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Research Article

Evaluation of Genetic Variability of Bamboo Varieties Cultivated in Gujarat Region using ISSR Markers

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ABSTRACT

Bamboo is an important plant of grass family belongs to the Family *Poaceae.* Bamboo genomes contain similar content of repetitive elements (36.2%) as rice. Bamboo exhibits high genomic synteny with rice and sorgum and both of these plants can be used as models for decoding bambusoideae genomes The molecular approach using inter simple sequence repeat (ISSR) markers was applied to analyse six bamboo species. From 7 ISSR primers, a total of 87 bands were obtained in which 62 bands (71.26%) were polymorphic and 25 bands were monomorphic (28.73%). The dendrogram was constructed by using UPGMA methods. Maximum genetic distance was observed between bambusa balcooa & dendrocalamus strictus (0.6592) & minimum genetic distance was observed between dendrocalamus strictus & bambusa vulgaris (0.2032). The mean Nei's genetic diversity (1973) across all loci was found moderate about 29% and Shannon Information Index was about 43.5%. Results not only can improve varieties and genetic breeding but also provide theory and technology basis about classification system of bamboos.

Keywords: ISSR markers, Bambusa balcooa, Molecular Markers, Population genetic analysis

INTRODUCTION

Bamboo is a fast growing, versatile and important plant of the grass family belonging to the Family *Poaceae* and Subfamily *Bambusoideae*. Bamboos originated about 30-40 million years ago and evolved in forests from an ancestral grass much similar to *Streptochaeta schrad* (Clark, 1996; Klinkenborg, 2001). These include 88 genera and more than 1400 species. About 22 species of bamboos reported from the Gujarat, among them *Bambusa arundinacea* (Katas/Thorny bamboo) and *Dendrocalamus strictus* (Manvel/Desi bamboo) being the dominant ones. The economy of India and so also of many Asian countries depends on bamboos and their uses. Besides, young edible shoots and culms are used for timber, furniture, handicrafts, and raw material for pulping. Bamboo is an efficient agent for preventing soil erosion and conserving soil moisture (Christanty *et al.*, 1997; Kleinhenz and Midmore, 2001). Lately, worldwide interest in bamboo as a source of biofuel or bioenergy has rapidly increased (Scurlock, 2000).

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To examine the genetic variation and differetiation of bamboo species and cultivar, several molecular marker approaches exploide, including random amplified polymorphic DNA (RAPD; Das et al., 2005; Ramnayake et al., 2007), simple sequence repeats (SSRs; Barkley et al., 2005; Sharma et al., 2008) and inter-simple sequence repeats (ISSRs; Lin et al., 2009). Becuase they are easy to apply and has high level of polymorphism and reproducibility, ISSRs are widely used for population genetics studies (Zhang and Dai, 2010). In ISSRs, 16-25 bp long microsatellite sequences as primers is used in a polymerase chain reaction to generate multilocus markers. These primers can be di-nucleotide, tri-nucleotide, tetra nucleotide or penta-nucleotide. ISSRs have high reproducibility, possibly due to the use of longer primers (16–25 bp) as compared to RAPD primers (10 bp) which permits the subsequent use of high annealing temperature (45–60ºC) leading to higher stringency. The present investigation aimed to estimate degree of genetic variation and differentiation among six populations of bamboo species. This information would help us to understand its genetic background and also provide a reference for the utilization of genetic resources when designing programmes for species protection and breeding.

MATERIALS AND METHODS

Plant Material

A total of six popular bamboo growing in different region of Gujarat were collected and maintained at Xcelris Labs Ltd., Ahmedabad. These were *Bambusa balcooa, Dendrocalamus strictus, Bambusa vulgaris, Bambusa ventricosa, Phyllostachys nigra, Bambusa multiplex f. variegate* and further designated as E1, E2, E3, E4, E5 and E6 respectively.

