



Traditional System Versus DNA Barcoding in Identification of Bamboo Species: A Systematic Review

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Received: 10 March 2021 / Accepted: 11 May 2021 / Published online: 17 May 2021

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Abstract

Bamboo, a gramineous plant belonging to the family *Poaceae*, comprises of 1575 species from 116 genera across the globe. It has the ability to grow and evolve on degraded land and hence, can be utilized in the various applications as an alternative for plastic and wood. DNA barcoding, a long genomic sequence, identifies barcode region which shows species-specific nucleotide differences. This technology is considered as advanced molecular technique utilized for characterization and classification of the various species by applying distinctive molecular markers. Recent investigations revealed the potential application of various barcode regions such as *matK*, *rbcL*, *rpoB*, *rpoC1*, *psbA-trnH*, and *ITS2*, in identification of many bamboo species from different genus. In this review we comprehensively discussed the relevance of DNA barcoding as a tool in classification/identification of various bamboo species. We highlighted the methodology, how this advance technology overcomes the challenges associated with traditional methods along with prospects for future research.

Keywords DNA barcode · Bamboo · Taxonomy · Phylogeny · Molecular technology

Introduction

Bamboo placed to monocotyledonous plant under *Poaceae* family and sub family *Bambusoideae* [1]. There have been diversities of bamboo species found across the globe including India (Fig. 1). Bamboo has three interesting characteristics: peak development pace of 1 m per day (unique to only a few species), blossoming cycle (3 to 120 years) and wide-spread rhizome (sympodial and monopodial) [2–6]. Bamboo from base to top, as its root (rhizome), stem (culm), and leaf is used in various applications like food, fire wood, hand crafts and therapeutic [7, 8]. Bamboo is widely utilized in pulp and paper industries because of its fibre content [9, 10]. Its natural physical structure of culms (pole) is an appealing

component for construction and building businesses, such as material for fortified cement beams [11, 12]. For the past two-decade bamboo has been utilized as fuel, electricity, biochar and charcoal which is an efficient way of controlling environmental contamination [13–17]. Previous studies on physico-chemical properties and mechanical strength of bamboo (rhizome, culm, and leaves) confirms that bamboo species are being utilized in numerous different ventures to deliver various items like furnishings, woven items, trimming/painstaking work, flooring, fibreboard, boards, mash, paper, development/building material, rustic uses, fuel, non-private/horticulture, pressing, and transport [2, 18–20]. Bamboo plantations and its biomass has been practiced in improvement of ecological restoration of degraded/contaminated lands and conversion of mine degraded sites into carbon sinks [11, 21]. Bamboo farming is done using rhizome, tissue culture, or small branch cutting directly without any further verification leads to mis identification and mis classification of bamboo species.

Several traditional identification and classification systems were deployed in the field of bamboo research previously. Researchers/investigators classified bamboo in to 67 genera in nine sub-tribes, on the basis of floral characters such as flower [22], inflorescence [23], ovary and seed [24]. Bamboo species have an unusual life cycle, which diverges

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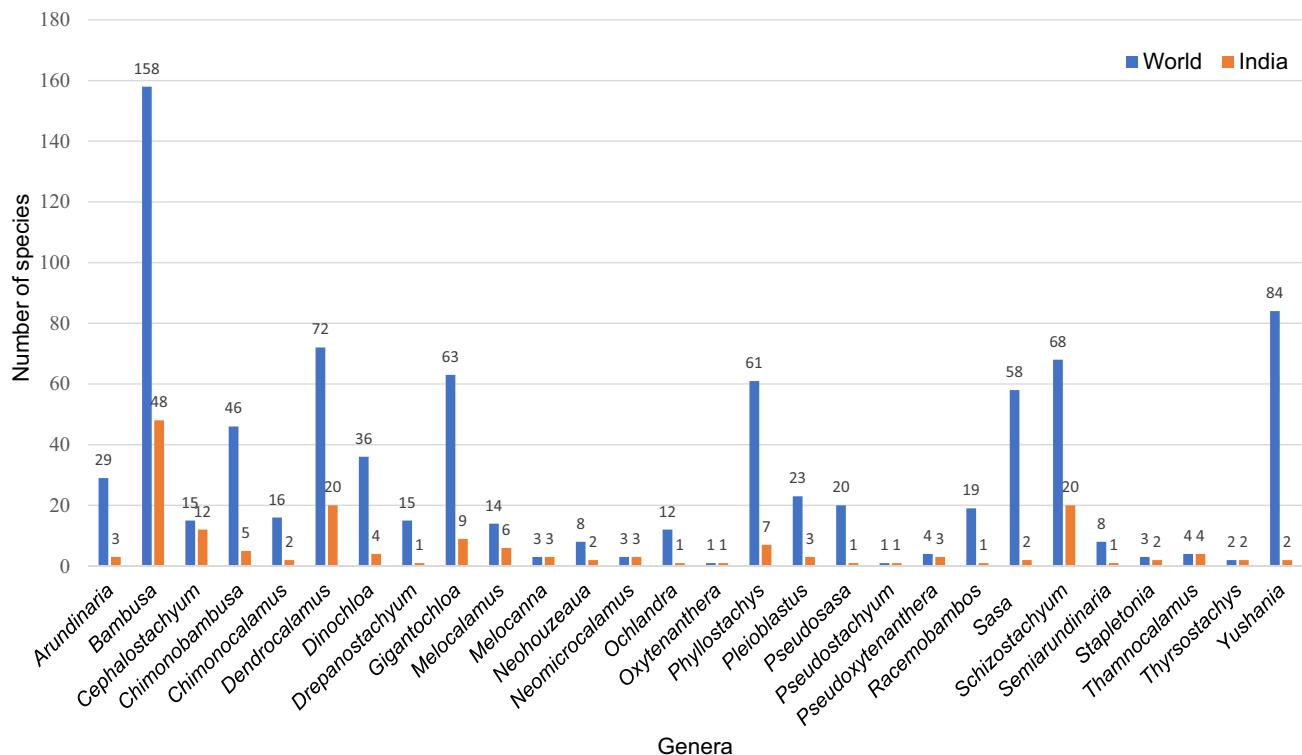


Fig. 1 Distribution of Bamboo species in India with respect to world

from one species to other; vast difference in vegetative growth phase with minimum from one year to maximum up to 120 years. Moreover, some of the species have not been reported flowering in their life cycle [25]. Further, floristic identification of these sterile bamboos is a serious problem and it does not have any solution in systematic classification, where, taxonomic studies are depended completely on floristic characters; moreover, vegetative characters could rapidly change or influenced by various environmental factors, such as soil, climatic factors etc., [26, 27]. Therefore, classification or identification of these bamboos using molecular approach is the best option to overcome problems in systematic classification [28]. Furthermore, utilizing DNA markers could provide solutions to systematic classification as an alternative approach in the studies of bamboo phylogeny and identification (Table 1). In 1997, Molecular identification studies by Clark and Kobayashi on woody bamboos have confirmed a linkage between temperate and tropical woody bamboos (New World and Old World) [29, 30].

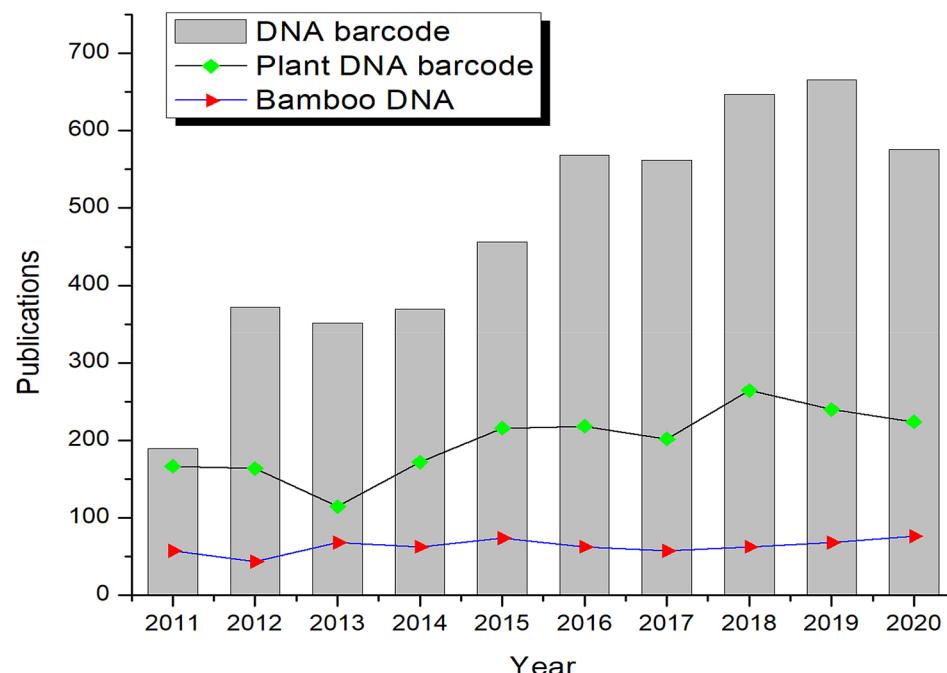
Currently, the application of advance molecular-based technology has become more prominent for characterization, taxonomic and systemic studies of organisms [46–48]. Also, several published researches emerge out in last decade related to application of barcode in identification of plants species (Fig. 2). These methodologies consist of RAPD (random amplified polymorphic DNA)

[49], AFLP (amplified fragment length polymorphism), RFLP (restriction fragment length polymorphism), ISSR (inter simple sequence repeat), iPBS (inter primer binding site), and SCoT (start codon targeted). These molecular markers give accurate and detailed information regarding the genetic diversity [41, 50, 51], phylogenetic interactions [52, 53] and species-specific marker [54]. DNA barcoding is only one of its kind methods for characterization and classification of several organisms at species level as well genus level. The last decade witnessed the potentialities of DNA barcoding as a promising tool to supplement the species recognition in plants as well as in animals. RFLP, RADP and AFLP markers used to investigate species identification and creating phylogenetic relationships between various species of *Bambusa*, *Dendrocalamus* and *Phyllostachys* species [55–58]. Some other researchers used ISSR markers and expressed sequence tag (EST) based primers to find molecular relationship among the bamboo species [59, 60].

The present review is designed to consider above facts and focus to prepare a framework with respect to the application and mechanism of DNA barcoding in identification and characterization of bamboo species. A detailed discussion on, how this method overrides the traditional identification and classification methods, along with opportunities, challenges and prospects for future research.

