2. REVIEW OF LITERATURE

Population Survey -

Singh (2004) surveyed the economically important, naturally occurring species of the Indian desert in the state of Rajasthan on the basis of the perception of the local plant scientist working in State Agriculture Department, District Forest Offices, Department of Botany in Colleges and Universities, Arid Forest Research Institute and Central Arid Zone Research Institute, Jodhpur. Personal interviews were conducted to identify the present status of naturally occurring species. Informations were collected on various points like economically naturally occurring plant species of the region, taxa that were at risk and reason for the same, taxa that had become rare, species that need to be protected, conservation priorities and general statement on strategy for conservation. The data was analyzed to calculate the percentage of persons favouring a perception on risk status of a species. Then status of risk was classified in to Red List categories of World Conservation Union (IUCN, 1995). About one-fourth of the total 84 taxa were seen to be facing varying stage of risk. Out of 84 species, 17 species and 8 botanical varieties were endemic to Indian desert. Eighteen species in Bikaner, 21 in Jaisalmer and 19 in Jodhpur divisions were reported to be at risk. A questionnaire survey was carried out by Siddique et al., (2004) for collection of indigenous knowledge and identification of endangered medicinal plants in Barind tract of Bangladesh. Over one hundred plants of ethno botanical importance were described, aiming at the preservation and perpetuation of knowledge of medicinal plants for the benefit of mankind. The data were collected by personal contact with the local people and herbal practitioners. The questionnaire were designed for collection of socioeconomic and ethno botanical data. Finally data were subjected to categorize in to IUCN Red List.
Semi structured interviews were carried out in order to examine the present use of wild medicinal plants growing in and around Lahore-Islamabad motorway by Ahmad(2007). The research process comprised of 81 plants belonging to 44 family which have been recorded for their medicinal use. Salvadora oleoides was vulnerable species in the region. Sher *et al.*, 2010 conducted ethnomedicinal survey in various parts of desert and semi desert ecosystem in Saudi Arabia. The aim of survey was to document ethno ecological and medicinal value of *Salvadora persica*. The data on folklore uses, indigenous knowledge of local people and traditional healing regarding S. persica were collected. Based on the results obtained, it was concluded that S. persica was a multipurpose plant with great ethnomedicinal value. Ethnomedicinal survey was carried out by Ameri and Kisangau (2010) on medicinal plants used to treat common illness. Data were recorded from traditional medical practitioners using open ended semi-structured questionnaire on diseases treated, methods of preparation, uses and habitat of medicinal plants. A total of 82 medicinal plants species belonging to 29 families were recorded during the study. Ethno medicinal surveys were conducted in 2006-2008, randomly selected village following the procedure as described by Jain,(1967). A total of 110 species of medicinal plants representing 88 genera and 43 families employed in the literature as traditional practice have been recorded from the studied region that are used to treat a variety of human and animals decrease. Rahmatullah *et al.* (2011) performed ethno -medicinal survey to know the use of plants by local people. Interviews of practitioner were conducted with the help of semi-structured questionnaire and guided field-walk method of Martin (1995) and Maudu (1995). Information were collected on various plants of medicinal uses from local people. Interviews were conducted with the help of semi structured questionnaire and the guided field walks to know the consumption of medicinal plants by local people in Bangladesh (Rahmatullah and Biswas, 2012).

**Major threats to plant-**

Gupta (1968) analyzed various anthropogenic disturbances in the form of direct and indirect influences. Direct influences were related to harvesting of vegetation for fuel and timber material. It was noticed that in north-west India natural vegetation forms
the chief source of fuel besides contributing to the requirement of small timber for agricultural implements and fencing material. Likewise the rapid growth in population had inflated the demand for fuel resulting in indiscriminate cutting of shrubs and tree like *Salvadora oleoides* and *Prosopis cineraria*. Overgrazing was a common problem because leaves of *Prosopis sp.*, *Salvadora oleoides* and *Ziziphus nummularia* were best top feed for cattle, sheep and camels.

Observations associated with loss of species or population in arid zone were made by Singh (2004). Many plant species were at risk in specific habitat or at divisional level, while others in the whole regions due to climatic as well as social, economic and development factors which were contributing to loss of species diversity. Among social factors, camels and goats were important as they were reported to eat away anything, preventing natural regeneration of the plants. Jain *et al.*, (2009) observed that several medicinal plants species are on the verge of extinction due to overgrazing, encroachment, unsustainable utilization and other developmental activities. Joshi *et al.*, 2009 investigated various type of threats to grassland and local species. Some of the locally highly preferred fodder yielding tree species were considered to be declining in the local environment in Bann,(Gujarat) due to fast spreading of exotic species such as *Prosopis juliflora*. *Salvadora persica* was suffered from serious environmental problem such as deforestation, soil degradation, loss of biodiversity and unsustainable livelihood. Conservation status of this plant was highly threatened in South Arabia (Sher *et al.*, 2010).

**Floral study and taxonomy-**

Structure of flowers, vegetative characters and systematic position of various species of angiospermic plants has been described time to time by various worker (Lindley, 1836; Engler, 1891; Hutchinson, 1927; Takhtajan, 1958; Cronquest, 1986; Thorn, 1968 and Stewart, 1972). Affinities and characters of Salvadoraceae has been described by Bentham and Hooker (1873). Bensel *et al.*, 1975 explained the floral morphology of Parnassioideae and Brexoideae. In family Parnassioideae, flowers were actinomorphic and pentamemorous with four carpels. Stamens were present opposite of
each sepal; number of locules was equal to number of carpels with parietal placentation. Style was short. Isomerous flowers were reported in Brexoideae. Flowers were with five fertile stamens, one in each sepal, one in each sepal plane. Ovary was pentalocular with axile placentation and five lobed stigmas. Various floral and vegetative characters of family Salvadoraceae were assessed by Qureshi, 1972. Bhandari (1978) studied various vegetative and reproductive feature of family Salvadoraceae. Rendle (1979) described flowers of family Salvadoraceae. Flowers were bisexual as well as unisexual, 4-5 merous with two carpels, forming one or two chambered ovary, each chamber containing one or two erect anatropous ovules with two integuments. The petals were reported fused, hypogynous stamens. The fruit was one seeded drupe. Parveen and Quiser (1996) studied the structure and behavior of pollens of *Salvadora* genus. Usher (2004) described various characters of family Salvadoraceae and supported its taxonomic position as assigned by earlier taxonomist. Korejo *et al.*, 2010 studied morphological and floral features of *Salvadora persica* and *Salvadora oleoides*. *Salvadora oleoides* was reported to have axillary inflorescence; branched panicked. Colour of flowers was greenish white, complete, actinomorphic. Petals and stamens were four. Style was reported with short stigma.

**Seed germination**-

Seed germination has been variously defined by different workers. Most of the authors call it “the sprouting of seeds” or “resumption of the growth by dormant embryo”. Mayer (1953) has defined germination as “that group of processes which cause the sudden transformation of dry seed in to the young seedlings.” Porter (1949) has suggested that the seed shall be considered to have germinated when it has developed those structures that are essential for normal seedlings. Miyamoto *et al.* (1982) indicated that seed germination may be reduced by approximately 25% due to salinity of salt solution ranging from 7 to 19 m mho/cm. This study was made in the nursery to test the effect of soil types and orientation of seeds on germination of seeds of tropical forest tree species of economic importance. Tahir *et al.* (1993) studied on the effect of NaCl concentrations and various soil types were undertaken on germination of Jojoba (*Simmondsia chilensis*) seeds. In high NaCl concentrations, germination was delayed
and reduced. In clay loam soil, germination was maximum followed by sandy loam soil. Difference in soil types has no significant effect on germination of *Cieba pentandra* *Leuceana leucocephala* (Lam) de Wit and *Gmelina arborea* as reported by Agboola *et al.*, 1993. Germination of *Tectona grandis* was significantly higher on loamy soil than in washed sterile river sand or non-sterile river sand.

Rao and Tarafdar (1998) demonstrated that mine spoil had supported significantly higher growth of *Prosopis juliflora*, *Salvadora oleoides* and *Cenchrus ciliaris* compared to normal soil. Seed of *Alseuosmia macrophylla* and *Solanum sp.* from freshly collected fruits were tested by Burrow (1999) to examine germination period in relation to season, germination rate, and degree of success, in conditions similar to those that could be experienced after dispersal in nature. Germination of a few *Alseuosmia macrophylla* seeds were reported delayed until spring of the second year. Success was high (97-60%) for *Alseuismia macrophylla* but 82% for *Solanum aviculare*. El-Darier and Youssef (2009) conducted experiments to elucidate the effect of soil type, salinity stress and allelochemicals on the germination efficiency, seedling growth and photosynthetic pigments of *Lepidium sativum*. The highest germination rate (69.9%) was recorded for seeds cultivated in sandy soil followed by that of clay soil (42%) and then loamy soil (19%). Mertia and Kunhamu (2000, 2003) predicted that natural regeneration of *Salvadora oleoides* was very rare through seeds. Depulping of fruits and pretreating the seeds promoted easy seed germination. 0.5 cm depth for seed sowing was more efficient in increasing the germination rate of *S.oleoides* seeds.

