until several years have passed. The present study evaluated the potentiality of seven Consortium for Barcode of Life (CBOL) recommended standard DNA barcode regions in commercially important bamboo species of India. Among the analyzed barcode regions, multiple sequence alignment (MSA) of *psbA-trnH* barcode region showed species-specific nucleotide differences in the studied bamboo taxa. The major nucleotide changes observed were transitions/transversions as well as insertions/deletions of nucleotides. Even though species-specific mononucleotide differences could be identified for most of the studied bamboo taxa, a small amount of sequence similarities were found in some of the *Dendrocalamus* and *Bambusa* species, which were grouped together in tree-based analysis. In subtribe Melocanninae, *Ochlandra travancorica, Melocanna baccifera* and *M. clarkei* showed unique species-specific *psbA-trnH* barcodes. Similarly, in the genus *Oxytenanthera*, unique species-specific *psbA-trnH* barcodes were obtained for *O. monadelpha* and *O. parvifolia.* Thus *psbA-trnH* barcode region generated distinct species-specific barcodes for commercial bamboo species in genera *Bambusa, Dendrocalamus, Melocanna, Oxytenanthera* as well as *Ochlandra*. Any national certification agency set up for the purpose can utilize *psbA-trnH* DNA barcode region to tag species identity and to establish the authenticity of multiplied planting materials in bamboos.

Keywords Commercial bamboos species · Planting stock · Species certification · DNA barcoding

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 one of the most suitable species for commercial forestry. Along with traditional uses of bamboo poles for rural building construction, fencing, agricultural purposes and household articles, in recent times, the 'green gold' has got immense demand as an industrial raw material (Khan and Hazra 2007). The multiple harvests possible around the year along with tremendous growth rate contribute towards 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 bamboo-based industries.

Even though India possesses 45% of global bamboo growth, the country contributes only 4% towards the global trade with an annual productivity of only 1 MT/ha (Tripathi

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13205-019-2018-8) contains supplementary material, which is available to authorized users.

✉ Suma Arun Dev
sumadev@kfri.res.in

[1] Forest Genetics and Biotechnology Division, Kerala Forest Research Institute, Peechi, Thrissur, Kerala, India

[2] Forest Ecology and Biodiversity Conservation Division, Kerala Forest Research Institute, Peechi, Thrissur, Kerala, India

et al. 2006). Overexploitation of available resources, poor performance of unsuitable species, forest fires, grazing, gregarious flowering, among others contributed to productivity decline albeit the existence of significant species diversity/growing habitats of bamboos in India (NBM 2013). National Bamboo Mission (NBM), Government of India, is trying to achieve a global share of 27% through improved productivity of bamboo plantations (Kumar et al. 2005). In this regard, NBM has recommended priority species based on their annual productivity and suitability in different agro-climatic zones throughout the country (https://www.nbm.nic.in). Guidelines issued by NBM aim at ensuring the use of superior planting stock for raising bamboo plantations through a network of certified high-tech bamboo nurseries in India. There are more than thousand nurseries in the country which provide planting stock to farmers for establishment of plantations. The crucial step in any productivity improvement program is proper identification, multiplication and supply of high-quality planting materials of suitable species with guaranteed productivity. It is a common observation, however, that plants being propagated in these nurseries are misidentified or that mixing of species occurs inadvertently. It is imperative to ensure correct species identity along with quality planting stock so as to make certain that it is suitable for a given site or purpose.

Unlike other plants, most bamboo species are semelparous, resulting in unpredictable flowering at long cycles after which all plants in the population die. Field identification of bamboo species thus traditionally rely on morphological characteristics of vegetative parts, mainly culm/culm sheath characteristics (Ohrnberger and Georrings 1986; Clark et al. 2007). Generally, in bamboo species, these characteristics manifest only when new culms emerge and juvenile plants do not possess these characteristics until after the 1st year or even later. Thus, identification of species is rendered difficult even for the best of nursery men. Further compounding the problem is the presence of variation between accessions within same species. Planting materials of bamboos handled in a typical bamboo nursery consist of plants in various age classes derived from seeds, macroproliferated seedlings (Kumar 1991), rooted culm or branch cuttings, rhizome transplants and micropropagated plantlets. Due to the absence of any consistent vegetative characteristics, unintentional mixing of species/clones is quite common at the nursery level. Therefore, to ensure that material used for planting conforms to the intended quality, NBM envisages a certification framework under which planting materials are characterized using various state-of-the-art tools (BTSG 2014). Methods to identify plant material at clonal level as well as at species level are equally important. Ideally these methods should be of the kind that is applicable to planting material at all stages of propagation, plantation and the harvested products. Morphology-based identification keys are very useful for quick identification at field, but it is well known that environmental plasticity in these characters is a serious limiting factor that prevents precise identification. DNA-based molecular tools are devoid of such environmental or developmental influences and 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 traceability systems in forest reproductive material employing molecular markers (Botta et al. 2001, 2004; Konnert and Behm 2006; Degen et al. 2010). The last decade had witnessed unraveling of potential of molecular techniques as a tool to supplement species identification methods in plants as well as in animals. DNA barcoding, process of species identification based on short standard conserved region of the genome (Hebert et al. 2004; CBOL 2009) is foremost when it comes to identification at species level and to resolve taxonomic problems. 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 species discrimination in bamboos has been reported only in a few instances. In temperate woody bamboos, four barcoding loci, namely *matK*, *rbcL*, *psbA-trnH* and *ITS2* were analyzed and the combination of *rbcL* + *ITS*2 suggested as a potential barcode region 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 core barcode region (*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 discriminant barcode loci in some temperate bamboos.

The present study therefore envisages developing DNA barcodes for commercially important priority species of bamboos recommended by NBM, Government of India for large-scale multiplication in accredited bamboo nurseries.