Table 1 List of markers along with its various characteristics [31–45]

Function	SCoT	RAPD	ISSR	iPBS	AFLP	RFLP
Prior seq. information required	No	No	No	No	No	Yes
Rapid/Speed	Fast	Fast	Fast	Fast	Slow	Rapid
Input sample DNA amount	Small	Small	Small	Small	Small	Large
Reproducibility	Highly	Low	High	—	Low	High
Informative	More informative	Less	Medium	Informative	More Informative	Informative
Reliability	More reliable	Low	Medium	Reliable	More Reliable	Highly Reliable
Cost	Expensive	Inexpensive	Low	Medium	Inexpensive	Expensive
Popularity	Less popular	Most Popular	More Popular	Less Popular	More Popular	Popular
Dominant Marker	Yes	Yes	Yes	—	Yes	Codominant
Simpler	Yes	Yes	Yes	—	No	No
Complexity	No	No	No	—	Yes	Yes
Radioactive labelling desirable	—	No	No	—	Yes	Yes
Restriction enzyme required	—	No	No	—	Yes	Yes
Gene-targeted	Yes	No	No	Yes	No	—
Primer length	Longer	Short	Long	—	—	Longer
DNA fingerprinting technique	Yes	Yes	Yes	Yes	Yes	Yes
Produces polymorphic fragments (bands)	High	Lowest	Lower	—	High	—

Fig. 2 Publication status of DNA barcode and Bamboo DNA in last decade

Traditional System of Classification Versus DNA Barcoding

Traditional system of plant identification i.e., floral taxonomy has several problems that hinder its progress. Few plant species are only known from their original descriptions and several others are yet to be identified [61]. Morphological categorization of plant species is generally

reliant on vegetative features that are simply governed by environmental variables [26]. Morphological systematic is based on morphological features, comparative anatomy and other vegetative characteristics [62]. Systematic identification would be beneficial in visual determination of species at various levels of classification by using their macromorphological characters, however, there are many drawbacks in the systematic [59]. Morphological characteristics, the properties of culm/culm sheaths and leaves

are highly crucial to distinguish or identify bamboo species only in its matured state or in the farming field but this method is not always reliable. Table 2 describes 7 key attributes of bamboo which are used for species identification in the traditional method by most of the people who work in the bamboo sector from farm to industry. Bamboo culms height, diameter, and internode length are the countable quality yet can't be used in species identification purpose (Fig. 3). These three attributes (culms height, width, and internode length) are not valuable to distinguish among same genus species like *Dendrocalamus* (*hamiltonii* and *latiflorus*) because these two species have roughly same tallness (20 m), same width (16 cm), same internode length (45 cm). Likewise, *Gigantochloa* (*apus* and *pseudoarundinacea*). Not only that, but this attribute is not adequate to compare and distinguish between different genus as (*Bambusa bambos* and *Gigantochloa levis*), (*Dendrocalamus asper* and *Bambusa balcooa*), (*Bambusa vulgaris* and *Cephalostachyum pergracile*), (*Bambusa tulda*, *Gigantochloa atrovirens* and *Melocanna baccifera*), (*Thyrsostachys oliveri* and *Bambusa cacharensis*) and (*Bambusa nana*, *Gigantochloa rostrata* and *Ochlandra scriptoria*) [7].

Therefore, the traditional method of bamboo species identification depends on these attributes is not reliable. In some species, the morphological characters typically used in

the conventional taxonomic classification of bamboos show similar affinities. In addition, recognition and grouping of bamboo using anatomical characteristics have failed to prove effective. Various taxonomists have identified the same bamboo species in separate genera or same species which have culminated in several revisions in different genera during the last several decades. Due to the high degree of morphological resemblance, in the field recognition of certain bamboo species is exceedingly challenging even for a taxonomy specialist. Therefore, identifying bamboo species on their physical and morphological characteristics is not reliable.

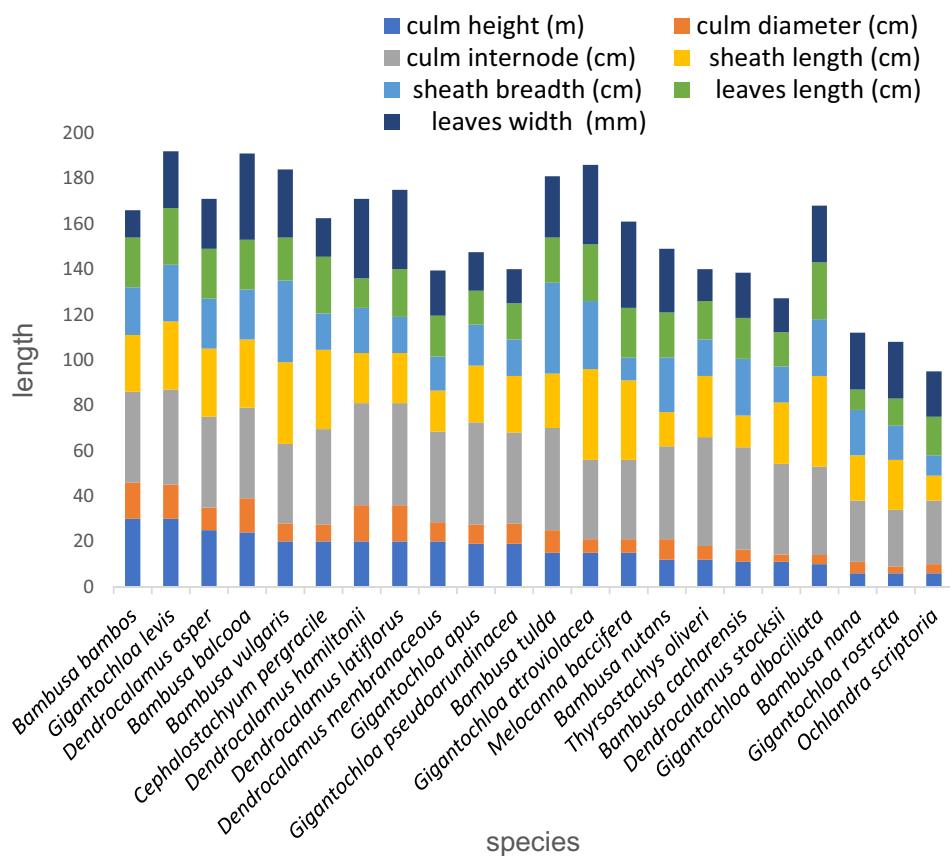
DNA polymorphism is the substitute for traditional systematic to differentiate individuals or species and classify them according to molecular approach to overcome systematic classification problems. DNA barcoding is best biotechnological approach to subsidise the complications of traditional taxonomy [67]. Short sequence of genome (< 1000 bp) acting as a “DNA barcode”, which could be able to identify a species or a taxonomical group. The study “DNA barcode” expresses that the uniform genetic sequences can categorize taxon in the identical manner as the 11-digit Worldwide merchandise Code recognizes retail merchandises in market [68].

Molecular operational taxonomic unit (MOTU) is a molecular system for identification of various taxa. It is fast and perfect identification tool, and also useful in solving

Table 2 A comparison of the 7 key attributes of culm, culm-sheath and leaves descriptors used to evaluate phylogenetic relationships among 22 bamboo species from 7 genera: A—average culm height (m), B—average culm diameter (cm), C—average culm internode length (cm), D—average culm-sheaths length at base (cm), E—average culm-sheaths breadth at base (cm), F—average leaves length (cm), G—average leaves width (mm)

Species	Culm			Culm sheaths		Leaves	
	A	B	C	D	E	F	G
<i>Bambusa balcooa</i>	24	15	40	30	22	22	38
<i>B. bambos</i>	30	16	40	25	21	22	12
<i>B. cacharensis</i>	11	5.5	45	14	25	18	20
<i>B. nana</i>	6	5	27	20	20	9	25
<i>B. nutans</i>	12	9	41	15	24	20	28
<i>B. tulda</i>	15	10	45	24	40	20	27
<i>B. vulgaris</i>	20	8	35	36	36	19	30
<i>Cephalostachyum pergracile</i>	20	7.5	42	35	16	25	17
<i>Dendrocalamus asper</i>	25	10	40	30	22	22	22
<i>D. hamiltonii</i>	20	16	45	22	20	13	35
<i>D. latiflorus</i>	20	16	45	22	16	21	35
<i>D. membranaceus</i>	20	8.5	40	18	15	18	20
<i>D. stocksii</i>	11	3.2	40	27	16	15	15
<i>Gigantochloa albociliata</i>	10	4	39	40	25	25	25
<i>G. apus</i>	19	8.5	45	25	18	15	17
<i>G. atrovirens</i>	15	6	35	40	30	25	35
<i>G. levis</i>	30	15	42	30	25	25	25
<i>G. pseudoarundinacea</i>	19	9	40	25	16	16	15
<i>G. rostrata</i>	6	3	25	22	15	12	25
<i>Melocanna baccifera</i>	15	6	35	35	10	22	38
<i>Ochlandra scriptoria</i>	6	4	28	11	9	17	20
<i>Thyrsostachys oliveri</i>	12	6	48	27	16	17	14

Fig. 3 Species from different genus which are showing similar value for attributes of culm (height, diameter and internode length), sheaths (length and breadth), leaves (length and width) [31, 63–66]



problems in species evolution point of view. Lambert et al., [69] studied diversity of the Earth's biota by using molecular approach by means of DNA barcoding. Generally, to study DNA barcode includes several key attributes, which include the DNA marker used as a barcode sequence should be appropriate for wide range of taxa, it should able to differentiate among various species and should be conserved within the species, therefore the intraspecific deviation will be non-significant [70, 71]. DNA barcode correspond to the sequence in the form of nucleotides ATCG, which representing adenine, thymine, cytosine and guanine. DNA barcoding could able to serve dual purpose as an advanced device in the new era taxonomists in the enhancement of systematic knowledge as well as being an modern tool for non-taxonomists who require to create a speedy classification [72]. In recent days, there are several researchers are attempting and creating multiple genomic databases, these databases are open for all and any one can use out of it for the classifying the organism/s [73]. The purpose of creating this molecular systematic approach is to facilitate and validate the existing morphological species identifications and support the speeding up of species discoveries and solving the problems in systematic approach through creation of these barcode databases [74]. Molecular database is relatively an easy approach to compare species in a group or identify individual species

and to determine distance correlation between the species [75].

