CHAPTER 2  
LITERATURE REVIEWED

Citrus fruits are one of the important horticultural crops with worldwide agricultural production over 100 million metric tons per year. Citrus fruits belong to the family of *Rutaceae*. The special feature of citrus fruits is the presence of juice sacs in them. Citrus fruits lack a firm pulp. Citrus originated from south-eastern Asia, China and the East of India is archipelago from at least 2000 BC (Swingle 1943; Webber et al 1967; Gmitter and Hu 1990).

*In-vitro* regeneration of plants is recognized via three patterns of differentiation viz. proliferation from pre-existing buds, differentiation of adventitious shoot buds and somatic embryo genesis (Bhojwani and Razdan 1983; Rao and Lee 1986). First pattern of morphogenesis has been suggested for clonal propagation of plants (Evans et al 1981; Rao and Lee 1986). Several attempts have been made to regenerate fruit plants using all three approaches of plant tissue culture and the salient research highlights have been reviewed by Nehra and Singh (1982), Bhojwani and Razdan (1983) and Hammerschlag et al (1992). It seems that tissue culture technique for clonal propagation of various temperate fruits trees has been refined to explants on commercial scale (Hammerschlag FA 1986; Zimmerman 1985). However, tissue culture technique for fruit crops like banana and pineapple has been successfully developed (Litz et al 1986).

Plant tissue culture has lot of scope for research in the plant science. It is however, a relatively new technique in commercial Horticulture. The two main applications of plant tissue culture in *citrus* production are being used in propagation and sanitation effectively. The relevant literature pertaining to these aspects have been reviewed in this context.

*In-vitro* propagation includessurface sterilization of explants, preparation of nutrient medium for *in-vitro* propagation, supplement media with different Plant Growth Regulators (PGRs) for *in-vitro* propagation.

Following the first attempts of Ball (1946) and Morel and Martin (1952) the regeneration of complete plants from apex culture has been successful and relatively rapid with numerous herbaceous species. On the other hand, the progress in perennial Horticultural crops, has been slow owing to the lack of basic genetic information and their recalcitrance to regenerate in cultures (Bitters and Murashige 1966; Jones and Vine 1968; Quak 1977; Jonard et al 1983;
Wilkins et al 1985; Waithaka 1988; Jones 1991; Sangwan-Norreel et al (1991). The techniques have greatly improved with the time as a result of development of more suitable basic nutrient media addition of various supplements and growth regulators, such as auxin, kinetin, etc., improved and controlled environmental conditions, and a better understanding, of the basic problems involved (Bitters and Murashige 1966).

The use of tissue from mature plants can be very difficult, since success in culturing their tissue is known to be inversely related to their age (Bhojwani and Razdan 1983; Rajmohan and Mohan Kumar 1988; Yadav et al 1990b; Das et al 1995). It has been realized that juvenile material has better potentiality to express totipotency under in-vitro condition than the explants taken from mature or adult phase of development (Yadav et al 1990b; Jordan and Oyanedel 1992).

The type of explants also influences the in-vitro regeneration. Nodal explant has been found superior to shoot explants to initiate the culture of various mature fruit and forest trees (Bhojwani and Razdan 1983; Yadav et al 1990a; Purohit and Tak 1992).

The position of the explants on the stock plant can interfere with the reaction in-vitro (Debergh and Maene 1985; Pierik 1987). In-vitro survival and adventitious shoot production were greater in explants from axillary shoot tips than from terminal position in grapes (Yu and Meredith 1986). New vegetative growth from the base of the main stem during the period of vigorous vegetative growth has been reported to be reliable source of explants in guava (Amin and Jaiswal 1987).

Nuceller embryos of polyemborny seeds were better explants for rapid, clonal propagation of polyemborny species, most of the Citrus are polyemborny and the identification of nuceller embryo is a problematic issue. On this aspect the method described by Ashari et al (1988) described a method of discrimination of zygotic and nuceller seedlings of five citrus species Isozyme analysis using eight loci of six enzyme systems was used to distinguish zygotic from nuceller seedlings resulting from pollination of five polyembryonic Citrus root stocks. Zygotic seedlings were detected by isozyme analysis in the root stocks rough lemon (2.1%), trifoliate orange (2.1%), Sweet orange (0.8%) and Troyer citrange (0.8%). No zygotic seedlings of Cleopatra mandarin were detected, but this may be due to the high proportion of homozygous loci. The zygotic seedlings have arisen by both self and cross pollination of the maternal parent. There was some morphological variability amongst the
zygotic seedlings but these characters could not be used to identify all of the zygotic seedlings detected by isozyme analysis.

2.1 Surface sterilization of explants

Many workers have encountered serious problems during the actual initiation phase, might be as a result of contamination of explants. Failure of the usual disinfection technique to eliminate bacteria and fungi is often attributed to heavy contamination and the location of the organisms deep within the explant (Murashige 1977).

