Introduction
The genus *Withania* is a member of family Solanaceae and consists of 23 species of which *W. somnifera* is known for its medicinal value along with its counterpart *W. coagulans* to some extent. *W. somnifera*, is commonly known as ‘Ashwagandha’ and referred to as Indian Ginseng. It is also known as ‘Amukkara’ in Tamil and as ‘Punir’ and ‘Asgandh’ in Hindi. It is commonly known as ‘Winter Cherry’ in English (Yoganarasimhan, 2000; Sangwan et al., 2004).

### 1.1. Description of the plant

*W. somnifera* plants are generally erect branching shrubs, up to one meter in height. All the above ground parts, especially the stem, the veins and the calyx, are covered with a sparsely hairy tomentum. The leaves are simple petiolate, ovate, exstipulate, entire, acute and glabrous. Flowers are simple, short-pedicellate, gamosepalous, sepals 5-parted, persistent with acute linear lobes. In the fruiting stage the calyx becomes enlarged, inflated and completely encloses the fruit. Corolla gamopetalous is 5-lobed, lobes spreading or recurved, acute pubescent and greenish yellow; stamens epipetalous, arising from the base of petals; filaments slender; anthers innate and oval. Gynoecium syncarpous is composed of minute swollen ovary subtended by a long slender style. The fruit is a berry enclosed in the green persistent calyx, green when unripe, orange-red when mature enclosing numerous small capsicum-like seeds. *W. somnifera* has a chromosome number of 2n=48 (Sivarajan and Balachandran, 1994).

### 1.2. Origin and distribution

*W. somnifera* is widely distributed in the drier parts of tropical and sub tropical zones, ranging from the Canary Islands, The Mediterranean region and Northern Africa to Southwest Asia including Israel, Jordan, Egypt, Sudan, Iran, Afganistan, Baluchistan, Pakistan and India. In India the plant grows wild in
North Western regions extending to mountainous regions of Punjab, Himachal Pradesh and Jammu up to an altitude of 1500 m (Singh and Kumar, 1998). It grows successfully in sandy loam or light red soils. A soil pH range of 7.5 to 8 is ideal. It is cultivated in an area of about 5000 hectares in India mainly in drier parts of Rajasthan, Madhya Pradesh andhra Pradesh and Uttar Pradesh (Kothari et al., 2003).

1.3. Ethnobotany

*W. somnifera* has been an important herb in Ayurvedha and indigenous medicinal system for over 3000 years. Punarvasu Atriya, an ancient scholar who taught medicine at the Taxila University in Punjab about 1000 B.C., mentioned numerous uses of this drug. Another famous physician, Charaka, who lived about 100 B.C., advocated various uses of this drug and prescribed it for hiccups and female disorders. The plant has been used as an antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, astringent and more recently to treat ulcers, bacterial infection, venom toxins and senile dementia. The roots are used as a nutrient and health restorative in pregnant women and aged people. Fruits and seeds of *W. somnifera* are diuretic, hypnotic, masticatory and employed in curdling plant milk to prepare vegetarian cheese (Saritha and Naidu, 2007). Clinical trials and animal research support the use of *W. somnifera* for anxiety, cognitive and neurological disorders, inflammation, hyperlipidemia and Parkinson’s disease (Kudhiraja et al., 2000; Sharatha et al., 2007).

1.4. Phytochemical importance

The medicinal properties of *W. somnifera* have been attributed to several classes of Withanolides. (Bhattacharya et al., 1987; Asthana, 1989; Uma et al., 1993; Davis et al., 2000; Mirjalili et al., 2009). Withania’s antitumor
mechanisms are most likely multifactoral. It exhibits both antioxidant and pro-oxidant activity. \textit{W. somnifera} showed increased glutathione (GSH), superoxide dismutase (SOD), catalase and glutathione S-transferase (GST) in the liver and skin of the treated animal models. These effects could clearly repair oxidative damage caused by tumor growth and inflammation, thus reducing the likelihood of disease progression. This antioxidant activity is enhanced by the potential of \textit{W. somnifera} to up-regulate phase II liver enzymes.

![Basic structure of Withanolides](image1)

**Basic structure of Withanolides**

The most important Withanolide is Withaferin-A, much of the pharmacological activity of \textit{W. somnifera} has been attributed to Withaferin-A, which is found in leaves. The most recent studies focusing on Withaferin-A is due to its two novel activities such as tumor inhibition and antiangiogenic properties (Mohan \textit{et al.}, 2004; Mirajalili \textit{et al.}, 2009).