Application of DNA Barcoding Technology in Bamboos

Utilization of molecular marker is one of best approaches to overcome the difficulties in identification of genotypes in bamboos [76]. Various DNA sequences were worked out to surmise phylogenetic interactions among the bamboos, however, deviation among the studied DNA sequence was very less or various DNA markers have showed similarity in the studied species [77–80]. Table 3 summarizes the molecular approaches consequently carry light to the challenging field in the taxonomical classification of several bamboos sp. at various systematic level.

Molecular markers techniques such as SSR, ISSR, RFLP, RAPD, SCoT, AFLP and SNP were in use by several researchers to distinguish the bamboos at species or generic levels. Glimpses of these techniques being used in identification, classification, characterization, cluster analysis or genetic mapping of bamboo species across the world as follows:

Table 3 Summary of work done on molecular taxonomy of different bamboos

Studied taxa of bamboo	Number of genera	Number of spp.	Work/regions compared/sampling strategy/recommendation	Country/year	Study
<i>Bambusa balcooa</i> , <i>B. bambos</i> , <i>B. nutans</i> , <i>B. pallida</i> , <i>B. tulda</i> , <i>B. vulgaris</i> ; <i>Dendrocalamus asper</i> , <i>D. giganteus</i> , <i>D. hamiltonii</i> , <i>D. strictus</i> ; <i>Melocanna baccifera</i> , <i>Ochlandra travancorica</i> and <i>Oxytenanthera parviflora</i>	5	13	Six of the seven barcode loci (four coding's: matK, rbcL, rpoB, rpoC1, and three intergenic spacers: psbA-trnH, psbK-psbI and atpF-atpH) display similar sequences in the <i>Bambusa</i> , <i>Dendrocalamus</i> , <i>Oxytenanthera</i> , <i>Melocanna</i> and <i>Ochlandra</i> groups. In the genus <i>Dendrocalamus</i> only <i>psbA-trnH</i> displayed species-specific nucleotide variations. Thus, <i>psbA-trnH</i> can be used as a DNA barcode region for species identification of many bamboo taxa	India/2020	[81]
<i>Bambusa cacharensis</i> , <i>B. mizoramensis</i> ; <i>Dendrocalamus manipureanus</i> , <i>D. hamiltonii</i> and <i>D. sikkimensis</i>	2	5	The comparative analysis of four markers (RAPD, ISSR, iPBS, and SCOT) systems using 10 primers of each marker used to examine genetic polymorphism and the association of 50 genotypes of 5 bamboos. According to polymorphic information content (PIC), effective multiplex ratio (EMR), and marker index (MI) values, SCOT is more descriptive than the other three markers with higher discriminating capacity	North East India/2020	[31]
<i>Bambusa manipureana</i> , <i>B. mizoramensis</i> , <i>B. nutan</i> , <i>B. vulgaris</i> , <i>B. tulda</i> ; <i>Dendrocalamus giganteus</i> , <i>D. hamiltonii</i> , <i>D. sikkimensis</i> , <i>D. hookeri</i> , <i>D. longispathus</i> , <i>D. manipureanus</i> ; <i>Schizotachyum dullooa</i> , <i>S. pergracile</i> , <i>S. munroi</i> , <i>S. fuchsianum</i>	3	15	By using ISSR marker, finding the phylogenetic connection between 5 different bamboo species from North-East India. The genetic association of these species as discovered by the dendrogram and PCoA study was fairly compatible with the conventional classification method, although there was some difference	Manipur, India/2018	[32]
<i>Bambusa balcooa</i> , <i>B. bambos</i> , <i>B. multiplex</i> , <i>B. tulda</i> , <i>B. vulgaris</i> ; <i>Phyllostachys vivax</i>	2	6	Analyse genetic diversity in 13 bamboo genotypes using RAPD and ISSR. Out of 183 (120 RAPD and 63 ISSR) primers, only 42 polymorphic primers (30 RAPD and 12 ISSR), gave reproducible amplification profile. The study shows the presence of large genetic diversity between different elite genotypes of bamboo	Gujarat, India/2015	[41]
<i>Acidosasa purpurea</i> , <i>Bambusa emeiensis</i> , <i>B. oldhamii</i> , <i>Chusquea circinata</i> , <i>Dendrocalamus latiflorus</i> , <i>Gelidocalamus tessellatus</i> , <i>Otatea glauca</i> , <i>Phyllostachys edulis</i>	7	8	There are 3 genomes (nuclear, mitochondrial, and plastid) coexisting in flowering plants. Study complete plastid genome sequences of six bamboos through sequencing of total genomic DNA or PCR-amplified plastid DNA using Illumina technology. Also shows the transfer of mitochondrial DNA to the plastid genome in bamboos	China/2015	[82]

Table 3 (continued)

Studied taxa of bamboo	Number of genera	Number of spp.	Work/regions compared/sampling strategy/recommendation	Country/year	Study
<i>Bambusa balcooa</i> , <i>B. multiplex</i> , <i>B. ventricosa</i> , <i>B. vulgaris</i> , <i>B.wamini</i> ; <i>Dendrocalamus strictus</i> ; <i>Melocanna baccifera</i> ; <i>Phyllostachys nigra</i> ; <i>Sasa fortuneii</i> , <i>S.palmata</i>	5	10	This study focuses on finding of genetic relationship between bamboo species using the ISSR marker. The genetic distance is independent of geographical distance in case of bamboo accessions. The genomic DNA of twenty bamboo accessions collected from different sites of India. Analyse genetic distance using marker ISSR with 8 primers. <i>Bambusa wamini</i> -Itanagar & <i>B. ventricosa</i> - Durg shows highest genetic distance (0.48221) while <i>Bambusa balcooa</i> -Modasa & <i>Bambusa balcooa</i> -Tripura have & minimum genetic distance (0.00787). Some species like <i>B. balcooa</i> and <i>B. vulgaris</i> were genetically close to as each other	India/2015	[83]
Moso bamboo (<i>Phyllostachys edulis</i>)	1	1	The first genome-wide microsatellites in <i>Moso bamboo</i> have been presented and their findings suggest that microsatellite markers are useful in bamboo genomic studies and investigations of associated grass species	China/2015	[84]
<i>Bambusa</i> and <i>Dendrocalamus</i>	2	–	The five barcode loci (<i>rbcL</i> , <i>matK</i> , <i>trnH-psbA</i> , <i>rpoB</i> , <i>rpoC</i>) are failed to demonstrate the differences but when the <i>trnH-psbA</i> combine with other loci then it is more effective for discriminating bamboos belonging to the genera <i>Bambusa</i> and <i>Dendrocalamus</i>	Kerala, India/2014	[85]
<i>Phyllostachys pubescens</i>	1	1	The relationship between SSR and transposable elements was examined, and the outcrossing levels in <i>Phyllostachys pubescens</i> were estimated. Using SSR markers for full-length cDNAs (FL-cDNAs) to distinguish parental stocks of interspecific bamboo hybrids inside and outside <i>P. pubescens</i> , and measure the outcrossing rate for <i>P. pubescens</i>	Zhejiang Province, China/2014	[86]
21 species of Poaceae family out of which 3 are belong to subfamily <i>Bambusoideae</i> (<i>Bambusa emerensis</i> ; <i>Dendrocalamus latiflorus</i> ; <i>Indocalamus longiauritus</i>)	3	3	The discriminatory influence of <i>matK</i> has been identified in the <i>Poaceae</i> family. The <i>matK</i> gene may be a successful DNA barcoding nominee for the <i>Poaceae</i> family of grasses	India/2014	[87]
<i>Bambusa lapidea</i> , <i>B. teres</i> , <i>Bonia amplexicaulis</i> , <i>Bonia saxatilis</i> ; <i>Dendrocalamus barbatus</i> , <i>D.birmanicus</i> , <i>D.brandisii</i> , <i>D.calostachyus</i>	3	8	The combined DNA barcodes loci (<i>rbcL</i> + <i>matK</i>) have quite low discriminatory power in bamboos, due to which its need to select the other effective DNA barcodes for bamboos. The <i>trnG-trnT(t)</i> + X can be used in DNA barcoding and molecular-phylogeny reconstruction study of bamboos. In the case of bamboos, the combined barcode region (<i>rbcL</i> + <i>matK</i>) has low discriminatory power whereas spacer <i>trnG-trnT</i> has shown high discriminatory power	China/2013	[88]

Table 3 (continued)

Studied taxa of bamboo	Number of genera	Number of spp.	Work/regions compared/sampling strategy/recommendation	Country/year	Study
<i>Bambusa bambos; Dendrocalamus strictus; Pseudoxytenanthera ritcheyi, P.stockii</i>	3	4	PCR-RAPD DNA profiling approach has proved to be outstanding for identification, phylogenetic study, population analyses, and genetic similarity mapping of several types of bamboo (<i>B. bambos</i> , <i>P. ritcheyi</i> , <i>P. stockii</i> and <i>D. strictus</i>). The findings will be helpful to build SCAR markers for each species	Maharashtra, India/2013	[89]
<i>Arthrostylidium excelsum, Aulonemia laxa; Chusquea aperta, C.bilimekii, C.circinata, C.coronalis, C.foliosa, C.glaucia, C.liebmanni, C.longifolia, C.mulleri, C.nelsonii, C.peroensis, C.pittieri, C.repens, C.sulcate; Guadua aculeata, G.amplexifolia, G.paniceolata, G.velutina; Olmecea clarkiae, O.fulgur O.recta, O.reflexa, O.zapotecorum; Otaea acuminata, Otaea carilloi, Otaea fimbriata, Otaea glauca, Otaea reynosana, Otaea transvolcanica, Otaea ximenesae; Rhipidochadum bartlettii, R.martinezi, R.pintieri, R.racemiflorum</i>	36	36	Created DNA barcode library for 36 species of bamboo. Evaluate and Analyse the efficiency of the barcode loci of <i>matK</i> and <i>rbcL</i> on bamboos. The <i>psbK</i> - <i>K</i> spacer obtained further polymorphic sites and mixing with <i>matK</i> it has been able to discriminate bamboos to at least the generic level. The performance and function of seven plastid DNA regions (three-gene: <i>matK</i> , <i>rbcL</i> , <i>rpoB</i> , <i>rpoC</i> , <i>I</i> and four spacers: <i>atpF-aphH</i> , <i>psbK-psbI</i> , <i>trnH-psbA</i>) has been controlled by Plant Working Group. In temperate bamboos, evaluated the efficiency and discriminant power of barcode region like (gene: <i>matK</i> & <i>rbcL</i>) or it can be the intergenic spacer (<i>psbK-psbI</i> & <i>matK+rbcL</i>)	Mexico/2013	[90]
<i>Bambusa balcooa, B.bambooos, B.nutans B.tulda, B.vulgaris; Dendrocalamus asper, D.giganteus, D.hamiltonii, D.strictus; Guadua angustifolia</i>	3	10	Analyse 25 markers (21 RAPD and 4 ISSR) that used for the identification of 10 bamboo species from three different genera. 10 primer used for the analysis and evaluation of genetic variation estimations. This PCR based marker (RAPD and AFLP) do not require any prior genome sequence knowledge to study. Evaluate and investigate the genetic relationship this bamboo species	Chhattisgarh, India/2013	[51]
<i>Bambusa arundinacea, B.balcooa, B.cacharensis, B.nutans Ochlandra travancorica</i>	1	4	Because of interspecific hybridization and polyploidy, the barcode region <i>matK</i> , failed to differentiate <i>Bambusa</i> species	Northeast India/2013	[91]
	1	1	Molecular analysis using AFLP and RAPD has been done. Fifty primers (8 AFLP & 42 RAPD) had 914 polymorphic loci identified. Cluster and PCA based on combined AFLP & RAPD data grouped all the random accessions into three separate populations	Kerala, India/2013	[92]