Giladi et al (1979) recommended the improved procedure for surface sterilization that includes-dipping of branchlets in 80 % v/v ethanol for 5 min, immerse in a filtered 9% (w/v) Ca(OCl)₂ solution contained an appropriate concentration of a non-ionic surfactant. Young et al (1984) reported that the treatment with aqueous sodium hypo chlorite or other disinfectants were often more damaging to the plant tissue than to the bacteria. They further indicated that at least some bacterial contaminants encountered in the tissue culture of woody plants eliminated by a careful choice of antibiotics-Gentamicin, Amikacin, Rifampicin, Tetracycline, Polymyxin B, Cefotaxime, etc.

Gill et al (1994, 1995) treated the in-vitro plantlets with fungicide (0.1-0.3 % Bavistin) solution for 5 min to prevent fungal and bacterial contamination during hardening. It has been reported that in certain plants, in-vitro propagation is severely handicapped by a morphological and physiological disease, called vitrification. In general, there has been no satisfactory way of avoiding vitrification, although in a number of cases, it has been prevented or decreased by increasing the agar concentrations in the medium (Debergh 1983; Kevers et al 1984; Gaspar et al 1987; Paques and Boxus 1987; Bottcher et al 1988; Pierik 1988a; Paques 1991; Das et al 2000). However, it has not been reported in Citrus species or its allied genera.

Meghwal et al (2001) standardized a method of quick establishment of aseptic culture in guava from mature field grown stock plants. The maximum 89% of contamination free explants as well as 80 % final aseptic culture established was obtained with combination of surface sterilizing agents involving hydrogen peroxide (10%) for five minutes, silver nitrate (0.25%) for five minutes and mercuric chloride (0.05%) for three minutes treatment of explants one by one.
Al-Khayri and Al-Bahrany (2001) conducted an experiment on surface sterilization techniques of Lime *Citrus aurantifalia* (Christem swing), using nodal explants of mature trees. The shoot branches about 10 cm long, were defoliated and washed with soapy water to remove any dust particles. These were treated for 30 seconds in 70 % Ethanol followed by 15 min shaking in 1 % w/v sodium hypochlorite (20 v/v chlorex) containing 3 drops of tween-20 per 100 ml of water. The twigs were rinsed three-four times in sterile distilled water.

Sudherson et al (2001) established axenic culture by shoot tip, collecting from field grown mature Ber (*Zizyphus mauritiana* Lamk cv. Umran plants). Explants were surface sterilized with 20% commercial Chlorox solution containing 1.05 % sodium hypochlorite and a drop of tween-20 per 100 ml of water for 15 minutes. The shoot tips rinsed in sterile distilled water and were dipped in 0.1 % mercuric chloride solution for 3 min followed by 3-4 rinsing with sterile double distilled water. The plant materials were then dipped in 70 % ethanol for one second and rinsed in sterile double distilled water.

Karwa (2003) reported the improved procedure for surface sterilization of Nagpur mandarin seeds. The mature and bold seeds were collected from rinsed fruit and washed thoroughly under running tap water. The seeds were immersed in 70 % ethyl alcohol for 5 minutes and rinsed with sterile double distilled water. Both seed coats were removed aseptically in laminar air flow hood with minimum damage of the cotyledons. Then these were immersed in 1 % NaOCl solution for 5 minutes followed by four washings with sterile double distilled water.

Mishra and Pandey (2006) carried out an experiment on Pansy to establish contamination free culture and plantlets preparation. The maximum aseptic culture was observed when shoot tips were treated with ethanol (70 %) for 1 minute followed by HgCl₂ (0.1 %) for 6 minutes resulted 80 % aseptic culture with 76.6 % survival of explants on basal MS media.

Syamal et al (2007) conducted an experiment on surface sterilization of nodal segments of field grown Kagzi lime. They found highest 82.51 % of aseptic culture with dipping in NaOCl (1%) for 20 min followed by dipping in HgCl₂ (0.1 %) for 10 min and finely in KCl (1 %) for one minute. Also the highest 72.43 % survival explants was recorded with the treatment of NaOCl (1 %) for 10 minutes, HgCl₂ (0.1 %) for 5 min KCl (1 %) for 1 minute.
Kour et al (2007) developed a protocol for surface sterilization of nodal segment of rough lemon (*Citrus jambhiri* Lush). Explants were collected from 8-10 year old mature tree. Explants were surface sterilized with 70 % ethanol for 30 seconds and 0.1 % mercuric chloride (HgCl$_2$) solution (w/v) for 8 minutes in laminar air flow cabinet and then were rinsed it for 3 minutes to sterile distilled water.