![Structure of Withaferin-A](image2)

**Structure of Withaferin-A**
Withaferin-A acts as a tumor inhibitor due to its ability to arrest dividing cells at metaphase. It has shown growth inhibitory activity against a number of cancerous cell lines such as Kb, Sarcoma 180 as well as Ehrlich ascites carcinoma and mammary carcinoma. It also exerts radiosensitizing effects on the tumors and carcinomas (Uma et al., 1993; Uma and Akagi, 1995).

1.5. Relevance of the study

Germplasm characterization is an important link between the conservation of plant genetic resources and utilization of bio-resources. Genetic improvement of plants has been the first and foremost aim of plant science research. Morphological characteristics are the earliest genetic markers used for assessment of variation and are still of greater importance. Usually, these characters are simple to score. The sharing of physical characteristic features is also often accepted as an indication of relatedness. There are several sets of physical character assessment for different plants at different developmental stages such as juvenile, vegetative, flowering and fruiting. However, these sets of character lack adequate coverage of the genome and are strongly influenced by environmental features (Wang and Tanksley, 1989).

Molecular markers have been replacing or complementing traditional morphological and agronomic characterization, since they are virtually unlimited, cover the whole genome and they are not influenced by the environment and are less time consuming. Each molecular marker has its own advantages and limitations. Choice of appropriate molecular marker depends on the accessibility and cost effectiveness.
Plants that are morphologically indistinguishable but differ in their chemical constituent are termed as chemotypes. Although occurrence of five distinct chemotypes of *W. somnifera* (three from Israel and one each from South Africa and India) were reported (Abraham *et al.*, 1968; Kirson *et al.*, 1971), there are evidences for the presence of more than one chemotype in India and the exact number of such chemotypes is yet to be ascertained through chemical profiling (Chakrabarti *et al.*, 1974; Kumar *et al.*, 2007). Systematic morphochemical characterization of *W. somnifera* is of great significance for programmes like quality enhancement. Earlier studies of genetic divergence of *W. somnifera* were very limited and restricted to North and Central India (Negi *et al.*, 2000; Dhar *et al.*, 2006; Kumar *et al.*, 2007). These studies have shown an extreme degree of variability with respect to growth habitat. Reports on occurrence and distribution of *W. somnifera* in Tamil Nadu are scarce and there is no extensive cultivation of this plant in this region. It is believed that *W. somnifera* is naturalized in Tamil Nadu and no extensive study is so far available for this species in this region (Sundari *et al.*, 1999).

Presently, Withanolides have been commercially obtained by solvent extraction of roots and leaves of the plant. Long gestation period between planting and harvesting, low yield from the natural source, genotypic and chemotypic variations, heterogeneity in content and non-economical chemical synthesis are major constraints in industrial production of Withanolides (Kulkarni *et al.*, 2000; Sangwan *et al.*, 2007; Sharada *et al.*, 2007). Therefore, production of a large number of superior selected *W. somnifera* chemotype without any seasonal constraints is desirable. For getting genetically uniform plants for better growth and productivity, micropropagation techniques are of special use. However, the regeneration is a genetically control trait (Bhojwani *et al.*, 1984), the development
of a micropropagated plants in superior selected chemotypes of *W. somnifera* would offer unique opportunities for producing drugs in the laboratory without depending the field cultivation.

Knowledge on *in vitro* flowering of plants for the formation of fruits and seeds is highly valuable. In *W. somnifera*, studies on *in vitro* flowering and fruiting help to overcome problems associated with premature fruit drop or poor seed set (Saritha and Naidu, 2007). Further this will offer opportunities to study the molecular physiology of fruit ripening under controlled conditions.

1.6. **Aim of the present work**

Not much is known about the genetic diversity of *W. somnifera* in Tamil Nadu compared to the Central and North region of India. Because of its increasing importance, it is imperative to do more research should be conducted in this relevance, that could be helpful for the genetic improvement. For uniform genotype and better productivity of its principle constituents, *in vitro* techniques are of special use. In this context, the present investigation was carried out with the following major objectives,

1. Collection and maintenance of wild accessions of *W. somnifera* from different parts of Tamil Nadu.
2. Developing a protocol to study the diversity among the collected accessions using important morphological traits and molecular markers including seed protein, esterases, peroxidases and RAPD.
3. Selection of the promising chemotypes using Withaferin-A as a marker
4. Developing a rapid and efficient protocol for *in vitro* regeneration of the selected chemotype.
5. Standardizing the culture conditions for *in vitro* flower induction and fruit development.