REVIEW
OF
LITERATURE
Technical innovations and resulting changes are the key to the process of economic development of our country. Dramatic advances in biological research augur a third agricultural revolution involving biotechnology, a catch-all term that include both cell and DNA manipulation. With regard to some of the applications of biotechnology, a mention can be made of micropropagation, induction of androgenic haploids and somatic hybridization as aids to crop improvement programmes.

2.1 MAJOR EVENTS IN THE HISTORY OF PLANT TISSUE CULTURE

As the basic structural unit of multicellular organisms, the cell is involved in a complex system of interrelationships. It is functionally autonomous and part of a graded morphological and physiological hierarchy of tissues, organ and organism. The concept of cell theory of Schleiden (1838) and Schwann (1839) forms the basis of plant cell, tissue and organ culture.

The last decade has witnessed a dramatic increase in our ability to manipulate the plant cells in culture. Cell culture studies have permitted investigations at many levels such as molecular, cellular, organal, which have been applied in a wide range of areas like agriculture, horticulture, cell biology, biochemistry, genetics and plant breeding.
Street (1977), Gautheret (1982, 1983), Cocking (1986) and Yeoman (1986) have reviewed the plant tissue culture in detail. Haberlandt (1902) for the first time attempted the cultivation of isolated plant cells in vitro and this led to many important discoveries.

This technique has been exploited both for basic and applied aspects of plant research encompassing haploidy, mutation and mutagenesis, somatic embryogenesis, somaclonal variation, protoplast fusion, genetic manipulation and molecular biology of plant genes (Bajaj, 1977, Barz et al., 1977, Lindsey and Yeoman, 1983; Mantell et al., 1985) and also has been commercially exploited (Murashige, 1974, Reinert and Bajaj 1977, Yeoman, 1986).

Krikorian (1982) reported that the concept of cell totipotency is inherent in the cell theory. Haberlandt (1902) had expressed faith in totipotency of plant cells and hoped that it would be possible to successfully cultivate artificial embryos from vegetative cells. Although the idea of culturing cells was well conceived, very little progress was made during the first three decades.

However, White (1934) established an actively growing clone of tomato roots. Gautheret (1983) considers "1939" as a significant year in which the possibility of culturing plant tissue for unlimited periods, was independently carried out by Gautheret and Nobecourt in Carrot and by White in tobacco.

There are numerous plants which have been cloned till date. It is roughly estimated that about 600 species have been cloned in vitro.
Major break through in plant tissue culture was achieved after the discovery of auxins and cytokinins. The identification and purification of Indole-3-acetic acid (IAA), the first known growth regulator (Kogl et al., 1934). Further Thimann (1935) made it possible to control the growth of plant cells and tissues. It was not until 1957 when Kinetin was discovered the idea of synergistic effects of auxins and cytokinins in promoting cell division in tobacco, triggered the imagination of physiologists (Skoog and Miller, 1957).

Steward et al., (1958) and Reincrt (1958, 1959) observed the organogenesis and development of somatic embryos from a culture mass of *Daucus carota*. Murashige and Skoog (1962) formulated a defined medium for tobacco culture which is now widely used for the culture of a wide range of plant species.

Guha and Maheshwari (1964) observed the embryoid-like structures from anther culture of *Datura*. But Nitsch and Nitsch (1969) formulated a method to grow hundreds of haploid tobacco plants from the pollen grains. The pioneering work of Guha and Maheshwari (1964) later culminated in an extensive programme of haploid plant production from cultured anthers, which is widely adopted in Chinese agriculture (Huo Han et al., 1983).

In 1965, Vasil and Hilderbrandt described growing of a wild type tobacco plant from a single cell. Interestingly, it took another twenty years to successfully obtain a whole plant from a single cell culture of tobacco protoplast (Pattnaik et al., 1981).