Materials and methods

Plant sampling

Leaf samples were collected from mature clumps of bamboo species from reserve forests and protected areas throughout the distribution zones in India (Table 1). For the preparation of voucher specimens, twigs with a few leaves and culm sheaths were collected. Thirteen commercially important bamboo species, viz., *Bambusa balcooa* Roxb., *B. bambos*

Table 1 Details on bamboo species and their distribution included in the present study

Name of species	No. of samples/species	Distribution	Propagules used
Bambusa balcooa Roxb.[a]	7	Northeastern India, Tripura, Nagaland, Meghalaya, Assam, West Bengal, Uttar Pradesh	Branch/culm cuttings Tissue culture plantlets
B. bambos Voss[a]	10	Wide distribution in India	Seedlings
B. nutans Wall ex Munro[a]	5	Himachal and Northeastern states, West Bengal, Orissa, Sikkim, UP	Culm/branch cuttings, Offset plantings
B. pallida Munro[a]	6	Northeast India, Bhutan, Myanmar	Seedlings Culm cuttings, rhizome/ Offset plantings
B. tulda Roxb[a]	10	Assam, Bihar, Meghalaya, Meghalaya, Mizoram, Tripura	Seedlings Culm/ branch/ rhizome cuttings Tissue culture plantlets
B. vulgaris var. *vulgaris* Schrad ex Wendle[a]	7	Northeast and central India	Culm/branch cuttings
B. mohanramii P. Kumari and P. Singh	5	Meghalaya	Culm/branch cuttings
B. jaintiana R.B. Majumdar	5	Assam, Meghalaya, Bangladesh, Myanmar, Bhutan	Culm/branch cuttings
B. multiplex (Lour.) Raeusch. ex Schult	5	Meghalaya, Assam	Culm/branch cuttings/Offset plantings
B. teres Munro	5	Meghalaya, Arunachal Pradesh, Assam, Manipur, West Bengal	Culm/branch/rhizome cuttings
Dendrocalamus asper Baker ex Heyne[a]	6	Exotic and cultivated in Northeast India	Culm/branch cuttings Tissue culture plantlets
D. giganteus Munro[a]	7	Exotic and cultivated in Northeastern India and West Bengal	Seedlings Culm/branch cuttings, rhizome/offset plantings
D. hamiltonii Ness and Arn ex Munro[a]	8	Central, Northeast India, Sikkim, West Bengal, Assam	Seedlings Culm/branch cuttings, rhizome/offset plantings
D. strictus Ness[a]	10	Throughout India	Seedlings Culm/branch cuttings, rhizome cuttings
D. stocksii (Munro) M. Kumar, Remesh and Unnikrishnan	6	Maharashtra, Karnataka, Kerala, Goa	Culm cuttings
D. longispathus (Kurz) Kurz	5	Meghalaya, Assam, Manipur, Mizoram, Tripura, West Bengal	Culm cuttings
D. brandisii (Munro) Kurz	7	Manipur, Burma	Culm/branch cuttings, rhizome/offset plantings
D. hookeri (Munro)	7	Meghalaya, Arunachal Pradesh, Assam, Manipur, Nagaland	
Melocanna baccifera Kurz[a]	8	Northeast India	Seedlings Rhizome cuttings
M. clarkei (Gamble ex Brandis) P. Kumari and P. Singh	6	Meghalaya, Assam, Manipur, Nagaland	Rhizome cuttings
Ochlandra travancorica Benth[a]		Kerala, Tamil Nadu	Seedlings Rhizome cuttings
Oxytenanthera parviflora Brandis ex Gamble[a]	5	Assam, Mizoram	Rhizome cuttings
O. monadelpha (Thwaites) Alston	4	Maharashtra, Karnataka, Kerala, Tamil Nadu	Culm cuttings
O. stocksii (Munro)	5	Kerala, Karnataka, Maharashtra	Culm cuttings

[a]National Bamboo Mission (NBM) recommended priority bamboo species

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* Nees and Arn ex Munro, *D. strictus* Nees, *Melocanna baccifera* Kurz., *Ochlandra travancorica* Benth and *Oxytenanthera parvifolia* Brandis ex Gamble were selected for the development of DNA barcodes. Leaf samples from multiple accessions were collected and dried in silica gel. In addition to NBM listed priority species, several species distributed in Northeastern India such as those of genera *Bambusa* (*B. jaintiana, B. mohanramii, B. teres, B. multiplex*), *Dendrocalamus* (*D. brandisii, D. hookeri, D. longispathus*), *Oxytenanthera* (*O. monadelpha*) as well as *Melocanna clarkei,* which are generally in cultivation were also considered for the development of DNA barcodes. Samples were authenticated at Kerala Forest Research Institute (KFRI) and voucher specimens were deposited at KFRI herbarium.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from either fresh or silica dried leaves using modified cetyltrimethylammonium 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, rpoC1* and three intergenic spacers namely *psbA-trnH, psbK-psbI* and *atpF-atpH* were evaluated to identify discriminant DNA barcodes for bamboo species. Primer details and reaction conditions standardized for DNA amplification through polymerase chain reaction (PCR) are listed in Table S1.

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 % 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) and quantified using Nanodrop (Thermo Scientific, USA). Sequencing was performed using Sanger dideoxy chemistry in both forward and reverse directions (Chromous, Bangalore).

Sequence analysis

Chromatograms were edited and trimmed using BioEdit software (Hall 1999). Edited sequences were aligned using ClustalX of Clustal W packages (Thompson et al. 1994) and submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) as well as BOLD https://www.barcodinglife.org.

For pairwise genetic distance (PWG) method, interspecific as well as intraspecific genetic distances were determined by MEGA v.6.0 using Kimura two-parameter distance model (K2P) adopting complete deletion option (Tamura et al. 2013). The interspecific divergence between species was calculated using three parameters: (1) average interspecific distance, (2) average theta prime (θ') and (3) minimum interspecific distances. Intraspecific parameters such as (4) average intraspecific distance, (5) theta (θ) and (6) coalescent depth were also calculated to characterize intraspecific divergences (Meyer and Paulay 2005). Barcoding gap was calculated by plotting intraspecific distances against interspecific divergences for each species (Meier et al. 2006). A blind sampling test was performed with twelve samples of unknown identity to check the efficiency of the selected barcode region in discriminating bamboo species.