DNA Isolation

Fully expanded leaves were collected from all six samples and then surface sterilized prior to DNA isolation. Genomic DNA was extracted using EZgene CP Plant Mini Kit (XcelGen, Xcelris Genomics) as per manufacturer's instructions. DNA concentration was measured using Nanodrop 8000 spectrophotometer (Thermo Scientific). The quality of DNA was determined using 0.8% agarose gel.

ISSR PCR Amplification

Genomic DNA was PCR-amplified using ISSR primers synthesized from PrimeX, Xcelri Genomics. Amplification was performed using Veriti 96 well thermal cycler (Applied Biosystem). The 25 µl mixer containing 50 ng of gDNA, 1 X Reaction buffer with 2.0 mM $MgCl_2$, 10 pM primer, 200µM dNTPs, 1 unit of *Taq* polymerase (Ferments) and nuclease free water (Ambion). The cycling conditions included an initial denaturation at 94˚**C** for 5 mins; then 35 cycles of 94˚**C** for 30 s, 44˚C for 45 s and 72˚C for 90 s with a final extension at 72˚C for 20 mins. The PCR products were electrophoretically separated using 2.0 % agarose gel electrophoresis for 90 minutes at 110 Volt. A 2kb DNA ladder (Rovalab) was used as a size marker. Gel picture was captured using The number and intensity of monomorphic and polymorphic bands were recorded using gel documentation system (Bio-rad) with the help of software QuantityOne. Only clear and reproducible bands were considered for data analysis.

Data Analysis

For ISSR analysis, only clear and unambiguous bands were visually scored as either present (1) or absent (0). Each band was interpreted as one allele. Bands with the same mobility were assumed to be homologous. Each marker was treated as an independent unit character. The genetic distance and genetic similarity from ISSR data were calculated among species using Nei's coefficient. Cluster analysis was based on a similarity matrix obtained with the Un-weighed Pair Group Method using Arithmetic Averages (UPGMA) and relationships between species were visualized as a dendrogram. All data were scored in the form of a binary matrix. For each pair of species, the Nei's coefficient was calculated. The calculations were performed with the POPGENE 1.32 software for data

analysis and MEGA 5.0 was used for dendrogram visualization.

RESULTS

Analysis of genetic diversity of six bamboo varieties

Of the 7 ISSR primer used, total 87 bands generated using six different bamboo varieties and the size of amplification products ranged from 100 bp to 2 kb. The number of bands generated per primer were ranged from 6 (primer 17898B) to 16 (primers 834 and 814) with an average of 12.425 bands per primer. Out of 87 bands generated, 62 bands (71.26%) were polymorphic and 25 bands (28.73%) were monomorphic (Table 1). The percentage of polymorphism per primer ranged from 31% to 100%. The primer that showed maximum polymorphic bands was primer 814 (16 bands out of 16) and the minimum number of polymorphic bands was primer 834 (5 bands out of 16). The mean Nei's genetic diversity (1973) across all loci was found moderate about 29% and Shannon Information Index was about 43.5% (Fig 1 and Fig 2).

Table 1. *The amplification profiles using ISSR primers*

S. No.	Primer	Sequence 5'→3'	No. of Amplified Bands	No. of Polymorphic Bands	% of Polymorphic Bands (PPB)
1	814	$(CT)_8TG$	16	16	100
2	834	$(AG)_8CTT$	16	5	31
3	841	$(GA)_8CTC$	12	8	66
4	HB10	$(GA)_8CC$	10	6	60
5	HB14	$(CTC)_3GC$	14	12	85
6	ISSR5	$(AC)_8TG$	13	10	77
7	17898B	$(CA)_8GT$	6	5	83
Average			12.42	8.85	71.98

Fig 1. *Genetic profile of six different bamboo varities using ISSR primers. (A) ISSR profile using ISSR primer "814" (B) ISSR profile using ISSR primer "834" (C) ISSR profile using primer "841" (D) ISSR profile using ISSR primer "HB10" (E) ISSR profile using ISSR primer "HB14" (F) ISSR profile using ISSR primer "ISSR5".*

