Introduction

Sericulture contributes four types of silk Mulberry, Tassar, Eri and Muga to the World of silk fabrics. Mulberry silk accounts for 90% of the total silk production in the country thus it has assumed prominent place in the agro–industrial activities. In the context of rural development, mulberry/sericulture served the social objectives like Providing off–farm employment and Preventing migration of rural people. The salient features like higher yield due to technological advancements, better returns in the domestic as well as international markets for the silk and silk products, and scope for frequent cash accrual round the year has brought mulberry sericulture to a comparable level with other agricultural cash crops grown in the similar agro–climatic condition. In addition to that, presently it was Morus spp. is utilized as a medicinal plant. It contains many biochemical compounds such as Moranolin (DNJ), Moran (glycopeptides), hydrophobic flavonoids (flavones and flavonone), 2-Arylbenzofuran, and Ethanolic extract, Flavonoids, Polyphenols, Carotenoids, Vitamins A, C, E, Ethyl acetate, γ -aminobutyric acid, Flavones are isolated from different parts of mulberry plants which play a vital role in Hypoglycemic activity, Anti-obesity action, Lipid-lowering action, antioxidants action, anti inflammatory actions, anti allergic action, vasoactive action, neuroprotective action, anticancer action (Saurabh et.al, 2012). Thus sericulture has become a highly remunerative not only as agro based cottage industry but also as pharmaceutical industry.

Silk production is expensive: consequently, silk is considered a fiber of luxury. It is thought that silks expense, beauty and hand contributed to the beginning of the manufactured fiber industry. People wanted fabrics that looked and felt like silk but without the cost so they tried to manufacture fibers similar to silk. Eventually rayon was developed from these efforts of trying to artificially produce silk. Cultivated silk is a beautiful luxurious fiber with a smooth luxurious hand. This type of silk can be dyed and printed in bright colors that are very pleasing to the eye. Wild silks are duller and have a more
coarse hand and texture. Silk’s abrasion resistance is moderate. However, it is ranked high in strength for natural fibers. Silk fabrics have good absorbency. Fabrics made from silk are comfortable in the summer and warm in the winter. Silk fabrics have only moderate resistance to wrinkling. It is often recommended that silk garments be dry–cleaned. When hand or machine washing washable silk garments, test for water spotting in an obscure place because silk water–spots easily. Perspiration and sunlight weakens and yellows silk fabrics. Upholstery and drapery fabrics that contain silk should be protected from prolonged exposure to direct sunlight.

Legend has it that silk was discovered around 2640 B.C. when a Chinese Empress, Hsi Ling Shi, became intrigued by silkworms. She was given the task of studying the silkworm by the Emperor. He wanted to know if the silkworm’s thread could create more happiness for his people. She then learned how to unwind the silk from the cocoon and make fabric from it. Thus, the silk industry in China began and China still holds the monopoly on the industry today with 54% of the silk production. The beautiful fabrics produced from silk fiber were often coveted in other countries. Eventually other countries began producing silk fabrics. The Silk industry has a unique position in India, and plays important role in Textile Industry and Export. According to Western historians, mulberry culture spread to India from China through Kotan (Tibet) by about 140 B.C. India is the 2nd largest producer of silk in world and contributes 18% of the total world raw silk production. In India Silk is available with varieties such as, Mulberry, Eri, Tasar, and Muga. Sericulture plays vital role in cottage industry in the country. It is most labor–intensive sector that combines both Agriculture and Industry. This sector operates around 54000 villages all over country and provides employment to 6 million people spread over rural areas. The production process of silk contains a large chain of interdependent operations that provides medium of livelihood to people, including, Silkworm Seed Producers, Farmers–cum–Rearers, Reelers, Twisters, Weavers, Spinners of silk Waste, Traders etc. Silk provides rich dividends with low investment, and it provides returns throughout the year. The total
production of silk during 2005–06 was 16,500 MT and Exports were Rs. 2,879.56 crores.

