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“Dreams are extremely important, you can’t do unless you imagine”

-George Lucas
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Biodiversity, as one of the resource ecosystems, is the totality of different organisms, the genes they contain and the ecosystems they form (New South Wales Environmental Protection Agency *Biodiversity*, 1990). Biodiversity encompasses three levels of diversity viz. *Ecosystem diversity* includes both biotic as well as abiotic components, being partly determined by soil parent material and climate, *Species diversity* as the number of species in a site or habitat and *Genetic diversity* which represents the heritable variation within and between populations of organisms.

Man is the crown of this biodiversity but unfortunately also an instrument of its destruction and depletion. The impact of humans upon biodiversity has gradually increased with growing technology, population, production and consumption rates. Consequently, there is a tremendous pressure on natural and biological resources which leads to the deforestation and wiping out the much more diversity day by day. Depletion of biodiversity and resultant loss is irreversible and dangerous to our planet earth. Protection of ecosystem and preservation of genetic resources is the need of the hour.

In order to bring out sustainable resource conservation and management, it is essential to adopt several different approaches for managing our forest and genetic resources.

Genetic conservation programmes are directed towards the long-term preservation of genetic resources either *in situ* or *ex situ* so that the potential for continuing evolution or improvement could be sustained. *In situ* conservation includes the organization and/or servicing of natural supplies where species are permitted to stay in maximum environments with the lowest of management. On the other hand, *ex situ* conservation includes the use of botanic landscapes, field farms, seeds shops and gene financial banks and germplasm. The whole set of genetic material of a species of plant is known as *Germplasm* of the organism. The characterization of germplasm is required to maintain
Ringal is socio-economically and ecologically important group of hill bamboo in Uttarakhand Garhwal Himalayas represented by four species namely: 

- *Sinarundinaria anceps* (Mitf.) Chao and Renvoize (old name *Sinarundinaria jaunsarensis*, Gamble) commonly known as Saura Ringal,
- *Arundinaria falcata* (Nees) Gol Ringal,
- *Thamnocalamus spathiflorus* (Trin.) Munro (Tham Ringal) and
- *Thamnocalamus falconeri* (Hook f. ex Munro), Dev Ringal cited in Plate 01. The species have wide variety of uses in the form of roofing, flooring material for houses, vegetables, medicines, fuel, fodder in periods of scarcity, stakes for cash crops, handicrafts, walking sticks, fishing rods, hookah pipes and baskets especially kiltas. These bamboos are widely used in Basketry that contributes significantly to the livelihood of poor rurals/artisans in this state.

The species are restricted to cooler, damper sites and thrive at different altitudes spanning from 1500m - 3000m, Gol Ringal existing at 1500m - 2700m, Dev Ringal (1700m - 2400m), Saura Ringal existing at 1800m - 2300m while Tham ringal is located at higher altitudes (2400m - 3000m). Like other bamboo species, Ringal also has erratic and long flowering cycles. *Arundinaria falcata* flowers irregaulry at times, gregarious over large areas, while few culms may be found in flowers almost every year. Stapf (1904) and Brandis (1906) reported *Thamnocalamus falconeri* gregariously flowering during 1846 and 1847 in upper Pindari River, North – West Kumaon. Ed. Madden sent quantities of seed to England, which was the origin of the plant that flowered all over Europe and in Algeria during 1875 and 1876. In Sikkim also it flowered in 1876 and at Darjeeling in 1890. The flowering during 1890 was also published by Gamble (1900) and Rogers (1901). In 2002 *T. falconeri* gregariously flowered throughout Uttarakhand and after seeding whole of the plants died (Naithani *et al.*, 2003). During 1966, *Sinarundinaria anceps* (Mitf.) Chao & Renvoize (old name *Arundinaria jaunsarensis* Gamble) it gregariously flowered in England. Bahadur & Naithani (1978) suggested its
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flowering cycle of 45-55 years. Thamnocalamus spathiflorus flowers both sporadically and gregariously. In the year 2001 - 2002, it gregariously flowered throughout Uttarakhand. After seeding the clumps died, (Naithani et al., 2003). Simultaneous flowering and subsequent death of entire populations increases the chances of their vulnerability. As they are at the end of their natural range, they are particularly sensitive to environmental degradation. Conscious management of forests to protect understorey and conservation and cultivation of these species is necessary if bamboo diversity is to be maintained under such conditions.