A significant development in plant tissue and cell culture in recent years has been the development of techniques for the isolation, culture and fusion of plant protoplasts. In 1970's, it was observed that protoplast isolated
from mesophyll cells could be induced to grow into complete plantlets (Takebe et al., 1971), and further under defined experimental conditions protoplasts could be stimulated to fuse (Power et al., 1970). Carlson (1972) created the first parasexual hybrid by fusing protoplasts from two tobacco species.

Through *in vitro* culture studies, the mechanisms of cell differentiation could be understood, thereby making an experimental approach to link genotype with phenotype. To a large extent the tissue culture revolution has occurred because of the need of this new biotechnological method and because of the fact that plant tissue culture and associated cloning techniques provide the foundation for exploitation of genetic engineering (Cocking, 1986). Recent development in transferring foreign genetic information into plants through protoplast culture has led to the appreciation of cell culture as an essential method of recovering modified plants. There are several potential practical applications of plant cell and tissue culture in agriculture, forestry and horticulture. The currently available tissue culture techniques could facilitate the following activities (Krikorian, 1982).

- Rapid multiplication of selected plants
- Elimination of virus and specific pathogens
- Virus indexing
- Germplasm introduction and evaluation
- Germplasm collection, preservation and management
- Production of polyploids, haploids, somaclonal variants for new crop production and use in breeding programmes.
- Elimination of certain breeding barriers
  (i) *in vitro* fertilization in ovule
  (ii) embryo rescue and/or storage
  (iii) androgeresis
  (iv) gynogenesis
- Selection for complex traits such as tolerance to temperature, salt and herbicides, diseases and pests
- *In vitro* mutation breeding
- Cryopreservation
- Genetic Engineering
  (i) transformation by selectable genes
  (ii) organelle transfer
  (iii) wide crosses-somatic hybridization

- Understanding controls in developmental and physiological process

2.2 MICROP propsagation

2.2.1 Definition and History of Micropropagation

Micropropagation represents the optimum efficiency in terms of vegetative plant propagation and allows large number of propagules to be produced in a relatively short period under controlled conditions in a relatively small space throughout the year.

The history of micropropagation was further dominated by the discovery of plant hormones like auxins and cytokinins. The discovery of first auxin (IAA) and cytokinin (kinetin) created the greatest opportunity for in vitro culture of higher plants in general and for micropropagation in particular.

Pierik (1987) explained various phases in micropropagation and also summarised several methods to propagate plants in vitro.

2.3 Tissue Culture in Carnation

Tremendous progress has been made with Carnation which is a herbaceous plant during the past two decades in several aspects of tissue culture and its development has attracted the interest of many biotechnologists at present. Perusal of literature reveals that lot of work has been done on herbaceous plant species with regard to shoot regeneration from leaf, nodes, internodes and callus using in vitro techniques.
Tissue culture studies in Carnation are confined to regeneration, effect of growth regulators, source of explants and micropropagation. Different explants such as stem, leaf, axillary bud (nodal explant), shoot tip, root and petals have been exploited for the purpose.

As early as 1963, Stone introduced meristem tip culture for Carnation micropropagation. Hackett and Anderson (1967) reported aseptic multiplication and maintenance of differentiated Carnation shoot tissue derived from shoot apices.

Earle and Langhans (1975) successfully propagated Carnation from shoot tips in liquid medium, when transferred to agar nutrient medium containing 0.5 mg l⁻¹ Kinetin and 0.1 mg l⁻¹ NAA formed multiple shoots.

Pennazio (1975) studied the effects of different concentrations of adenine and kinetin on the development of Carnation meristem tips. Kinetin at 0.1 ppm reduced root formation, whereas 1.0 ppm Kinetin completely inhibit root formation. However kinetin promoted lateral bud development. Adenine at 10 ppm also inhibited root formation.

