PREFACE

Bamboo grows fast and matures early. The output of bamboo plantation is great and the use of bamboo stem is wide. Once successfully planted, bamboo plants keep on rhizoming, shooting and maturing every year. The annual selective cutting and sustainable utilization can be implemented without damaging ecological environment. The world is facing rapid decrease in forest resources and is suffering serious deterioration of ecological environment. Therefore, the development and exploitation of bamboo resources is of considerable importance. The morphology, structure and chemical components of bamboo differ from those of timber. Consequently, the methods, technology and equipment for timber processing can not be applied indiscriminately in bamboo utilization. This is one of the direct reasons why bamboo is utilized only for manual weaving, and simple ware making for hundreds of years.

It is a fast growing woody grass which is one of the most beautiful and useful plants on this Earth, mainly found in the tropical and sub-tropical regions of the world. It plays a vital role in the protection of soil and water resources in forest catchment areas. Bamboo has been and continues to be a material of choice for traditional crafts throughout the world and is also important for construction, fencing, basketry and many other uses. These uses of bamboo makes a significant contribution to rural income and employment, although the rapidly diminishing supplies of forest bamboos through indiscriminate clearing of natural forests and the lack of priority in its development join forces to erode its status. Because of such factors and the rapid growth in human population, the demand for bamboo has increased. This has also led to reduction in bamboo cover to make land available for human settlement and overexploitation especially in the more accessible forest areas.

Conventional propagation through seeds is limited due to long flowering cycle of upto 120 years, seed sterility and short seed viability. Infrequent and unpredictable flowering events coupled with peculiar monocarpic behaviour i.e. flowering once before culm death, and extensive genome polyploidization are additional challenges for this woody group. Similarly, vegetative propagation by cuttings, offsets and rhizomes are also inadequate to cope up with the demand of planting stock due to large propagule size, limited availability, seasonal dependence, low multiplication rate and rooting percentage. Therefore, attempts have been made to propagate bamboos through in vitro techniques. But micropropagation through tissue culture itself has a major limitation regarding true to type nature of the in vitro originated propagules in comparison to their mother plant due to chromosomal abnormality, gene
amplification, point mutations etc., especially in long term in vitro cultures. It is therefore extremely important to ascertain the suitability of a particular micropropagation protocol developed for a particular species, where commercial success in micropropagation depends solely on the maintenance of clonal uniformity.

Considering the above facts, present study was undertaken with following objective - development of micropropagation protocol at commercial level followed by assessment of the probability of induction of somaclonal variation in in vitro cultures and in vitro-raised plantlets maintained and propagated in vitro for over two years via enhanced axillary branching, using a combination of arbitrary (RAPD), semi-arbitrary (ISSR, AFLP) and sequence based (SSR) markers.

The present study was carried out at Plant Molecular Biology Lab of Centre for Plant Biotechnology (CPB), Department of Science and Technology, Govt. of Haryana, CCS Haryana Agriculture University, New Campus, Hisar-125004 (Haryana), India and Department of Biotechnology, Kurukshetra University, Kurukshetra-136119 (Haryana), India. The thesis has been divided into following five chapters along with a summary and list of references.

**CHAPTER 1: INTRODUCTION**
The first chapter gives a broad overview of the general background and literature available on bamboo and particularly about the two species (D. asper and D. hamiltonii) of the genus *Dendrocalamus*. The chapter describes the areas of distribution, diversity, germplasm availability, taxonomy, uses, natural and conventional propagation methods, constraints in conventional propagation and potential of biotechnology applied in the micropropagation till date. Finally the objectives of the present study have been discussed.

**CHAPTER 2: REVIEW OF LITERATURE**
This chapter presents the present scenario of the recent developments made in improvement of bamboos through in vitro propagation, molecular marker technologies, cloning, and transformation and transgenics. The future potential of improvement of bamboos using modern biotechnological tools has also been discussed.

**CHAPTER 3: IN VITRO PLANT REGENERATION VIA AXILLARY BUD PROLIFERATION**
Regeneration potential of the nodal explants taken from the mature culms of *D. asper* and *D. hamiltonii* has been evaluated in the two sub-sections of this chapter. This chapter also
describes the effect of various PGRs like auxins, cytokinins, other adjuvants etc., different media and season of explant collection on the response of axillary bud proliferation followed by impact of PGRs, carbon source and propagule size on the shoot multiplication rate. The impact of various auxins and medium strength on in vitro rooting, acclimatization of in vitro raised plantlets followed by their field transfer and evaluation in the field are also described in this chapter.

CHAPTER 4: OPTIMIZATION OF DNA EXTRACTION PROTOCOL
In this chapter, comparison of various methods has been evaluated to identify the best method for DNA isolation in bamboos for the genus *Dendrocalamus* using *D. asper* and *D. hamiltonii* as representative species. In addition, four types of leaf tissues collected from two sources namely tissue culture raised (in vitro shoot cultures and in vitro raised plants transferred to greenhouse) and field grown plants (young and mature leaves) were subjected to five DNA extraction methods to assess the combinational effect of leaf source and extraction method if any in *Dendrocalamus* sp. The isolated DNA was subjected to amplification using RAPD, ISSR and SSR primers to study its sensitivity towards PCR.

CHAPTER 5: GENETIC FIDELITY TESTING OF IN VITRO-RAISED PLANTLETS
An evaluation was made to ascertain the effect of length of in vitro culture age on clonal fidelity of the in vitro raised plants using Random Amplified Polymorphic DNA (RAPD); Inter-Simple Sequence Repeat (ISSR); Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeat (SSR) markers in two sub-sections of this chapter. The PCR amplification of the genomic DNA of samples (mother plant, in vitro raised shoots from 3rd - 30th passage, and in vitro raised plants transferred to the field) using 90 primer combinations (25 each of RAPD, ISSR, SSR and 15 AFLP primer combinations) has also been discussed.

CHAPTER 6: SUMMARY AND CONCLUSION
The main findings of the present study conducted on *D. asper* and *D. hamiltonii* micropropagation through axillary bud proliferation followed by genetic fidelity testing of in vitro-raised clones are summarized in this section.