Mulberry is grown for its foliage, which is used for rearing silkworm (*Bombayx mori* L.). Mulberry (*Morus* Species) is an economically important crop belonging to the family Moraceae. The genus *Morus* L, known as mulberry, is native to temperate Asia and North America. Presently it grows in warm climatic zones between 50° N latitude and 10° S latitude (Yokoyama, 1962) which includes Sino–Japanese region of the old world and Rocky Mountains southwards to Andes of continental America in the new world and distributed over 29 countries or more. So far there are about 150 species of genus *Morus* described (Vide *Index Kewensis*, London) and presently 50% of them have been reduced as synonymous or varieties of the same species. Mulberry is presumed to be a native either of India or China and it is known to be originated on the lower slopes of the Himalayas. According to Qadri (2008) the seri–germplasm resources (SGRs) should be protected from being getting extinct and towards this the biodiversity available should be explored and conserved following suitable and cost effective management practices.

The production cost of mulberry leaves accounts to more than 60 percent of cocoon production and reflects the importance of Mulberry leaf in silk industry. Improved mulberry varieties with higher leaf productivity are very much essential for enhancing and sustaining profitability in Sericulture. In India different mulberry varieties suitable for different agro climatic regions and agro economic practices have been developed, among which a few promising clones are M₅, S₃₆, V₁, Anantha. Micropropagation of some of these is often cited as the most successful example in sericulture. It has changed from the laboratory curiosity to a commercial industry and offers many advantages over the conventional method of multiplication. It is the most widely and successfully used technology for private companies for the mass production of mulberry. The success has been relatively rapid with herbaceous crops but the progress in perennial sericulture and woody trees has been slow. Nevertheless,
the potentials are there, and much more effort is needed to get 100% success in mulberry plants of value.

Tissue culture methods will considerable shorten the breeding cycle time and make the way for genetically altered plants. Plants tissue culture is based on two principles: first that plant cells may be cultured axenically in vitro and second, that they are inherently totipotent, so that given the correct conditions they are able to regenerate to form new plants. In herbaceous dicot species, regenerable tissue cultures can be established a variety of plant parts, including relatively mature organs. Tissue culture, the technique of growing plant cells, tissues and organs in an artificially prepared solid or liquid nutrient media under aseptic conditions is now a well-established in vitro technique. It has been proved to be a scientific tool to achieve the improvement in any crop. One of the main methods of tissue culture, which is widely used in inducing variation towards crop improvement is Induction of callus through endosperm has been accomplished by many workers (La Rue, 1949; Bhojwani and Johri, 1971; Seki et al., 1974; Boj’wani and Razdan, 1996; Thomas et al., 2000). Though callus or cell cultures could be easily induced from genotypes, plant regeneration is the real problem that has limited the application of these techniques in mulberry improvement. Nevertheless, it is required and important to study its feasibilities in varieties of current importance on which not much work has been carried out in this regard Hence, the present study has been undertaken, primarily to study the possibility of developing a viable protocol for callus induction, regeneration from endosperm callus and also to induce roots from the in vitro shoots.

Mulberry is propagated by asexual means such as stem cuttings and root grafts. Since cross pollination is the rule rather than an exception, enormous heterozygosity occurs in the plant (Das, 1983). The speed of improvement of this crop is restricted because of its perennial nature and prolonged juvenile period. Although it is sexually fertile it is not possible to propagate commercially through seeds because of high degree of heterozygosity. The
drawback of seed propagation is by bring about varied populations. There is a paucity of information about the inheritance pattern of yield contributing characters which is a limiting factor in choosing the parents further, propagation through seeds also limited by the ploidy. Among the polyploids of mulberry the triploids have many desirable traits including faster to growth rate, superior quality of leaf and resistance to cold and diseases (Ho-Rak Kim et al., 1985). But the production and multiplication of triploids is time consuming (Das, 1983).

Mulberry tree improvement through conventional breeding takes many years to evolve a desirable clone from economic and commercial point of view by conventional hybridization methods and also difficult due to its heterozygotic nature (Rao et al., 1989; Ravindran and Lakshmi, 1994; Song and Sink, 2004). Many elite varieties have poor rooting ability, and also propagation through cuttings is restricted to only certain months of the year (Narayan et al., 1989). One more limitation of good quality and quantity of mulberry is poor tolerance of mulberry verities to adverse environmental conditions. These include acidity, alkalinity, drought etc. Considerable progress has recently been made in the development of new techniques such as tissue and cell cultures which is an answer to many unsolved problems in plant breeding, development of homozygous lines, genetic manipulation, production of resistance varieties in plants including mulberry.

