REVIEW OF LITERATURE

Plant tissue culture technique which paved the way for the development of modern biotechnology has become a powerful tool for studying and solving various problems of plant biotechnology. As our traditional wealth on plant genetic resources has been decreasing tremendously, these techniques have gained greater momentum on commercial application in the field of plant propagation. A considerable account of in vitro studies had been undertaken both nationally and internationally in agricultural and horticultural fields. Now-a-days the method for plant micropropagation has been well developed (Paranjothy et al., 1990; Machey et al., 1995).

Rapid clonal propagation is possible through bud or shoot proliferation (Pierik, 1990), induction of bulbs or corms (Ziv, 1990), or somatic embryogenesis (Ammirato, 1989). Using culture techniques it has been possible to regenerate propagules with better qualities, greater vigor, higher yield and disease resistance. A number of physiological and morphological changes have been reported in unorganized callus tissue including habituation, changes in biochemical sensitivity and requirements, alteration of growth habit and modification of cellular constituents. Only certain cells are capable of normal embryo development (Barba and Nitchell, 1969). Somatic embryogenesis has tremendous potential for large scale production of plant material.

A general phenomenon of tissue culture is the ability to produce embryoids, organs, tissues etc. Murashige (1974) described plant regeneration accomplished from explants like leaves, stem, cotyledons, microsporocytes and shoot tips. According to Gamborg et al. (1974), most important determinant of plant multiplication and quality of regenerated plants is the initial explant used.
Micropropagated plants may or may not show variations from the parent plant. Variations in plant tissue culture also include changes in growth habit, rates, appearance and requirements. Callus cultures of *Picea glauca* (Reinert, 1956), pea (Torrey and Shigemura, 1957), tobacco (Sievert and Hildebrandt, 1965), carrot (Blakely and Steward, 1964; Muir, 1965) and wheat (Nakai and Shimada, 1975) *etc.* are some of the reports on differences in growth habit with some clones. Nickell and Maretzki (1972) and Nickell (1973) reported the difference in growth rate of yellow and white clones of sugar cane.

Intact plants isolated from tissue culture have been reported to be variant in many cases. Ibrahim (1969) obtained variants in carrot after high kinetin treatments *in vitro*. Altered phenotype in *Digitalis purpurea* plants regenerated from kinetin containing media was reported by Corduan and Spix (1974).

Geranium (*Pelargonium hortorum*) plants derived from stem and anther culture differ in variegation, number of petals and stamens and phyllotaxy (Abo El-Nil and Hildebrandt, 1972). Clonal plants obtained from callus culture of *Coptis* were reported to produce more jatrrhine than the parent plants (Ikuta *et al*., 1975).

Molecular genetics can be used as a tool for crop improvement. For the application of molecular genetics, plant tissue culture is one of the essentials. Regeneration may be achieved through organogenesis or somatic embryogenesis and progress has been made in defining the conditions required as well as the physiological and biochemical changes accompanying these types of developments (Ammirato, 1987; Christianson, 1987; Thorpe, 1990).

The suitability of sucrose as a carbon source for uptake and utilization of plants was analysed by Khuri and Moorby (1995). There are reports that during subculturing, the number of variant plantlets increases (Ziv *et al*., 1983; Leshem *et al*., 1998; Safrazbekyam *et al*., 1990).
The effect of growth hormones cannot be generalized because different plants behave differently and may have different requirements. Cytokinins are found to be very effective for both direct and indirect shoot bud initiation. BA is the most commonly used cytokinin (Moreno et al., 1985; Bonabdallah and Branchard, 1986; Misra and Bhatnagar, 1995). Endogenous cytokinin is known to occur in plant tissue culture and exhibit a change in concentration during cell growth (Mackenzie and Street, 1972; Ernst et al., 1984). Somaclonal variation for both quantitative and qualitative characters had been reported in many species of higher plants (Bajaj, 1990). Plant cells cultured in vitro produced wide range of primary and secondary metabolites of economic importance. Partially differentiated callus cultures of Ruta graveolens, chamomile, Coriandrum and peppermint synthesized flavor compounds. Callus cultures of saffron produced flavor and pigments (Ravishankar and Venkataraman, 1990).

Plant hormones at different combinations affect the growth of plants differently (Rojina, 1991). Agarwal et al. (1987) successfully developed callus using 2, 4-D. Addition of NAA and KIN to IAA containing medium resulted in initiation of organogenesis and formation of semi-differentiated callus.

Several reports indicate that plants regenerated from callus or suspension cultures may show genetic changes like phenotypic variability (Nishi et al., 1968; Williams and Collins, 1976), chromosomal rearrangements (Cummings et al., 1976), polyploidy or aneuploid plants (Murashige and Nakano, 1965; Sacristan and Melchers, 1969). But culture raised through meristem explants successfully regenerate large number of genetically uniform plants (Murashige, 1974) suggesting that significant chromosomal changes occur principally in dedifferentiated callus or suspension cultures (Malnassy and Ellison, 1970).

More variation can be induced in plants raised through florets, than those raised through leaf and stem explants (Khalid et al., 1989).
Suryanarayanan and Pai (1988) reported _in vitro_ propagation of _Coleus forskohlii_ using flowers as explants and were of the opinion that flowers are a better alternative for regeneration from callus. Khalid _et al._ (1989) reported that flowers of _Chrysanthemum_ are an ideal explant for inducing somaclonal variation.

Scanning of literature reveals that several workers had conducted micropropagation studies on the fruit yielding members of Rosaceae. Pereira-Netto (1996), conducted studies on the _in vitro_ propagation of _Hancornia speciosa_ on Murashige and Skoog medium supplemented with growth regulators. _In vitro_ studies were carried out, comparing rooting of almond, apple, plum, _Pyrus pyraster_ and two hybrid rootstocks, after coculture with _Agrobacterium rhizogenes_ strain 1855, with and without the addition of hormones. Studies reveal that infection at the base of microcuttings _in vitro_ can improve the rooting of these fruit species (Damiano and Monticelli, 1998).

_In vitro_ culture and evaluation of development interdependence in fruits, seeds and embryos, _etc._ were made on some fruit and berry yielding members of Rosaceae like, _Ribes, Fragaria, Malus, Prunus_ and _Chaenomeles_ by Rugienius _et al._ (2003). Breeding of _Rosa rugosa_ using different tissue culture methods was done by Jakobsone _et al._ (2006).

Wide spectrum of literature is available on the micropropagation and related aspects of strawberry plants. Successful plant regeneration from leaf explants in strawberry (_Fragaria x ananassa_) has been reported (http). Plant regeneration from leaf explants _i.e._, petioles and leaf blades at various concentrations of 6-benzylaminopurine (BAP) 0; 1.6; 3.2; 6.4 mg/l in Murashige and Skoog (MS) medium were found to be effective.

_In vitro_ culture work of _Fragaria x ananassa_ was attempted by Oda (1989). He studied the effect of light intensity, temperature and CO₂ concentration on photosynthesis and growth of strawberry plantlets cultivated _in vitro_. Mohamed (1990) made a detailed study of the role of
various plant growth regulators in tissue culture induced rejuvenation of strawberry plants. Shoot organogenesis studies in strawberry (*Fragaria x ananassa*) were made by Rashid (1991). The effects of various growth regulators, explant age and size were also studied.

Study on hardening and field survival of micropropagated plants of strawberry (*Fragaria x ananassa*) was made under Punjab conditions (8http). The experimental plant material of cv. Chandler was raised through tissue culture on Murashige and Skoog (MS) nutrient medium using runner tips as explant. The MS medium supplemented with 6-benzyladenine (0.5 mg/l) + indole-3-butyric acid (0.10 mg/l) was used for shoot multiplication. The rooting was obtained on the same basal medium fortified with indole-3-butyric acid (1 mg/l) alone.

Scanning of literature reveals a low cost medium for the *in vitro* propagation of strawberry (*Fragaria x ananassa* cv. Chandler) (9http). Vegetative buds were cultured on Murashige and Skoog (MS) medium supplemented with low cost medium components. The major medium manipulations were made by replacing agar-agar, sucrose and distilled water with tapioca granules, table sugar and tap water, respectively. Maximum *in vitro* multiplication of shoots was obtained on MS medium supplemented with KIN 0.5 mg/l, BAP 1 mg/l and GA3 2.0 mg/l and table sugar in place of sucrose and maximum *in vitro* rooting was induced on one fourth MS medium supplemented with IBA 1 mg/l, charcoal 200 mg/l and table sugar 20 gm/l in place of sucrose. Hundred per cent survival of micropropagated plants was recorded in the field. Plants grown through cost effective micropropagated protocol showed good field adaptability.