Davis *et al.*, (1977) showed three stages of clonal multiplication, i) shoot tip culture as initiation stages ii) shoot multiplication stage and iii) rooting stage. In the initiation stage for multiple shoots they examined the uses of various inorganic salts, vitamins, carbohydrates, growth regulators, agar, pH and additional supplements to study their effect on growth and development. They found that in shoot tips grown on modified MS medium with 10 µM kinetin and 1 µM of NAA, apical dominance was counteracted and morphologically similar shoots differentiated rapidly.
Shabde and Murashige (1977) observed that in a medium containing both IAA and kinetin apical meristem dome explants produced highest frequency of plants. Further, in an unsupplemented MS medium they observed continuous development of the explants.

Thimann (1977) reported *in vitro* plantlets should have sufficient number of roots for absorption of nutrients and to provide anchorage. In most of the instances the presence of high cytokinin/auxin ratio in stage II inhibits root formation and therefore a separate medium for induction of roots has to be used at stage III. Since auxin is essential for root initiation normally the medium at stage III contains auxins. At the same time very high concentration of auxin inhibits root elongation.

Dabbski and Malinowska (1979) studied the influence of kinetin in combination with different concentrations of NAA (0.5, 1.5, 4.5 and 13.5μM) on *in vitro* growth of meristem tips of Cv. Scania 3C. The highest fresh weight of shoots was reported on MS medium supplemented with 13.5μM kinetin and 0.5μM NAA.

Hempel (1979) found that shoot apices of Carnation cultured on MS medium with 6μM BAP for 20 days and transferred to the medium with further 4μM kinetin was found to be the best for the multiple shoot production.

Densco (1984) reported that for every 100 meristems, approximately 1300-1500 plants were obtained after 15 weeks. He had also observed that Carnations were not sensitive to the microelements of the nutrient medium but the proportion of kinetin to NAA was important in the propagation stage.
Ioannov (1990) studied the production of Carnation plants by shoot tip culture in *in vitro* using modified MS medium supplemented with NAA at 0.02 mg/l and kinetin at 0.5, 1.0, of 2.0 mg/l and observed highest multiplication rate of 33% obtained with the highest concentration of kinetin (2.0 mg/l).

Can and Koc (1992) reported that Carnation meristems cultured on MS medium containing 5.0 mg/l BAP and 1.0 mg/l IAA produced more axillary buds than other combinations of growth regulators.

Frey and Janick (1991) reported that shoot regeneration in Carnation was influenced by genotype, explant source and different growth regulators.

Opera and Pamfil (1982) produced virus free Carnation planting material from meristem cultures. They selected (0.2 to 0.8mm) sized apical or axillary meristem explants from three cultivars of Carnation cultured on modified MS medium. They observed that explant regeneration increased from 20 percent to 70 percent in MS medium. Duration of explant regeneration also decreased with the size and age of the meristem.

Moshari and Danesh (1983) studied Carnation meristem tip culture for production of Carnation mottle virus free plants when 0.6 to 1mm long meristem tips were cultured on MS medium containing 1 mg/l of NAA.

Roset and Bokelmann (1979) cultured Carnation stem sections, obtained from node, on a medium containing 1 mg/l BAP and 0.1 mg/l IAA to induce shoots.
Rosset and Bokelmann, (1981) studied vegetative propagation of Carnation in \textit{in vitro} through multiple shoots. The vegetative shoot cuttings of 6 cultivars of Carnation were irradiated with 30 or 60 Gy X-rays and obtained multiple shoots from cultured nodal stem explants. The \textit{in vitro} propagation of irradiated 209 stem segments resulted in the production of 2,220 plantlets within four months after incubation.

Ghosh and Ram (1986) studied multiplication of axillary buds cultured on Gamborg's B5 medium containing 3 percent sucrose and NAA 10^{-4}M and BAP 10^{-3}M. Each bud proliferated to yield 13 shoot buds in 2 weeks. Thus beginning with a single axillary bud, it was possible to obtain 12 - 13 shoot buds of Carnation in 6 months time.