Ramoliya and Pandey (2002) studied the effect of salinization of soil on emergence, growth and physiological attributes of seedlings of *Salvadora oleoides Decne* (Salvadoraceae). Seed germination exhibited a negative relationship with increasing concentration of salts. Results concluded that *Salvadora oleoides* was salt tolerant at seed germination stage. A study was conducted by Evers and Parson (2003) to determine the influence of soil texture and moisture on ‘Alamo’ switchgrass emergence and seedling growth. More than 90% seedlings survived in all soils followed by watering interval of 7 days. Trials were performed to test germination and emergence characteristics of Jimson weed (*Datura stramonium* L.) seeds buried in 10 different soil
types with sandy or clay textures. Results indicated that clay soil had low germination rate (Benvenuti, 2003). Dagar et al. (2004) found maximum seed germination percentage at pH 8.0 in *Salvadora persica* and *jatropha curcas*. Seed germination was faster at pH 6-8 when compared with other level. Maximum germination was reported in natural light in both species followed by yellow, green, red and blue light respectively. Ramoliya et al. (2004) reported 93% seed germination over a period of 16 days under control (4.1 dsm\(^{-1}\)) salinity conditions. There was a significant reduction in germination of seed with increasing salt stress. Cancell (2004) predicted that frugivores seemed to be essential for germination and dispersal of *Coremna album*.

Jindal et al. (2006) noticed eighty seven percent seed germination in *Salvadora oleoides*. Seedling showed significant variations for plant height, nodes per plant, number of layers per plant, leaf length and width. Plant height had positive and significant association with number of leaves, leaf length and leaf width. Al-Khalifa (2006) reported that for seed storage, there was no significant differences among types of soil or concentration used, after six and nine months of storage. Gresta et al. (2007) investigated changes in germination determined by storage time and temperature on seeds of two population of *S. subvillosus*. Germination of *S. subvillosus* seeds was tested in relation to four storage time (30, 130, 200 and 360 days after harvest (DAH)). Seed stored for 200 days or more time showed reduction in germination percentage. The effects of soils were studied on the growth of *leucaena leucocephala* under natural environmental conditions in a greenhouse by Rehman and Iqbal, 2007. Different soils showed variable results on root length, shoot length, seedling length and plant cover, number of leaflets, leaf area and dry weight of roots, shoot and leaf and total dry weight of plant. Jafarzadeh and Aliasgharzad (2007) performed experiments to determine the effect of water salinity levels and salt composition on germination and seedlings root length of four sugar beet cultivars. Seed germination percentage and root length were adversely effected by high salt concentration in irrigated water. Al-sherif (2007) showed the effect of chemical scarification, salinity and preheating on the germination of *Prosopis farcta* as a primary step for its propagation. Moderate salinity, 75 and 100 m\(\mu\) NaCl did not affect the final germination percent, while the high salinity levels (150 and 200 m\(\mu\)) decreased it by
about 30% and 70% respectively. The findings confirm the ability of *Prosopis farcata* to grow in salt affected soil but its seeds need scarification.

Alshammary, (2008) carried out field experiment to determine growth characteristics and natural composition of *Atriplex halimus* and *Salvadora persica* under saline irrigation levels of 2000,8000,12000 and 16000 (mg l⁻¹ TDS), in sandy soil. Mean fresh biomass yield and fresh plant root weight of *A. halimus* increased while that of *Salvadora persica* decreased significantly with increasing irrigation water salinity in all treatments. Seed germination percentage decreased considerably when shown after 120 days of harvesting. Seed productivity and the time of emergence of sprouts depended on ecological conditions of plant growth in *Adonis vernalis* L. as reported by Poluyanova and Lyubarskii,2008. Domenech and villa (2008) conducted an array of germination tests on *Cortaderia selloana* with different degrees of shading, soil textures and water availability. Seed germination was reported high in sandy soil which contained increased levels of clay. Youssef (2009) explained the effects of temperature regime and pH levels on the behavior of seed germination of desert plants in order to define the optimum germination conditions for *Ononis serrata* and *Mesembryanthemum crystallinum* and *Retama raetam*. Seeds of all species did not germinated at buffered solution of pH level of 4 and 10. Chad et al. (2009) conducted experiments to determine germination rate of prairie drop seed under different conditions in an attempt to potentially increase its successful establishment under field conditions. Seed germination rate was reported high in field soil as compared to mixture of soil, peat and perlite. Experiments in green house were conducted by Vaghela et al.(2009) to assess the effect of supplemental calcium in salinized soil on the response of germination and seedlings growth of *Salvadora oleoides* Decne. Salinity retarded the seed germination and seedling growth. Injurious effect of NaCl on seed germination was ameliorated and seedling growth was reported with calcium supply. Suthar (2009) described seed attributes, seed storage, and dormancy, effect of temperature, pH and sowing depth on seed germination in *Solanum nigrum*. Mature freshly collected air-dried seeds showed 52% germination. The maximum percent of seed germination was observed at room temperature. Neutral pH showed high percentage of seed germination. Germination response of seeds of *Medicago sativa* was
evaluated to study the effect of salt stress by different salt solutions by Azhdari et al. (2009). The results indicated that the effect of salinity levels were significant for percent seed germination, seed germination rate, mean time to germination, length of stem and seed vigour. Seed germination decreased significantly by increasing salinity levels. Experiments were conducted to assess the salinity tolerance and effect of salinity-fertility interaction on growth and dry matter production of four mature thorn forest species viz. *Salvadora oleoides*, *Prosopis cineraria*, *Capparis decidua* and *Tamarix aphylla* by Sharif and Khan (2009). The results showed a negative linear relationship between gain in plant height, dry matter production and increasing salinity. All growth characters decreased with increasing salinity levels.

Hu et al. (2010) reported that seed germination rate decreased as storage time increased in *Bletilla striata*. Okunomo (2010) studied the effect of soil amendments on germination of *Annona muricata* seeds like poultry droppings, cow dung, pig manure, wood ash and top soil. It was recommended that *A. muricata* seed should be grown on a top soil without any pretreatment. Mathew et al. (2011) tested germination percentage of different *Ficus* sp. at 1, 6, 12 and 18 months after storage under ordinary conditions whereas the seed under refrigerated condition were tested at 12 and 24 months after storage. In *Ficus racemosa*, it was reported that germination percentage decreased drastically on storage. Urgessa (2011) identified nutrient media that support optimum generation of *Ficus vallischaude* seeds. The germination percentage of *Ficus vallischaude* varied significantly among different media. Accordingly the germination percentage was 100% for control followed by sand and forest soil with 88% and 72% respectively. Rodrigo et al. (2011) observed in *Prosopis* species, the digestion of fruits by wild and domestic animals could promote and accelerate germination. It was further hypothesized that fruit might have some germination inhibitors. Dickens, (2011) evaluated the effect of media on the germination and seedling performance of *Irvingia wombolu*. The results revealed that rooting percentage with river sand was 21.8% followed by sawdust 18.1%, clay soil 17.6%. A pronounced effect of media on leaf area was observed with river sand than the rest treatments.
Vegetative propagation-