*In vitro* studies were conducted to evaluate the performance of somaclonal strawberry (*Fragaria x ananassa* cv. Brighton) variants for susceptibility to *Phytophthora cactorum* (Battistini and Rosati, 1991). Experimental studies were conducted to evaluate the micropropagated seedlings of strawberry (*Fragaria spp.*) for salt tolerance by Esensee et al.
The different aspects and conditions of tissue culture regeneration of strawberry (Fragaria vesca) plants from leaf explants were reported by Greene and Davis (1991). Genetic transformation studies in cultivated strawberry, Fragaria x ananassa was conducted by Jelenkovic et al. (1991).

Studies on the resistant lines of strawberry (Fragaria x ananassa) to the fungal wilt disease caused by Fusarium oxysporum f. sp. fragariae were made by Toyoda et al. (1991). These strawberry plants were regenerated from leaf derived callus tissues. The study demonstrated the existence of somaclonal variation for disease resistance against a soil borne fungal pathogen.

In vitro culture of Fragaria x ananassa was performed along with various other plants by Riquelme et al. (1991). They also studied the preconditioning effects and acclimatization of these micropropagated plantlets in greenhouse conditions.

Photosynthesis and growth of in vitro cultured strawberry plantlets (Fragaria x ananassa cv. Kent) were investigated during a 4 week in vitro culture in a rooting medium and a 4 week ex vitro period by Yue et al. (1993). The leaves formed in vitro on a medium containing sucrose developed a positive photosynthetic capacity. Shoot tip culture studies of Fragaria x ananassa was done by Hider and Desjardins (1994). They also studied changes in ribulose-1, 5-bisphosphate carboxylase/oxygenase and phosphoenolpyruvate carboxylase activities and $^{14}$CO$_2$ fixation during the rooting of shoots in vitro.

Production of anthocyanins from strawberry cell suspension cultures was investigated by Mori and Sakurai (1994). The effect of controlling sugar concentration and ratio of ammonium : nitrate in culture medium of strawberry, Fragaria ananassa cv Shikinari was also analysed. Short term studies of $^{15}$NO-3 and $^{15}$NH4+ uptake by micropropagated strawberry (Fragaria x ananassa) shoots cultured with or without CO$_2$ enrichment was made by Hider et al. (1994).
Micropropagation studies of strawberry (Fragaria x ananassa) plantlets were made by Hudier and Desjardins (1995). They also studied the reduction of ribulose-1, 5-bisphosphate carboxylase/oxygenase efficiency by the presence of sucrose during the tissue culture of these plants.

Studies were conducted by Yang et al. (1995) on the micropropagation of Fragaria x ananassa. The effect of tonic composition and strength of culture medium on the photoautotrophic growth, transpiration and net photosynthetic rates of the strawberry plantlets were also analyzed in vitro.

Studies conducted by Motomori et al. (1995) revealed that hairy roots of Fragaria x ananassa cv. Reikou, induced with Agrobacterium rhizogenes, grew well in hormone free Murashige and Skoog (MS) and Gamborg B5 liquid media. Particularly, in MS medium, hairy roots showed maximum growth producing high contents of polyphenols. Polyphenol contents in the intact plant (leaf blade, petiole, calyx, receptacle and root) were also investigated.

A study was conducted by Owen and Miller (1996) to maximize plant regeneration frequencies from cultured anthers of 'Chandler', 'Honeoye', and 'Redchief' strawberries (Fragaria x ananassa). A comparison of auxins (IAA, NAA), cytokinins (BA, BAP, KIN) and carbohydrates (sucrose, glucose, maltose) in MS medium showed that the highest shoot regeneration across cultivars (8%) occurred when using a medium containing 2 mg/l IAA, 1 mg/l BA, and 0.2 M glucose.

The biosynthesis of 6-deoxy-D-fructose has been investigated by Zabetakis and Holder (1996) in order to improve the flavour of cultivated strawberries (Fragaria x ananassa cv. Elsanta). Callus cultures of strawberries have been established. Methylpentoses were found to be the key compounds for the biosynthesis of 6-deoxy-D-fructose.
An efficient and reliable method for shoot regeneration from leaf disks of *Fragaria vesca* has been developed by El Mansouri *et al.* (1996). This protocol has been successfully employed to obtain transformed plants using *Agrobacterium tumefaciens* as gene vector. Murashige and Skoog basal medium supplemented with benzyladenine (4 mg/l) and indole-3-butyric acid (0.25 mg/l) induced maximum percentage of shoot regeneration (98%) and the highest number of shoot colonies per explant (4.6) after 8 weeks of culture. Isolated shoots would elongate and proliferate when the benzyladenine concentration was lowered to 0.5 mg/l.

Experimental work had been conducted by Irkaeva and Matveeva (1997) to find out the response of different strains of strawberry (*Fragaria vesca*) micropropagated plants to varying combinations of cytokinins.

Experimental studies were conducted to estimate the growth of *in vitro* cultured callus of musk strawberry (*Fragaria moschata*) by Infante *et al.* (1998). They have also shown that plant regeneration could be made from leaf disk and petiole derived callus sub cultured for 18 months.

An efficient method of micropropagation leading to an increased percentage survival of explants and reduced phenol induced browning in wild strawberry (*Fragaria indica*) has been developed by Bhatt and Dhar (2000). Nodal segments cultured on Murashige and Skoog medium supplemented with 6-benzyl adenine (4.0 μM) and α-naphthalene acetic acid (0.1 μM) gave the best (94.4%) explant establishment and shoot number (22.3) per explant. Of the cytokinins tested, 6-benzyl adenine was found to be more effective than kinetin and N⁵-(γ,γ dimethylallylamino) purine. Excised shoots rooted on half strength agar gelled medium with 1.0 μM α-naphthalene acetic acid.

*In vitro* studies were conducted to find out the suitability of strawberry (*Fragaria x ananassa*) microplants to field cultivation by Zebrowska *et al.* (2003). The method of *in vitro* selection for increased salt tolerance during seed germination and early growth phase of strawberry seedlings was
proposed by Dziadczyk et al. (2003). Adventitious shoot regeneration studies were conducted on seven commercial strawberry cultivars of *Fragaria x ananassa*, viz. Calypso, Pegasus, Bolero, Tango, Elsanta, Eros and Emily, using a range of explant types (leaf discs, petioles, roots and stipules) and culture conditions by Passey et al. (2003).

The influence of inbreeding on the micropropagation process in the strawberry (*Fragaria x ananassa*) was estimated by Zebrowska (2004). The explants of this plant material were proliferated in two subcultures. The micropropagation has been described by the number and weight of microplants derived from individual explants. The results showed that inbreeding influenced on the parameters of micropropagation. Khan and Spoor (2004) conducted a study on the *in vitro* callus culture from the leaf disc explants in strawberry (*Fragaria x ananassa cv. Tango*). They reported a high percentage of regeneration and established a new protocol for the speedy micro propagation of strawberry.

Agrobacterium mediated leaf disc technique is currently the main strawberry genetic transformation method applied. Tang (2004) established a stable, efficient and renewable system of genetic transformation in strawberry.

Three strawberry (*Fragaria spp.*) cultivars (Clea, Irvine and Paros) useful for South Italy pedoclimatic conditions and several genotypes of wild strawberry, *Fragaria vesca* were tested for plant regeneration, somatic embryogenesis and genetic transformation (http). High percentage of organogenesis and plant regeneration was obtained in strawberry and *Fragaria vesca* by using MS medium supplemented with 1 mg/l IBA and 1 mg/l BAP. Somatic embryogenesis events were observed when the explants were maintained in darkness during callus induction step. A sufficient number of genetic transformation events were obtained either in strawberry or in *Fragaria vesca*. Studies on the morphogenesis in *Fragaria* tissues *in vitro* after biolistic treatment with nitrogen fixing bacteria had been conducted
recently (http). Both somatic embryogenesis and organogenesis occurred in high frequency on modified MS medium.