### 2.1.2 Nutrient Medium for *in-vitro* propagation

Virtually all media contain inorganic salts and sucrose as the carbon source typically at concentrations between 1 and 3 %. It has been reported that 5 % sucrose is optimum for culture of tissue and organ of *citrus*. The concentrations of these ingredients in a basal medium depend on the type of a plant being cultured and the stage of culture development (Murashige and Tucker 1969; Rangaswamy 1975; Styer and Chin 1983; Waithaka 1988; Nagao et al 1994). Most of the media have pH value lies Between 5.5-6.2. MS medium has been widely used with appropriate modifications for culturing many *Citrus* species and cultivars for regeneration and proliferation, sub-culturing, and its subsequent rooting (Murashige and Tucker 1969; Murashige 1974; Chaturvedi and Mitra 1975; Gamborg et al 1976; Kochba and Spiegel Roy 1997; Altman and Goren 1987; Raj Bhansali and Arya 1978a; Styer and Chin 1983; Yong NB 1983; Grosser & Chandler 1986; Nel 1987). The based MS medium contains a relatively high level of nitrogen in the form of ammonium and nitrate ions in addition to high concentration of calcium than the other media. The most commonly included vitamins are thiamine, inositol, nicotinic acid and pyridoxine. Thiamine is critical, and it is usually provided in the range 0.1 - 0.4 mg l$^{-1}$. Inositol is not essential; nevertheless, it has been clearly beneficial and has been used at the rate of 100 mg l$^{-1}$ (Murashige 1974).

### 2.1.3 Plant Growth Regulators for *in-vitro* propagation

Murashige (1974) mentioned that, the auxin and cytokinin were critical organic components of plant propagation media. A relatively high concentration of auxin favoured root initiation and depressed shoot formation. In contrast relatively high concentration of cytokinins induced shoot initiation and suppressed rooting. The control of root and shoot initiation by auxin and cytokinin balance appeared to be a general phenomenon among plants. However type and concentration of the growth regulator in the nutrient medium may vary according to *citrus* species, explants age, size of explants and type of response required for culturing.
Grinblat (1972) reported maximum bud initiation at 10 mg l\(^{-1}\) benzyl adenine (BA), 0.1 mg l\(^{-1}\) naphthalic acetic acid (NAA) and 500 mg l\(^{-1}\) mait extract. The result indicated that the BAP/NAA ratio at the concentration used stimulated bud initiation and inhibited their development contrary to this findings, Chaturvedi and Mitra (1974) reported that developed leafy shoots inhibited neither the development of other shoot buds nor the initiation of new shoot buds and produced maximum number of shoot buds in a treatment contained a 0.25 mg l\(^{-1}\) BAP with 0.1 mg l\(^{-1}\) NAA. Similarly, Chaturvedi and Mitra (1975) reported that BAP was highly effective in inducing shoot bud formation but suppressed rooting in *C. grandis* (L.) Osbeck. Roots were induced on a medium which contained a high level of auxin like NAA and IBA the later species, *C. microcarpa* Bunge was equally amenable for clonal propagation due to its shoot apices (0.5 mm or Longer), internodal segments and excised leaves (both 1-3 mm Long) which exhibited capacity for organogenesis *in-vitro* (Rangaswamy 1975; Song et al 1991).

It has been reported that adventitious bud explants of sweet orange. Shamouti developed multiple shoots with proliferation and was influenced by genotype, explants source, cytokinin, sucrose level and light intensity (Bouzid 1975). Altman and Goren (1987) reported multiple shoots with BAP (10\(^{-5}\) urn), at the higher levels. Regeneration was slower but continued for a longer period. Among the other cytokinin, kinetin, adenine (Ad) and adenine sulphate (Ads) was ineffective to induce shoot bud formation and the effect of the 2, 4-D on organogenesis was inhibitory. However, regeneration and formation of new buds was controlled by the action of different growth hormones added in the culture medium (Raj-Bhansali and Arya 1978a, 1978b; Bouzid and Belcouna 1987). Roots were formed in 2, 4-D free medium with higher level of NAA (2.5 ppm) (Raj-Bhansali and Arya 1978c). Although the organogenesis was observed in the absence of auxin, the length of shoots and leaves was reduced (Raj-Bhansali and Arya 1979).