Green cuttings of *Alnus incana* consisting of one internodes and one leaf with its axillary bud had been rooted in aerated liquid substrate under growth chamber conditions by Danell,(1981). Sanchez *et al.*(1996) predicted that the concentration and exposure time of the indole 3-butyric acid(iba)treatments were critical for root induction in *Quercus robur* and *Q. rubra*. Best rooting efficiency was achieved by culture in medium containing 25 mg l\(^{-1}\) IBA for 24 hours and subsequent transfer to auxins free medium containing 1% activated charcoal. Hartmann *et al.*, (1997) reported that vegetative propagation was the most important method for multiplication of agricultural, horticultural, agaroforestry system and woody trees. IBA application is one of the most common and possibly most effective methods to enhance root formation in cuttings. IBA increases the percentage of cuttings that form roots in a wide range of trees and shrubs (Hartmann *et al.*,1997; leaky, 2004 and Husen and Pal, 2007). However cutting of some species appear unresponsive to auxins (Shiembo *et al.*,1996; Atangana *et al.*, 2006; Trueman and Peters, 2006 and Arya *et al.*,2007) and auxins doses can cause death of stem cuttings ( Perry and Trueman,1999 ; Zuffellato-Ribas and Rodrigues, 2001; Wendling and Xavier, 2005; Trueman and Richardson, 2008). Cutting must survive physiological stress severances from the stock plant with little water or nutrient uptake until roots penetrate the propagation medium(Grange and Loach, 1983;Blazich, 1988; Hartmann,1997). Brennan and Mudge (1998) evaluated cutting and air layering as mean of air vegetative propagation of the tropical woody tree, *Inga feuillei*. Effect of moisture management system, leafless, auxins application and stem diameter on rooting of semi hardwood cuttings were investigated. Leafless cuttings did not root regardless of moisture management system or auxins pretreatment whereas 55% rooting of leafy cuttings was observed. Likewise, hundred percent of air layering shoots rooted within five weeks with or without auxins treatments, and all rooted layers survived transplanting in soil. Large scale propagation of three mangrove species was attempted using cuttings and air layering techniques by Eganathan *et al.*(2000). Maximum rooting was recorded when the cuttings and air layers were treated with IBA alone up to 2500 ppm October was found to be best followed by January for plantation of cuttings and initiation of air
layer. Many plants were reported unable to produce roots through cutting and layering methods as reported by Hussain (1994) and Abuetgasim (2000) while studying on *Ziziphus spina* and species of tropical trees respectively.

Rifaki *et al.* (2002) investigated the effects of auxins treatments, collecting date, crown position and age of orlet on rooting of stem cuttings. Application of IBA was reported having a strong influence on rooting percentage, route number and length of roots. It was further found that cuttings of young tree rooted better than those of mature trees. Propagation of the ornamental cherry species *Prunus subhirtella* was carried out by Osterc and Tamper, (2003) using softwood cutting. The rooting percentage ranged between 29 to 45%; with in average ten or more roots. Mascarlo *et al.* (2003) carried out propagation trials on the physiological role related to season and several growth hormones treatments. The best period for vegetative growth propagation by cutting was January to April. The effect of different concentrations of IBA, cutting length and cutting positions on the rooting of leafy stem cuttings of *Pausinystalia johimbe* were investigated in three experiments using non-mist propagators by Mpeck *et al.* (2003). A range of five IBA concentrations (0, 50,100,150 and 200 μg per 10μl solution) was examined to investigate the auxins requirements for rooting of stem cutting. It was further observed that stem cuttings tested with high IBA concentrations (100,150 and 200mg l⁻¹) were most responsive in terms of number of roots per cuttings. An attempt was made to propagate *Rhinacanthus nasutus* through stem cuttings by Das (2006). Maximum rooting percentage was observed in apical shoot cuttings treated with 2000 ppm of IBA. Ozel *et al.* (2006) investigated vegetative propagation of juvenile and mature softwood stem cuttings removed from mother explants. The cuttings were treated with 0, 500, 1000 ppm IBA and NAA for 5, 10 and 15 minutes. Sand was served as rooting medium. The highest frequency of rooting, mean number of roots per plant and root length was achieved from juvenile cuttings treated with 500ppm IBA for 10 minutes. No rooting was observed on NAA treated cuttings. Vegetative propagation of *Dalbergia melanoxylon* by shoot cuttings was investigated using IBA and NAA by Al-Khliifa (2006). Cutting of *Dalbergia* did not root well under the condition of that study.
Sharma et al. (2007) demonstrated that propagation through stem cutting was the most convenient and cheap methods of obtaining true to type, fully developed plants of *Punica granatum* in considerably lesser time. Maximum percentage of rooting, root number and root length was observed with 500ppm IBA+borax 1% both in semi-hard and hard wood cuttings. Hard wood cuttings responded better to the hormonal treatments as compared to semi hard wood cuttings. Reproductive inefficiency of air-layering and stem cutting was reported in *Merremia biosiana* by Mingguang et al. (2009). Adventitious roots formation was not observed in stem cutting and air layering experiments with and without rooting hormones treatments. Study was carried out to evaluate the effectiveness of stem cuttings to regenerate roots in *Macadamia* by Gitonga et al. (2009). Single node cuttings were set in moist sand in green house tunnels or in pots and covering with polythene sheet and maintained under green house conditions for three months. All findings of stem cutting experiments indicated difficulty in rooting of *Macadamia*. The use of rooting hormones was not significantly beneficial. IBA application promoted a significant increase in rooting percentage over control cuttings as reported by Canli and Bozkurt, (2009). The best rooting percentage (87.5%) was achieved by 1500 mg l⁻¹ of IBA application whereas the rooting percentage of the untreated control cuttings was only 10.8%.

Gamlath et al. (2010) tested three different concentration (1%, 3% and 5% w/v) for their root growth promotion properties in air layering of three popular ornamental *Ficus* species. 3%(w/v) chitosan treatment resulted in the highest mean root length as compared to control (tap Water). Sulusoglu and Cavusoglu, (2010) investigated the effects of different IBA doses on rooting capacity of cherry laurel (*Prunus lauroerasus*) types in green house conditions under mist propagation. Different concentrations of IBA gave variable results on rate of rooting percentage, average root number and average root length (cm). Gautam et al. (2010) conducted nursery experiments over three years on standardization of clonal propagation technique in *Psidium* (Guava) through rooting of cuttings. Influence of various factors such as potting mixtures (vermiculite, sand and soil). Cutting size (5, 10 and 15 cm) and seasonal changes (summer, rainy and winter), were studied on adventitious root formation in the guava cuttings. The study
revealed significantly higher root induction in vermiculite followed by sand. There was slight difference between 10 and 15 long cuttings in terms of root induction. The effect of IBA and NAA on the rooting of Rohida (*T. undulata*) in the late winter and late autumn was evaluated in order to determine the rooting ability of *Tecomella undulata* cuttings (Karami and Salehi, 2010). It was further noticed that both NAA and IBA had significant effect on rooting from semi-hardwood and hard wood cuttings. Mathew *et al.* (2011) studied on vegetative propagation using stem cuttings of *Ficus sp.* It was reported that all species had poor rooting response of stem cutting. The percent success of rooting was 10-12% in all species. However air layering was found very successful in all species as percent of success was 80-90%. Effective techniques for vegetative propagation of *Picconia azorica* by rooting of stem cutting or by air layering was invented by Martin *et al.* (2011). Rooting substrate, IBA concentration and portion of area of the terminal leaf pair kept on stem cuttings, when tested in early spring (semi-hard wood cuttings) and autumn (hard wood cuttings) failed to produce any rooting cuttings. However, air layering experiments performed in the autumn on lateral branches of adult tree was successful, favouring propagation by air layering. Yeboah *et al.* (2011) reported that high rooting performance and low infection rate could be achieved when cuttings had been irrigated once daily. Significantly, high percentage of rooting per cutting was observed for cutting with petiole. Studies were conducted to investigate the effect of IBA application, rooting system, rooting substrate, supplementary lighting and shading upon rooting of *Ilex aquifolium* stem cuttings by Rifaki *et al.* (2011). Treatments with IBA had a strong influence on the percentage of rooting, number and length of roots. Akwatulira *et al.* (2011) investigated an appropriate technique for propagation of *Warburgia ugndensis* using stem cuttings. The highest percentage of callusing, rooting and shoot regeneration was recorded in softwood cuttings which also produced the highest number of roots.

**Micropropagation**

Micropropagation refers to *in vitro* regeneration of plants, using plants parts (tissues, organs, embryos, single cell, and protoplast) on nutrient media under aseptic, controlled and artificial conditions. It includes axillary budding, induction of adventitious
buds, callus formation and somatic embryogenesis. Haberlandt (1902) developed the concept of culturing of isolated cell of Tradescantia in artificial conditions, though his experiment failed to divide the cells. He gave the concept of totipotency. The technique of micropropagation is based on this concept. Every cell of the plant body is totipotent i.e. capable of giving rise to a new plant under proper nutritional conditions. His pioneering experiments inspired other botanists to conduct further works on the morphogenetic potentialities of living cells and abilities of tissue and organ to develop in to complete plant. Hanning (1904) initiated a new line of investigation involving the culture of embryonic tissue of crucifers. Segments of string bean and observed some cell divisions but no proliferation. Kotte (1922a,1922b), was successful in establishment of excised plant root tips in vitro. The first successful organ culture was achieved by White (1934) with the demonstration of potentially unlimited growth of excised tomato root tips. Gautheret (1934) is credited with the first successful attempt of callus induction on woody cambial explants of some tree species such as willow and popular on Knop’s solution. This was followed by the formation of continuous callus cultures in carrot and tobacco by Gautheret, Nobecourt and White in 1939 independently of each other. Snow(1935) demonstrated that indole acetic acid (IAA - a growth substance discovered by Went in 1926) stimulated cambial activity. Skoog (1944) ,Skoog and Tsui (1948 )for the first time indicated the possibility of chemical regulation of in vitro organogenesis in Tobacco plant. Ball (1946) demonstrated the possibilities of regenerating plants from isolated explants of some angiosperm shoots apices like Lupinus and Trapaeolum.

