Plants are indispensable to man for his life. Plants have always been a common source of medicament either in the form of traditional preparations or pure active principles. The history of herbal medicine is as old as human civilization (Devgun et al., 2009). According to an all India ethno biological survey carried out by the Ministry of Environment & Forest, Government of India, there are over 8000 species of plants being used by the people of India. While the demand for medicinal plants is growing, some of them are increasing being threatened in their natural habitat (Sharma et al., 2010). In India, drugs of herbal origin have been used in traditional system of medicine such as Unani and Ayurveda since ancient times. Ayurveda is one of the oldest known Indian books on plants, vedas, recorded the medicinal and religious use of herbs and plants. It is a system of medicinal uses about 700 species, Unani 700 and, modern medicine around 30 species (Jawla et al., 2009).

The time has come when western countries also showing special interest in taking treatment by Ayurvedic and Unani system. Therefore, looking to the requirement of local population and of the world, it has become necessary to concentrate on our traditional Ayurvedic and Unani system of therapy (Harilal, 2009). The medicinal value of natural and allopathic drug is due to the presence of certain active substance such as alkaloids, steroids, flavonoids, glycosides, resins, volatile oils, gums, tannins and saponins etc. The active principles of the plants drugs are commonly more concentrated in storage organs of the plant such as bark, root, seeds and leaves etc. The preparation and administration of the drug should be held in the hand of skilled pharmacists and physician, because some of these materials are powerful poisons. Natural drug products, many of which have been derived from higher plants, play an important role as useful tools in pharmacological studies.

Thus to overcome the ruthless exploitation of plants, there is a great need for use of modern advanced technologies. Advanced biotechnological methods of culturing plant cells and tissue should provide new means of conserving and rapidly
propagating valuable, rare and endangered medicinal plants (Nalawade et al., 2003). Plant cell suspension and immobilized cells are being utilized for large scale production of valuable medicinal compound in vitro. Plant tissue culture is an alternative method of propagation (George and Sherrington, 1984) and is being used widely for the commercial propagation of a large number of plant species (Rout et al., 2000).

1.1 PLANT TISSUE CULTURE

“Plant Tissue Culture” is the maintenance and propagation of plant parts, as small as single cell, in axenic under controlled environmental condition (Pauls et al., 1995). The regeneration of whole organisms depends upon two concepts: that all plants cells, given the correct stimuli, can express the total genetic potential of the parent plant. This maintenance of genetic potential is called ‘Totipotency’. On the other hand, the ability to initiate cell division from almost any tissue of the plant and to regenerate lost organs or undergo different developmental pathways in response to particular stimuli is ‘Plasticity’. When plant cell and tissues are cultured in vitro they generally exhibit a very high degree of plasticity, which allows one type of tissue or organ to be initiated from another type in practical terms though, indentifying the culture condition and stimuli required to manifest the totipotency can be extremely difficult for woody plant species as compared herbaceous species and it is still a largely empirical process.

HISTORICAL BACKGROUND

The science of plant tissue culture takes its roots from the discovery of cell followed by propounding of cell theory. In 1838, Schleiden and Schwann proposed that cell is the basic structural unit of all living organisms. They visualized that cell is capable of autonomy and therefore it should be possible for each cell if given an environment to regenerate into whole plant. Based on this premise, in 1902, a German physiologist, Gottlieb Haberlandt for the first time attempted to culture isolated single palisade cells from leaves in knop’s salt solution enriched with sucrose. The cells remained alive for up to one month, increased in size, accumulated starch but failed to divide. Though he was unsuccessful but laid down
the foundation of tissue culture technology for which he is regarded as the father of plant tissue culture. White in 1934 cultured aseptically the excised roots of tomato. For the first time Gautheret (1934) reported extensive work on the tree species (woody trees). He cultured cambial tissue of *Ulmus campestris* and obtained adventitious buds. Further studies led to independent demonstration by Gautheret (1939), Nobecourt (1939) and White (1939) that the cells in culture, can be made to proliferate continuously which can undergo differentiation. Mathes (1964) was probably the first researcher to achieve growth of both shoots and roots in callus culture of *Populus tremuloides*. During 1960s a new era began and new approaches were tried such as the eradication of virus through meristem culture (Morel and Martin, 1952), culture of single cells and establishment of cell suspension culture (Muir et al., 1954), the auxin cytokinin basis of organogenesis (Skoog and Miller, 1957), somatic embryogenesis (Reinert,1958), production of protoplast (Cocking, 1960), anther culture (Guha & Maheshwari, 1964), regeneration of plants from single cell and DNA uptake by plant cells.