Though, Rechin Ger (1893) reported that the formation of callus on isolated fragments of stems and root, the culture of plant tissues on a nutrient medium was first performed by Haberlandt (1902). According to cell theory proposed by Schleiden (1838) and Schwaan (1839), the genetic information necessary for the development of the entire organism is contained in all the living cells. When such cells are released from the developmental controls, they are able to divide and differentiate into organs and whole organisms. Scientists exploited this totipotent nature of the cells and developed various media and cultural conditions for the proliferation of callus, which was
described as an unorganized proliferating mass of tissue. The callus can be again differentiated into whole plants by manipulating the media. Earlier investigators were under the impression that the regenerated plants are true to type. It is not universally the case. On the basis of intensive survey of literature, Larkin and Scrowcroft (1981) came to the conclusion that changes could be induced in the genetic material during culture. These can be described as somaclonal variants which was observed in many plants. Induced changes include most of the economically important characters such as male sterility, resistance to diseases, plant type and increase in yield. Rapid progress has been made in plant tissue culture and its application in agriculture and horticulture during the past fifty years. The importance of tissue culture technique, for clonal propagation of tree species has been amply stressed by Geissbuhler and Skoog (1957) and Haissig (1965). In most of the trees which are not amenable to vegetative multiplication or where the conventional methods are time consuming, it has become imperative to devise methods by which large scale populations of selected trees can be raised (Mehra et al., 1974). The history of regeneration of complete plants in tree species through tissue culture is only about a decade old. The immense possibilities offered by application of technique of tissue culture for genetic upgrading of economically important plants have been emphasized by Murashige (1977).

The use of shoot apex cultures and auxiliary bud cultures to clonal propagation provides an alternative to routine vegetative propagation which in turn provides an alternative to routine vegetative propagation of woody species. Micro propagation can be described as the *in vitro* multiplication of a plant and it normally involves the hormonal release of dormancy of the auxiliary buds and their outgrowth. These released auxiliary buds then are subcultured into a similar medium and the whole process is repeated. It is clear that by using this technique large numbers of plantlets can be produced in a short time. The advantage of a micro propagation system over conventional seed propagation is that it is possible clonally to multiply plants with a desired
genotype (Jones et al., 1982). Micro propagation can speed up the production of planting

Induced changes include most of the economically important characters such as male sterility, resistance to diseases, plant type and increase in yield. Tissue and cell cultures are going to play a key role in this new ERA of research activity by providing advanced technology such as single cell cultures, protoplast cultures and somatic hybridization. The unique features to be appreciated include the ability of wide range of plant cells to be cultured indefinitely in fully defined media. Successful work, in fundamental research can stimulate applied studies. Hence, there is every need to develop right cultural conditions, for callus initiation, shoot initiation and root initiation in mulberry which provides an anchor for developing advanced technology.

In the first decade of 1900, when sericulture industry in Japan began to prosper, existence of a large number of natural triploids and their significant contribution to the quality and quantity of silk produced was realized. These findings have opened new vistas in the sphere of mulberry breeding throughout the world. As a result, large numbers of superior triploids were developed in Japan and China. Triploids exhibit better rooting ability, survival percentage (71.81), shoot ratio (0.92), higher rate of growth and development, inter nodal length is in between that of diploids and tetraploids. Triploid variety viz. AzN sh9 was resistant to Pseudomonas syringe pv mori and yielded 42% more leaf than the diploid (Dzhafarov et al., 1970). Triploids exhibit 11.5% more leaf thickness than diploids and the number of stomatal chloroplast and size of stomata increased while the number of stomata per unit area decreased in triploids. Biochemical studies revealed that total nitrogen, protein, carbohydrate, vitamins and mineral contents (Fe, B, Mo, Cu and Co) are more in triploids than diploids (Das and Prasad 1973). The rate of reduction of most of the trace elements during growth was low in triploids, which indicates the possibility of triploids as a source of late food for silkworms. The activity and functional, composition of esterase, lactase, malate, glutamate and glucose-6-
phosphate dehydrogenase, exhibits an increase in triploids and tetraploids than diploids. The changes in the enzyme activity were proportional to the gene dose (Talyshinskii, 1990). The relative content of DNA and RNA in growing triploid leaves increase more rapidly than those in the diploid leaves (Das and Prasad, 1970). Crossing diploids with tetraploids usually produce triploids. Some of the existing triploids in India are Tr4, Tr8 and Tr10 popular in hilly areas of West Bengal and Uttar Pradesh. S 1635 variety is in demand in West Bengal, while C 1730 popular in Koraput (Orissa) and S 1730 and V-5 triploid varieties in Assam.