Morel (1950) successfully cultured monocot tissue with the help of coconut milk. Virus free dahlia plants were first time obtained by culturing of healthy shoot tips from diseased individuals (Morel and Martin,1952). In 1952, Steward initiated work on cultured carrot explants and used coconut milk as a nutrient (Steward and Caplin, 1952) that ultimately led to the discovery of embryogenesis(Steward et al.,1958). Muir (1953, 1954) developed nurse culture method to culture single cell. The work of Miller and Skoog (1953) on bud formation from cultured pith explants of tobacco led to the discovery of kinetin. Miller et al. (1955) finally isolated from yeast extract, a derivative of adenine (6-furfylaminopurine), named kinetin. A substance with kinetin like propertied
was also detected in young maize endosperm (Miller, 1961), which was isolated by Letham (1963) and named zeatin. The proliferation of single cell into callus was demonstrated by Torrey (1957) and Muir et al., (1958). Skoog and Miller (1957) suggested that shoot and root initiation in cultured callus can be regulated by varying ratio of auxins and cytokinins in the medium. The growth of dormant axillary buds can be initiated by exogenous application of cytokinins (Wickson and Thimann, 1958). Steward (1958) and Reinert (1959) published the initial reports of formation of somatic embryos from tissue of carrot.

Morel (1960) recovered virus free plants by the technique of shoot tips culture. He had developed rapid clonal propagation by which an estimated 4 million genetically identical plants can be produced in a year starting from a single shoot tip measuring less than 1mm. Murashige (1961) demonstrated usefulness of the technique of in vitro culture for propagation of various plant species. According to Murashige and Skoog (1962), even tissue from different parts of a plant may have different requirements for satisfactory growth. They gave the formulation of MS medium, which is one of the most widely used salts composition for the purpose of in vitro plant propagation. Hildebrandt et al. (1963) found that chlorophyllous callus was dependent on an exogenous sugar for continued growth, even with adequate light intensities. In 1964, development of embryoids in anther cultures of Datura innoxia and subsequently their origin from pollen grains was confirmed by Guha and Maheswari (1966). Vasil and Hildebrandt (1965) demonstrated that a single isolated cell could divide and ultimately give rise to whole plant in Nicotiana. The ability of mechanically isolated fully differentiated mesophyll cell of Macleoya cordata to yield an embryogenic callus was reported by Kohlenbach (1966). It is known fact now after the discovery made by Skoog and Miller (1957) while working on tobacco that the root and shoot initiation was basically regulated by interaction between the two hormonal substances i.e. auxins and cytokinins. The pattern of organogenesis depends upon their concentrations and sequence of application in the nutrient medium. Relatively higher concentrations of auxins favours root initiation by suppressing the shoot initiation. On the contrary, relatively higher concentration of cytokinin induced shoot initiation by suppressing root induction. When a proper ratio of
cytokinin and auxin is used that has helped in both the shoot and root formation (Murashige, 1974).

indicus (Sree Kumar et al., 2000), Cardiospermum halicacabum (Babber et al., 2001), Lippia alba (Gupta et al., 2001), Chukrasia tabularis (Nagalakshmi and Pullaiah, 2001), Melia azedarach (Shahzad and Siddique, 2001), Alnus nepalensis (Thakur et al., 2001), Solanum aculeatissumum (Manjula and Nair, 2002), Rauvolfia tetraphylla (Faisal et al., 2002), Entada Phaseoloides (Rao and Vishnupriya, 2002), Jatropha curcas (Rajore et al., 2002), Cassia alata (Ramamurthy and Savithramma, 2002), Gloriosa superba (Sachdev et al., 2002), Azadirachta indica (Shekhawat et al., 2002), Santolina canescens (Casado et al., 2002), Lepitademia reticulate (Arya, et al., 2003), Ceropogia candelabrum (Beena et al., 2003), Spilanthes acmella (Haw and King, 2003), Saussurea aobvallata (Joshi and Dhar, 2003), Eclipta alba (Gawde and Paratkar, 2004), Phyllanthus amarus (Ghanti et al., 2004), Pterocarpus marsupium (Chand and Singh, 2004), Sesbania rostrata (Jha et al., 2004), Mentha piperita (Ghanti et al., 2004), Pterocarpus marsupium (Anis et al., 2005), Wedelia chinensis (Kameri et al., 2005), Maerua oblongifilia (Rathore et al., 2005), Vitex negundo (Sharma et al., 2006), Peristrophe bicalyculata (Sharma and Devi, 2006), Ruta graveolens (Faisal et al., 2006), Macuna pruriens (Faisal et al., 2006), Catharanthus roseus (Satdive et al., 2006), Basilicum polystachyon (Chakraborti et al., 2006), Gymnema sylvestare (Reddy et al., 2006), Rauwolfia serpentine (Tomar and Tiwari, 2006), Amomum microsteplanum (Thoyajaksa and Rai, 2006), Capsicum annum (Rao et al., 2006), Coleus blumei (Rani et al., 2006), Musa spp. (Rahman et al., 2006), Azadirachta indica (Reddy et al., 2006), Solanum album (Sanjaya et al., 2006), Nyctanthes arbor-tristis (Sddique et al., 2006), Prospis laevigata (Gonzalez et al., 2007), Epidendrum radicans (Gayatriand Kavashree, 2007), Dianthus caryophyllus (Pareek and Kothari, 2007), Prosopis cineraria (Kumar and Singh, 2007), Aegle marmelos (Nayak et al., 2007), Curcuma angustifolia (Sukla et al., 2007), Musa sapientum (Kalimuthu, 2007), Citrullus colocynthis (Meena and Patni, 2007) Bupleurum distichophyllum (Karuppusamy and Pullaiah, 2007), Cornus mas (D’urkovic, 2008), Clitoria ternatea (Singh and Tiwari, 2010), Sapindus mukorossi (Singh et al., 2010a), Commmiphora mukul (Singh et al., 2010b) Celastrus paniculatus (Lal and Singh, 2010) Spilanthes acmella (Yadav and Singh, 2010), Croton bonplandianum (Ashish and Sharma, 2011) Nepenthes albomarginata (Sukamto et al., 2011), Cassia auriculata (Negi et al., 2011) and Lippia nodiflora (Evelyne and Ravindharan, 2011).
Nutrition-

The *in vitro* culturing of tissue depends mainly on the composition of the media. The nutritional requirements differ with type of tissue; each tissue type requires a different media formulation to induce organogenesis. An important breakthrough for continuously growing root tip cultures came from White (1934), who initially used yeast extract medium containing inorganic salts and sucrose but later replaced yeast extract by three B vitamins, namely pyridoxine, thiamine and nicotinic acid. White’s synthetic medium later proved to be one of the basic media for a variety of cell and tissue cultures. Initially, tissue culturist used Knop’s mineral solution (Gautheret, 1942, Nobecourt, 1939) and white’s medium with various trace elements. Gautheret (1955) emphasized the importance of nutrition in plant tissue culture. The various media formulated have been modified time to time to complete the particular requirements of tissue to be cultured (White, 1943; Nitsch, 1951; Murashige and Skoog, 1962; Mc Cown and Lloyd, 1981). Among the above describe media, MS (Murashige and Skoog, 1962) medium was most common and frequently used one. The MS medium was used either as described originally or with little variation and combination of phytohormones and vitamins e.g. *Plumbago rosea* (Harikrishnan and Hariharan, 1996), *Alpinia galanga* (Mustafa and Hariharan, 1997), *Terminalia arjuna* (Kumari et al., 1998), *Sapindus mukorossi* (Philomena and Rao, 1999), *Jatropha curcas* (Sardana et al., 2000), *Melia azedarach* (Shahzad and Siddique, 2001), *Lilium nepalense* (Wawrosch et al., 2001), *Jatropha curcas* (Rajore et al., 2002), *Azadirachta indica* (Shekhwat et al., 2002), *Cassia alata* (Ramamurthy and Savithramma et al., 2002). There were some cases where modified MS medium had been used e.g. *Prosopis Juliflora* (Nandwani and Ramawat, 1991), *Dalbergia latifolia* (Raghavaswamy et al., 1992), *Hemidesmus indicus* (Sarasan et al., 1994), *Punica granatum* (Sharon and Sinha, 2000). MS (Murashige and Skoog, 1962) and B5 (Gamborg et al., 1968) media were used for callus initiation and organogenesis in *Jatropha curcas* (Sardana et al., 2000). Raghavaswamy et al. (1992) observed that axillary bud initiation in *Dalbergia latifolia* was better on MS medium while multiple shoot induction was better on WPM or MS (reduced major salts) medium. Goyal et al. (1999) successfully cultured *Solanum nigrum* on GD and Pushplata (1999) cultured
Tylophora asthmatica on MS medium. In Camptotheca acuminata multiple shoots were best achieved on B₅ medium (Liu et al., 2001). Multiple shoots were induced directly from auxillary buds on MS medium in Cassia alata (Ramamurthy and Savithramma et al., 2002). Increased concentration of reduced nitrogen in the medium also promotes better organogenesis (Thomas and Street, 1972).