**BASICS OF PLANT CELL AND TISSUE CULTURE**

In plant cell culture, plant tissues and organs are grown *in vitro* on artificial media, under aseptic and controlled environment. The technique depends mainly on the concept of totipotency of plant cells (Haberlandt, 1902) which refers to the ability of a single cell to express the full genome by cell division. Along with the totipotent potential of plant cell, the capacity of cells to alter their metabolism, growth and development is also equally important and crucial to regenerate the entire plant (Thorpe, 2007). Plant tissue culture medium contains all the nutrients required for the normal growth and development of plants. It is mainly composed of macronutrients, micronutrients, vitamins, other organic components, plant growth regulators, carbon source and some gelling agents in case of solid medium (Murashige and Skoog, 1962). Murashige and Skoog medium (MS medium) is most extensively used for the vegetative propagation of many plant species *in vitro*. The pH of the media is also important that affects both the growth of plants and activity of plant growth regulators. It is adjusted to the value between 5.4 - 5.8. Both the solid and liquid medium can be used for culturing. The composition of the medium,
particularly the plant hormones and the nitrogen source has profound effects on the response of the initial explant. Plant growth regulators (PGR’s) play an essential role in determining the development pathway of plant cells and tissues in culture medium. The auxins, cytokinins and gibberellins are most commonly used plant growth regulators. The type and the concentration of hormones used depend mainly on the species of the plant, the tissue or organ cultured and the objective of the experiment (Ting, 1982). Auxins and cytokinins are most widely used plant growth regulators in plant tissue culture and their amount determined the type of culture established or regenerated. The high concentration of auxins generally favors root formation, whereas the high concentration of cytokinins promotes shoot regeneration.

TYPES OF TISSUE CULTURE

**Callus culture:** Callus culture may be defending as production and maintenance of an unorganized mass of proliferative cell from isolated plant cell, tissue or organ by growing them on artificial nutrient medium in glass vials under controlled aseptic conditions.

**Organ culture:** The maintenance or growth of tissue, organ primordial, or the parts or whole of the organ in such a way as to allow differentiation or preservation of the architecture or function. The organ culture refers to the *in vitro* culture and maintenance of an excised organ primordial or whole or part of an organ in way and function.

**Single cell culture:** Single cell culture is a method of growing isolated single cell aseptically on nutrient medium under controlled condition.

**Suspension culture:** Suspension culture is a type of culture in which single cell or small aggregates of cell multiply while suspended in agitated liquid medium. Suspension cultures are used in induction of somatic embryos and shoots, production of secondary metabolites, *in vitro* mutagenesis, selection of mutants and genetic transformation studies.

**Embryo culture:** Embryo culture may be defined as aseptic isolation of embryo (of different developmental stages) from the bulk of maternal tissue of
mature seed or capsule and in vitro culture under aseptic and controlled physical condition in glass vials containing nutrient solid or liquid medium to grow directly into plantlet.

Anther culture: Androgenesis is the in vitro, development of haploid plants originating from potent pollen grains through a series of cell division and differentiation.

Pollen culture: Pollen culture is the in vitro technique by which the pollen-grains (preferably at the microscope stages) are squeezed from the intact anther and then cultured on nutrient medium where the microspores without producing male gametes.

Somatic Embryogenesis: Somatic embryogenesis is the process of a single or group of cells initiating the development pathway that leads to reproducible regeneration of non zygotic embryos capable of germinating to form complete plants.

Protoplast Culture: It is the culture of isolated protoplasts which are naked plant cells surrounded by plasma membrane which is potentially capable of cell wall regeneration, cell division, growth and plant regeneration on suitable medium under aseptic condition

Shoot tip and meristem culture: The tips of shoots (which contain the shoot apical meristem) can be cultured in vitro, producing clumps of shoots from either axillary or adventitious buds. This method can, be used for clonal propagation.

Explant Culture: There are variety of forms of seed plants viz., trees, herbs, grasses, which exhibit the basic morphological units i.e. roots and leaves. Parenchyma is the most versatile of all types of tissues. They are capable of division and growth.

TECHNIQUES OF PLANT TISSUE CULTURE

Micropropagation

Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture
method. The main advantage of micropropagation is that this technique, is alternative method of vegetative propagation and is applied with the objectives of enhancing the rate of multiplication. The objective of plant propagation via tissue culture, termed micropropagation, is to propagate plants in large number acquiring less space and that too rapidly.

**Somatic cell genetics** - Contribution of *in vitro* methods to plant breeding i.e. somatic cell genetics is most significant, mostly in terms of haploid production and somatic hybridization.