Kim and Kang (1986) studied \textit{in vitro} multiplication of Carnation by using nodes, leaf buds and shoot tips from plantlets of Cv. Scarlet bell cultured on solid MS medium containing various concentrations (0.01 mg l^{-1}) of NAA and BAP. They obtained multiple shoots from the above explant. Further, they compared production of multiple shoots from axillary buds in liquid culture medium.

Lubomski and Jerzy (1989) studied the effects of BAP (0:10^{-7}M) and IAA (0:10^{-5}M) on regeneration capabilities in internodal segments. They obtained both adventitious shoots and roots at 10^{-3}M BAP and 10^{-4}M IAA.

Nugent \textit{et al.}, (1991) reported that in stem explants, morphogenetic capacity was determined mostly by the development stage of the explant. Highest percentage of shoot formation was observed in the youngest stem segment on all the cytokinins tested.
Altvorst et al., (1994) showed that when two leaves from one node of in vitro aseptic plants were cultured on solid MS media with 4 μM BAP and 1.6 μM NAA, they responded with different shoot forming potential. The leaf removed second from the stem formed more shoots and also had a large amount of adhering stem tissues.

Kakehi (1979) and Gimelli et al., (1984) observed that the immature petals and basal flower segments are morphogenetic in a determined floral development pattern.

Miller et al., (1991) regenerated adventitious shoots from flower buds that is individual petals and receptacles on MS basal medium supplemented with 4 - 8 μM BAP. They found that the major site of shoot formation was the sub-epidermal cells at the proximal end of petal.

Frey and Janick, (1991) regenerated plants from petals, calyces, nodes, internodes and leaves from three cultivars (Scania, Improved White Sim and Sandra). They found that only petals, calyces and nodes were regenerative. Maximum proliferation was achieved with petals on MS medium supplemented with 0.05 μM Thidiazuron and 0.5 μM NAA.

Nugent et al., (1991) studied the effect of different cytokinin on regeneration from petal and stem explants of cultivar White Sim. They reported that Thidiazuron was more effective than BAP or kinetin and also stem derived plants grew faster than petal or receptacle derived plants.

Mubarack and Choudhary, (1992) used the base of the petal explant for direct shoot differentiation and obtained 60 percent of differentiation when cultured on MS medium supplemented with 2.0 mg l⁻¹ IAA and 1.0 mg l⁻¹ BAP.
Fisher et al., (1993) developed an efficient procedure for adventitious shoot regeneration from various parts of Carnation petals in liquid medium. Shoot regeneration was obtained from nearly all parts of the plant on liquid medium. The adventitious origin of the regenerative shoot was confirmed by scanning under electron microscope.

Messeguer et al., (1993) reported the adventitious shoot regeneration from leaves, basal segment of flower and petals. It was observed by them that petals and floral segments exhibited a high morphogenetic capacity in a wide range of growth regulator treatments. Lowering the BAP concentration to 0.01 mg l\(^{-1}\) and NAA to 0.001 mg l\(^{-1}\) resulted in the suppression of floral development of petal explants and the regeneration of vegetative shoots.

Nakano et al., (1994) studied adventitious shoot regeneration of vegetative shoots among the leaf, stem and petal explants on MS medium containing different concentrations of BAP and NAA. Regeneration was found to be high in media containing 5-10 \(\mu\)M BAP with or without 5\(\mu\)M NAA. Among the cytokinins tested N-2 chloro-4 pyrindyl induced organogenesis and petal explants regenerated on N'phenyl urea and Zeatin.

Engvild (1972) cultured callus and cell suspensions of Carnation stem using half strength MS medium with \(3 \times 10^{-6}\)M IAA combined with \(3 \times 10^{-4}\)M BAP and \(10^{-4}\)M 2,4-D. He observed 100 fold increase in fresh weight with IAA and BAP and also in 2,4-D where cell suspension cultures had a doubling time of about 2 days.