In the tree-based analysis, neighbor-joining (NJ) trees were constructed for the studied bamboo taxa using most discriminant *psbA-trnH* spacer barcode region, adopting K2P parameter in MEGA v.6.0. Bootstrap support was estimated with 1000 heuristic replicates (100 random addition cycles per replicate, with tree bisection reconnection and branch swapping) to test the reliability of inferred phylograms. All positions containing gaps and missing data were eliminated from dataset (complete deletion option).

Results

DNA barcode amplification, sequencing and alignment

An ideal DNA barcode is expected to have adequate conserved regions, high PCR amplification efficiency, and enough variability for species identification (CBOL Plant Working Group 2009). All the evaluated DNA barcode regions (*matK*, *rbcL*, *rpoC1, rpoB, psbK-psbI, atpF-atpH* and *psbA-trnH*) were successfully amplified with 100 % PCR efficiency using CBOL (2009) recommended primers (Fig. S1). The edited sequences after homology searches were deposited in the GenBank and accession numbers were provided (Table 2).

Six of the DNA barcode regions, viz., *rbcL, matK, rpoB, rpoC1, psbK-psbI* and *atpF-atpH* displayed exactly identical sequences in all analyzed species of *Bambusa*, *Dendrocalamus, Oxytenanthera* as well as *Melocanna* and *Ochlandra*. Hence, these DNA barcode regions cannot be useful for species certification of planting materials in bamboos. Multiple sequence alignment (MSA) of *psbA-trnH* intergenic spacer barcode region showed species-specific nucleotide differences in most studied bamboo taxa, viz., *Bambusa, Dendrocalamus, Melocanna* and *Ochlandra.* The blind sampling test carried out proved the effectiveness of this barcode

Table 2 GenBank accession numbers and barcode regions of *Dendrocalamus, Bambusa, Melocanna, Oxytenanthera* genera

Sl. no.	Barcode regions	Accession number			
		Genus *Dendrocalamus*	Genus *Bambusa*	Genus *Melocanna*	Genus *Oxytenanthera*
1	*RbcL*	MH1855639–MH185696	MH170547–MH170611	MH185450–MH185463	MH256570–MH256587
2	*matK*	MH185581–MH185638	MH170482–MH170546	MH238490–MH238503	MH249834–MH249851
3	*rpoB*	MH185523–MH185580	MH185697–MH185761	MH189392–MH189405	MH249852–MH249869
4	*rpoC1*	MH185464–MH185522	MH304513–MH304578	MH241037–MH241050	MH256588–MH256605
5	*atpF-atpH*	MH185392–MH185449	MH185269–MH185333	MH240992–MH241005	MH256606–MH256623
6	*psbK-psbI*	MH185334–MH185391	MH185204–MH185268	MH240978–MH241005	MH256624–MH256641
7	*psbA-trnH*	MH230004–MH230061	MH240913–MH240977	MH234677–MH234690	MH249816–MH249833

region. Two specimens from each of the six bamboo species (*Bambusa tulda, B. vulgaris, B. bambos, Dendrocalamus strictus, D. stocksii*, *Melocanna baccifera*) randomly collected by a third party from a bamboo nursery maintained at KFRI, Peechi Campus were correctly identified to the species level. Additionally, the intergenic spacer *psbK-psbI* also showed species discrimination in the genus *Melocanna.*

Sequence length and basic sequence statistics like conserved sites, variable sites, singletons and informative sites of *psbA-trnH* spacer region based on CLUSTALX alignment as well as with alignment explorer in MEGA v.6.0 are provided in Table S2. The maximum number of variable sites among species was in genus *Bambusa* (23) followed by *Dendrocalamus* (12) and the least was in genus *Melocanna* (8).

Sequence analysis of *Bambusa*

Multiple sequence alignment (MSA) of *psbA-trnH* barcode showed nucleotide differences unique to species in most cases (Fig. 1). The major nucleotide changes were transitions/transversions as well as insertions/deletions of nucleotides in the analyzed barcode region. In most species, deletions of mononucleotide thymine repeats in various numbers were obvious. Among ten species of the genus *Bambusa*, *B.*

Fig. 1 Multiple sequence alignment of *psbA-trnH* barcode region in the genus *Bambusa*

multiplex had maximum level of unique nucleotide changes. *B. balcooa*/*B. vulgaris/B. pallida* as well as *B. nutans*/*B. teres* shared the type of deletion of thymine mononucleotides along with other specific nucleotide changes. *B. balcooa, B. tulda* and *B. vulgaris* had a similar transition event (*G*>*A*). Similarly, *B. nutans, B. tulda, B. vulgaris, B. multiplex* and *B. jaintiana* had a similar transition event (*C*>*T*) (Table 3).

In distance-based analysis using *psbA-trnH*, basic statistical parameters (average interspecific distance, theta prime and minimum interspecific distance) were employed to characterize interspecific divergence. The intraspecific variations were calculated by employing average intraspecific distance, mean theta and coalescent depth (Table 4). Average interspecific distance was 0.0509 and DNA barcoding gap was 0.0485 for the genus *Bambusa*. Even though species-specific nucleotide differences could be identified for each *Bambusa* species, differences were located in the non-coding intergenic spacer regions or in mononucleotide repeats.

Table 3 Nucleotide differences in *psbA-trnH* spacer region in the genus *Bambusa*

Sl. no.	Species	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. mohanramii*	Insertion of TT mononucleotide repeats
		Transversion *T* > *G* at 2 places

Sequence analysis of *Dendrocalamus*

Out of the seven analyzed barcode regions, only *psbA-trnH* showed species-specific nucleotide differences in the genus *Dendrocalamus* (Fig. 2). Basic statistical parameters used to characterize interspecific and intraspecific distances are provided in Table 4. The average interspecific distance was 0.0267 and DNA barcoding gap was 0.0264 for the genus *Dendrocalamus*.