**Transgenic plants** - The plants, in which a functional foreign gene has been incorporated by any biotechnological methods that generally not present in plant, are called transgenic plants. The methods used in gene transfer are electroporation, particle bombardment, microinjection, *Agrobacterium*-mediated gene transfer, virus-mediated transformation, pollen-mediated transformation, liposome-mediated transformation, leaf-disc transformation method. One of the successful approaches has been to engineer resistance, against insects, virus and other pathogens as well as herbicides.

**Morphogenesis in cell and tissue culture** - Plant cell and tissue culture system has been recognized as a powerful tool for the study of the process and control of developmental pathways, including cell differentiation and morphogenesis. The process of morphogenesis is regulated by many factors like culture media, culture conditions, type of explant, hormones have been recognized as a modulators of plant growth and development. Propagation of plant cell requires both cytokinin and auxin. The importance of these two hormones, auxin and cytokinin in promoting the development of shoots and roots from undifferentiated cells was first demonstrated with cultured tobacco callus cells.

**Biodiversity and strategies to preserve biodiversity** - Biodiversity in common place is defining as species richness in given habitat. Biodiversity exits as three major levels Genetic biodiversity, species biodiversity and ecosystem biodiversity. To
preserve biodiversity, strategies are two types, in situ and ex situ conservation. In situ conservation applies to wild fauna and flora in their natural habitat. Plant tissue culture plays keen role in ex situ conservation of endangered species. These applications include plant propagation, storage and maintenance germplasm. Somatic embryogenesis and automated micropropagation system have the potential for rapidly multiplying high value genotype for reforestation, which will help the race to increase forest productivity.

1.2 INTRODUCTION TO MEDICINAL PLANTS

Medicinal plants are staging a comeback and herbal ‘renaissance’ is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that regarded as unsafe to human and environment. Although medicinal plants have been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with of safety and security.

According to the World Health Organization (WHO), approximately 80% of the world’s population currently uses herbal medicines directly as teas, decots or extracts with easily accessible liquids such as water, milk, or alcohol (Farnsworth, 1990). More than 30% of the entire plant species, at one time or the other was used for medicinal purpose, while in fast developing countries such as China and India, the contribution is as much 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world.

India has probably the oldest, richest and most diverse culture traditions in the use of medicinal plants (Ved et al., 2001). The forest in India is the principle repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. Out of 17,000 species of higher plants reported to occur within India, 7500 are known to have medicinal uses. This proportion of medicinal plants is the highest known in any other country against the existing flora of that country (Shiva, 1996; Kala et al.,
Ayurveda, the oldest medicinal system in the Indian subcontinent, has alone reported approximately 2000 medicinal plant species, followed by the Siddha and Unani medicinal systems. The Charak Samhita, an age-old written document on herbal therapy, reports on the production of 340 herbal drugs for curing various diseases (Prajapati et al., 2003). Approximately 25% of drugs is derived from plants and many others are synthetic analogues built on prototype compounds isolated from plant species in modern pharmacopoeia (Rao et al., 2004).

Continuous exploitation of several medicinal plant species from the wild and substantial loss of their habitats during the past years have resulted in the population decline of many high value medicinal plant species over the years (Farnsworth and Soejarto, 1991; Kala, 2005). Surprisingly the bulk of the traded material is still from the wild and a very small number of species are cultivated. To meet demands of the Indian herbal industry, which has an annual turnover of about US$ 300 million, medicinal plants are being harvested every year from some of 1,65,000 hectares of forest (Ved, 1998).

An integrated approach for the cultivation, conservation and preservation of important plant species through plant molecular biology, plant tissue culture; research on the rationale and methodology of ayurvedic medicinal practice; isolation of active constituents and their development into new therapeutics; standardization and validation of known herbal medicinal and other related aspects need to be focused upon (Kumar, 1997). With a similar view and to strengthen the medicinal plants sectors all over the country as well as conserve the wild stock, the National Medicinal Plants Board (NMPB) was set up by the Government of India (Rawat, 2003). The prime objective of NMPB is conservation, cultivation, harvesting, marketing, processing, drug developing, etc.