Table 3 (continued)

Studied taxa of bamboo	Number of genera	Number of spp.	Work/regions compared/sampling strategy/recommendation	Country/year	Study
<i>Acidosasa purpurea</i> , <i>Ampelocalamus microphyllus</i> , <i>Arundinaria fargesii</i> ; <i>Chimonobambusa angustifolia</i> , <i>C.ningnanica</i> , <i>C.convoluta</i> , <i>C.utilis</i> ; <i>Chimonocala-</i> <i>mus fimbriatus</i> , <i>C.griffithianus</i> ; <i>Fargesia macclureana</i> , <i>F.yunnanensis</i> ; <i>Indocalamus bashanensis</i> , <i>I.longiauritus</i> , <i>I.pseudostimicus</i> , <i>I.tessellatus</i> ; <i>Indosasa shibataeoides</i> , <i>I.sinica</i> ; <i>Oligostachyum scaberriflorum</i> ; <i>Phyllostachys edulis</i> , <i>P.nidularia</i> ; <i>Pleioblastus macrurus</i> ; <i>Pseudosasa amabilis</i> , <i>P.hinakii</i> ; <i>Yushania confusa</i> , <i>Y.grammatica</i> , <i>Y.polytricha</i> , <i>Y.yadongensis</i>	13	27	The feasibility of the four (<i>matK</i> , <i>rbcL</i> , <i>psbA-trnH</i> , <i>ITS2</i>) proposed barcoding loci are tested. In temperate woody bamboos, a combination of <i>rbcL</i> + <i>ITS2</i> has a potential barcode for species identification and discrimination	China/2012	[93]
<i>Dendrocalamus membranaceus</i>	1	1	ISSR marker is used for analysing the genetic diversity of this species in Yunnan, China. This molecular variance study showed that there is a substantial genetic difference across populations, i.e. (78.95%) variation within the populations and only (21.05%) are found among populations. Mantel tests revealed no clear association of genetic and geographic distances between populations	China/2012	[94]
<i>Bambusa balcooa</i> ; <i>Dendrocalamus asper</i> , <i>D.brandisii</i> , <i>D.calostachyus</i> , <i>D.giganteus</i> , <i>D.hamiltonii</i> , <i>D.membranaceus</i> , <i>D.sahni</i> , <i>D.sikkimensis</i> , <i>D.somdevai</i> , <i>D.strictus</i> , <i>Dinochloa macclellandii</i> ; <i>Melocalamus compac-</i> <i>tiflora</i> ; <i>Oxytenanthera abyssinica</i> ; <i>Thysostachys siamensis</i>	6	15	In the present study AFLPs, generated using five primer combinations, were used to investigate relationships between 10 <i>Dendrocalamus</i> and 5 outgroup species	India/2011	[95]
<i>Actidosasa purpurea</i> ; <i>Bambusa emeiensis</i> ; <i>Terracalamus rimosivaginus</i> ; <i>Indocalamus longiauritus</i> ; <i>Phyllostachys edulis</i> , <i>P.nigra</i> var. <i>henonis</i>	5	6	Relation between three subfamilies (<i>Bambusoideae</i> , <i>Pooideae</i> and <i>Ehrhartoideae</i>) of <i>Poaceae</i> are controversial. This research utilizes Illumina sequencing to reveal the entire nucleotide sequences of six woody bamboo chloroplast (cp) genomes. These cp genomes can be used for the development of biotechnological applications and it describe the evolutionary history of the whole grass family	China/2011	[96]
<i>Bambusa bambos</i> , <i>B.blumei</i> , <i>B.glaucescens</i> , <i>B.heterostachya</i> , <i>B.multiplex</i> , <i>B.variegata</i> , <i>B.texilis</i> , <i>B.tulda</i> , <i>B.tuldoides</i> , <i>B.vulgaris</i> , <i>B.vulgaris</i> cv <i>vittata</i> , <i>B.vulgaris</i> cv <i>wavamin</i> ; <i>Ceph-</i> <i>alostachyum pergracile</i> ; <i>Dendrocalamus asper</i> , <i>D.brandisii</i> , <i>D.giganteus</i> , <i>D.strictus</i> ; <i>Dendrochloe levii</i> ; <i>Gigantochloa apus</i> , <i>G.arrovillacea</i> , <i>G.ligulata</i> , <i>G.ridleyi</i> , <i>G.rostrata</i> , <i>G.sceriechnii</i> , <i>G.verticillata</i> ; <i>Melocanna baccifera</i> ; <i>Phyllos-</i> <i>tachys pubescens</i> , <i>P.glaucua</i> , <i>P.viridi-glaucescens</i> ; <i>Schizos-</i> <i>tachym brachycladum</i> , <i>S.jaculans</i> ; <i>Teinostachyum dullooa</i> ; <i>Thysostachys siamensis</i>	10	33	The nuclear DNA content (genome size) has been calculated. The examined genome size was between 2.5 and 5.9 pg DNA per 2C nucleus. Such knowledge would be valuable for researchers employed in numerous fields including biotechnology, bioinformatics, ecology, gene study, plant propagation, physiology, and molecular biology	Singapore, Malaysia, Thailand/2011	[97]

Table 3 (continued)

Studied taxa of bamboo	Number of genera	Number of spp.	Work/regions compared/sampling strategy/recommendation	Country/year	Study
<i>Bambuseae</i>	--	--	Described microsatellites in the <i>Bambuseae</i> tribe to research morphologically distinct bamboo species, overcome taxonomic identification, and consider genome architecture. This new set of SSR primers identified in this study will facilitate the use of a DNA marker tool for the identification of species	India/2011 [98]	
<i>Bambusa arundinacea</i> , <i>B. glaucescens</i> , <i>B. sp. (Nangai)</i> , <i>B. nutans</i> , <i>B. sp. (Tapi)</i> , <i>B. tulda</i> , <i>B. ventricosa</i> , <i>B. vulgaris</i> , <i>B. vulgaris</i> var. <i>vittata</i> ; <i>Dendrocalamus giganteus</i> , <i>D. hamiltonii</i> , <i>D. membranaceus</i> , <i>D. strictus</i> ; <i>Melocanna baccifera</i> ; <i>Oxytenanthera nigrovillosa</i> ; <i>Phyllostachys nigra</i> ; <i>Pleioblastus fortunei</i> , <i>P. pumilus</i> ; <i>Schizostachyum pergracile</i> ; <i>Sasa auricoma</i> ; <i>Thysanostachys oliveri</i>	9	22	Using ISSR-PCR marker with expressed sequence tag (EST) dependent random primers, determine the genetic variance and relationships between various bamboo taxa, resulting in an amplification of 220 loci	Bangladesh, Bhutan, China, India, Japan, Myanmar/2010 [60]	
<i>Thamnochalamus spathifloru</i>	1	1	Statistical analyses of the quantitative vegetative character showed considerable high variation between communities, though not within communities. DNA fingerprinting study, however, could not detect any polymorphism between populations or inside populations by applying highly polymorphic random primers	India/2009 [99]	
<i>Bambusa balcooa</i> , <i>B. bambos</i> , <i>B. mugalba</i> , <i>B. nutans</i> , <i>B. pallida</i> , <i>B. polymorpha</i> , <i>B. tulda</i> , <i>B. ventricosa</i> , <i>Dendrocalamus asper</i> , <i>D. brandisii</i> , <i>D. giganteus</i> , <i>D. hamiltonii</i> , <i>D. hookeri</i> , <i>D. sikkimensis</i> , <i>D. stockii</i> , <i>D. strictus</i> ; <i>Melocanna baccifera</i> ; <i>Ochlandra ebracteata</i> , <i>O. scriptoria</i> , <i>O. travancorica</i> ; <i>Oxytenanthera stockii</i> ; <i>Phyllostachys bambusoides</i> ; <i>Schizostachyum dullooa</i> ; <i>Teinostachyum dullooa</i> ; <i>Thyrostachys oliveri</i> , <i>T. siamensis</i>	9	26	RAPD was used in this analysis to distinguish between 26 varieties of bamboo. The screening was performed with 50 random primers and with about 10 reliable tests were obtained. Varieties of <i>Dendrocalamus</i> stand out from other species of bamboo	India/2008 [100]	
<i>Bambusa bambos</i> , <i>B. balcooa</i> , <i>B. multiplex</i> , <i>B. nana</i> , <i>B. nutans</i> , <i>B. polymorpha</i> , <i>B. tulda</i> , <i>B. ventricosa</i> , <i>B. vulgaris</i> ; <i>Dendrocalamus asper</i> , <i>D. giganteus</i> , <i>D. hamiltonii</i> , <i>D. hookeri</i> , <i>D. membranaceus</i> ; <i>Melocanna baccifera</i> ; <i>Ochlandra scriptoria</i> , <i>O. travancorica</i> ; <i>Phyllostachys aurea</i> , <i>P. heteroclada</i> , <i>P. nigra</i> , <i>P. pubescens</i> ; <i>Sasa auricoma</i>	6	23	Genome-based SSR markers for field crops such as rice (<i>Oryza sativa</i>) and sugar cane (<i>Saccharum</i> spp.) can be used for bamboo genome research. To check this, the transferability of 23 bamboo species was tested using 98 mapped SSR primers serving 12 rice linkage classes and 20 SSR primers derived from EST sugarcane. Of the checked markers, 44 (44.9%) rice and 15 (75%) sugarcane SSR primers displayed repeatable replication in at least one bamboo species and were also widely used for phylogenetic and genetic diversity research	India, Thailand, China/2008 [101]	