Kitto and Young (1981) reported that the degree of proliferation in Carrizo citrange was proportional to BAP concentration and NAA enhanced rooting (80 %) as compared to IBA or IAA whereas Barlass and Skene (1982) reported 10 % rooting on IBA (5 µm), 25 % (1 root per shoot) at NAA (0.5 urn) and 80 % rooting at NAA (5 um). It was noted that 'Carrizo' citrange had regeneration capacity much higher than close relatives of its parents. Sauton et al (1982) reported that root growth of sweet orange cv. 'Trovita' was relatively difficult and MT + Adenine sulphate (25 mg l\(^{-1}\)) + GA\(_3\) (1.0 mg l\(^{-1}\)) was only medium which ensured a noticeable growth. Pontikis and Sapoutzaki (1984) reported that shoot proliferation in Troyer citrange
significantly increased with phloro-glucinol. Edriss and Burger (1984a) was able to induce roots from regenerated shoots of Troyer' citrangeon NAA (2.0 mg l⁻¹) alone but diminished the gradient of bud forming potential in sweet orange or Valencia (Burger and Hacket 1986). Similarly, Moore (1986) found inhibitory effect of NAA in the medium to shoot production in long term cultures.

The inability of explants to induce root and shoot formation on a single media limits the efficiency and cost effectiveness of *in-vitro* multiplication of *citrus* root stocks. Working on this line, Grosser and Chandler (1986) recovered whole plants from 'Swingle' citrulmeo epicotyl segments (0.5 cm) on coumarin (90-150 µm). Similarly, Tusa and Geraci (1988) produced vigorous shoots of *Sacatori citrulmeo* Troyer' citrange and sour orange at low levels of coumarin but at this level (10 and 15 urn), no explants of any root stock produced roots.

Rogers et al (1990) Siiltured Sour orange (*Citrus aurentium*) shoot tips and Lime (*Citrus aurenifolia*) shoot tips (1-1.5 mm long) excised from *in-vitro* generated seedlings on semi solid MS medium gelled with 0.2 % Gelrite without growth factors. After 8-10 weeks, shoots and leaves developed in 60 % of the sour orange explants, and in 77 % of the swingle explants. Some of the plantlets rooted after 11-12 weeks and were established in soil.

Usually an excessively high cytokinin level in the culture medium caused strong branching habit when transferred to soil. Therefore, Pierik (1988a) advocated low level or without cytokinin media for the rooting *in-vitro* but root growth and shoot elongation were inhibited in media with 22.2 or 44.4 µm BAP (Bhat et al 1992). Parthasarathy and Nagaraju (1993b) reported that BAP at 0.75 mg l⁻¹ alone induced more number of shoots of good size which has rooted in media contained 0.05 mg l⁻¹ NAA or media without auxin. It hasten indicated that combination of two or more cytokinin was generally more effective than single cytokinin in producing shoot for micro propagation (Bowman 1994; Baruah et al 1995). Similarly, multiple shoots were obtained on a combination of BAP, Kinetin and NAA in different *citrus* species and a combination of NAA, IBA and BAP was found to be essential for root development (Singh et al 1994). It has been noted that plantlet survival *in-vitro* was directly proportional to the number of roots produced per shoot and larger sized shoot showed higher rooting %age and took less time for rooting (Gill et al 1994, 1995; Baruah et al 1996b).

Moore (1986) cultured inter-nodal seedling stem sections of three *citrus* rootstocks using sour orange (*Citrus aurentium*), 'Carrizo' citrange (*C. sinensis* Osb. X *Poncirus trifoliate*
Raf.) and 'Cleopatra' mandarin (Citrus reshni Hort. ex Tanaka) on Murashige and Tucker (1969) medium with 5% sucrose and 0.8% agar and various combinations of benzyl adenine (BAP) and naphthalene acetic acid (NAA). In all cases, 'Carrizo' citrange was much more responsive to in-vitro culture than 'Cleopatra' mandarin and sour orange. The NAA in medium was inhibitory to shoot production in long term cultures.

Baruah et al (1995) conducted an experiment on effect of cytokinin on shoot proliferation in pummalo (Citrus paradisi) from in-vitro grown seedlings with two cytokinins namely 6-Benzyl amino purine (BAP) and 6 furfuryal amino purine (kinetin) in MS medium. There were variations between BAP and kinetin for shoot proliferation, while kinetin improved the size of the plantlets. It is also concluded that 0.75mg BAP produced maximum number of shoot.

Baruah et al (1996a) were successful in in-vitro rooting and simultaneous acclimatization of micro-shoots of C. assamensis, C. Latipes and C. indica proliferated in-vitro in soil rite. The rooted plantlets were transferred to green house in polythene bags containing soil. The plantlets well acclimatized availing lengthy and cumbersome process of hardening (Rooting % and time. Jafeen for rooting was dependent on size of the shoot placed for rooting. Large sized shoot showed higher rooting % and took less time for rooting).

Parthasarathy and Nagaraju (1996a) studies were conducted on four Citrus species viz., Mandarin (Citrus reticulata Blanco cv. Khasi Mandarin), Calamondrin (C. madurensis Lour), Sweet Orange (C. sinensis Osbeck cv. Musambi) and Lemon (C. limon Burmann cv. Assam lemon) to ascertain the optimum concentration of NAA required for rooting of micro-cuttings. Results revealed that NAA at 0.5 mg 1⁻¹ supplemented in the MS medium was best for all citrus spices. However, the best concentration for Musambi was 0.2 mg 1⁻¹ NAA. Duranvila et al (1989) reported that response of micro cuttings to NAA, to induce rooting at Citrus sinensis cultivars were 10 mg/l while for C. aurentifolia it was only 1.0 mg 1⁻¹ and NAA above 50 mg 1⁻¹ was toxic to Citrus.