Studies on the phenotypic stability of in vitro regenerated plants of strawberry (Fragaria x ananassa) had been made by Singh et al. (2004). The effects of various culture conditions on the shoot growth of Fragaria x ananassa were examined by Takayama and Takizawa (2004). To multiply the shoots efficiently, modified MS medium (with the concentrations of NH₄NO₃, KNO₃ and CaCl₂·2H₂O halved) supplemented with 1 mg/l 4PU (N-(2-chloro-4-pyridyl)-N'-phenylurea), 30 gm/l sucrose and 8 gm/l agar was selected as the optimum medium. Studies on the effects of different culture conditions on regeneration of plantlets from leaf explants of strawberry (Fragaria x ananassa cv. Toyonoka) were made by Wu et al. (2004).

The influence of three photoperiods: a) 16/8 (d/n) - control, b) 4/2 (d/n) (4 cycles per 24 h), and c) 22/2 (d/n) on the growth of in vitro cultures of strawberry (Fragaria x ananassa) cultivars ‘Senga Sengana’ and ‘Elsanta’ were investigated by Litwinczuk and Zubel (2005). Kaushal et al. (2006) established a new protocol for the maintenance of callus cultures and plant regeneration in strawberry (Fragaria x ananassa cv. Chandler). Lucyszyn et al. (2006) formulated a new combination of media containing blends of agar/galactomannan for the micropropagation of strawberry (Fragaria x ananassa cv. Pelican).

Micropropagation studies of strawberry (Fragaria x ananassa) plants newly introduced in Bangladesh was made by Sakila et al. (2007). Nodal segments of strawberry gave rise to multiple shoots when cultured on MS medium supplemented with different concentration of BA with KIN or GA. The highest response of shoot multiplication was obtained in MS medium containing 1.5 mg/l BA + 0.5-0.1 mg/l KIN. The regenerated shootlets were rooted on MS basal medium with different concentrations IBA and IAA. Studies on somatic embryogenesis from callus derived out of leaf and nodal segments of strawberry were made by Biswas et al. (2007). The highest
percentage of cultures with somatic embryos was achieved on MS medium supplemented with 1.0 mg/l 2,4-D, 0.5 mg/l BAP and 50% proline.

Experimental works on the somaclonal variation in strawberry (*Fragaria x ananassa*) cultivars is available in literature (http). A combination of BAP (0.50 mg/l) and NAA (0.75 mg/l) was found to be best for induction and multiplication of callus cultures in cv. Chandler and cv. Fern. It was 88.89% in cv. Chandler and 83.55% in cv. Fern. Plant regeneration was obtained only in cv. Chandler in MS medium containing a combination of BAP (2 mg/l), NAA (0.25 mg/l) and kinetin (0.5 mg/l).

Recently studies were conducted to reveal somaclonal variation in plants regenerated by organogenesis from callus culture of strawberry (*Fragaria x ananassa*) (http). Somaclonal variation was induced in strawberry plants regenerated from leaf and petiole derived callus by increasing the intervals for subcultures to 12 weeks, and also by transferring the calli, starting with the first subculture, to media containing combinations of BA (4.4, 13.3 or 22.2 μM) and 2,4-D (4.5 μM). It was suggested that both genotype and type of explant strongly influence the occurrence of somaclonal variation. Several somaclones exhibiting useful variation in plant and fruit characteristics have been identified so far. A variant having a modified (white) colour of flesh for all fruits, induced from petiole derived callus of cv. Gorella, is reported for the first time. Since most of the useful variations affecting plant vigour, fruit yield and runnering ability occurred in strawberry somaclones regenerated from leaf derived callus, it has been suggested that the type of variation in plants regenerated by organogenesis, is related to the type of explant.

All tissue culture systems exhibit some degree of somaclonal variation during culture (Larkin and Scowcroft, 1981). Tissue culture plants are different from the normal plants because of the environmental conditions in the tissue culture container. The water retention capacity (WRC) of the head space in the container is responsible for the different physiological response.
Debergh et al. (1992) showed that by controlling the WRC of head space, the physiology and anatomy of the cultured plants can be improved to resemble normal plants. The rapidly dividing and differentiated cells fail to age and therefore clones obtained by vegetative propagation could be viable for an unlimited period of time (Kazaryan, 1969; Libbert, 1974).

The ordered process of chromosomal replication and division is the basis of growth and development. Any change in the process, irregularities or imperfections lead to variation and evolution. In tissue culture, as the cells are grown in artificial environment, possibilities of change in the genetic level are common. The changes include doubling of basic set of chromosome complement, 2x, 4x, 8x, 16x and so on. This phenomenon had been discussed in detail by Partenen (1963; 1965). A change in chromosome number through breakage almost invariably results from the formation of ring, dicentric or tricentric chromosomes. The occurrence of such chromosomes in tissue and cell culture was common and reported in several species (Torrey, 1959; Mitra et al., 1960; Mitra and Steward, 1961; Norstog et al., 1969). Cells growing in an artificial environment may have many genetic changes such as increased frequencies of single gene mutations, chromosome breakages, transposable element activation, quantitative trait variation and variation of normal DNA methylation patterns (Kaeppler and Phillips, 1993b; Do et al., 1999).

There are many reports on the instability of chromosome behavior during culture (D’Amato, 1952, 1978; Skirvin, 1978; Constantin, 1981). Variation in morphology and chromosome numbers of callus derived plants is a common observation. Changes in the ploidy level can be directly detected by chromosome counting in the cells of the strains or the regenerated plants. Karyotype analysis of metaphase chromosomes was used to determine rearrangements of chromosomes of somaclones (Bhojwany et al., 1986). Chromosomal abnormalities, especially
chromosomal doubling is a common feature associated with tissue culture (Morel, 1971).

Variations occurring in plant genome in response to passage of plant cells through cycles of tissue culture and regenerated plants have been mentioned by Constantin (1981). As per his view the genomic changes usually occur in the highly repetitive fraction of the genome and are limited to a specific subset of these sequences which may be localized at particular chromosomal sites.

Ghosh and Sharma (1979) reported chromosomal variation in Vigna and Pisum cultures. This type of observation was also found in Vicia cultures (Jha and Roy, 1982). Chromosomal variation and frequency of spontaneous mutation associated with in vitro cultures was assessed by Edallo et al. (1981). According to Morel (1971), chromosomal abnormalities, especially chromosome doubling is a common feature associated with tissue culture. Rearrangements involving chromosome breaks at heterochromatin was described by Sacristan (1971) in Crepis capillaris. Three callus lines of Allium fistulosum culture and their regenerated plantlets were observed by Lee and Ono (1999) to elucidate the relationships between chromosomal aberration and morphogenetic potential. Cytological methods have been employed to assess the somaclonal variants within garlic callus culture (Dolezel and Novak, 1985).

Cytological studies have been conducted on several fruit yielding plants of Rosaceae. Various estimates suggests that as many as 30 - 70% of flowering plants are of polyploid origin (Grant, 1971; Goldblatt, 1980). Polyploidy had been reported in several members of Rosaceae. In many genera, different species will have different ploidy levels (multiples of a base number) representing a series of polyploids. The Rosaceae family has been traditionally divided into four subfamilies, grouped by fruit type and also on the basis of basic chromosome numbers.
The Maloideae subfamily is characterized by a distinctive fruit, the pome, and a base chromosome number (x) of 17. Most other members of the family have x = 7, 8, or 9. Subfamily Rosoideae, which contains plants such as roses, strawberries, and raspberries, have x = 7 (rarely 8). Amygdaloideae, best known for cherries, apricots, peaches, plums, and almonds, have x = 8. The fourth traditional subfamily, Spiraeoideae, or bridlewreath subfamily, is heterogeneous and has x = 9 or, in a few genera, x = 15 or 17 (Goldblatt, 1976).

Maloideae are hypothesized to have originated through an ancient polyploidization event as their base chromosome number is x = 17; all other Rosaceae are primarily x = 7, 8, or 9. Two most popular hypotheses for the origin of the Maloideae are: (1) allotetraploidization following an ancient hybridization event between Amygdaloideae (x = 8) and Spiraeoideae (x = 9) ancestors and (2) a polyploidization event (allo or auto) within the Spiraeoideae (http).

The plants in the rosaceous subfamily Maloideae (Malus, Pyrus, Photinia, Chaenomeles, etc.) are believed to have originated from an ancient allopolyploid since they have n = 17 base chromosomes whereas plants in other rosaceous subfamilies have n = 8 or 9 (Rowley, 1993).

A literature survey of chromosome number counts for Rubus species was made by Thompson (1997). Numbers are presented for 387 species, representing about 40 percent of the total number, and including 11 of the 12 subgenera. The basic number is universally 7 and ploidy levels include 2x, 3x, 4x, 5x, 6x, 8x, 9x, 10x, 11x, 12x, 14x, and, questionably, 13x and 18x. In a few species, more than one chromosome number has been reported.

