Review of Literature
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Agriculture is expected to feed an increasing population, forecasted to reach 8 billion by 2020, out of whom 6.7 billion will be in developing countries where the carrying capacity of agricultural lands will soon be reached (FAO, 1999). Plant biotechnological applications must respond to this increasing demands in terms of food scarcity, socioeconomic development and promote the conservation, diversification and sustainable use of plant genetic resources as basic inputs for the future agriculture (Izquierdo, 1999). As suggested by the Nobel Peace Laureate, Bourlag (1997), Biotechnology and plant genetic engineering, complemented with conventional plant breeding, are needed to boost crop yields to feed the growing world.

About 30,000 species of the 250,000 so far identified are edible and about 7,000 have been estimated or collected by humans for food at one time or another (Wilson, 1992). Although our diet primarily relies on just 22 crops, more than 20,000 edible plants exist worldwide (Louder, 1988). For species conservation, the degree of extinction totally or locally as suggested by Heywood and Wyse Jackson (1991) is one among many criteria to consider, others being value, actual and potential usage, cultural importance and uniqueness. Estimates suggest that as many as 3226 of the 4752 communities in India representing 70% of the population are dependent on traditional plant-based medicines (Gadgil and Rao, 1998). Some of the most potent remedies used today (e.g., aspirin, digoxin, codeine, morphine, vinblastine,
vincristine, pilocarpine, cocaine, atropine, emetine and ephedrine) are derived from the herbal wealth of the world. (Natesh, 1999).

This unexploited biodiversity would generate economic value from extractable products obtained from individual species (Wilson, 1992). In recent times, modern medicine, which usually uses non-plant based drugs or synthesized drugs has shown an interest in natural drugs of plant origin. Efforts have been made in many countries to identify plants having natural drugs, which are effective against various modern diseases (Cordell et al., 1991). The value of yet undiscovered pharmaceuticals in tropical forests is at US $ 3-4 billion for a private pharmaceutical company and as much as US $ 47 billion to a society as a whole (Mendelson and Balick, 1995).

The application of plant tissue culture in ex situ conservation of many rare and endangered medicinal plants is emphasized in several reports (Sharma and Chandel, 1992; Sudha and Seeni, 1994). The scientific basis for the development of cell and tissue culture system is derived from the cell theory of Schleiden (1838) and Schwann (1839) which states that individual cells with in an organism have the “capacity for independent life”. Although attempts to culture isolated cells and tissues in nutrient solutions were made as early as 1902 (Haberlandt, 1902) formal organized and detailed studies on plant tissue culture began only after 1930. These were greatly influenced by the discovery in 1934/1935 of the first naturally occurring plant growth substances the auxin indole-3-acetic acid, and its effects on plant growth (Gautheret, 1939; Thimann, 1977).

Totipotency of plant cells enables fertile plants to be regenerated from individual cells and tissues, when the latter are given appropriate stimuli from growth regulators, such as auxins and cytokinins, supplied exogenously in the culture medium, combined with the correct physical parameters. The discovery of cytokinins and the observation that in combination with auxins they modulate shoot morphogenesis (Skoog and Miller, 1957) were important milestones in the development of methods for regeneration of Plants from cultured cells. The classical work of Skoog and Miller (1957) on callus tissues of Nicotiana tobbacum was the initial breakthrough in the understanding of the control of
morphogenesis. The ability to regenerate plants directly from explants is fundamental to clonal multiplication of elite germplasms via micropropagation.

Induction of multiple shoots through axillary branching is now recognized as a useful technique for propagation and *in vitro* conservation of threatened plants especially those in which roots or rhizomes contain the active compound (Constable, 1990). Interest in the application of tissue culture techniques as an alternative means of asexual propagation was greatly stimulated by the work of Morel (1965), who demonstrated that orchids could be multiplied rapidly by shoot meristem culture *in vitro*. Cytokinins trigger a variety of developmental events both in whole plants and in *in vitro* cultures (Kaminek, 1992; Brazobohaty *et al.*, 1994). Determination, which refers to an irreversible commitment of specialized cells or tissues to a specific morphogenetic pathway is very essential for any successful micropropagation system (Sussex, 1989).