Parthasarathy and Nagaraju (1996b) reported that culture without BAP produced single shoot with more number of leaves and thin roots, where as BAP in the medium completely inhibited the rooting. However, BAP (up to 0.75 mg 1⁻¹) accelerated the proliferation but at higher concentrations both the culture growth and the shoot number were negatively affected (Parthasarathy and Nagaraju 1996c). Similarly, Rahaman et al (1996)
concluded that optimum level of cytokinin for plantlet growth and proliferation varied between 0.25-0.75 mg l⁻¹ and kinetin was shown to have better effect for most of the characters and indicated the superiority of kinetin to BAP. Thirumalai and Thamburaj (1997) reported that GA₃ along with BAP in the culture medium favoured the production of multiple shoots. But BAP responded slightly better in the absent of GA₃. Mishra et al (1999) reported that micro propagated citrus species exhibited better growth over seedling plantlets in terms of plant height, stem girth, total canopy, number of branches and number of leaves. Pattnaik and Chand (1997) developed on protocol for rapid multiplication of mulberries. High frequency bud break and multiple shoots were induced in apical shoot buds and nodal explants of Mows cathayana, M. Lhou and M. serrata in murshige and skoog medium containing 0.5-1.0 mg l⁻¹ BAP. Addition of Gibberellic acid (0.4 mg l⁻¹) along with BAP induced faster bud break both in apical shoot buds and nodal explants and also enhanced the frequency of bud break in all three species. The shoot initiation was greatly influenced by explant type age and season. The shoots were successfully rooted on half strength MS medium containing a combination of Indole-3-acitic acid, Indole-3-butyric acid and Indole-3- propionic acid, each at 1.0 mg l⁻¹.


Karwa (2003) developed a protocol for in-vitro propagation of Nagpur mandarin through in-vitro generated shoot taken as explant. The explants were inoculated on MS medium supplemented with different combinations of 6-Benzyl ammopurine (2.22 µm - 8.88 µm), kinetin (2.32µm - 4.65µm) and alfa-Naphthaline acetic acid (1.34µm - 5.37µm). The study demonstrated that MS medium fortified with BAP (8.88 µm), NAA (2.69 µm) and kinetin (2.32 µm) recorded maximum shoot induction (80%) as compared to other hormonal combinations and maximum number of multiple shoot (16.8 + 0.96 per explant) were also regenerated in the same treatment combination. However, IBA (4.92µm) + BAP (1.11µm) induced maximum (78%) rooting with an average of 5.8 ± 0.86 root per micro shoot.

Begum et al (2004) conducted an experiment on axillary shoot proliferation by using nodal segment of pummelo. A large number of shoot buds were produced when such four weeks old culture were sub-cultured on half strength of MS medium containing 1.0 mg l⁻¹ BAP.
Thakur et al. (2004) conducted an experiment for in-vitro multiplication of 'Kozak' and 'Gold Disk' cultivars of gerbera (Gerbera jamesonii) using shoot tips as explants. Among the different concentrations of PGRs used, the maximum establishment was observed in MS media supplemented with 2.0 and 2.5 mg l⁻¹ BAP in 'Kozak' and 'Gold Disk' cultivars respectively. Maximum number of multiple shoots was observed in the treatment consisting of MS basal medium modified with 1.0 and 1.5 mg l⁻¹ BAP in 'Kozak' and 'Gold Disk' cultivars respectively. In both cultivars, maximum rooting was observed in a medium supplemented with 0.4 mg l⁻¹ of IAA.

Mehatre et al. (2004) attempted to study the technique of in-vitro multiplication of Sarpagendra using axillary buds as explants. The maximum establishment was observed in MS medium supplemented with NAA (0.5 mg l⁻¹) with kinetin (0.1 mg l⁻¹). However, the highest multiple shoots were produced on MS basal medium fortified with BAP (2.0 mg l⁻¹) with NAA (3.0 mg l⁻¹). The initiation of roots to the multiple shoots was observed on 1/2 strength MS medium enriched with NAA 1.0 mg l⁻¹.