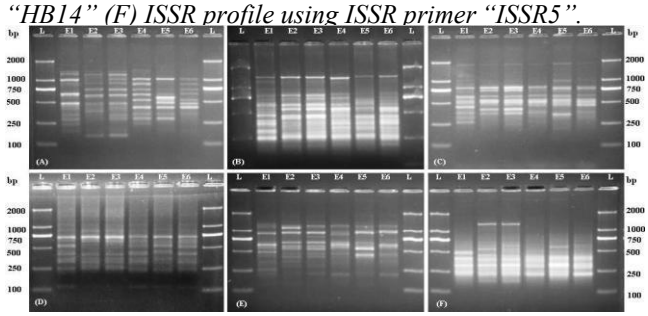
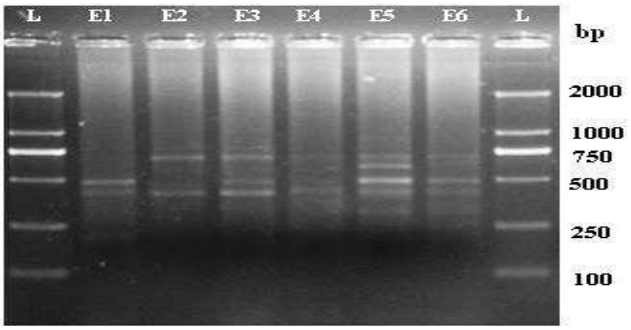


Fig 2. *Genetic profile of six different bamboo varities using ISSR primers "17898B"*



Analysis of Genetic distances

Genetic distances for all possible pairs based on Nei's coefficient using ISSR data were calculated (Table 2). The population pair of *Dendrocalamus strictus* (E2) and *Bambusa vulgaris* (E3) showed minimum genetic distance (GD = 0.2032) and population pair *Bambusa balcooa* (E1) and *Dendrocalamus strictus* (E2) showed maximum genetic distance (GD = 0.6592) (Table 2).

Table 2. *Genetic Distances among six Bamboo varieties as per Nie's coefficient.*

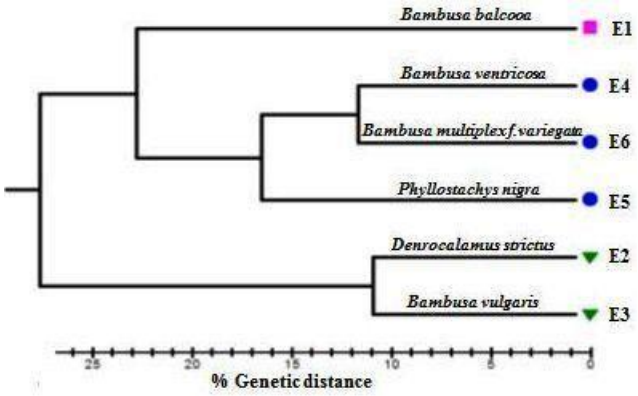
Population ID	E1	E2	E3	E4	E5	E6
E1	****					
E2	0.6592	****				
E3	0.5341	0.2032	****			
E4	0.3388	0.4769	0.4055	****		
E5	0.5947	0.6373	0.5147	0.3228	****	
E6	0.3884	0.5741	0.4956	0.2174	0.3070	****

Cluster Analysis

Phylogenetic tree for 6 bamboo varieties were constructed based on Nei's coefficient (1972) and UPGMA tree, based on the values for the genetic distance, was generated (Fig 3). At 73% genetic similarity, the phylogenetic tree was divided into 2 major groups. The first major group includes varieties of bamboo *Dendrocalamus strictus* and *Bambusa vulgaris*. The second major group includes varieties of *Bambusa balcooa, Bambusa ventricosa*, *Bambusa multiplex f. variegata* and *Phyllostachys nigra*. At 78% genetic similarity, the second major group was

subdivided into twp groups i.e. *Bambusa balcooa* in the first group and *Bambusa ventricosa, Bambusa multiplex f. variegata* and *Phyllostachys nigra* were placed in the another group. *Bambusa balcooa* was found highly diverse from rest of the varieties.