Plant growth regulators play an important role in plant tissue culture. They directly or indirectly affect the growth and differentiation of plant tissue. Various studies have been carried out on the influence of the concentrations of various growth regulators. Different plant growth regulators have different effects and vary with the type and quantity to be applied. It has been reported that low concentration of auxins and a high concentration of cytokinins in medium favoured shoot induction whereas reverse proportional promoted root formation and an intermediate concentration caused callus development. Skoog and Miller (1957) proposed the concept of hormonal control of organ formation. Therefore, a critical role of auxin/cytokinins ratio is inevitable for inducing roots and shoots proliferation (Murashige, 1974). Wolter and Skoog (1966) obtained continuous growth of callus cultures of Fraxinus pennsylvanica on modified Rienert and White’s medium containing myo-inositol, pyrodoxin and an auxin. Bud formation in callus of Ulmus campestris depends on proper balance of auxins and meso-inositol (Jacquiot, 1966). Coconut milk and casein hydrolysate were essential for callusing and differentiation in Prunus amygdalus, (Mehra and Mehra, 1974). Burron and Butha (1975) reported that coconut milk stimulated embryogenesis. Addition of yeast extract to MS medium supplemented with NAA and kinetin increased the number of differentiated roots in Dalbergia lanceolaria (Anand and Bir, 1984). Simola (1984) obtained plantlets in Betula pendula on a new medium N₇ supplemented with casein hydrolysate, zeatin or zeatin riboside. Multiple shoots were obtained in Eucalyptus grandis on MS medium supplemented with additional thiamine (Laksmi Sita and Soba Rani, 1985). Mittal et al., (1989) obtained multiple shoots from axillary buds of Accacia auriculiformis on Gamborg’s (B₅) basal medium supplemented with coconut milk and BAP. Gamborg’s medium was used by Mukhopadhayay and Mohan Ram (1981) for culturing of Dalbergia sissoo. Supplementation of B₅ medium with NAA alone or in combination with BAP
resulted in shoot production and rooting was successful on B5 medium. Whereas multiple shoots were obtained in MS medium + vitamins of Gamborg’s B5 medium containing NAA (Datta and Datta, 1983). In case of Delbergia latifolia MS medium supplemented with NAA and BAP was used. The decrease in NAA ensured shoots formation (Rao et al., 1984; Sudha Devi and Natreja, 1987). Goyal and Arya (1979) observed the regeneration in Prosopis cineraria on MS medium supplemented with different concentrations and combinations of kinetin, BAP, IAA, NAA and IBA. Plant regeneration was reported from cultured axillary buds in a medium with BA and IAA in Albizzia lebeck by Sharma and Chandra (1987). Pierik (1987) and Murthi et al. (1998) discovered that N,N’-diphenylurea (DPU), thidiazuron, N-2-chloro-4-pyridyl-N-phenyl urea (CPPU) and other other derivatives of diphenyle urea which show the cytokinins like activity.

Full strength MS medium with combination of cytokinins are mainly used for regeneration of shoots. Among cytokinins, BAP in concentration up to 25 μm for shoot regeneration is most commonly used in a variety of explants (Mc Cown and Amos, 1979; Singh et al., 2010a and Lal and Singh, 2010). Kopp and Nataraj (1990) regenerated plantlets by supplementing 2.0 mg l\(^{-1}\) BAP in Tamarindus indica. Rumary and Thorpe (1984) reported that in some cases mixed cytokinins have beneficial role. Banerjee et al. (1999) reported in Centella asiatica that initial sprouting required the presence of 2.0 mg l\(^{-1}\) BAP and 0.1 mg l\(^{-1}\) IBA; however for multiple shoot induction a higher concentration of BAP (3.0 mg l\(^{-1}\)) and a lower concentration of NAA (0.05 mg l\(^{-1}\)) are required. Multiple shoots were obtained from shoot tips (1-2 cm) of Bacopa monnieri in MS medium fortified with 0.5 mg l\(^{-1}\) BAP within 6 days of culture, whereas in the case of Paederia foetida and Centella asiatica multiple shoots were obtained in MS medium supplemented with 1.0 mg l\(^{-1}\) BAP within seven days of culture (Singh et al., 1999). Supplementation of plant growth regulators such as 0.3 mg l\(^{-1}\) BAP and 0.2 mg l\(^{-1}\) Kn have been found to show a good response of shoot proliferation in Withania somnifera with a regeneration rate of eighty five percent (Kulkarni et al., 2000). The optimal medium for maximum shoot formation was MS + 10 mg l\(^{-1}\) BA + 160 mg l\(^{-1}\) Ads + 0.1 mg l\(^{-1}\) IBA in Aloe vera (Chaudhuri and Mukunadan, 2001). Haw and Keng (2003) found that only BA was needed to be supplemented into the MS medium for the induction of
multiple shoot formation in *Spilanthes acmella*. Thoyajaksha and Rai (2006) reported that MS medium supplemented with 2.0 mg l$^{-1}$ BA gave maximum shoot initiation in *Amomum microstephanum*.

Among auxins, 2,4-D and NAA mainly initiate callus formation in leaf, nodal and internodal segments. Sarsan *et al.* (1994) reported that in *Hemidesmus indicus* callus obtained in 2,4-D containing medium was yellowish and friable while those of NAA containing medium was green and compact and regeneration potential of both the calli were different. Mirghis *et al.* (1995) also reported hundred percent callus induction on medium containing NAA and BA in *Lycopersicon esculentum*. Optimal callus was also derived from mature leaves of *Coleus forskohlii* on MS media supplemented with 0.54 µm kinetin (Reddy *et al*., 2001) where as in same investigation the callus obtained in the presence of either 2,4-D or NAA was pale yellow and friable. The highest amount of callus was found in MS medium with 2,4-D (3.0 mg l$^{-1}$) in *Stevia rebaudiana* and 2,4-D (5.0 mg l$^{-1}$) gave the poorest callus (Uddain *et al*., 2006). Singh and Lal (2007) reported that media supplemented with BAP (1.0 mg l$^{-1}$) + NAA (2.0 mg l$^{-1}$) supported hundred percent callus induction in hypocotyl and cotyledonary leaves explants in *Leucaena leucocephala*. This medium also supported maximum growth of callus after subculturing. The potential of NAA in combination with BA for callus induction in tomato has been recognized by Lalage *et al.* (2007). Ashis *et al.* (2011) achieved callus formation from nodal explants of *croton bonplandianum* on MS medium fortified with 0.5 mg l$^{-1}$ BAP.

There are some complex substances like coconut milk (CM), casein hydrolysate (CH), adenine sulphate (Ads), activated charcoal (AC), which are also required sometimes in addition to growth hormones for callus induction and regeneration. Coconut milk of green nut is very effective in providing an undefined mixture of organic nutrients and growth factors (Gamborg and Phillips, 1995). Mustafa and Hariharan (1997) used coconut milk for regeneration of rhizome bud explants in *Alpinia galanga*. Positive effects of CM in nutrients medium are also reported by Roy (1998) in *Elaeocarpus robustus*. Similarly Niranjjan and Sudarshana (2000) used 10% coconut water (CW) for callus formation in *Nymphoides cristatum*. Addition of coconut water (10% V/V), malt extract (0.1%), yeast extract (0.1%) into MS medium did not show any
promotory effect on shoot regeneration in *Chukrasia tabularis* (Nagalakshmi and Pullaiah, 2001). However, yeast and malt extract, casein hydrolysate and coconut milk were failed to support the continuous growth of subcultured callus in *Prosopis Juliflora* (Nandwani and Ramawat, 1991).