An improved technique for counting chromosomes in the root tips of almond has been developed (http), where the pretreatment was effected with colchicine, fixation in 6 methanol : 3 propionic acid : 2 chloroform, hydrolysis in 1N HCl, followed by staining in acetic acid - orcein. This
technique has been successfully applied to other *Prunus* species including peach and apricot, utilizing either root tips or pollen mother cells.

Lim *et al.* (1998) reported genomic *in situ* hybridization (GISH) and fluorescent *in situ* hybridization (FISH) data for chromosomes of raspberry (*Rubus idaeus* 2n = 2x = 14), blackberry (*Rubus aggregate* subgenus *Eubatus* 2n = 2 - 12x = 14 - 84) and their allopolyploid derivatives used in fruit breeding programmes. GISH analysis of an aneuoctaploid blackberry cv. Aurora (2n = 8x = 58) showed that both whole and translocated raspberry chromosomes were present.

Somatic chromosome numbers and morphology in three species of *Spiraea* collected in China was studied by Oginuma *et al.* (1999). Karyomorphology is consistent between two diploid species, *S. japonica* (2n = 18) and *S. rosthornii* (2n = 18). Somatic chromosome number of 2n = 72 of *S. schneideriana* var. *amphidoxa* collected in high mountainous regions at an altitude of 3,800 m in Sichuan Province is octoploid with x = 9 and was found to be the highest number reported within the genus.

According to Potter *et al.* (2002), the basic chromosome data of the four subfamilies of the family Rosaceae are as follows: Rosoideae (*Rosa, Fragaria, Potentilla*, and *Rubus*; fruit = achene; x = 7, 8 or 9), Prunoideae (*Prunus*; fruit = drupe; x = 8), Spiraeoideae (*Spirea*; fruit = follicle or capsule; x = 9), and Maloideae (*Malus, Pyrus*, and *Cotoneaster*; fruit = pome; x = 17).

Zhao-Yang *et al.* (2002) reported a karyomorphological study on one natural population of each of eight varieties in the *Spiraea japonica* complex. The species *S. japonica* var. *japonica*, *S. japonica* var. *acuta*, *S. japonica* var. *incisa*, *S. japonica* var. *stellaris*, *S. japonica* var. *acuminate*, *S. japonica* var. *ovatifolia* and *S. japonica* var. *glabra* were found to be with 2n = 18 chromosomes. Whereas, *S. japonica* var. *fortunei* possesses 2n = 36.

The localization of ribosomal RNA genes on chromosomes of almond (*Prunus amygdalus*, 2n = 16) was studied with the help of fluorescence *in
situ hybridization by Corredor et al. (2005). Hayirhoglu-Ayaz et al. (2006) conducted karyological analysis of 14 species of *Alchemilla* collected from SW Europe (Spain and French Pyrenees). About 75% of the analyzed species have chromosome numbers $2n = 95 – 136$. A few species have lower chromosome numbers. The chromosome numbers of 13 species are presented for the first time.

Recent phylogenetic analyses of combined sequence data from six nuclear and four chloroplast loci together with the basic chromosome number data provided strong support for the division of Rosaceae into three subfamilies (Potter et al., 2007). These include Dryadoideae (including *Cercocarpus*, *Dryas*, and *Purshia*; $x = 9$), Rosoideae (including *Fragaria*, *Potentilla*, *Rosa*, *Rubus*, and others; $x = 7$), and Spiraeoideae (including *Kerria*, *Spiraea* and others; $x = 8, 9, 15, \text{or } 17$).

Biosystematic studies of *Sorbus meinichii* (Rosaceae) was made by Bolstad and Salvesen (2008). Morphometric and cytological data were used to test the variation within the apomictic hybrid *Sorbus meinichii* from its type locality, in the Moster area, Bomlo in Sunnhordland, Norway. Cytological studies showed *S. meinichii* to be triploid, with $2n = 51$ chromosomes.

Cytological and karyotype studies had been reported on strawberry plants by several earlier workers. Yarnell (1929) revealed the somatic chromosome number of *Fragaria collina*, *F. maxima*, and *F. nilgerrensis*, all belonging to the diploid group with $2n = 14$ somatic chromosomes. Detailed genetic and cytological studies on the various polyploid and aneuploid forms in *Fragaria* had been conducted by Kihara (1930) and also by Yarnell (1931a; 1931b). Karyological and genetic studies of some species of *Fragaria* conducted by Lilienfeld (1933) revealed the chromosome numbers of *F. nipponica* ($n = 7$) and *F. elatior* ($n = 21$). Similar studies conducted by Lilienfeld (1936), revealed that autopolyplidy might have occurred during the evolution of the strawberry, *Fragaria elatior*. 
Fedorova (1934) conducted studies on the polyploid forms shown by all *Fragaria* species. He had found 16 cases of doubling of the chromosome number in sexual cells of hybrids, and 5 cases of doubling in pure species. According to him the higher polyploid series of *Fragaria*, with 14, 28, 42, 56 somatic chromosomes, may arise as a result of allopolyploidy or autopolyploidy.

Cytological studies were conducted by Scott (1951) on a colchicine-induced autotetraploid, *Fragaria vesca* and on a strawberry plant derived from crosses of the autotetraploid *F. vesca* and cultivated strawberry. The hybrid hexaploid plants when crossed with the cultivated octoploids gave seedlings with chromosome numbers of 49, 70, and 77. The 70 chromosome plants (decaploids) are new types which are relatively fertile and some have the high aroma characteristic of *F. vesca*. These plants have 14 chromosomes from *F. vesca* and 56 from the cultivated strawberry.

Reports are also available regarding the cytogenetical and karyological studies in different species of *Fragaria* (Staudt, 1952; 1955). Accessions of the cultivars two octaploid progenitor species, *F. chiloensis* and *F. virginiana*, are valued by strawberry breeders as sources of novel traits, especially pest resistance and abiotic stress tolerance. Because strawberry is a relatively new crop, dating to the 1700s (Darrow, 1966), as few as three introgressive backcrosses can yield selections of cultivar quality.

A natural nonaploid hybrid (2n = 63) from fusion of a reduced *F. vesca* male gamete with an unreduced *F. chiloensis* gamete, and a partially fertile natural hexaploid hybrid (2n = 42) from fusion of an unreduced *F. vesca* (2n = 14) male gamete with a reduced *F. chiloensis* (2n = 56) gamete were discovered in separate mixed colonies along with over 20 additional pentaploid (2n = 35) hybrids (Bringhurst and Senanayake, 1966). This justifies the postulation that other euploid levels also may occur naturally, including triploids, tetraploids, decaploids, 12 ploids, and 16 ploids.
Of these, the even multiples tetraploids, decaploids, 12 ploids, and 16 ploids should be at least partially fertile.

Studies on the relationship between production of albino berries and mixoploidy in the strawberry (*Fragaria x ananassa* cv. Kent) was done during summer of 1997 and 1998 (http). As clonal origin seemed to be correlated with this disorder, they characterized normal and abnormal plants by RAPD and chromosome numbers. RAPD analysis did not yield polymorphism. However, chromosome counts revealed mixoploidy in 63% of the cells evaluated in the abnormal plants when compared to 40% in the normal plants; numbers of chromosomes was found to be varying from 44 to 106 and 51 to 105 respectively. The results suggest that there could be a critical level for the presence of mixoploid cells, which seems to be associated with the appearance of phenotypic or biochemical abnormalities like albinism.

Studies on the ecological differentiation in perennial, octoploid species of *Fragaria* (2n = 56) had been conducted by Hancock and Bringhurst (1979). It has been suggested that ecological differentiation plays a minor role in determining the eco-geographical range of allopolyploids due to the effects of polyploidy on the generation of mutational and recombinational variability, which has not been the case in *Fragaria*.

Recently karyotype and ribosomal gene mapping had been made in *Fragaria vesca* (http). Fluorescent *in situ* hybridisation (FISH), with cloned probes for the ribosomal RNA genes 45S and 5S, were used for detecting the location of the rDNA gene sequences on the chromosomes of *Fragaria vesca* (2n = 2x = 14). Three pairs of 45S loci and a pair at 5S locus were identified and these provided 3 chromosomal markers for the karyotype of the seven chromosome pairs.