When compared to seedling tissue, mature flowering plants exhibit a reduced competence for organogenesis. Regeneration from seedling tissue has met with some success but is of little significance for breeding purposes. The use of various explants for the production of friable callus and subsequent regeneration of shoot buds in herbaceous plants is well documented by Routh *et al.* (1992). Differences in the ability of regeneration were observed among different plant organs. For instance in *Pinelis temara*, bulbils show the highest regenerative ability followed by leaf blades and petioles. The distal explants produced more shoots than the proximal explants.

Adventitious buds could be regenerated from different source organs of medicinal herbs for e.g. terminal or lateral buds, bulbils, immature leaf blades, petioles, stem segments and root tips. (Gau *et al.*, 1993). Adventitious shoot proliferation is the most frequently used multiplication technique in micropropagation systems (Chu, 1992). The Primary target of a micropropagation system especially for the conservation of rare and endangered species is the best acclimation and field establishment of regenerated plants. Various factors like carbon dioxide fixation efficiency, formation of leafy wax, humidity are certain essential factors for plant establishment (Wardle *et al.*, 1983 and Grout, 1988).
Regeneration of shoot buds was reported from *Aconitum carmichaeli* (Hatano *et al.*, 1988), *Elletaria cardamomum* (Regahunath and Bajaj, 1992) from young shoots of tirucalli (Yamamoto, 1992) stem of *Phyllanthus* (Unander, 1991). Meristem culture technology has been applied to many crops, especially vegetatively propagated crops such as potato, over the last 40 years to eliminate issues from important cultivars (Hartmann *et al.*, 1990). Micropropagation through axillary shoots and shoot apices is quite successful in *Camellia sinensis* (Agarwal *et al.*, 1992). In temperate plants where shoots are newly initiated each year from resting buds, shoots can be said to become progressively older as the season advances. Explants for shoot tip or meristem explants are usually best dissected from young shoots (Kaul, 1986) or from buds just before the spring flush (Kurz, 1986). Nodal explants were also used as a suitable explant to produce multiple shoot cultures in many aromatic and medicinal plants (Patnaik and Birah Kishore, 1996).

Until relatively recently, axillary shoot production represented the most advanced micropropagation technology. However, organization can be brought about in tissue culture by controlled initiation of an organ primordium through manipulation of the nutrient and hormonal constituents in the culture media. A recent review has emphasized the basis and practical application of *in vitro* conservation of plant genetic resources (Withers and Engelmann, 1998). Interest in somatic embryogenesis increased dramatically (Gray, 1990) with an impressive number of over 200 species reported to have produced somatic embryos (Ammirato, 1983) in culture. Finer (1994), reported formation of somatic embryos or embryogenic tissue directly from the explant with or without the, formation of an intermediate callus phase.

According to Carman (1990), embryogenic cells from which somatic embryos form are developmentally plastic, not determined, their commitment to a particular pathway is strongly influenced by environmental factors and their behavior conforms to principles of differential gene expression. Nayak *et al.* (1996) have reported that somatic embryos can also be induced from callus cultures. These somatic embryos have developed in to plantlets when plated on a medium supplemented with comparatively lower levels of growth regulators. Choi and Soh (1997) have reported the origin and early developmental Patterns of somatic embryo from freely suspended single cells in cell culture of celery.
Based on friable embryogenic callus in the ornamental *Alstroemeria*, Lin et al., (2000) have established a plant regeneration system. The type of explant as well as its physiological state when entering storage an influence the duration of storage achieved. Roxas et al., (1995) indicate that, in the case of chrysanthemum, nodal segments showed higher survival rates than apical buds. The type of culture vessel, its volume as well as the type of closure of the closure vessel can greatly influence the survival of stored cultures (Engelmann, 1991; Withers, 1992).