Ruffoni et al., (1990) described shoot regeneration from callus and cell cultures of Carnation. The friable callus was obtained by culturing stem segments
on solid MS medium with 1 ppm of BAP and NAA. They obtained highest regenerants in liquid medium containing 7 ppm and 2 ppm NAA from callus.

Radojevic et al., (1990) found variation in leaf rosette formation and axillary bud induction in different Carnation cultivars. Tangerina, Scania, Dark Purple, Arthur Sim, Lena and Telestar gave 42, 50, 73, 76, 90 and 93 per cent regeneration respectively from stem segments. They also showed that the choice of cytokinin depended on the cultivar response and kinetin was more favourable for shoot multiplication of Telestar, Lena and Scania While BAP was better for Arthur Sim, Dark Purple, Tangerina and White Sim.

Choudhary and Mubarak (1991) investigated plant regeneration from the callus of leaf explants of three varieties viz., Shocking Pink, William Sim and Arthur Sim. Early callus initiation was observed in 0.5 mg l\(^{-1}\) 2,4-D and 0.5 mg l\(^{-1}\) NAA whereas presence of BAP in the medium delayed callus initiation. Shoot regeneration from callus occurred only in case of shocking pink callus when medium was supplemented with either 0.5 mg l\(^{-1}\) kinetin and 0.1 mg l\(^{-1}\) NAA or 0.25 mg l\(^{-1}\) 2,4-D and 0.5 mg l\(^{-1}\) NAA and 0.5 mg l\(^{-1}\) BAP.

Fal et al., (1991) used Scania, Improved White, Barbaret, Antares, Pink Calipso and Angeline for mass propagation by using meristem cultures and obtained varietal difference in the co-efficient of multiplication which ranged from 3 to 6.

Malczewska et al., (1979) cultured callus tissues obtained from shoot apices of young plants on half strength MS medium. Adventitious shoots regenerated best on 0.03 µM NAA and 3.0µM BAP.
Frey et al., (1992) induced somatic embryos from Carnation internodal callus in MS liquid medium supplemented with 3.0μM 2,4-D. Further they showed by transferring to liquid basal medium without 2,4-D obtained embryo development.

Nakano and Mii (1993) showed that antibiotics stimulate embryogenesis from leaf explants in Carnations without plant growth regulators. They tested a range of antibiotics on several cultivars. Among them Cefotaxine at 500 mg l⁻¹ was most effective in inducing somatic embryogenesis in 30.7 per cent of cultures after two months.

Choudhary and Chin (1995) reported that the occurrence of somatic embryogenesis in cell suspension cultures of White Sim. Callus was generated on MS medium supplemented with 2,4-D (5 or 10 μM). During subsequent subcultures in the absence of 2,4-D formation of somatic embryos resulted.

2.4 PROBLEMS ENCOUNTERED IN TISSUE CULTURE OF CARNATION

2.4.1 VITRIFICATION

Debergh et al., (1981) made the first scientific examination of vitrification in *in vitro* plants. Vitrification is a physiological disorder frequently affecting both herbaceous and woody plants cultured *in vitro*. Vitrified shoots develop abnormally producing short stem and thick fragile leaves with a translucent or glassy appearance. Further, their investigation reveals that the explant chosen from the mother plant has not effect on vitrification.

Recently Debergh et al., (1992) reconsidered the term vitrification and substituted it by "hyperhydricity". Kataeva et al., 1991; and Ziv, 1991; described the causes and prevention methods for vitrification.
Hakkart and Versluijs (1981) studied the effect of agar concentration, and temperature on vitrification. They found that increasing the agar concentration from 6 g l⁻¹ to 12 g l⁻¹ leads to decreased glassiness, but at the same time reduced plant growth. The type of caps used for the tubes was also found to affect glassiness, the looser caps showed less glassiness. Exposure of Carnation meristem tips to high temperature during the initial periods of culture favoured the development of normal plants.