Fridberg *et al.* (1978) reported that charcoal had an important role during culture by absorbing toxic compound released by inoculated explants. Pierik (1987) showed that the addition of AC often has a promoting effect on growth and organogenesis in plant species. Beneficial effects of activated charcoal were also found in *Muscari armeniacum* by (Pierik, 1987). Charcoal has been used in regeneration medium for trees like *Dalbergia sissoo* (Gulati and Jaiwal, 1996) and *Areca catechu* (Mathew and Philip, 2000) to prevent browning of culture due to phenolic exudation released by the explants. Cotyledon and hypocotyl explants resulted in shoot elongation with the addition of charcoal (Lu and Thorpe, 1991). Similarly, Figueiredo *et al.*, (2001) noticed addition of charcoal resulted in shoots elongation in *Rollinia mucosa*. Addition of 15% (v/v) CW and 2 mg l\(^{-1}\) activated charcoal increased the number of shoots (up to 15) per shoot culture in *Gloriosa superba* (Sayeed Hassan and Roy, 2005).

During culture, carbohydrates play an important role and act as an energy source required for growth, maintenance and for synthesis of cell constituents. Most commonly used carbohydrate source is sucrose, but others sugars like glucose, fructose, dextrose, mannitol, sorbitol etc are also occasionally used. Sucrose plays an important role as it serves as a source of carbon and energy (Kishore, 1983). In most of plants 2-3% sucrose is found very effective for optimal growth and morphogenesis. MS medium with 2 percent sucrose was optimal for culturing of shoot tips in *Tamarindus indica* (Kopp and Nataraja, 1990). However, in *Eucalyptus sideroxylon*, observed that 4 to 6% sucrose caused more callus formation during culturing of axillary shoots while 2-6 percent sucrose in MS medium supported roots development (Cheng *et al.*, 1992). It is found that 3% sucrose is effective for shoot initiation from cotyledonary node explant in *Stryphnodendron polyphythum* (Franca *et al.*, 1995). According to Kumari *et al.*, (1998) 20% sucrose concentration is more effective for development of globular embryos of *Terminalia arjuna*. In *Alnus nepalensis*, 1.5% sucrose in WPM medium was optimal for
shoot proliferation from terminal axillary buds (Thakur et al., 2001). Likewise in *Lippia alba* and *Melia azedarach*, Gupta et al. (2001) and Shahzad and Siddiqui (2001) respectively reported that 3% sucrose is required for callus as well as for shoot proliferation respectively. Similarly, Shekhawat et al.(2002) in *Azadirachta indica* and Ramamurthy and Savithramma (2002) in *Cassia alata* also advocated the use of 2-3% sugar to obtain multiple shoots. Gayatri (2007) reported the effect of sorbitol, sucrose, glucose, fructose, maltose, lactose and galactose as carbon source at various concentrations on *in vitro* seed germinations and protocorm formation in *Epidendrum radicans*. Agar-agar is used as a solidifying agent and assumed to be that of neutral support for callus growth and multiplication. Normally, 0.8 percent agar is used for culture medium. It was reported by Pasqualatto et al. (1986) that a higher concentration of solidifying agent in the medium reduced vitrification. But in certain cases an increase in agar amount causes adverse effect as observed by Lal and Singh (1995). It was reported by Selby et al. (1989) that combination of lower agar-agar (0.6%) and pH (5.6) gives better growth and proliferation in many coniferous plants.

**Choice of explants**-

Type of explant has a prime importance in their response to tissue culture for callus initiation and regeneration. The successful production of callus and subsequent plant regeneration is partially dependent upon the number of factors associated the explant used. These involves selection of organs that serve as tissue source, physiological and ontogenic stage of organ, the season in which explant is being obtained; the size of explant and overall quality of plant from which explants are to be obtained (Murashige, 1974). In *Albizia lebbeck*, stem, root, leaf, rachis, leaflets, hypocotyl and axillary buds were used for regeneration (Arya et al., 1978, Vergees and Kaur, 1988, Gharyal and Maheshwari, 1990). *Croton bonplandianum* was micropropagated using various explants like leaves, nodal and internodal segments by Ashish et al.(2011).

Nodal explants from mature tree of *Heavea Brasiliensis* failed to produce plantlets while explants taken from 6 to 8 weeks old plant regenerated plantlets (Rehman et al., 1981). Similarly explants from mature tree of *Eucalyptus citriodora* required
pretreatment for induction of shoot buds but explants from seedlings did not require any pretreatment (Gupta et al., 1981). Gulati and Jaiwal (1996) reported that nodal explants taken from coppied shoots of mature *Dalbergia sissou* exhibited least phenolic exudation and responded better shoot regeneration, while it was not observed so in explants taken from mature tree. This was probably due to differences in the physiological states of two explants. Nodal segments of mature plants have been however used in most cases i.e. *Centella asiatica* (Srivastava et al., 1997; Patra et al., 1998; Banerjee et al., 1999), *Bacopa monnieri* (Tiwari et al., 2000), *Eclipta alba* (Gawde and Paratkar, 2004). Micropropagation had been reported through shoot tip, nodal and intermodal segments in *Phyllanthus amasus* (Ghanti et al., 2004). Sukamto et al. (2011) developed a protocol for mass multiplication of *Nepenthes albomarginata* using shoot tip explants. Nandwani and Ramawat (1991) reported callus formation and regeneration of plantlets from nodal explants in *Prosopis juliflora*. Raghvaswamy et al. (1992) used nodal explants of *in vitro* grown root suckers from 60-80 years old tree of *Dalbergia latifolia* for direct organogenesis. In *Fraxinus angustifolia* shoot tips or nodal segments were used for micropropagation (Perez-Parron et al., 1994). Harikrishnan and Hariharan (1996) obtained direct shoot regeneration from nodes and leaves of *Plumbago rosea*. Nangia and Singh (1996) reported regeneration of multiple shoots from cotyledonary node and nodal explants excised from 7-10 days *in vitro* grown seedling of *Leucaena leucocephala* on MS basal medium fortified with (0.5, 1.0 and 2.0 mg l⁻¹) BAP. Rajendra and D’Souja (1998) obtained direct organogenesis from internodal segments of *Murraya koenigii*. Sardana et al. (2000) reported regeneration via somatic embryogenesis in leaf explants culture of *Jatropha curcas*. Indirect organogenesis from nodal explants of *Melia azedarach* was observed by Shahzad and Siddiqui (2001). Gupta et al. (2001) obtained direct shoot regeneration from nodal explants of *Lippia alba*.

Multiple shoots from axillary buds of *Cassia alata* were obtained by Ramamurthy and Savithramma (2002). In *Jatropha curcas* multiple shoots from nodal segments was also developed (Rajore et al., 2002). Sharma et al. (2006) reported *in vitro* mass propagation of a medicinally potent plant species *Vitex negundo* via nodal segments. Half strength of MS medium supplemented with BA (1.0 mg l⁻¹) + Kn (2.0 mg l⁻¹) + IBA
(0.02 mg l\(^{-1}\)) + GA\(_3\) (0.2 mg l\(^{-1}\)) + Ads (20 mg l\(^{-1}\)) supported good bud break as compared to other composition tried in *Glycyrrhiza glabra* (Vadodaria *et al.*, 2007). Adventitious shoot formation from embryos of *Areca catechu* was reported by Mathew and Philip (2000). Singh *et al.* (2010a) produced multiple shoots by culturing of nodal segments of *Sapindus mukorossi* on MS medium augmented with 2.0 mg l\(^{-1}\). Leaf explants proved better for callus formation than root and hypocotyl explants in *Vigna sinensis* (Pandey and Bansal, 1989). Rozga *et al.* (1993) found highest frequency of adventitious shoot regeneration from cotyledonous derived callus as compared to that of hypocotyls and shoot tip in *Paulownia tomentosa*. Seasonal conditions at the time of explant collection may influence the *In vitro* growth of explants, phenolics exudation and degree of contamination. The nodal segments of *Eucalyptus tereticornis* collected during July to September were more responsive to micropropagation because of negligible phenolic exudation from explants as compared to that collected in October- November and May-June due to high amount of phenolic exudation (Das and Mitra, 1990). Bonga and Pond (1991) reported fewer shoot initiation in nodal explants of *Larix spp.* collected from October to February than during the time proceeding or following that period. Bonneau *et al.* (1994) observed higher percentage of embryonic callus production from the zygotic embryo explants in *Euonymus europaeus* taken during May to September than before and after this period. Similarly, Thakur *et al.* (2001) observed optimal establishment of axillary and terminal buds of *Alnus nepalensis* cultured during February and March; therefore, the percent establishment showed a declining order.