Intensity of light influences the total light energy received by plant cultures. From experiments with shoot tip propagation of several different kinds of plants Murashige (1974) suggested that an optimum level of illumination for stages I and II might be 1000 lux (ca 14-15 μ mol, m⁻² s⁻¹) from white fluorescent tubes. Although light is invariably essential for the growth of normal green shoots and plantlets, unorganized cell and tissue cultures can frequently be grown in its absence and darkness may be beneficial to growth and morphogenesis (Jagannathan and Marcotrigiano, 1986). Cathey and Campbell (1980) have reviewed on aspects of light and lightning systems for horticultural crops.

A significant variation in growth pattern exists between embryos from different cell lines (Jalonen and Von Arnold, 1991). The effect of donor plant’s age is also important. In most woody plants, explants taken from the juvenile material were more responsive. In *Hedera helix* the juvenile stems maintained more than 20 per cent higher growth rate compared to their adult type (Stoutmeyer and Britt, 1969). Organogenic capacity decreased in mature stems of *Chrysanthemum X Morifolium* (Lu et al., 1990). In *Halesia carolina* seasonal fluctuations dramatically affected the shoot proliferating potential of the explants in vitro (Brand and Lineberger, 1986). Compton (1994) has discussed statistical methods suitable for the analysis of plant tissue culture data.

The nutrient medium also effects explants response to PGR in shoot organogenesis. Changing the nutrient medium can dramatically alter the percentage of explants forming adventitious shoots and/or the number of adventitious shoots or buds. The change can be more dramatic than simply altering the PGR in the medium. Gomez and Segura
(1994) doubled the number of buds on leaf explants by using SH rather than MS medium. Mencuccini and Rugini (1993) found that when full-strength MS was used, about twice as many olive petiole explants formed adventitious shoots than when half-strength MS was tested.

The storage of in vitro cultures of excised meristems has enabled short and medium-term maintenance of germplasm (Bajaj, 1993). Extensive work has been done on various species and the refined technique enables almost 100% survival/regeneration (Tannoury et. al., 1991). Having a long-term cryogenic preservation system offers immense advantages over other vegetative propagation systems, which attempt to maintain the juvenility of stock plants. Today synthetic seeds have been prepared by using a suitable encapsulating matrix in many plants. The main thrust is to prepare a simple inexpensive delivery unit. The concept of 'synthetic seed' was first introduced by Redenbaugh et. al.,(1984). Since then several researches throughout the world have been working on syn seeds with different crop species (Gray, 1990; Senaratna, 1992; Redenbaugh, 1993). In the last few years, this technique has been revealed to be a practical and efficient method to cryopreserve meristems, somatic embryos or cell suspensions from a wide range of plant species (Nino and Sakai, 1992; Blakesley et al., 1995; Bachini et al., 1995 and Martinez et al., 1999).

Debergh et al., (1981) coined the term vitrification to describe a morphological appearance of organs or tissues, particularly leaves that appeared water soaked transluscent and 'glassy'. As the phenomenon was more widely studied, it became clear that changes in the physiology of the explant proceeded the appearance of the visual symptoms. Therefore Debergh et al. (1992) reconsidered the usage of term vitrification and have proposed the term hyperhydricity which is now widely adopted.

Appearance of variation in tissue culture-raised plants (Somaclonal variation) is a major limitation in widespread acceptance of the technology for clonal propagation of plants of medicinal and commercial importance. The heritable variation obtained through tissue culture has been attributed to various factors (Bajaj, 1990). Molecular markers like RAPDs and RFLPs have been used by many investigators to monitor tissue culture induced variation in plants.
Initially, research in tissue culture focussed on aspects of plant developments following the recognition that cultured tissues enabled mass propagation of plants and provided the baseline for the introduction of agronomically and economically valuable traits into plants. Tissue culture has now progressed to an enabling technology, underpinning a spectrum of disciplines from plant genetic engineering through to conservation and bioremediation. Genetic engineering of plant cells through tissue culture, linked to molecular biology and biochemistry, has the potential to improve the yield and spectrum of biotechnologically important secondary metabolites and to modify plant metabolism directed at vaccine production (Mason and Arntzen, 1995).