According to Hancock and Luby (1993) the cultivated strawberry (*Fragaria x ananassa*) is a hybrid derived from two New world species, *F. chiloensis* and *F. virginiana*, in the mid 18th century and all three species are octaploids (2n = 8x = 56).
Ahokas (1998) described a method for inducing polyploids in detached stolons using an aqueous solution of colchicine, DMSO and glycerol. About 10% of the treated stolons, starting with diploid and tetraploid strawberry, are converted, but 50% non viability is caused by the treatment. The method can be applied to some other species and shoot types, using other chemicals with chromosome doubling or reducing effect, or even mutagens.

An interspecific hybrid was obtained from the cross of *Fragaria nilgerrensis* (2n = 2x = 14), a diploid species derived from China, and two cultivars of octaploid *F. ananassa*. (2n = 8x = 56) by the means of immature embryo culture technique with the latter as the pollen parent (Ma and Chen, 2004). The hybrids were pentaploids and have poor fertility. The hybrids showed expected 2n = 35 chromosomes in somatic cells and shows abnormal meiotic chromosome associations forming aberrant microspores.

A recent work reports cytological studies on unreduced gamete formation of strawberries (*Fragaria*) (http). A diploid strawberry (*Fragaria vesca*) which can naturally produce unreduced gametes (2n pollen) and doubled unreduced gametes (4n pollen) was used to study the cytological mechanism of 2n and 4n gamete formation. The result showed that the formation of 2n gamete was mainly due to the abnormal orientation of spindles at metaphase.

The phenotypical and cytogenetical characterization of intergeneric hybrids of *Fragaria x Potentilla* and some ornamental varieties of *Fragaria* with unknown origin were made by Sutan and Popescu (2006). The ploidy level of the intergeneric hybrids of *Fragaria x Potentilla* was found to be different, depending on their origin.

In strawberry the octoploids are diploidized allopolyploids that have descended from four diploid ancestors. Although the diploid ancestry of the octoploid has yet to be definitively established, the leading ancestral candidate diploids are *F. vesca* and *F. iinumae*, with *F. bucharica* and *F.*
mandshurica as additional intriguing possibilities (Folta and Davis, 2006). Currently, 23 species are recognized in the genus *Fragaria*, including 13 diploids ($2n = 2x = 14$), four tetraploids, one hexaploid, and four octaploids (Folta and Davis, 2006).

According to Davis *et al.* (2007), the cultivated strawberry is genomically complex due to its octaploid ($2n = 8x = 56$) composition. Chromosome studies conducted by Preeda *et al.* (2007) revealed somatic chromosomes in *Fragaria vesca* ($2n = 14$) and *Fragaria x ananassa* ($2n = 56$). They used 0.002 M 8-hydroxyquinoline as cytostatic solution, fixed using 3 : 1 absolute alcohol : glacial acetic acid for 40 min, hydrolyzed in the 1N HCl solution at room temperature for 2 h, macerated using an enzyme solution for 25 min at 42 °C, and stained in 1.5% lacto-propionic orcein solution. On the other hand, in case of DAPI staining, the macerated root tips of *F. x ananassa* were soaked in 60% acetic acid for 5 min. before staining. Their respective 14 (diploid) and 56 (octaploid) chromosomes were counted.

Very recently Ahokas (2008) had described a tetraploid ($2n = 28$) clone of *Fragaria* from SW Finland. The following alternatives are considered as possible reasons for the tetraploidy; unreduced gametes in an interspecific hybrid or hybrid derivative; hybridization followed by induction of tetraploidy either by viruses, or by industrial pollutants, *e.g.* chlorophenols and their congener compounds in the soil. A hexaploid (*F. moschata*) diploid hybrid leads directly to tetraploidy and a tetraploid derivative from an octoploid (*F. x ananassa*) diploid hybridization can also be obtained.

The karyotypes and chromosome associations at meiosis in two types of natural hybrids, 7x and 8x, between *Duchesnea chrysanthaa* (2x) and *D. indica* (12x) were investigated by Naruhashi and Iwatsubo (1991). The 7x hybrid had a haploid chromosome set from each parent plant, whereas the 8x hybrid was considered to have a full set of *D. chrysanthaa* and half a set of *D. indica*. In the two hybrids, the chromosomes of *D. chrysanthaa* and *D. indica* conjugated only slightly at meiosis. It is probable that no common
genome set between the diploid *D. chrysantha* and the dodecaploid *D. indica* exists. They concluded that *D. chrysantha* and *D. indica* should be considered to be distinct species, although they have sometimes been treated as a single species.

RAPD analysis proves to be very useful to detect the genomic changes that usually occur in the highly repetitive fraction of the genome and are limited to a specific subset of these sequences which may be localized at particular chromosomal sites. RAPD markers were used for distinguishing various plant species by several workers. Major *et al.* (1998) compared the isozyme and RAPD analysis to identify the variability among the clones of *Robinia pseudoacacia*. There are reports on the use of RAPD in determining the genetic relationship and variation in many plant species (Yu and Pauls, 1993; Liu *et al*., 1994; Swobodha and Bhalla, 1997; De Bustos *et al*., 1999). This technique had been used for genetic analysis of micropropagated plants (Rani *et al*., 1995; Shoyama *et al*., 1997; Goto *et al*., 1998; Watanabe *et al*., 1998). Several workers applied RAPD to detect somaclonal variation (Bohm and Zyprian, 1998; Al-Zahim *et al*., 1999; De Verno *et al*., 1999) and to identify micropropagated plants and cultivars (Ho *et al*., 1997). RAPD markers were used for distinguishing plant species such as cereals (Ko *et al*., 1994) and *Festuca* (Valles *et al*., 1993) and also for genetic mapping (Williams *et al*., 1990; Yu and Pauls, 1993). Valles *et al*. (1993) used this technique for analyzing genetic stability of tissue cultured plants. Study of DNA polymorphism, marker assisted breeding programmes *etc.* also rely upon RAPD studies. Other applications are in creating linkage maps, locating defective and disease resistant genes, identification of chromosome specific markers *etc.* According to Gallego and Martinez (1997), RAPD is found to be technically easier with low statistical errors.

There are reports on the use of RAPD based technology for studying genetic relationships and diversity in some of the members of Rosaceae. Molecular analysis of a random sample of roots obtained from almond,
apple, plum, *Pyrus pyraster* and two hybrid rootstocks, after co-culture with *Agrobacterium rhizogenes*, with and without the addition of hormones was done by (Damiano and Monticelli, 1998). Amplification of the sequences of *rolB* and *vir* genes was done using PCR.

Forty one of the major strawberry (*Fragaria x ananassa*) cultivars grown in the United States and Canada were examined by RAPD (randomly amplified polymorphic DNA) marker polymorphisms using 10mer primers (> 50 % GC content) by Degani et al. (1998). A set of 10 primers produced 15 polymorphic fragments ranging in size between 450 and 1200 bp, which were more than sufficient to distinguish among all tested cultivars. Ten of the markers derived from seven primers were absolutely required for distinguishing the cultivars. They demonstrated that RAPD markers can be used effectively for strawberry cultivar identification.

Bartish et al. (1999) studied genetic relatedness in *Chaenomeles* (Rosaceae) by RAPD analysis in 42 plants representing accessions of three wild species and one hybrid taxon. Amplification with 17 primers yielded a total of 156 polymorphic RAPD bands. Genetic relatedness was estimated among the different species of *Chaenomeles*, suggesting the most distantly related species, the most closely related species and which takes an intermediate position between these two.

Shimada et al. (1999) investigated the genetic diversity of 42 plum varieties by RAPD analysis. Twenty primers discriminated all plum varieties thereby revealing the genetic distinctness of each variety from the other plums by cluster analysis.

The combination studies of *in vitro* embryo culture with the use of molecular markers were made by Hormaza (1999). In this work the combination of those two techniques has been used to assess, with certainty, the paternity of embryos obtained after mixed pollinations with pollen from three cultivars of cherry (*Prunus avium*).
The taxonomy of the dog-roses (*Rosa* sect. *Caninae*) were analysed with the help of molecular markers by Olsson *et al.* (2000). They used a novel combination of random amplified polymorphic DNA (RAPD) markers and elliptic Fourier analysis of leaflet shape to investigate relationships within and between the seven common dog-rose taxa in the Nordic countries.

Genetic relationships among four taxa in the genus *Chaenomeles* using isozyme analysis were studied by Garkava *et al.* (2000). The band patterns obtained with six polymorphic isozyme systems provided 108 reliable markers, which were scored as unordered multistate traits. A cluster analysis as well as a multidimensional scaling analysis grouped the taxa in agreement with previously published results obtained with RAPD (random amplified polymorphic DNA) analysis. However, the isozyme data were less efficient than the RAPD data for intraspecific grouping of the genotypes according to the origin of the plant material.

