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OPTIMIZATION OF PCR CONDITIONS AND SCREENING OF POLYMORPHIC ISSR MARKER IN *BAMBUSA BALCOOA* ROXB.

MOHD IBRAHIM[1]*, N. RAVI[2], M.K. MODI[3], RITASHREE KHANIKAR[1] AND MONPI HAZARIKA[1]

[1]Rain Forest Research Institute, Jorhat, Assam
[2]Institute of Wood Science and Technology, Bangalore
[3]Depatment of Biotechnology, Assam Agriculture University, Jorhat, Assam
**Corresponding author email: ibrahim@icfre.org*

ABSTRACT: Inter-Simple Sequence Repeat (ISSR) marker is considered as an efficient and inexpensive way to provide molecular data in those species where genomic information is lacking. A laboratory experiment was conducted to optimize PCR conditions and screening of ISSR markers for polymorphism to study the genetic variation and phylogenetic relationship of *Bambusa balcooa.* The PCR condition for ISSR markers was standardized using different concentrations of $MgCl_2$ (1.5-2.5 mM), dNTPs (0.25-0.5mM), Taq DNA polymerase (0.07-0.25 unit) and varying concentration of DNA templates (20-50 ng/µl). Reproducible amplification of DNA with the different pairs of ISSR marker was obtained using 3 mM $MgCl_2$, 0.33 mM dNTPs and 20ng DNA. The annealing temperature of sets of primers was also optimized in a gradient PCR machine. Using the optimized protocol, 40 sets of ISSR primers were screened for polymorphism in a set of 24 diverse accessions of *B. balcooa*. Out of 40 primer sets, 7 primers produced reproducible polymorphism and 10 produced monomorphic bands. The Polymorphic Information Content (PIC) of markers range from 0.076 to 0.324. The optimized PCR protocol and the identified polymorphic marker will be useful for genetic diversity estimation and other molecular techniques in *B. balcooa*.

Keywords: *Bambusa balcooa, ISSR marker, polymorphism*

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INTRODUCTION

Bambusa balcooa Roxb. is one of the most economically important sympodial densely tufted bamboo of Northeast India. The species is generally chosen for construction purposes and fiber-based mat board and panel manufacture (Ganapathy, 1997). Owing to high mechanical strength, it is considered as one of the best and strongest bamboo used for construction and building purposes (Das *et al*., 2005). For proper utilization and effective conservation of germplasm of a species, characterization is an important component (Stapleton and Rao, 1995; Nayak *et al*., 2003). Molecular marker plays an important role in the characterization of a species/germplasm. Different types of molecular marker are available for characterization of a species. Among the different types of molecular markers, ISSR markers having advantage that no prior sequenced data is required for primer construction. ISSR markers are highly polymorphic and are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping, and evolutionary biology (Reddy *et al*., 2002). The technique use random microsatellite repeats as primers and amplify the region between oppositely oriented microsatellites and product size ranges from 100- 3000bp. The primer can be di-nucleotide, tri-nucleotide, tetra- nucleotide or penta-nucleotide. The primers used can be either unanchored (Gupta *et al*., 1994; Meyer *et al*., 1993; Wu *et al*., 1994) or more usually anchored at 3′ or 5′ end with 1 to 4 degenerate bases extended into the flanking sequences (Zietkiewicz *et al*., 1994). ISSR marker are widely used in population genetic studies due to its high reproducibility and longer size (16-25 bp) then RAPD primers (10 bp) (Zhang and Dai, 2010). Study of phylogenetic relationship and diversity estimation in many crop and tree species including bamboo have been worked out using ISSR technique (Amom *et al*., 2018; Nagaoka and Ogihara, 1997; Wolfe *et al*., 1998; Blair *et al*., 1999; Chaudhary *et al*., 2015; Lalhruaitluanga and Prasad, 2009; Lin *et al*., 2011; Tsumura *et al*., 1996; Vijayan, 2005; Joshi *et al*., 2000; Qian *et al*., 2001). Study of genetic relationship in