Kanjilal (2006) developed a protocol for rapid mass propagation of Rangpur lime (Citrus limonia Osbeak) using nodal segments from in-vitro raised seedlings used as explants. The stem segments of one year old in-vitro raised seedlings were cultured in Murashige and Skoog medium supplemented with various combinations of 6-benzyl amino purine (BAP) and naphthalene acetic acid (NAA). The best result for multiple shoot regeneration was obtained when MS media was modified with (1.0 mg l⁻¹) BAP with 0.5 mg l⁻¹ NAA. An average of 5-7 shoots were regenerated from each explant with the 1.0 mg l⁻¹ BAP and 0.5 mg l⁻¹ NAA was added in MS medium. The elongated shoots were cultured in liquid MS medium supplemented with various concentrations of NAA for rooting. The maximum number of roots (3-4 per micro shoot) were recorded when 0.2 mg l⁻¹ NAA was added with MS medium. Well rooted shoots were hardened and exhibited normal growth under greenhouse and field conditions.

Vashist et al. (2006) carried out a study to develop a protocol for micro propagation in Gurmar (Gymnema sylvestre R. Br). The shoot tips and axillary buds were used as explants. The highest shoot proliferation 90% was observed on MS medium containing BAP 5.0 mg l⁻¹, NAA 0.2 mg l⁻¹ and AgNO₃ 10 µl in shoot tip explants. The shoot tip explants showed maximum 90% shoot regeneration. The highest multiple shoot formation response 18.37 was recorded on MS medium containing BAP 5.0 mg l⁻¹ while 26.6% explants showed multiple shoot formation in media supplemented with AgNO₃. The callus formation was observed in
axillary bud explants, the best response for callus formation from axillary bud explants was found to be on medium supplemented with BAP 5.0 mg l\(^{-1}\), NAA 2.0 mg l\(^{-1}\) TDZ 4.9 mg l\(^{-1}\) producing 77.5 % embryogenic calli. 20% of proliferated shoots showed root formation in MS medium supplemented with NAA (0.1 mg l\(^{-1}\)).

Kour et al (2007) conduct an experiment on in-vitro multiplication of Rough lemon (Citrus jambhiri Lush). They concluded that BAP (1.5 mg l\(^{-1}\)) + malt extract (500 mg l\(^{-1}\)) + NAA (0.25 mg l\(^{-1}\)) gave maximum shoot proliferation, shoot number and length of shoot.

Syamal et al (2007) conducted an experiment on Kagzi lime using nodal segment as explants. These explants were inoculated in MS medium supplemented with various growth regulator via BAP (0.5, 1.0, 2.0, 3.0 mg l\(^{-1}\)) NAA (0.1, 0.2 mg l\(^{-1}\)) and GA\(_3\) (0.1, 0.2 mg l\(^{-1}\)) in all possible combinations. The treatment with BAP 2.0 mg l\(^{-1}\)+ NAA 0.10 mg l\(^{-1}\)+ GA\(_3\) 0.10 mg l\(^{-1}\) gave the maximum shoot tip sprouting, highest number of shoots, length of shoot and leaves per explants.

De-Souza et al (2007) conducted an experiment on micro-propagation of arnica (Lychnophora pinaster). It is a medicinal plant of the Cerrado ecosystem in Brazil. It is widely used in the form of alcoholic extract for its anti-inflammatory and anesthetic and healing effects on sprains, bruises, and inflammation. The best medium for germination of arnica embryos and plantlet growth was quarter strength semisolid Murashige and Skoog medium containing 0.75% (W/V) sucrose. For shoot induction, the best results were obtained on MS with 2.76 μm of 6-benzyl amino purine. Maximum shoot elongation before rooting occurred in the presence of 8.67 μm Gibberellic acid for 19 days. Micro shoots were successfully rooted in the presence of 10.7 μm of naphthalene acetic acid for 15 days.

Rosal et al (2007) done a work on establish appropriate condition's for the in-vitro micropropagation of Eremanthus erythropappus (DC) Macleish through shoot multiplication on apical and nodal bud explants were excised from in-vitro grown seedlings and inoculated on Murashige and Skoog medium containing different combination of 6-benzyl amino purine (BAP) and naphthalene acetic acid (NAA) for apical buds. Proliferation of apical shoots was successfully achieved in the presence of BAP and NAA, each at 1.0 mg l\(^{-1}\), while the elongation of apical shoots could only be attained\(^5\) on medium containing NAA at 1.0 mg l\(^{-1}\). Elongation of nodal shoots was induced in the presence of NAA at 2.0 mg l\(^{-1}\). The MS medium forted by 1.0 mg l\(^{-1}\) NAA was best for root production in E. erythropappus.
Devi and Sarma (2007) developed a procedure for micro-propagation in Brahmi by culturing leaf explants in Murashige and Skoog medium. Nodal segments of Brahmi were first cultured on hormone free medium for five days. Then leaves from disease free shoots were used as explants in different combinations of PGRs in MS medium. PGRs used were kinetin and NAA, and also kinetin, NAA and IBA in combination. Better results in terms of plantlet production and time required for generation were observed in MS medium with 0.1µg/ml Kinetin plus 0.5µg/ml NAA. Addition of IBA exhibited variation in results and best results were obtained in 0.1 µg/ml of kinetin, 0.5 µg/ml of NAA and 1.0 µg/ml of IBA.