TISSUE CULTURE FOR RAPID PROPAGATION OF MEDICINAL PLANTS

In vitro propagation of medicinal plants offers not only a mean for the mass multiplication of existing stock of germplasm for biomass energy production but also
for the conservation of important elite or rare species that are threatened with the danger of extinction. Plant cell culture systems represent a potential renewable, and colorant, which cannot be produced by microbial cells or chemical synthesis (Sree et al., 2010). Plant cell culture is being utilized for large scale production of valuable medicinal plants. The principle advantage of this technology is that it may provide continuous, reliable source of plant pharmaceuticals and can be used for the large scale culture of plant cells from which these metabolite can be extracted. Plant tissue culture is an alternative method of propagation (Badoni and Chauhan, 2010) and is being used widely for the commercial propagation of a large number of endangered medicinal plants (Sharma et al., 2010).

1.3 *Pterocarpus marsupium*

*Pterocarpus marsupium* (Roxb.) is a deciduous tree, commonly called as Indian Kino tree or Malabar Kino, belonging to the family fabaceae. It is a medium to large sized tree reaching height up to 15-20 meter with dark brown to grey bark having swallow cracks. The bark exudes a red gummy substance called ‘Gum Kino’ when injured. Leaves are compound and imparipinate. Flowers are yellow in terminal panicles. Fruit is circular, flat, winged pod. Seed is convex and bony (Warrier, 1995). Tree flowers and fruits in the month of March to June (Yadav and Sardesai, 2002).

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**Pterocarpus marsupium** Roxb.

**Taxonomic classification**

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Class: Magnoliopsida
Order: Fabales
Family: Fabaceae
Genus: Pterocarpus
Species: marsupium

Vernacular Names
Eng.- Indian kino tree, Malabar kino tree; Hindi- Bijasal, Vijayasar, Bia; Beng.- Pitashal, Piyasal; Gujar. -Biyo, Hirdakhan; Kan.- Hannemara, Honne, Bijasara, Asana; Mal. - Venna, Venga; Mar.- Dhorbenla, Asan, Bibla; Punj. - Chandanlal, Channanlal; Tam. - vengai; Tel.- Peddagi, Asana; Oriya- Byasa, Piashala; Assam- Ajar; Kash.-Lal Chandur; Urdu- Bijasar.

GEOGRAPHIC DISTRIBUTION

Pterocarpus marsupium is distributed in deciduous forest throughout the India (Varghese, 1996). It is found to grow in parts of states such as Andhra Pradesh, Bihar, Gujarat, Karnataka, Kerala, Madhaya Pradesh, Maharashtra, Orissa, Rajasthan, Tamilnadu, Uttar Pradesh, West Bengal and Goa (Sanjappa, 2000).

ETHNOBOTANY

Leaves are used for food and manure. The flowers are used in the treatment of fever. Pterocarpus marsupium is a multipurpose leguminous tree. Heart wood is astringent, bitter, acrid, cooling, anti-inflammatory, depurative, haemostatic, anthelmintic, constipating and rejuvenating (Warrier, 1995). The wood is useful in chest pain, body pain, and indigestion (Bressers, 1951). The paste of seed and wood is useful in diabetic anaemia (Trivedi, 2006). The paste of heart wood is useful in body pain and diabetes (Yesodharan and Sujana, 2007). Wood of the tree is useful in making the water glasses of the diabetic patients (Reddy, 2008). Bark is useful in vitiating condition of kapha and pitta, elephantiasis, erysipelas, urethrorrhea, rectalgia, opthalmopathy, hemorrhages, dysentery, cough and grayness of hair. Aqueous infusions of the bark possess antidiabetic potential (Anonymous, 1968). The powdered bark is mixed with Schleichera oleosa and taken with cold water to treat
dysentery (Mohanta, 2006). The juice of the bark is applied in the mouth (Prusti and Behera, 2007). Tribal people residing in the Jodhalal forest of Karnataka use stem bark to treat the wounds, fever, stomachache, diabetes and elephantiasis (Mankani et al., 2005). Bark is useful in urinary discharge and piles. The gum Kino is externally applied to leucorrhoea (Pullaiah, 1999). Gum Kino is used in the treatment of polyurea and inordinate night sweat and Phthisis plumonalis. The Kino powder may be dusted on ulcers and bleeding surfaces. The gum is used in the toothache and leaves paste is applied in wounds (Chopra et al., 1956). Bruised leaves are useful in boils sores, skin diseases, stomachic and cholera (Jain, 1991). Leaf juice is given in purulent discharges from ear, plant is useful in snakebite and scorpion sting. Fruit cures biliousness and kapha (Kirtikar and Basu, 1975). Flowers are bitter, sweet, cooling, appetizing and febrifuge (Warrier, 1995) and used in fever (Pullaiah, 1999).