Unlike in *Bambusa*, *Dendrocalamus* species had a lower 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 both in *D. hookeri* and *D. brandisii*. An inversion of GTA nucleotides was specifically observed in *psbA-trnH* sequence of *D. stocksii.* Both *D. asper* and *D. longispathus* showed only a deletion of T mononucleotide (Table 5).

Sequence analysis of *Melocanna* and *Ochlandra*

Among genus *Melocanna* and *Ochlandra* of subtribe Melocanninae, only *psbA-trnH* and *atpF-atpH* spacer regions showed nucleotide differences which were species specific out of the seven analyzed barcode regions. Both *M. baccifera* and *M. clarkei* differed in terms of *C*>*T* and *T*>*C* transitions and *G*>*C* transversion in their *psbA-trnH* sequences. Additionally, *M. baccifera* had two specific deletions of GTATTG and TTATTTT sequences (Fig. 3). *psbA-trnH* sequence of *O. 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

Table 4 Genetic divergence parameters in the genus *Bambusa, Dendrocalamus* and *Oxytenanthera* using MEGA v 6.0

Parameters	*psbA-trnH*		
	Bambusa	*Dendrocalamus*	*Oxytenanthera*
Average intraspecific distance	0.0024 ± 0.0010	0.0003 ± 0.0002	0.0000 ± 0.0000
Average theta	0.0008 ± 0.0003	0.0015 ± 0.0001	0.0000 ± 0.0000
Average coalescent depth	0.0004 ± 0.0001	0.0022 ± 0.0026	0.0000 ± 0.0000
Average interspecific divergence	0.0509 ± 0.0012	0.0267 ± 0.0006	0.0014 ± 0.0006
Minimum interspecific distance	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000
Average theta prime	0.0086 ± 0.0033	0.0043 ± 0.0021	0.0060 ± 0.0002
Barcoding gap	0.0485 ± 0.0002	0.0264 ± 0.0004	0.0014 ± 0.0006

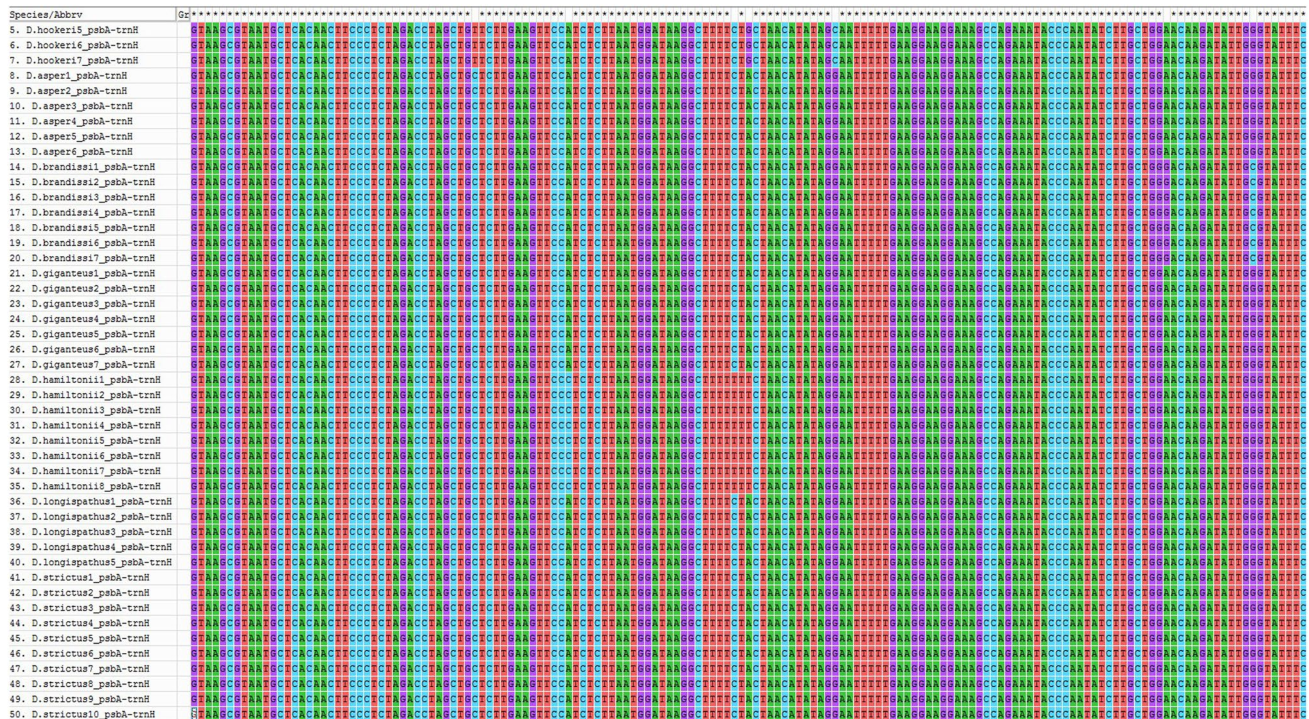

Fig. 2 Multiple sequence alignment of *psbA-trnH* barcode region in the genus *Dendrocalamus*

Table 5 Nucleotide differences in *psbA-trnH* spacer region in the genus *Dendrocalamus* using MEGA v 6.0

Sl. no.	Species	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	*D. asper*	Deletion of T in mononucleotide repeats
7	*D. longispathus*	Deletion of T in mononucleotide repeats
8	*D. brandisii*	Deletion of GTTTTT mononucleotide repeats
		Insertion of TG nucleotides
		Transversion *G*> *C*

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 GTGGGTATTTTTTTTT (Fig. 3). Even though, two genera shared many nucleotide changes among them, each of them had unique species-specific nucleotide changes as well. Statistical parameters were employed to characterize interspecific divergence and average intraspecific distances (Table 6).

Sequence analysis of *Oxytenanthera*

Among seven analyzed barcode regions, only *psbA-trnH* barcode showed species-specific nucleotide differences in the genus *Oxytenanthera*. Species-specific differences were mostly in mononucleotide repeats of *psbA-trnH*, which consist of three base pair and single base pair deletions, respectively, in *O. monadelpha* and *O. parvifolia* (Fig. 4). Statistical parameters to characterize interspecific/intraspecific distances were employed (Table 4).