Most of the studies in mulberry tissue culture are confined to regeneration and micro propagation. The most responsive explants which have been exploited for micro propagation are shoot tips, auxiliary buds and winter buds (Oka and Ohyama, 1986; Ohyama and Oka, 1987). Oka and Ohayma (1974, 1975) got induction of complete platelets from different age winter buds and studied the effect of BAP and NAA combinations on the regeneration. Complete plantlets and multiple shoots were reported from auxiliary buds (nodal explants) of different mulberry genotypes viz., M. alba, M. indica by Mhatre et al. (1985), Chattopadhyay et al. (1990), Jain et al. (1990) Raghunath et al. (1992) and Ravindran and Lakshmi Sita (1994) using different concentrations of BAP, GA3, 2.4-D and IBA in MS media. High BAP concentration was found to be useful in the regeneration and micro propagation of M. laevigata (Hossain et al. (1992). Zaman et al. (1992a) studied the effects of different sugars on in vitro shoot proliferation of Morus alba cv S-1. The best results were obtained from the medium containing 30g/l sucrose followed by 30 g/l fructose. Further, Zaman et al., (1992b) observed maximum shoot growth (6.03 shoots /culture) when pH level of the medium was 5.5. Also sprouting of axillary buds and growth of proliferation shoots were faster on media gelled with 6g/l of agar agar. According to Zaman et al., (1993) adventitious root formation was improved in the shoots cultured at ambient temperature of 30°C.
Islam et al. (1993) investigated about the effects of cytokinins types and tyrosine for *in vitro* propagation of mulberry (*M. alba*). *In vitro* clonal propagation of *Morus alba* cv S-1 was achieved by culturing nodal explants and shoot apices of mature trees on MS medium supplemented with different cytokinins alone or combination with tyrosine. Maximum frequency of explants producing shoots and highest number of shoots/ explant were obtained from cultures grown on media supplemented with 2mg/l BAP + 50 mg/l tyrosine. Response was better in nodal explants than from shoot apices. The shoots were excised and cultured on half strength of MS media using IBA, IAA & NAA each 1.0 mg/l. Root induction was best in 1.0 mg/l IBA. In NAA a considerable amount of callus formed at the base portion of the micro-shoots, and could not survive after transplantation. The rooted plantlets were taken out of the culture tube and washed and planted in small plastic pot filled with garden soil and compost (1:1). Plantlets taken from IBA supplemented media survived much better (70%) as compared to IAA supplemented media (15%).