The size of explants plays a key role in expressing the morphogenetic potentiality. Okazava *et al.* (1967) reported that small explants are more likely to form callus while larger explants maintain greater morphogenetic potentiality. This may be due to the available food reserves and growth regulators which proved useful in the initiation of new growth (Anderson, 1980). It was observed in *Populus wilsoni* that the time required obtaining culture decreased as the size of meristems tips used as explants increased (Rutledge and Douglas 1988). The orientation of the explant also plays an important role in giving morphogenic response. The horizontal position of the explant has been reported to promote adventitious shoot formation in many higher plants (Frett and Smagula, 1953).
and Pierik, 1987). Highest multiplication rate of the mature material was obtained in *Fraxinus angustifolia* when the nodal segment was placed horizontally (Parez- Parron *et al.* 1994).

Direct shoot bud regeneration was reported when proximal cotyledon halves were placed with their petioles laying outside the medium in *Cucumis melo* (Singh *et al.*, 1996). Isolated embryos gave rise to many shoot buds on MS + TDZ (0.2 – 0.3 mg l⁻¹) when mature bulbs of *Lilium nepalense* were used as explants on MS medium supplemented with factorial combinations of cytokinins and auxins, it gave 7 shoots/explant (Wawrosch *et al.*, 2001). It is the difference in the physiological age of the explants that cause the variations in regeneration characteristics. During culture studies of *Citrus vulgaris*, it was found that young cotyledons were physiologically very active and their morphogenetic response could easily be influenced by exogenous supply of hormones (Dong and Jia, 1991). Komalavalli and Rao (2000) noticed that young and juvenile seedlings gave better results in *Gymnema sylvestris*. Juvenile nodal explants resulted into high efficiency shoots proliferation in *Macuna pruriens* in half strength MS medium supplemented with BA(5.0 mg l⁻¹) and NAA(0.5 mg l⁻¹) in Half strength MS medium (Faisal *et al.*, 2005). Yadav *et al.* (2010) noticed that cultured nodal segments responded better to 1.0 mg l⁻¹ BAP fortified in full strength MS medium.

**Culture conditions**-

The major environmental factors in tissue culture are light and temperature. The illumination of cultures is considered in terms of intensity, length of the daily exposure period and the quality. Most of the cultures grow well within a wide range of photoperiod, light intensities and optimal temperature (White and Risser, 1964). Murashige (1977) reported optimum root and shoot formation from tobacco callus which involved a 16hours daily light period with intensity of 1000 lux. Gupta *et al.*, (1981) reported multiple shoots production when terminal buds from twenty years old tree of *Eucalyptus citridora* were cultured on MS medium at 15°C in continuous light followed by culture at 25°C with 16 hours photoperiod. The effect of light and cytokinins interaction on cultured cotyledon explants of Radiata pine was studied by Victor *et al.* (1984). A
high rate of multiplication had been achieved on MS medium with long day photoperiod in *Ranunculus asiaticus* (Pugliesi et al., 1992). A continuous light elicited only a low response (20%) in somatic embryogenesis from leaf explant of *Jatropha curcas*, 40% in complete darkness and optimum under a photoperiod of 16 hours (Sardana *et al*., 2000). In *Eucalyptus tereticonis*, a high rate of multiplication has been achieved on MS medium at a slightly higher temperature (30-32°C) (Das and Mitra, 1990). Calleberg and Johansson (1993) studied that direct regeneration was mostly stimulated when the anther cultured was incubated at 20°C. Rajore *et al.* (2002) reported multiple shoot formation on MS medium at 25°C ± 2°C in 16 hours photoperiod in *Jatropha curcas*. Formation of multiple shoots at 25 ± 2°C and 16 hours light and 8 hours dark period under light intensity of 3000-4000 lux has also been reported in *Azadirachta indica* (Shekhawat *et al*., 2002).

**Organogenesis**-

Organogenesis is the term which is used for differentiation of cultured tissue into well organized organs like shoot, root and leaf. It deals through two pathways i.e. direct pathway and indirect pathway. Direct pathway occurs through the continuous development of shoot meristems activity from lateral or axillary buds. Indirect pathways deal with the shoot formation via callus formation.

**Direct organogenesis**-

Direct organogenesis i.e. without callus formation has also been reported in many herbaceous and tree species. This method involves utilization of shoot tips, lateral buds and small nodal and internodal cutting as an explant and establishes genetically stable culture without any callus formation. The earlier report of organogenesis under *in vitro* conditions was given by White (1939) who obtained shoots form callus of *Nicotiana glauco* and *N. langsdorffii* hybrid on a agar-agar solidified medium. The lateral and axillary bud production system is described as conservative because of its relative ability to produce true to type plants without genetic change (Dunstan and Thorp, 1984). Multiple shoots could be induced from nodal segments of *Eucalyptus grandis* (Cresswell...
and Nitch, 1975 and Lakashmisita and Vaidtnathan, 1979). Mittal et al. (1989) observed the formation of multiple shoots from axillary buds from in vitro grown seedlings of Accacia auriculiformes. Shoot tip explant from in vitro grown seedlings of Tamarindus indica regenerated plantlets (Kopp and Natraja, 1990). Dass and Mitra (1990) reported 18-22 shoots per explant in Eucalyptus tereticornis when nodal explants were grown on MS medium with BAP (mg l⁻¹) and NAA (0.1 mg l⁻¹). Singh et al. (1993) observed sprouting of axillary bud in Acacia nilotica in MS and WP medium fortified with BAP (1.0 mg l⁻¹). Patnaik and Debata (1996) developed a protocol for in vitro propagation of an aromatic and medicinal plant Hemidesmus indicus from nodal segments on MS medium supplemented with NAA (0.054ppm) + K(1.5ppm). Khan et al. (1998) reported clonal multiplication of Syzygium alternifolium from mature nodal segments supplemented with BAP (4ppm) + NAA (0.5ppm). Similarly, Kumar and Seeni (1998) achieved rapid clonal multiplication of Aegle marmelos by enhanced axillary bud proliferation in single node segment of a twenty five years old tree on MS medium supplemented with BAP (2.5 mg l⁻¹)+ IAA (1 mg l⁻¹). Komalavalli and Rao (2000) established in vitro micropropagation of Gymnema sylvestre. According to them the nature of the explant, seedling age, medium type, plant growth regulators, complex extracts (casein hydrolysate, coconut milk, malt extract and yeast extract) and antioxidants (activated charcoal, ascorbic acid, citric acid and polyvinyl pyrrolidone) markedly influenced in vitro propagation of plant. Rajore et al., (2002) reported multiple shoots from nodal segments of Jatropha curcas on MS medium fortified with Kn (2.0 mg l⁻¹) and IBA (1.5 mg l⁻¹).MS medium fortified with growth regulators such as BAP (0.5 mg l⁻¹) in combination with NAA (0.01 mg l⁻¹) had been reported to give optimum results in Utleria salcifolia (Gangaprasad et al., 2003). Walia et al. (2003) reported direct shoots regeneration of Pandorina using nodal explants. Martin et al. (2003) developed protocol for rapid micropropagation of Wedilia chilensis through axillary bud proliferation from nodal segments cultured on MS medium with BA and IBA. Muthu kumar et al. (2004) micropropagated Datura metal by direct multiple shoots formation from nodal explants cultured on MS medium supplemented with BAP and NAA. Rapid shoots proliferation from shoot tip and nodal explants on MS medium fortified with Kn and IBA was achieved by Gururej et al. (2004). Maruthi et al. (2004) developed protocol for in vitro
propagation of *Celestrus paniculatus* on LS medium supplemented with fructose, Kn and IBA. Direct shoots regeneration and in vitro flowering was studied in *Solanum nigrum* by Jabeen *et al.* (2005). Kumari *et al.* (2005) reported multiple shoots formation from nodal and shoot tip explants in *Wedelia chilensis* under in vitro conditions. The highest efficiency of shoot proliferation in *Mucuna pruriens* through the nodal segment was reported in half strength MS medium fortified with BA (5 mg l\(^{-1}\)) and NAA (0.5 mg l\(^{-1}\)) by Faisal *et al.*, 2005. Rathore *et al.* (2005) developed protocol for in vitro propagation of *Maerua oblongifolia* using nodal shoots segments on MS medium and achieved high rate of shoots multiplication. A rapid protocol was developed for high frequency shoots regeneration from the nodal explants of *Spilanthes paniculata* and *Spilanthes acmella* on MS medium supplemented with various growth hormones by Mahendran *et al.* (2006) and Yadav and Singh (2010) respectively.