The application of micropropagation techniques for medicinal plants gives many benefits to the breeders as it enables to increase the rate of shoot multiplication. However, the intricacies involved in the micropropagation of medicinal plants have been completely elaborated periodically by Murashige (1978); Ammirato (1983); Hussey (1986) and Bajaj (1986). Successful *in vitro* regeneration of medicinal plants could be made possible through the use of varied explants such as leaf and stem segments, shoot buds, hypocotyl, cotyledons, roots, anthers and seedlings. Leaf segments of *Centaureum erythrea* (Baresova *et al.*, 1985) is a good example of successful *in vitro* culture, through direct regeneration.


As far as the family Umbelliferae is concerned methods of clonal multiplication were established for a wide number of species. A perusal of literature shows that *Daucus carota* (Carrot) the first plant material which was successfully cultured and regenerated in large numbers indicated the possibility of using the same technique for other members of the family Umbelliferae. Carrot tissues showed the maximum potentialities and become the

Of the six cultivated Indian species viz., cumin, coriander, fennel, dill, celery and jowar, only 2 have so far been investigated. Other species that have been worked on include *Coriandrum sativum* (Steward *et al.*, 1970) *Apium graveolens* (Reinert *et al.*, 1966; William & Collins, 1976a, b) *Carum carvi* (Ammirato, 1974), *Pimpinella anisum* (Huber *et al.*, 1978) and *Conium maculatum* (Steward *et al.*, 1970). The work on *in vitro* culture has progressed in case of *Cuminum cuminum* (Jha, 1980) and very recently protoplasts have been successfully isolated from leaves of *in vitro* regenerated shoots.

Moon and Lee (1994) have induced embryogenic callus from petiole segments of *Pimpinella brachycarpa*. Plantlets of *P. brachycarpa* were also regenerated through secondary embryogenesis (Kim *et al.*, 1996). Kudielka and Theimer (1983) and Ernst and Oesterhelt (1984) have reported a detailed description of anise (*P. anisum*) embryogenesis. Recently *in vitro* flowering and seed setting in coriander (*Coriander sativum* L.) were reported by Stephen and Jayabalan (1998). Analysis and characterization of genetic variation is fundamental to any conservation strategy, whether *in situ* or *ex situ*, (Westmann and Kresovich, 1997). Application of marker technology can pinpoint gaps in collections, decide which genotype is of high priority for conservation and determine optimum size of gene bank accessions (Callow *et al.*, 1997).

The diversity inherent in seeds and other propagules are plant science's greatest natural heritage. Very small populations (the extreme being single plants) are particularly at risk, as are populations growing at the limits of geographic or altitudinal range. In such instances, breeding programmes to maximize variation by controlled crossing between particular sets of individuals may have to be considered. Nonetheless, some example of extremely low genetic variability among narrow range endemics has been recorded. *Pinus torreyana*, of California, is known from two populations and electrophoretic studies have revealed no significant intra population variation (Ledig, 1986). Our ability to survey for
genetic diversity at the molecular level has increased by orders of magnitude over the past few decades. Recently, DNA makers have been employed to study variation in rare species. For example, low variation was detected in the endangered island endemic *Malacothamnus fasciculata var nesioticus* of Santa Cruz Island, California. (Swenson et al., 1995) and in Swedish populations of the rare species *Vicia pisiformis* (Gustafsson and Gustafsson, 1994).

Germlasm of each of these species and many other economically important fiber, medicinal and commercial crops must be continually preserved. The strategies to do this include exploration, conservation, genebank development (Plucknett et al., 1987; Plucknett and Smith, 1989) evaluation, data storage retrieval utilization, training and global coordination (Hawkes, 1981).