Tree-based analysis

In the tree-based analysis, ten species of the genus *Bambusa* showed species-specific clustering with two major groups except for *B. balcooa* and *B. teres* (Fig. 5). Similarly, out of the seven species in genus *Dendrocalamus*, three species such as *D. asper, D. giganteus* and *D. longispathus* were clustered together as a complex, while the remaining species formed monophyletic clusters with greater than 60 %

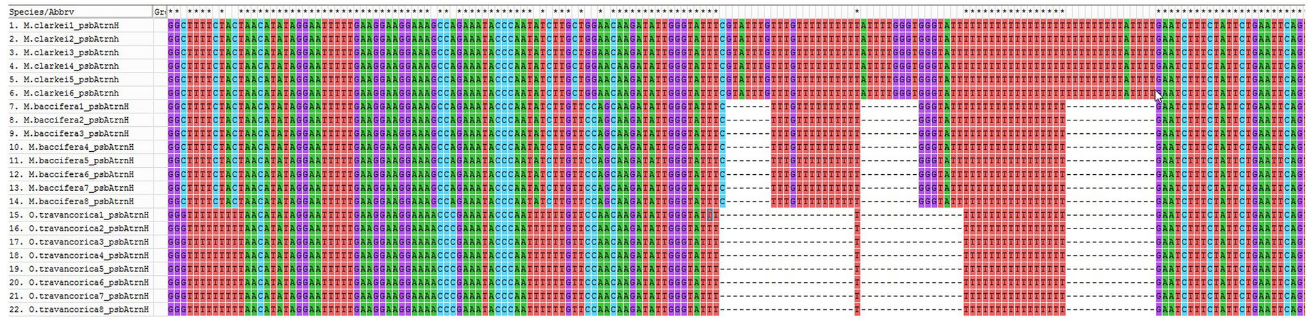

Fig. 3 Multiple sequence alignment of *psbA-trnH* barcode region in the genera *Ochlandra* and *Melocanna*

Table 6 Genetic divergence parameters in the genus *Melocanna* using MEGA v 6.0

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

bootstrap support (Fig. S2). Three taxa of Melocanninae subtribe such as *M. clarkei, M. baccifera* and *O. travancorica* formed monophyletic clusters with 64 %, 96 % and 99 % bootstrap support values, respectively, thus *psbA-trnH* spacer region can be effectively utilized as a discriminant barcode for these species (Fig. S3). Similarly, *O. parviflora* and *O. monadelpha* grouped into two separate monophyletic clusters with 100 % bootstrap support (Fig. S4).

Discussion

Bamboo is very fast growing with 20 times more yield than any other timber tree species and is the most preferred industrial raw material (https://life.gaiam.com/article/how-eco-friendly-bamboo). Over the last few decades, commercial bamboo plantations in India have significantly increased and the planters are really concerned about reliability and identity of species/clonal material multiplied in nurseries all over India. Among the hurdles that nursery managers face in producing quality planting material, the difficulty in precise identification of species is particularly vexing. A further confounding problem is the relative lack of discriminatory morphological features in juvenile plants maintained in nurseries. The misidentification of species suitable for different agro-climatic zones can lead to a significant decline in the productivity (Sharma 2008). It can also lead to significant loss to farmers if wrong species identity is discovered much later especially when the species is unsuitable for a specific end use for which it was grown. This has necessitated the need for a certification agency at national level and with Bamboo Technical Support Group (BTSG), Kerala Forest Research Institute (KFRI) proposed to National Bamboo Mission (NBM), Government of India, a certification framework and guidelines and recommends an integrated approach which includes DNA barcoding for precise species identification (BTSG 2014). A species-specific DNA barcode thus can serve as a valid certification tool to ensure species identity and productivity in the commercial bamboo plantations.

Out of the seven recommended DNA barcode regions evaluated, six DNA barcodes were substantially the same across all species of genera *Bambusa, Dendrocalamus, Ochlandra* and *Melocanna*. Because of low ability for species discrimination, most working groups had 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),

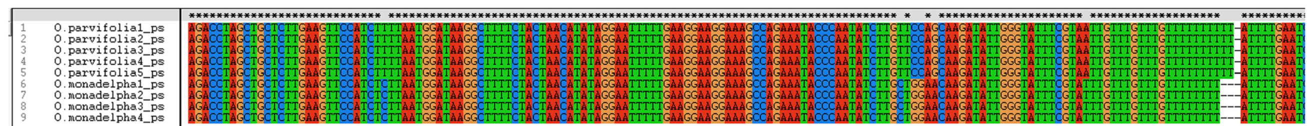

Fig. 4 Multiple sequence alignment of *psbA-trnH* barcode region in the genus *Oxytenanthera*