Table 3 (continued)

Studied taxa of bamboo	Number of genera	Number of spp.	Work/regions compared/sampling strategy/recommendation	Country/year	Study
Moso bamboo (<i>Phyllostachys pubescens</i>)	1	1	Bamboo's genome size (~2034 Mb) is comparable to maize. Around 1000 genome survey sequences (GSS) were created to evaluate the high proportion of repeat elements in the bamboo and maize genomes. Sequence review found a 23.3 percent proportion of repeat elements in the bamboo genome and a maize genome (65.7 percent) respectively [102]	China/2007	
<i>Arundinaria hindsii</i> ; <i>Bambusa atra</i> , <i>B. bambos</i> , <i>B. ventricosa</i> , <i>B. vulgaris</i> ; <i>Dendrocalamus asper</i> ; <i>D. giganteus</i> ; <i>D. longispathus</i> ; <i>Gigantochloa atroviolacea</i>	4	9	The genetic distance is used for maintaining the genetic variation and relationships among the nine bamboo species using RAPD DNA marker. This marker showed a high degree of polymorphism [26]	Sri Lanka/2007	
<i>Bambusa affinis</i> , <i>B. arundinacea</i> , <i>B. atra</i> , <i>B. auriculata</i> , <i>B. balcooa</i> , <i>B. multiplex</i> , <i>B. oliveriana</i> , <i>B. striata</i> , <i>B. tulda</i> , <i>B. vulgaris</i> , <i>B. wamini</i> ; <i>Dendrocalamus giganteus</i> , <i>D. strictus</i> ; <i>Gigantochloa atrovioletacea</i> ; <i>Pseudobambusa kurzii</i>	4	15	Analysed phylogenetic relationships between 15 bamboo species using 32 key morphological attributes (15 culms + 17 culm sheath) and 120 polymorphic loci of genomic DNA produced using RAPD technique. Here the phylogenetic relationship is exposed by the use of dendrogram, PCA and UPGMA which are a valid, commonly used method for the classification of bamboo. RAPD produces accurate markers which are useful in forming relationships between species [103]	Kolkata, India/2007	
<i>Guadua angustifolia</i> , <i>G. amplifolia</i> , <i>G. uncinata</i> , <i>G. superba</i> , <i>G. macroscopula</i> , <i>Bambusa balcooa</i> and <i>B. tulda</i>	1	4	The genetic richness of <i>Guadua angustifolia</i> is measure using microsatellite sequences of rice and sugarcane species [104]	Colombia and central Andes/2007	
<i>Arundinaria manii</i> ; <i>Bambusa arundinacea</i> (<i>B. bambos</i>), <i>B. balcooa</i> , <i>B. clarata</i> , <i>B. multiplex</i> , <i>B. nana</i> , <i>B. nutans</i> , <i>B. tulda</i> , <i>B. ventricosa</i> , <i>B. vulgaris</i> , <i>B. vulgaris</i> var. <i>striata</i> ; <i>Cephalostachyum pergracil</i> ; <i>Dendrocalamus giganteus</i> , <i>D. hamiltoni</i> , <i>D. hookeri</i> , <i>D. patellaris</i> , <i>D. sikkimensis</i> , <i>D. strictus</i> ; <i>Dinochlea macclellandii</i>	5	19	For proper identification of <i>Bambusa balcooa</i> and <i>B. tulda</i> develop a species-specific molecular marker in order to avoid unknowingly adulteration that affects the competence and quantum of paper pulp production. The conversion of species-specific RAPD markers into reliable SCAR markers to identify <i>B. balcooa</i> and <i>B. tulda</i> [54]	Kolkata, India/2005	
			Within a bamboo genus, for the first time six microsatellites, three polymorphic and three monomorphic, were characterized. The importance of the SSR loci in the analysis of genetic diversity of <i>B. arundinacea</i> and other types of cross amplified bamboo were discussed [105]	India/2005	

Table 3 (continued)

Studied taxa of bamboo	Number of genera	Number of spp.	Work/regions compared/sampling strategy/recommendation	Country/year	Study
<i>Acidosasa purpurea</i> ; <i>Ampelocalamus scandens</i> , <i>An. patellaris</i> , <i>An. Actinorrhicus</i> ; <i>Arundinaria alpina</i> , <i>A. gigantea</i> ; <i>Chimonocalamus pallens</i> , <i>C. fimbriati</i> ; <i>Drepanostachyum hookerianum</i> ; <i>Fargesia altior</i> , <i>F. porphyrea</i> , <i>F. yunnanensis</i> , <i>F. sylvestris</i> , <i>F. graciliflora</i> , <i>F. yulongshanensis</i> , <i>F. frigida</i> , <i>F. yunjianjiangensis</i> , <i>F. edulis</i> , <i>F. fungosa</i> , <i>F. communis</i> , <i>F. hygrophila</i> , <i>F. spathacea</i> , <i>F. nitida</i> , <i>F. lishuiensis</i> , <i>F. setosa</i> , <i>F. murielae</i> ; <i>Gauligongshania megalophyra</i> ; <i>Thinnocalamus spathiflora</i> , <i>T. spathiflorus</i> var. <i>crassinodus</i> , <i>T. tessellatae</i> ; <i>Yushania bojeiana</i> , <i>Y. falcatiaurita</i> , <i>Y. oblonga</i> , <i>Y. polysticha</i> , <i>Y. niitakayamensis</i>	11	35	The present paper addressed the phylogenetic relationship among 33 species (35 species in the ITS analysis) of the <i>Thinnocalamus</i> group and its allies inferred from partial sequences of the nuclear granule-bound starch synthase (GBSS1) gene and from those of the nuclear ribosomal ITS spacer was discussed	China/2004	[106]
<i>Bambusa arundinacea</i> , <i>B. balcooa</i> , <i>B. multiplex</i> , <i>B. ventricosa</i> , <i>B. vulgaris</i> , <i>B. vulgaris</i> var. <i>striata</i> ; <i>D. giganteus</i> , <i>D. strictus</i> ; <i>Dinoecoa macclllandii</i> ; <i>Cephalostachyum pergracil</i> ; <i>Sasa species Makino & Shibata</i>	5	12	The identification and genetic associations of 12 bamboo species were investigated using RAPD. The research began with the use of thirty 10-mer primers that helped us to discern twelve species and pick a reduced collection of primers. The RAPD methodology is capable of being used in species recognition and genetic associations between taxa and bamboo species for the breeding program	India/2003	[107]
<i>Guadua angustifolia</i> , <i>G. angustifolia</i> , <i>G. uncinata</i> , <i>G. superba</i> , <i>G. amplexfolia</i> , <i>G. macrospiculata</i> , <i>G. angustifolia</i> var <i>nigra</i> <i>Ampelocalamus actinofractus</i> ; <i>Thinnocalamus spathiflorus</i> ; <i>Fargesia altior</i> , <i>F. communis</i> , <i>F. edulis</i> , <i>F. graciliflora</i> , <i>F. jungosa</i> , <i>F. hygrophila</i> , <i>F. lishuiensis</i> , <i>F. muritiae</i> , <i>F. nitida</i> , <i>F. porphyrea</i> , <i>F. setosa</i> , <i>F. spathacea</i> , <i>F. syvestris</i> , <i>F. yunjianjiangensis</i> , <i>F. yulongshanensis</i> , <i>F. yunnanensis</i> ; <i>Yushania bojeiana</i> , <i>Y. falcatiaurita</i> , <i>Y. niitakayamensis</i> , <i>Y. oblonga</i> , <i>Y. polysticha</i>	1	7	AFLP used for finding genetic variation and links among different species and biotypes of <i>Guadua</i>	Colombia/2002	[107]
<i>Bambusa longispiculata</i> , <i>B. blako</i> , <i>B. multiplex</i> , <i>B. textilis</i> , <i>B. ulda</i> , <i>B. ventricosa</i> , <i>B. vulgaris</i> ; <i>Dendrocalamus brandisii</i> , <i>D. giganteus</i> ; <i>Gigantochloa rideleyi</i> , <i>G. rostrata</i> , <i>G. scoreichii</i> , <i>G. verticillata</i> , <i>G. atroviridis</i> ; <i>Thysostachys siamensis</i>	4	23	The ITS sequence data were used in this paper to investigate the degree of genetic diversity in alpine bamboos and to analyse their phylogeny	China/2001	[78]
<i>Arundinaria gigantea</i> ; <i>Bambusa longispiculata</i> ; <i>Glaziopteron mirabile</i> ; <i>Nastus elatus</i> ; <i>Neurolepis aperta</i> ; <i>Oreaea acuminata</i> ; <i>Phyllostachys pubescens</i> ; <i>Pseudosasa japonica</i> ; <i>Rhipidocladum pittieri</i> ; <i>Schizostachyum luzonicum</i>	10	10	AFLP analysis using 8 primer combinations was carried out on 15 species from four genera of bamboo. It used unique banding patterns for distinguishing the different species. This AFLPs is used for particular bamboo species identification as well as useful for industrial purposes and systematic studies	Singapore/2000	[108]
<i>Dendrocalamus strictus</i> , <i>D. giganteus</i>	1	2	Sequences of plastid genes such as <i>rbcL</i> , <i>matK</i> , <i>rpl34</i> , <i>rpl16</i> , and others have significantly led to the modern knowledge of bamboo systematics and phylogeny	1997	[109]

Table 3 (continued)

Studied taxa of bamboo	Number of genera	Number of spp.	Work/regions compared/sampling strategy/recommendation	Country/year	Study
<i>Arthrostylidium naibunensis</i> ; <i>Bambusa multiplex</i> ; <i>B. oldhamii</i> ; <i>Dendrocalamus giganteus</i> , <i>D. latiflorus</i> ; <i>Gigantochloa levis</i> ; <i>Melocanna baccifera</i> ; <i>Phyllostachys bambusoides</i> ; <i>Pleioblastus chino</i> ; <i>P. linearis</i> ; <i>Pseudosasa japonica</i> ; <i>Sasa Veitchii</i> ; <i>Schizostachyum difusum</i> ; <i>Semiarundinaria fastuosa</i> ; <i>Shibataea kumasasa</i> ; <i>Sinobambusa Tootsik</i> ; <i>Thyrostachys stamensis</i> ; <i>Yushania nftakayamensis</i>	16	19	Estimate phylogenetic relationships among species of bamboo genera <i>Arundinarieae</i> , <i>Bambusa</i> , <i>Dendrocalamus</i> , and <i>Melocanna</i> , examined restriction site mutations of cpDNA for these Asian genera. Phylogeny focused on cpDNA does not establish a history of introgression by paternal ancestors	Japan/1994 [110]	
<i>Phyllostachys bambusoides</i>	1	1	RAPD has been used for the genetic profiling of <i>Yushania</i> . The aim of this research was to examine this species' genetic structure on Mt Hohuan, in central Taiwan	Taiwan/1994 [111]	
	1	1	A library of random genomic probes from a <i>Phyllostachys nigra PsII</i> sample was built to determine the usefulness RFLP analysis in bamboo systematics and germplasm screening. There was considerable variability in RFLP, and species-specific trends were readily collected. Chloroplast DNA shows little difference across analysed bamboo accessions	USA/1991 [58]	

