CHAPTER-1
INTRODUCTION

Fungi have been known from the fossil record as far back in time as the Silurian period, 408 to 438 million years ago in the Paleozoic era. Today, it has become hugely influential group amongst all other organisms. It offers tremendous applications in health, agriculture and food industry, biotechnology, ecosystem functions and as model organisms for basic scientific investigations (Mueller et al., 2004; Xu, 2006). The ‘mushroom’ is a macrofungus with distinctive fruiting bodies or large context fungi with stalks and caps. Mushrooms have been part of the fungal diversity for around 300 million years. Prehistoric humans probably used mushrooms collected in the wild as food and possibly for medicinal purposes. It represents world’s one of the greatest untapped resources of nutritious foods which can substantiate the sufferings from malnutrition partly, owing to its richness in minerals, vitamins, fibers and essential amino acids (Chadha and Sharma, 1995; Sadler, 2003). Additionally, mushrooms can be produced artificially in the controlled cropping rooms in large quantities within a short time and provide more protein per unit area than other crops (Gupta, 1986).

Apart from the nutritional value, some mushrooms have medicinal importance, e.g., *Ganoderma lucidum, Cordyceps sinensis, Lentinula edodes* etc. *Ganoderma* is a mushroom with the longest record of medicinal use. Currently, it is among the most sorts after medicinal mushrooms in the world market. The generic name *Ganoderma* derives from the Greek *ganos* means "brightness; sheen", hence "shining" and *derma* means "skin" (Liddell and Scott, 1980). It is an annual mushroom and characterized by the presence of pores instead of the gills on the lower side of the fruiting body. The mushroom belongs to family *Ganodermataceae* having double walled basidiospores (Donk, 1964). The genus *Ganoderma* has been reported to have a worldwide distribution based on gross similarity of morphological features, e.g., Europe (Ryvarden and Gilbertson, 1993), Asia (Nunez and Ryvarden, 2000), America (Gilbertson and Ryvarden, 1986), Oceania (Quanten, 1997), and Africa (Ryvarden and Johansen, 1980). To date more than 250 species of *Ganoderma* have been described worldwide (Gottlieb
et al., 1998), of which *G. lucidum* (W. Curt.; Fr.) P. Karsten has come to forefront of intensive research (Russell and Paterson, 2006) and is the type species (Moncalvo, 2000). *Ganoderma* is spread primarily through basidiospores (Pilotti, 2005; Sanderson, 2005) or by root to root contact among trees (Ariffin et al., 2000).

Fruit bodies of *Ganoderma* are quite tough and bitter in taste, for this reason the special attention was paid to its medicinal properties rather than its value as a source of food. It is known to possess multiple health benefits such as anti-thrombosis (Choi and Sa, 2000), neuroprotective (Zhao et al., 2005), lowering blood pressure (Ansor et al., 2013), improving immunity (Kuo et al., 2006; Liang et al., 2008), preventing and treating various cancers (Zhang et al., 2010; Gao et al., 2011) and chronic bronchitis (Singh et al., 1995), diabetes mellitus (Seto et al., 2009; Xiao et al., 2011), gastric ulcers (Rony et al., 2011), hepatitis (Li and Wang, 2006), hyperlipidemia (Chen et al., 2005; Zhu et al., 2013) and hypertension (Morigiwa et al., 1986) with apparent absence of side effects (De Silva et al., 2012). Importantly, the mushroom has been demonstrated to posses anti-ageing (Saltarelli et al., 2009; Wu and Wang, 2009) and antimicrobial activities (Eo et al., 2000; Li et al., 2005; Wang and Ng, 2006), including anti-human immune deficiency virus (HIV) activity (Sahar et al., 1998; El-Mekkawy et al., 1998). All these pharmacological properties are correlated to large pool of bioactive compounds produced by fruit bodies, mycelia and spores of medicinal *Ganoderma* (Shiao, 2003). Bioactive compounds mainly include polysaccharides, triterpenoids, fatty acids, nucleotides, protein/ peptides, sterols, vitamins and minerals etc. (Chang and Miles, 2004), each having their own outstanding medicinal effects (Mizuno, 1995). In particular, the polysaccharides from *Ganoderma* have gained great attention, especially in food and drug industries throughout the world (Shao et al., 2004; Lin, 2005; Kuo et al., 2006). Its polysaccharide fractions have been recognised as effective antitumor agents (Wang et al., 2012), with immunomodulating, antiviral and other promising effects. High content of polysaccharides makes this mushroom particularly potent (Komoda et al., 1989).

Lai et al. (2004) reported the total global production of *G. lucidum* mushroom in 2002 as about 5000 tonnes, of which 3800 tonnes were produced in China. In India, the market for *Ganoderma* based nutriceuticals is growing very rapidly and was estimated to
be about US $20 million in 2000-2001 (Thakur, 2005), and more than 100 brands of *Ganoderma* are sold on the market (Lin, 2000). Even though the cultivation technology has advanced in many Asian countries, very little is known about the cultivation aspect of this mushroom in India and it is one the countries that import *Ganoderma* products from China, beside the fact that there are indigenous *Ganoderma* species in India.

On the other hand, *Ganoderma* is rare in nature due to very low frequency of basidiospore germination (Seo, 1995); consequently the amount of wild mushroom is insufficient for commercial exploitation. The higher price (Rs 600-9000/kg) received for the mushroom reflects, partly, the less developed and less reliable technology available to growers for its cultivation. Thus, growers need potentially higher incomes as its cultivation is more capital intensive. To meet the high demand in the domestic and export markets augmentation of its cultivation has become an essential aspect. Alternatively, indoor cultivation technology permits better environmental control and more concentrated production year-round. *G. lucidum* being non-chlorophyllous can’t photosynthesize to make organic matters but can make use of the large variety of substrates including lignocellulosic components (cellulose, hemicellulose, and lignin) of plant to get soluble inorganic and organic materials. High degradability potential of *Ganoderma* is a result of its ability to synthesize relevant hydrolytic and oxidative extracellular enzymes which aids in absorptive or osmotrophic nutrition (Chang and Miles, 2004). These enzymes are low substrate specific and can oxidize a wide range of compounds with structural similarities to lignin (Winquist *et al.*, 2008).