RAPD analysis was applied to reveal the genetic diversities of 4 species of subg. *Lithocerasus* within the genus *Prunus* using 40 accessions representing the subgenera *Prunophora*, *Amygdalus*, *Lithocerasus* and *Cerasus* (Takehiko *et al.*, 2001).

The extent of clonality and genetic diversity in *Lyonothamnus floribundus*, or island ironwood (Rosaceae), an endemic species found on only four of the eight California Channel Islands had been analysed by Bushakra *et al.* (2003). They have used random amplified polymorphic DNA (RAPD) analysis to examine clonality and genetic diversity in *L. floribundus*.

Genomic studies of Rosaceous fruit trees conducted by Arus and Gardiner (2007) have concentrated on two species; peach (*Prunus persica*), which has served as a model for other species of the same genus, such as the stone fruits (apricot, cherry and plum) and almond; and apple (*Malus x domestica*), which itself is a model for other close species such as pear, quince and loquat.
Several earlier workers had conducted molecular research on the different strawberry species. Few isoenzymatic markers have been used in the identification of variants and in evaluation of the genetic diversity of strawberries. Apparently, the studies on isoenzymes are not highly effective (Arulsekar et al., 1981; Bringhurst et al., 1981).

Genetic diversity studies can be very effectively conducted with the help of RAPD, which has been proved to be successful in Chilean *Fragaria chiloensis* accessions. (15http). The genetic diversity of a Chilean *Fragaria chiloensis* collection (82 accessions collected between 34° and 48° S) was assessed with a genomic analysis technique known as RAPD. Out of 160 primers evaluated only 10% turned out to be informative, yielding a total of 38 polymorphic bands.

In recent years genetic markers associated with usable properties have proved particularly useful in plant breeding. Genetic markers enable observation of regrouping in strawberry genomes and permanent and relatively quick analysis of the segregation of alleles, which facilitates the selection of mixed species (Levi et al., 1994).

Graham et al. (1996) analysed genetic similarities between 8 cultivars of strawberry. Using 10 primers for their analysis, they obtained 116 bands, 79 (68%) of which were polymorphic and 37 (32%) were monomorphic.

The level of genetic diversity between 16 cultivars of strawberry (*Fragaria x ananassa*) and the wild species of *Fragaria virginiana* was studied on the basis of the analysis of their DNA by RAPD reaction (16http). Six 10-nucleotide primers generated jointly 354 bands, of which 94.8% were polymorphic and 5.2% monomorphic.

Relationships among 37 North American octaploid strawberry populations were studied by evaluating 44 morphological traits and 36 randomly amplified polymorphic DNA (RAPD) markers (Harrison et al., 1997). RAPD data revealed that all octaploid North American strawberries
have likely derived from a common ancestor and have differentiated into *F. chiloensis* and *F. virginiana* by adapting to moister and drier environments, respectively.

RAPD analysis had been conducted to find out the relationship of North and South American subspecies of *Fragaria chiloensis* (Porebski and Catling, 1998). To improve the intraspecific classification of *Fragaria chiloensis*, 35 plants including 5 North American subsp. *lucida*, 15 North American subsp. *pacific*, and 15 South American subsp. *chiloensis* were analysed. From 100 primers screened, 12 provided 62 scorable polymorphic bands.

Phylogenetic studies can be very effectively done by using random amplified hybridized fragment polymorphism (RAHFPs) as has been found in the case of some *Fragaria* spp. (http) with the aim to understand better the genomic structure of the cultivated strawberry and the genetic relationship among the octaploid *Fragaria* species. Out of the 92 random primers of 10bp, several DNA fragments were obtained and 84 out of 213 were utilized like probes. The hybridizations were performed either on amplification products or on digested genomic DNA fragments. The polymorphisms obtained indicated that the class of molecular marker named RAHFPs are more informative than RAPDs.

Micropropagated strawberry plants (*Fragaria x ananassa*) grown on 5 μM and 15 μM BA medium or cold-stored were grown in the field to examine morphological variation (Kumar et al., 1999). Except for plant height, morphological characteristics did not differ for field grown plants micropropagated on 5 μM and 15 μM BA medium. Cold-stored plants were less vigorous, both vegetatively and reproductively, than BA treated plants. Random amplified polymorphic DNA (RAPD) markers were used to determine if cold storage or supra-optimal levels of N⁶-benzyladenine (BA) in the culture medium caused genetic changes leading to somaclonal variation. Analysis was made in 246 loci amplified by the 29 primers tested. Possible
changes in methylation patterns of ribosomal DNA genes of strawberries were also examined.

Morphological, anatomical and molecular techniques were used to characterize wild strawberry and wild strawberry-like species in northwest Argentina (Ontivero et al., 2000). Characteristics of leaves, flowers, runners, achenes, and genomic DNA polymorphisms were used to analyze similarities among Potentilla tucumanensis, Duchesnea indica, and Fragaria vesca. Comparison of phenograms obtained by using morphological and anatomical traits or genomic DNA characters revealed similar clustering of the species. Both phenograms suggest that D. indica is more closely related to P. tucumanensis than to F. vesca. Using the randomly amplified polymorphic DNA (RAPD) technique with specific primers, they detected polymorphic bands that permit the identification of P. tucumanensis, D. indica, and F. vesca. In addition, they report new morphological and anatomical characters that can be used as diagnostic traits for better identification of species in reproductive and vegetative states.

Congiu et al. (2000) used random amplified polymorphic DNA (RAPD) technique to settle a lawsuit involving unauthorized commercialization of a patented strawberry variety of high economical relevance (Marmolada). Because of economical involvements, the molecular approach was added to the more traditional morphological examination in a double-blind test. All plants belonging to the patented variety were unambiguously identified (13 plants among a total of 31 plants examined). The results were accepted as evidence in the court. This study confirms that the RAPD technique is especially suitable for identification of asexually reproduced plant varieties for forensic or agricultural purposes.

A detailed work was conducted to determine the genetic diversity of a representative sample of the Chilean strawberry (Fragaria chiloensis) using biochemical and molecular markers (Becerra et al., 2001). They compared the information generated by random amplified polymorphic DNA (RAPD)
diversity within the same accessions and also to determine the feasibility of using this information for a strawberry breeding and improvement program.

Nineteen of the major strawberry (*Fragaria x ananassa*) cultivars grown in the United States and Canada were examined for AFLP and RAPD marker polymorphisms (Degani *et al.*, 2001). The RAPD markers had been specifically selected for fingerprinting purposes because they successfully distinguish 41 strawberry cultivars, including the 19 cultivars analyzed. Separate dendrograms were constructed based on analysis of the AFLP and RAPD marker data using a neighbor-joining algorithm. A detailed comparison of genetic relationship measures in strawberry (*Fragaria x ananassa*) had been made based on AFLPs, RAPDs and pedigree data.

Kuras *et al.* (2004) used two PCR based techniques, RAPD and ISSR, for determination of genetic relationship of 24 strawberry cultivars used in breeding program at the Research Institute of Pomology and Floriculture in Poland. Polymorphism of investigated genotypes was observed in reactions with 23 out of 48 tested RAPD primers and 41 from 90 tested ISSR primers. Recently molecular characterization and genetic diversity in *Fragaria* genotypes were revealed by randomly amplified DNA polymorphisms (18http). The efficiency of RAPD markers for undertaking *Fragaria* DNA fingerprinting and the estimation of genetic relatedness was evaluated. Thirteen cultivars of *Fragaria x ananassa* and two wild species, *Fragaria indica* and *Fragaria vesca* were analyzed with twenty 10 bp primers. Only thirteen yielded scorable polymorphic amplification patterns based on discernible bands, however, no single primer out of these thirteen could produce a unique fingerprint for all the fifteen genotypes.

Gambardella *et al.* (2005) conducted studies on the molecular and morphological characterization of wild and cultivated *Fragaria* growing in southern Chile. Hokanson *et al.* (2006) conducted studies to find out the relationships among the new world octaploid strawberry species, *Fragaria virginiana* and *Fragaria chiloensis*, based on simple sequence repeat marker
analysis. Marker analysis revealed that these two new world octaploid strawberry species are closely related. Experimental studies were conducted by Sugimoto et al. (2006) to identify markers for the everbearing gene in strawberries (*Fragaria x ananassa*). Seventy one primers, which produced 89 polymorphic fragments between the two parents, were identified from a total of 175 primers.