Simple Sequence Repeat (SSR)

Microsatellites also known as simple sequence repeats (SSR), which are comprised of short tandem repeated sequence motifs (1–6 bp in length). Genomic SSR markers have become the most common markers of choice for mapping the genes, fingerprinting of genomes and study the genetic diversity with highly reproducible data [112]. In previous studies, by Nayak and Rout, demonstrated the polymorphism of different bamboo species by Locus Ba10 amplified and characterized four bamboo species such as *Bamboosa clarata*, *B. vulgaris*, *B. vulgaris var striata* and *B. ventricosa*; further, Locus Ba20 was amplified in *Dendrocalamus strictus*. In recent studies, the species diversity was demonstrated among 803 moso bamboo genotypes from 34 populations with the help of 20 fluorescently labelled SSR markers [56]. Previous studies have developed 15 caespitose bamboo EST-SSR markers to identify the sequence polymorphism across the bamboo species of *Bambusa edulis*, *Bambusa oldhamii*, *Phyllostachys pubescens* and related species [113]. Moreover, the transferability among various bamboo species varies from 30–100%. Characteristics of 20 polymorphic microsatellite loci for *Phyllostachys edulis* was established with primer sequences, which will be useful in study the population genetics and conservation of the species [114]. Biogeographic history of *Bambusa arnhemica* from Australia was established using SSR primers [115]. SSR markers will be useful as a tool in understanding the relationship between various intra/inter bamboo species, phylogeny, ecological structure and its diversity. Characteristics of some of SSR markers being used in identification and classification of bamboo species were tabulated in Table 4.

Inter Simple Sequence Repeat (ISSR)

ISSR markers are more resourceful and reliable as it is based on longer primer sequence. ISSR markers have been widely used to identify taxonomic classification of closely resembled species, population genetics studies and phylogenetic characterization. Amom et al. [32] constructed a phylogenetic tree using ten ISSR primers and found similarity matrix between 15 North-East Indian bamboos such as *Bambusa tulda*, *B. nutan*, *B. mizoramiana*, *B. vulgaris* and *B. manipureana*; *Dendrocalamus giganteus*, *D. hamiltonii*, *D. sikkimensis*, *D. hookeri*, *D. Longispathus* and *D. Manipurianus*; and *Schizotachyum dullooa*, *S. pergracile*, *S. Munroi* and *S. fuchsonianum*. They constructed a genetic relationship between the genus and between the species and expressed disagreement with traditional system of classification. ISSR markers also found beneficial in identifying cross bred of *Phyllostachys kwangsiensis* and *Phyllostachys bambusoides* [116]. Mukherjee et al., [60] studied genetic relationships among 22 taxa of bamboo by using twelve ISSR random

primers, resulting in amplification of 220 loci and classified several bamboo species (Table 5). Gami et al., [83] collected twenty bamboo accessions from different regions of India and classified bamboo species based on eight ISSR primers. Some known ISSR primers have been used in previous studies to classify bamboo species are given in Table 5.

Random Amplified Polymorphic DNA (RAPD)

RAPD is rapid and efficient technique to screen polymorphism among the species as it is directed with a single, arbitrary and short oligonucleotide primer. In this technique arbitrary primers will be used as a tool for the detection of DNA polymorphism. RAPD technique has showed positive results in identification of genetic relationships between various bamboo genera and species such as, *Cephalostachyum*, *Dendrocalamus*, *Dinocloa* and *Sasa* using 10 RAPD primers [117]. Several other researchers studied the phylogenetic relationships among the bamboo species viz., *Bambusa*, *Denderocalamus*, *Melocana*, *Oxytenanthera*, *Phyllostachys*, *Schizostachyum*, *Teinostachyum*, *Thrysostachys* by with the help of polymorphic loci of the genomic DNA generated

by RAPD [100]. Further, Annisa et al., [118] also studied the genetic relationship between 25 bamboo species of Indonesia such as *Bambusa*, *Dendrocalamus*, *Dinocloa*, *Gigantochloa* and *Schizostachyum* by using RAPD technique. Moreover, RAPD technique could be used to draw phylogenetic tree between closely resembled species and to eliminate distantly related species of bamboos [117]. Some characteristics of known RAPD primer sequences have been given in Table 6.

Amplified Fragment Length Polymorphism (AFLP)

AFLP method is a robust and reliable assay to detect genetic polymorphism. Moreover, this technique will be of great use in comparing a large number of genetic loci parallel being functioning under rigorous conditions of experimentation and thereby providing high reproducible results with good precision rate. In previous studies, AFLP was used to prepare cluster analysis of species among bamboo genera such as *Bambusa*, *Dendrocalamus*, *Gigantochloa* and *Thrysostachys* [55]. Several researchers are in the opinion that AFLPs will be the best tool in identification of inter/inter

Table 4 Various SSR primers used in identification of bamboo species

Locus	Primer sequences (5'-3')	Repeat motif	Gen Bank accession no
Phe01	F: CACCTTTCGTCATCAACC R: ATCTAACGGCCCAAATGC	(AG) 29	FP093322
Phe10	F: TAAGGCCACGTTGCCAG R: CGCTGAAATCCACCCAGAAG	(AG) 19	FP095585
Phe23	F: CCCCATGTTACCTATCCC R: GCATCCTCTTGCCTTAC	(TC) 14	FP091611
Phe34	F: ACATACCCGCACCAACAA R: CGACCACCTCGAAACAA	(AG) 14	FP092058
Phe40	F: AGGTTCGTGTCCGTGGGT R: TTAGGCGCAGGAAGGTTGG	(GA) 12	FP097227
Phe51	F: GTCCCGTCTCAAGGAGT R: GTTGACCATCGGGATT	(CT) 11	FP093298
Phe100	F: GACATTAGGCAGGTTCGG R: GGGAGATGGACAGGTTGCT	(CTT) 8	FP094809
Boes-3	F: AAACCTGTCGTGCCAGC R: ATTACCGCCTTGAGTGAG	(ATG)23	EE-6615
Boes-4	F: GCGAATGAGAGACCTGCA R: CTCGCATAAGCCACATCTG	(TAA)8	EE-661510
Boes-5	F: ACGGTGGAAACTTGAAG R: CTCTCGATAAGCCACATCT	(CTCG)12	EE-661498
Boes-10	F: GCAATAGCATAGCACCAAC R: CAAGAACAGAGATGGAGCAG	(CTGTG)5	EE-661477
Boes-11	F: GGTACTGTTGAGGAAGG R: TCCCACAACCTCATTCTGC	(TC)16	EE-661410
Boes-12	F: GGCAAACAACGGTACATCA R: AGTCATCTCCGACATGC	(GA)23	EE-661366
Boes-13	F: ACTGATCCCTTGTGTACG R: GTTCATCTCCGACATGC	(GA)85	EE-661316

specific bamboo species and useful in systematic studies and cluster analysis within the sub tribe. In China, *Phyllostachys violascens* cultivars were studied by using fifteen primer combinations of AFLP markers to identify genetic diversity among cultivars and found it is more efficient than other genomic techniques [119]. Some known AFLP markers have been presented in Table 7.

Restriction Fragment Length Polymorphism (RFLP)

The technique RFLP uses the combination of restriction enzymes and finds the variation in the banding pattern and reveals the genetic diversity. It is a significant molecular tool in the field of fingerprinting, mapping and paternity testing. Konzen et al., [120] studied variation among species of *Bambusa*, *Dendrocalamus*, *Guadua* and *Phyllostachys* by using RFLP techniques and constructed genomic library. Previously, Friar and Kochert distinguished 20 species of *Phyllostachys* by using RFLP and examined the degree of genetic variation, genetic distances and constructed dendograms for 61 accessions [121].

Start Codon Targeted (SCoT)

SCoT technique will find the polymorphisms using reproducible markers based on the short-conserved region of the genomic sequence surrounding the ATG codon translation start or initiation codon. Previous studies showed that SCoT markers have been useful in discriminating the Bamboo species compared to other molecular techniques [31]. Recently, Amom et al., [2020], used SCoT technique to identify genetic relationship between commercial Bamboo species such as *Bambusa cacharensis*, *B. mizorameana*, *Dendrocalamus manipureanus*, *D. hamiltonii* and *D. Sikimensis* from North-East India. Furthermore, dendrogram

analysis based on UPGMA (Unweighted Pair Group Arithmetic Mean Method) demonstrated species-specific clustering of this five bamboo species and found that all molecular techniques used for this study were having close association except RAPD [31].

Single-Nucleotide Polymorphism (SNP)

These are position in genome where some individuals have one nucleotide (G) and others have a different nucleotide (T) and it is referred as single-nucleotide polymorphism (SNP) or ‘Snips’ [122]. For example, two sequenced DNA fragments from different individuals—CTTAGGTTCGAA and CTGAGGTTCGAA—contain variation in a single nucleotide, that is, G or T. It is genes specific marker and its detection is more rapid because it is based on oligonucleotide hybridization analysis. Species identification was achieved by detecting allelic variations of these type of markers.

Complete phylogeny of bamboo which included bamboo tribes and its subtribes was presented in the form of molecular database by using chloroplast genome of 6.7 kb of coding nucleotide and noncoding nucleotide sequence data and characteristics of 37 microstructure [123]. Classical taxonomy contributes a major role in classifying various bamboo species. However, molecular approach i.e., DNA barcoding can certainly support the traditional system of classification and also make possible identification process more consistent and easier. In India, several researchers are working on preparation of barcodes belong to various plant and animal species (Table 8).