Ram Niwas et al (2007) conducted a study on elite genotype of spineless Cactus pear. The effect of different levels of BA (0.0, 3.0, 6.0 and 9.0 mg l⁻¹) and NAA (0.0, 0.1 mg l⁻¹) alone or in combination were evaluated for in-vitro culture establishment, axillary bud breaking, micro-shoot formation and root initiation. The early axillary bud breaking was induced with 9.0 mg l⁻¹ BA along with 0.1 mg l⁻¹ NAA in 19.47 days, whereas all NAA concentrations failed to induce bud breaking in the explants. However, root initiation was noticed with NAA 0.1and1.0mg l⁻¹. The maximum 80.0% culture establishment was recorded in MS medium modified with 6.0 mg l⁻¹ BA + 0.1 mg l⁻¹ NAA as compared to minimum 26.67 % at 3.0 mg l⁻¹ BA and 1.0 mg l⁻¹ NAA. All treatments of BA alone and in combination with NAA found to induce micro-shoots in single bud areole of cladode explants. The proliferated micro-shoots were subjected to root formation with different concentrations of NAA (0.0, 2.0, 4.0 and 5.0 mg l⁻¹) and IBA (2.0, 4.0 and 6.0 mg l⁻¹). The maximum number of roots 6.0 and length of roots3.80 cm was recorded with 6.0 mg l⁻¹ IBA.

Singh et al. (2007a) carried out an experiment on Ker to influence survival factors such as season (autumn and spring), explants source (fruit bearing mature tree pruned or un-pruned) BA concentrations (0.0, 1.0, 2.0 and 4.0 mg l⁻¹) and IBA levels (0.0, 0.1 and 1.0 mg l⁻¹) with MS basal medium were evaluated for in-vitro culture establishment and multiple shoot formation. The autumn season was found better as compared to spring season. The influence of season, explants source, BAP and IAA levels and their interactions on the number of days to bud breaking in the nodal explants was found to be significant. Early bud breaking was observed with explants taken from pruned stock plant in autumn season. The mean maximum rate of shoot proliferation 6.26 per explants was recorded at BA 2.0 mg l⁻¹ + IAA 0.1 mg l⁻¹. Proliferated shoot clumps were subjected for elongation to get rootable size of micro-shoots. Elongated micro-shoots 1.5-3.0 cm length were excised and transferred to rooting media.
containing $V_z$ strength MS medium, supplemented with 2.0 % sucrose 0.8 % and fortified by auxin mixtures of IBA and NAA at 0, 0.5, 1.0 or 2.0 mg l$^{-1}$). The rooting %age in micro-shoots were significantly influenced by different levels of auxin mixture and maximum %age of micro-shoots with root was recorded with 0.5 mg l$^{-1}$ IBA + 0.5 mg l$^{-1}$ NAA auxin mixture either alone or together significantly affected the rooting of micro-shoots. The maximum rooting 80 % and better quality of root 5.1 cm and 3.60 roots per micro-shoots were recorded with same treatment combination.

Singh et al. (2007b) conducted an experiment on rapid colonel propagation of mulberry. The nodal segment having 1-2 nodes were taken as explants. The medium composition used for micro-shoot formation was MS medium supplemented with 30 g l$^{-1}$ sucrose 8 mg l$^{-1}$ agar and different levels of BA (0.0, 3.0, 6.0 and 9.0 mg l$^{-1}$) alone or in combination of NAA (0.0, 0.1 and 1.0 mg l$^{-1}$). In-vitro morphogenesis in terms of micro-shoot formation was remarkably influenced by different media composition. The MS medium containing 6.0 mg l$^{-1}$ BA was best treatment for shoot induction. Adventitious root formation in micro-shoot or directly on to nodal explants were observed in half strength MS medium supplemented by different levels of IBA, NAA and 2,4-D (0.0, 0.5, 1.0 and 2.0 mg l$^{-1}$). Better adventitious root formation was observed in all concentration of IBA. Higher concentration of IBA resulted in thick and short root 3.0 cm whereas low concentration (0.5 mg l$^{-1}$) produced longest roots 5.0 cm.

Maas EV et al 1993 investigated that Soil salinity significantly limits citrus production in many areas worldwide. Although data on fruit yields in response to salinity are limited they indicate that grapefruit, lemons, and oranges are among the most sensitive of all agricultural crops. Fruit yields decrease about 13% for each 1.0 dS m $^{-1}$ increase in electrical conductivity of the saturated-soil extract (EC (e)) once soil salinity exceeds a threshold EC (e) of 1.4 dS m $^{-1}$). Accumulation of excess Cl (-) and Na (+) can cause specific ion toxicities but this problem can be minimized by selecting rootstocks that restrict the uptake of these ions. This these research reports and discusses differences in the role of different rootstocks, the causes of salt injury and the interactions of other environmental conditions or stresses with salinity.