Random amplified polymorphic DNA (RAPD) markers were effectively used to evaluate genetic variability among populations of an Italian strawberry ecotype, and to determinate genetic relationships between genotypes and their putative ancestor (Milella et al., 2006). A total of 222 RAPD markers were obtained using 16 decamer primers and 6 longer primers, 90.8% of the markers obtained by selected primers were turned to be polymorphic at least within analysed genotypes. The results obtained confirm that RAPD markers could be used as reliable markers to perform phylogenetic studies in *Fragaria x ananassa*.

One hundred and two accessions of wild and cultivated *Fragaria chiloensis*, collected in southern Chile were studied (*http*). They were characterized using isozymes and RAPD markers. Out of the 80 Operon primers used, 17 were selected because they provided a greater number of polymorphic bands. The information was used to evaluate the genetic variability among wild clones. The results indicated that there exists a high degree of genetic variability among the different accessions and that four genetically related groups can be identified.

Cytogenetics and RAPD analysis of interspecific hybrids of two *Fragaria* spp. had been conducted by Ma et al. (2007). Embryo rescue obtained interspecific hybrids of *Fragaria mandschurica* and *F. x ananassa* were used as materials for the identification of somatic cell hybridization between the species. RAPD analysis showed that the hybrid offspring showed similarities with parents. Individual genetic heterogeneity existed among the generations were also revealed.
Previous reports reveal that the members of Rosaceae are blessed with an array of aromatic compounds, which are stored in various parts of these plants. Vollmann and Schultze (1995) studied the root essential oils of several members of the genera *Geum*, *Waldsteinia* and *Coluria*, of the subtribe *Geinae* (Rosaceae) by GC-MS. The qualitative composition of these oils was relatively similar with eugenol and some pinane derivatives, *e.g.* myrtanals, myrtenal, myrtenols and myrtenol, as characteristic constituents. The oils differed considerably concerning the quantitative composition, *e.g.* two groups of *Geum* species could be observed, one with a high (> 65%) and one with a low (< 5%) content of eugenol. In the root oil of *Waldsteinia ternata*, only traces of this phenyl propanoid were found. Headspace analysis of a root extract of *W. geoides* showed a high percentage of cinnamyl alcohol.

Volatile constituents of the essential oil of flowers of *Rosa brunonii* was studied by Kaul *et al.* (1999). The essential oil of the flowers of *Rosa brunonii* (Rosaceae) was prepared by hydrodistillation and studied by capillary GC-FID and GC-MS; 35 constituents were identified, accounting for 78.4% of the total oil. The essential oil consisted mainly of eugenol (30%), citronellol (2.65%), geraniol (10.5%) and terpinen-4-ol (13.7%) as the major compounds.

Demetzos *et al.* (2002) reported the chemical composition of the essential oils of twenty five populations of *Cistus creticus* subsp. *creticus* (Rosaceae) from the island of Crete (Greece) and their interpopulation variability were analysed in detail by GC-MS. 142 compounds were identified representing an average of 56.8 - 89.8% of the oil composition. The components are represented here by homologous series of monoterpenes, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, diterpenes, labdane diterpenes, aldehydes, alkanes, esters, fatty acids, ketones, and others. Labdane diterpenes were detected and identified in the essential oils and have been found in high percentage composition.
Essential oil analysis of three species of “Chamae Roses” was made by Tucker et al. (2003). The essential oil of *Chamaebatia millefolium* from California is dominated by 24.90 ± 4.46% camphor, 17.36 ± 4.23% borneol, 11.17 ± 4.26% camphene, and 10.95 ± 4.59% α-pinene. The essential oil of *Chamaebatia australis* from California is dominated by no constituent greater than 10% but contains 7.07 ± 0.97% δ-cadinene, 6.84 ± 1.47% terpinen-4-ol, and 5.46 ± 1.50% linalool. The essential oil of *Chamaebatia foliolosa* from California is dominated by 12.90 ± 3.67% unidentified sesquiterpene alcohol and 15.96 ± 6.61% viridiflorol.

Eutuxia and Loannis (2005) analysed the volatile constituents of the jam of *Crataegus azarolus* (Rosaceae) using simultaneous distillation and extraction and identified by gas chromatography - mass spectroscopy. Forty four volatile compounds were identified for the first time in this jam, while 12 peaks were not identified. The volatile compounds were quantitatively determined by the use of an internal standard. The major classes of compounds identified included aliphatic and aromatic aldehydes, ketones, alcohols, monoterpenes, sesquiterpenes and hydrocarbons. The major constituent identified was 2-furaldehyde. Physicochemical properties of the seed oils of three species of Rosaceae family, namely *Prunus armeniaca* (apricot), *P. cerasifera* (prune), and *P. persica* (peach) have been determined by Javed et al. (2006).

Previous reports show that considerable amount of work had been done to evaluate the chemical components present in the strawberry plants. Larsen et al. (1992) evaluated the aroma composition of some strawberry (*Fragaria ananassa*) cultivars by the use of odor threshold values.

Hamilton Kemp et al. (1993) isolated headspace compounds from detached strawberry (*Fragaria ananassa*) foliage by using both air and nitrogen as entrainment gases and trapping on the porous polymer Tenax. Compounds were eluted from traps with hexane, analyzed by GC and GC -MS, and identified by comparison with authentic standards. The profile
of volatiles entrained with nitrogen differed considerably from that obtained with air; the former yielded more aliphatic alcohols, esters, and aromatics and the latter yielded greater quantities of terpene hydrocarbons.

GC – MS studies of the essential oil composition of three strawberry genotypes, *Fragaria x ananassa* were made by Khanizadeh and Belanger (1993). Thirty seven compounds were detected of which sixteen were identified. The major components were linalool (16.08 - 18.80%) and nonanal (5.89 - 16.63%). Many of the other constituents were aliphatic in nature. Differences in oil composition among the three cultivars were observed.

Two isomeric methyl jasmonates were isolated from extracts of strawberry fruit (*Fragaria x ananassa* cv. Kent) by means of micropreparative gas chromatography (Gansser *et al*., 1997). It was found that the levels of methyl jasmonates in strawberry may affect aroma formation and further events during fruit development.

The aroma compositions of *Fragaria x ananassa* varieties are assessed by purge and trap high-resolution gas chromatography by Gomes da Silva and Chaves das Neves (1999). Gas chromatography - mass spectrometry and gas chromatography - Fourier transform infrared spectroscopy allow the identification of 93 components from which 21 are for the first time described as constituents of strawberry aroma.

Volatile flavour components of two strawberry (*Fragaria x ananassa*) varieties, Bogyojosaeng and Suhong, are extracted by SDE (Simultaneous steam distillation and extraction) using a mixture of n-pentane and diethylether (1 : 1, v/v) as an extract solvent (Eun-Ryong *et al*., 2000). Analysis of the concentrate by capillary gas chromatography and gas chromatography - mass spectrometry led to the identification of 146 and 153 components in Bogyojosaeng and Suhong respectively. Among these, (E)-2-hexenyl acetate (4.56%) in Bogyojosaeng and (E)-nerolidol (12.38%) in Suhong were major compounds and acetic acid, (E)-2-hexenal, hexyl
acetate, ethyl acetate, ethyl butanoate, methyl butanoate, ethyl hexanoate and γ-dodecalactone were the main components in each sample, though there were several differences in composition and threshold of volatile compounds.

Studies conducted by Ruan et al. (2001) reveal the chemical constituents from the fruit of *Fragaria x ananassa*. They used chromatographic methods to isolate compounds and followed chemical and spectral methods to elucidate their structures. Three compounds, 9, 19-cyclolanost-24-en-3-ol, 14-methyl-stigmasta-7, 24(28)-dien-3-ol and beta-sitosterol were isolated from the freeze dried powder.

Total soluble phenols, soluble flavanols, (+)-catechin, ferulic acid and 1-O-feruloyl-beta-d-glucose were analyzed during the development of a strawberry (*Fragaria x ananassa*, cv. Chandler) callus culture by Lopez Arnaldos et al. (2001). The time course changes of the different phenols assayed were well correlated with callus growth and morphology.