In India, large amounts of freely available agro-forestry waste offer a potential alternative substrate source for its indoor cultivation. Thus, its cultivation also offers a unique opportunity to bio-convert agricultural pollutants into economically valuable medicinal products, which in turn minimize health hazards in the environment and serve to generate incentives to Indian economy. This way, cultivation of *Ganoderma* may become one of the most inexpensive and profitable agri-businesses. One more crucial issue is the lack of knowledge of *Ganoderma* species diversity exists in India. This information is very important for determining if North-Western Himalayan regions have specific *Ganoderma* species that are of medicinal importance and to formulate
conservation measures to protect this medicinally valuable genus before it becomes extinct. Better understanding of the diversity and taxonomy of this genus would be a starting point for the Himachal Pradesh and Punjab government to formulate disciplines for exploitation of these mushrooms at state level.

Numerical taxonomy is a classification system in biological systematic, which can generate closely related groups by numerical methods of taxonomic units based on morphology only. Taxonomy of the species within genus *Ganoderma* has been considered to be in disarray due to the fact that basidiocarp morphological characters are highly versatile due to influence of environmental variations and many species within the genus share the same characteristics which make it difficult to distinguish between them (Gottlieb and Wright, 1999; Smith and Sivasithamparam, 2003a; Pilotti *et al.*, 2004). The morphological variability led many taxonomists to explore various methods to distinguish between species of *Ganoderma* or to ensure the accuracy (Hseu *et al.*, 1996; Gottlieb *et al.*, 2000). Several researchers have used different alternative methods such as cultural characteristics (Adaskaveg and Gilbertson, 1989), sexual compatibility studies (Adaskaveg and Gilbertson, 1986; Pilotti *et al.*, 2003), isozyme studies (Gottlieb *et al.*, 1998; Smith and Sivasithamparam, 2000b) and molecular techniques to determine the identity of *Ganoderma* species. Molecular techniques include internal transcribed spacer (ITS) sequence analysis (Smith and Sivasithamparam, 2000a; Lee *et al.*, 2006; Zheng *et al.*, 2007), random amplified polymorphic DNA (RAPD) (Hseu *et al.*, 1996), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Gottlieb *et al.*, 2000; Lee *et al.*, 2006) and amplified fragment length polymorphism (AFLP) (Zheng *et al.*, 2007).

Internal transcribed spacer (ITS) refers to a piece of non-functional region situated between structural ribosomal RNAs (rRNA) on a common precursor transcript contains 5’ external transcribed sequence (5’ETS), 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA and 3’ ETS. The non-transcribed spacers (NTS) are between pieces of rDNA tandem. These ITS region in fungi are highly variable, hence are probably important for molecular systematic in order to distinguish the species or strains of fungi (Moncalvo *et al.*, 1995). The ITS sequence analysis is performed by PCR amplification of 5.8S r RNA,
their sequencing and sequence comparison by Basic Local Alignment Search Tool (BLAST). There have been many reports published on analysis if ITS regions to establish taxonomic relationship within *Ganoderma* species (Moncalvo *et al.*, 1995; Smith and Sivasithamparam, 2000a; Gottlieb *et al.*, 2000). Random amplified polymorphic DNA (RAPD) is a PCR based molecular marker that employ several arbitrary and short primers to create polymorphism in DNA. It is not a specific PCR amplification, the primers randomly prime on complimentary DNA template. It is sensitive and efficient method, which is currently available for distinguish between different strains of different mushrooms (Zheng *et al.*, 2007).

To know about the genetic diversity of genus *Ganoderma* is very crucial. Low genetic diversity in a population indicates greater risks of extinction. This is because the individuals have nearly one form of information and are less likely to adapt to new environment especially if there is occurrence of environmental disaster (such as forest fires). Therefore, quantifying the genetic diversity between *Ganoderma* species plays a major role in the conservation of this important genus. On the other hand, diversity reflects much of the genetic universe within which the tools of selection, breeding and genetic engineering can be applied to provide strains adapted to a wider range of substrates, environments and cultivation methods (Kapoor *et al.*, 1996; Ikegaya, 1997). Greater genetic diversity also suggests greater variation in mushroom yield characteristics such as mushroom size, colour, shape, flavour and texture, as well as season of fruiting and biological efficiency (BE, a measure of the efficiency with which a strain converts substrate mass into mushroom mass).

At present our knowledge about the diversity and the cultivation aspects of *G. lucidum* in North-Western Himalayan region of India is limited. The study of genetic diversity of *Ganoderma* species in North-Western Himalaya will lead to better understanding of genetic relatedness up to strain level and conservation of *Ganoderma* germplasm which might lead to utilization of indigenous *Ganoderma* mushrooms in India. Due to its high demand and multiple health benefits, in current study we attempted its cultivation in cost effective manner, as well as studied genetic diversity in this genus.
1.1 AIM AND OBJECTIVES

The aim of the present investigation was “To study genetic variability and bioactive molecules production by *Ganoderma* species.” This research work was planned with following objectives:

i) To study the morphological and cultural characteristics of *Ganoderma* isolates.

ii) To study the genetic diversity of *Ganoderma* isolates on the basis of DNA profile.

iii) To screen different substrates for production of fruit bodies.

iv) Extraction and analysis of bioactive compounds from *Ganoderma* isolates.