Kevers et al., (1984) hypothesized that initiation of vitrification occurred due to stress induced ethylene production, which is controlled by peroxidase - IAA oxidase system. Excess of ethylene in the atmosphere of stressed plants decreases activity of PAL and acidic peroxidases which hinders the lignification process. Deficiency of both cellulose and lignin could allow more water uptake due to reduced wall pressure and bring about vitrification.

Leshem and Sachs (1985) reported high concentration of NAA in the culture medium increased and benzylaminopurine decreased the proportion of vitrified shoots.

Leshem (1986) reported that Carnation plants obtained from vitrified plantlets serve as a source of somaclonal variation.

Gasper et al., (1987) reviewed extensively about the causes, reactions, physiological, biochemical and ecological events of the vitrification.

Werker and Leshem (1987) studied the structural changes that occurred during vitrification of Carnation plantlets. They showed three main differences from normal structure viz., i) reduction in the period in which cells remain meristematic in developing parts of the shoot, up to full maturation of
2.5 VARIETAL PERFORMANCE

medium eliminates or minimizes vitrification.

Hu (1961) observed that use of high concentration of penicillin in the

vitrification.

and selection of appropriate media and cultural environment could minimize

in addition high hormone concentration with a high cytoxin to auxin ratio

superculture. Use of loose filling cap was culture index is also beneficial.

the explain on a medium containing high concentration of agar and by frequent

Miller et al. (1961) showed that vitrification can be controlled by culturing

when the CaCl2 concentration was doubled.

reduce by lowering the KNO3 and NH4NO3 concentrations but was increased

proliferation. They also reported that the proportion of vitrified plants was

was optimal, above which aches developed into small shoots instead of

Choudhury and Prakash (1961) showed that use of 0.2% agar or agar

fluorescent plants.

1.5% in MS medium or the addition of AB.A (10-9M) produced normal

Khim et al. (1968) showed that use of higher concentration of agar

bushy and normal plants.

Leschen et al. (1968) showed that use of high cytoxin resulted in

certain regions to form cavities.

of elongation and (iii) defective cell walls in which disintegration occurs at

shoot apex itself. (ii) Hypertrophy of the cell's with a loss of a clear axis.
methods used in variety trials and evaluation. Important characters for cultivar evaluation are, keeping quality of the flowers, *Fusarium* resistance and productivity.

Behvarova *et al.*, (1988) selected 6 cultivars grown in a greenhouse and assessed for productivity, cut flower quality and resistance to *Fusarium*. Cultivars Orange Triumph, Hellas and Palma were the best followed by Roma, Verona and Pirana.

Wilfret (1971) reported after evaluating 40 standard and 13 spray type Carnation cultivars under sub-tropical conditions, that the length of miniature sprays ranged between 40 cm and 64 cm representing Royalette and GRA Goldlocks, respectively.

Similarly, Loeser (1986) reported that out of the Standard Carnation studied, Cv. Castellano was the tallest (140 cm) with long stem 60 cm.

Kim *et al.*, (1992) studied 15 Carnation cultivars to assess their suitability for cut flower production. They found that, Galil, Rimon, Beta of the Standards, and Carmit, Darling of the sprays had longer flower stems.

Snijbloementelt (1987) tested large flowered Sim type and Mediterranean type Carnation cultivars for their suitability to cut flower production. The Mediterranean type cultivars had fewer side shoots compared to Sim type.

Miske (1982) found that the earliest flowering Carnation cultivars were Ministar, Goldstar, Minigold, Pepito, Lanceruso and Londrino flowered early, the late flowering was observed in Royal Red, Yellow Stone, and Jolvitte cultivars.
Oszkinis and Kus, (1971) reported that the Carnation cultivars Caravelle, Arthur Sim, Clear Yellow Sim and Caravelle Saugus were outstanding with respect to flower diameter. Loeser (1986) found that Cv. Castellano had the largest flower diameter of 76mm.