Fig 3. *UPGMA dendogram based on Nei's (1972) genetic distances among 6 different varieties of Bamboo.*



DISCUSSION

Bamboo is a fastest growing, most versatile plant. The economy of India and many Asian countries depends on bamboos and their uses. In recent years, worldwide interest in bamboo as a source of biofuel or bioenergy has rapidly increased (Scurlock, 2000). DNA-based markers provide precise information on genetic diversity and identification of variety-specific markers because of the independence of the confounding effects of environmental factors. In recent years, DNA profiling through ISSR technique has been used for the analysis of genetic diversity, phylogenetic relationship, and varietal identification. In the present investigation, seven ISSR primers were used to detect DNA polymorphism in six varieties of bamboo. ISSR primers produced 87 amplified fragments varying from size range of 100bp to 2000bp. ISSR marker system revealed high levels of polymorphism of about 71.98 % among the bamboo varieties.

Cluster analysis was performed using the unweighted pair group method with arithmetic averages (UPGMA). Dendrograms were constructed using the UPGMA algorithms in the MEGA 4.0 software (Tamura *et al.*, 2007) and showed that at 73% similarity the phylogenetic tree was divided into two major clusters. The first major group includes varieties of bamboo *Denrocalamus strictus* and *Bambusa vulgaris*. The second major group includes *Bambusa balcooa, Bambusa ventricosa, Bambusa multiplex f. variegata* and *Phyllostachys nigra*. Nayak *et al.* (2003) studies showed that *Bambusa vulgaris, Bambusa ventricosa, Bambusa balcooa,* were placed in the same cluster and *Dendrocalamus strictus* and B*ambusa multiplex* were placed in the different cluster at 80% genetic similarity. While in our study at 80% similarity, *Bambusa vulgaris* and *Dendrocalamus strictus* were placed in one cluster, *Bambusa multiplex* and *Bambusa ventricosa* in second cluster and *Bambusa balcooa* was placed individually in a separate cluster. The difference may be due to different techniques were used for analysis. In RAPD analysis random primers may cause artifacts in the results due to their short length, whereas in ISSR technique primers have enough length (16–25 bp) that permits the subsequent use of high annealing temperature (45–60ºC) leading to higher stringency. Inter-simple sequence repeat permits detection of polymorphisms in inter-microsatellite loci, using a primer designed from dinucleotide or trinucleotide simple sequence repeats. ISSR markers have been extensively used for DNA finger-printing (Moreno *et al.*, 1998), population genetics studies (Nebauer *et al.*, 1999) and phylogenetic studies (Hess *et al.*, 2000). Because of the multilocus fingerprinting profiles obtained, ISSR analysis is also be applied in studies involving genetic identity, parentage, clone and strain identification, and taxonomic studies of closely related species (Godwin *et al.*, 1997; Gupta *et al.*, 1994). Sun *et al.* (2006) revealed diversity similarity between Bambusa species. They were placed *Bambusa multiplex* and *Bambusa ventricosa* in one cluster and *Bambusa vulgaris* was placed in other cluster and it also shows more relevance to the *Dendrocalamus* genus. Thus, population pair of *Dendrocalamus strictus* (E2) and *Bambusa vulgaris* (E3) showed minimum genetic distance (GD = 0.2032) and population pair *Bambusa balcooa* (E1) and *Dendrocalamus strictus* (E2) showed maximum genetic distance (GD = 0.6592).

In conclusion, high genetic diversity and lower genetic differentiation were detected among different bamboo variety used. On the basis of UPGMA analysis using ISSR marker system, *Dendrocalamus strictus* and *Bambusa vulgaris* varieties are observed genetically more similar and grouped together and also *Bambusa ventricosa*, *Phyllostachys nigra* and *Bambusa multiplex* varieties are genetically more similar which are grouped together. Thus, our study indicates that ISSR technique is a useful tool for varietal identification and genetic relationships among the bamboo species has been observed by several other workers with other plant species.

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