Despite the great economic importance of citrus, its phylogeny and taxonomy remain a matter of controversy. Moreover pathogens of increased virulence and dramatic environmental changes are currently spreading or emerging to measuring genetic variability and studying its pattern of distribution, were crucial steps to optimize sampling strategies in the search of
genotypes that tolerate or resist these threatening factors within the huge array of Citrus and Citrus related species. Their intraspecific and intra-generic variability was studied comparatively by means of ten enzymatic systems using eight different measures. The analysis of ten enzymatic systems allowed us to distinguish all the species and all but one artificial hybrid. The species with the lowest genotypic variability are *C. myrtifolia*, *C. deliciosa* (willow leaf mandarin), *C. paradisi* (grapefruit), *C. limon* (lemon) and *C. sinensis* (sweet orange), while *Severinia buxifolia* shows the highest value. A broad spectrum of heterozygosity values was found in the collection. Lemons (*C. limon, C. meyeri, C. karna, C. volkameriana*), limes (*C. aurantifolia, C. limettioides, C. lattifolia*) and *C. bergamia* show a very high percentage of heterozygosity which indicates an origin through interspecific hybridization. (Herrero et al 1996)

Guo LQ et al 2000 reported that with juices of grapefruit and related fruits, possible relationships between contents of six different furanocoumarins and extents of inhibition of microsomal CYP3A activity *in vitro*. Microsomal CYP3A-mediated testosterone 6beta-hydroxylation was inhibited by the addition of a fruit juice (2.5%, v/v) from eight different grapefruit sources, two sweeties, three pomelos, and one sour orange, whereas no clear inhibition was observed with two sweet orange juices. The inhibitory component in grapefruit juice resides mainly in the precipitate rather than in the supernatant after centrifugation.

High levels of MS vitamins enhanced callus initiation and growth. These calli could be maintained in culture for up to two years and plantlets regenerated from them on transfer to light. Greening of callus and regeneration of shoot-buds from callus occurred on transfer to MS media with BAP. These shoots grew further in media with both, BAP and GA3. Rooting of shoots occurred on media with NAA or IBA and the rooted plantlets could be transferred to soil. Regeneration of shoots and roots decreased with increase in age of callus. (Chakravarty B et al 1999).

Kirbalsar G et al 2009 reported that the samples of the Citrus fruits viz., lemon (*Citrus limon* (L.) Burm. f.), grapefruit (*Citrus paradisi* Macfayden), bergamot (*Citrus bergamia* RissoetPoit.), bitter orange (*Citrus aurantium* L.), sweet orange (*Citrus sinensis* (L.) Osbeck), Mandarin (*Citrus reticulata* Blanco) was collected from southern Turkey (Antalya) in November 2006 and their peel oils were obtained by cold pressing process. The antimicrobial activities of Turkish Citrus peel oils were evaluated using the disk diffusion method toward 9
bacteria and the results compared with those for penicillin-g, ampicillin, cefotaxime, 
vancomycin, oflaxacin and tetracycline. Antifungal activities were reported for 
*Kluyveromyces fragilis, Rhodotorularubra, Candida albicans, Hansenia sporaguilliermondii*
 and *Debaryomyces hansenii* yeasts, and the results were referenced against nystatin, 
ketaconazole and clotrimazole antifungal agents. The Citrus peel oils showed strong 
antimicrobial activity against the test organisms. Lemon and bergamot peel oils have a little 
higher activity than the other citrus peel oils.

Sharma MC & Sharma 2010 conducted a study on antimicrobial properties of the combined 
essential oil of *Citrus paradisi* var. star ruby and *Ficus carica* extracts. The *Citrus paradisi* 
and *Ficus carica* were tested against pathogenic microorganisms; *S. aureus, E. coli, K.* 
*pneumoniae, B. subtilis, M. luteus and Candida albicans*. The extracts tested exhibited good 
antimicrobial activity against all the clinical isolates when compared with standard. The 
different extracts showed remarkable inhibitory action against various Gram positive and 
Gram negative bacteria and two fungal species. The methanolic, petroleum ether, chloroform, 
ethyl ether, ethanol extract *Citrus paradisi* and *Ficus carica* was screened for its 
antimicrobial activity. Antimicrobial activity was detected by observing the growth response 
of different organisms to the methanolic extract. It was generally based on the inhibition of 
growth of microorganisms which were measured with a desired concentration of the plant 
extract of *Citrus paradisi* to be examined with the standard concentration preparation.