Hiroyuki (1992) reported about the possibility to develop an *in vitro* propagation system using immature leaves of mulberry. Immature leaves in winter buds were collected precultured on MS medium containing 10 mg/l of BAP. The largest number of shoots was obtained from adventitious buds when 1 mg/l BA was added to the MS medium. Hayatesakari mulberry varity developed the largest number of shoots followed by Minamisakari mulberry varity and Kenmochi mulberry varity. These shoots could be successfully rooted and acclimatized. When they were transplanted to nursery soil, 90% of them grew into saplings. Katase (1993) conducted an experiment on *in vitro* shoot proliferation in *Morous bombycis* cv. Mitsushiughri in response to carbon sources, explant preparation and incubation conditions. Optimum proliferation was obtained on MS medium supplemented with 1.0 mg/l BAP and 25% fructose under a light intensity of 3000 - 6000 lux at 25°C. Successive subcultures resulted in 6-fold proliferation for every 30 days.
Islam et al. (1992) reported on micro propagation method of mulberry (*M. alba* cv S-1). They used nodal explants of mature trees. Multiple shoots were proliferated in MS medium supplemented with 2 mg/l BAP. By repeated subculture a large number of shoots were built up. Then the shoots were rooted in half strength of MS salts and 1.5% sucrose. Best root formation was in 0.1 mg/l IBA. For transfer to the soil, the *in vitro* regenerated plantlets were removed, washed thoroughly and transplanted in poly bags with 1:1 non-sterile garden soil and compost. The transplantation success was 85%. Tewary et al. (1995) reported the protocols of rapid *in vitro* multiplication of high yielding mulberry (*Morus spp.*) genotypes V-1 and S-34. They used the apical shoots of those varieties as explants and cultured on MS (1962) media fortified with 0.5-3 mg/l BAP alone and it combination with 0.1 - 0.5 mg/l NAA. For rooting, regenerated shoots after attaining about 3cm were clipped off the transferred to rooting media (MS +NAA or IBA ranging from 1 -2 mg/l). The rooted plantlets of 5 - 6 cm height were hardened for 20-30 days on vermiculite + sand mixture and thereafter transferred to field. Scanning electron Microscopic studies and qualitative estimations of field established tissue cultured plants proved its true to type nature with mother plants. The best shoot induction response was 2 mg/l BAP + NAA 0.1 mg/l of V - 1 and 2 mg/l BAP alone for S -34. Kazuyohayashi (1995) experimented with shoot tips of *in vitro* grown seedlings of mulberry which were cultured in a liquid MS medium, using tubes (24 x 120 mm) that were rotated vertically at 2 rpm under a moderate light intensity. Multiple bud bodies (MBBs) were induced after 1 -2 months in the presence of 2 mg/l Phenyl Urea, a cytokinin like urea derivative. BA and Zeatin promoted the development of a few leaves but did not induce MBB. NAA had no effect on the MBB induction but stimulated the callus formation. MBB has a central parenchymatous tissue associated with many bud primordial on its surface. Thus, MBB could be subdivided into small parts and each could be cultured at intervals of one week. By this way the MBBs from mulberry, produced normal shoots when cultured on soiled medium containing 1 mg/l BA. Fructose stimulated the shoot formation from MBB more effectively than sucrose.
shoots could be rooted in a medium with 0.01 mg/l NAA. MBB requires neither large space nor medium solidifying agent for maintenance and is a satisfactory material for plant propagation.

Tewary et al. (1996) reported about genotypic differences to in vitro shoot development in different genotypes of mulberry (Morus spp.). They used nodal explants of ten selected mulberry genotypes on MS medium fortified with different concentration and combinations of BAP IBA, and GA₃. Differential response in terms sprouting percentage ranging from 70-80 were recorded in Sujanpur -5 on BAP 1mg/l; S-799 on BAP 2mg /l; K-2 & LF -2 on BAP 3 mg/l Tr-10, MS-3, RFS-135 and Mr-2 on BAP 1mg/l +IBA 0.5mg/l; S-41 and C-763 in BAP 1mg/l + IBA 0.5 mg/l + GAS 0.5 mg/l media. Incorporation of kinetin either alone or in combination with IBA and GAS did not induce any response. Sprouted explants or the tested genotypes gave and average shoots length of 4.5 cm within 6 weeks of culture. Kamili and Shah. (1998) studied on micro propagation from auxiliary buds of Morus alba and Morus latifolia. The auxiliary buds were cultured invitro on a number of auxin and cytokine combinations. In M. alba 40% of the cultures initiated inflorescence. L Addition of growth adjuvant like Yeast extract (YE), coconut milk (CM), malt extract (ME), peptone and casamino acids with 2,4-D(1mg/l) promoted hundred percent sprouting of auxiliary buds in both the varieties of mulberry. Plantlets with profuse root system were obtained in M. latifolia var. in 60% cultures when MS medium full strength was supplemented with NAA (0.5 mg/l) + BA (1mg/l). In case of M. alba robust shoots were obtained in culture with NAA (1 mg/l)+Kn (1 mg/l) and rooting was best in full strength MS containing NAA (0.5 mg/l) + kn (1 mg/l) where both root and shoot formation occurred in about 60% of the cultures. The plantlest were transferred successfully to soil and 80% survival was observed in both the varieties.