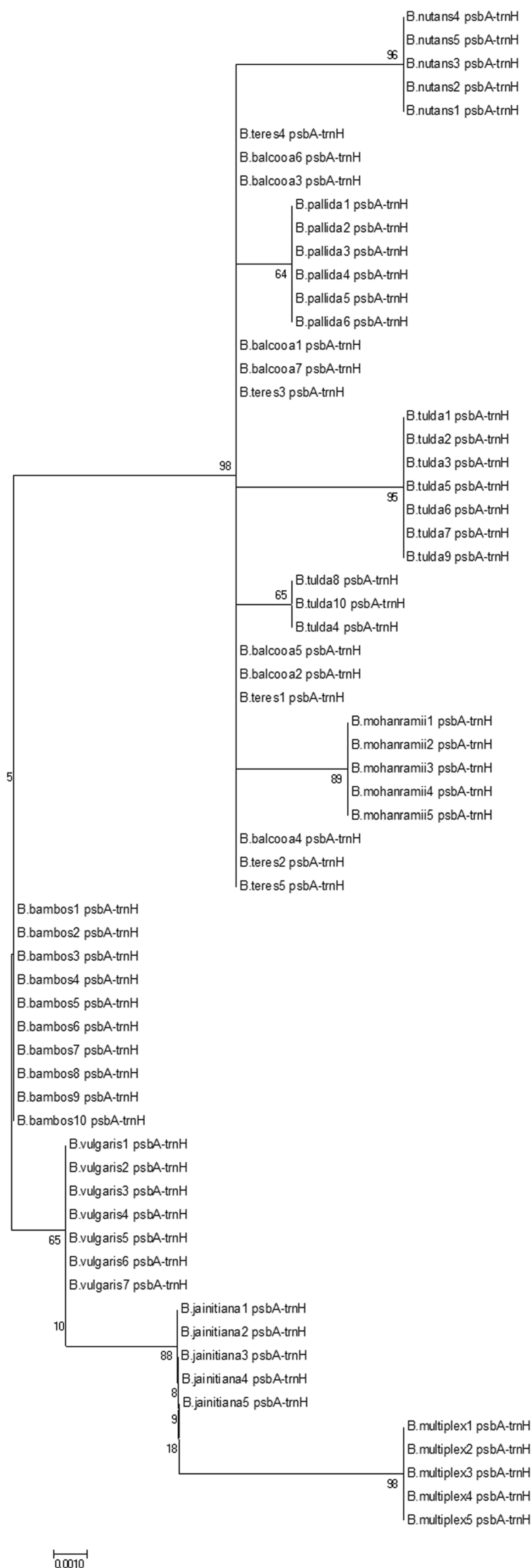

Fig. 5 Neighbor-joining tree of selected *Bambusa* species

orchids (Lahaye et al. 2008), sedges (Starr et al. 2009) and *Acacia* (Newmaster and Ragupathy 2009), but universality of this barcode region remains uncertain in various taxa. In this study, *rbcL* and *matK* could not differentiate bamboo species. Even though *rpoB* and *rpoC1* has been recommended as suitable barcodes, due to low interspecific divergence, these barcode regions were reported as inappropriate supplementary barcode loci (Lahaye et al. 2008). The present barcode analysis also revealed low discriminatory power of *rpoB* and *rpoC1*. In addition to the candidate barcode regions described above, other plastid barcoding regions such as *atpF-atpH*, *psbK-psbI* and *trnT-trnL* were also recommended for species identification (Taberlet et al. 2007*). psbA-trnH* has the potentiality as a suitable marker for species discrimination between closely related taxa due to high rate of sequence variation present generally in this intergenic spacer region (Kress and Erickson 2007; Newmaster et al. 2007). It has also been recommended as one of the best performing loci for various taxa in terms of PCR amplification success, sequencing and species resolution (Lahaye et al. 2008). In the present analysis, species-specific nucleotide differences were observed in *psbA-trnH* barcode region of genera, *Bambusa* and *Dendrocalamus*, *Ochlandra* and *Melocanna.* Thus, *psbA-trnH* can serve as a DNA barcode region for species identification of various bamboo taxa taken up for this study.

Bambusa balcooa and *B. vulgaris* shared the same type of deletion in thymine mononucleotides. These species are widely cultivated and morphological features are greatly influenced by selection process. Both the species are different in 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. *B. vulgaris* formed a very distinct cluster in tree-based analysis. Likewise, B. *balcooa* and *B. tulda* had a similar transversion event (*G* > *A*). In *B. balcooa* and *B. tulda,* inflorescence is clustered at nodes and glumes persistent and shorter than spikelet. In NJ tree, *B. balcooa, B. tulda* and *B. teres* grouped together into a complex. Both *B. teres* and *B. balcooa* are arborescent densely tufted clump-forming species with glabrous culm sheaths. Even though *B. teres* is distinct 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, Majumdar (1989) treated *B. teres* under synonym of *B. tulda*. *B. tulda* is a widely distributed species and morphologically highly variable in vegetative and reproductive characters.

Morphologically distinct species like *B. multiplex, B. mohanramii, B. jaintiana* as well as *B. pallida* displayed unique barcodes and also showed distinct species-specific clusters in the derived NJ tree. In mature state, *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 sheath proper and lanceolate spikelets with 3–8 fertile florets. *B. jaintiana* is a shrubby erect bamboo found in loose clumps. Culms are green becoming orange with age and while young ones are white powdery. *B. mohanramii* differs remarkably in other vegetative and floral aspects like culm sheath short than internodes, auricles with short rounded shape with somewhat matching culm sheath to *B. balcooa* (Kumari and Singh 2009). *Bambusa multiplex* is a morphologically variable widely cultivated perennial species with slender and erect woody culms, nodal roots, bractiferous inflorescence and caryopsis fruit. Among ten species of the genus, *B. multiplex* had maximum level of unique nucleotide changes.

Dendrocalamus brandisii and *D. giganteus* had same type of GTTTTT nucleotide deletions and both of which have morphologically prominent auricles in culm sheath and its sheath is glabrous. Some unique nucleotide changes present in *D. stocksii* such as inversion of GTA to ATG and insertion of AA nucleotides are absent in other *Dendrocalamus* species. *D. stocksii* was initially known as *Oxytenanthera stocksii* which was shifted later on to genus *Dendrocalamus* based on similar morphological features such as basal nodes bearing aerial roots, erect culms and short internodes, large panicle of spicate heads, keeled palea, among others (Kumar et al. 2004). *D. brandisii* with its unique nucleotide changes is a morphologically distinct species with its mature culm smooth ashy-gray to greenish-gray colored, loosely spaced and thornless which formed a well-defined clade in the tree-based analysis. *D. asper*, *D. giganteus* and *D. longispathus* have morphologically distinct characteristics but they grouped together in the phylogenetic tree generated.