Recognizing bamboo species by DNA barcode is fundamental, since it can precisely distinguish bamboo species in their adolescent stage [81]. Along with these, recognizable proof of the species is useful in bamboo industries as well as in commercial bamboo farming. Surrounding atmosphere plays a critical role in the development and growth of bamboo, hence, this barcoding technology can help the farmer in choosing the species that are the best for growth and development of bamboo in specific climatic conditions [137]. DNA standardized barcode is additionally utilized for building DNA barcode library which further utilized for contrasting and recognizing DNA barcode sequences recuperated from obscure DNA samples [138]. This technology is not only used for identification and classification of species but also has to protect endangered species, preserving natural resources, pharmaceutical, wildlife forensic, authentication of natural health products, illegitimate trading and even amusing activities [74, 90, 139, 140].

Bamboo identification is regularly troublesome because of its sporadic flowering pattern and its long reproducing cycle which changes in each species [141], yet by utilizing DNA barcoding, identification can be made with much simplicity and accuracy. Bamboo has genetic material as

Table 5 Various ISSR primers used in identification of bamboo species

Primer	Primer sequences	Fragment size (bp)	Primer index
ISSR1	(AG)8C	550–3000	3.19
ISSR5	C(AG)8	200–1500	3.198
ISSR6	(GACA)4	500–2000	2.69
ISSR8	T(GACA)4	500–2000	2.58
ISSR9	G(GACA)4	700–2400	4.53
ISSR10	(GACA)4G	550–3000	4.07
ISSR14	(GGAT)4	600–2800	3.181
ISSR17	G(CT)8	600–2200	4.157
ISSR18	GGGT(GGGGT)2G	500–2500	4.413
ISSR19	TGT(G)4 T(G)7	400–2000	3.214
AK8	(GAA)6	200–1700	2.566
12PT20C4	(GACAC)4	400–2500	3.897

Table 6 Various known RAPD primers used in identification of bamboo species

Primer	Primer sequence (5'-3')	Fragment size (bp)	Polymorphic information content (PIC)
OPA 1	CAGGCCCTTC	250–2000	0.381
OPA 2	TGCCGAGCTG	327–2247	0.97
OPN 2	ACCAGGGGCA	436–1000	0.98
OPA 3	AGTCAGCCAC	250–2000	0.318
OPN 3	GGTACTCCCC	235–1191	0.98
OPA 4	AATCGGGCTG	250–2000	0.291
OPN 4	GACCGACCCA	382–1866	0.96
OPA 5	AGGGGTCTTG	394–1504	0.315
OPN 5	ACTGAACGCC	367–1196	0.98
OPA 6	GGTCCCTGAC	755–2022	0.9
OPA 7	GAAACGGGTG	297–2201	0.98
OPC 7	TGCCCGACGA	250–2000	0.312
OPE 7	AGATGCAGCC	250–2000	0.333
OPA 8	GTGACGTAGG	489–1090	0.94
OPC 8	TGGACCGGTG	250–2000	0.333
OPA 9	GGGTAACGCC	221–830	0.97
OPA 10	GTGATCGCAG	254–1560	0.98
OPN 10	ACAACCTGGG	216–1825	0.96
OPF 14	TGCTGCAGGT	250–1500	0.394
PPG 15	ACTGGGACTC	250–2000	0.201

Table 7 Various known primer and adapter sequences for AFLP analysis of bamboo species

Name/abbreviation	Enzyme	Type	Sequence (5'-3')
GYY101/EA +	<i>Eco</i> RI	Adapter +	CTCGTAGACTGC GTACC
GYY102/EA -	<i>Mse</i> I	Adapter -	AATTGGTACGCAGTC TAC
GYY103/MA +	<i>Mse</i> I	Adapter +	GACGATGAGTCC TGAG
GYY104/MA -	<i>Eco</i> RI	Adapter -	TACTCAGGACTCAT
GYY105/E-A	<i>Eco</i> RI	Primer +1	GACTGCGTACCA ATTCA
GYY107/E-AAC	<i>Eco</i> RI	Primer +3	GACTGCGTACCAATT CAAC
GYY108/E-AAG	<i>Eco</i> RI	Primer +3	GACTGCGTACCAATT CAAG
GYY109/E-ACA	<i>Eco</i> RI	Primer +3	GACTGCGTACCAATT CACA
GYY110/E-ACT	<i>Eco</i> RI	Primer +3	GACTGCGTACCAATT CACT
GYY111/E-ACC	<i>Eco</i> RI	Primer +3	GACTGCGTACCAATT CACC
GYY112/E-ACG	<i>Eco</i> RI	Primer +3	GACTGCGTACCAATT CACG
GYY113/E-AGC	<i>Eco</i> RI	Primer +3	GACTGCGTACCAATT CAGC
GYY114/E-AGG	<i>Eco</i> RI	Primer +3	GACTGCGTACCAATT CAGG

DNA, and despite the fact that two closely species have a place with a similar family or genera have contrasted in their DNA [142]. DNA is the genomic material which differs in size from millions to billion according to species and their family, the Moso bamboo's (*Phyllostachys pubescens*) whole genome size has been evaluated to be around 2034 Mb [143]. DNA barcoding isn't new, yet the first run through came well known in the year 2003, when Paul Hebert's published a research namely 'Biological identifications through DNA barcodes', that presented a global biological distinguishing proof framework for creatures utilizing mitochondrial gene cytochrome c oxidase I (COI) [67]. The COI has been broadly exploited in the animal kingdom and suggested to be an appropriate DNA barcode at several taxonomical levels [144, 145]. But due to its lowering rate of evolution, it is not considered as a suitable DNA marker [146]. A variety of DNA markers have been assessed for their performance as a barcode for a variety of land plants [147]. Numerous combinations of DNA markers have been recommended for the barcoding of plants [148].

The technique of DNA barcoding has been advanced for most of the taxonomic challenging lineages in the *Poaceae* family. DNA barcoding has been proposed a great technique for the identification of grasses species that can be challenging to recognize at the species level [67]. In 2011 Drumwright et al., [149] demonstrated the identification of grass species with 95% accuracy with the use of

matK + rbcL as the main core barcode. The maximum coverage of genes in gene bank to study the species diversity of grass and barcoding included *gbss1*, *matK*, *ndhF*, *nrITS*, *phB*, *rps16*, *rpoA*, *rbcL*, *rpoC2*, and *trnL*, etc. [150–152].

In Bamboo, chloroplast region could be utilized as a marker region for identification. In previous study, seven up-and-comer barcoding loci from chloroplast region used to make barcoding with four coding genes such as *matK*, *rbcL*, *rpoB* and *rpoC1*, and non-coding inserts such as *atpF-atpH*, *psbA-trnH* and *psbK-psbI*. These 7 barcode loci show diverse oppressive force and their position as *rpoC1 < rpoB < atpF-atpH < rbcL < matK < psbK-psbI < psbA-trnH* [153]. Consortium for the Barcode of Life (CBOL) mentioned that loci *rbcL* and *matK* genes are well-known barcodes for the characterization of terrestrial plant species [71, 148]. Moreover in bamboo species, *ITS2* and *psbA-trnH* barcode would be considered as effective barcodes [154]. In previous study by Cai et al., [93] it is demonstrated that some barcoding loci would be useful in species identification in bamboos viz., *ITS2*, *matK*, *psbA-trnH*, and *rbcL*. Further, the combination of these loci example *rbcL + ITS2* would be better barcodes for identification of bamboo species [88]. Figure 4 illustrates the general procedures of DNA barcoding.

Methodology involved in DNA barcoding

The methodology of DNA barcoding includes the following phases starting from DNA extraction, amplification of DNA by polymerase chain reaction (PCR) with the help of universal barcode primers has been shown in Fig. 5. Combination of several primers has been reported to be useful in identification and discrimination of various taxa at species level as well at genus level in the system of molecular approach (Table 9).

The grouping of the primers sequence from coding region (*rbcL*, *matK* and *rpoC1*) and non-coding region (*trnH2-psbA*) displayed potential differential insight among the chosen species. Some molecular studies confirmed to identify 48 phylogenetically diverse plant genera by molecular approach i.e. DNA barcoding using nine 9 loci such as, *accD*, *ITS1*, *matK*, *rpoC1*, *ycf5*, *rbcL-a*, *rpoB2*, *trnH-psbA* and *ndhJ* [152, 155]. The DNA barcoding practices involves two fundamental steps: (1) construction of barcode library of identified species and (2) blast the barcode sequence of unidentified species compared to the barcode library intended for identification. Gene Bank has databases of some commercial bamboo species of various genera as *Bambusa*, *Dendrocalamus*, *Melocanna*, *Oxytenanthera* with their standardized identification for the species-specific region both

Table 8 Barcoding efforts in India made so far

Organism	Organization/institute	References
DNA barcoding freshwater and marine finfishes and shellfishes	Indian Council of Agricultural Research- National Bureau of Fish Genetic Resources (ICAR-NBFGR), Lucknow	[124]
Barcoding Anurans of India	Wildlife Institute of India (WII), Dehradun; Centre for Cellular and Molecular Biology (CCMB), Hyderabad; and North Orissa University (NoU), Baripada	[125]
DNA barcoding family Zingiberaceae: <i>Alpinia</i> , <i>Zingiber</i> and <i>Globba</i>	Rajiv Gandhi Centre for Biotechnology (RGCB), Kerala; and University of Calicut (UoC), Kerala	[126]
DNA barcoding of Rattans and <i>Phyllanthus</i>	University of Agricultural Sciences (UAS), Bangalore; Botanical Survey of India (BSI), Kolkata; and Ashoka Trust For Research In Ecology And The Environment (ATREE), Bengaluru	[127–129]
DNA barcoding butterflies from Western Ghats	National Centre for Cell Science (NCCS), Pune	[130]
DNA barcoding <i>Dendrobium</i>	Delhi University (DU), Delhi; and Tropical Botanical Garden and Research institute (TBGRI), Kerala	[131]
DNA barcoding in Indian taxa of <i>Berberis</i>	CSIR-National Botanical Research Institute (NBRI)	[132]
DNA barcoding in <i>Bambusa</i> species	The Energy and Resources Institute (TERI), Delhi; and Tropical Botanical Garden and Research institute (TBGRI), Kerala	[81]
Development of DNA barcode for amphibian fauna of Western Ghats	Centre for Environmental Management of Degraded Ecosystems (CEMDE), New Delhi; and National Centre for Cell Science (NCCS), Pune	[133]
Identification of Satyrine butterflies of Peninsular India through DNA barcodes	Indian Institute of Science (IISc), Bengaluru; and Kerala Forest Research Institute (KFRI), Kerala	[134]
DNA barcoding of <i>Dalbergia</i> species	National Bureau of Plant Genetic Resources (NBPGR), New Delhi; CSIR-National Chemical Laboratory (NCL), Pune; and Kerala Forest Research Institute (KFRI), Kerala	[135, 136]

essential and optional (*rbcL*, *matK* and *psbA-trnH*) (<https://www.ncbi.nlm.nih.gov/genbank/>). Google has an additional desktop search engine for DNA barcoding. This barcode library can be useful to various government and private agencies for providing agriculture certification to different crops [81, 156–158].