Srinivasa et al., (2000) standardized the protocol for the micro propagation of M.alba (Berhampore local) through auxiliary buds in vitro and field grown plants. The explants were inoculated on two different basal media.
MSBM (Murashige and Skoog, 1962) and LSBM (Linsmaier and Skoog, 1965). MSBM was found to be best when compared to LSBM for bud multiplication. Further studies were conducted on MSBM with different concentrations of BAP (1.0, 2.0, 2.5 and 3.5 mg/l) and compared with the responses of LSBM. After three weeks the auxiliary buds of aseptic plants gave more multiple shoots on MS medium, supplemented with 2.5 mg/l BAP, whereas explants from field grown plants gave comparatively lesser number of multiple shoots. Tewary et al. (2001) reported that expensive agar agar (8g/l) could be replaced by China grass (12 g/l) for invitro shoot development and by sago (150 g/l) for in vitro rhizogenesis. Bhatnagar et al. (2002) studied on rapid in vitro TDZ mediated micro propagation of Morus indica cv. C-176 and C-76 through auxiliary buds. The explants were vertically placed on the slants of MS basal media. After 15 days when bud break was observed they were transferred to a shoot elongation medium (SEM = MS +0.5 mg/l BAP + 0.5 mg/l GA3 + 2 mg/l Ag NO3) for further growth of shots. The elongated shoots bearing 6-7 leaves were transferred to MS + NAA + activated charcoal for rooting. Shoots, which produced at least one root, were scored as rooted. Plantlets with well developed roots were transferred to earthen post containing autoclaved soil and soil rite (1:1) and maintained in the culture room for about one month before transferring to field. The shoot formation was better in MS +TDZ (0.1 mg/l) in both varieties. Rooting was best in MS + NAA (1 mg/l + activated charcoal (1%). The rooting percentage was 72 for C-176 and 6 for c-776. The survival rate of the transplanted plantlets was 80% for C-176 and 75% for C-776.

Most of the sericulturists are traditionally practicing local mulberry (Mysore local) varieties for plantation which gives low leaf yield and the low quality. Central Sericultural Research and Training Institute, Mysore and Regional Sericultural Research Station, Anantapur District have evolved following High yielding mulberry varieties like M5, V1, S13, S30, S36, S54 and Ananta. M5 is an open pollinated hybrid (OPH) selection from among the breeding population of Mysore Local cultivator. Bushes open type, branches simple, erect and greenish to grayish. Slightly hairy, semi-glossy, dark green
Leaf thickness (µm) 210.00 Leaf yield (MT/ha/yr) 22.4 MT/ha/yr against 35.7 MT/ha/yr in putative diploid. When grown under irrigated condition yields 12 MT/ha/yr and grown under rainfed condition yields 35 MT/ha/yr under irrigated condition. It is recommended for South India under irrigated conditions. V1 hybrid variety is developed by Central Sericultural Research and Training Institute, Mysore with combination of S30 and Ber C 776. The nutritious dark green boat shaped leaves are thick and shiny in nature. The branches grow long and straight. When grown under irrigated condition with 2’ X 2’ spacing the leaf yields 55 tonnes per hectare per year. It is convenient to feed all stages of silkworms. S 36 Variety is having light green coloured leaves are big and succulent. The branches grow straight. It is convenient to feed all stages of silkworms. When grown under irrigated condition the leaf yield will be 45 tons per hectare per year. Ananta variety yielding mulberry variety developed at Regional Sericultural Research Station, Anantapur. It is drought resistant. Leaves are very big in size with light green in color. The leaves are succulent and good in quality. The leaves can be fed to both Chawkie and Late age silkworms. This is pest and disease resistant and yields more during summer. With 2’ X 2’ spacing, this variety gives around 65 to 70 tonnes of leaf per hectare per year.

Most of the above desired varieties do not root easily or have low rooting ability. Such varieties difficult to root could be multiplied by using tissue culture techniques. And also, propagation via cuttings is restricted to only certain months of the year and the saplings obtained by cuttings show inferior morphogenic vigor when compared with micropropagated plants (Zaman et al., 1997). Mulberry improvement through seeds propagation is undesirable owing to cross-pollination and heterozygosity (Das, 1983), polyploidy in the plants and the dioecious nature of the genus is a serious barrier to genetic improvement by conventional hybridization technique. Asexual multiplication is preferred over sexual means as the genetic characters of the parent are maintained and population variation is minimized. Clonal
propagation of mulberry using tissue culture techniques has many useful applications in silk industry (Ravindaran et al., 1988).