So far, only universal molecular markers were used for species/cultivar certification of tree species. For example, RAPD markers were employed for certification of *Populus* species (Sanchez et al. 1998), ISSR markers for *Picea* species (Nkongolo et al. 2005), *Eucalyptus* species (Balasaravanan et al. 2006), certification of lupine cultivars (Nam et al. 2014) as well as for genetic fidelity testing in *Saccharum officinarum* (Thorat et al. 2018). A reliable and affordable certification tool based on SSRs was reported for the certification of chestnut varieties to prevent its commercial misuse (Botta et al. 2001), commercial cultivars of *Populus* (de-Lucas et al. 2007), characterization of olive cultivars (Muzzalupo et al. 2009), registration and certification of planting materials in *Eucalyptus* (Torres-Dini et al. 2011), to differentiate Chilean and foreign commercial rice varieties (Becerra et al. 2015), discrimination of *Panax* species/cultivars (Jo et al. 2016) and for the certification of Albania olive (Muzzalupo et al. 2018).

Forest certification schemes, state agencies such as customs offices, forest enterprises producing timber have relied on molecular methods to improve the traceability of timber and offering opportunities to identify false declarations of timber origin (Finkeldey et al. 2010). Even though, the necessity of a viable molecular method for the certification of planting material through vegetative propagation has been discussed and suggested by various research groups (Alvarez et al. 2001; Rajora and Rahman 2003; Fossati et al. 2005), no reports are available on actual use and implementation of DNA barcode tool for certification of vegetative propagation material.

Conclusion

A molecular tool that is not influenced by age, phenological and physiological status, is useful for species certification of planting stock produced in bamboo nurseries. This study could demonstrate the efficiency of DNA barcoding as a reliable supplementary tool in an integrated approach for the proposed certification system in bamboos. *psbA-trnH* DNA barcode region can thus be utilized to authenticate species identity against a database of barcodes of bamboo species generated for this purpose. The tool can be integrated into the framework to comply with requirement for confirming species identity under the certification program and give farmers an assurance of the quality intended in label issued by accredited nurseries. To achieve assured productivity in bamboo species, any national certification agency set up for this purpose can utilize *psbA-trnH* DNA barcode region to tag species identity and to prove the authenticity of multiplied planting materials in all NBM recommended priority bamboo species.

Acknowledgements We are grateful to the Forest Department (KFD), Govt. of Kerala and Karnataka as well as Forest Departments of the States of Arunachal Pradesh, Meghalaya, Mizoram for their permission to collect leaf samples. The financial support received from National Bamboo Mission (NBM), Govt. of India is gratefully acknowledged.

Author contributions SAD: developing the concept, getting financial support, designing the wet lab experiments and writing paper. SK: conducting wet lab experiments and writing paper. PPS: conducting wet lab experiments. SVB: involved in sample collection and writing paper. EMM: involved in sample collection and writing paper.

Funding National Bamboo Mission (NBM), Government of India Grant (F.NO. 44-73/2018-NBM/KFRI 688.12/2014).

Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

Data archiving statements All the generated barcode gene sequences are submitted to GenBank and are available in the following (https://www.ncbi.nlm.nih.gov/genbank/). GenBank accession numbers are provided in Table 2.

References

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

Balasaravanan T, Chezhian P, Kamalakannan R, Yasodha R, Varghese M, Gurumurthi K, Ghosh M (2006) Identification of species-diagnostic ISSR markers for six Eucalyptus species. Silvae Genetica 55:1–6. https://doi.org/10.1515/sg-2006-0017

Becerra V, Paredes M, Gutiérrez E, Rojo C (2015) Genetic diversity, identification, and certification of Chilean rice varieties using molecular markers. Chilean J Agri Res. https://doi.org/10.4067/S0718-58392015000400001

Botta R, Marinoni D, Beccaro G, Akkak A, Bounous G (2001) Development of a DNA typing technique for the genetic certification of the chestnut cultivars. For Snow Landsc Res 76:425–428

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

BTSG (2014) Certification of Bamboo Planting Material for Area Expansion Programme under National Bamboo Mission, Bamboo Technical Support Group- KFRI, Kerala Forest Research Institute, Peechi. https://nbm.nic.in/PDF/CertificationNBM15.05.2014.pdf.

Cai ZM, Zhang YX, Zhang LN, Gao LM, Li DH (2012) Testing four candidate barcoding markers in temperate woody bamboos (Poaceae: Bambusoideae). J Syst Evol 50(6):527–539. https://doi.org/10.1111/j.1759-6831.2012.00216.x

Cbol PWG (2009) A DNA barcode for land plants. Proc Natl Acad Sci USA 106(31):12794–12797. https://doi.org/10.1073/pnas.0905845106

Chase MW, Cowan RS, Hollingsworth PM, van den Berg C, Madrinan S (2007) A proposal for a standardised protocol to barcode all land plants. Taxon 56 (2): 295–299. https://www.jstor.org/stable/25065788

Clark LG, Dransfield S, Triplett JK, Sanchez-Ken JG (2007) Phylogenetic relationships among the one-flowered, determinate genera of Bambuseae (Poaceae: Bambusoideae). Aliso 23(1):315–332. https://doi.org/10.5642/aliso.20072301.26

Das MM, Mahadani P, Singh R, Karmakar K, Ghosh SK (2013) MatK sequence based plant DNA Barcoding failed to identify *Bambusa* (Family: Poaceae) species from Northeast India. J Env Sociobiol 10(1):49–54

Degen B, Holtken A, Rogge M (2010) Use of DNA-fingerprints to control the origin of forest reproductive material. Silvae Genetica 59:268–273. https://doi.org/10.1515/sg-2010-0038

de-Lucas AI, Santana JC, Recio P, Hidalgo E (2007) SSR-based tool for identification and certification of commercial *Populus* clones in Spain. Ann For Sci 65(1):107. https://doi.org/10.1051/forest:2007079

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

Finkeldey R, Leinemann L, Gailing O (2010) Molecular genetic tools to infer the origin of forest plants and wood. Appl Microbiol Biotechnol 85:1251–1258. https://doi.org/10.1007/s00253-009-2328-6

FSI (2011) State of forest report. Forest Survey of India (Ministry of Environment & Forests), Dehradun

Fossati T, Zapelli I, Bisoffi S, Micheletti A, Vietto L, Sala F, Castiglione S (2005) Genetic relationships and clonal identity in a collection of commercially relevant poplar cultivars assessed by AFLP and SSR. Tree Genet Genom 1:11–19. https://doi.org/10.1007/s11295-004-0002-9