Due to over usage of genetic resources and heavy extraction of the material from natural forests, the species are depleting at an alarming rate, gregarious flowering in turn intensifying the depletion of Ringal resources since flowering result into death of entire clumps following seeding. If a single regeneration event coincides with unsuitable conditions, the entire population might lose its chance to reproduce. Thus the factors that are likely to lead to erosion of Ringal diversity are varied. Heavy forest degradation and agricultural encroachment, forest fires, human impact, heavy grazing pressures, etc. narrows the genetic base and eventually degradation of the gene pool of these species. Because bamboo seed is produced infrequently and can be stored only for few years, in situ and ex situ conservation efforts are needed to complement the current emphasis on conservation through seed storage.

Responding to the urgency for conservation of depleting genetic resources of Ringal, Forest Research Institute had taken initiatives and established a Germplasm of these four hill bamboos collected from different ecological zones, at Khirsu (Pauri), Uttarakhand, India in the year 2008 for evaluation, collection, utilization and conservation under a project “Bamboo improvement for rural and tribal communities, integrating new technologies” funded by National Bamboo Mission, New Delhi.

Genetic variation is the fundamental component of adaptation and thus of stability of forest ecosystems particularly when the stability of forest ecosystem is increasingly threatened by environmental stress and mismanagement. Genetic variation within and between populations is essential to exploit their improvement potential and is considered
to be a substantial determinant of adaptive abilities of population which is best indicated with the knowledge of the extent of variations available within and among populations (Subramanian et al., 1992).

Phenotypic variability and identification of bamboo taxa are usually based upon vegetative characters due to unusually long sexual cycles (1 to 120 years) and unavailability of reproductive structures, hence often lead to systematics conflicts (Stapleton, 1994). Moreover, environmental variability in vegetative characters may mislead in distinguishing between pairs of closely allied species (Wu, 1962), geographical races or ecotypes because not all genetic differentiation results in morphological variation.

The traditional method of identifying species and their genetic relationships is now gradually being replaced by protein that is more reliable or DNA profiling largely because of several limitations of morphological data.

Genetic markers have frequently been used for genetic diversity studies in a number of species (Ransom et al., 1998; Dias et al., 2008; Saxena and Chandra, 2010). Genetic markers are of two classes. One that is derived from direct analysis of polymorphism in DNA sequences called as Molecular Markers while the other derived from studies of chemical product (terpenes) or proteins/enzymes of gene expression referred as Biochemical Markers.

Isozymes are commonly used as biochemical markers as detectably different enzymes, which catalyze the same reaction (Hamrick et al., 1992). Enzymatic analyses are added tools for detecting diversity (Zeidler, 2000). The relationship between observed phenotypes and unobserved genotypes is simpler and better understood for electrophoretic evidence. Allozymes are the biochemical consequence of the substitution, deletion, or addition of amino acids in the polypeptides that comprise the enzymes and they can be distinguished if these changes affect their electrophoretic migration (Gottlieb, 1977). Since the amino acid sequence of a polypeptide is collinear to the nucleotide sequence of its coding structural gene locus, allozymes result from gene mutation. Thus,
isozyme analysis which is an analysis of protein structure using electrophoresis is an analysis of a gene (Crawford, 1990).

A major advantage of isozyme analysis is that it is a codominant, single-locus genetic marker because it allows individuals to be identified as homozygous or heterozygous at a given locus (Hamrick, 1989; Parker et al., 1998). A second significant advantage is that isozyme analysis avoids the problem of convergence and functional correlation, as it allows systematic comparisons between products of genes which are homologous (have a common origin). This is due to the simpler relationship between amino acid sequence and nucleotide sequence (Weeden and Wendel, 1990). A third significant advantage is that electrophoresis evidence is precise and directly quantifiable in terms of the number and kinds of enzymes studied, permitting the amount of genetic information utilized to be stated exactly (Gottlieb, 1977).