PHYTOCHEMISTRY

Mitra and Joshi (1982) isolated an isoflavon glycoside from the heart wood of the Pterocarpus marsupium and identified it as 5, 4’-dimethoxy-8-methylisoflavone. Three isoflavon glycosides namely retusin-7-glucoside, irisolidone-7-rhamnoside and 5,7-dihydroxy-6-methoxyisoflavone-7-rhamnoside, eudesmane type sesquiterpene alcohol, Selin-4(15)-en-1,11-diol was reported from the heart wood of the P. marsupium (Adinarayana and Syamsundar, 1982). Subba Rao and Mathew (1982) characterized a naturally occurring hydrobenzoin, marsupol, 4,4’-dihydroxy-a-methylhydrobenzoin and a novel 2-hydroxy-2-benzylcoumaranone, carpucin, characterized as 2-benzyl-2,4’,6-trihydroxy-4-methoxybenzo(b) furan-3(2H) from the P. marsupium heart wood.

PHYTOPHARMACOLOGY

In the Indian system of medicine many plants have been used to treat diabetes; of which, Pterocarpus marsupium popularly known as Bijasar is one of the most potent species (Rastogi and Mehrotra, 1989; Sivarajan and Balchandran, 1994; and Grover et al., 2002a). Its heart wood is used as antidiabetic drug (Shah, 1967). Many workers have studied the antidiabetic potential of Pterocarpus marsupium.
According to Joshi et al., (2004) *P. marsupium* decreased the blood glucose levels both in normal and non-insulin dependent diabetic (NIDDM) rats.

**USES & BENEFITS OF *PTEROCARPUS MARSUPIUM***

*Pterocarpus marsupium* has many therapeutic and medicinal properties and is used in treating conditions like elephantiasis, diarrhea, leucoderma, rectalgia, cough, dysentery, and grey hair.

- The crushed leaves of the plant are used externally for the treatment of boils, sores and certain types of skin diseases.
- Consumption of the herb helps to lower the lipid and glucose levels of the body.
- It leads to the significant reduction in the blood sugar levels, in diabetic patients.
- One of the major effects of *P. marsupium* in human body is that it helps to prevent cell proliferation and induces apoptotic effect in tumor cell lines.
- It is also used as an astringent and helps to cure dental problems, like toothache.
- The herb also has anthelmintic properties and is a bitter acrid and an anodyne.
- *P. marsupium* prevents the leakage of electrolytes, caused by herbicide-induced oxidative damage.
- The herb cures ailments like leprosy, leucoderma, rectalgia, constipation, hemorrhages and even rheumatoid arthritis.
- *P. marsupium* exhibits strong anti hyperlipidemic properties and helps in reducing total cholesterol, LDL, VLDL and serum triglyceride levels in the human body. It, however, does not cause any change in the HDL cholesterol levels.
- *P. marsupium* also has antimicrobial properties and is used to act against certain types of bacteria and virus.

**ROLE OF PLANT TISSUE CULTURE**
The application of biotechnological techniques to medicinal plants has received considerable interest especially when the final product is defined, purified, and natural. Biotechnology tools are important for multiplication and genetic enhancement of the medicinal plants by adopting techniques such as in vitro regeneration and genetic transformations. It can also be harnessed for production of secondary metabolites using plants as bioreactors. In vitro propagation of plants holds tremendous potential for the production of high quality plant-based medicines (Murch et al., 2000). This can be achieved through different methods including micropropagation. Plant tissue culture is an alternative method of commercial propagation (George and Sherrington, 1984) and is being used widely for the commercial propagation of a large number of medicinal plants (Rout et al., 2000; Mehrotra et al., 2007). Nevertheless, implementations of tissue culture techniques are not so simple. The regeneration protocols are not universal and highly genotypic and species specific. Therefore, there is a need to develop tissue culture technique for important medicinal plants.

Tissue culture method has been proved to be a promising technique for conservation and rapid multiplication of several forest rare woody species. However, Fabaceae members have been found to be difficult to culture in vitro due to their recalcitrant nature (Trigiano et al., 1992; Jha et al., 2004). In vitro regeneration protocols have been reported in Pterocarpus marsupium using different explant sources including cotyledonary nodes, nodes and hypocotyl (Chand and Singh, 2004; Tiwari et al., 2004; Anis et al., 2005; Husain et al., 2007, 2008, 2010). The present work is taken with aim to establish aseptic cultures of Pterocarpus marsupium from nodal segments of mature tree.

**OBJECTIVE**

Objective of the research work is to develop in vitro propagation technique for *Pterocarpus marsupium* Roxb. This includes:

(a) Axillary bud proliferation

(b) In vitro shoot multiplication
(c) In vitro rooting

(d) Hardening and acclimatization