Volatile components of strawberries (*Fragaria x ananassa* cv. Korona) kept under low oxygen conditions had been evaluated by Rosenfeld et al. (2003). Compounds responsible for off-flavors in fruits kept in anaerobic atmosphere have partly been identified by means of GC-sniff. Ethyl acetate, ethanol, ethyl butanoate, butyl acetate and ethyl hexanoate showed relatively high concentrations in samples stored at 0.5 - 2% of oxygen and may together with ethylene cause off-flavors like fermented flavor.

Twenty six compounds, mainly flavanoids, coumarins and phenolcarbolic acids, were observed in *F. vesca* leaves by HPLC and TLC (Bubenchicova and Drozdova, 2003). Seven of these compounds were identified for the first time. Results showed that the carbohydrate complex of the leaves consists of water soluble polysaccharides, pectins and hemicelluloses. Pectins and hemicellulloses are isolated from *F. vesca* leaves for the first time.
High performance liquid chromatography combined with diode array and electro spray ionization mass spectrometric detection was used to study soluble and insoluble forms of phenolic compounds in strawberries, raspberries (red and yellow cultivated and red wild), arctic bramble, and cloudberries (Maatta-Riihinen et al., 2004). Hydroxy-cinnamic acids were present as free forms in cloudberries and mainly as sugar esters in the other berries. Quercetin 3-glucuronide was the typical flavanol glycoside in all of the berries studied.

Strawberry fruits (Fragaria x ananassa cv. Elsanta) were harvested at the ideal stage of maturity and their volatile compound profile was analysed using both Atmospheric Pressure Chemical Ionisation–Mass Spectrometry (APCI–MS) and Gas Chromatograph – Mass Spectrometry (GC – MS) by Modise et al. (2004). There was good agreement on the compound identity using the two techniques for nine out of fourteen selected volatile compounds.

The fruits of 23 strawberry cultivars (Fragaria x ananassa) were studied to determine the contents of soluble solids, organic acids, ascorbic acid, sugar and anthocyanins (Zmuda et al., 2004). The cultivars contained 7.14 (Dukat) to 9.84% (Mara des Bois) soluble solids. The sugar and total organic acid contents ranged from 4.09 (Dukat) to 6.21% (Calypso) and 0.67 (Senga Sengana) to 1.06% (Mara des Bois), respectively. Elsanta and Gerida had the highest ascorbic acid content (~60 mg). Kama and Honeoye had the highest anthocyanin content (more than 800 mg/kg fresh weight).

Aaby et al. (2005) measured total phenolics and total monomeric anthocyanin content in the flesh and achenes of strawberries (Fragaria x ananassa). Ellagic acid, ellagic acid glycosides, and ellagitannins were the main contributors to the antioxidant activities of achenes. The major anthocyanin in flesh was pelargonidin-3-glucoside, whereas achenes consisted of nearly equal amounts of cyanidin-3-glucoside and pelargonidin-3-glucoside.
Carrasco et al. (2005) revealed the chemical constituents responsible for the most popular strawberry aroma. Esters and furanones are the main aroma determinants in fresh strawberries. They also identified the inheritance patterns of the aroma trait in segregating populations of strawberries.

A detailed chemical and sensory analysis of the fruits of strawberry was conducted by Lawson et al. (2006). They have detected diacetyl compounds in strawberry fruit extract, which contributes to the quality of strawberry juice. A review on the biosynthesis of three major classes of flavor compounds in strawberry, namely carbohydrates, esters, and furanones were made by Bood and Zabetakis (2006). They are qualitatively discussed with respect to their importance in fruit flavor, their biochemical formation, and the biochemical relationships between each other and fruit ripening.

Phenolic compounds in strawberry (Fragaria x ananassa) fruits were identified by Aaby et al. (2007). They characterized about 40 phenolic compounds including glycosides of quercetin, kaempferol, cyanidin, pelargonidin, and ellagic acid, together with flavanols, derivatives of p-coumaric acid and ellagitannins. Quercetin-3-malonylhexoside and a deoxyhexoside of ellagic acid were reported for the first time.

The total phenolic, flavonoid and anthocyanin content of achenes (true fruit) and thalamus (receptacle) from the native South American Fragaria chiloensis subsp. chiloensis (F. patagonica and F. chiloensis), Fragaria vesca and Fragaria x ananassa cv. Chandler was determined by Cheel et al. (2007). Highest phenolic content was found in F. vesca while lowest content was measured for white strawberry (F. chiloensis subsp. chiloensis). The total anthocyanin and total flavanoid contents in the samples investigated was lower for the white strawberry and higher in F. x ananassa cv. Chandler.
The phenolics from different strawberry cultivars (Aromas, Camarosa, Diamante, Medina and Ventana) cultivated in two different soil less systems (with and without recycling nutrient solution) were quantified to assess differences in their profiles as a function of both the variety and the cultivation system (Hernanz et al., 2007). Considering groups of phenols, it was found that either anthocyanins (including pelargonidin - 3 - glucoside, cyaniding - 3 - glucoside, pelargonidin – 3 - rutinoside, pelargonidin – 3 - acetylglucoside and two unidentified pelargonidin derivatives) or phenolic acids (including caffeic, ferulic, p-coumaric, p-hydroxybenzoic, and ellagic acid) were quantitatively more important than those of flavanols (quercetin and kaempferol).

Ozgen et al. (2007) conducted studies on the determination of total phenolics. They found that strawberry plants are rich in phenolic compounds and possess potent antioxidant activity. Ulrich et al. (2007) conducted prospective analysis of four accessions of four wild strawberry accessions in comparison to a standard cultivar of Fragaria x ananassa by using human sensory, gas chromatography - mass spectrometry (GC - MS) and gas chromatography – olfactometry (GCO) analyses. The wild species have higher aroma intensities compared with the cultivated one. The flavour quality differs significantly. Semi quantitative GC analysis revealed that F. x ananassa cv. ‘Elsanta’ has the lowest content of volatile compounds whereas Fragaria moschata cv. Cotta has the highest. The aroma impressions, measured by GCO, support the findings of GC - MS analyses.

Tung et al. (2007) studied the chemical constituents of the wild strawberry plant (Duchesnea indica) from China. By employing a variety of chromatographic techniques like the chemical constituent purification and spectral data analysis etc. Several compounds were identified as short leaf hematoxylin acid, short leaf hematoxylin methyl phenol, short leaf hematoxylin phenol, Hill kaempferol, kaempferol, oleic acid, oleanolic acid, β - sitosterol and 6 - β hydroxyl - 2, 4 - ethyl - cholesteric - 4 – en - 3 - ketone.

The inheritance of important aroma compounds, especially the ester methyl anthranilate in strawberry is demonstrated by the use of a model.
population of *Fragaria x ananassa* (Olbricht *et al*., 2008). Studies conducted by Pinto *et al*. (2008) revealed that strawberry fruits are rich in various bioactive compounds. They quantified total ellagic acid present in the fruits of strawberries (*Fragaria x ananassa*).

Aroma components of amphiploid strawberries derived from interspecific hybrids of *Fragaria x ananassa* and diploid wild species were detected recently (http). The aroma components of amphiploids of *F. vesca* x *F. x ananassa* and *F. x ananassa* x *F. nilgerrensis* method were analyzed by GC - MS. Major aroma compounds of *F. x ananassa* were methyl butylate, ethyl butylate, methyl butyric acid, acetic acid and 2, 5 – dimethyl - 4-hydroxy (2H) furanone. *F. vesca* contained a high amount of ethyl acetate, but amounts of methyl butylate, ethyl butylate, organic acids and furanones were low. *Fragaria nilgerrensis* contained a high level of ethyl acetate and furanone, but amounts of methyl butylate, ethyl butylate and organic acids were low.

Recently a detailed analysis of volatile aromatics in the fruit extracts of ‘Toyonoka’ strawberries (*Fragaria x ananassa*) was made (http). 52 volatile aromatic compounds were detected, out of which 2,5-dimethyl - 4 – hydroxy - 2H – furan – 3 - one (DMHF) was found to be the prominent one.

Even though Indian strawberry is closely related to cultivated commercial strawberries (*Fragaria* spp.), reports on research work of *Duchesnea indica* is scanty. However, the wide spectrum of literature collected regarding the work done on the various aspects of some rosaceous plants and commercial strawberries may help in comparing *D. indica* with its close allies. Apart from a very few reports cited earlier (Naruhashi and Iwatsubo, 1991; Ontivero *et al*., 2000; Tung *et al*., 2007) cytological, RAPD and GC -MS analyses of the *in vivo* and *in vitro* plants of Indian strawberry, *D. indica* had not been attempted. Hence the present investigation is carried out on all these aspects in *D. indica*. 