The genetic diversity identified through isozyme analysis is important for breeding and crop improvement programmes (Getinet Alemaw and Sharma, 1996). It also enables to detect the rate of erosion and irreversible loss of genetic variability and structure of crop varieties at the centers of genetic diversity which would facilitate the conservation of genetic diversity in landrace crop populations (Frankel, 1974; Mulatu Geleta, 2001). Isozyme electrophoresis is used to describe population structure, breeding structure and gene flow; to know species boundaries, and to document adaptive differences in allozymes; and to investigate phylogenetic relationships, rates of evolution, origin of polyploid plants and ploidy levels (Murphy et al., 1996).

In recent years, DNA profiling through RAPD technique has been used for the analysis of diversity and identification of duplicates within the large Germplasm populations (Virk et al., 1995), phylogenetic relationship (Millan et al., 1996) and management of genetic resources (Bretting and Widerelechner, 1995). With the advent of restriction enzymes and Polymerase Chain Reaction (PCR), the assessment of genetic variation directly at DNA level is possible. RFLPs (Restriction Fragment Length Polymorphism), RAPDs (Randomly Amplified Polymorphic DNAs) AFLP (Amplified Fragment Length Polymorphism) are simple Mendalian molecular markers used to assess...
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the polymorphism at gene loci. As these class of markers are based on DNA sequences, they offer advantages such as heritable through generations; availability of standard protocol; easily extractable from any plant part; required in small quantity (in nanograms) and easy to store and handle. Molecular DNA techniques allow researchers to identify genotypes at the taxonomic level, assess the relative diversity within and among the species and locate diverse accessions for breeding purposes. Moreover, the commercial value associated with identifying useful traits creates a direct value on gene banks ensuring long-term preservation of a collection. The development of randomly amplified polymorphic DNA (RAPD) markers, generated by the polymerase chain reaction (PCR) using arbitrary primers, has provided a new tool for the detection of DNA polymorphism (Williams et al., 1990). RAPD assay is the cheapest method for identifying the genotypes within a short period and also requires only limited amount of DNA. RAPD analysis has been used to study genetic relationship in a number of grasses (Huff et al., 1993; Gunter et al., 1996; Kolliker et al., 1999; Nair et al., 1999).

Marker technologies are directly applied to the area of germplasm acquisition, plant diversity assessment, stability assessment of conserved germplasm and gene bank management. The mechanism of gene flow and the distribution of genetic variation within and among populations have practical importance in terms of conservation since both factors affect the genetic structure of the populations (Dawson et al., 1997).

The advent of these highly sophisticated and informative techniques, attracting researchers, need to have some alliance with morphological studies to gain far deeper impetus of diversity, to delineate phylogeny and evolutionary biology. A combination of thus both morphological and molecular descriptors will be helpful in refining the classical taxonomic studies (Campbell et al., 1995 and Endress et al., 1996).

The elevational distribution of Ringal species ranges between 1500m to 3100m. Populations and individuals of Ringal are expected to exhibit distinct phenotypic variations which are likely to be reflected in its genetic constitution. Identification and characterization of its genetic resources and the amount of diversity present in a
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germplasm would be beneficial to all phases of improvement including identification of potential germplasm groups and for optimizing hybridization and selection procedures.

The present work was therefore, undertaken to assess variability among and within accessions of four Ringal species growing in a Germplasm at Khirsu (Pauri) through morphological and genetic tools with the following objectives:

1. To study the genetic polymorphism in four hill bamboo species on the basis of morphological traits.
2. To study genetic and molecular diversity through biochemical and molecular traits.
3. To draw phylogenetic relationship based on above studies.
PHOTOPLATE.01. PLANT TAXA (RINGAL)

A. falcata

T. falconeri

S. anceps

T. spathiflorus