In France, Nitsch succeeded in the culture of excised ovaries and tissues of fruit trees. This was the first attempt of the culture of fruits in vitro. Ohyama (1970) was the first to report tissue culture in mulberry plants. *Morus alba* L., *M. bombycis* L. and *M. indica* L have since been cultured (Ohyama, 1970; Ghugale et al., 1971; Oka and Ohyama, 1974, 1975, 1981; Patel et al., 1983). In mulberry, few attempts have been made for direct regeneration from leaf (Oka and Ohyama, 1981; Vijayan et al., 2000; Machii, 1990; Saito and Katagiri, 1989; Hossain et al., 1992; Ivanica, 1987; Jain and Datta, 1992), cotyledon (Wang et al., 1996) and anther (Shoukang et al., 1987) explants were carried out and indirect regeneration (Srinivasa et al., 2001; Narayanan et al., 1989) was also reported.

Recently, tissue culture technology has been applied to mulberry and fruitful results have been obtained. There are reports available regarding the development of highly efficient protocol for direct regeneration from axillary buds of some elite Indian mulberry cultivars, *viz.*, RFS _175_, S _1_, K _2_, DD and a Japanese cultivar *Morus multicaulis* cv. Goshoerami using thidiazuron and activated charcoal (Tewary et al., 1999; Bhatnagar et al., 2001). Earlier studies on plant induction from winter buds (Oka, 1985), axillary buds (Jain and Datta, 1992), leaf explants (Wen et al., 1990), inter nodal callus (Narayan et al., 1989), immature embryo (Kirn et al., 1985), cotyledons (Wang et al., 1996) and from anthers (Shoukang et al., 1987) of mulberry genotypes reflected that the regeneration potential is genotype dependent. Attempts have been made to micropropagate mulberry through bud culture (Islam et al., 1993; Katase, 1993a).

**Aim of the study**

The present investigation is having worth scientific utility and aimed: To standardize protocols for the initiation of callus mainly from explants of
different varieties of mulberry varities (Morus Spp.). The family Moraceae is one of the most responsive and diverse plant groups in the context of the application of tissue culture techniques. It has long been recognized that cells, tissues and organs from members of this family undergo morphogenesis and *invitro* plant regeneration easily. However tissue culture of certain mulberry varieties (Morus Spp.) are lagged behind, most likely due to the lack of the success in early attempts to regenerate plants from different explant cultures. Most works on *invitro* techniques in mulberry are concerned with direct organogenesis. Detailed investigation on variability aspects of callus formation, shoot formation, rooting and biochemical changes during their regeneration and multiplication in Indian varieties is very meager.

**Significance of the study**

Genotypes variations in magnitude of *invitro* responses in several crop plants have provided significant information concerning their adaptability, callus formation, plantlet regeneration, rooting and acclimatization and biochemical changes during organogenesis/plantlet regeneration in culture media. Preliminary studies have been conducted to screen some of the promising varieties in the Rayalaseema region of Andhra Pradesh and based on these studies four mulberry varieties were selected for tissue culture. Hence, the studies on evaluation of suitable media, callus, plantlet formation, rooting and acclimatization were carried out using different media with different combinations of hormones in four mulberry varieties of *Morus Spp*. Further, biochemical studies were carried out in the leaf / explant tissue of the regenerates of four Mulberry varieties for the characterization of the active growth patterns and to identify somaclonal variability in regenerates.
**Objectives**

In order to employ tissue culture techniques for the improvement of mulberry, four varieties viz. M₅₅, V-1, S₃₆ and Anantha were chosen for the study with the following objectives.

1. Optimization of surface sterilant for the culture initiative of Mulberry varieties M₅₅, V₁, S₃₆ and Anantha.

2. Optimization of nutrient media, plant growth regulators and carbon source for achieving initiation of callus mainly from auxiliary bud explants of four varieties of *Morus Spp.* (M₅₅, V-1 S₃₆ and Anantha)

3. Establishment of primary sprouts and subsequent initiation as well as secondary sprouts of Mulberry varieties M₅₅, V₁, S₃₆ and Anantha.

4. Extrapolations of the standardized protocol for shoot *in vitro* multiplication and *in vitro* rooting and subsequent acclimatization of plantlets of Mulberry varieties M₅₅, V₁, S₃₆ and Anantha.

5. To confirm the plantlet regenerations based on biochemical changes.