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

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc Natl Acad Sci USA 101:14812–14817. https://doi.org/10.1073/pnas.0406166101

Hollingsworth PM, Clark A, Forrest LL, Richardson J, Pennington RT (2009) Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. Mol Ecol Resour 9:439–457. https://doi.org/10.1111/j.1755-0998.2008.02439.x

Jo IH, Kim YC, Kim DH, Kim KH, Hyun TK, Ryu H, Bang KH (2016) Applications of molecular markers in the discrimination of Panax species and Korean ginseng cultivars (Panax ginseng). J Ginseng Res 41(4):444–449. https://doi.org/10.1016/j.jgr.2016.09.001

Kelchner SA, Clark LG (1997) Molecular evolution and phylogenetic utility of the rpl16 intron in *Chusquea* and the Bambusoideae (Poaceae). Mol Phylogenet Evol 8(3):385–397. https://doi.org/10.1006/mpev.1997.0432

Khan AU, Hazra A (2007) Industrialisation of the bamboo sector: Challenges and Opportunities. India Development Foundation, Publication 15. Confederation of Indian Industry (CII), New Delhi

Konnert M, 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, 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(1):e508. https://doi.org/10.1371/journal.pone.0000508

Kumar M (1991) Mass production of field planting stock of *Dendrocalamus strictus* through macroproliferation—a technology. Ind For 117(12):146–152

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

Kumar M, Remesh N, Unnikrishnan N (2004) A new combination in *Dendrocalamus* (Poaceae: Bambusoideae). SIDA Contrib Bot 21:93–96

Kumari P, Singh P (2009) Two new species of *Bambusa* (Poaceae) from India. Kew Bull 64:565–569

Lahaye R, van der Bank M, Bogarin D, Warner J, Pupulin F (2008) DNA barcoding the floras of biodiversity hotspots. Proc Natl Acad Sci USA 105(8):2923–2928. https://doi.org/10.1073/pnas.0709936105

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

Meier R, Shiyang K, Vaidya G, Ng PK (2006) DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. Syst Biol 55(1):715–728. https://doi.org/10.1080/10635150600969864

Meyer CP, Paulay G (2005) DNA barcoding: error rates based on comprehensive sampling. PLoS Biol 3(12):2229–2238. https://doi.org/10.1371/journal.pbio.0030422

Muzzalupo I, Muto A, Badolati G, Veizi A, Chiappetta A (2018) Genotyping of Albania olive (*Olea europaea*) germplasm by SSR molecular marker. Emir J Food Agric 30(7):573–580. https://doi.org/10.9755/ejfa.2018.v30.i7.1740

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

Nam IY, Zayakin VV, Artjuchova AV, Lukashevich MI, Kupzov NS (2014) Identification and certification of lupine cultivars using

molecular markers. World Appl Sci J 30:796–801. https://doi.org/10.5829/idosi.wasj.2014.30.07.14076

NBM (National Bamboo Mission) (2013) This information is retrieved on 17 December 2013 from https://www.nbm.nic.in

Newmaster SG, Fazekas AJ, Steeves RAD, Janovec J (2007) Testing candidate plant barcode regions in the Myristicaceae. Mol Ecol Notes 8(3):480–490. https://doi.org/10.1111/j.1471-8286.2007.02002.x

Newmaster SG, Ragupathy S (2009) Testing plant barcoding in a sister species complex of pantropical Acacia (Mimosoideae, Fabaceae). Mol Ecol Res 9:172–180. https://doi.org/10.1111/j.1755-0998.2009.02642.x

Nkongolo KK, Michael P, Demers T (2005) Application of ISSR, RAPD, and cytological markers to the certification of *Picea mariana*, *P. glauca*, and *P. engelmannii* trees, and their putative hybrids. Genome 48(2):302–311. https://doi.org/10.1139/g04-118

Ohrnberger D, Georrings J (1986) The bamboos of the World. International Book Publishers, Dehra Dun

Rajora OP, Rahman MH (2003) Microsatellite DNA and RAPD fingerprinting, identification and genetic relationships of hybrid poplar (*Populus* × *canadensis*) cultivars. Theoretical Appl Genet 106:470–477. https://doi.org/10.1007/s00122-002-1082-2

Sanchez N, Grau JM, Bueno MA (1998) RAPD markers for the identification of *Populous* species. Silvae Genet 47:67–71

Sharma A (2008) Bamboo Industry Eyes Slice of $7.5 Bn World Market. The Financial Express, p,20. https://www.financialexpress.com/news/bamboo-industry-eyes-slice-of-7.5-bn-worldmkt/299457/

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

Starr JR, Naczi RFC (2009) Plant DNA barcodes and species resolution in sedges (*Carex*, Cyperaceae). Mol Ecol Res. https://doi.org/10.1111/j.1755-0998.2009.02640.x

Taberlet P, Coissac E, Pompanon F, Gielly L, Miquel C (2007) Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. Nuc Acids Res 35(3):e14. https://doi.org/10.1093/nar/gkl938

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA 6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. https://doi.org/10.1093/molbev/mst197

Torres-Dini D, Bennadji Z, Cabrera M, Centurion C, Resquin F, Balmelli G (2011) Use of SSR-tools for clone certification in Uruguayan *Eucalyptus grandis* and *Eucalyptus dunnii* breeding programs. BMC Proc 5(Suppl 7):P58. https://doi.org/10.1186/1753-6561-5-S7-P58

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nuc Acids Res 22:4673–4680. https://doi.org/10.1093/nar/22.22.4673

Thorat AS, Sonone NA, Choudhari VV, Devarumath RM, Babu KH (2018) Plant regeneration from direct and indirect organogenesis and assessment of genetic fidelity in Saccharum officinarum using DNA based markers. Biosci Biotech Res Commun. https://doi.org/10.21786/bbrc/11.1/9

Tripathi SK, Sumida A, Shibata H, Ono K, Uemura S, Kodama Y, 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. Biol Fertili Soils 43(2):237–436. https://doi.org/10.1007/s00374-006-0100-y

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