Concluding remarks and future prospects

Among the huge diversity of bamboo species, some are more commercial and ecologically important therefore it is necessary to identify such species accurately. Bamboo species recognition, based only on morphology, is a difficult task because it continuously changing and dependent on the environment and mainly due to the absence or long flowering cycle in their entire lifespan. Bamboo plant species characterization with recognizable proof is one of the troublesome errands in agribusiness because of its assortment and distinctive field conditions. Incorrect identification and classification of bamboo species appropriate for various agro-climatic zones can prompt a noteworthy decrease in profitability. It can likewise prompt striking misfortune to farmers if an inappropriate species personality is found a lot later particularly when the species is inadmissible for a particular end-use it was grown for.

DNA barcoding a well-known molecular technique has been observed to be an alternative tool for addressing several taxonomical complexities predominant in bamboo species. DNA barcoding is short and novel for every species of bamboo and its outcome is increasingly accurate, unlike morphological identification. It recognizes species as well as builds up connections between various bamboo species. In addition, it characterizes and recognizes bamboo species without knowing its age, physico-chemical and mechanical properties. This technique has the probability to serve as an innovative method for identifying taxonomically distinct species of bamboo. When, DNA barcode library is well-known and the classification and identification of commercial bamboo species are affordable, it can be used for standardization and certification. DNA barcode can also help to identify the genes which are responsible for quick development, high compressive strength, and high fiber content. DNA barcoding is used to handle the complexity at a taxonomic level not only in the identification of species but also for the selection of planting materials. This technology can act as an innovative molecular tool complementing modern approaches for distinguishing taxonomically difficult species of bamboo. For DNA barcoding, the entire genome sequence and expressive region must be notable ahead of time. Building a DNA barcode library for all bamboo species is expensive and challenging since it has 1575 species and tremendous

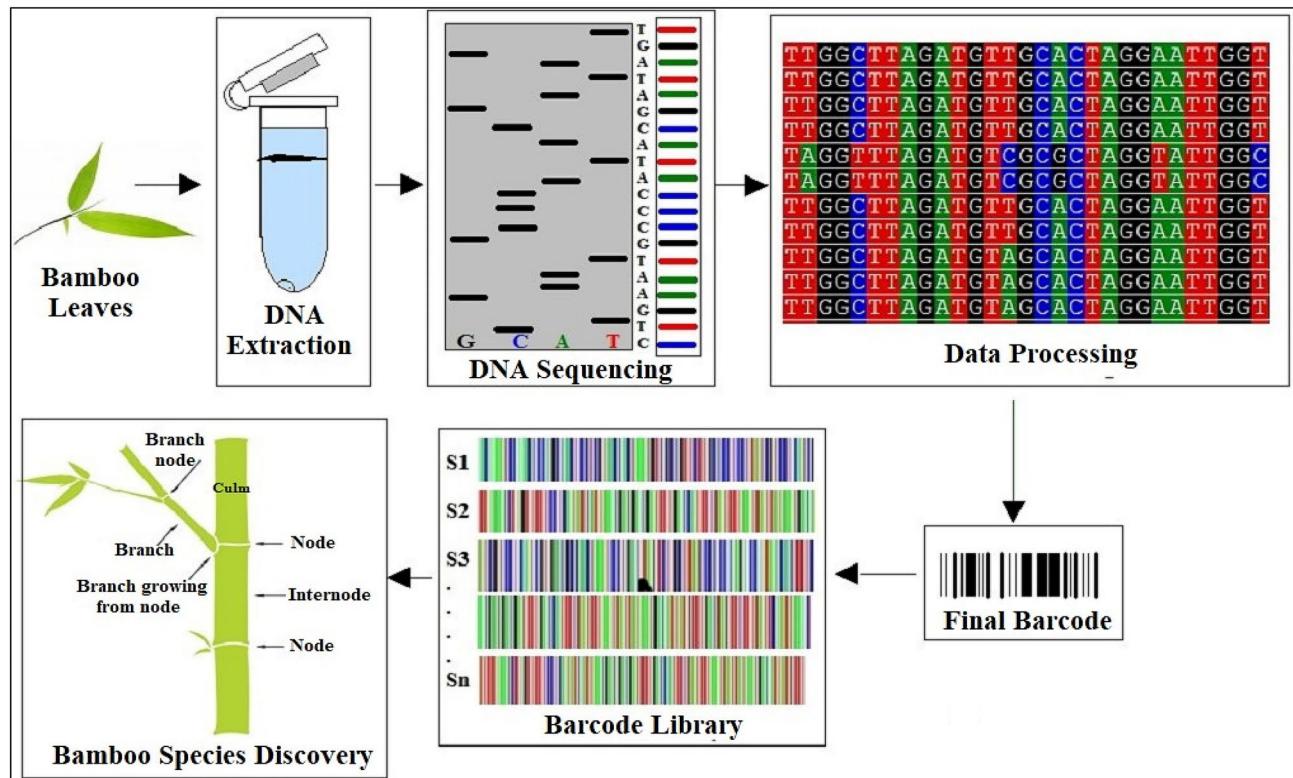


Fig. 4 Schematic representation of DNA barcoding

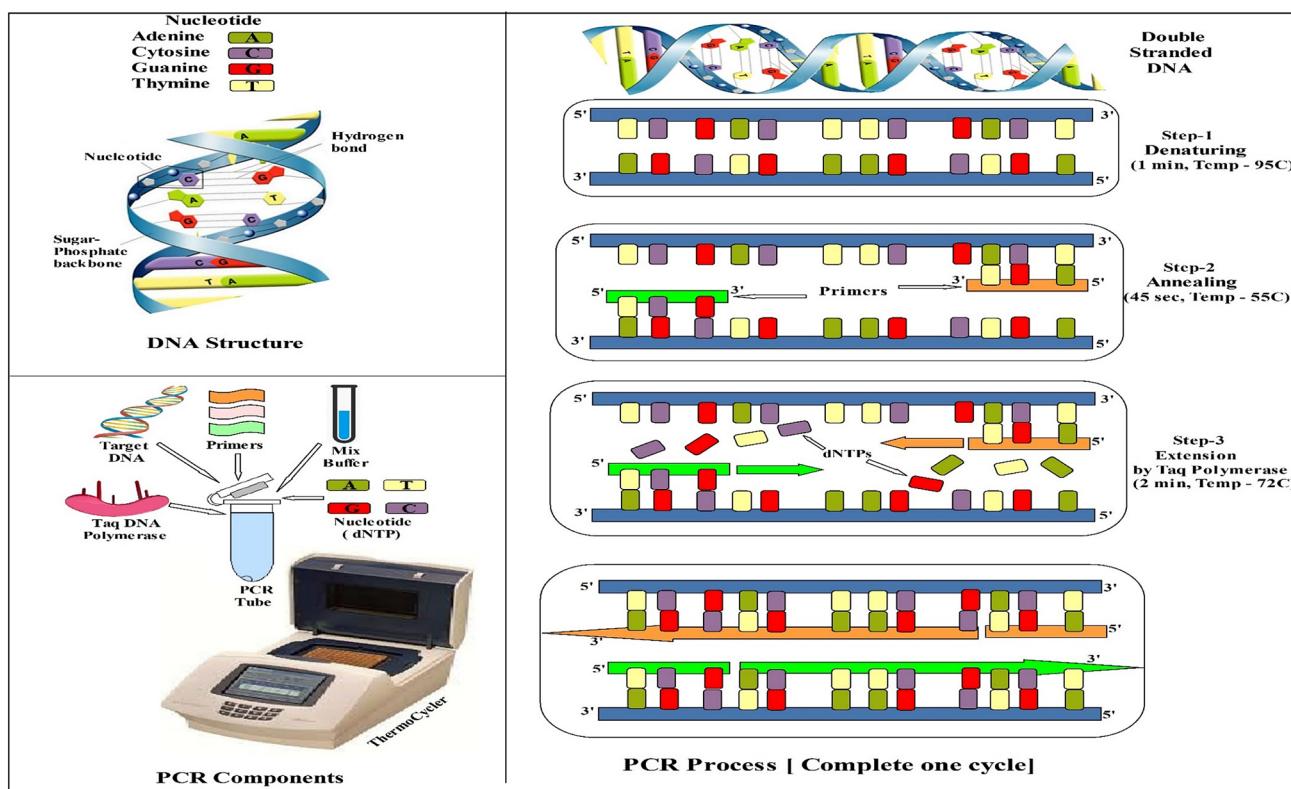


Fig. 5 Schematic representation of PCR components with cycle involve in DNA barcoding

Table 9 List of routinely used primers in DNA barcoding

Primer name	Forward primer	Reverse primer
<i>rbcL</i>	ATGTCACCACAAACAGAAC	TCGCATGTACCTGCAGTAGC
<i>matK</i>	CCCRTYCCTGGAAATCTTGGTT	GCTRTRATAATGAGAAAGATTCTGC
<i>psbA-trnH</i>	GTWATGCAYGAACGTAATGCTC	CGCGCATGGTGGATTACAATCC
<i>rpoC</i>	GGCAAAGAGGGAAGATTCG	CCATAAGCATATCTTGAGTTGG
<i>rpoB</i>	AAGTGCATTGTTGAACTGG	GATCCCAGCATCACAAATTCC
<i>atpF-atpH</i>	ACTCGCACACACTCCCTTCC	GCTTTATGGAAGCTTAACAAT
<i>psbK-psbI</i>	TTAGCCTTGTGGCAAG	AGAGTTGAGAGTAAGCAT

genome sizes extending from 100 Mbp to 150 Gbp. However, the technology is accurate and reliable, yet expensive because of the inaccessibility of the standardized barcode library for all species. At full scale, this method can be utilized by all bamboo ventures and ranchers for distinguishing species according to their requirements. The government or private agency may give accreditation data modest rate that improves the bamboo trade.

Acknowledgements The authors are thankful to Director, CSIR-National Environmental Engineering Research Institute, Nagpur and Department of Computer Science and Engineering, Visvesvaraya National Institute of Technology (VNIT), Nagpur for providing necessary facilities for this work

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This review was approved by research committee of CSIR-NEERI, Nagpur and registered in library.

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