**Indirect organogenesis -**

Lakshamisita and Vaidyanathan (1979) raised plantlets from cotyledonary callus of *Eucalyptus citriodora*. Adventitious shoots regeneration was reported through callus culture in *E. camaldulensis* taken from shoot culture of mature tree (Murlidharan and Mascarenhas,1987). Callus formation had been reported in nodal segments and subsequently plantlets were obtained in Chinese gooseberry (*Actinidia chinesis*) by Gui(1979). Gharal and Maheshwary (1990) observed plantlets regeneration on callus culture derived from stem and petiole explants of *Albizia lebbeck*, *casia fistula* and *C.siamea*. Similarly, Axillary bud derived leaf explants of an alpine medicinal herb *Aconitum balfourii* favoured callus on medium containing BAP (4.5ppm) and NAA(2.7ppm) and these calli turned organogenic by lowering the NAA concentration to 1.4 ppm (Pandey *et al.*, 2002). Thomas and Philip (2005) reported high frequency shoot organogenesis from leaf derived callus of a medicinal climber *Tylophora indica*. Similarly, a protocol has been developed for high-frequency shoot regeneration and plant establishment of *Tylophora indica* from petiole derived callus by Faisal and Anis (2005). Organogenic callus was developed from stem explant of *Ruta graveolens* on MS medium composed of 2.5µM BA +10 µM 2.4-D. Thereafter maximum number of shoots
per callus clumps were obtained on MS medium supplemented with 7.5 μM BA + 2.5 μM NAA (Faisal et al., 2006). Deepa et al. (2006) noticed profuse callusing from cotyledon and shoot tip explants in Pseudarthria viscida on MS medium supplemented with 1.5-2 mg l⁻¹ 2,4-D and 1-1.5 mg l⁻¹ BAP. Subsequently shoot regeneration was achieved in MS medium supplemented with 2 mg l⁻¹ BAP. Shoot regeneration via formation of morphogenic calli could be achieved in Rauwolfia serpentina on MS medium supplemented with NAA and BA in combination (Tomar and Tiwari, 2006). Pareek and Kothari (2007) reported plant regeneration via callus formation in Dianthus caryophyllus in MS medium supplemented with 1 mg l⁻¹ BAP, 2 mg l⁻¹ 2,4-D and 2 mg l⁻¹ NAA.

Rooting-

Nutritive medium has been shown to vary from tissue to tissue as well as species to species. Among auxins, IBA is being commonly used to induce rooting of shoots on MS medium in plant like Dalber gia latifolia (Raghvaswamy et al., 1992), Prosopis cineraria (Nandwani and Ramawat, 1993), Melia Azedarach, (Shahazad and Siddiqui, 2001). Half strength MS medium supplemented with IAA (0.5 mg l⁻¹) developed rooting in Centella asiatica (Patra et al., 1998), Leptadenia reticulate (Hariharan et al., 2002) and Rotula aquatica (Sebastian et al., 2002). Medium fortified with 1.0 mg l⁻¹ IBA was found effective in Dalber gia lanceolaria (Dewari and Chand, 1996), Centella asiatica (Banerjee et al., 1999) and Rhinacanthus nasutus (Sudhakar et al., 2006). In Alpinia galanga, 2.2 mg l⁻¹ NAA was reported to induce rooting in excised shoots (Mustafa and Hariharan, 1997). Root induction was observed after 12 and 21 days of culture on MS media fortified with BAP (0.25 mg l⁻¹) + IBA (0.5 mg l⁻¹) and BAP (0.5 mg l⁻¹) + IBA (1.5 mg l⁻¹) in case of Paederia foetida and Centella asiatica respectively (Singh et al., 1999). Tiwari et al. (2000) achieved rooting on full and half strength MS medium with or without auxins in Bacopa monosperma and 90% rooting was achieved when shoots were cultured on full strength MS medium supplemented 2.46μm IBA. Half Strength MS medium supplemented with 9.84μm of IBA gave rise to (5.1 ± 0.49) roots per shoot in case of Withania somnifera (Manickam et al., 2000). IBA (2.0 mg l⁻¹)
was found to be effective for root formation of excised shoots in plant like *Alnus nepalensis* (Thakur et al., 2001) and *Capsicum annum* (Rao et al., 2006). Rooting was also observed by some worker in hormone free media as reported in *Cardiospermum halicacabum* (Babber et al., 2001) and *Peristrophe bicalyculata* (Sharma and Devi, 2006). Martin (2002) observed that half strength MS medium supplemented with 0.5 mg l\(^{-1}\) NAA was effective for roots formation in *Rotula aquatica*. Similar observations were made in *Citrus sinensis* by Dass et al., (1995). Lattoo et al. (2006) also reported vigorous rooting in the half strength MS medium supplemented with 5×10\(^{-6}\)M IBA in *Chlorophytum arundinaceum*.

MS medium fortified with 9.84μM of IBA resulted in vigours rooting in case of *Withnia somnifera* (Manickam et al., 2000) and 1.0 μM IBA supported better rooting in *Artemisia judaica* (Liu et al., 2003). MS medium supplemented with IBA (0.5 mg l\(^{-1}\)) induced rooting in *Phyllanthus amarus* (Ghani et al., 2004) and *Accacia magnum* (Nanda et al., 2004). In *Amomum microstephanum* rooting of excised shoots was achieved in MS medium (3% sucrose) fortified with IAA (1.0 mg l\(^{-1}\)) (Thoyajaksha and Rai, 2006). Vadodaria et al. (2007) observed rooting on medium containing 1% sucrose and fortified with NAA (0.1 mg l\(^{-1}\)) in *Glycyrrhiza glabra*. The frequency of roots in *Spilanthes acmella* was obtained on 1.0 mg l\(^{-1}\) IBA fortified half strength MS medium by Yadav and Singh (2010). Singh et al. (2010 a) noticed forty percent rooting in *Sapindus mukorossi* cultured on MS half strength medium supplemented with 2.0 mg l\(^{-1}\) IBA after 22 days of inoculation. Half strength MS medium containing IBA of 1.0 mg l\(^{-1}\) produced the maximum number of roots in *Lippa nodiflora* as reported by Evelyne and Ravindhran (2011).

**Hardening of Plantlets**

After regeneration of roots under *in vitro* conditions, hardening of plantlets prior to transfer in the soil enhances the survival rate of plantlets. Various types of substrates had been used during acclimatization such as soil vermiculites mixture (Goyal and Arya, 1981; Gulati and Jaiwal, 1996; Philomina and Rao, 1999), sterilized sand (Thakur et al., 2001) and soil Sunaina and Goyal, (2000). *In vitro* developed plantlets in *Dalbergia*
*latifolia* were successfully transferred by Raghvaswamy *et al.* (1992) by keeping the roots in tap water and high humidity. After this, plantlets were exposed to fresh air for few hours daily and after 14 days these were transferred to 1:3 courses- sand: soil mixture. Hardening of plantlets in *Jatropha curcas* had been done by using soil and vermiculite (3:1) by Rajore *et al.* (2002). All the plantlets of *Kaempferia galanga* were transferred to soil: sand (1:3) mixture with good survival rate (Rubin Jose *et al.*, 2002). Plantlets were transferred to autoclaved vermiculites wetted with half strength MS liquid medium without sugar. The plantlets of 4-5 cm long with fresh leaves were slowly transferred to field conditions (Chaudhary *et al.*, 2004). Sharma *et al.* (2006) transferred well developed plantlets of *Ruta graveolens* into pot containing sterile vermiculite and covered with polythene bags to ensure high humidity in order to ensure to acclimatize the plants to the field conditions. The survived plants were transplanted in the field after 2 months. Sharma *et al.* (2006) used sterilized vermi-compost and soil (1:3) mixture in pots to acclimatize plantlets of *Vitex negundo* by maintaining 85% relative humidity for 15 days. Equal ratio of soil and vermiculite has been used by Reddy *et al.* (2006) in *Azadirachta indica* and Rao *et al.* (2006) in *Capsicum annum* for successful establishment of *in vitro* regenerated plants. *In vitro* regenerated complete plants of *Sapindus mukorossi* were transferred in pots containing sterilized soil and sand mixture (1:1) with sixty per cent survival rate under field conditions by Singh *et al.* (2010a). Evelyne and Ravindhran (2011) observed that addition of coco pith and sand(1:1) had increased the survival rate (95-98 per cent ) of the acclimatized plantlets under field conditions.